Understanding the mechanism(s) of hydro-priming to improve seed vigour and seedling establishment of *Solanum lycopersicum*

Submitted by Isabelle Véronique Marie Camu to the University of Exeter as a thesis for the degree of Doctor of Philosophy in Biological Sciences
In September 2017

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

(Signature) ……

Abstract

Seeds are very complex and diverse plant organs. Seed germination is the most sensitive stage of plant life and is influenced by various environmental signals including phytohormones, salt, light, temperature and water potential. Seeds have an innate mechanism called dormancy that blocks germination, and plants have developed several dormancy-inducing strategies to optimise the timing of germination.

Seed germination vigour is an important factor in crop yield. Seedling vigour is defined as the sum of the seed properties which determine the level of activity and performance during germination and seedling emergence. A poor seed lot can be improved by post-harvest treatment such as hydro-priming as it is used in the seed industry, but the biological mechanism is unknown. The aim of this study is to understand the mechanism(s) of hydro-priming in order to improve seed vigour and seed germination. I set seeds at different temperatures to produce variation in seed vigour and it showed that germination of seeds with low vigour can be improved by hydro-priming. Using LC-IT-ToF/MS I characterised compounds that leach from seeds during hydro-priming, and showed that some of these are putative germination inhibitors. Adding these compounds to the water during hydro-priming showed that the inhibitory effect of these compounds is not the main mechanism that regulates germination. Also, transcriptomic analysis showed that genes involved in OPDA pathway are expressed during hydro-priming as well as during endosperm weakening cap associated genes. I concluded that hydro-priming improves the speed of germination of low temperature set and its efficiency is dependent of activation of metabolic activity.

Table of Contents

List of figures

List of tables

Acknowledgments

I would like to thank Steve Penfield for his supervision and his guidance during these years. I am thankful to the Penfield lab for the support as I carried out this project. In particular, I would like to thank Dana McGregor, Albor Dobon, Min Chen and Carmel O'Neill for sharing knowledge and ideas and discussing hypotheses.

Also, I would like to thank the people I collaborated with: Fernando Goffman for his guidance on tomato knowledge, I am thankful to Minako Kaneda for her support and being so enthusiastic during my training at Enza Zaden.

I would like to thank the molecular analysis department in John Innes Centre, in particularly, Lionel Hill, Baldeep Kular and Paul Brett.

Also, my friends have been an important resource for these years, especially Fei-Yian, Joyita, Cyrielle, Bogna, Rita and Jessica who empathise with me in different occasions on various situations.

Least but not last, thanks to my family who provided support and help during this challenging and demanding years. Special thanks goes to Cyrille to understand challenging periods.

Declaration

I declare that I am the sole author of the work in this thesis and that it is the original except where indicated by special reference in the text. No part of this degree has been submitted for any other degree.

The ionomic analysis outlined in section 2.9 was performed by ALcontrol Laboratories.

Running of RNA samples for RNA-sequencing on Illumina (section 2.14) was performed by Richard Feron. RNA-sequencing and preliminary analyses (section 2.14) was performed by Minako Kaneda and Richard Feron.

The seeds of *acx1a* mutant, *spr2* mutant and 35S::SlRNA were a gift of Bettina Hause.

Abbreviations

AAO	ABA aldehyde oxidase
ABA	Abscisic acid
ABI	ABA INSENSITIVE
ACC	1-aminocyclopropane 1-carboxylic acid
ACO	1-aminocyclopropane 1-carboxylic acid oxidase
ACS	1-aminocyclopropane 1-carboxylic acid synthase
ACX	Acyl CoA oxidase
AGO	ARGONAUTE
AOC	Allene oxide cyclase
AOS	Allene oxide synthase
AR	After-ripening
ATXR	ARABIDOPSIS TRITHORAX-RELATED
bZIP	Basic leucine zipper
Ca	Calcium
CIPK	CBL-INTERACTING PROTEIN KINASE
COI1	CORONATINE INSENSITIVE1
CON	Control without added hormone
CPS	*ent*-copalyl diphosphate synthase
CTS	COMATOSE
Cv	Cultivar
Cvi	Cape Verde Island
CYP707A	Cytochrome monoxygenase 707A (p450)
DAD1	DEFECTIVE IN ANTHER DEHISCENCE1
DAS	Days after sowing
DEG	Differentially Expressed Gene
DEP	DESPIERTO
DGL	DONGLE
DOG1	DELAY OF GERMINATION
DNA	Deoxyribonucleic acid
EC	Electrical conductivity
EDTA	Ethylene Diamine Tetraacetic Acid
EGTA	Ethylene Glycerol Tetraacetic Acid
EIN	ETHYLENE INSENSITIVE
ERF	ETHYLENE RESPONSE FACTOR
ETR	ETHYLENE RESISTANT
EXP	Expansin
FD	FLOWERING LOCUS D
Fe	Iron
FIS	FERTILIZATION INDEPENDENT SEED
FLC	FLOWERING LOCUS C
FPKM	Fragment Per Kilobase of exon per Million fragment

FRI	FRIGIDA
FT	FLOWERING LOCUS T
FUS3	FUSCA3
GA	Gibberellin
GA2ox	GA2 oxidase
GA3ox	GA3 oxidase
GA20ox	GA20 oxidase
GAI	GA INSENSITIVE
GID	GIBBERELLIN INSENSITIVE DWARF
GGDP	Geranylgeranyl diphosphate
GluB	endo-β-1,3-glucanase
HCl	Hydrochloride
HD2B	HD2 histone deacetylase
HDA	Histone deacetylase
HSL	HIGH-LEVEL EXPRESSION OF SUGAR-INDUCIBLE GENE2-LIKE
HUB	H2B MONOUBIQUITINATION
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectrometry
JA	Jasmonic Acid
JA-Ile	Jasmonoyl-*L*-isoleucine
JAZ	Jasmonate ZIM-domain
K	Potassium
KAT	3-l-ketoacyl-CoA-thiolase
KMB	2-keto-4-methylbutyrate
KO	*ent*-kaurene oxidase
KS	*ent*-kaurene synthase
KYP	KRYPTONITE
L1L	LEC1-LIKE
LAFL	LEC1/AFL
LC–IT–ToF MS	Liquid Chromatography Ion–Trap–Time of Flight Mass Spectrometry
LD	Long day
LDL	LYSINE SPECIFIC DEMETHYLASE LIKE
LEA	Late embryogenesis abundant
LEC1	LEAFY COTYLEDON1
LEC2	LEAFY COTYLEDON2
LOD	Limit of detection
LOX	Lipoxygenase
MACC	Malonyl-1-aminocyclopropane 1-carboxylic acid
MAN	endo-β-1,4-mannanase
MAP3K	Mitogen-activated protein kinase kinase kinase
MeOH	Methanol
MFP	Multifunctional protein

MFT	MOTHER OF FLOWERING LOCUS T AND TERMINAL-FLOWER
µg	Microgram
µl	Microliter
ml	Milliliter
µm	Micrometre
mm	Millimetre
mmol	Millimole
Mn	Manganese
MPa	Megapascal
MTR	5-methylthioribose
N	Nitrogen
Na	Sodium
NaAc	Sodium acetate
NaTFA	Sodium trifluoroacetate
NCED	9-cis-epoxycarotenoid dioxygenase
NRT	NITRATE TRANSPORTER
OPC-8:0	3-oxo-2-(2'-[Z]-pentenyl)cyclopentane-1-octanoic acid
OPCL	OPC-8:CoA ligase
OPDA	12-oxo-phytodienoic acid
OPR3	12-oxophytodienoate reductase 3
P	Phosphorus
PA	Proanthocyanidins
PAC	Paclobutrazol
PCR	Polymerase Chain Reaction
PG2	Polygalacturonase 2
PIF	PHYTOCHROME INTERACTING FACTOR
PIL	PHYTOCHROME-INTERACTING FACTOR3-LIKE
QC	Quality control
RDO	REDUCED DORMANCY
RGA	REPRESSOR OF GA1-3
RGL2	RGA-LIKE 2
RNA	Ribonucleic acid
RNAseq	RNA-sequencing
RPKM	Reads Per Kilobase Million
ROS	Reactive Oxygen Species
rpm	Rotation per minute
RT	Room temperature or Retention time (for LC-IT-ToF MS section)
SAM	S-adenosylmethionine
SD	Standard Deviation
SDR	short-chain alcohol dehydrogenase/reductase
SDS	Sodium dodecyl sulphate
SLY	SLEEPY

SNL	SWI-INDEPENDENT-LIKE
SnRK2	Snf1-related protein kinase 2
SOC1	SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1
SPT	SPATULA
TF	Transcription factor
TFIIS	Transcription elongation factor S-II
TT	TRANSPARENT TESTA
TTG	TRANSPARENT TESTA GLABRA
TZ	Tetrazolium salt
UPLC	Ultra Performance Liquid Chromatography
VDE	Violaxanthin de-epoxidase
VIN	VERNALIZATION INSENSITIVE
XTH/XET	Xyloglucan endotransglucosylase/hydrolase
ZEP	Zeaxanthin epoxidase

Chapter 1

General Introduction

1.1 Introduction

1.1.1 Seed germination

Seeds are very complex and diverse plant organs; they store nutrients and allow far-distance dispersal as well as persistence of a species in the local habitat. Seeds are able to survive for long periods and harsh environments until weather conditions are favorable for germination and seedling development.

Seed germination is the most sensitive stage of plant life and is influenced by various environmental signals including salt, light, temperature and water potential (Foolad *et al.*, 2007; Mancinelli *et al.*, 1966; Shichijo *et al*., 2001). These factors may delay the onset, rate and uniformity of germination. Nevertheless, the impact of the environment depends to a large extent on the interaction between the genetic makeup of the plant and the environment and it is believed that the plant's response to environmental condition is controlled by many genes (Foolad *et al.*, 2007). The beginning of seed germination is defined by the imbibition phase or water uptake and finishes by the elongation of the embryonic axis (Berrie and Drennan, 1971; Bewley, 1997). Water uptake by dry seeds in favourable conditions permits germination and radicle protrusion. The imbibition phase is divided into three phases of water uptake that allow the re-hydration of the seed and germination (Figure 1.1; Weitbrecht *et al.*, 2011). Dry seeds have a very low water potential and phase I is a rapid uptake, followed by a plateau (phase II) and by another increase in water uptake, phase III, which permits the elongation of the embryo and the breaking of testa to complete germination (Figure 1.1; Finch-Savage and Leubner-Metzger, 2006; Schopfer and Plachy, 1984; Weitbrecht *et al.*, 2011).

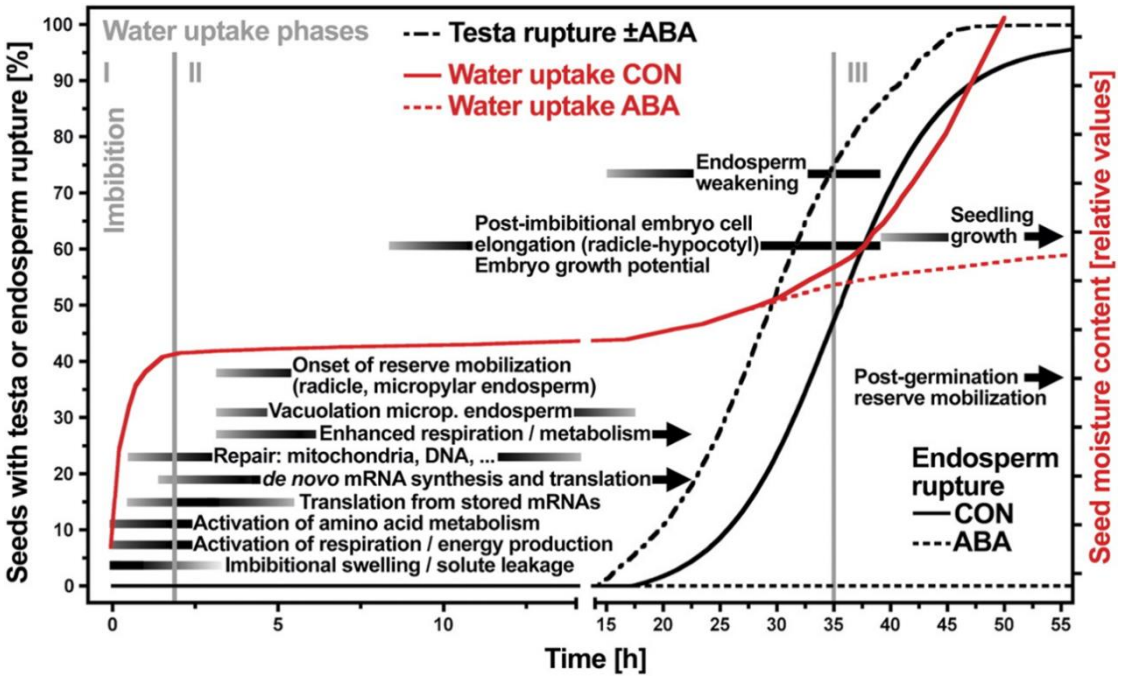

Figure 1. 1 **Key processes during germination of typical endospermic eudicot seeds with separate testa and endosperm ruptures**. Time-course of *B. napus* seed water uptake, testa rupture, radicle growth >2 mm, and the effect of abscisic acid (ABA); control without added hormone (CON). Data from Weitbrecht *et al.*, 2011. *Journal of Experimental Botany*. 2011;**62**(10):3289-3309

Modification of water potential alters the speed of tomato seed germination (Liptay and Schopfer,1983) and Bradford lab have shown that seed germination and speed of germination depend of water availability (Figure 1.2; Cheng and Bradford, 1999; Dahal and Bradford, 1994). During the imbibition phase, water penetrates into seeds through permeable seed coat by the micropyle as major entry point; this uptake modifies seed size and shape (Preston *et al.*, 2009; Robert *et al.*, 2008). It activates the biochemistry by the resumption of the metabolic activity and stimulates respiration and other mechanisms that deal with the damage imposed during the dehydration, storage or fast and inhomogeneous rehydration such as DNA repair, cell wall repair, activation of DNA ligase, DNA synthesis (Figure 1.1, Weitbrecht *et al.*, 2011). The inability to repair DNA damage during the imbibition period contribute to loss of seed vigour (Elder and Osborne, 1993; Powell and Matthews, 2012).

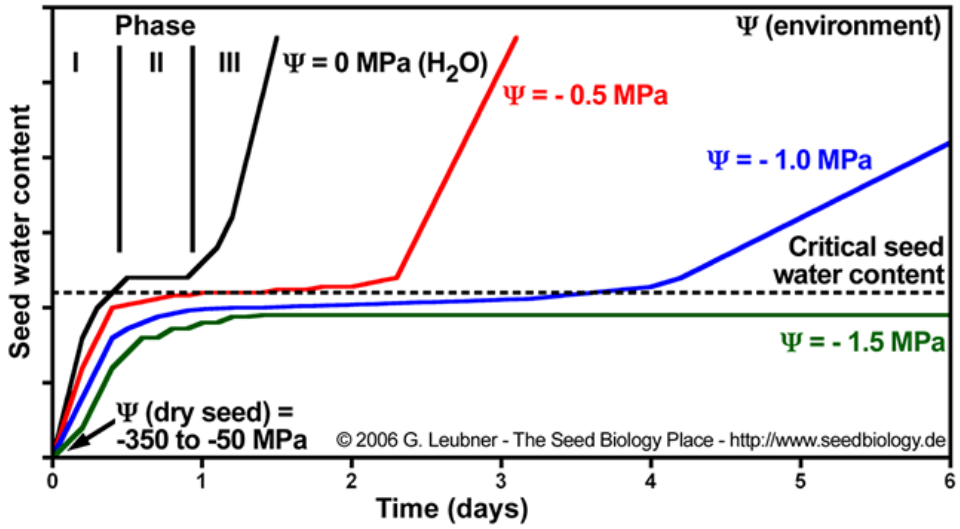

Figure 1. 2 **Seed water uptake and ambient water potential**. Data from Leubner-Metzger – The seed biology place (2006).

1.1.2 Seed dormancy, a process that blocks the completion of germination

Many plant seeds have an innate mechanism called dormancy that blocks germination. Seed dormancy is defined as a failure of an intact viable seed to complete germination under favourable conditions, and plants have developed several dormancy-inducing mechanisms to optimise the timing of germination (Bewley, 1997; Foley, 2001). Harper established three categories of dormancy: innate, induced and enforced (Harper, 1957); then Baskin and Baskin defined and classed dormancy into new groups: physiological dormancy, morphological dormancy, morphophysiological dormancy, physical dormancy and combinational dormancy (Baskin and Baskin, 2004). In many plant species, seed dormancy strongly influences fitness by delaying germination until conditions are appropriate for growth (Hepher and Roberts, 1985; Hilhorst, 1995; Vleeshouwers *et al.*, 1995; Li and Foley, 1997). Practical methods to release dormancy and promote germination include after-ripening, temperature treatment, hormone application, scarification and seed enhancement (Benech-Arnold, 2004; Halmer, 2004). The period of dry storage of freshly harvested and mature seeds, usually several months, at room temperature is named after-ripening (Bewley, 1997). During this period, biochemical or physical changes occur in the mature dormant seed to ensure germination. In crops, the delay of germination or sporadic germination is undesirable for growers or seed companies but the absence of dormancy is unwanted too; especially for cereals which could germinate on mother plant, this phenomenon is known as pre-harvest sprouting (Bewley,

1997). Sprouting or vivipary can be observed in tomatoes during seed maturation (Wang *et al.*, 2016); effectively tomato seeds are quiescent with a non-deep physiological dormancy which is often broken when seeds are exposed to low temperatures called chilling then exposed to elevated temperature (Bewley and Black, 1982; de Castro and Hilhorst, 2000; Hilhorst and Downie, 1995). Moreover, ABA-deficient mutant tomato seeds show that abscisic acid (ABA) prevents germination and plays a role in the induction of dormancy during seed development (Berry and Bewley, 1992; Groot and Karssen, 1992; Wang *et al.*, 2016).

1.1.2.1 Environmental signals in dormancy

Developing seeds rely on environmental signals to break or induce the dormancy such as temperature, light, nitrate, hormones, or chemical products (Figure 1.3; Holdsworth *et al.*, 2008a; Seo *et al*., 2009; Nonogaki, 2014). Seed germination or dormancy is controlled by the phytohormones gibberellic acid (GA) and ABA; a change in balance of ABA/GA levels could break the dormancy (Figure 1.1; Debeaujon and Koorneef, 2000). ABA is a major inducer of seed dormancy and regulates negatively germination.

1.1.2.2 Hormonal regulation of dormancy

There are two main hormone pathways that are associated with dormancy: ABA and GA. Others hormones such as ethylene and jasmonic acid regulate the major pathways.

The ABA biosynthesis pathway is now well understood and the germination phenotypes of a large number of mutants of the biosynthetic pathway have been characterised (Figure 1.3; Schwartz *et al.*, 2003). The first step is the epoxidation of zeaxanthin and antheraxanthin to violaxanthin, which occurs in plastids. This step is catalyzed by a zeaxanthin epoxidase (ZEP; Marin *et al.*, 1996). After a series of structural modifications, violaxanthin is converted to 9-cis-epoxycarotenoid. Oxidative cleavage of the major epoxycarotenoid 9-cis-neoxanthin by the 9-cis-epoxycarotenoid dioxygenase (NCED) yields a C15 intermediate, xanthoxin (Schwartz *et al.*, 1997). Xanthoxin is exported to the cytosol, where it is converted to ABA through a two-step reaction via ABA-aldehyde. A short-chain alcohol dehydrogenase/reductase (SDR), encoded by

the abscisic acid biosynthesis ABA*2* (*ABA2)* gene (Rook *et al.*, 2001; Cheng *et al.*, 2002; Gonzalez-Guzman *et al*., 2002), catalyzes the first step of this reaction and generates ABA aldehyde. ABA aldehyde oxidase (AAO) catalyzes the last step in the biosynthesis pathway.

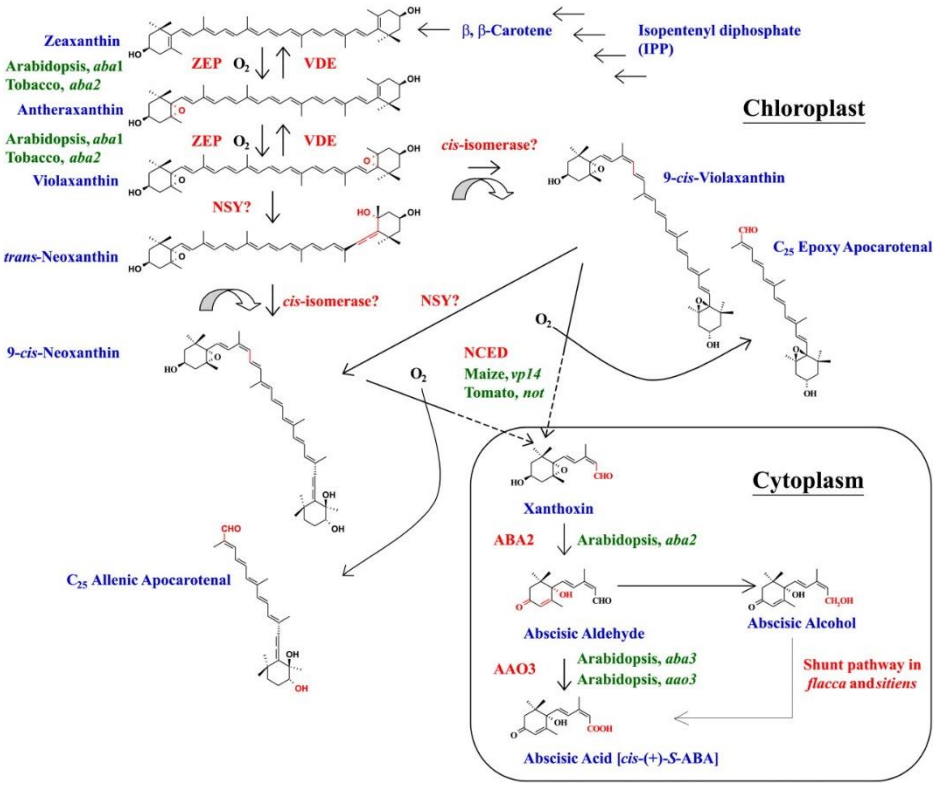

Figure 1. 3 **ABA biosynthesis pathway**. The pathway of ABA synthesis beginning with violaxanthin which is catalysed by zeaxanthin epoxidase (ZEP) through antheraxanthin. Isomers of neoxanthin and violaxanthin are cleaved by 9-cis-epoxycarotenoid dioxygenases (NCED) to form xanthonin. Xanthonin is converted to abscisic aldehyde by alcohol deshydrogenase (ABA2). Then the abscisic aldehyde is catalysed by an abscisic aldehyde oxidase (AOO3) to form abscisic acid. From Schwartz *et al.*, Elucidation of the indirect pathway of abscisic acid biosynthesis by mutants, genes, and enzymes. *Plant Physiology*. 2003 Apr;**131**(4):1591-1601

ABA is involved in regulating a number of developmental and growth processes under non-stressful conditions. ABA in developing seeds can either be derived from maternal tissues or be synthesized *de novo* in the embryo. Studies in *Arabidopsis thaliana* suggest that during seed development, two peaks of ABA appear (Karssen *et al.,* 1983; Kanno *et al.,* 2010). The first one occurs at mid-maturation, promotes the synthesis of storage proteins and is derived from maternal tissues (Phillips *et al.*, 1997). The second peak of ABA is from the biosynthesis of the embryo and activates the synthesis of late embryogenesis abundant (LEA) proteins. This peak also initiates seed dormancy. Seed

dormancy is affected by the seed maturation program which is controlled by LAFL regulators in *Arabidopsis*. The LAFL regulatory network includes *LEAFY COTYLEDON1* (*LEC1*) and *LEC1-LIKE* (*L1L*) of the NF-YB gene family, and *LEAFY COTYLEDON2* (*LEC2*), *FUSCA3* (*FUS3*), *ABA INSENSITIVE 3 (*ABI3; AFL) of the B3-*AFL* gene family (Luerssen *et al.*, 1998; Kirkbride *et al.*, 2013; Jia *et al.*, 2014). The expression of *LEC1* can activate *LEC2*, *FUS3* and *ABI3* which are a major component of ABA signalling and *ABI3* is a main regulator of seed dormancy and germination (Bentsink and Koorneef, 2008). *ABI3* expression is regulated by *DESPIERTO* (*DEP*) which is involved in ABA sensitivity during seed development and *dep* mutant seeds show complete dormancy loss (Barrero *et al.*, 2010). Cytochrome p450 707A1 (*CYP707A1*) and *CYP707A2* encode 8'-hydroxylases which are considered to be the key enzymes involved in ABA catabolism during seed development and germination (Kushiro *et al.*, 2004, Saito *et al.*, 2004). *CYP707A2* plays a role in the decrease of ABA levels prior to seed germination. A key gene in dormancy is *DELAY OF GERMINATION 1* (*DOG1*). *DOG1* is expressed in seeds during the maturation stage and loss of function *DOG1* results in no dormancy (Bentsink *et al.*, 2006). The amount of DOG1 protein in seeds determines the time they have to be stored to release dormancy (Nakabayashi *et al.*, 2012). The regulation of DOG1 is complex and involves polyadenylation and splicing (Cyrek *et al.*, 2016; Nakabayashi *et al.*, 2015). DOG1 is predominantly located in the nucleus suggesting that it might be a transcriptional factor. *DOG1* mediates a conserved coat-dormancy mechanism including temperature and GA-dependent pathway (Graeber *et al*., 2014). The epigenetic regulation of *DOG1* is important (see below 1.1.2.4). During seed imbibition, the embryo maintains the seed in a reversible state between dormancy and germination by regulating the basic leucine zipper transcription (bZIP) factor *ABI5* which is activated by Snf1-related protein kinase 2 (SnRK2; Lopez-Molina *et al.*, 2001; Piskurewicz *et al.*, 2008). *ABI5* maintains seed osmotolerance by stimulating *de novo* expression of the late maturation genes including LEA genes (Finkelstein and Lynch; 2000). Although *ABI5* has no effect on seed dormancy and does not affect dormancy level (Finkelstein, 1994), this transcription factor negatively regulates seed germination (Piskurewicz *et al.*, 2008; Kanai *et al.*, 2010), suggesting the distinct signalling pathways for ABA-mediated seed dormancy and ABA-inhibited seed germination. Seeds of typical ABA-deficient mutants germinate faster than wild-type (Koornneef *et al.*, 1984, Finkelstein,

1994) and transgenic plants constitutively expressing ABA biosynthesis gene maintain deep seed dormancy. WRKY41, a transcription factor, and RAF10/11, a mitogen-activated protein kinase kinase kinase (MAP3K), regulate Arabidopsis seed dormancy also through directly controlling *ABI3* and *ABI5* transcription during seed maturation and germination (Finkelstein, 1994; Ding *et al.*, 2014; Lee *et al.*, 2015c). Another key component in the ABA-signalling pathway, *ABI4*, was also described as a positive regulator of primary seed dormancy (Shu *et al.*, 2013). Furthermore, a study showed that calcium also regulates seed germination by affecting *ABI4* transcription that controls ABA signalling (Kong *et al.*, 2015). MYB96, the ABA-responsive R2R3-type MYB transcription factor, positively regulates seed dormancy and negatively regulates germination through mediating expression of *ABI4* and ABA biogenesis genes, including *NCED2* and *NCED6* (Lee *et al.*, 2015a, 2015b).

In contrast with ABA, GA levels are initially low in dry seed; and GAs are required after imbibition for radicle emergence (Ogawa *et al.*, 2003; Piskurewicz *et al.*, 2008). GA-deficient mutants (*ga1-3* and *ga2-1*) are defective in an early step of GA biosynthesis pathway and as *ga1-3* seeds did not germinate without exogenous GAs application, GAs are suggested to be required for seed germination (Koornneef and van der Veen, 1980; Ogawa *et al.*, 2003). The GA biosynthesis pathway is known and reviewed by Yamaguchi (Figure 1.4; Yamaguchi, 2008). Briefly, GAs are biosynthesised from *trans*-geranylgeranyl diphosphate (GGDP), formed in plastids through the methylerythritol phosphate pathway (Kasahara *et al.*, 2002), then it converted by two plastid-localised terpene cyclases, followed by oxidation on the endoplasmic reticulum by cytochrome monooxygenases p450 which leads to the production of GA_{12} (Yamaguchi, 2008). The dioxygenases comprise small families of GA 20-oxidase (GA20ox) and GA 3-oxidase (GA3ox) isozymes which convert GA_{12} to the active form GA_4. A third class of dioxygenases, the GA 2-oxidases (GA2ox), produce inactive products and function to enable GA turnover. The GA signalling pathway is regulated by DELLA proteins which are transcriptional regulators through targeting GAs receptor named *GIBBERELLIN INSENSITIVE DWARF1* (*GID1*). This binding changes the conformation of *GID1* which is recognized by *SLEEPY1* (*SLY1*), an F-box component of the SCF^{SLY1} E3 ubiquitin ligase and address *GID1*-GA-DELLA for degradation through the 26S proteasome (Tyler *et al.*,

2004). Mutation in DELLA *RGA-LIKE2* (*RGL2*) is sufficient to rescue *ga1-3* germination (Piskurewicz *et al.*, 2008) suggesting that *RGL2* represses seed germination. Moreover, ABA promotes DELLA accumulation (Penfield *et al.*, 2006) and *RGL2* stimulates *ABI5* expression by increasing ABA synthesis (Piskurewicz *et al.*, 2008). Cold stratification releases seed dormancy through an increase in GA levels which degrades *RGL2* (Lee *et al.*, 2002). Another DELLA factor, *REPRESSOR OF GA1-3* (*RGA*), is expressed during seed germination and stimulates the expression of *XERICO* which promotes ABA accumulation (Tyler *et al.*, 2004; Ko *et al.*, 2006).

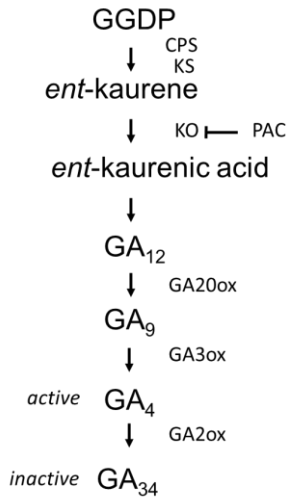

Figure 1. 4 **GA biosynthesis pathway**. *trans*-geranylgeranyl diphosphate (GGDP) is converted by terpene synthase and p450 enzyme in GA_{12} through the intermediate *ent*-kaurene and *ent*-kaurenic acid. GA_{12} is converted to a bioactive form GA_4 by GA 20-oxidase (GA20ox) and GA 3-oxidase (GA3ox). Adapted from Yamaguchi, 2008. Gibberellin metabolism and its regulation. *Annual Review of Plant Biology*. 2008;**59**:225-251.

The role and molecular mechanisms of jasmonic acid (JA) and its precursor 12-oxo-phytodienoic acid (OPDA) in seed inhibition or germination activation have been less studied in contrast to ABA in relation to seed dormancy and germination. The pathway for jasmonate (JA) biosynthesis has first been described in *Vicia faba* by Vick and Zimmerman (1983). JA has been studied primarily for its role in plant responses to wounding stress and herbivore feeding (Wasternack, 2007; Wu and Baldwin, 2010). The biosynthetic precursor of JA,OPDA, is also a hormonal signal in physiological reactions and developmental processes. JA synthesis is initiated in the chloroplast with the conversion of α-linoleic acid to OPDA which is mediated by 13-lipoxygenase (LOX), allene oxide synthase (AOS) and allene oxide cyclase (AOC) (Fonseca *et al.,* 2009). Then

OPDA is transported to the peroxisome where is converted to 3-oxo-2-(2'-[Z]-pentenyl)cyclopentane-1-octanoic acid (OPC-8:0) before entering 3 cycles of β-oxidation to produce JA (Figure 1.5; Wasternack, 2007; Acosta and Farmer, 2010). Jasmonic acid is converted to a JA-isoleucine conjugate (JA-Ile) in the cytoplasm, and JA-Ile binds to CORONATINE INSENSITIVE1 (*COI1*) which leads to the degradation of JASMONATE ZIM-DOMAIN (JAZ) proteins, which are key transcription repressors of JA-dependent responses (Boter *et al.*, 2004; Chini *et al.*, 2007). Dave *et al*. (2011, 2012) proposed, based on crossing several mutants impaired at several steps during ß-oxidation, that in fact OPDA, and not JA, has the stronger effect in inhibiting seed germination of *A. thaliana*. They found that OPDA was approximately ten times more efficient than JA to inhibit seed germination. They further found a synergistic effect of OPDA and ABA on the germination inhibition of *A. thaliana seeds* (Dave and Graham, 2011; Dave *et al.*, 2012).

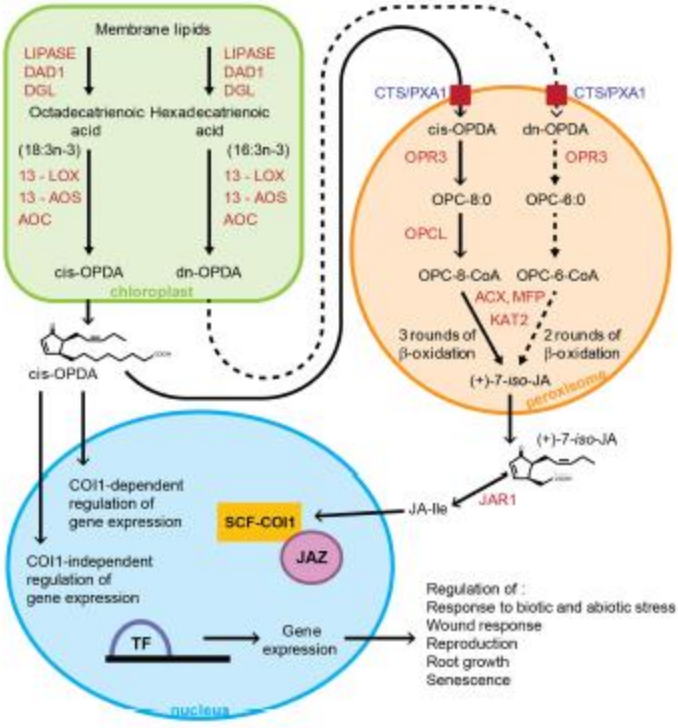

Figure 1. 5 **Oxylipin biosynthesis pathway and signal transduction in *Arabidopsis thaliana***. Jasmonic acid biosynthesis initiates in the plastid which release from membrane lipids. cis-OPDA and dn-OPDA are formed following sequential steps and is transported to the peroxixome where after reduction JA is formed and is transported in the cytosol. Active form of JA (JA-Ile) and cis-OPDA are addressed to the nucleus. Figure from Dave and Graham (2012). *Frontiers in Plant Science* **3**:42.

Ethylene (C_2H_4) acts positively on seed germination in *Arabidopsis thaliana* (Kepczynski and Kepczynska, 1997). The ethylene biosynthesis pathway is well characterized (Figure 1.6; Yang and Hoffman, 1984; Arc et al., 2013). Briefly, S-adenosyl-methionine synthesized from methionine by the S-adenosyl-methionine synthetase (or SAM synthetase), is converted to 1-aminocyclopropane 1-carboxylic acid (ACC) by ACC synthase (S-adenosyl-L-methionine methylthioadenosine-lyase, ACS). Ethylene production results from the oxidation of ACC by ACC oxidase (ACO), which also generates CO_2 and hydrogen cyanide. Breaking dormancy by GA shows an increase of ethylene production (Kepczynski and Kepczynska, 1997). Moreover, mutant lines altered in ethylene pathway such as *ETHYLENE INSENSITIVE1* (*ein1*), *ETHYLENE RESISTANT1* (*etr1*) and *ETHYLENE RESPONSE FACTOR* (*erf*) demonstrated the role of ethylene in seed germination (Wang *et al.*, 2007). For example, *etr1* enhances primary dormancy relative to wild type (Chiwocha *et al.*, 2005). Ethylene biosynthesis gene such as *ACO1*, *ACO4*, *ACO5* and *ERF9*, *ERF105*, *ERF112* induced seed germination by activating genes involved in the endosperm rupture (Linkies *et al.*, 2009; Arc *et al.*, 2013; Wang *et al.*, 2013). The major role of ethylene in seed germination is the rupture of endosperm cap by counteracting the role of ABA (Linkies *et al.*, 2009).

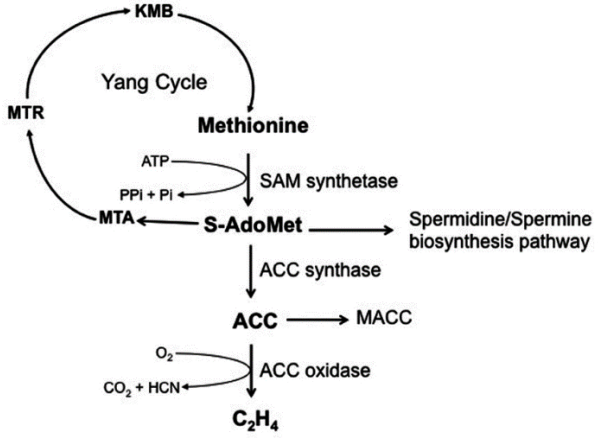

Figure 1. 6 **Ethylene biosynthesis pathway**. S-adenosyl-methionine (S-AdoMet) is synthetized from the methionine by the S-adenosyl-methionine synthetase (SAM synthetase). S-AdoMet is then converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase. 5′-methylthioadenosine (MTA) is recycled to methionine by successive enzymatic reactions involving 5-methylthioribose (MTR) and 2-keto-4-methylthiobutyrate (KMB). S-AdoMet is also the precursor of the spermidine/spermine biosynthesis pathway. Ethylene production is catalyzed by the ACC oxidase using ACC as substrate. Ethylene production is reduced by malonylation of ACC content to malonyl-ACC (MACC). From Arc *et al*., 2013. ABA crosstalk with ethylene and nitric oxide in seed dormancy and germination. *Frontiers in Plant Science*. 2013 Mar;**4**:63.

1.1.2.3 Histone regulation in dormancy

The regulation of transcription elongation and chromatin remodelling are involved in dormancy mechanisms (Figure 1.7). For example, the transcription elongation factor S-II (TFIIS) assists RNA polymerase II to overcome the temporal arrest during elongation and enhances RNA synthesis (Kim *et al.*, 2010). A mutation in TFIIS resulted in reduced seed dormancy (Grasser et al., 2009). A mutation in the *ARABIDOPSIS TRITHORAX-RELATED 7* (*ATXR7*) and *H2B MONOUBIQUITINATION1* (*HUB1*) causes reduced dormancy in seeds (Liu *et al.*, 2011). Histone demethylases *LYSINE SPECIFIC DEMETHYLASE LIKE 1* (*LDL1*) and *LDL2* repress seed dormancy by regulating *DOG1* (Zhao *et al.*, 2015) and chromatin remodelling of *DOG1* is also involved in dormancy cycling (Footitt *et al.*, 2015). Furthermore, the histone methyltransferases KRYPTONITE (KYP)/SUVH4 and SUVH5 repress *DOG1* and *ABI3* transcription during seed maturation (Zheng et al., 2012). These studies demonstrated that the *DOG1*-mediated regulation pathway might be distinct from the ABA and/or GA pathway. Moreover, ABA stimulates *SWI-INDEPENDENT3* (*SIN3*)-*LIKE1* (*SNL1*) and *SIN3-LIKE2* (*SNL2*) expression, which suggests that there is positive feedback regulation to maintain high levels of ABA through the histone deacetylation pathway (Wang *et al.*, 2013). The four separate hormone pathways (ethylene/JA, ABA and GA) associated with dormancy are regulated by a HD2 histone deacetylase (*HD2B*; Yano *et al.,* 2013; Zhou *et al.*, 2005, 2013). Indeed, *HD2B* regulates *DOG1* and *ABI3* which are involved in ABA pathway, then *HD2B* affects GA_4 accumulation that increase GA level in imbibed seeds. Moreover, JA and ethylene induce histone deacetylase19 (*HDA19*) which represses seed germination by binding to HIGH-LEVEL EXPRESSION OF SUGAR-INDUCIBLE GENE2-LIKE1 (*HSL1*; Zhou *et al.*, 2005, 2013). Histone methylation also affects seed dormancy. For example, the methylation of H3K4 and H3K79 activates gene expression and causes seed dormancy while *KRYPTONITE* (*KYP*) caused the methylation of H3K9me2 and suppresses seed dormancy genes (Jackson *et al.*, 2002; Kim *et al.*, 2012).

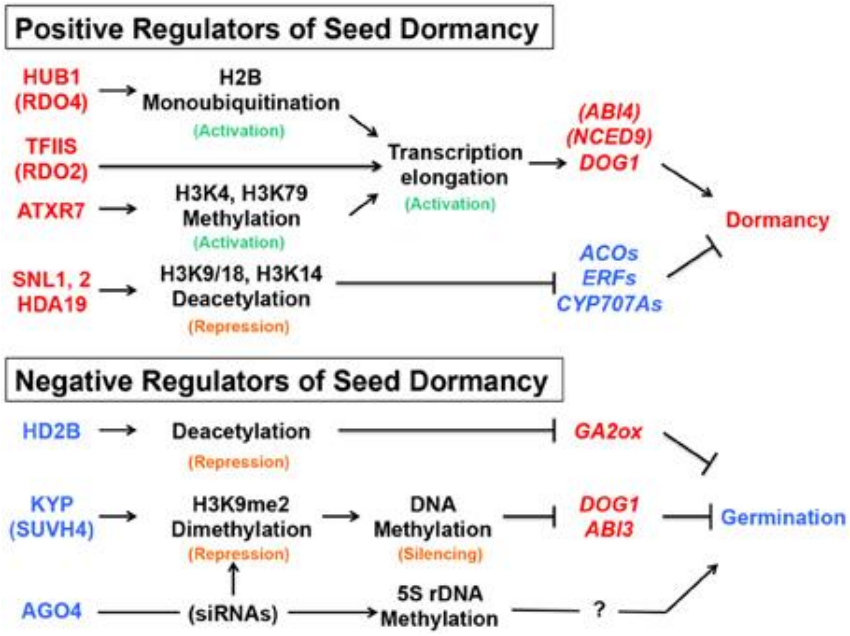

Figure 1. 7 **Schematic presentation of the regulation of dormancy and germination.** *Frontiers in Plant Science*. 2014 May;**5**:233. The positive (red) and negative (blue) regulators of seed dormancy and their roles in the chromatin remodelling, DNA modification or siRNA pathways are indicated, together with promotive (arrows) or suppressive (blocked arrows) effects on the downstream genes (italics). Active (green) or repressive (orange) marks on histones or DNA are also indicated. Gene and protein abbreviations: *ABI3*, *ABA INSENSITIVE 3; ABI4, ABA INSENSITIVE 4; ACO, 1-AMINOCYCLOPROPANE-1-CARBOXYLATE OXIDASE; AGO4, ARGONAUTE 4; ATXR7, ARABIDOPSIS TRITHORAX-RELATED 7*; CYP707As, Cytochrome p450 707A; *DOG1, DELAY OF GERMINATION 1; ERF, ETHYLENE RESPONSE FACTOR*; Ga2ox, GA2-oxidase; *HDA19, HISTONE DEACETYLASE 2B 19*; *HUB1, H2B MONOBIQUTINATION 1*; *KYP, KRYPTONITE; RDO, REDUCED DORMANCY* (=TFIIS); *SNL, SIN3-LIKE*; TFIIS, Transcription elongation factor S-II. From Nonogaki (2014).

1.1.2.4 Role of the light in the dormancy

Light is perceived by photoreceptors PHYA and PHYB which stimulate seed germination by increasing GA levels and decreasing ABA levels through the degradation of the *PHYTOCHROME-INTERACTING FACTOR3-LIKE5* (*PIL5*) also known as *PHYTOCHROME INTERACTING FACTOR 1* (*PIF1*; Oh *et al.*, 2006, 2007). *PIL5* is a phytochrome-interacting basic helix-loop-helix transcription factor (Oh *et al.*, 2006) which promotes higher ABA levels by activating *ABA1*, *NCED6* and *NCED9* (Figure 1.5) and repressing *CYP707A2* (Oh *et al.*, 2007). Then *PIL5* promotes lower GA levels by repressing *GA3ox1* and *GA3ox2* and activating *GA2ox2* (Oh *et al.*, 2007). Moreover, *PIL5* regulates GA levels through the activation of *GA INSENSITIVE* (*GAI)* and *RGA* (Piskurewicz *et al.*, 2008). The darkness permits the accumulation of *PIL5*. Light

regulates seed germination with *PIF1* and *PIF4*. DELLA binds *PIF1* to activate PIF1-regulated transcripts in the light and binds *PIF4* to repress PIF4-regulated transcripts in the dark (Cheminant *et al.*, 2011). Furthermore, an early far-red light pulse that blocks germination involves phytochrome B (phyB) inactivation in the endosperm, which blocks the expression in GA biosynthesis genes in the endosperm by stabilization of *PIF1*, and induces ABA release towards the embryo (Oh *et al.*, 2006; Lee *et al*., 2012).

1.1.2.5 Role of nitrate in dormancy

Nitrate (NO_3^-) is one of the most abundant nitrogen (N) sources and NO_3^- impacts plant development, growth and seed dormancy (Hilhorst, 1990; Alboresi *et al.*, 2005; Vidal and Gutierrez, 2008; Wang *et al.*, 2012). In Arabidopsis seeds, nitrate content is negatively related to the depth of dormancy (Alboresi *et al.*, 2005). Plant have developed nitrate transporters to ensure its supply, these nitrate transporters are encoded by *NRT1* and *NRT2* (Desikan *et al*., 2002). During seed imbibition *NITRATE TRANSPORTER 1* (*NRT1.1*), which encodes a dual-affinity low/high nitrate transporter, is expressed at low concentration of NO_3^- (Alboresi *et al.*, 2005). This response to nitrate acts via *CBL-INTERACTING PROTEIN KINASE 23* (*CIPK23*) on the phosphorylation/dephosphorylation of *NRT1.1* (Ho *et al.*, 2009). The regulation of *CIPK23* regulates dormancy via temperature signals. The response to nitrate accumulation in *nrt2* mutant did not change the germination, suggesting that this transporter has no role in seed germination. Even if *NRT2* is not directly involved in seed germination, *nrt2.7* mutants are affected in proanthocyanidins (PAs) and have a lower content of NO_3^- than wild-type (WT; Chopin *et al*., 2007; David *et al.*, 2014). *nrt2.7* mutant have a similar phenotype to *transparent testa10* (*tt10*) and have higher levels of soluble PAs. Debeaujon *et al.*, have shown that PA content in seed is correlated with dormancy levels suggesting that *nrt2.7* is dormant (Debeaujon *et al.*, 2000).

1.1.2.6 Role of temperature in dormancy

Temperature is an environmental factor which acts on seed germination. A period of storage at cold temperature may overcome the seed dormancy. The sensitivity of seeds to cold was dependent upon the length of time that seeds had been dry after-ripened (Finch-Savage *et al.*, 2007). Moreover, temperature acts with light. For example, *hy2-1* mutant (deficient in phytochrome) mutant did not

overcome the dormancy during cold temperature (Donohue *et al.*, 2007). The genetic mechanism for temperature regulation has been identified (Sidaway-Lee *et al.*, 2010). Cold temperatures stimulate GA biosynthesis by inducing *GA3ox1* and *GA3ox2* (Yamauchi *et al.*, 2004). This regulation is mediated by the bHLH transcription factor SPATULA (SPT) which suppresses the expression of these genes (Penfield *et al.,* 2005). Furthermore, the *NCED*s (*NCED2*, *NCED5* and *NCED9*) contribute to the thermo-inhibition of germination by high temperatures by increasing ABA levels (Toh *et al.,* 2008). This results show that high temperatures act on GA biosynthesis as well with the repression of *GA3ox1* and *GA3ox2*. *MOTHER OF FLOWERING LOCUS T AND TERMINAL-FLOWER 1* (*MFT*) plays a role in dormancy cycling through *DOG1* (Dave *et al.*, 2016). *MFT* and *DOG1* expression is correlated with *CIPK2*3 and PHYA expression and the changes in *CIPK23* and *PHYA* permit to the germination through the sensitivity of nitrate and light signals (Footitt *et al*., 2013). Moreover, *mft2* loses its sensitivity to ABA confirming the role of *MFT* in ABA signalling (Footitt *et al.*, 2017).

1.1.2.7 After-ripening in dormancy

After-ripening (AR) is the period of dry storage where dormancy is released after its induction during seed maturation. The regulation of after-ripening is poorly understood (Finch-Savage *et al.*, 2007; Carrera *et al.*, 2008). Changes in dry seeds during AR are associated with ABA genes. Indeed, *NCED* and *ABA1* are down-regulated in imbibed AR seeds (Cadman *et al.,* 2006). Moreover during after-ripening, JA pathway is induced through *COI1* (Ellis and Turner, 2002; Barrero *et al.*, 2009). After-ripening permits to adjust the balance of ABA/GA to induce germination or dormancy. Major environmental signals act on the ratio of ABA/GA to release the dormancy by decreasing ABA levels or to enhance GA levels as shown on the Figure 1.8 (Bentsink and Koornneef, 2008).

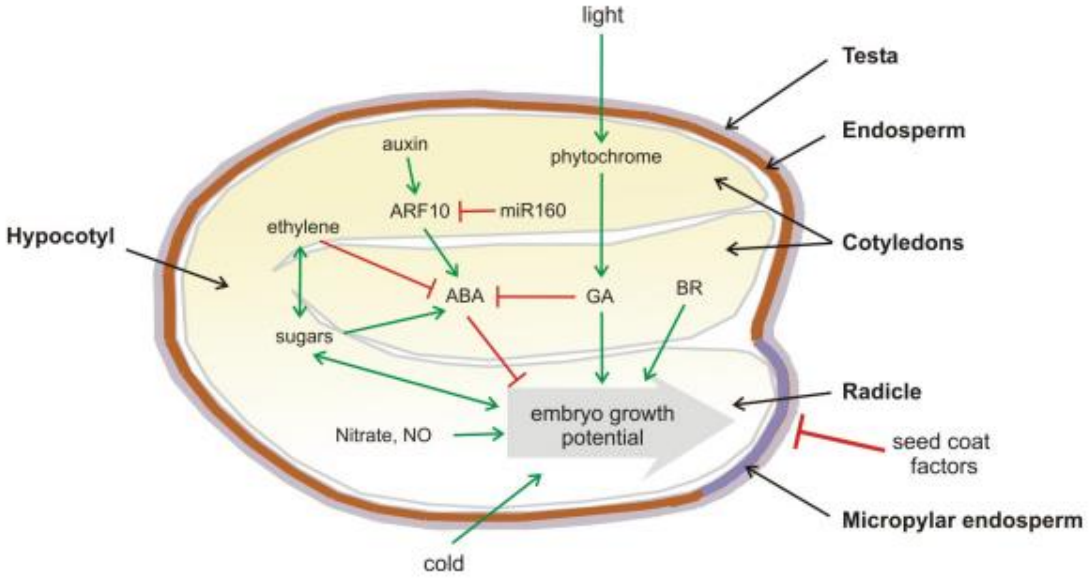

Figure 1. 8 **Schematic presentation of processes controlling seed dormancy and germination in an *Arabidopsis* seed** – From Bentsink and Koornneef (2008). *Arabidopsis book* doi: 10.1199/tab.0119. The Arabidopsis seed is characterized by the embryo with two cotyledons and a single layer endosperm. Germination promoting (green arrow) and inhibiting factors (red arrows) are indicated.

1.1.3 Seed structure and role of endosperm and seed coat in dormancy

The structure of the seed derives from the ovule. After fertilization, the carpel begins to develop into fruit and seed development commences (Ho and Hewitt, 1986). The endosperm is a product of double fertilization and serves as a nutrient source for the embryo during seed development and for the emerging embryo during seed germination and seedling establishment. Mature tomato seeds are composed of an embryo surrounded by an endosperm which is covered by a seed coat composed of testa and tegmen. Each of these structures is genetically distinct (Figure 1.9). The embryo develops from the egg cell and contains equal representation of the maternal and paternal genomes.

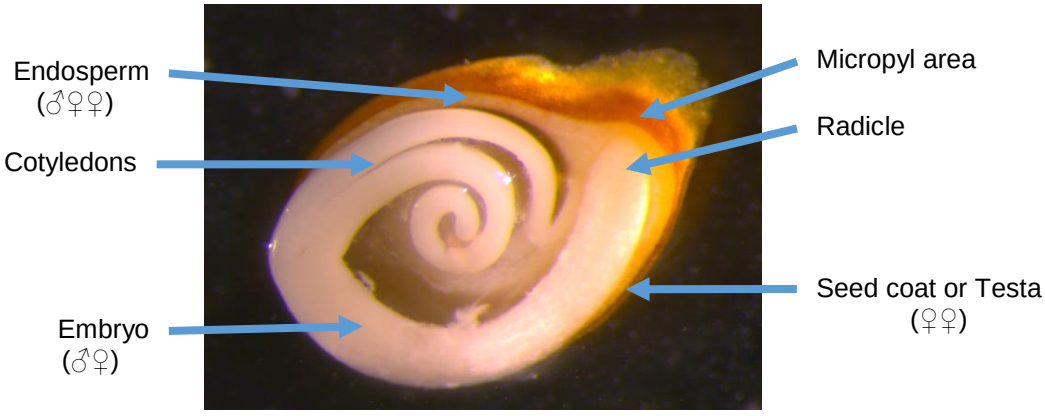

Figure 1. 9 **Cross section of a tomato seed**. Male or female symbols present the genome's origin of the tissue.

The endosperm has an important role in the regulation of germination. The triploid endosperm is formed by the fusion of the two polar nuclei and one sperm nucleus. The organisation of endosperm is controlled by DNA methylation and by *FERTILIZATION INDEPENDENT SEED* (*FIS*) which repressed the formation of seeds in the absence of the fertilization (Köhler and Makarevich, 2006; Hehenberger *et al.*, 2012; Hands *et al.*, 2016). The *FIS* genes encode Polycomb group proteins which ensure the stable propagation of H3K27me3 through mitotic cell cycles. In solanaceous species such as tomato, mature seeds have a substantial thick endosperm layer which is broken down during germination. Endosperm weakening has been shown to be biphasic: the first phase is ABA-insensitive, and this is followed by a second phase which is inhibited by ABA (Bewley, 1997; Müller *et al.,* 2006; Ni and Bradford, 1993; Toorop *et al.*, 2000; da Silva *et al.*, 2004). Endosperm tissue weakening is associated with the action of cell wall remodelling protein (CWRPs; Nonogaki *et al.*, 2000; Finch-Savage and Leubner-Metzger, 2006; Holdsworth *et al.*, 2008a). Cell wall remodeling can also be promoted by reactive oxygen species (ROS), including short-lived molecules such as superoxide ($O_2^{\cdot -}$), hydroxyl radicals ($^{\cdot}OH$) and hydrogen peroxide (H_2O_2), which are also known to play a role in the regulation of germination timing (Bailly, 2004). In tomato seeds, the endosperm cap is equivalent to the micropylar region of endosperm in Arabidopsis seeds which surrounds the radicle tip. The resistance of the endosperm cap is due to the rigid and thick cell walls of the tissue (Bewley *et al.*, 2013). Genes encoding cell-wall modifying proteins such as xyloglucan endotransglycolase/hydrolases (*XTH*) and expansins (*EXPA4*, *EXPA8* and *EXPA10*) are expressed exclusively in the micropylar region of the endosperm in Arabidopsis and tomato during seed germination (Dekkers *et al.*, 2013; Chen and Bradford, 2000; Chen *et al.*, 2002). In chickpea, xyloglucan endotransglucosylase/hydrolase (*XTH*), renamed *XET4* in tomato, controls germination (Chen *et al.*, 2002; Hernandez-Nistal *et al.*, 2010). Moreover, it has been shown that polygalacturonase A (*PG2*), endo-ß-1,4-mannanase (*MAN1* and *MAN2*) and endo-ß-1,3-glucanase (*GluB*) are specifically expressed in the micropylar endosperm during germination and are involved in hydrolysis of the mannan-rich cell walls (Chen and Bradford 2000; Nonogaki *et al.*, 2000; Chen *et al.*, 2001; Leubner-Metzger 2003). In tomato seeds, the degradation of the cell

wall is controlled by the embryo and is accompanied by the disappearance of storage vacuole and lipid bodies.

The seed coat develops from the integuments of the ovule and plays an important role in embryo nutrition during seed development and forms a barrier between the embryo and its immediate environment. The seed coat can impose dormancy by being impermeable to water or oxygen, or by its mechanical resistance to radicle protrusion. Between plant taxa structural features controlling water permeability in seed coat vary, and the presence of one or more layers of impermeable palisade cells are observed in legume seeds (Baskin and Baskin, 1998). It has previously been proposed that the cuticle is the key factor of the permeability (Shao *et al.*, 2007; De Giorgi *et al.*, 2015). Moreover, the composition of carbohydrates (such as xylan), hydroxylated fatty acid or phenols compounds in seed coat affect the level of permeability (Weber *et al.*, 1996; Aparicio-Fernandez *et al.*, 2005; Shao *et al.*, 2007). The phenolic compounds such as proanthocyanidins (PAs) colour the seed coat and decrease its permeability (Debeaujon *et al.*, 2000). For example, red seeds of *Sinapis arvensis* exhibit a reduced dormancy compared with black seeds (Duran and Retamal, 1989). Genes affecting flavonoid metabolism have been characterized in detail (Winkel-Shirley, 2001). Flavonoids are sub-classified into several families including flavonols, flavones, phlobaphese, isoflavonoids, anthocyanidin and condensed tannins (Winkel-Shirley, 2001). In Arabidopsis, mutation in genes encoding PAs biosynthesis proteins showed an alterations in seed coat pigmentations or in flower pigmentations (Koornneef, 1990). PAs biosynthesis and its regulation have been studied in *Arabidopsis* using *transparent testa glabra* (*ttg*) mutants and *transparent testa* (*tt*) mutants which regulate production, transport and storage of PAs. *ttg* and *tt* mutants which are blocked at different steps in flavonoid biosynthesis pathway have a range of seed coat colour from yellow to brown. Plants having an alteration in PAs pathways exhibited a reduction of seed dormancy and the reduced dormancy of the mutant is related to the degree of paleness of seed coat (Gfeller and Svejda, 1960; Debeaujon *et al.*, 2000; Himi *et al.*, 2002), suggesting a role of proanthocyanidins in dormancy. Authors have investigated the role of PAs as seed germination inhibitors and have shown a relationship between proanthocyanidins and ABA (Jia *et al.*, 2012). Indeed, PAs

inhibition of seed dormancy is affected by ABA signaling even if the mechanism is still unclear.

1.1.4 Seed vigour

Seed vigour is defined as the performance of seed to germinate and complete germination until developing into a plant. Seed vigour is dependent on the seed maturation conditions and time in dry storage (Hampton *et al.*, 1995). Moreover, the environment during seed production exerts great influence on seed quality (Horii *et al.*, 2007). Seed vigour can vary between seed lots, cultivars or varieties. Seed vigour, among the other attributes, is affected by the amount and composition of protein (albumin, globulins and proalbumins), starch and oil (triacylglycerols), which are functionally dependent on the carbon–nitrogen (C-N) balance, central metabolism and sink-source interaction during development on the mother plant (Wobus and Weber, 1999; Castro *et al.*, 2006; Toubiana *et al.*, 2012). Most of the reserves are accumulated in the endosperm (Bewley *et al.*, 2013). Tomato contains high levels of protein (22-33%) and lipids (20-29%) but low levels of starch (0.5-2%; Schauer *et al.*, 2005; Sheoran *et al.*, 2005). The quantity of the storage reserve is influenced by the availability of carbon and nitrogen to the parents (Singletary and Below, 1989). These reserves are important because they support early seedling growth after being degraded upon germination and participate in the crop establishment. The success of seedling establishment is determined by the quality of the seed, the interaction with the environment and the food reserves until seed becomes independent, autotrophic and can use light energy. Seed vigour is dependant of the accumulation of protecting proteins during maturation such as LEA, peroxiredoxins and heat shock proteins which increase seed storability and dessication tolerance (Delseny *et al.*, 2001).

1.1.5 Seed longevity

Seed longevity is defined as the total time that seeds remain viable. Seed longevity is a major challenge for the conservation of plant biodiversity and for crop success. Moreover, longevity is variable between species. For example, the seed longevity is higher than 2,000 years for *Phoenix dactylifera* while the seed longevity for *Capsicum annuum* L. is around two years (Sallon *et al.*, 2008).

Seeds possess a wide range of systems (protection, detoxification, repair) allowing them to survive in the dry state and to preserve a high germination ability (Sano *et al.*, 2016). Knowledge of the seed longevity permit for seed companies to control the storage and avoid problem with storability. Indeed, seed longevity is affected by dry storage; dry seeds slowly deteriorate and lose vigor which results in germination failure. The seed viability decreases due to the ageing processes. Research on seed longevity under high relative humidity and high temperature (artificial ageing) to accelerate deterioration have shown that these conditions mimics molecular and biochemical events that occurs during natural seed ageing (Rajjou *et al.*, 2008). Mutation in seed maturation and dormancy genes, such as *LEAFY COTYLEDON1* (*LEC1*) and *ABSCISSIC ACID INSENSITIVE3* (*ABI3*) lead to reduction in seed viability (Debeaujon *et al.*, 2000). The testa-defective mutants, such as *transparent testa* (*tt*) and *aberrant testa shape* (*ats*), display a reduced seed longevity (Debeaujon *et al.*, 2000). Waterworth lab showed that *DNA LIGASEIV* and *DNA LIGASEVI*, which are essential to maintain genome integrity, are determinant in seed longevity (Waterworth *et al.*, 2015). In addition, the accumulation of oxidative damage in seeds is correlated with the loss of germination vigor (Rajjou *et al.*, 2008). The accumulation of cellular oxidative damage induces a loss of seed vigor and a loss of germination capacity until irreversible death of the embryo. To remove excess ROS accumulated during dry storage, seeds use a set of anti-oxidant enzymes (Bailly, 2004). Loss of seed longevity is associated with the accumulation of DNA lesions (Cheah and Osborn, 1978; Waterworth *et al.*, 2015). Heat stress protein overaccumulated improved tolerance to ageing (Prieto-Dapena *et al.*, 2006). In conclusion, changes in the regulation of proteins synthesis, post-translational modifications and proteins turn-over are crucial determinants of the age-related decline in the maintenance, repair and survival of the seed (Figure 1.10; Sano *et al.*, 2016; Prieto-Dapena *et al.*, 2006; Waterworth *et al.*, 2015).

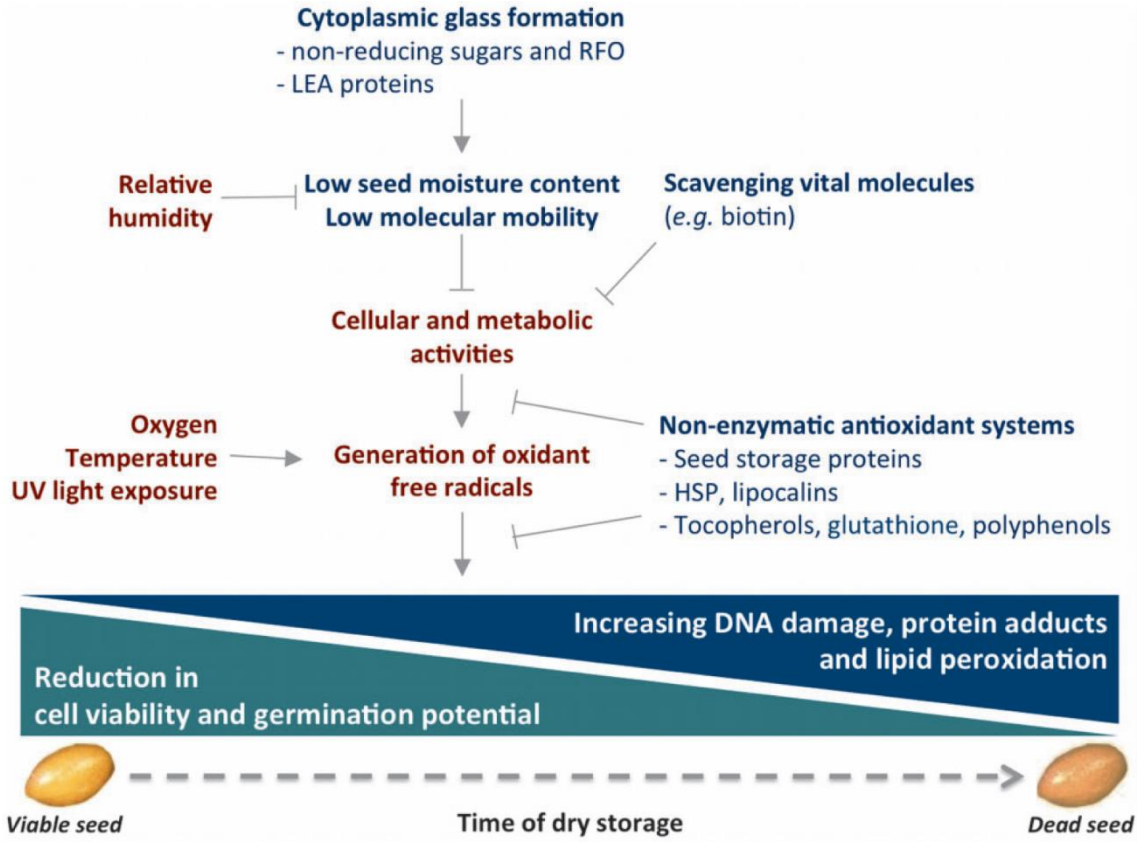

Figure 1. 10 **Model for the cellular systems protecting seeds from injury by oxidizing free radicals during dry storage and anhydrobiosis**. In *Plant & Cell Physiology*. From Sano *et al.,* 2016 Apr;**57**(4):660-674. HSP, heat-shock protein; LEA, late embryogenesis abundant; RFO, raffinose family oligosaccharides. In dark red are factors that negatively influence seed viability during dry storage. In blue are factors that positively influence viability during dry storage.

1.1.6 Maternal environment effects seed vigour

Maternal environmental effects refer to the particular phenomenon in which the external ecological environment of the maternal parent influences the phenotype of its progeny (Roach and Wulff, 1987; Roff, 1998; Donohue, 2009). Even though paternal and maternal environments may contribute to transgenerational plasticity such as the accumulation of food reserve during seed maturation stage, maternal effects, such as quantity and quality of offspring, are greater than paternal effect (i.e. prior to fertilization such as flowering time) (Roach and Wulff, 1987; Schmid and Dolt, 1994; Lacey, 1996; Etterson and Galloway, 2002). Maternal effects enhance offspring fitness by increasing the germination rate and intensifying performance in the habitats their offspring are likely to experience (Galloway, 2005). Maternal effects in plants include effects caused by maternal inheritance of organelles, the effect of endosperm, the effect

of the seed coat (a maternal tissue), the effect of maternal provisioning during seed development and maternal determination of the progeny environment by seed dispersal at times of favourable photoperiod and temperature for growth (Donohue, 2009; Chen *et al.*, 2014; Postma and Agren; 2015; MacGregor *et al.*, 2015). Seed dormancy level is influenced by the temperature experience by the mother plant (Kendall *et al.*, 2011; MacGregor *et al.,* 2015). Cold temperatures induce higher dormancy in Arabidopsis mature seeds (Kendall *et al.,* 2011) and this response is maternally controlled by *FLOWERING LOCUS C* (*FLC*) and *FLOWERING LOCUS T* (*FT*; Chen *et al.*, 2014). FT is known to play a role in temperature responses and high *FT* expression is associated with low dormancy (Chen *et al*., 2014). In Arabidopsis, the major repressor of flowering is a MADS-box transcription factor *FLC* (Michaels and Amasino, 1999). After a prolonged period of low temperature (named vernalization), *FLC* expression is repressed and plants are able to initiate flowering. This repression of *FLC* is associated with chromatin change (Bastow *et al.*, 2004). *FLC* is regulated by *FRIGIDA* (*FRI*) and by *VERNALIZATION INSENSITIVE 3* (*VIN3*; Johanson *et al*., 2000; Sung and Amasino, 2004). *FRI* is responsible for the production of *FLC* protein and *VIN3,* which is induced at low temperature, reduces *FLC* activity during vernalization (Sheldon *et al.*, 2000). The binding of *FLC* to the *FT*, *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*), and *FLOWERING LOCUS D* (*FD*) permits the floral transition (Searle *et al.*, 2006; Helliwell *et al.*, 2006). This binding represses the induction of these genes. Flowering is also regulated by phytochrome through *FT*. In long day conditions, GAs promote flowering by increasing *FT* expression (Porri *et al.*, 2012) whereas, under short day conditions, ABA inhibits flowering time by the regulation of *FLC* through the *ABI4* which activates *FLC* (Shu *et al.*, 2016).

All of these results confirm that the main regulator of the germination or dormancy is the ratio ABA/GA which is regulated at different levels (hormonal, temperature....).

1.1.7 Effects of priming on germination and growth

The performance of a seed is a function of the complex interaction between the genome and the environment; seed vigour can be enhanced during all of the different steps of the production process. As it is difficult to influence the

production environment, even under greenhouse conditions, plant breeders or/and seed companies try to acquire the best possible quality of seeds mainly by varying the location and method of harvest, and particularly by using post-harvest treatments such as cleaning, sorting, coating and priming and by controlling the storage conditions.

The seed industry and growers want rapid and uniform seedling establishment of vigorous plants because this can affect the yield and the quality of the final product. Indeed, good crop establishment is vital in the production of annual crops from seed because patchy stands results in low yields. Several approaches have been employed to precondition seeds to improve germination and seedling growth of various crops, including seed priming, seed soaking (e.g. hydro-priming) and seed coating with plant growth regulators and nutrients (Basra *et al.*, 2003). During coating a thin and permeable layer of emulsion is stuck to the seed surface. Priming is controlling the hydration level within seeds to allow seedlings to emerge more quickly and to help them all emerge at the same time. Priming is a physiological enhancement method in which seeds are pre-soaked in liquid then dried to re-attain their original moisture content (Heydecker, 1973). Initial imbibition is often accompanied by a massive leakage of cellular solutes from the rearrangement of the phospholipid bilayer at low temperature (Hoekstra *et al.*, 1999; Matthews and Powell, 2006; Weitbrecht *et al.*, 2011). Seeds go through the first stages of germination but do not begin radicle emergence. Seeds can be dried back to their original water content and will generally exhibit more rapid rate of radicle emergence upon subsequent imbibition (Heydecker and Coolbear, 1977, Bradford, 1986, Khan, 1992; Harris *et al.*, 2002). It ensures rapid and uniform germination accompanied with low abnormal seedling percentage for most plants (Singh, 1995; Shivankar *et al.*, 2003). The beneficial effects of priming have being associated with various biochemical, cellular and molecular events including synthesis of ribonucleic acid (RNA) and proteins (Bray *et al.*, 1989; Dell'Aquila and Bewley, 1989; Davison and Bray, 1991). Several studies on priming have observed an increase of the nuclear DNA content of radicle meristem cells from 2C to 4C indicating the progression of the cell cycle and reduced time to the radicle emergence following priming was correlated with this ratio of nuclei (Lanteri *et al.*, 1994, 1996). Additional nuclear processes related to cell cycle are involved in germination advancement such as

DNA repair (Ashraf and Bray, 1993). Others work have shown that initial radicle protusion is dependent of cell expansion and not cell division (Haigh, 1988; Górnik *et al.*, 1997). Moreover, both temperature and osmotic pre-treatment of tomato seeds have advanced germination but only the latter resulted in an increase of DNA content prior to radicle emergence (Coolbear and Grierson, 1979; Coolbear *et al.*, 1990).

Priming of seeds has proved to be a successful strategy to increase the rate of radicle emergence and the uniformity of seedling emergence (Kaya *et al.*, 2006). Several types of priming are used by the seed industry. Seed performance is improved by osmotic adjustment and leakage of cellular solutes for some field crops (Harris, 1996; Matthews and Powell, 2006). Osmo-priming is defined by a soaking in osmotic solution (Parera and Cantliffe, 1994; Li *et al.*, 2011). A high water potential solution is used to permit the absorption of water by seeds by reduction of the osmotic potential of the seed. As osmotic solution is more concentrated than seeds, ions from the solution can penetrate inside the seeds. Halo-priming is a treatment of seed with salt in order to improve germination and generally, a solution with sodium chloride enhances more the germination than other salt treatments (Gholami *et al.*, 2015). The effect of halo-priming on seed germination has been studied in some vegetables (Bradford, 1986; Matsushima and Sakagami, 2013; Passam and Kakouriotis, 1994; Tarquis and Bradford, 1992).

Finally, hydro-priming is a technique that involves soaking of seeds in an oxygenated water solution followed by a step of drying. Hydro-priming allows the seeds to quickly reach a high level of moisture with a constant supply of oxygen as seeds are in liquid solution under movement through rotation axis. Thus, the level of metabolites associated with the germination process (intermediate metabolites) and enzymes associated with the production of energy increase; this enhancement is due to the imbibition phase which activates seed metabolism. Furthermore, hydro-priming minimizes the use of chemicals and avoids discarding materials that may be undesirable to the environment (McDonald, 2000).

1.1.8 Seedling establishment as seed performance criteria

Seedling establishment is defined as the proportion of germinated seeds that develop into normal seedlings. It is an important trait for good, sustainable and profitable crop production and final yield of the crop. During seedling establishment before cotyledon emergence, seeds used stored reserves to produce energy to develop in the new crop. The quality of the seed and seedling is determined by its interaction with the environment and food reserves until seed becomes an independent, autotrophic organism, able to use light energy (Castro *et al.*, 2006; Ellis, 1992). Heavy seeds may lead to a better root architecture and seed size appears to have an essential role in an increased growth rate during its initial stage of seedling growth (Westoby *et al.*, 1992). The length of the main root and the density of the lateral roots determine the architecture of the root system in tomato and other dicotyledons and plays a crucial role in determining whether a plant will survive in a particular environment (Malamy and Ryan, 2001). This may have a major impact required inputs, and could also have direct influence on the marketing quality of a crop (Finch-Savage, 1995). Inadequate seedling growth will reduce total crop yield at harvest (Bleasdale, 1967). Abnormality at the time of seedling emergence can also affect the uniformity in plant size at harvest, which reduces the proportion of the crop in high-value size grades (Benjamin, 1990). In such a case the gross production may be high but the net profit of the crop can be greatly reduced due to low marketable yield. Seed vigour is therefore an important key factor which not only contributes directly to the economic success of commercial crops, but can also contribute in a number of indirect ways on crop production (Finch-Savage, 1995). For example, timing and uniformity of seedling emergence has an immediate impact upon the efficacy of herbicide applications, weeding strategies and other aspects of crop production that determine cost effectiveness. Poor seed quality also has a direct financial penalty for the production of plant from seed for vegetables and ornamentals in the glasshouse through wasted space, materials and reduced product quality resulting from non-uniformity.

For tomato, seedling establishment is determined 14 days after sowing (DAS) and is classified into four categories: normal seedling, abnormal seedlings, small seedlings and non-germinated seeds (Rao *et al.*, 2006). Normal seedlings have the essential structures (roots, shoots and sufficient food reserves) and are

capable of development into reproductively mature plant. The primary root is at least three time longer than the hypocotyl and plants have two cotyledons (Figure 1.11 A). Small seedlings have a normal structure but are smaller than those from the normal category (Figure 1.11 C). Abnormal seedlings have a stunted primary root, or a root which can be stubby, missing, broken, split from the tip, spindly or trapped in seed coat and the hypocotyl is short and thick, split right through, missing, constricted, twisted or glassy. The terminal bud or leaves are deformed, damaged or missing and cotyledons are swollen, deformed, necrotic, glassy, separated or missing (Figure 1.11 D). Non-germinated seeds don't germinate (Figure 1.11 B).

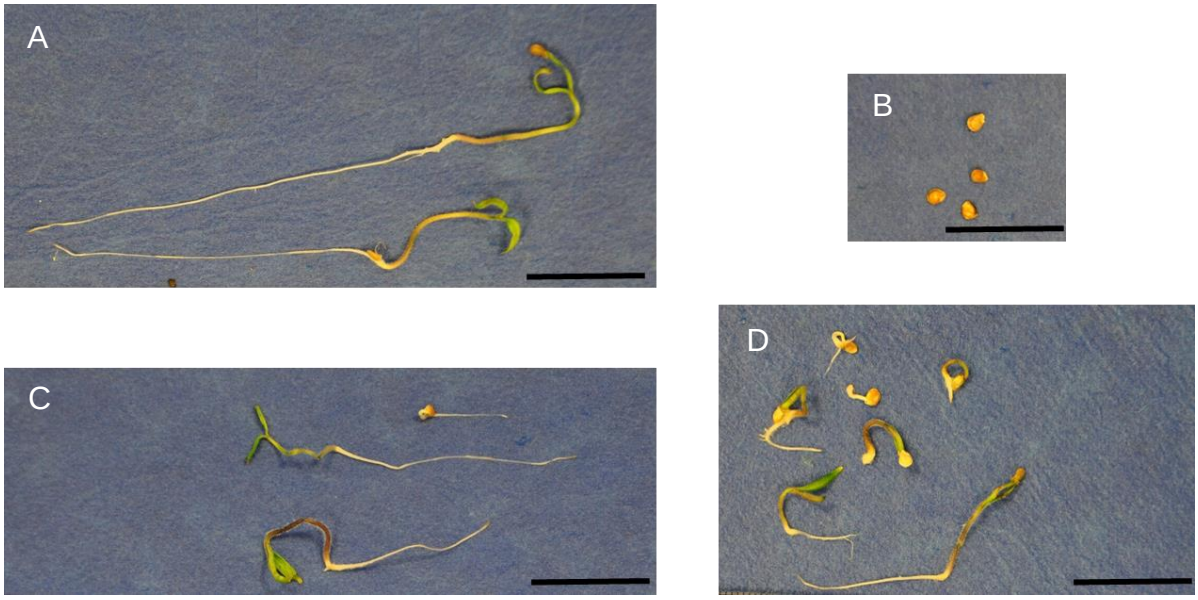

Figure 1. 11 **Seedling establishment 14 days after sowing**. A. Normal seedlings. B. Non-germinated seeds. C. Small seedlings. D. Abnormal seedlings

1.2 Thesis outline

The aim of this project is to understand the basic mechanism of the effectiveness of hydro-priming in germination improvement, and whether hydro-priming can overcome variation in seed vigour caused by changes in the maternal environment during seed production. My objective is to facilitate new methods for enhancement of seed vigour by integrating the physiology, transcriptomics and metabolomics of seeds during post-harvest treatment to identify the mechanism controlling seed germination in tomato seeds.

This thesis is divided in seven chapters including this general introduction (Chapter 1).

Chapter 2 introduces the materials and methods used for this work.

Chapter 3 explores the effect of maternal environment on vigour, hydro-priming and seed coat properties. This chapter also presents the impact of temperature during seed development on progeny and the optimal condition of hydro-priming.

Chapter 4 shows the role of nutrient balance during hydro-priming treatments. Some elements were leaked, allowing the osmosis diffusion during hydro-priming. Moreover, the role of iron in germination and during hydro-priming is studied.

Chapter 5 uses metabolomics to understand the various ways in which the seeds interact with the hydro-priming flow-through liquid called hydro-priming workflow. The work describes the relationship between the metabolites leaked into the flow through liquid and the effectiveness of hydro-priming treatments differing in duration and efficacy. Seed germination inhibitors were identified in liquid after hydro-priming treatments, supporting the concept that metabolites leakage during hydro-priming treatment could help it in the enhancement of seed germination.

Chapter 6 assesses the transcriptomic variations during hydro-priming treatment. This chapter also presents a review of the genes involved during this treatment which may be the key to understand the potential pathways involved in seed germination during hydro-priming.

Chapter 7 discusses the main findings and overall contribution of the thesis and a final critical opinion about present and future research needed to follow up for a better understanding of complex seed vigour and hydro-priming treatments.

Chapter 2

Materials and methods

2.1 Plant materials and condition of growth

Tomato plants (Appendices, Table 1), *Solanum lycopersicum*, were grown in temperate climate across the world in open fields, net house or greenhouse. Seeds were provides by Enza Zaden B.V. (Enkhuizen) and Microtom seeds were bought (www.myseeds.co). To produce temperature set batches, normal seedlings of microtom tomatoes were sown in compost Levington F2 (base fertilizer consists of 144 parts for the nitrogen (N), 73 parts for the phosphorus (P) for roots and 239 parts for the potassium sulphate (K) for flowers and fruit) until anthesis then plantlets were sowing in John Innes Seed compost (2N, 1K, 2P). During the growth period, seedlings were entrained to long days photoperiods: 16L:8D (LD) in white light at 120 $mmol.m^{-2}.s^{-1}$ at 19°C, 22°C or 25°C.

2.2 Seed extraction

Mature fruit was based on the colour of the skin which should be truly red to be sure to have mature seed inside the fruit. Freshly harvested tomatoes were smashed in a deionised water solution of Viscozyme (Sigma-Aldrich), an enzymatic mixture containing pectinase, cellulose and others enzymes, at 0.5% v/v in a 1:10 weight fruit / volume solution ratio. The mixture was placed at 27°C for at least 2h. After maceration, kitchen sieves were used to clean and remove the pulp from seeds under a flow of water. Collected seeds were dried at 25°C and 30% Relative Humidity (RH) with high air blowing-fan until seeds were reached 6 – 7% moisture content.

2.3 Seed germination

For experiments on the speed of germination and seedling establishment, seeds were taken from at last ten individual of one large population and mixed before use. Fifty dry seeds of tomato, surface sterilized, were sprinkled on two 9 cm diameter filter discs (Whatman paper) wetted with 15.0 ml sterilized water and placed into 9 cm diameter Petri dish sealed with micropore tap and incubated in a growth chamber at 22°C, LD at 200 $mmol.m^{-2}.s^{-1}$ during 14 days.

The percentage of germination was calculated with the following formula according to the International Seed Testing Association (ISTA) rules (ISTA, 1996):

$$(\%) = n/N \times 100$$

Where *n* is the number of germinated seeds and *N* is the number of total seeds.

The speed of germination was determined with the following formula:

$$\sum D \times n/N$$

Where *n* is the number of germinated seeds at each day, *D* is the numbers of days after the start of the experiment and *N* is the number of total seeds.

The accuracy and reproducibility of the germination result were dependent on the quality of paper used for germination testing. Indeed, filter paper must meet the following basic requirements:

- It should be non-toxic to the germinating seedlings,
- It should be free from other microorganisms,
- It should provide adequate, aeration and moisture to the germinating seeds,
- It should be easy to handle and use,
- It should make good contrast for judging the seedlings.

2.4 Seeding establishment

As described in Chapter 1 (Chapter 1, section 1.1.7, Figure 1.10), seedling establishment is determined after the termination of the experiment (14 days after sowing (DAS)) and seedlings from each replicates were separated into 4 categories: normal seedlings, abnormal seedlings, small seedlings and non-germinated seeds. Normal seedlings have the essential structures capable of development into reproductively mature plant. Abnormal seedlings were defined as plant having a default in essential structure and small seedlings have the essential structure as normal seedlings but they are smaller (ISTA, 1996). The percentage of normal seedlings on the total seedlings was calculated with the followed formula:

$$Normal\ seedlings\ (\%) = \frac{Normal\ seedlings}{Total\ seedlings}\ x\ 100$$

2.5 Priming

2.5.1 Hydro-priming

20% of seeds were primed in deionized water at 15°C for 24h at 200 rpm. After hydro-priming, water was discarded and seeds were dried at 25°C with high air blowing-fan until seeds reached 6 – 7% of moisture content before sowing for germination test.

For experiments on the hydro-priming liquid and more exactly for conductivity measurement and Liquid Chromatography Ion-Trap Time-of-Flight Mass Spectrometry (LC-IT-ToF/MS), seeds were primed few seconds for negative control (T0) to determine the potential presence of compounds on the outer seed coat before hydro-priming. Duration of hydro-priming had defined time:

Hydro-priming (h)	Duration
0	2 – 3 seconds
3	3 hours
6	6 hours
12	12 hours
24	24 hours

2.5.2 Hydro-priming with addition in the solution

For the experiment on the effect of external iron in hydro-priming flow, seeds were soaked in ferric sulfate ($Fe_2(SO_4)_3$), in ferrous sulfate ($FeSO_4$) solution (10 µM, 100 µM or 1 mM) or only in water at 15°C during 24h. After priming, seeds were dried until they reached the initial water content then seeds were stored until germination assay.

For the experiment with adding external 12-oxo-phytodienoic acid (OPDA) in hydro-priming flow, seeds were soaked in deionized water + 1µg/ml OPDA at 15°C for 24h. After priming, seeds were dried at 25°C and 30% RH with high air blowing-fan until seeds reached 6 – 7% of moisture content. Seeds were stored until for germination assay.

2.6 Conductivity measurement

Three replicates of 20% pre-weighed seeds of each seed lot were soaked in deionized water in plastic containers covered with a cap to prevent evaporation

loss and entry of foreign matter. A container of deionized water without seeds was prepared as the control and a container of deionized water with seeds in during 2-3 seconds was prepared as T0. This latter step is crucial because conductivity measures the leachates coming from the inside of the seeds, and leachates can also be present on the seed coat. All the containers were placed at 15°C for a time-course: 3h (T3), 6h (T6), 16h (T16) and 24h (T24). Conductivity was measured with an electrical conductivity meter (Oakton PCSTestr 35).

2.7 Tetrazolium assay

The tetrazolium salt assay (TZ) is a colorimetric assay for assessing seed coat permeability. It can give an early and quick snapshot of seed viability but it is not a replacement for the seed germination test. The protocol is adapted from Debeaujon *et al.* (Debeaujon *et al.*, 2000) in which seeds need to be alive and permeable. Dehydrogenase enzymes present in living tissue reduce the tetrazolium chloride to formazan, a reddish, water-insoluble compound. This reaction occurs in or near living cells, which are releasing hydrogen in respiration processes. Seeds are soaked in water for negative control and 1% TZ solution.

The staining is quantified with image analysis by ImageJ software (program developed at the National Institute of Health). Captured image of seeds is converted on black and white backgrounds, image type is changed into RGB stack, and threshold is adjusted with automatic setting for upper and lower limit for first time point in series. Around each sample, a circle is drawn and stack is measured (area, area fraction, limit to threshold and display label). The values are saved into Excel worksheet (Microsoft), area percent value for blue channel (background) is subtracted from red (all seeds) and green values (seeds not red) to eliminate non-specific information and the red staining are quantified with the following formulas:

$$\text{Staining}\ (\%) = 100 - \left\{\frac{(\text{Not red} - \text{Background})}{(\text{All seeds} - \text{Background})}\right\} * 100$$

Or

$$\text{Staining}\ (\%) = 100 - \left\{\frac{(\text{Green} - \text{Blue})}{(\text{Red} - \text{Blue})}\right\} * 100$$

The red colouration is determined by incubation in TZ solution and by subtracting the value for seeds in water only from the one from in TZ. To observe the staining on the endosperm or embryo, seeds could be dissected, either longitudinally or transversely, with a scalpel and one half of this seed is used for the test and the other half is discarded.

2.8 Determination of proanthocyanidins content

The method used to determine proanthocyanidins (PAs) content was adapted from Routaboul (Routaboul *et al.,* 2012). PAs were extracted from frozen ground seeds using methanol/acetone/water/acetic acid (30/42/28/0.05, v/v/v/v) as an extraction solvent. The first extraction was spiked with 3 µg of apigenin (Sigma-Aldrich) as an internal standard. The supernatant was removed and stored at -20°C, the pellet was extracted furthermore with 1 ml of the same extraction buffer overnight at 4°C. The solutions from both extractions were pooled and dried. The insoluble PAs which remained in the pellets during both extractions were kept at -20°C until required. Soluble PAs was redissolved in 300 µl of extraction buffer, 10 µl were used for colorimetic acid butanol assay according to Porter (Porter *et al.*, 1985) using 600 µl of butanol-HCl reagent (butanol-concentrated HCl, 95/5, v/v) and 20 µl of the ferric reagent (2% ferric ammonium sulphate in 2N HCl). Samples were heated to 95°C for 60 minutes and measured at 550 nm using a spectrophotometer.

2.9 Mineral analysis using Inductively Coupled Plasma-Mass Spectrometry

Pre-weighed seeds were primed in deionized water during 3h, 6h, 16h and 24h at 15°C. Water was retained after hydro-priming and was analysed by an external supplier (ALcontrol laboratories) which is ISO17025 and mCERTS accredited. The concentration of the elements were measured using inductively coupled plasma-mass spectrometry (ICP-MS) using the method 3125B (APHA/AWWA/WEF, 1999) and major cations in water were determined by iCap 6500 Duo inductively coupled plasma-optical emission spectrometry (ICP-OES) using the method 6010B (US EPA). Ionized atoms were carried out to sampling and were transferred to quadrupole mass analyzer in which they passed through a series of ions lenses according to their mass to charge ratio. Ions were detected

and quantified by comparing with reference standards for each elements and above the Limit of Detection (LOD) (Table 2.1). The total amount of element in hydro-priming flow through liquid was established by means of ICP-MS data and was obtained from three independent experiments and the mean value was calculated for each mineral. All statistical analyses were performed using Minitab 17 (Minitab) and Microsoft Excel.

Table 2. 1 **Analysis method using ICP-MS or ICP-OES**.

Elements	**Method**	**Units**	**LOD**
Aluminium	TM 152	µg/l	<2.9
Antimony	TM 152	µg/l	<0.16
Barium	TM 152	µg/l	<0.03
Boron	TM 152	µg/l	<9.4
Calcium	TM 228	µg/l	<12
Chromium	TM 152	µg/l	<0.22
Copper	TM 152	µg/l	<0.85
Iron	TM 228	µg/l	<19
Lead	TM 152	µg/l	<0.02
Lithium	TM 152	µg/l	<1.92
Magnesium	TM 228	µg/l	<36
Manganese	TM 152	µg/l	<0.04
Molybdenum	TM 152	µg/l	<0.24
Nickel	TM 152	µg/l	<0.15
Phosphorus	TM 152	µg/l	<6.3
Potassium	TM 228	µg/l	<1000
Sodium	TM 228	mg/l	<0.076
Strontium	TM 152	µg/l	<0.05
Titanium	TM 152	µg/l	<1.5
Vanadium	TM 152	µg/l	<0.24
Zinc	TM 152	µg/l	<0.41

2.10 Iron determination with Ferene

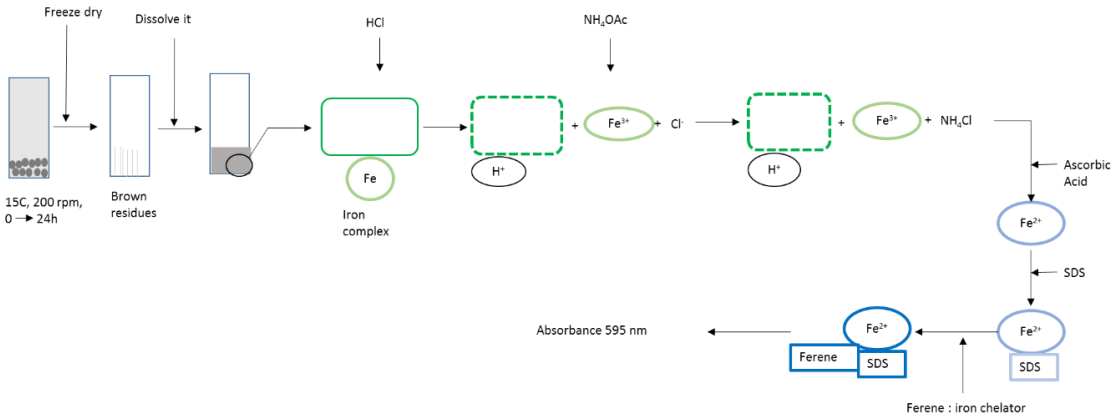

Figure 2. 1 **Flowchart summarizing the main steps taken in this thesis in the determination of iron with Ferene**. The details of each steps are given below.

For iron content determination on seed coat, seeds were primed during 24h at 15°C, then the seed coat was removed and ground. Samples were concentrated in 100 µl of deionized water or stored at – 20°C. Iron quantification protocol was adapted from Smith (Smith *et al.*, 1981). The iron within a protein complex is released by 1% (w/v) aqueous HCl solution. Excess acid is neutralized with 7.5% (w/v) aqueous ammonium acetate solution, iron(III), Fe^{3+}, is converted into iron(II), Fe^{2+}, by reduction with 4% (w/v) aqueous ascorbic acid solution. Precipitated protein is complexed with 2.5% (w/v) aqueous sodium dodecyl sulphate (SDS) solution. Finally, a 1.5% (w/v) aqueous iron chelator solution (3-(2-pyridyl)-5,6-bis(5-sulfo-2-furyl)-1,2,4-triazine or Ferene) is added to form a blue complex and is measured at 593 nm using a spectrophotometer.

2.11 Extraction of metabolites from hydro-priming liquid for LC-IT-Tof/MS detection with untargeted method

Metabolite analysis was performed using a liquid chromatography ion-trap time-of-flight mass-spectrometer (LC-IT-ToF/MS or LC-MS) as summarized in Figure 2.2.

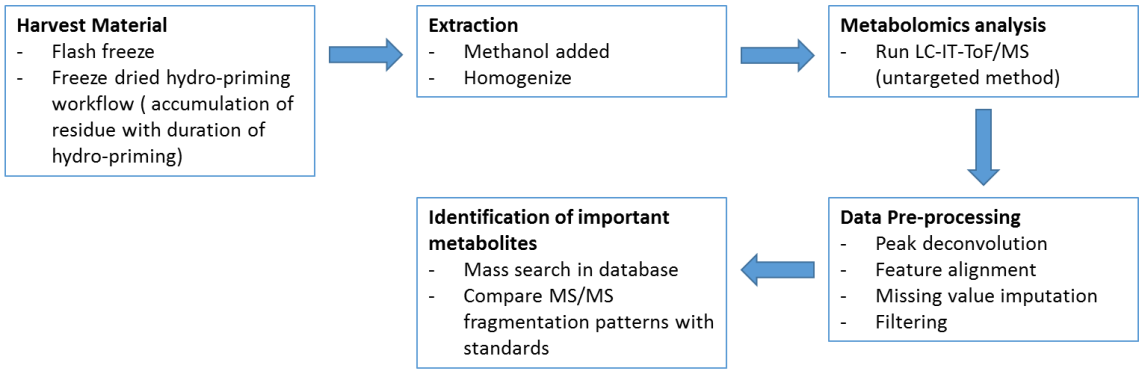

Figure 2. 2 **Flowchart summarizing the main steps taken in this thesis in the metabolomic analysis of water retained from hydro-priming solution using LC-IT-ToF MS**. The details of each steps are given below.

Hydro-priming flow through liquid was freezed in liquid nitrogen after each time-point then frozen samples were freeze-dried during 2 days in freeze-dryer (Freeze Drying Solutions). Samples were stored at -80°C until needed for analysis. Samples were reconstitued in 20% methanol then were mixed throughtly. After centrifugation (2 minutes (min) at room temperature (RT) at 14 000 rotation per minute (rpm)), samples were transferred in LC-MS screw cap vials and analysed using a Prominence/Nexera Ultra-Performance Liquid Chromatography (UPLC) system attached to an ion-trap ToF mass spectrometer (Shimadzu). Metabolomic features were detected with two ionization modes: positive and negative. These two modes of ionization analyses were separated to broaden the range of target analytes. Separation was on a 100×2.1mm 2.6µ Kinetex XB-C18 column (Phenomenex) and samples ran at 0.4 $mL.min^{-1}$, 40ºC using the following gradient of acetonitrile versus 0.1% formic acid in water:

Time (minutes)	% acetonitrile
0	4
0.5	4
15.0	95
17.0	95
17.2	4
22.4	4

The injection volume was 10 µL. The instrument was set up to collect visible and ultra-violet (UV) absorbance, and electrospray mass spectrometry (MS). Absorbance spectra were collected from 200-600 nm at 12.5 Hz with a time-constant of 0.16 sec. Full spectra were collected from *m/z* 80-800 (*m/z*

represents mass divided by charge number), *m/z* 200-2000 and *m/z* 50-2000 with a maximum ion accumulation time of 20 msec and automatic sensitivity control set to a target of 70% of optimal base peak intensity. The instrument also collected fragmentation MS-MS data for the most abundant ions in the range *m/z* 50-2000 at an isolation width of *m/z* 3.0, 50% collision energy, and 50% collision gas, and 10 msec ion accumulation time. At least three spectra were collected for each precursor ion, after which the precursor was ignored in favour of the next most abundant, for 2.5sec.

An LC-MS solution software was used for instrument control and data acquisition in profile mode (Shimadzu, Europe). Quality control (QC) samples used as positive controls were prepared by pooling all samples and control samples. Negative control samples were prepared using deionized water only following the same procedure as hydro-priming. The analysis order of samples, negative control and QC were randomized within the experiment batch. The untargeted metabolites were analyzed with LCMSsolution V380 and Profiling solution softwares. Metabolites in the hydro-priming work flow were separated by retention time (RT), and mass signals corresponding to the peak area was used to quantify abundance of compounds. An internal standard, sodium trifluoroacetate (NaTFA), was used to calibrate samples. Signals present in less than 3 replicates, or less than 5 controls were discarded. The mass spectra (ion *m/z*) of those signals corresponding to a compound were identified with a tolerance of 10 ppm from an online database (METLIN: Metabolite and Tandem MS Database).

2.12 Phytohormone extraction from hydro-priming flow-through using LC-IT-ToF/MS detection

OPDA analysis was performed using LC-IT-ToF/MS method described in this chapter, section 2.11. Briefly, hydro-priming flow-through was reconstituted in 20% methanol (MeOH), samples were run using a Prominence/Nexera UPLC system attached to an ion-trap ToF mass spectrometer (Shimadzu). Separation was achieved on a Kinetex XB-C18 100×2.1mm 2.6µ column (Phenomenex) using the gradient of acetonitrile versus 0.1% formic acid in water, run at 0.4mL.min-1 and 40ºC, as used in untargeted method (section 2.11).

LC-IT-ToF/MS data were collected in full MS scan mode over the mass range *m/z* 80 to 2000 in positive ionization mode and in negative ionization mode.

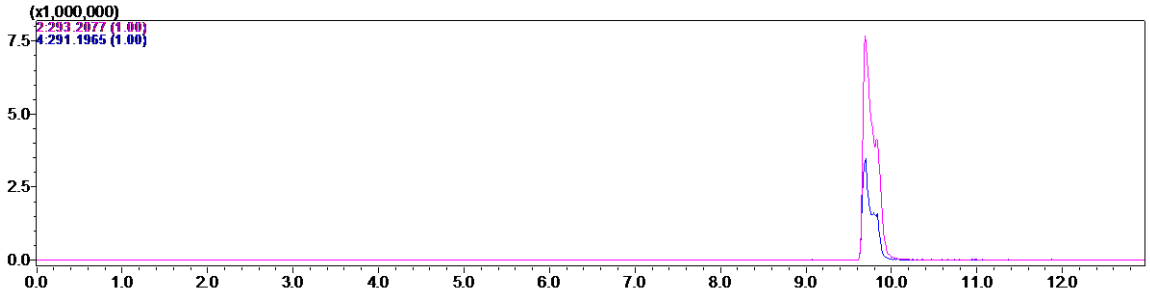

Figure 2. 3 **OPDA ion chromatograms after tuning.** Peaks were identified based on retention times and m/z for specific ions for negative scan mode and positive scan mode.

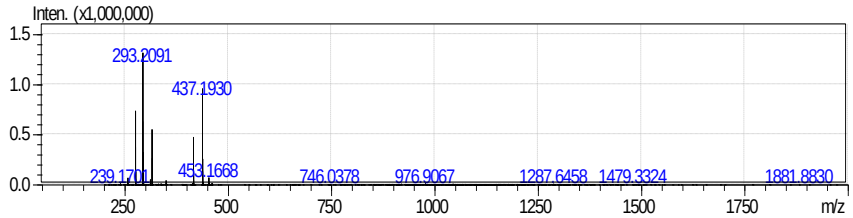

Figure 2. 4 **OPDA spectrum obtained with positive ionization mode**.

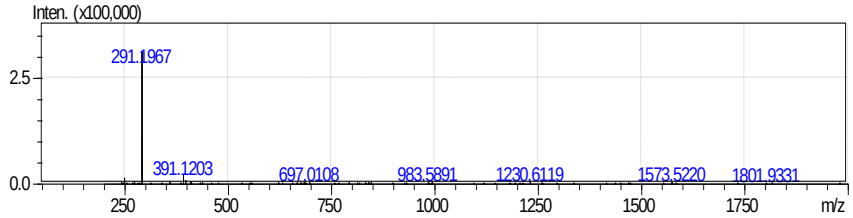

Figure 2. 5 **OPDA spectrum obtained with negative ionization mode**.

Extracted ion chromatograms (XIC) are obtained for the OPDA peaks from external standards and extracts using m/z for specific ions (Figure 2.3 – 2.5). The peak area of XIC obtained for each samples and standards were integrated using LC-MS solution software. NaTFA was used as internal standard and OPDA was used as external standard (Figures 2.3-2.5). This software was used for instrument control and data acquisition in profile mode (Shimadzu, Europe). Quality control (QC) samples were used as positive controls and were prepared by pooling all samples and control samples. Negative control samples were prepared using deionized water only following the same procedure as hydro-priming experiment. The analysis order of samples, negative control and QC were randomized within the experiment batch. Metabolites in the water retained from hydro-priming were separated by retention time, mass spectra and mass signal

corresponding to the peak area was used to identify and quantify the abundance of compound. Signals present in less than 3 replicates, or less than 5 controls were discarded.

2.13 Phytohormone extraction from dry seeds using LC-IT-ToF/MS detection

Oxilipin analysis was performed using LC-IT-ToF MS method described by Theodoulou (Theodoulou *et al.*, 2005). For seed analysis, seeds from five biological replicates were used. Briefly, 80 to 100 mg dry seed tissue was ground and extracted with 1.9ml of Extraction solvent (isopropanol (Thermo Fisher Scientific) plus 1% acetic acid (Sigma-Aldrich)) on shaker overnight in cold room in dark at 250 rpm with 10 µl internal standard mix (2 µg/ml Prostaglandin A1 (Sigma-Aldrich) and 0.2 µg/ml Jasmonic acid (Sigma-Aldrich)) added as an internal standard. The samples were centrifuged at 12 000 rpm for 5 min at 4°C. Supernatant was dried in GeneVac low boiling point and lamp off settings selected then samples were re-extracted with 1 ml of extraction solvent for 60 min on shaker in cold room at 250 rpm then samples were centrifuged at 12 000 rpm for 5 min at 4°C. Supernatant was dried and samples were stored at -80°C.

Samples were re-suspended in 100 µl of 20% methanol and extract was transferred into the LC-MS vial for analysis. Samples were run using a Prominence/Nexera UPLC system attached to an ion-trap ToF mass spectrometer (Shimadzu). Separation was achieved on a Kinetex XB-C18 100×2.1mm 2.6µ column (Phenomenex) using a gradient of mobile phase water + 0.1% formic acid and acetonitrile with a flow rate 0.8 mL/minutes.

Time (minutes)	% acetonitrile
0.01	4
0.10	4
3.00	95
3.10	95
3.15	4
4.02	4

LC-IT-ToF/MS data were collected in full MS scan mode over the mass range *m/z* 80 to 2000 in positive ionization mode and in negative ionization. OPDA was quantified using authentic OPDA standard (Larodan) as external standard. Extracted ion chromatograms (XIC) are obtained for the oxilipins peaks

from internal standards and extracts using m/z for specific ions. The peak area of XIC obtained for each samples and standard were integrated using LC-MS solution software. This software was used for instrument control and data acquisition in profile mode (Shimadzu, Europe). Quality control (QC) samples were used as positive controls and were prepared by pooling all samples and control samples. Negative control samples were prepared using deionized water only following the same procedure as steeping experiment. The analysis order of samples, negative control and QC were randomized within the experiment batch. Metabolites compounds in the steeping work flow were separated by retention time (RT), mass spectra and mass signal corresponding to the peak area was used. Signals present in less than 3 replicates, or less than 5 controls (QC) were discarded.

2.14 RNA-Sequencing

2.14.1 Samples preparation for RNA-sequencing

Seeds of Kanavaro 547.392 were primed at 15°C in water for 3h (T3), 6h (T6), 16h (T16), 24h (T24) or un-primed (T0). After hydro-priming, seeds were frozen in liquid nitrogen and stored at -80°C until needed for experiment.

All samples were performed from five or more biological replicates and in two technical replicates each (A and B) except for the negative control that was performed in three replicates (from A to C).

2.14.2 Library preparation for RNA-sequencing

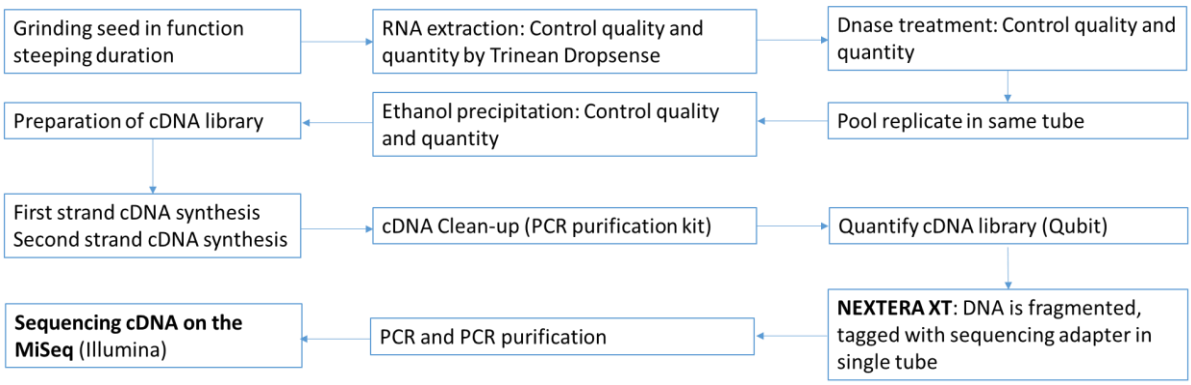

Figure 2. 6 **Flowchart summarizing the main steps taken in this thesis in the library preparation for RNAseq**. The details of each steps are given below.

Total RNA (Ribonucleic acid) was prepared from 100 mg of seed tissue using the Kit Total RNA isolation NucleoSpin 96 RNA (Macharey-Nagel) with

rDNase digestion step according to the manufacturer's protocol (Macharey-Nagel). Total RNA quality and quantity control were performed by spectrophotometry using Trinean DropSense (Trinean). An additional DNase digestion step was performed according to the manufacturer's protocol (Rnase-free Dnase set; Qiagen) then the quality and the quantity control were performed by spectrophotometry using Trinean DropSense. Replicates were pooled in the same tube. To purify, de-salt and concentrate acid nucleic, an additional ethanol precipitation step was performed. 3M of sodium acetate (NaAc) pH 5.2 was added (1/10 (v/v) NaAc/RNA) then 5 mg/ml of glycogen was added to obtain a bigger and more adhesive pellet. 630 µl of 100% ethanol was added then samples were incubated for 15 min at -80°C. Samples were centrifuged at 14 000 rpm for 15 min at 4°C then ethanol was discarded. 150 µl of fresh 70% ethanol was added then samples were centrifuged at 15 000 rpm for 5 min at 4°C. Supernatant was removed and pellet was dried. Pellet was re-suspended in 25 µl of Rnase-free water. Quality and quantity of total RNA was controlled by spectrophotometry.

First strand cDNA (complementary Deoxyribonucleic acid) was synthesized from 1-10 µg DNA-free total RNA with M-Superscript II reverse transcriptase (200 U/µl) according to the manufacturer's protocol (Thermofisher) using oligo $(dT)_{20}$ primer, following the manufacturer's instructions. Second strand of cDNA was synthesized using 10X second strand synthesis Reaction buffer and a second strand synthesis enzyme mix (*E. coli* DNA Ligase, *E. coli* DNA Polymerase I and *E. coli* Rnase H) for 2h at 16°C. After incubation 2 µl of T4 DNA polymerase was added then sample were incubated for 5 min at 16°C. 10 µl of 0.5M EDTA (ethylene-diamineteraacetic acid) was added to samples to stop reaction. cDNA clean-up was performed using MinElute PCR (Polymerase Chain Reaction) purification kit (Qiagen) according to the manufacturer's protocol. cDNA was eluted into 20 µl of Rnase-free water then cDNA quantity was controlled using fluorometric quantification method with qubit (Qubit*).* DNA library preparation kit was prepared with Nextera XT Index kit (Illumina) according to the manufacturer's protocol. Samples were prepared with different couples of index primers:

T0-A	T0-B	T0-C	T3-A	T3-B	T6-A	T6-B	T16-A	T16-B	T24-A	T24-B
S701	S702	S703	S704	S705	S706	S707	S708	S709	S710	S711
S502	S503	S504	S505	S506	S507	S508	S502	S503	S504	S505

The reactions were performed in RT-PCR (Real Time-Polymerase Chain Reaction) system with the following program: 72°C for 3 min and 95°C for 30 sec, followed by 15 cycles of 95°C for 10 sec, 55°C for 30 sec and 72°C for 30 sec, and 72°C for 5 min. PCR products were purified for high-throughput sequencing (Hiseq) with 1X of Agencourt AMPure XP bead solutions (Beckman Coulter) for 5 min at room temperature (RT). Samples were mixed and placed on magnetic tube holder for 5 min. Supernatant was drained without disturbing the beads, was washed with 150 µl of 80% ethanol for 1 min twice and was dried. After drying, 20 µl of Rnase-free water was adding on sample. After vortexing, samples were incubated on the magnet holder at RT. If the incubation at RT exceeds 5 min, cDNA may be damaged and changed the results on transcript levels. 15 µl of cDNA library was used to control quality and quantity with Qubit. cDNA library was pooled to obtain a concentration of 1 ng/µl. The concentration was controlled using Qubit method. Volume of cDNA library was reduced by heating samples at 65°C for 60 min. Samples were stored at -20°C until needed for sequencing.

cDNA library was sequenced using MiSeq Reagent kit and MiSeq Reagent kit V3 (Illumina) according to the manufacturer's protocol. cDNA library stock was pooled at 17 pM and 600 µl of solution was loaded.

2.14.3 RNA-seq analysis workflow

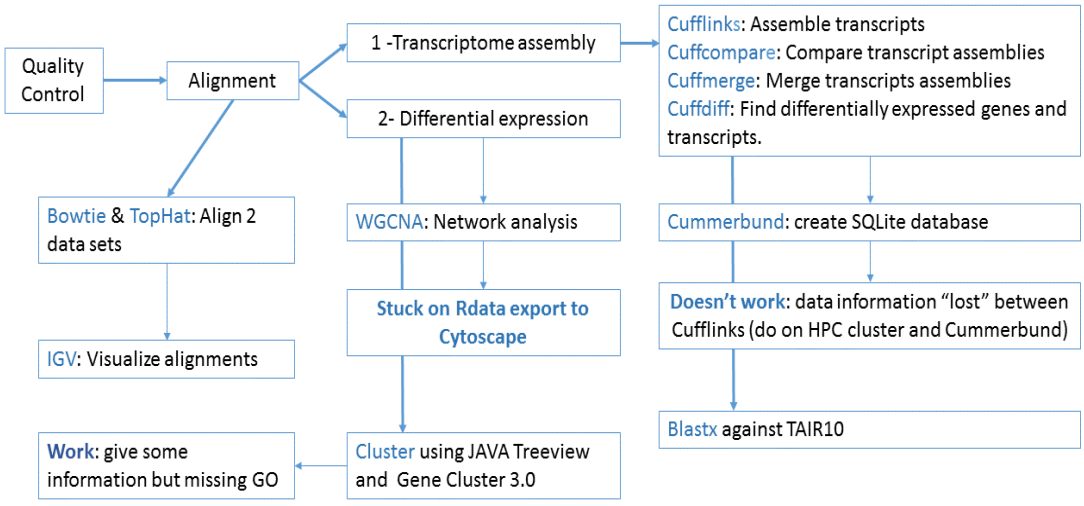

Figure 2. 7 **Flowchart summarizing the main steps taken in this thesis in the transcriptomic analysis.** The details of each steps are given below.

Sequencing was performed on the Illumina platform and was generated 76 bp (base pair) paired-end sequence data using index primers selected on cDNA. Illumina high-throughput sequencing was provided high sequence data and all data analysis was performed on UNIX system. Data quality was investigated for each dataset to visualize the quality score. FastQC was processed to generate a visual quality control report of the reads. Based on the results, the sequence data was processed using trimmomatic, a toolkit to trim the reads (Bolger *et al.*, 2014).

For all samples, quality score (Q(A)) was between 30-40 giving an error probability (P(~A)) of 0.001 according to the relationship:

$$Q(A) = -10 \log 0(P(\sim A))$$

Table 2. 2 **Pre-processed RNA-sequencing data using Trimmomatic.** Each sample represents seeds in function hydro-priming time-course. All statistics were calculated based on the paired-end reads.

Sample	# of surviving reads	Overall mapping rate (%)	Alignment rate (%)
T0-A	2,217,646	83.2	64.2
T0-B	2,087,510	24.4	11.5
T0-C	2,087,510	84.6	67.6
T3-A	2,652,244	85.5	69.4
T3-B	2,537,893	85.8	69.8
T6-A	1,525,210	85.6	71.3
T6-B	2,474,229	85.7	68.6
T16-A	1,909,126	85.9	69.8
T16-B	2,572,299	83.6	61.6
T24-A	2,592,124	85.4	67.1
T24-B	2,645,169	87.3	75.1

After trimming, read 1 and read 2 of the same dataset were aligned together on the reference genome provided by Tomato Genome Consortium (Solgenomics) using tophat-toolkit based on the protocol published by Trapnell (Trapnell *et al.*, 2012). The reference genome was built on SL2.50 and was annotated on February, 2014 by ITAG2.4 (Tomato Genome Annotation). The annotation was covered approximately 84% of the genome with 37 725 gene models.

Tophat-toolkit was produced several files:

- accepted_hits.bam
- align_summary.txt
- deletions.bed
- insertions.bed
- junctions.bed
- prep_reads.info
- unmapped.bam

As some part of the genome were not unique (common, repeated motifs or regions), samtools-toolkit was used to manipulate alignments created previously with the BAM file. Samtools sort was an option to sort alignment according to position on reference genome by producing accepted_hits.sorted.bam file, samtools flagstat was another option to collect and calculate statistics from BAM files and outputs in a text format. The last option of samtools was samtools index, it was used to coordinate sorted BAM file by producing accepted_hits.sorted.bai file. In order to assembl transcripts and test their abundance in sample, cufflinks-toolkit was used on accepted_hits.bam file.

This steps was produced 4 files:

- genes.fpkm_tracking
- isoforms.fpkm_tracking
- skipped.gtf
- transcript.gtf

To find differential expressed genes and transcripts, cuffdiff-toolkit was used on accepted_hits.bam file. This option was produced many files:

- bias_params.info
- cds.count_tracking
- cds.diff
- cds.fpkm_tracking
- cds.read_group_tracking
- cds_exp.diff
- gene_exp.diff
- genes.count_tracking
- genes.fpkm_tracking
- genes.read_group_tracking
- isoform_exp.diff
- isoforms.count_tracking
- isoforms.fpkm_tracking
- isoforms.read_group_tracking
- promoters.diff
- read_groups.info
- run.info
- splicing.diff
- tss_group_exp.diff
- tss_groups.count_tracking
- tss_groups.fpkm_tracking
- tss_groups.read_group_tracking
- var_model.info

To compare transcript assemblies to annotation, cuffcompare-toolkit was used. This steps was produced several files:

- cuffcmp.combined.gtf
- cuffcmp.loci
- cuffcmp.stats
- cuffcmp.tracking

After transcriptome assembly, values were normalized by FPKM (Fragment Per Kilobase of exon per Million fragment) method. Values were normalized by length of exon, total number of mapped fragments. Differential expression analysis was performed using Weighted Gene Co-expression Network Analysis (WGCNA) package on R (Langfelder and Horvath, 2008) and using the open source software bioconductor. Expressed genes were described as being differentially expressed between primed and un-primed if the expression was two fold or more different.

2.14.4 Clustering

Clustering array was processed following Eisen protocol (Eisen *et al.*, 1998) using Gene Cluster 3.0 and Java TreeView. On Cluster 3.0, data were log transformed in log2 counts/intensities and low expressed or invariant genes were removed. Relative expression of the gene from each experiment was represented by substracting the mean values of the gene from each experiment. Data were clustered for both genes and array. The results of clustering was visualized using Java TreeView. To avoid problems with taking the log of zero, +1 is adding to all read counts in Excel before importing the data into Gene Cluster 3.0 and replicates were clustered together to minimize the source of variation and permit to identify key genes.

2.15 Statistical analysis

To determine whether the differences between means were statistically significant, a two-tailed Student's t-test was performed. Means are described as being significantly different throughout the thesis when $P \leq 0.05$.

Analysis of variance (ANOVA) was performed using Excel (Microsoft) to determine the difference among group means.

The standard error (SE) is used to determine the dispersion of sample means around the sample data.

Chapter 3

Effect of maternal environment on vigour, hydro-priming and seed coat properties

3.1 Introduction

3.1.1 Maternal environment effects seed vigour

In different *a. thaliana* accessions, seeds can have different requirement for dormancy breakage and therefore may often vary in response to the same cues. For example under the same conditions, *Landsberg erecta* (*Ler*) seeds which have a low dormancy level needed about 6 weeks of dry storage to obtain 100% of germination whereas Cape Verde Island (Cvi) strain which have a strong dormancy needed 15 weeks of dry storage to reach 100% of germination (Alonso-Blanco *et al.*, 2003). Genetic variation of seed dormancy is controlled by the *DELAY OF GERMINATION 1* (*DOG1*) gene. *DOG1* is regulated by temperature during maturation. Ecotypes having a high transcript level of *DOG1* gene showed a strong dormancy (Bentsink *et al.*, 2006). Moreover, the maternal environment may influence the phenotype of the offspring directly by maternal provisionning of resources and hormones and, maternal control of germination can operate throught seed coat or endosperm. Indeed, non-dormant seed are more permeable than the dormant ones (Debeaujon *et al.,* 2000; MacGregor *et al.,* 2015). In *Arabidopsis thaliana* seeds, the depth of dormancy in fully matured seeds is determined by environmental conditions, particularly temperature and light, experienced by the mother plant (Dobrovolna and Cetl, 1966; Goto, 1982; Roach and Wulff, 1987; Biere, 1991; Platenkamp and Shaw, 1993; León-Kloosterziel *et al.*, 1994; Lacey *et al.*, 1997; Baskin and Baskin, 1998). Seed dormancy is coat-imposed, with both the testa and endosperm playing a critical role in dormancy maintenance. The seed coat plays a role in dormancy through the accumulation of tannins in the inner integument (Debeaujon *et al.*, 2000; MacGregor *et al.*, 2015).

3.1.2 Maternal effects on the seed coat

The seed coat, composed of maternal tissue (see chapter 1), creates the environment that the embryo experiences (Schmitt *et al.*, 1992; Platenkamp and Shaw, 1993; Donohue and Schmitt, 1998). It imposes mechanical constraints to germination (Kugler, 1951; Dobrovolna and Cetl, 1966; Goto, 1982; Biere, 1991; Platenkamp and Shaw, 1993; León-Kloosterzeil *et al.,* 1994) and can determine permeability (Baskin and Baskin, 1998) and alters light environments

experienced by the embryo (Botto *et al.*, 1996). Thus, the evolution of seasonal dormancy may involve selection on genetic variation in germination responses to offspring environments among maternal parents, as well as among individual seeds. For example, in the desert annual *Ononis sicula*, day-length variation caused changes in the development of the seed coat and its surface structure, modifying the permeability of the seed coat, its fungal resistance, and the seed longevity (Gutterman, 1992).

3.1.3 Effect of hydro-priming on seed germination

Hydro-priming uses only water to hydrate the seeds before radicle protrusion followed by drying of seeds to prevent radicle emergence (Cantliffe *et al.*, 1984, Farooq *et al.,* 2006). When seeds are primed, water content is limited and the metabolic steps necessary for germination can occur without the irreversible act of radicle emergence. Duration of soaking and temperature of hydro-priming are optimised for each crop variety (Kaya *et al*., 2006; Khan *et al.*, 2012; Dastanpoor *et al.*, 2013).

It has been previously shown that variation in maternal environment affects seed dormancy, but little is known about the effects on seed germination vigour in species with little or no dormancy, and whether these effects can be altered by priming. The compounds that are imported from the mother plant and also factors that are produced by the embryo itself are controlled by a large number of genes which are affected by both developmental and environmental factors. In tomato (*Solanum lycopersicum*) seeds, seedling emergence is frequently slow and not uniform. The objective of this study is to assess the influence of seed production temperature on tomato seed vigour and to optimise the maternal environment for production of tomato seeds that respond to hydro-priming on germination and seedling emergence of tomato, thus creating a study system for assessing the effect of hydro-priming on seed germination.

3.2 Results

3.2.1 Hydro-priming decreases the time necessary for germination and emergence

Beef tomatoes have the lowest speed of germination in comparison with other tomatoes varieties and, to optimise a hydro-priming procedure for tomato, post-harvest treatment such as hydro-priming was tested at different temperatures and durations (Figure 3.1). Freshly harvested seeds that were matured in the field showed low germination rate without post-harvest treatment. The results showed that hydro-priming at cold temperatures, either 10°C or 15°C, increased the germination rate whilst hydro-priming at warm temperature, 20°C, reduced the germination rate. The duration of hydro-priming was tested to determine the best condition of hydro-priming. Germination rate was significantly higher in seeds with hydro-priming treatments at 10°C or 15°C. Two treatments had a higher germination rate: priming at 10°C during 24h and priming at 15°C during 24h, but only the second had more than 80% of germination. Therefore, for all experiences in this thesis, seeds will be primed at 15°C during 24h then dried for two days at 25°C before sowing for germination test.

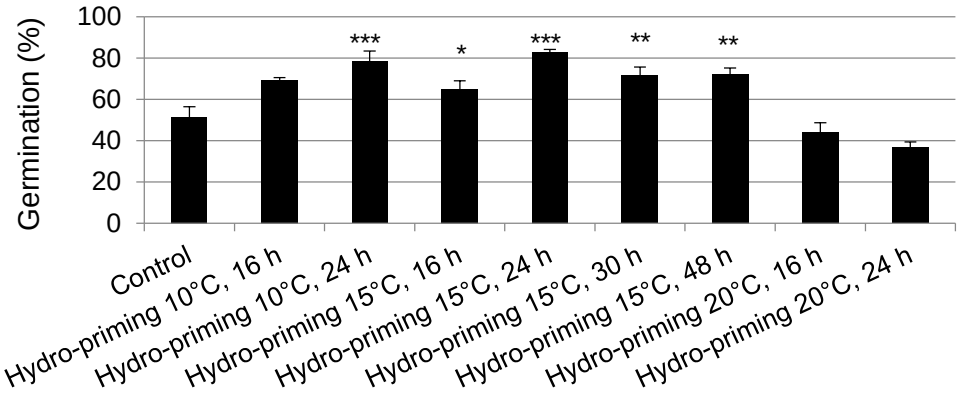

Figure 3. 1 **Effect of hydro-priming temperature on beef tomato seed germination**. Germination rate of seeds 3 days after sowing (DAS) at the indicated temperatures and duration of treatment. Data presented are the averages of four biological replicates ± SE of 50 seeds each. Significant differences by ANOVA: *, P<0.05; **, P<0.01; ***, P<0.001.

In this thesis, seed performance is defined by two criteria: germination speed and seedling establishment. To try to understand the effect of maternal environment on seed performance, plants were grown at different temperatures under laboratory conditions. This temperature treatment is referred to as the seed “maturation temperature”.

S. lycopersicum cultivar (cv.) Microtom seeds were used and the temperatures were chosen to sample the range of behaviours of seed set under standard conditions and also under conditions that are higher or lower in temperature compared to the standard (Figure 3.2). To grow tomatoes, optimal temperature of seed maturation is between 22°C-25°C (Adams *et al.*, 2001). The increase in the seed maturation temperature from 12°C to 28°C have an effect on the speed of germination (Figure 3.2A). In general, little dormancy is induced when tomato seeds are matured at 25°C. In contrast, when seeds are matured at lower temperature, a delay in the speed of germination is observed. These results show that Microtom seeds display a sensitivity to the decrease of temperature maturation. Germination of a seed lot was assessed by a standard germination test in which the number of seeds capable of producing normal seedlings are recorded (Figure 3.2B). The frequency of normal seedlings produced from seed matured at 19°C were similar to the one of normal seedlings produced from seeds matured at 22°C. When seeds are matured at higher or lower temperature, the number of normal seedlings per batches decreased. These results show that by reducing maturation temperature or increasing the maturation temperature, the number of normal seedlings is negatively affected, even though radicle emergence frequencies remain high.

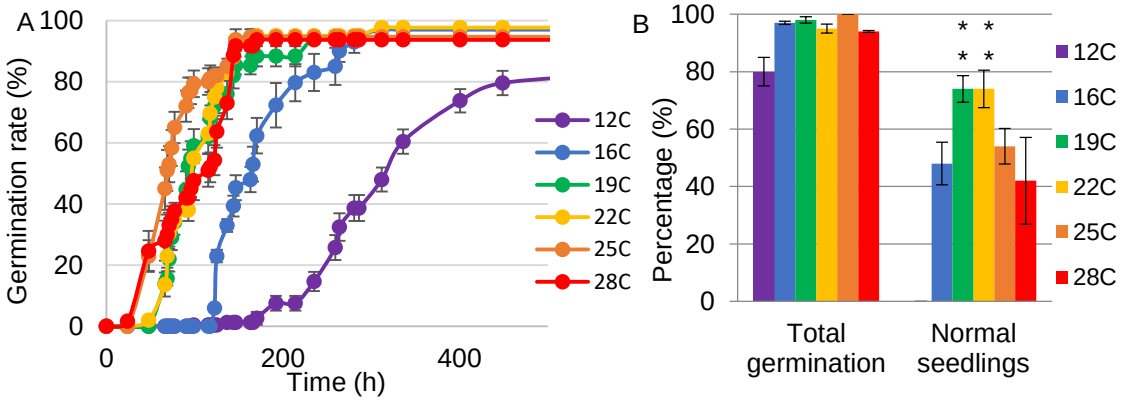

Figure 3. 2 **Effect of maturation temperature on seed vigour for *S. lycopersicum* cv. Microtom line**. A. Germination time-course of Microtom seeds under different maturation temperatures as indicated. B. Seedlings establishment of Microtom seeds under different maturation temperatures as indicated. No normal seedlings are observed at 12°C. Data presented are the averages of three biological replicates ± SE of 50 seeds each. Significant differences between seeds matued at 28°C and other temperatures of maturation by a Student's *t-test*: *, P<0.05; **, P<0.01.

In order to check if the maturation temperature affects the seeds, I measured and weighed seeds set from different temperatures and results showed that maturation temperature acts on seeds (Figure 3.3). Therefore I concluded that maturation temperature affects seed size and seeds matured at higher temperatures have higher weight than seeds matured at low temperatures.

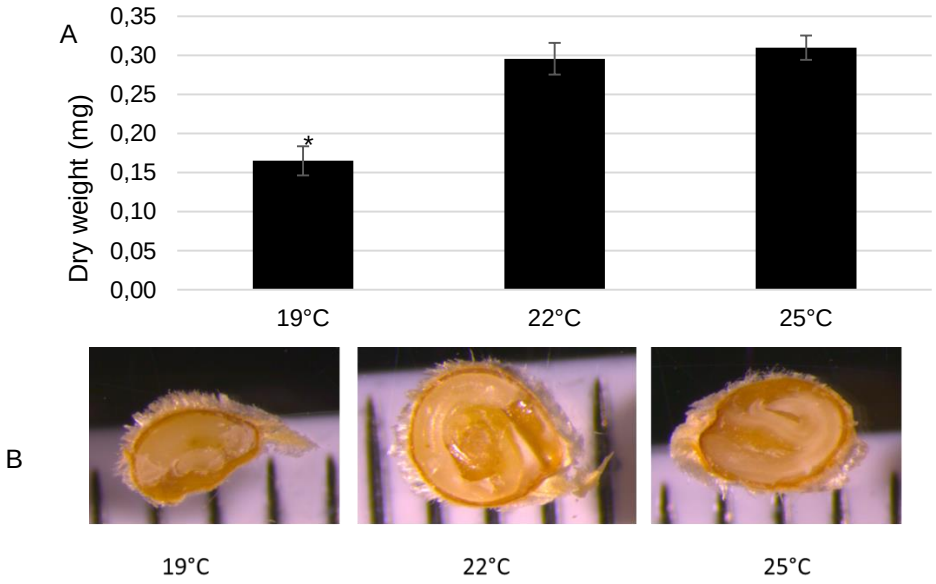

Figure 3. 3 **Effect of maternal temperature on seeds weight for *S. lycopersicum* cv. Microtom line**. A. Dry weight per seeds from seeds matured at different temperatures. B. Seeds cut longitudinally. The data presented are mean values ± SE of 3 replicates of 50 seeds each. Significant differences against 19°C by a Student's *t-test*: *, P<0.01.

3.2.2 Influence of hydro-priming on seed performance

To optimise a hydro-priming protocol for Microtom seeds, a time-course experiment was conducted on freshly harvested seeds matured at 22°C (Figure 3.4, Table 3.1). Germination speed, number of hours to 50% of germination (T50) and seedling establishment are measured for each condition: un-primed seeds, 3h, 6h, 16h or 24h of hydro-priming. Primed seeds germinated significantly more rapidly than the controls ones. Hydro-priming treatments improved speed of germination. Indeed, seeds primed during 16h or 24h had a higher speed of germination than seeds primed during 3h, 6h or even the controls. Hydro-priming did not affect the seedling establishment frequency of Microtom seeds.

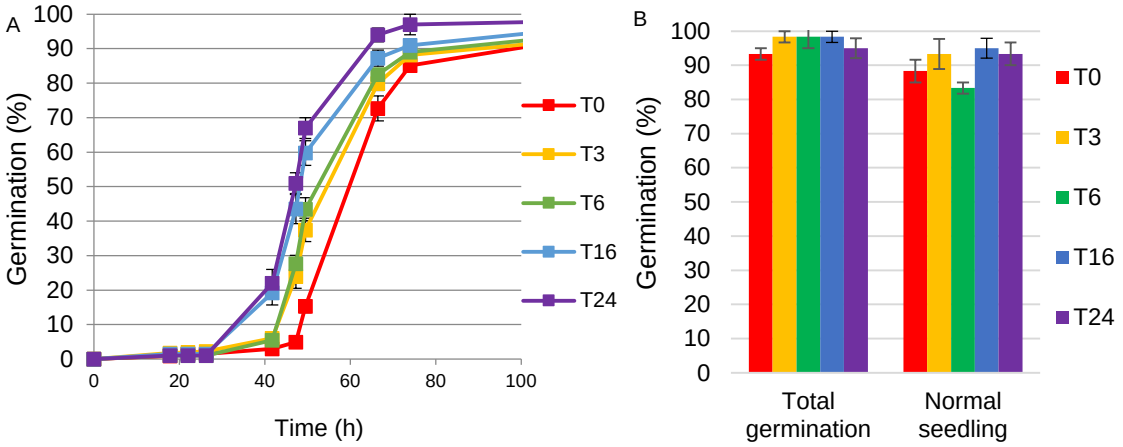

Figure 3. 4 **Effect of hydro-priming for *S. lycopersicum* cv. Microtom line**. A. Effect of duration of hydro-priming on speed of germination. B. Effect of hydro-priming on seed vigour. Duration of hydro-priming is indicated on the legend: un-primed seeds (T0), seeds primed for 3h (T3), 6h (T6), 16h (T16) and 24h (T24). The data presented are mean values ± SE of 3 replicates of 50 seeds each.

Table 3. 1 **T50 of hydro-priming time-course for *S. lycopersicum* cv. Microtom line.** Reciprocal of time to respectively 50% of viable seeds to germinate (h) in the control (T0), 3h (T3), 6h (T6), 16h (T16) and 24h (T24) of hydro-priming treatments. F values for Student's *t-test* against T0 and T-value for Student's *t-test*.

Treatment	T50	SD	F-Values	T-Values
T0	60	± 1.598	-	-
T3	52	± 3.576	0.49686	≤0.05
T6	52	± 3.412	0.063245	≤0.01
T16	46	± 2.000	0.56823	≤0.0001
T24	46	± 1.195	0.46139	≤0.0001

3.2.3 Effect of seed set temperature on germination and seedling vigour

The question tested in this study was whether hydro-priming could improve seed vigour of seed set matured at low temperatures (Figures 3.5 and 3.6). Tomato cv. Microtom seeds were set at three different temperatures: cool (19°C), intermediate (22°C) and warm (25°C). Fresh seeds from each condition were harvested and dried for two days at 25°C before the germination test. Germination took place at two temperatures (19°C and 22°C); half seeds were germinated at 19°C with or without hydro-priming (Figure 3.5) and other half at 22°C with or without hydro-priming (Figure 3.6).

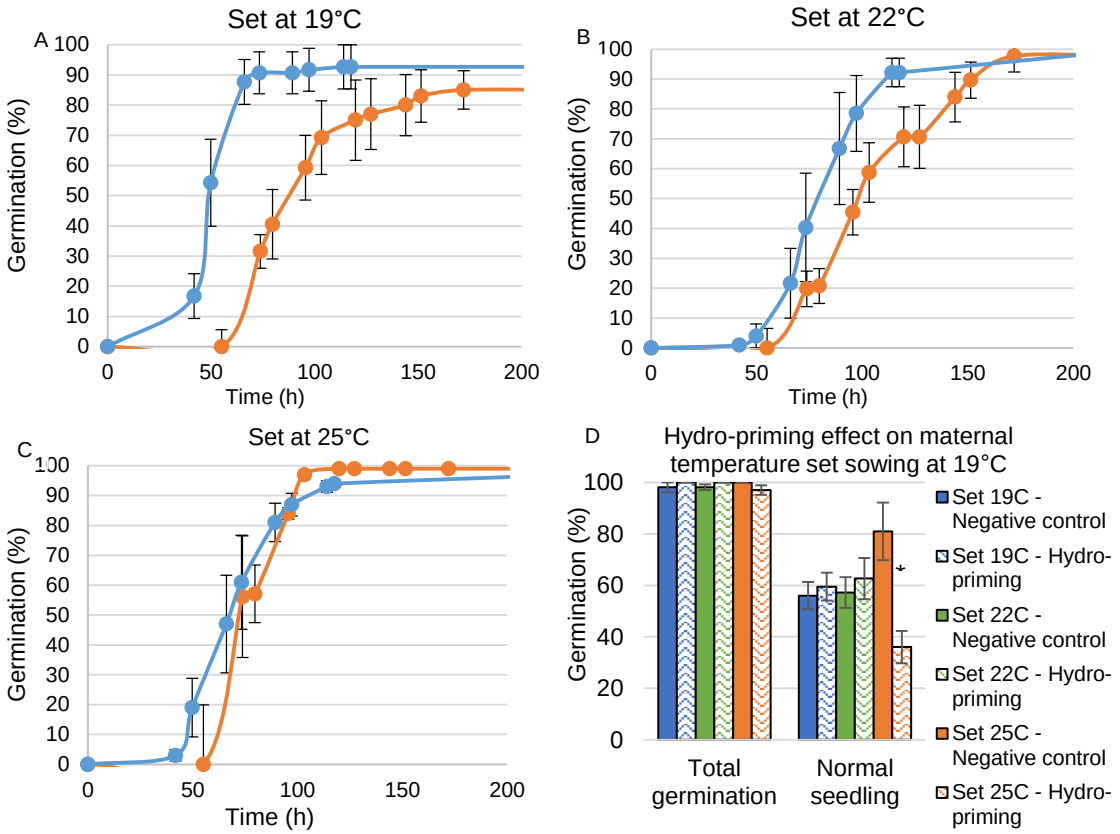

Figure 3. 5 **Effect of hydro-priming and temperature on seed germination at 19°C for *S. lycopersicum* cv. Microtom line**. A. Seeds set at 19°C. B. Seeds set at 22°C. C. Seeds set at 25°C. Germination of un-primed (orange) and primed seeds (blue). D. Seedling establishment 14 DAS. The data presented are mean values ± SE of 3 replicates of 50 seeds each. Significant differences between both un-primed / primed seeds at normal seedling establishment by a Student's *t-test*: *, P<0.01.

Table 3. 2 **T50 of primed and un-primed temperature seed set for *S. lycopersicum* cv. Microtom line at 19°C.** Reciprocal of time to respectively 50% of viable seeds to germinate (h) in primed or un-primed (control) seeds set at 19°C, 22°C and 25°C. F values for Student's *t-test* and T-value for Student's *t-test*.

Treatment	T50 (h)	SD	F-Values	T-Values
19°C control	86	± 6.364	-	-
19°C primed	48	± 2.121	0.104	≤0.001
22°C control	101	± 10.008	-	-
22°C primed	79	± 5.543	0.644	≤0.05
25°C control	74	± 5.991	-	-
25°C primed	67	± 7.263	0.759	≥0.05

For seeds germinated at 19°C, the results show that hydro-priming improves significantly the speed of germination without affecting seedling establishment (Figures 3.5 A, B and D). However, hydro-priming had no effect on the speed germination of seed set at 25°C, and had a negative effect on seedling establishment (Figures 3.5 C, D). These results showed that there are no correlation between size of seeds (Figure 3.3 B) and the effect of hydro-priming when seeds germinate at 19°C (Figure 3.5). I concluded that the effect of hydro-priming on the speed of germination decreased when the seed set temperature increased. By comparison, hydro-priming had a similar effect on seeds germinated at 22°C and 19°C for speed of germination (Figure 3.6 A and B), whereas it had a significantly positive effect both on the speed of germination and it improved seedling establishment of set matured at warm temperature (Figures 3.6 C and D). These results showed that hydro-priming enhanced seed performance of all sets when sown at optimal temperature (22°C).

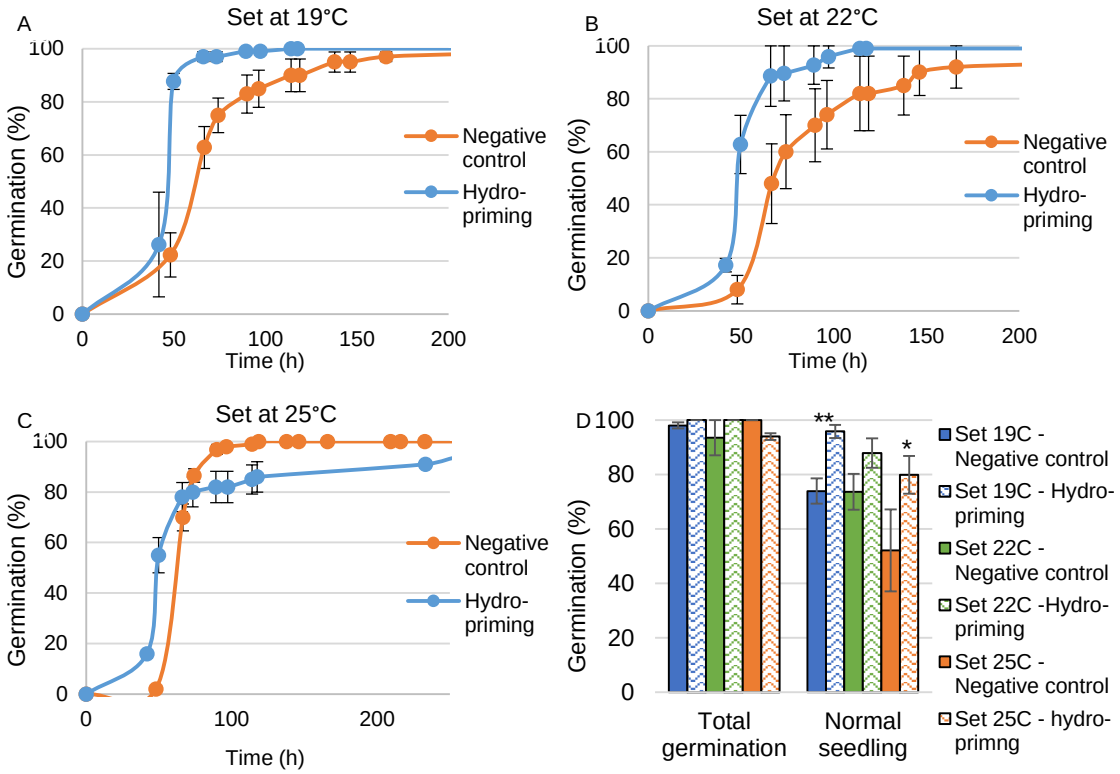

Figure 3. 6 **Effect of hydro-priming and temperature on seed germination at 22°C for *S. lycopersicum* cv. Microtom line**. A. Maternal temperature sowing at 19°C. B. Maternal temperature sowing 22°C. C. Maternal temperature sowing at 25°C. D. Seedling establishment. The data presented are mean values ± SE of 3 replicates of 50 seeds each. Significant differences between both un-primed / primed seeds at normal seedling establishment by a Student's *t-test*: *, P<0.05; **, P<0.01.

Table 3. 3 **T50 of primed and un-primed temperature seed set for *S. lycopersicum* cv. Microtom line at 22°C.** Reciprocal of time to respectively 50% of viable seeds to germinate (h) in primed or un-primed (control) seeds set at 19°C, 22°C and 25°C. F values for Student's *t-test* and T-value for Student's *t-test*.

	Negative control		Hydro-priming			
Treatment	**T50 (h)**	**SD**	**T50 (h)**	**SD**	**F-Values**	**T-Values**
19°C	59	± 2.449	43	± 5.001	0.1753	≤0.05
22°C	71	± 3.667	50	± 2.872	0.8181	≤0.05
25°C	59	± 0.629	55	± 1.548	0.1734	≤0.05

3.2.4 Effect of hydro-priming on Enza varieties seeds

In order to test the effect of maternal environment on speed of germination, I sowed two Enza varieties, Predator and Fame, which were harvested at different location sites and at different years (Figure 3.7). Seeds batches did not mature in the same conditions (Appendices: Table 1), and did not germinate at the same speed. Predator 4 had the lowest speed of germination and Predator 5 had the fastest germination speed (Table 3.3). There is a significant difference in the speed of germination of the Fame lines. Therefore, I concluded that maternal environment during seed maturation is important for speed germination.

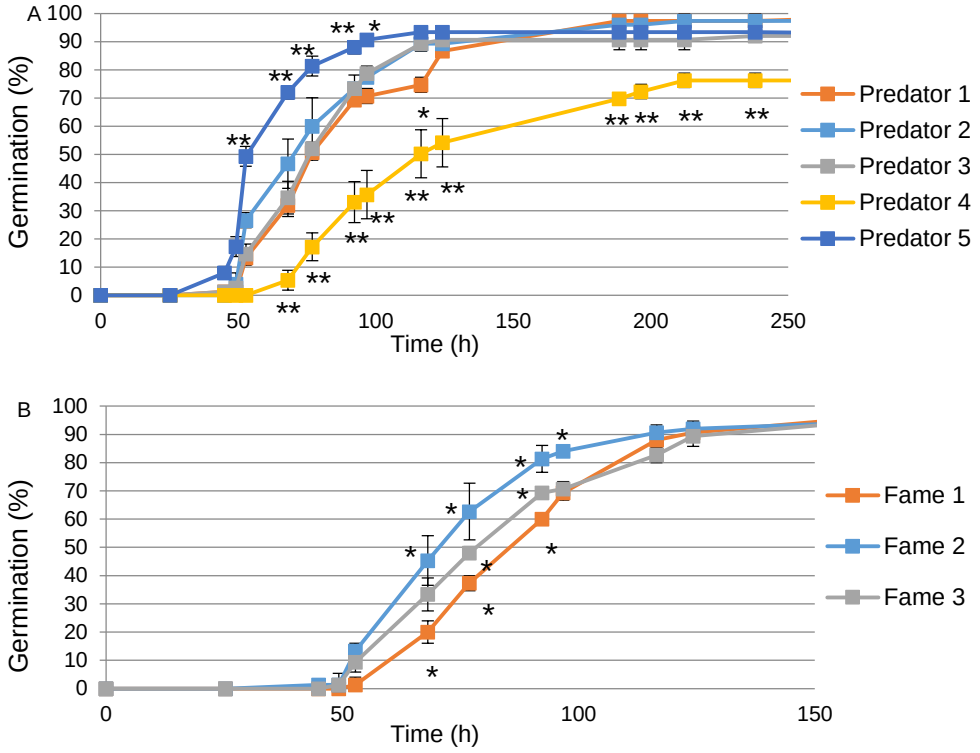

Figure 3. 7 **Effect of maternal environment on seed germination at 22°C for *S. lycopersicum* cv. Predator and cv. Fame lines**. A. Predator lines sowing at 22°C. B. Fame lines sowing at 22°C. The data presented are mean values ± SE of 3 replicates of 50 seeds each. Significant difference by ANOVA: *, P<0.05; **, P<0.01.

After testing the effect of maternal environment on Enza varieties, I tested the effect of hydro-priming on both varieties (Figure 3.8, Table 3.3). I compared the speed of germination, T50 and seedling establishment between primed and un-primed seeds for each batches. Generally, hydro-priming improves the speed of germination for these batches except for two batches: Predator 4 (Figure 3.8D) and Fame 1 (Figure 3.8H). In these cases, hydro-priming improves significantly the speed of germination at T50 except for Predator 4. I concluded that hydro-priming is efficient to improve the speed of germination for most seed lots.

Table 3. 4 **T50 of primed and un-primed Enza seeds for *S. lycopersicum* cv. Predator lines and cv. Fame lines.** Reciprocal of time to respectively 50% of viable seeds to germinate (h) in primed or un-primed (control) seeds. F values for Student's *t-test* and T-value for Student's *t-test*.

	Negative control		**Hydro-priming**			
Lines	**T50 (h)**	**SD**	**T50 (h)**	**SD**	**F-Values**	**T-Values**
Predator 1	77	± 2.667	44	± 3.333	0.7805	**≤0.01**
Predator 2	68	± 0.333	48	± 3.180	0.0217	**≤0.01**
Predator 3	77	± 1.333	44	± 4.000	0.2000	**≤0.001**
Predator 4	116	± 15.377	132	± 25.465	0.5344	≥ 0.05
Predator 5	53	± 1.202	30	± 2.000	0.5306	**≤0.001**
Fame 1	77	± 4.485	60	± 3.000	0.6183	**≤0.01**
Fame 2	68	± 2.309	44	± 1.453	0.5672	**≤0.001**
Fame 3	85	± 1.155	56	± 1.453	0.7742	**≤0.001**

A positive effect of hydro-priming on seed germination did not predict a positive effect on seedling establishment (Figure 3.9). Generally, in seeds batches in which hydro-priming had a positive effect on speed of germination, there was no effect of hydro-priming on seedling establishment (Figure 3.9 A and B). By contrast, in seed batches in which hydro-priming had negative effect on speed of germination, there was a significant reduction of the seedling establishment (Figure 3.9 C). These results suggest that hydro-priming enhances only the speed of germination only. In general, primed seeds germinated one day earlier, or more than un-primed seeds without affecting seedling establishment except for Predator 4, Fame 1 and Fame 3.

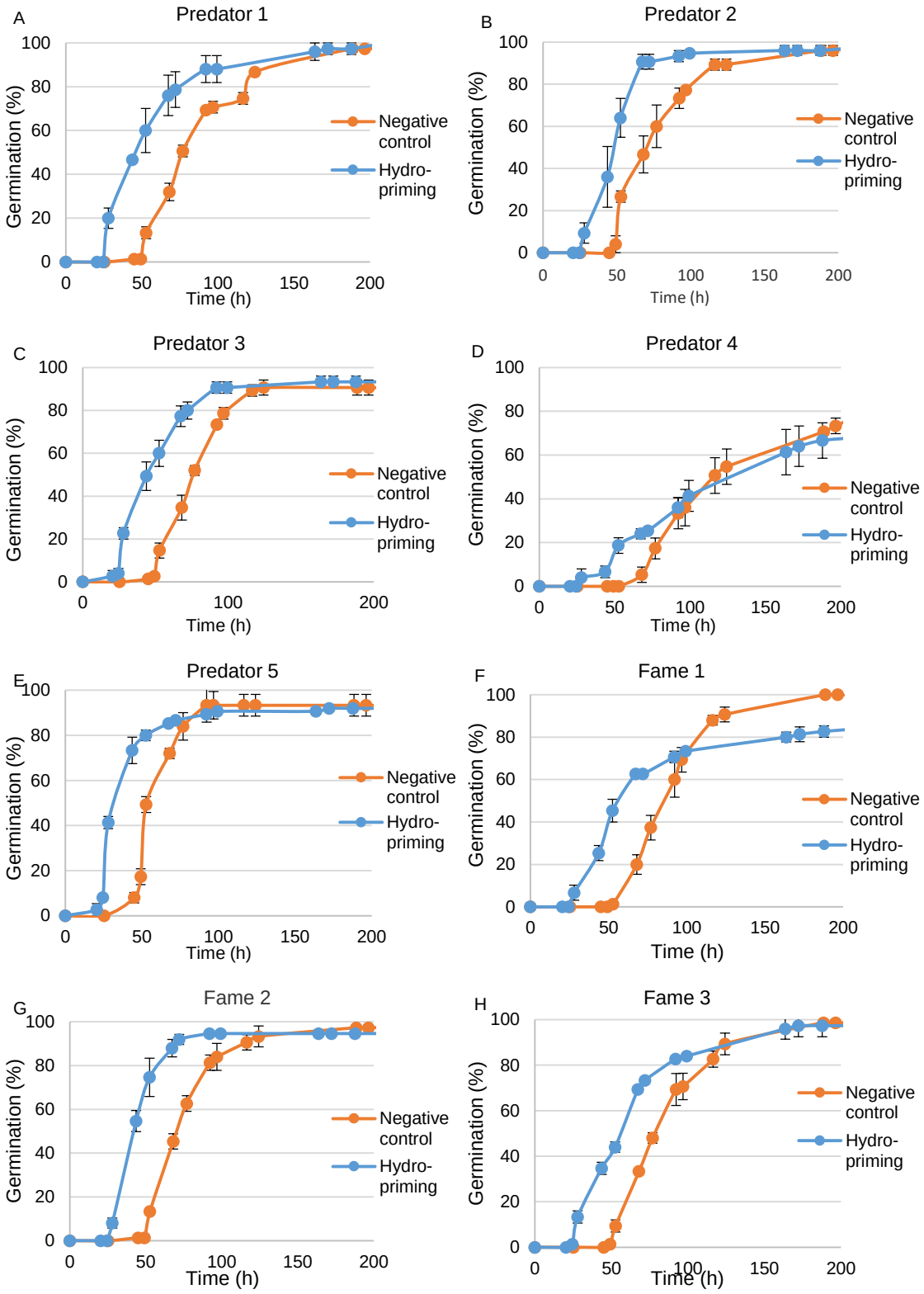

Figure 3. 8 **Effect of hydro-priming on seed vigour for *S. lycopersicum* cv. Fame and cv. Predator lines**. Time-course of seed germination of tomato seeds from different batches of Predator and Fame. Germination of un-primed (orange) and primed seeds (blue). The data presented are mean values ± SE of 3 replicates of 50 seeds each.

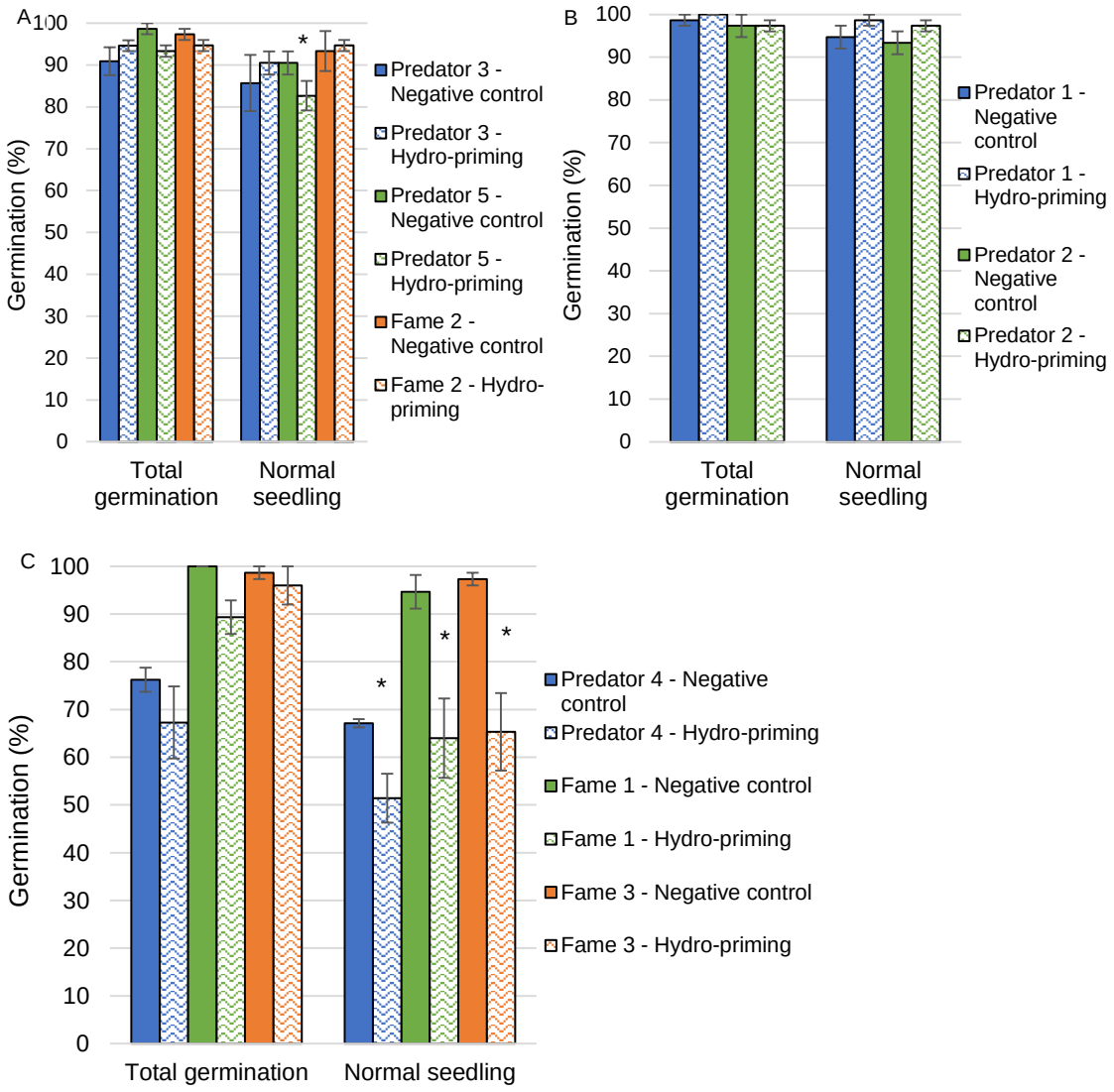

Figure 3. 9 **Effect of hydro-priming on tomato seedling vigour for Predator and Fame batches**. Total germination and seedlings vigour 14 DAS. The data presented are mean values ± SE of 3 replicates of 50 seeds each. Significant differences between un-primed seeds and primed seeds by a Student's *t-test*: *, P<0.05.

3.2.5 Effect of varietal differences on tomato responses to hydro-priming

Hydro-priming was optimised on laboratory variety (Microtom) and on Enza varieties seeds (Predator and Fame) produced at different locations and years. To determine if hydro-priming has a general use as post-harvest treatment in tomato, it was tested on a panel of tomato varieties including the main morphotypes plum, cherry, round, beef and rootstock tomatoes (Appendices, Table 1). All seeds were produced by Enza Zaden Seed Operations B.V. (Enkhuizen) and came from several production sites around the world.

These results show that hydro-priming is effective in most of varieties for speed of germination but not for seedling establishment (Table 3.4 and Figure 3.10). For some varieties including Babette 608.564, Emma 616.831, Kiki 543.998, Kiki 547.392 and Sofia 446.883, hydro-priming significantly improves the speed of germination but hydro-priming reduces the speed of germination of Feline 626.590 (Table 3.4). This result for Feline 626.590 may be explained by the fact that pathogens were located on the seeds. For Kiki 547.392, Nienke 276.611, Sofia 446.684 and rootstock N408488, hydro-priming improves significantly the seedling establishment. Therefore I concluded that hydro-priming is effective to improve germination speed of the main tomato morphotypes.

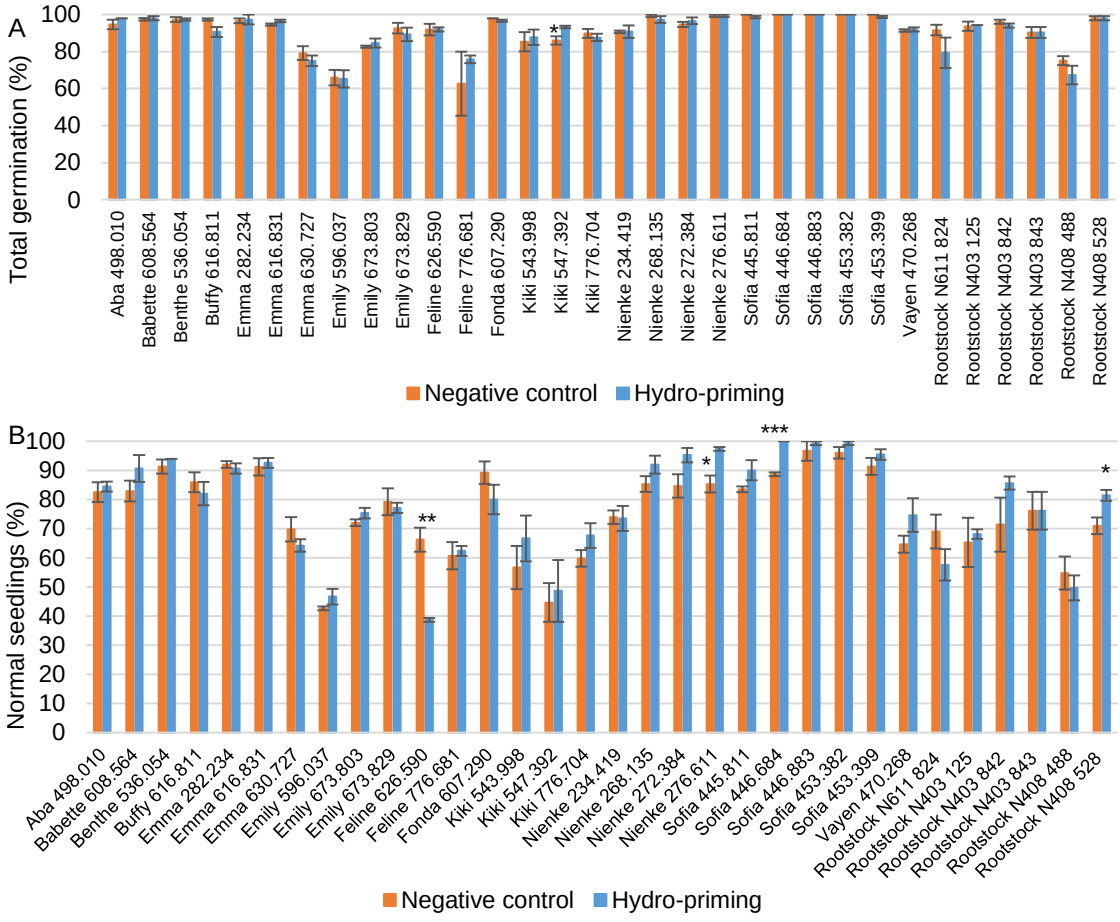

Figure 3. 10 **Effect of hydro-priming on tomato seed vigour.** A. Total germination of all cultivars was done 14 DAS. B. Seedling establishment was done 14 DAS. Total germination and seedlings vigours were compared between un-primed and primed seeds. The data presented are mean values ± SE of 3 replicates of 50 seeds each. Significant differences between un-primed and primed seeds by a Student's *t-test*: *, P<0.05; **, P<0.01; ***, P<0.001.

Table 3. 5 **T50 of primed and un-primed enza seeds for *S. lycopersicum*.** Reciprocal of time to respectively 50% of viable seeds to germinate (h) in primed (hydro-priming) or un-primed (negative control) seeds. F values for Student's *t-test* and T-value for Student's *t-test*.

	Negative control		Hydro-priming			
	T50 (h)	**SE**	**T50 (h)**	**SE**	**F-Value**	**T-Value**
Aba 498.010	91	± 1,333	86	± 1,202	0,8966	**≤ 0.05**
Babette 608.564	77	± 3,712	70	± 2,028	0,4596	**≤ 0.05**
Benthe 536.054	59	± 1,667	62	± 1,667	1,0000	≥ 0.05
Buffy 616.811	82	± 1,453	75	± 5,812	0,1176	≥ 0.05
Emma 282.234	88	± 2,728	88	± 1,667	0,5435	≥ 0.05
Emma 616.831	82	± 0,333	79	± 1,202	0,1429	**≤ 0.05**
Emma 630.727	141	± 11,552	141	± 7,333	0,5745	≥ 0.05
Emily 596.037	239	± 5,207	236	± 23,438	0,0941	≥ 0.05
Emily 673.803	141	± 10,088	141	± 6,960	0,6450	≥ 0.05
Emily 673.829	136	± 2,000	119	± 4,041	0,3934	≥ 0.05
Feline 626.590	46	± 4,163	83	± 4,667	0,8864	**≤ 0.01**
Feline 776.681	81	± 1,764	85	± 2,667	0,6087	≥ 0.05
Fonda 607.290	74	± 1,856	72	± 2,000	0,9254	≥ 0.05
Kiki 543.998	102	± 4,410	90	± 1,453	0,1959	**≤ 0.05**
Kiki 547.392	93	± 1,333	66	± 1,202	0,8966	**≤ 0.001**
Kiki 776.704	71	± 1,764	68	± 0,577	0,1935	≥ 0.05
Nienke 234.419	111	± 4,041	112	± 3,055	0,7273	≥ 0.05
Nienke 268.135	90	± 1,000	98	± 1,155	0,8571	**≤ 0.01**
Nienke 272.384	79	± 1,333	82	± 0,882	0,6087	≥ 0.05
Nienke 276.611	74	± 1,000	73	± 0,333	0,2000	≥ 0.05
Sofia 445.811	43	± 0,667	43	± 0,667	1,0000	≥ 0.05
Sofia 446.684	52	± 2,848	54	± 1,764	0,5545	≥ 0.05
Sofia 446.883	49	± 1,202	44	± 0,882	0,7000	**≤ 0.05**
Sofia 453.382	46	± 1,453	44	± 2,333	0,5588	≥ 0.05
Sofia 453.399	55	± 0,667	50	± 5,175	0,0327	≥ 0.05
Vayen 470.268	85	± 1,764	80	± 1,202	0,6341	≥ 0.05
Rootstock N611 824	196	± 10,263	208	± 17,321	0,5197	≥ 0.05
Rootstock N403 125	107	± 1,764	91	± 1,202	0,0282	≥ 0.05
Rootstock N403 842	123	± 5,925	107	± 3,712	0,5636	≥ 0.05
Rootstock N403 843	113	± 2,906	104	± 3,844	0,7273	≥ 0.05
Rootstock N408 488	171	± 9,404	223	± 28,113	0,2013	≥ 0.05
Rootstock N408 528	86	± 1,453	154	± 33,005	0,0039	≥ 0.05

To investigate how hydro-priming improves the speed of germination, three varieties in which the hydro-priming was efficient (Babette 608.564, Kiki 543.998 and Kiki 547.392) and three others varieties in which hydro-priming was not efficient were chosen (Fonda 607.290, Nienke 272.384 and Sofia 453.399). I

chose these varieties because seed coat colours, sizes and shapes were different (Figure 3.11).

Figure 3. 11 **Photos of seed for hydro-priming candidates**. Photos taken by Andrew Davis (JIC). Bar = 1 cm.

3.2.6 Role of permeability in tomato seed germination

In order to test the hypothesis that hydro-priming changes seed coat permeability using a Tetrazolium salt assay (TZ), I initially tested the permeability of the tomato seed coat on Microtom seeds.

In Arabidopsis seeds, TZ penetrates into the seeds through the micropyle area (MacGregor *et al.,* 2015) and the mechanism is similar on tomato seeds (Figure 3.12). Seeds were stained into TZ solution then seed coat was removed to visualize the red staining in the embryo and endosperm. The time-course of TZ penetration showed that red staining began through the endosperm close to the micropyle area after 9h of imbibition for Microtom seeds then staining propagated from endosperm to the cotyledon and micropyle areas (from 15h to 24h). After 48h of imbibtion, results showed clearly that TZ is propagated on embryo through two directions: via the endosperm area at the level of cotyledon and via the micropyle area. I concluded that TZ assay is efficient to test seed coat permeability on tomato seeds.

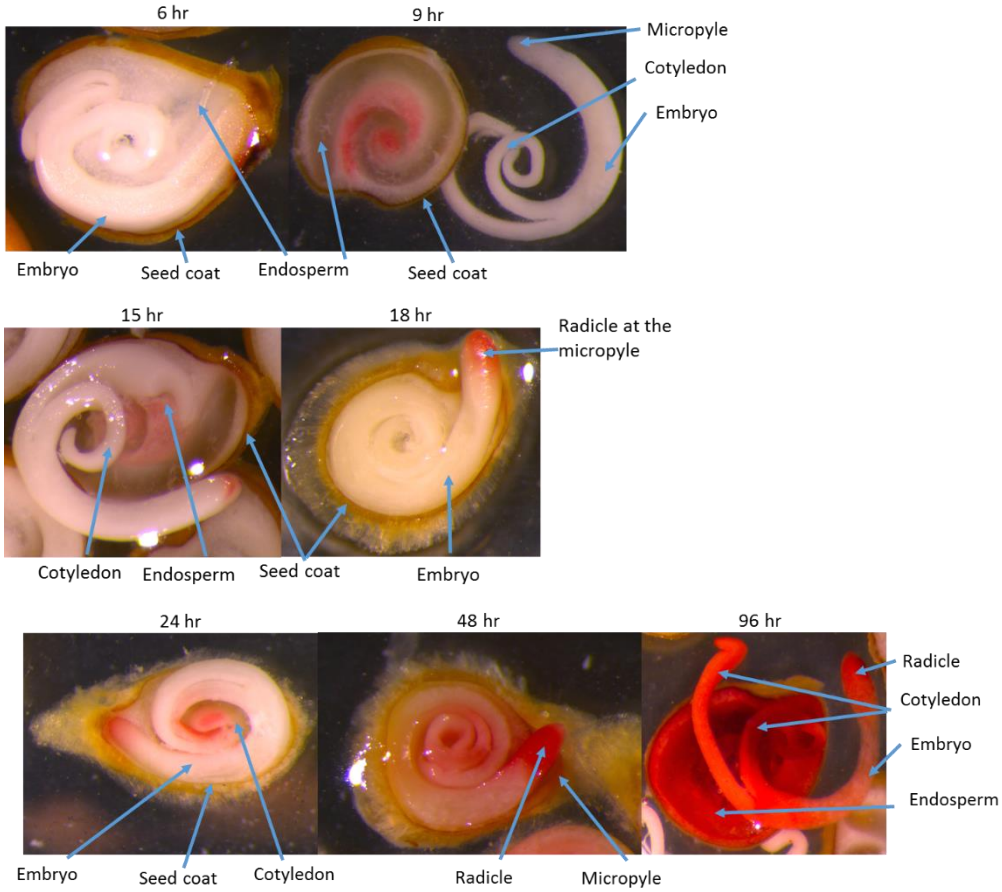

Figure 3. 12 **Tetrazolium progression into Microtom seeds**. Time-course of tetrazolium penetration into Microtom seeds.

Secondly, to learn if tomato varieties sensitive to hydro-priming were more permeable than varieties insensitive to hydro-priming, I tested TZ uptake on the six hydro-priming candidates (Figure 3.13). Varieties insensitive to hydro-priming have significantly a higher TZ uptake rates for primed seeds than controls. Varieties sensitive to hydro-priming had the same TZ uptake rates in both treatments controls and hydro-priming, except for Kiki 547.392. This variety had a higher TZ uptake rates when seeds are primed. On Arabidopsis seeds, MacGregor *et al*. showed that TZ uptake is independent of seed coat integrity and temperature during seed maturation affects seed coat permeability by changing the flavonoid content of seeds (MacGregor *et al.,* 2015). Therefore, I concluded that insensitive seeds to hydro-priming absorbed more TZ so they are more permeable than sensitive seeds.

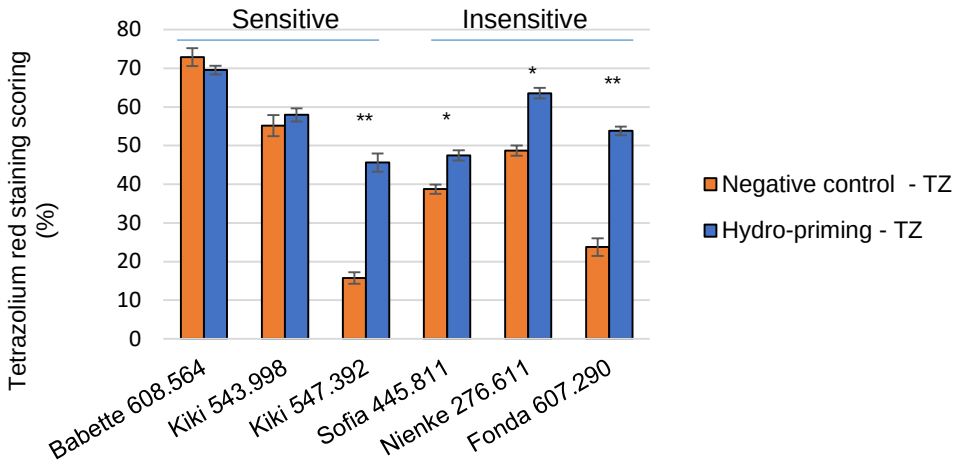

Figure 3. 13 **Tetrazolium salt uptake on six candidates chosen for hydro-priming experiment**. Primed seeds incubated in water for 24h at 15°C then dried 2 days at 25°C before staining (hydro-priming). Seeds incubated in 1% tetrazolium (TZ) for 4 days at 30°C in darkness. The data presented are mean values ± SE of 3 replicates of 50 seeds each. Significant differences between un-primed and primed seeds by a Student's *t-test*: *, P<0.01; **, P<0.001.

3.3 Discussion

Previous studies have shown the correlation between the maternal environment and the offspring genotype (Roach and Wulff, 1987; Roff, 1998; Donohue, 2009). Works on *Arabidopsis* have shown that low temperatures during seed maturation increased seed dormancy (Donohue, 2009, Kendall *et al.*, 2011; Schmuths *et al.*, 2006). Most of studies on the effect of maturation temperature accentuated their work on seed vigour and did not study the effect of maturation temperature on seedling establishment. My results show that maternal temperature affects germination speed and seedling establishment in tomato (Figure 3.2). Indeed, lower temperatures during seed maturation slows the speed of germination and reduces the number of normal seedlings. Moreover, maternal temperature significantly affects seed size and affects seedling vigour (Figure 3.3). Seedlings growing under a wide range of environments need to produce alive seeds to perpetuate species (Finch-Savage, 1995). Moreover, seedling establishment is an important factor for seed industry, the success to produce normal seedling is important for yield (Khan *et al.,* 2012).

The effectiveness of hydro-priming decreased when the seed set temperature increased (Figures 3.5 and 3.6), suggesting hydro-priming mechanism may act on germination process. In general, the maturation of seeds

at 19°C or 22°C lead to a higher level of normal seedlings (77 %). Nevertheless, for seed maturated at 28°C, only 42% of seed germinated develop into normal seedlings (Figure 3.2 B). It possible that seeds matured at 25°C or 28°C suffered of drought during maturation that may decrease seedling vigour.

Post-harvest treatments are used to improve the vigour of seed lots. It has recently been shown that hydro-priming ensures rapid and uniform germination, but the mechanism underlying the effectiveness of hydro-priming remains unclear (Berrie and Drennan, 1971; Ahmadi *et al.*, 2007; Dastanpoor *et al.*, 2013; de Souza *et al.*, 2016). The analysis of hydro-priming conditions on bad seed lots shows that hydro-priming for 24h at 15°C is optimal for high performance of germination, improving germination rates by 30% (Figure 3.1). As un-primed seeds need additional time to germinate, it is possible that hydro-priming improves seed lot by activating the germination process. It is important to note that the hydro-priming mechanism may act on earlier initiation of metabolic processes, better synthesis of DNA, RNA and protein, intermediate metabolites, enzymes associated with the production of energy, increasing the level of moisture or supply of oxygen without emergence of the radicle. Another explanation could be that the hydro-priming mechanism may be linked with the germination process. Indeed, seed priming may help in germination possibly by acting on the embryo development and/or leaching of emergence inhibitors during priming.

The efficiency of hydro-priming on the speed of germination of uncommercial seeds was similar at this observed previously (Figure 3.8 and Table 3.4). Though, the analysis of hydro-priming on several varities of tomato seeds shows that hydro-priming was not an efficient post-harvest treatment (Figure 3.10), suggesting that hydro-priming had a positive effect on germination speed when seeds were matured at low temperature. This highlighted that the reduction of speed of germination of seeds matured at low temperature does not represent a loss of viability and seeds are able to germinate.

The permeability of the seed coat was tested by tetrazolium salt assay (Figures 3.12 and Figure 3.13). The penetration of the dye on Microtom seeds is observed by scarifying the seed coats and by removing the embryo of the

endosperm (Figure 3.12). In Arabidopsis seeds, TZ uptake penetrates into seeds through micropyle area (MacGregor *et al.*, 2015). The same pattern is observed in tomato seeds, TZ goes through endosperm and micropyle area to stain embryo both from radicle to cotyledon and from cotyledon to radicle. Furthermore, seed coat permeability tested on hydro-priming candidates show that insensitive candidates have higher permeability after hydro-priming (Figure 3.13). It is possible that the contrast between seed coat colour and TZ uptake was not enough different for image analysis for some tomato line (Kiki 547.392). Moreover, tomato seeds germinated very well in TZ solution preventing to test the TZ uptake rate with GA to prove that insensitive candidates were more permeable to tetrazolium salt. Further work will be necessary to test the seed coat permeability with seeds matured at several temperatures.

In wheat, sage or physalis, seed priming was efficient when seeds were matured in stressful conditions (Ahmadi *et al.*, 2007; Dastanpoor *et al.*, 2013; de Souza *et al.*, 2016). In Arabidopsis, seeds matured at low temperature showed increased dormancy which can be broken by cold stratification (Kendall *et al.*, 2011). To conclude, it is possible that tomato seeds matured at low temperature have a delay in germination caused by a residual activity of dormancy. This dormancy process is not sufficient to completely block progression to germination.

Taken together, these results in this chapter highlighted a new discovery on hydro-priming; it has shown that the efficiency of hydro-priming is releated to the seed maturation environment. Indeed, seeds matured at low temperature are more vigorous after hydro-priming treatment; their germination were faster than un-primed seeds. In general, hydro-priming improved speed of germination when seeds are matured at 22°C, suggesting that this treatment is favourable for enhancement of germination. Hydro-priming may change seed coat integrity but this it is not clear whether this is linked to the hydro-priming process.

Chapter 4

Ionomic analysis of hydro-priming flow through

4.1 Introduction

4.1.1 Role of nutrient in plants

With regard to the provision of plants with nutrients, the germination and seedling establishment of plants are a critical developmental phase (Holdsworth *et al.*, 2008b; Koorneef *et al.*, 2002). As long as the root system is not established and nutrient uptake is not fully functional yet, nutrient provision of the early seedling depends on stored nutrient reserves. The ability of the seed to germinate quickly despite adverse environmental conditions is encompassed in the term of seed vigour and is dependent on the physiological constitution of the seeds. The knowledge on the role and on distribution of mineral elements in seeds is very limited (Holdsworth *et al.*, 2008b; Rajjou *et al.*, 2012; Weitbrecht *et al.*, 2011). Essential mineral elements support important biochemical functions in plants, they are of particular importance during germination and seedling establishment (Eggert and von Wiren, 2013). Some of them act as cofactors of stress-related proteins (Cu, Fe, Mg, Mn, Mo, Ni, S, Zn), stabilize cell walls and allow cell elongation (B, Ca), form bio-membranes and energy carriers (P), or play an important role in turgor stability and osmoregulation (K) of plant cells (Husted *et al.*, 2011; Hepler, 2005; Tanaka and Fujiwara, 2008; Hansch and Mendel, 2009). For rice, micronutrients are primarily localized in the embryo but also in the aleurone layer (Walker and Waters, 2011). The imbibition of seed in nutrient-enriched solution and their re-drying have been proved to be an efficient measure to stimulate germination and seedling establishment (Taylor *et al.*, 1998; Hassanpouraghdam *et al.*, 2009).

The plant system has an inbuilt mechanism for ion homeostasis in which it regulates ion accumulation. Nutrients are sequestered into several pools such as in vacuoles of the embryo, endoplasmic reticulum and vacuolar compartments of the chalazal endosperm, and nutrients are remobilized during germination (Lobréaux and Briat, 1991; Otegui *et al.*, 2002; Grillet *et al.*, 2014). Seeds store minerals in the form of mineral deposits then, during maturation, nutrients are decationized and hydrolyzed to serve as sources of food for embryo (Loewus and Murthy, 2000). Iron is one of the most important elements on Earth, and it is involved in many biological process such as respiration, glyoxylate cycle, photosynthesis, and co-factor of enzyme (Harrison and Arosio, 1996; Arosio *et*

al., 2009; Balk and Schaedler, 2014). Previous studies have shown that iron played a role in germination and seeds stored different forms of iron (Wada and Lott, 1997; Lobréaux and Briat, 1991; Ravet *et al.*, 2009; Grillet *et al.*, 2014). In plants, ferritin is a ubiquitinous iron storage protein, and it is found in seeds (Waldo *et al.*, 1995, Chasteen and Harrison, 1999; Conte and Walker, 2011). For exemple in *Phaseolus* seeds within the same species, it was shown that different genotypes accumulate a different proportion of iron content in the seed coat, the embryonic axis and the cotyledon tissues (Cvitanich *et al.*, 2010). Iron is supplied by releasing from ferritin and more especially from phytoferritin (Deng *et al.*, 2010; Conte and Walker; 2011) and iron(II) (Fe^{2+}) is known to be more toxic than iron(III) (Fe^{3+}) because it induces oxidative stress (Deng *et al.*, 2010; Ravet *et al.*, 2009).

4.1.2 Electrical conductivity as vigour test

The electrical conductivity test is a quantitative and repeatable test which is related to field emergence for peas (Matthews and Powell, 1981; ISTA, 2006). This test is one of the tests used for the evaluation of the loss of cell membrane integrity through the concentration of electrolytes released by seeds during imbibition (Halloin, 1975; Simon and Raja-Harun, 1972). Cell membrane integrity is considered as the physiological events of seed deterioration processes (Delouche, 1976). Consequently low vigour seed lots exhibit higher losses of cellular constituents such as inorganic ions. Often ion leakage is used to measure vigour without germinating the seeds. For legumes, high level of leakage are a characteristic of low vigour lots with high levels of laboratory germination but low field emergence (Pandita and Nagarajan, 2002; Burcu and Peksen, 2008). Moreover, few studies have determined which specific cations are leached from imbibing seeds (Weges and Karssen, 1990). Potassium (K) has been shown to be the main ion leached by seeds during imbibition, followed by sodium and calcium, and may be used as an indicator of cell membrane integrity. The potassium leachate test is based on the same principle of the electrical conductivity test and it focus on a specific ion (potassium), while the electrical conductivity test evaluates a set of electrolytes release (Panobianco and Marcos-Filho, 2001). Although this test is internationally standardized for peas and soybeans, it continues to require adjustement of methodology for application to others species.

The purpose of this study was to explore different strategies to identify nutrients regulating seed vigour during hydro-priming. A better understanding of micronutrients leakage from tomato seeds could lead to improved hydro-priming strategies.

4.2 Results

4.2.1 Determination of element leaks during hydro-priming

The hypothesis is that the hydro-priming causes the movement of charged solutes out of the seeds. To investigate this, conductivity was measured at each time-point of hydro-priming for a few varieties (Figure 4.1) such as Microtom seeds set at 19°C, 22°C or 25°C (Figure 4.1 A), seeds sensitive or not to hydro-priming (Figure 4.1 B) and rootstock lines (Figure 4.1 C) which had low seedling vigour without hydro-priming. Seeds were primed during 3h, 6h, 16h or 24h and conductivity was measured at each time-point to measure the kinetics of ion leakage. Data showed significant effects of hydro-priming duration on electrical conductivity of seed leachates, and that leakage occurs within the first three hours of imbibition. Furthermore, during seed set the temperature is modifying quantity of leachates, and temperature seeds set matured at higher temperature had a higher conductivity measurement (Figure 4.1 A). These results showed the opposite than expected and confirmed that during hydro-priming, metabolites leak out the seeds. The effect of genotype can affect the results on electrical conductivity test. Contrary to peas, high level of leakage are not characteristic of low vigour tomato seeds.

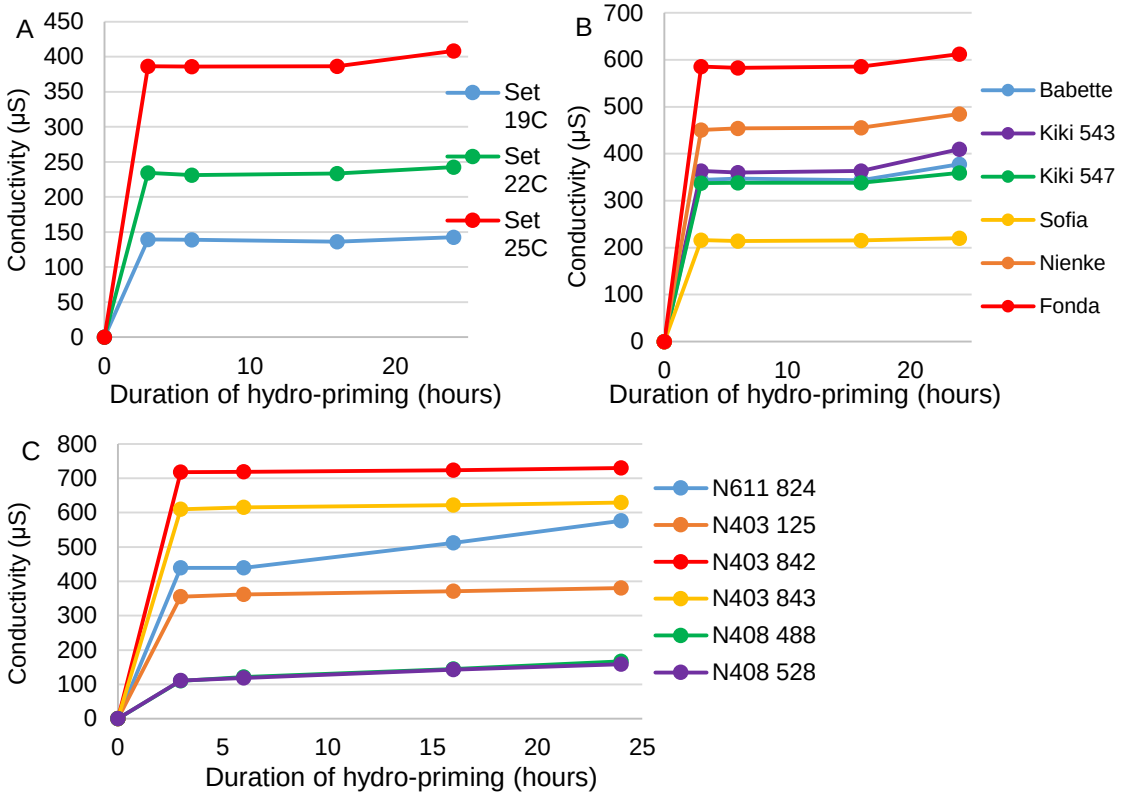

Figure 4. 1 **Conductivity measurement**. A. Microtom set at 19°C, set 22°C and set at 25°C. B. Hydro-priming candidates. C. Rootstock lines. The data presented are mean values ± SE of 3 replicates in hydro-priming solution; error bars are too small to see it.

4.2.2 Presence of insoluble residues after freeze drying of hydro-priming flow through liquid

Insoluble residues were found in water retained from hydro-priming after freeze drying. Residue colour observed were similar in colour to seed coat pigmentation (Figure 4.2 A) and the quantity of insoluble residues increased with the duration of hydro-priming (Figure 4.2 B). I hypothesised that residues came from the seed coat and candidates were tannins, melanin or iron.

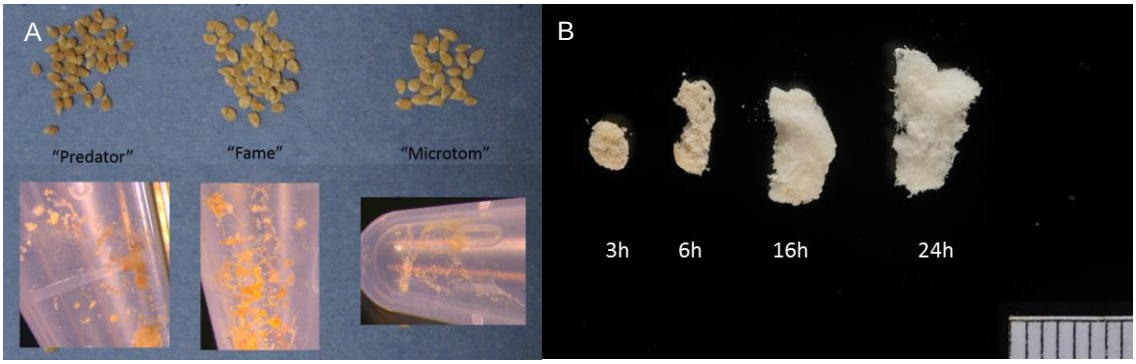

Figure 4. 2 **Photos of insoluble residue after freeze drying of hydro-priming flow through liquid**. A. Brown residue are the same color as the seeds coat. B. Time-course of hydro-priming flow through liquid on Microtom seeds then presence of insoluble residue after freeze drying.

Several studies showed that the colour of the seed coat in *Arabidopsis thaliana* correlated with the presence of tannins and plants having a reduction in proanthocyanidins pathways exhibited a reduction of seed dormancy (Debeaujon *et al.*, 2000). Plant polyphenols (tannins) constitute a group of natural polymers and are divided into two groups: hydrolyzable and condensed tannins (see chapter 1, Winkel-Shirley, 2001). One method to establish the presence of tannins is to determine the presence of PAs which are contained in seeds. A protocol adapted from Routaboul *et al.* (2012) was used with some modifications to extract seed flavonoids then, with colorimetric acid butanol analysis, I measured soluble and insoluble proanthocyanins (Porter *et al.*, 1985; Makkar *et al.*, 1999). The role of PAs as seed germination inhibitors was investigated in three tomato varieties: Microtom, Predator and Fame. No PAs (soluble nor insoluble) were found in tomato seed extracts (Figure 4.3). After 24h of hydro-priming, presence of proanthocyanidins was only observed in the control Arabidopsis seed extract. Therefore, residue found in hydro-priming flow through liquid did not originate from seed coat proanthocyanidins. Two remaining hypotheses remain: the residue could be come from iron or melanin that is deposited in the seed coat.

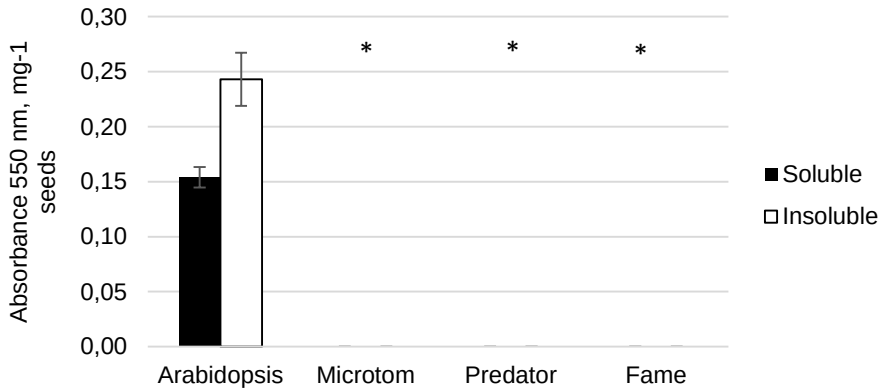

Figure 4. 3 **Proanthocyanidins determination by acid butanol assay in tomato seeds**. Soluble (black) and insoluble (white) proanthocyanidins. The data presented are mean values ± SE of 4 replicates of more 25 seeds each. Significant differences by a Student's *t-test*: *, P<0.001.

4.2.4 Iron determination in hydro-priming flow through liquid

Iron is essential for embryo development and is stored under several forms in seeds and localization differed between species (Grillet *et al.*, 2014; Roschzttardtz *et al.*, 2011; Zhang *et al.*, 2013). In *Arabidopsis* seeds, iron loaded

into seeds circulates via xylem and phloem around the seed coat. In *Phaseolus* seed, iron and ferritin were accumulated near to seed coat (Cvitanish *et al.*, 2010). Iron is found under diverse forms and, in legume seeds, ferritin is most important iron storage form. As ferritin is an important protein for seed development, I hypothesed that iron in the seed coat and could be a source of residue found. Very little knowledge is known on iron release during soaking. Three different tomato varieties (Predator, Fame and Microtom) were used and these varieties had a range scale of brown seed coat colour. Predator have a darker seed coat than Fame, the latter being darker than Microtom (Figure 4.4). I supposed that the colour of the insoluble residue (Figure 4.2) came from the seed coat, and the darker the seed coat is, the more iron is present in the seed coat.

Figure 4. 4 **Photos of tomato seeds**. A. Predator seeds. B. Fame seeds. C. Microtom seeds. Photos taken by Andrew Davis (JIC). Bar = 1 cm.

To quantify the amount of iron in the seed coat and water from hydro-priming flow through liquid, standard curve with a known amount of iron is used (Smith *et al.,*1981; Smith *et al.*, 1984). This method converts iron(III), Fe^{3+}, into iron(II), Fe^{2+}, then it is complexed with an iron chelator, Ferene, to measure the absorbance (Figure 4.5). The results showed that iron leakage increased with the duration of the hydro-priming and that the concentration of iron leaked from seeds differed between varieties (Figure 4.5 A). Indeed, iron concentration in flow through liquid from darker colour seeds (Predator) was significantly higher than lighter seed coat colour (Microtom). Additionally, Fame seeds with an intermediate seed coat colour had an intermediate concentration of iron which was present in the hydro-priming flow through liquid. Therefore, iron may be the brown residue found in water remaining after hydro-priming. Seed coats were dissected and isolated before iron extraction and quantification with Ferene. The concentration of iron in water retained from hydro-priming was similar to T24 and

did not exceed the amount in seed coat (Figure 4.5 B). These results suggested that the iron which is present in the water retained from hydro-priming can come from the seed coat.

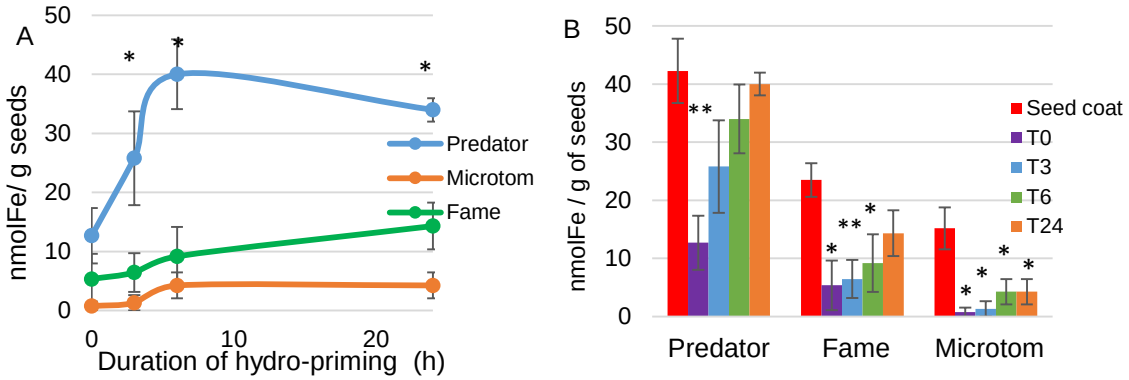

Figure 4. 5 **Iron measurement in water retained from hydro-priming for *S. lycopersicum*.** A. Iron quantification in water retained from hydro-priming. Hydro-priming flow through liquid from Predator seeds (blue), from Fame seeds (green) and from Microtom seeds (orange). B. Hydro-priming time-course on the iron leakage in the water retained from hydro-priming in comparison with iron amount in seed coat. The data presented are mean values ± SE of 3 replicates of 50 seeds each. A. Significant difference by ANOVA: *, P<0.05. B. Significant differences between seed coat and hydro-priming flow through liquid by a Student's *t-test*: *, P<0.05; **, P<0.01.

4.2.3 Role of exogenous iron in hydro-priming solution

In order to check if iron (Fe) leakage permits the germination and affects germination speed, I added iron in hydro-priming solution. Seeds were soaked in iron(II) sulphate ($FeSO_4$) solution (Figure 4.6) or in iron(III) sulphate ($Fe_2(SO_4)_3$) solution (Figure 4.7) at 10 µM, 100 µM or 1 mM, or only in water in order to obtain a treatment allowing Fe uptake into seeds. The effect of exogenous iron(II) sulphate on seedling vigour was observed on seeds matured at 19°C or 22°C (Figures 4.6). Adding exogenous ferrous sulfate did not enhance speed of germination for seeds matured at 19°C or 22°C (Figures 4.6 A and C). High concentration (1 mM) of ferrous sulfate was toxic for seedling vigour for each temperature sets but low concentration of ferrous sulfate improved seedling vigour (Figures 4.6 B and D). These results have shown that adding exogenous iron(II) sulphate in low concentration improved significantly seedling establishment rate for seed set at 19°C.

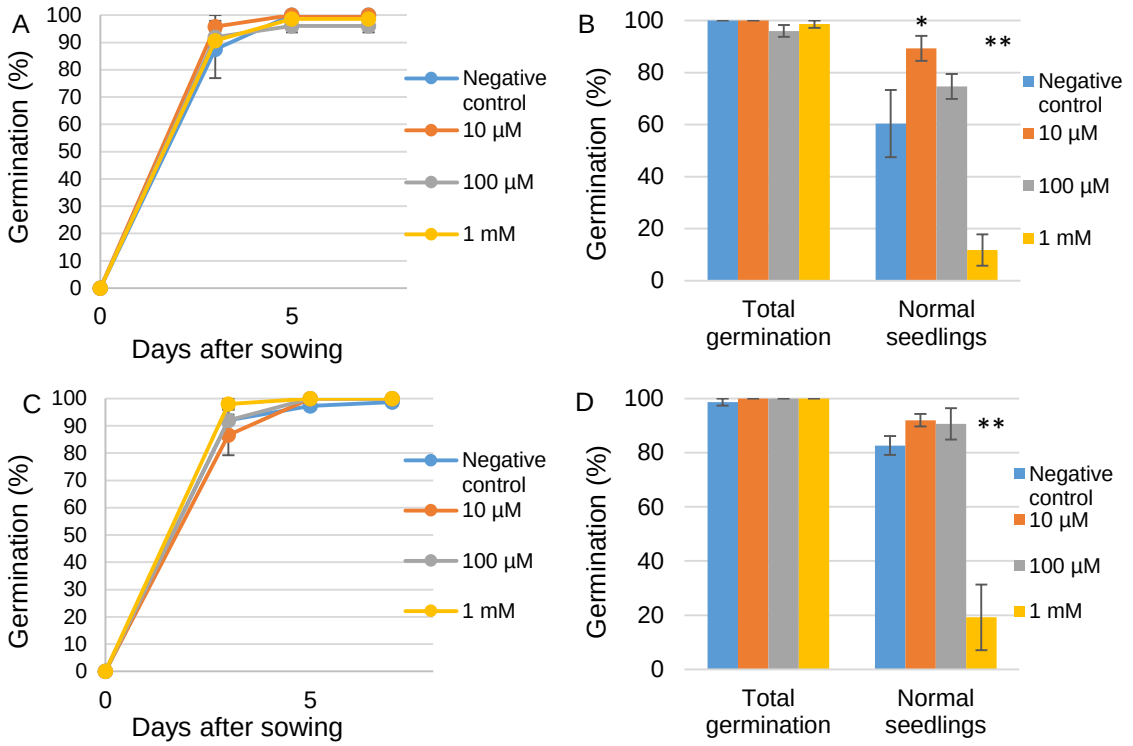

Figure 4. 6 **Effect of exogenous ferrous sulfate ($FeSO_4$ or iron(II) sulfate) on seed vigour for *S. lycopersicum* cv. Microtom.** A. Germination time-course of Microtom seeds matured at 19°C. B. Seedling establishment of Microtom seeds matured at 19°C. C. Germination time-course of Microtom seeds matured at 22°C. D. Seedling establishment of Microtom seeds matured at 22°C. Un-primed seeds (blue), primed seeds with 10 µM of iron(II) sulphate (orange), primed seeds with 100 µM of iron(II) sulphate (grey) and primed seeds with 1 mM of iron(II) sulphate (yellow). The data presented are mean values ± SE of 3 replicates of 50 seeds each. Significant differences between negative control and iron(II) by a Student's *t-test*: *, P<0.05; **, P<0.001.

After having tested the contribution of iron(II) sulphate during the hydro-priming on seed vigour, the addition of iron(III) sulphate during hydro-priming is tested (Figure 4.7). Exogenous ferric sulfate in flow through liquid did not modify the speed of germination for seeds matured at 19°C or 22°C (Figures 4.7 A and C). As observed previously, high concentration of iron was toxic for seed germination, but low concentration of ferric sulfate improved seedling establishment of seeds matured at low temperature (Figure 4.7 B). These results have shown that adding exogenous iron(III) at low concentration improved significantly seedling establishment for the set matured at low temperature.

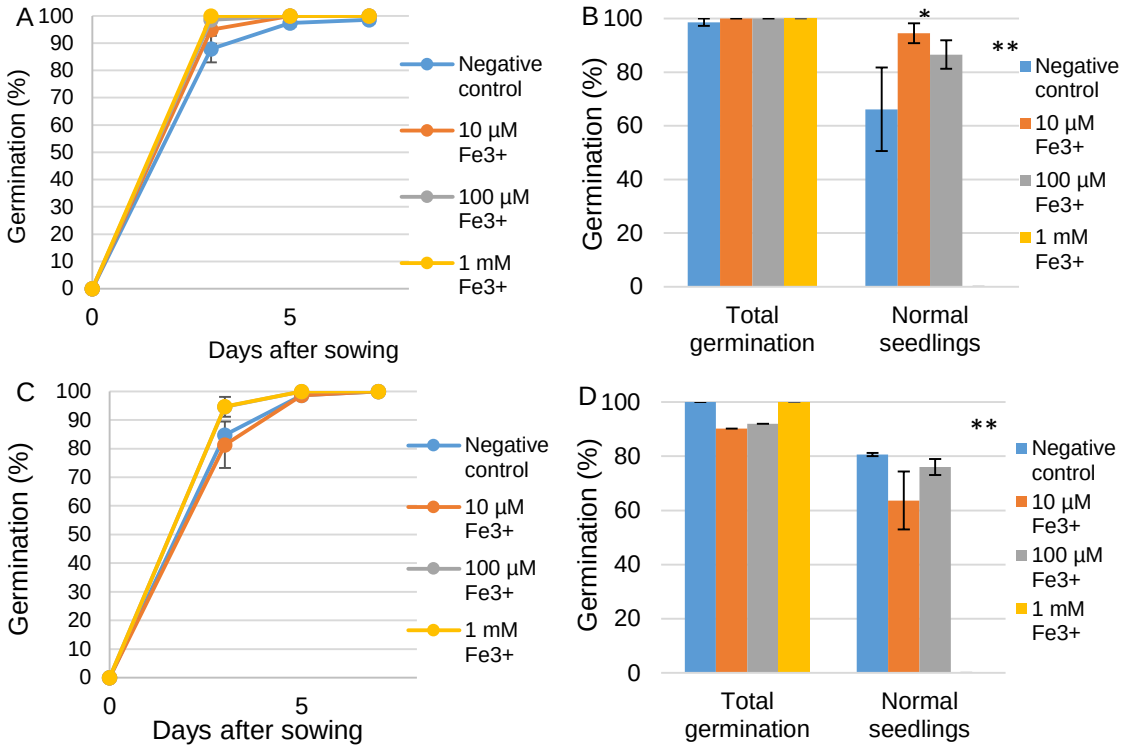

Figure 4. 7 **Effect of ferric sulfate ($Fe_2(SO_4)_3$ or iron(III) sulfate on seed vigour for *S. lycopersicum* cv. Microtom.** A. Germination time-course of Microtom seeds matured at 19°C. B. Seedling establishment of Microtom seeds matured at 19°C. C. Germination time-course of Microtom seeds matured at 22°C. D. Seedling establishment of Microtom seeds matured at 22°C. Un-primed seeds (blue), primed seeds with 10 µM of iron(III) sulfate (orange), primed seeds with 100 µM of iron(III) sulfate (grey) and primed seeds with 1 mM of iron(III) sulfate (yellow). The data presented are mean values ± SE of 3 replicates of 50 seeds each. None normal seedling grew with 1mM Fe^{3+}. Significant differences between negative control and iron(III) by a Student's *t-test*: *, P<0.05; **, P<0.0001.

To confirm that the enhancement of seed performance comes from exogenous iron during hydro-priming and not from sulfate, ferric EDTA (Fe-EDTA) was used to chelate the iron that were released from seeds, and sodium sulfate (Na_2SO_4) was used as a control. For this experiment, I used Microtom seed set maturated at 19°C (Figure 4.8). The results showed that hydro-priming with deionised water enhanced significantly the speed of germination (Figure 4.8 A, Table 4.1). Seeds primed with Fe-EDTA had a significantly lower seedling establishment rate than un-primed seeds and seeds primed with exogenous iron or sodium sulphate had no significant difference with un-primed seeds. Nevertheless, seedling establishment was higher in seed primed with water (Figure 4.8 B). These results showed that iron is not essential in hydro-priming solution to improve the seed performance.

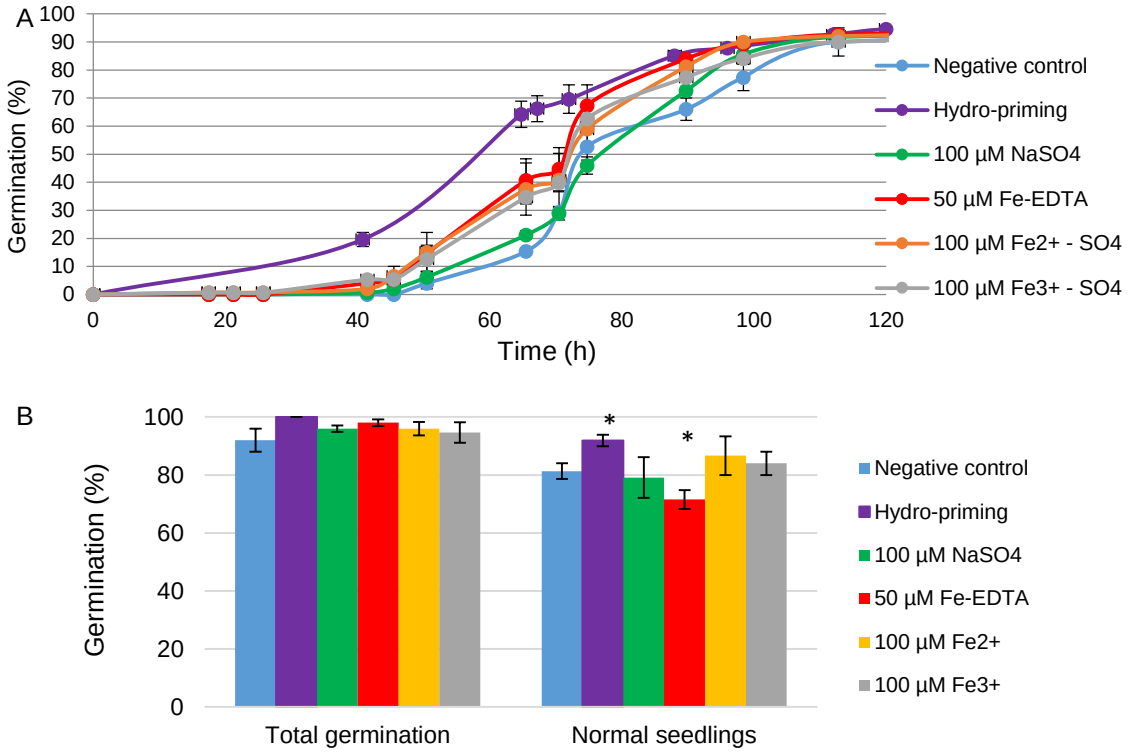

Figure 4. 8 **Effect of iron hydro-priming on seed vigour for *S. lycopersicum* cv. Microtom**. A. Germination time-course of Microtom seeds under different treatments. B. Seedlings establishment of Microtom seeds under different treatments. The data presented are mean values ± SE of 3 replicates of 50 seeds each. Significant differences between negative control and treatment by a Student's *t-test*: *, P<0.05.

Table 4. 1 **T50 of hydro-priming treatments for *S. lycopersicum* cv. Microtom line.** Reciprocal of time to respectively 50% of viable seeds to germinate (h) without hydro-priming (negative control), primed in water (hydro-priming), primed in 100 µM of sodium sulphate (100 µM $NaSO_4$), primed in 50 µM of ferric-EDTA (50 µM Fe-EDTA), primed with 100 µM of iron(II) sulphate (100 µM Fe^{2+}) and primed in 100 µM of iron(III) sulphate (100 µM Fe^{3+}) of hydro-priming treatments. F values for Student's t-test and T-value for Student's *t-test.* Significant differences between negative control and treatments.

Treatments	T50 (h)	SD	F-Values	T-Values
Negative control	75	± 2.000	-	-
Hydro-priming	52	± 2.529	0.7217	**≤ 0.01**
100 µM $NaSO_4$	75	± 3.111	0.0861	≥ 0.05
50 µM Fe-EDTA	71	± 7.688	0.3491	≥ 0.05
100 µM Fe^{2+}	73	± 3.203	0.1747	≥ 0.05
100 µM Fe^{3+}	73	± 2.404	0.0974	≥ 0.05

4.2.5 Presence of metals in hydro-priming flow through liquid

The ionome is involved in a huge range of important biological processes such as respiration, photosynthesis, osmoregulation and transport (Marschner, 1995). The conductivity measurements showed a leakage of charged solutes in

hydro-priming flow through liquid. In order to understand how hydro-priming promotes germination, ionomic analysis by ICP-AES was undertaken by an external laboratory on the flow through liquid of Microtom set at 19°C (Figures 4.9 and 4.10). In total 20 elements were present in sufficient concentrations to be detected in hydro-priming flow through liquid above the Limit of Detection (LOD) (Table 2.1). Figure 4.9 is a histogram showing the mean concentrations (µg/g seeds ± SE) of metals in hydro-priming flow through liquid after 24h of hydro-priming of microtom seeds. The results indicated that sodium (Na), potassium (K) and phosphorus (P) are among the most dominant nutrients effluxed during hydro-priming. Boron (B), magnesium (Mg), iron (Fe), titanium (Ti) and aluminum (Al) were exuded after hydro-priming treatment in higher quantity than other mineral ions leached (Figure 4.9). As electrical conductivity is based on the fact that the seeds, when soaked in deionised water, exude ions, sugars and other metabolites due to changes in the integrity of the cell membrane, these results showed that ions moved out of the seeds.

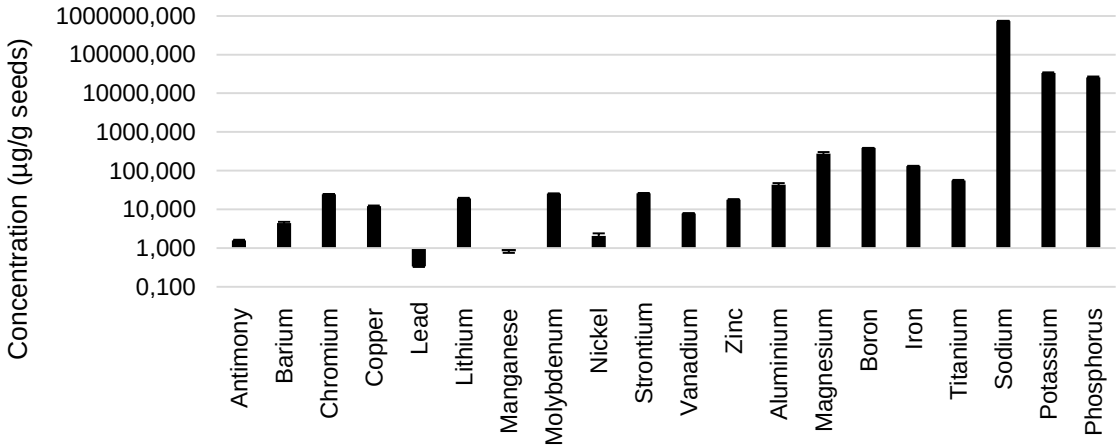

Figure 4. 9 **Determination of elements present in flow through liquid after hydro-priming**. A. Metals in hydro-priming flow through liquid (µg/g seeds). The data presented are mean values ± SE of 3 replicates.

Data from time-course of the hydro-priming flow through liquid provided an analysis of the kinetics of the leakage (Figure 4.10). Data showed that the leakage was the most rapid during the first moments of imbibition and the rate slowed down until 6h and a steady state condition was reached in the next 24h, in agreement with the results observed on conductivity (Figure 4.1), while hydro-priming took 24h to be efficient. Therefore ion leakage can not be the mechanism of hydro-priming.

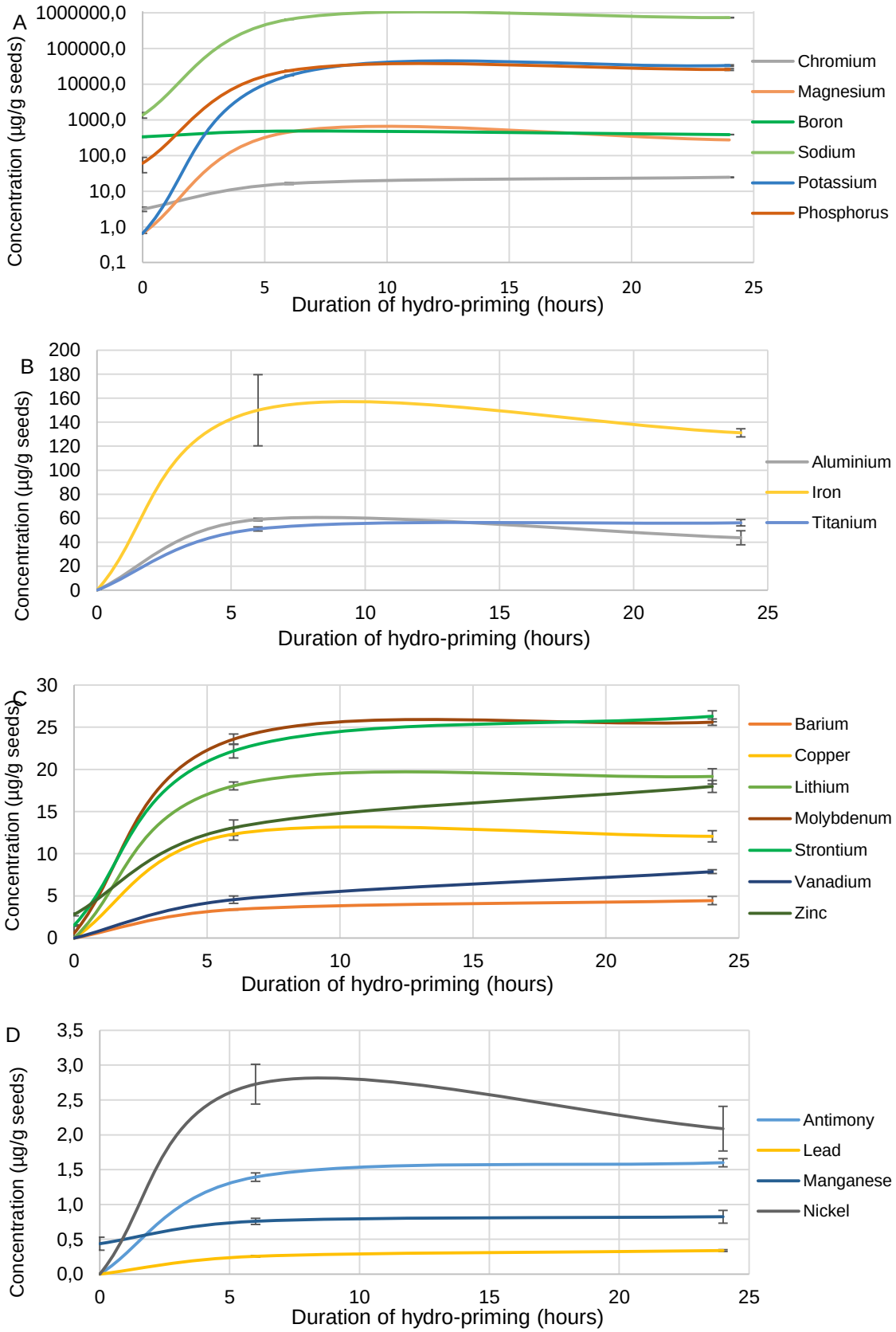

Figure 4. 10 **Hydro-priming time-course on element leakage**. A. Elements that are the most abundant in hydro-priming flow through (µg/g of seeds). B. Elements abundance in hydro-priming flow through (µg/g of seeds). C. Elements presence in hydro-priming flow through (µg/g of seeds). D. Trace of elements in hydro-priming flow through (µg/g of seeds). The data presented are mean values ± SD of 3 replicates.

4.3 Discussion

Previous studies showed that the process of imbibition involved repair and restoration of damaged cell membrane integrity during the early minutes of contact between the seed and the surrounding water (Simon and Raja-Harun, 1972; Powell and Matthews, 1978; Simon, 1984; Bewley and Black; 1986). Membrane damage occurring during seed maturation and desiccation is higher in low vigour seeds and cell death may take place due to the rapid uptake of water. This is the time during which solutes leak out of the cells. Many substances such as amino acids, organic acids, sugars, phenolics, phosphate and potassium ions, gibberellic acid and proteins, are leaked and rate of leakage is not the same for each substance. The increasing in electrical conductivity (E.C.) in leachates of imbibing seeds is due to the increasing leakage of these electrolytes (Simon, 1984). It may be possible that the increased leakage of organic metabolites from seeds might indirectly enhance the growth of rhizosphere microorganisms providing nitrogen and carbon sources.

Hydro-priming improves the speed of germination and permits an important leakage of elements during the first three hours of hydro-priming (Figures 4.1 and 4.10) It is possible that the cause of the leakage is the deterioration of membranes resulting from physiological ageing or deterioration, which can be defined as the loss of quality, viability and vigour either due to effect of adverse environmental factors or ageing (i.e. the progressive deterioration of the structures and functions of the seed over time). These ion concentrations are due to changes in the integrity of the cell membranes as a function of water amount and the level of seed deterioration. In deteriorated seeds, the repair mechanism is absent or inefficient, or the membranes are completely damaged thus permitting the leakage of larger electrolyte amounts (Bewley and Black, 1986; McDonald, 1999). And, also during imbibition phase, water uptake reached a plateau (Bewley, 1997) and, permits a relative equilibrium by homeostasis, the permeability of the seed coat leads to increased conductivity. To tomato seeds, conductivity measurement was higher for varieties insensitive to hydro-priming treatment (Figure 4.1). The presence of compounds in solute leakage may differ from one variety to one other. Moreover, the difference between varieties in conductivity come from the fact that each variety does not absorb the same

quantity of water. Electrical conductivity as vigour test does not allow the detection of seed lots with low vigour in tomato seeds contrarily to large-seeded legumes such as beans, chickpeas and peas (Matthews and Powell; 2006).

Kinetic analysis of the solute leakage out of the seed have shown that ion leakage occurred by 3h while hydro-priming took 24h (Figures 3.4, 4.1 and 4.10). This leakage has been used to understand what happens during imbibition phase. One explanation is that the initial water entry into seeds permits the passage of substance out of the tissue under condition where membrane reorganization can occur. Results showed that mineral elements present in hydro-priming flow were leaked at different concentrations and a diversity of elemental leakage is observed (Figures 4.9 and 4.10). Na, K and P were the dominant effluxed metals and potassium leakage was used as an indicator of membrane cell integrity on soybean seeds (Custodio and Marcos-Filho, 1997). Results also showed that the leachate contains a large number of nutrients and Fe, Mn, Na and Ca are known to play an important role in seed germination (Sethy and Ghosh, 2013; Singh and Barthi, 1985; Hakala *et al.*, 2006; Millaleo *et al.*, 2010). Futher work can be done on solute leakage from imbibiting seeds where hydro-priming is not effective to determine if solute leakage by cellular membrane can be used in improving tomato seed vigour.

Iron is an essential ion in plant nutrition and is involved in many processes. Chapter 3 showed that hydro-priming is more suitable for seeds that are set at cold temperature. Previous work has showed that adding external iron is not sufficient to improve priming. Ravet *et al.*, showed that iron and more especially ferritins are important for seed germination by the role of iron in protection against oxidative stress (Ravet *et al.*, 2009). In this chapter, results showed that exogenous iron adding in solution at low concentration (10 µM) during soaking improved seedling establishment rate for seed set at 19°C (Figures 4.6 and 4.7). Fe content was not measured in seeds but previous studies on several crop used Fe priming as an increase of nutritional Fe available for health benefit to the plant (Afify *et al.,* 2011; Wei *et al.*, 2013; Zielińska-Dawidziak and Siger, 2012). These results suggested that iron sulphate may be added during priming at low

concentration not to improve the speed of germination but to improve seedling vigour even if it is not the main compound which improves seed performance.

The role of iron in seedling establishment is not essential (Figure 4.8). A lack on iron by adding of chelating agent reduced significantly the seedling establishment rate without modify speed of germination. As hydro-priming improved significantly the speed of germination and the seedling establishment rate, in contrast to iron which improved only seedling establishment, I concluded that iron was not an essential element in germination. Moreover, iron played a role against oxidative stress (Doria *et al.*, 2009). Ferritin protein accumulates a high concentration of iron which could be used in several biological processes when needed (Harrison and Arosio, 1996, Cvitanich *et al.,* 2010). To reduce the toxicity of iron in the seeds, iron released out and it leachates found in hydro-priming flow through came from seed coat (Figure 4.5). Iron and more especially ferritin played a protective role against reactive oxygen species (ROS; Ravet *et al.*, 2009). But, ROS are produced during completion of seed germination (Bailly, 2004). The availability of ferritin into seeds may be keeping seeds safe by detoxifying ROS during seed germination.

In Arabidopsis seeds, flavonoids are abundant secondary compounds (Winkel-Shirley, 2001) and, among them, proanthocyanidins are particularly important. Debeaujon *et al.* have shown the role of PAs in seed coat and in dormancy (Debeaujon *et al.,* 2000); moreover, the relationship between PAs and ABA was highlighted by Jia *et al*. (Jia *et al.*, 2012). The role of PAs as seed germination inhibitors was investigated in tomato seeds and no PAs (soluble or insoluble) were found in tomato seeds (Figure 4.3). Indeed in tomato (*Solanum lycopersicum*), PAs are only synthetised in vegetative tissues and seeds only accumulate flavonols (Torres *et al.*, 2005).

Taken together, the results in this chapter highlight that the origin of the insoluble residue comes from the iron which comes from the seed coat. Moreover, ion leakage can not be the hydro-priming mechanism.

Chapter 5

Characterisation of hydro-priming mechanism(s) by metabolomic analysis

Chapter 5: Characterisation of hydro-priming mechanism(s) by metabolomic analysis

5.1 Introduction

As seen in chapter 1, many studies have been conducted on seed physiology during hydro-priming but there is no information available concerning the metabolites that are present into hydro-priming flow through. Matthews and Powell have linked the conductivity test with seed vigour by the concentration of charged metabolites in the solution (Matthews and Powell, 1981). Hoekstra *et al.* have shown that cellular solutes were leaked when seeds are placed in water. Mostly, seeds leaked low molecular weight elements such as ions, amino acids, sugars, etc (Hoekstra *et al.*, 1999). The mechanism of leakage was explained by a disorganization of the membrane which is reassembled during imbibition phase (Buttrose, 1973; Webster and Leopold, 1977; Morrison-Baird *et al.*, 1979). In legumes, seeds are well protected against the damaging effect of a membrane phase change during imbibition by the seed coats which restrict penetration of liquid water (Duke and Kakefuda, 1981). The limited amount of water that eventually penetrates may create a sort of pre-hydration (Matthews and Powell, 1981*;* Hoekstra *et al.,* 1992*;* Tetteroo *et al.,* 1996) and leakage involved diffusion through an intact bilayer (Duke *et al.*, 1983; Senaratna and McKersie, 1983).

Seeds contain thousands of metabolites, some of which are secondary metabolites that have different functions as defence function against bacteria or fungi, or seed germination inhibitors. Metabolites can be either “constitutive” which means high level of the metabolites are maintained in the seed, or “induced” which means the metabolite is changed in abundance when it is required. For example in yeast, animal and plant, the sucrose non-fermenting-1-related protein kinase (SnRK1) acts as a major component of the sugar-sensing and response mechanism. In tomato seeds, the α-subunit is expressed constitutively and by contrast the β-subunit is induced during maturation (Bradford *et al.*, 2003). Only the regulatory γ-subunit (LeSNF4) is responsive to GA, ABA, and stress (Bradford *et al.*, 2003). Thus, hormonal signalling pathways played critical roles in the physiology of organisms. Measuring metabolic fluxes in seed is difficult, most pathways are interconnected and there are alternative routes and product turnover. Moreover, there is a rapid exchange of metabolites between the seed and the hydro-priming flow through liquid (Murphy and Noland, 1982; Welbaum and Bradford, 1990). To understand the hydro-priming

mechanism, the metabolites leaked from seeds during hydro-priming were analysed by LC-IT-ToF/MS.

Metabolomics is an emerging approach in plant and food science (Shu *et al.*, 2008; Barvkar *et al.*, 2012). Metabolites represent the end products of the interaction between the genome, the transcriptome, the proteome and the environment (Fiehn, 2002). Metabolite profiling techniques aim at extracting, identifying and quantifying a broad spectrum of these metabolites with liquid chromatography (LC) coupled to mass spectrometry (MS). Only one method to determine the presence of strigolactones in root exudate has been reported by using LC-MS/MS (Sato *et al.*, 2003). Indeed, the isolation and characterization of natural strigolactones were difficult because they were unstable and present in very low concentration. There are two ways to do LC-MS: one method is a targeted approach, users start with standards, they look for limited numbers of compounds and compared their peaks with standards. The other method is an untargeted approach, in this case users aim to set up methods able to measure a large set of targets. Genetic and environmental influences on crop metabolite profiles have been investigated (Frank *et al.*, 2012). Metabolite profiling was used to show the importance of energy metabolism to support germination and seedling growth (Fait *et al.*, 2006). Metabolite profiling is also considered to provide valuable data metabolic engineering and is used as a tool to improve agronomic characteristics (Dixon *et al.*, 2006).

The aim of this study was firstly to apply LC/MS-based metabolite profiling to tomato seeds in the course of the hydro-priming process to analysis of a broad spectrum of low molecular weight metabolites from a wide range of chemical classes, secondly to test the employed approach regarding its suitability to reflect the hydro-priming process by a time-dependent clustering based on multivariate analysis, and last but not least to identify and to quantify major contributors leaked into the hydro-priming solution.

5.2 Results

A metabolite profiling approach based on liquid chromatography-ion trap-time of flight-mass spectrometry (LC-IT-ToF/MS) was used to investigate time-dependent metabolic leakage during hydro-priming (Chapter 2.11; Figure 5.1). Because of a lack of information on solutes leaking from seeds into hydro-priming flow through liquid, an untargeted approach was used on samples.

Samples taken in the course of hydro-priming were subjected to an extraction and fractionation procedure covered a broad spectrum (mass spectra: *m/z* 80-800, *m/z* 200-2000 and *m/z* 50-2000) of lipophilic and hydrophilic low molecular weight tomato constituents. Metabolites in the water retained from hydro-priming were separated by retention time (RT), and mass signals corresponding to the peak area was used to quantify the abundance of compounds. The mass spectra (ion *m/z*) of those signals corresponding to a compound were identified with a tolerance of 10 ppm from an online database (METLIN: Metabolite and Tandem MS Database). All peaks identified in samples were present in all replicates.

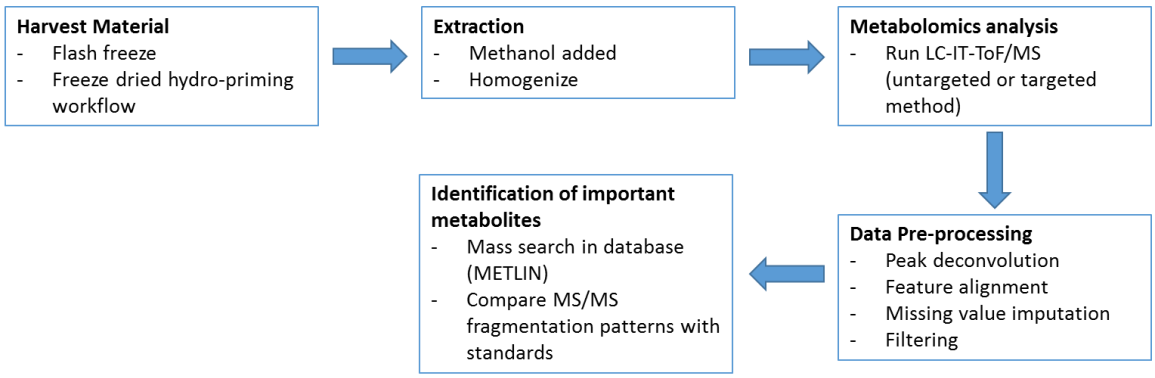

Figure 5. 1 **Flowchart summarizing the main steps taken in this thesis in the metabolomic analysis of hydro-priming flow through liquid using LC-IT-ToF/MS**.

5.2.1 Application and testing of LC-IT-ToF/MS-based metabolite profiling on hydro-priming flow through

To identify the metabolites involved during hydro-priming, the experiment was done on seeds from Microtom set at 19°C. To distinguish metabolites that are already present on the seed coat, seeds were soaked for a few seconds (T0) before analysis in deionized water and, for the others time points, hydro-priming flow through liquid was used. Metabolites leaked into the hydro-priming flow

through liquid were quantified (Appendices, Tables 2 and 3). Quantifications based on standardised peak heights revealed dynamic changes of the metabolites in the course of the different hydro-priming stages.

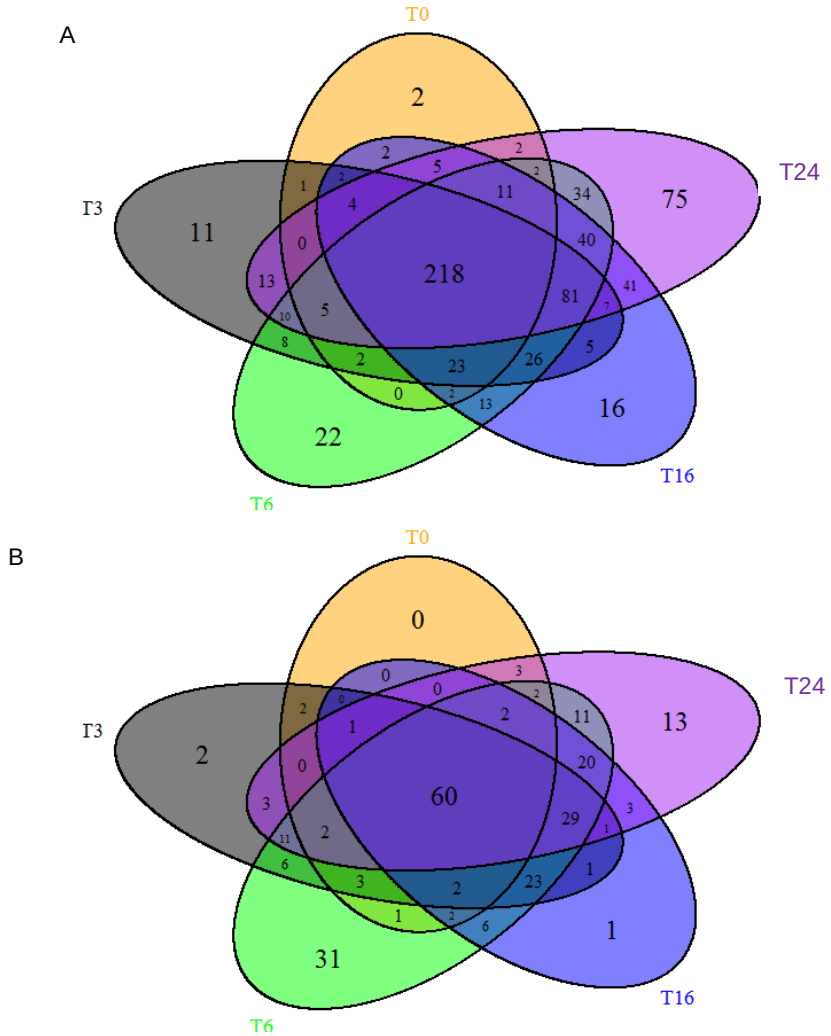

Figure 5. 2 **Venn diagram of summarising the number of shared and unique metabolites found in water retained from hydro-priming after soaking Microtom seeds**. Metabolites measured in hydro-priming liquid flow through liquid after 3h of hydro-priming (T3), 6h of hydro-priming (T6), 16h of hydro-priming (T16), 24h of hydro-priming (T24) and in control before hydro-priming (T0). A. Positive ionization mode. B. Negative ionization mode. The data presented are mean values ± SE of 3 replicates of 50 seeds each.

The LC metabolite profiling approach allowed the detection of a total of 744 peaks in the five fractions of water retained from hydro-priming with the positive ionization mode (Figure 5.2 A) and a total of 254 peaks in the five fractions of hydro-priming flow through with the negative ionization mode (Figure 5.2 B). A set of peaks was identified in all samples, I found 218 peaks that were present in all samples in positive ionization mode and 60 peaks in negative

ionization mode. At T0, 281 peaks were identified in positive ionization and 80 peaks were identified in negative ionization. At T3, 417 peaks were identified in positive ionization mode and 146 peaks were identified in negative ionization mode. At T6, 497 peaks were identified in positive ionization mode and 211 peaks were identified in negative ionization mode. At T16, 496 peaks were identified in positive ionization mode and 154 peaks were identified in negative ionization mode. At T24, 548 peaks were identifed in positive ionization mode and 161 peaks were identified in negative ionization mode. Some metabolites were identified at particular time-points. In positive ionization mode, I found only 11 metabolites at T3, 22 metabolites at T6, 16 metabolites at T16 and 75 metabolites at T24. In negative ionization mode, I found only 2 metabolites at T3, 31 metabolites at T6, 1 metabolite at T16 and 13 metabolites at T24. Some peaks appeared and disappeared (Appendices, Tables 2 and 3). These results obtained may permit a preliminary identification of metabolites leaking from seeds that may play a role in the hydro-priming process.

5.2.2 Identification of major peaks of metabolites involved in hydro-priming flow through liquid

The major metabolites leaked in the five fractions were preliminarily identified as alkaloids (e.g. dormantinone, ß1-tomatidine), flavonols (e.g. quercetin, kaempferol) and 12-oxo-phytodienoic acid (OPDA) in positive ionisation mode (Figure 5.3; Appendices: Table 2).

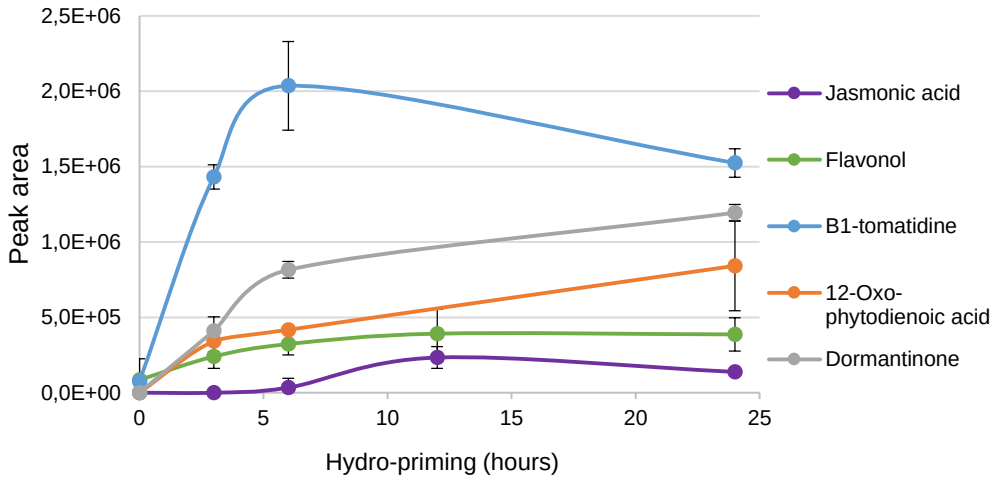

Figure 5. 3 **Main metabolites identified in hydro-priming flow through by LC-IT-Tof/MS on Microtom**. Average abundance of metabolites identified with METLIN database in function RT, chromatogram and spectra. The data presented are mean values ± SE of 3 replicates of 50 seeds each.

5.2.3 Presence of OPDA in hydro-priming flow through

After discovering OPDA in the metabolomic experiment with the untargeted approach, I used a targeted approach to confirm the presence of OPDA in hydro-priming workflow. OPDA content was quantified in water retained from hydro-priming by LC-IT-ToF/MS on Microtom set at 19°C and 25°C (Figure 5.4). The Jasmonic Acid (JA) precursor 12-oxo-phytodienoic acid (OPDA) has been shown to be a key negative regulator of germination (Dave *et al.*, 2011; Wasternack *et al.*, 2012). It was hypothesised that levels of OPDA may differ with maturation temperature and leakage of OPDA content in hydro-priming flow was identified. These chromatograms confirmed the presence of OPDA in hydro-priming flow throughof seed set at 19°C and 25°C.

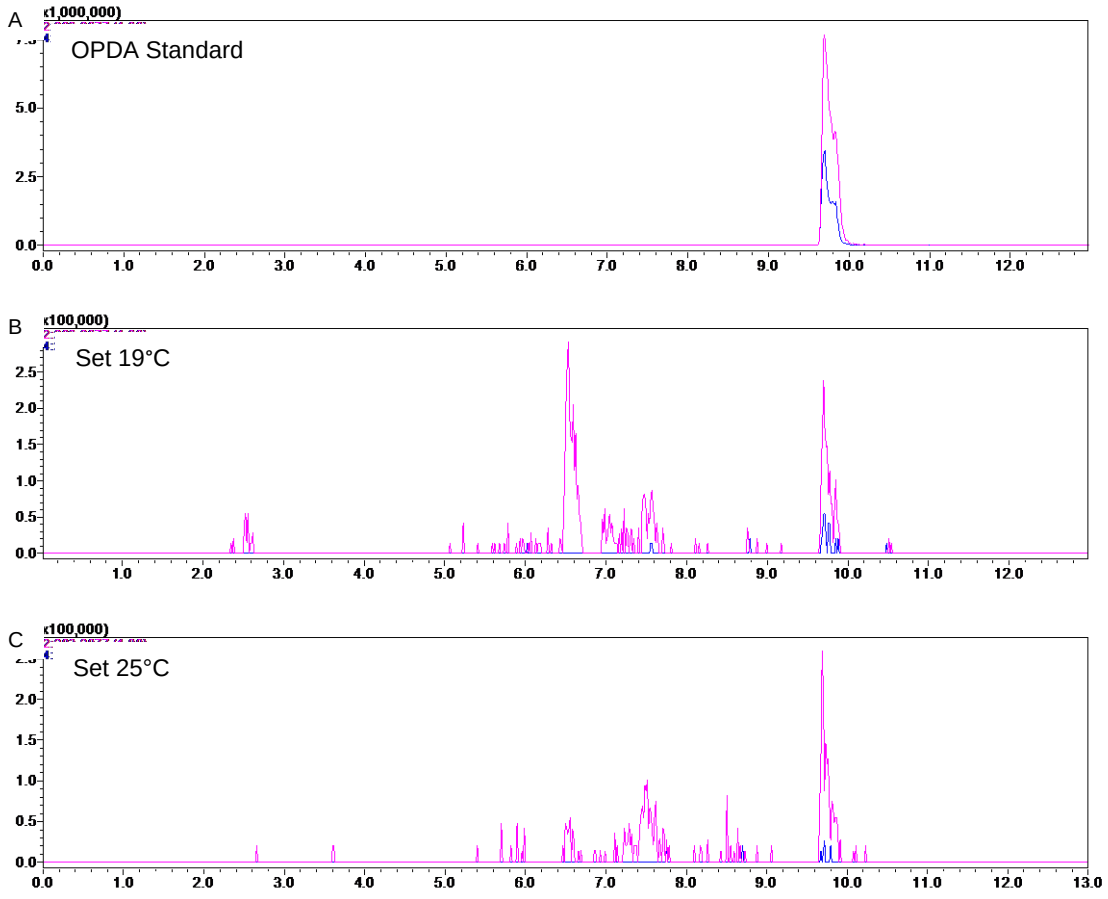

Figure 5. 4 **Chromatograms of OPDA detection in water retained from hydro-priming on *S. lycopersicum* cv. Microtom**. A) OPDA standard (10 µg/ml OPDA). B) Chromatogram of Microtom set at 19°C. C) Chromatogram of Microtom set at 25°C. Positive ionization (purple), negative ionization (blue).

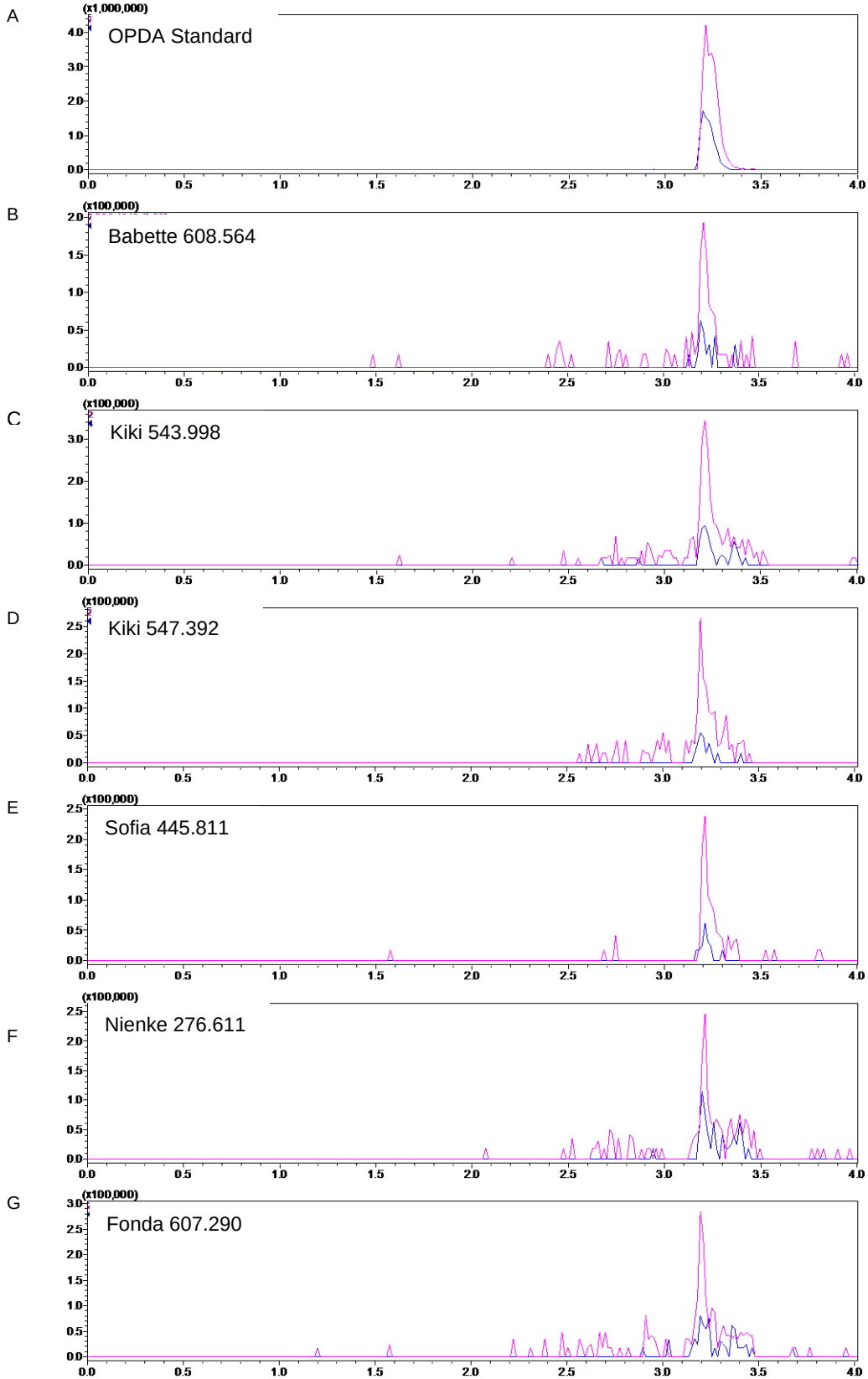

Figure 5. 5 **Chromatograms of OPDA detection in hydro-priming flow through on *S. lycopersicum***. A) OPDA standard (10 µg/ml OPDA). B) Chromatogram of Babette 608.564. C) Chromatogram of Kiki 543.998. D) Chromatogram of Kiki 547.392. E) Chromatogram of Sofia 445.811. F) Chromatogram of Nienke 276.611. G) Chromatogram of Fonda 607.290. Positive ionization (purple) and negative ionization (blue).

I hypothesised that OPDA was present in hydro-priming flow of the Enza varieties, both sensitive and insensitive to hydro-priming. OPDA content was identified and quantified by LC-IT-ToF/MS using internal and external standard (Figure 5.5). Chromatograms confirmed the presence of OPDA in all Enza varieties. Therefore, I concluded that OPDA was leaked out the seed during hydro-priming.

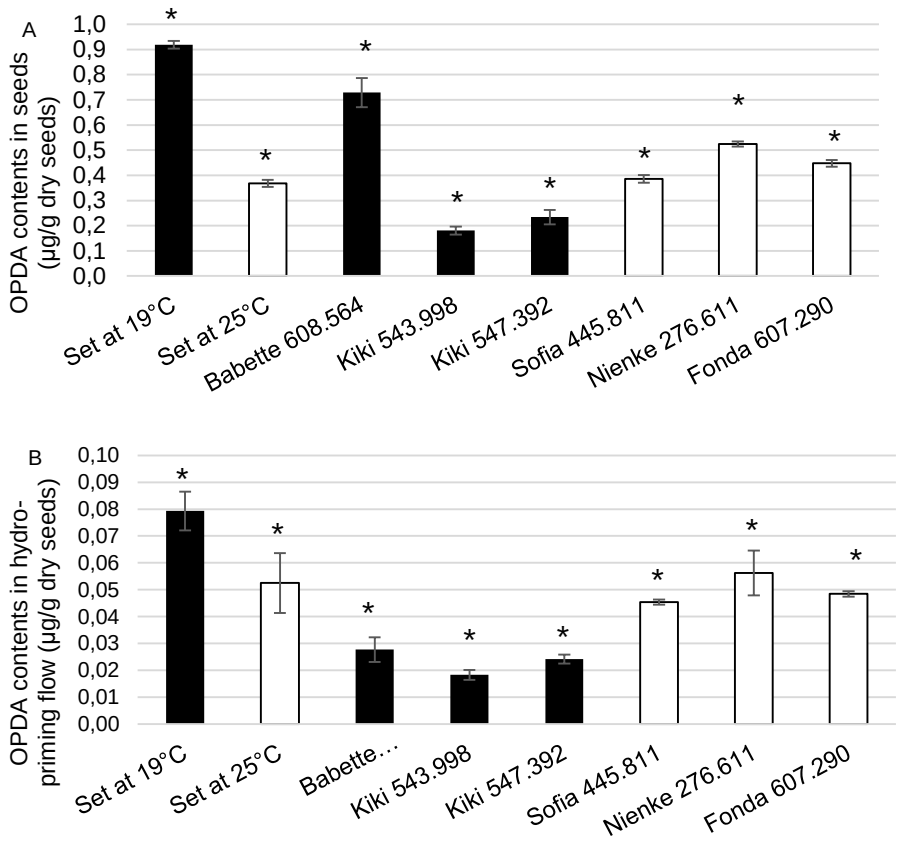

Figure 5. 6 **OPDA content**. Seeds that were sensitive to hydro-priming (black) and insensitive to hydro-priming (white). A. Proportion of OPDA, in dry seeds freshly harvested for each cultivar, collected in positive ionisation mode. B. Proportion of OPDA, in water retained from hydro-priming for each cultivar, collected in positive ionisation mode. The data presented are mean values ± SE of 3 replicates. Significant differences by ANOVA: *, P<0.05.

In order to understand the role of OPDA, which is a biologically active precursor of JA, on efficiency of hydro-priming, I supposed that seeds matured at low temperature (set 19°C) or that were sensitive to hydro-priming which have lower speed of germination without hydro-priming, would contain higher levels of OPDA in comparison to seeds matured at warm temperatures (set 25°C) or that were insensitive to hydro-priming. I measured OPDA content in freshly harvested

dry seeds and hydro-priming leachate by LC-IT-ToF/MS (Figure 5.6). OPDA levels were significantly lower in seeds that were matured at 25°C in comparison to seed set at 19°C. Then, I found the same pattern of OPDA content into dry seeds and hydro-priming leachate except for Babette which had more OPDA into seed. Therefore, these results highlight that there is a correlation between OPDA levels and seed maturation temperature.

5.2.3 Role of OPDA on the speed of germination

a. thaliana mutants of the ß-oxidation pathway, such as mutation of ATP-binding cassette (ABC) transporter *COMATOSE* (*cts*), mutation of acyl-CoA oxidase (*acx1*) or mutation of 3-ketoacyl-coenzyme A thiolase (*kat2*), are impaired in their ability to catabolize fatty acids derived from storage oil and to synthesize jasmonate and are not only defective in seedling establishment but are also impaired in their germination potential by reduction of viable seeds and by a high rate of ovule abortion (Castillo *et al.*, 2004; Theodoulou *et al.*, 2005; Dave *et al.*, 2011, 2012). In tomato, mutants of the ß-oxidation pathway were constructed on two different cultivars, Microtom and Castlemart, and were a gift of Dr. Hause. The *ALLENE OXIDE CYCLASE* (*AOC*), an important gene encoding a JA biosynthetic enzyme (Kallenbach *et al.*, 2010) were transformed in double-stranded RNA interference line under the promoter 35S (35S::SlAOC RNAi) in Microtom cultivar and the transgenic line has very low levels of both OPDA and JA (Wasternack *et al.*, 2012). The tomato *Suppressor of Prosystemin-mediated Responses2* (*SPR2*) encoded a fatty desaturase involved in the synthesis of the octadecatrienoic acid (18:3) was mutated in Castlermart cultivart (Wasternack *et al.*, 2012). Loss of *SPR2* function prevents the biosynthesis of both OPDA and JA. The third mutant used in this study is *acx1a* mutant in Castlemart cultivar and loss of function of an acyl-CoA oxidase (*ACX1A*) disrupts the production of JA but not the production of OPDA (Wasternack *et al.*, 2012). To link the role of OPDA with the seed performance, I used mutant seeds deficient in OPDA pathways and wild-type seeds as control. Mutant lines *spr2* and *acx1a* and the corresponding wild-type background cv. Castlemart were used. I tested the speed of germination of these mutants with or without hydro-priming treatment (Figure 5.7). Hydro-priming was not clearly effective in the wild-type (WT) Castlemart. For *spr2* mutant, the speed of germination is faster than

the wild-type and no difference at T50 with or without hydro-priming is observed (Table 5.1). For *acx1a* mutant, the speed of germination is delayed in comparison with the WT. Therefore, these results showed that loss of both OPDA and JA improved the speed of germination in tomato and increased of OPDA levels in *acx1a* negatively affected the speed of germination. Moreover, it is likely that OPDA levels affect the germination speed in tomato.

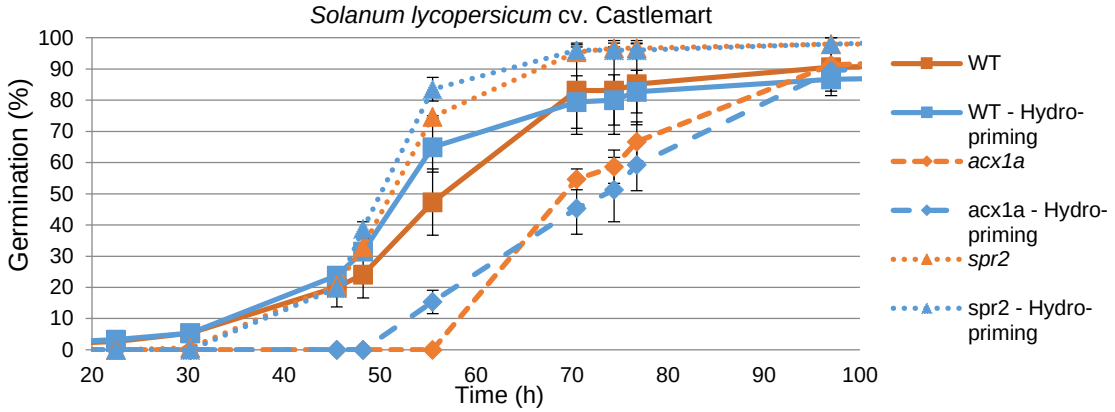

Figure 5. 7 **Effect of hydro-priming on speed of germination of *Solanum lycopersicum* cv. Castlemart.** Germination time-course of tomato seeds; wild-type (WT), *spr2* mutant (*spr2*) and *acx1a* mutant (*acx1a*), as a function of hydro-priming treatment. The data presented are mean values ± SE of 3 replicates of 50 seeds each.

Table 5. 1 **T50 of primed and un-primed for *S. lycopersicum* cv. Castlemart.** Reciprocal of time to respectively 50% of viable seeds to germinate (h) in primed (hydro-priming) or un-primed (negative control) seeds. F values for Student's *t-test* and T-value for Student's *t-test*.

	Negative control		**Hydro-priming**			
	T50	SD	T50	SD	F-Values	T-Values
Wild-Type	56	± 10,611	50	± 1,538	0,7184	≥ 0.05
acx1a	70	± 3,333	74	± 10,349	0,4197	≥ 0.05
spr2	50	± 2,586	50	± 2,200	0,8397	≥ 0.05

For WT, hydro-priming reduced significantly the seedling establishment rate while hydro-priming did not affect seedling establishment rate for *spr2* and *acx1a* (Figure 5.8A). Moreover, hydro-priming reduced significantly the size of the main root in WT but there was no change in *spr2* and *acx1a* (Figure 5.8B). Therefore, I concluded that loss of both OPDA and JA does not improve seedling establishment and hydro-priming had a negative effect seedling establishment and the length of root on wild-type cv. Castlemart.

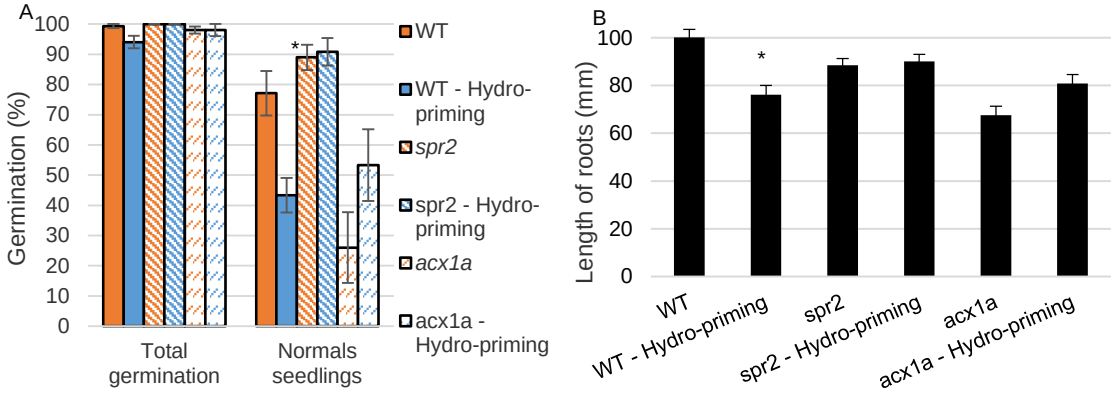

Figure 5. 8 **Effect of hydro-priming on seed vigour of *Solanum lycopersicum* cv. Castlemart.** A. Seedling vigour of tomato seeds; wild-type (WT), *spr* mutant (*spr2*) and *acx1a* mutant (*acx1a*), in function of hydro-priming treatment. B. Effect of hydro-priming on the length of root. The data presented are mean values ± SE of 3 replicates of 50 seeds each. Significant difference between un-primed and primed seeds by a Student's *t-test*: *, P<0.05.

The effect of OPDA on seed performance was tested on mutant lines 35S::SlAOC RNAi which are independent transgenic AOC overexpression line and the corresponding wild-type background cv. Microtom (Figure 5.9, Table 5.2). Hydro-priming improved significantly the speed of germination in wild-type (WT) but hydro-priming did not improve significantly the speed of germination of seeds of 35S::SlAOC RNAi. Moreover, 35S::SlAOC RNAi had a faster speed of germination than WT. These results confirmed the conclusion observed in Castlemart cultivar, that loss of both OPDA and JA improved the speed of germination.

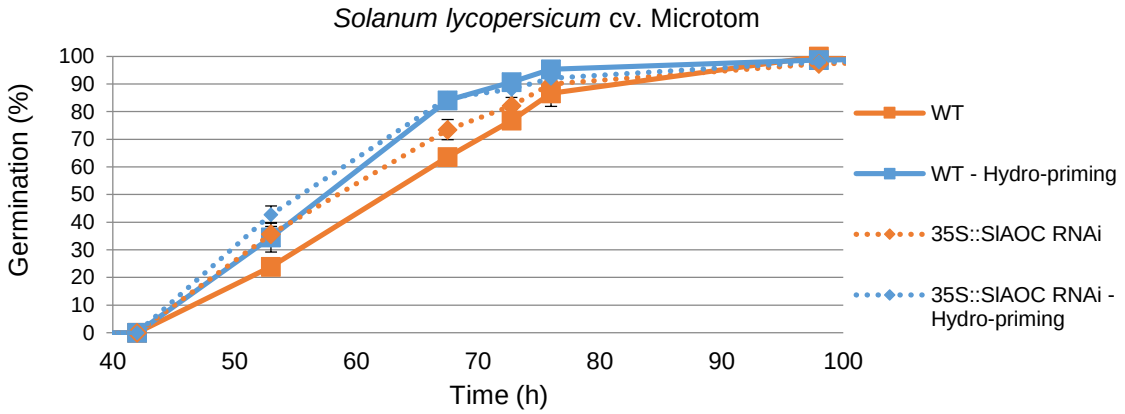

Figure 5. 9 **Effect of hydro-priming on speed of germination on *Solanum lycopersicum* cv. Microtom.** Germination time-course of tomato seeds; wild-type (WT) and 35S::SlAOC RNAi line 16-5-1, in function of hydro-priming treatment. The data presented are mean values ± SE of 4 replicates of 50 seeds each.

Table 5. 2 **T50 of primed and un-primed for *S. lycopersicum* cv. Microtom.** Reciprocal of time to respectively 50% of viable seeds to germinate (h) in primed (hydro-priming) or un-primed (negative control) seeds. F values for Student's *t-test* and T-value for Student's *t-test* between primed and un-primed seeds.

	Negative control		**Hydro-priming**			
	T50	SD	T50	SD	F-Values	T-Values
Wild-Type	62	± 1,817	56	± 3,349	0,5966	**≤ 0.05**
35S :SlRNAi	58	± 2,786	56	± 3,145	0,5797	≥ 0.05

The seedling establishment rate was tested for seeds from Microtom background (Figure 5.10). Hydro-priming did not affect seedling establishment rate and the length of the roots. To conclude, hydro-priming affects only the speed of germination in Microtom cultivar.

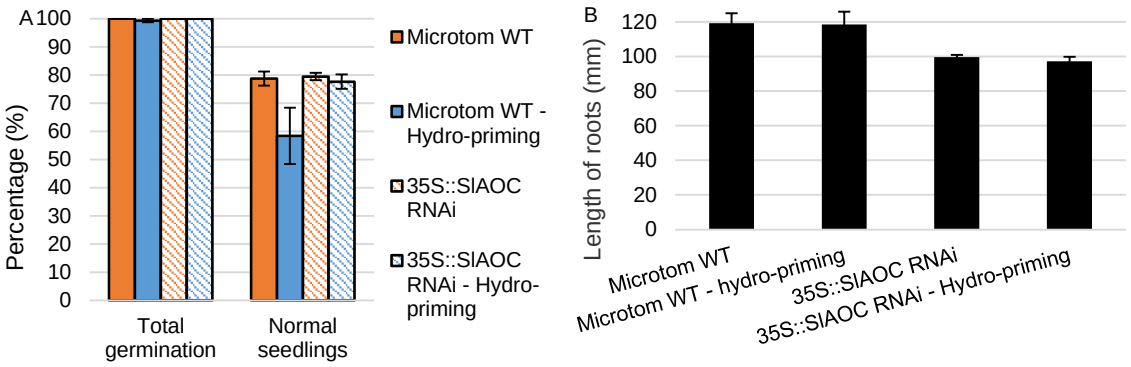

Figure 5. 10 **Effect of hydro-priming on seed vigour of *Solanum lycopersicum* cv. Microtom.** A. Seedling vigour of tomato seeds, wild-type (WT) and 35S::SlAOC RNAi, in function of hydro-priming treatment. B. Effect of hydro-priming on the length of root. The data presented are mean values ± SE of 3 replicates of 50 seeds each. Significant difference by a Student's *t-test*.

5.2.4 Role of OPDA in hydro-priming solution

To test whether OPDA could counteract the effects of hydro-priming, I added exogenous OPDA (1µg/ml) to the hydro-priming solution (Figure 5.11 and 5.12). As it was shown previously, primed seeds had a significantly higher speed of germination than un-primed seeds. On WT, adding exogenous OPDA in hydro-priming liquid did not modify the speed of germination in comparison with the negative control but abolished hydro-priming effect (Figure 5.11, Table 5.3). In OPDA deficient mutant, hydro-priming improved significantly the speed of germination and OPDA reduced significantly the speed of germination. Therefore, the conclusion on OPDA was that exogenous OPDA in liquid was not

sufficient to overcome the promoting effects of hydro-priming on speed of germination.

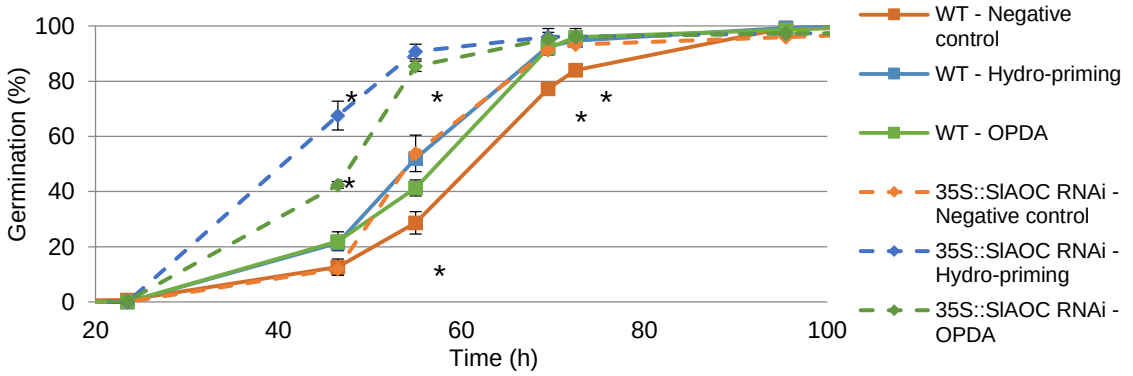

Figure 5. 11 **Effect of adding exogenous OPDA in hydro-priming leachate on *S. lycopersicum* cv. Microtom.** Germination time-course of tomato seeds; wild-type (WT), *acx1a* mutant (*acx1a*) and *spr2* mutant (*spr2*), in function of hydro-priming treatment. The data presented are mean values ± SE of 3 replicates of 50 seeds each. Significant difference by Student's *t-test*: *, P<0.05.

Table 5. 3 **T50 of primed and un-primed enza seeds for *S. lycopersicum*.** A. Reciprocal of time to respectively 50% of viable seeds to germinate (h) in primed (hydro-priming) or un-primed (negative control) seeds. B. Reciprocal of time to respectively 50% of viable seeds to germinate (h) in un-primed seeds (negative control) or primed with OPDA (OPDA). F values for Student's *t-test* and T-value for Student's *t-test*.

A	Negative control		Hydro-priming			
	T50	SD	T50	SD	F-Values	T-Values
Wild-Type	62	± 4,055	55	± 1,493	0,2388	**≤ 0,05**
35S::SlAOC RNAi	55	± 2,407	40	± 2,628	0,2757	**≤ 0,01**

B	Negative control		OPDA			
	T50	SD	T50	SD	F-Values	T-Values
Wild-Type	62	± 4,055	60	± 2,906	0,6785	≥ 0,05
35S::SlAOC RNAi	55	± 2,407	50	± 1,824	0,1430	**≤ 0,05**

The effect of external OPDA in hydro-priming solution was tested on seedling establishment rate as well (Figure 5.12). Seedling establishment is not affected by hydro-priming or exogenous OPDA in hydro-priming solution. Moreover, hydro-priming or OPDA did not affect the size of the main root of normal seedling. With this complementary experiment, the conclusion on OPDA was that OPDA did not affect germination.

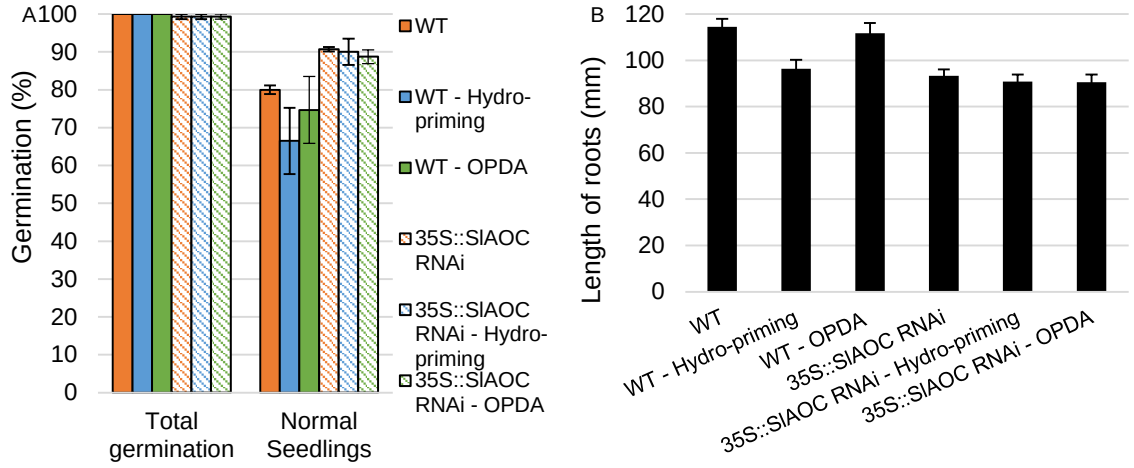

Figure 5. 12 **Effect of hydro-priming on seed vigour of *Solanum lycopersicum* cv. Microtom.** A. Seedling vigour of tomato seeds, wild-type (WT) and 35S::SlAOC RNAi, in function of hydro-priming treatment with or without adding exogenous OPDA in water retained from hydro-priming. B. Effect of hydro-priming or adding exogenous OPDA during hydro-priming on the length of root. The data presented are mean values ± SE of 3 replicates of 50 seeds each. Siginificant difference between negative control and hydro-priming treatment by a Student's *t-test*.

5.3 Discussion

Liquid chromatography-mass spectrometry (LC-MS) is an extremely sensitive analytical technique that enables the detection of metabolites with a vast range of chemistries and molecular masses. Metabolomic data can be described as 'noisy' with many features that do not represent real metabolites, and also real metabolites that are not recorded in every replicate due to technological failings (Hrydziuszko and Viant, 2012). These problems were circumvented by filtering out the 'noise' so that any remaining features are more likely to represent metabolites. In instances where a metabolite was not recorded in all replicate samples, these zero values in the data were removed to enable more robust statistical analyses. In addition, the relatively large number of biological replicates had been maximised to enable better filtering of the data and to increase the robustness of the measurements. Data sets from metabolic analysis contain thousands of data points and require various statistical and descriptive analyses to summarise this information. Appropriate statistics include specialist methods such as a combination of different approaches to interpret the data. Using both univariate and multivariate approaches together can give a clearer picture of an organism metabolome, and how the metabolites in an organism change in response to biotic and abiotic factors. This demonstrates how

careful consideration should be given to the statistical and descriptive analyses employed to fully explore the questions being asked.

As identity of metabolites leaked from seeds during hydro-priming was not known an untargeted approach was used (Figures 5.2, 5.3, Appendices: Tables 2 and 3). There are three crucial steps in this technique: sample extraction, chromatography separation and MS detection (Lu *et al.*, 2008; Vuckovic, 2012). An inadequate protocol on preparation could cause loss of metabolites or degradation. Moreover, sometimes metabolites need to have specialized protocols to optimize their extraction (Lu *et al.*, 2008). To follow-up the untargeted approach, I used a targeted approach to confirm the identity of compounds by comparison with external standard and check it matches in retention time, mass and fragmentation pattern (Figures 5.4 and 5.5).

At the end of the hydro-priming, the tomato seeds released hundreds of metabolites were putatively identified as alkaloid, flavonols and oxidised acid (Figure 5.3, Appendices: Tables 2 and 3). In *Solanaceae*, alkaloids have been studied for their diverse biological activities such as phytoanticipins that protect plants against pathogens (Itkin *et al.*, 2011). Dormantinone has been reported to be a steroidal alkaloid (Eich, 2008) and its role in germination is not known.Tomatidine is the main glycoalkaloid in tomato seeds and acts on pathogens by disruption of the membranes without being toxic in tomato cells (Keukens *et al.*, 1995). Tomatidine leakage could indeed provide a chemical defence against the pathogen attack. Other major compounds found in hydro-priming flow through are flavonols (Figure 5.3). Flavonoids are abundant as secondary products by their role in responses to environmental factors (Winkel-Shirley, 2001). Flavonoids are sub-classified into several families including flavonols, flavones, phlobaphese, isoflavonoids, anthocyanidin and condensed tannins (Winkel-Shirley, 2001). Phenylpropanoid biosynthesis pathway leading to the synthesis of phenols or phytoalexins which have defence function in plants, such as the reinforcement of plant cell walls, antimicrobial activity and synthesis of signaling compounds such like salicylic acid (Wen *et al.*, 2005). Moreover, some studies have reported that production of flavonoids compounds such as kaempferol or quercetin may serve as antimicrobial agents (Cushnie and Lamb, 2011). Quercetin was found to be induced and accumulated in some other plants

after MeJA treatment (Rudell *et al.*, 2002). Compared to the distinctiveness of metabolites leaked at different time-points, the biologically interesting compounds found in the hydro-priming liquid linked to the germination was OPDA. Indeed, OPDA had a role as seed germination inhibitor (Dave *et al.*, 2011, 2012).

The identification and profiling of plant metabolites using untargeted LC-MS or targeted LC-MS could be employd to identify the specific metabolites that are affected by hydro-priming. With this current study on Castlemart variety, mutants on the OPDA/JA pathway had shown a delay in germination (*acx1a*) or an enhancement of the speed of germination (*spr2*) demonstrating the role of the OPDA in the inhibition of the speed of germination (Figure 5.7). RNAi technology was used to disrupt OPDA function in Microtom (35S::SlAOC RNAi line) (Wasternack *et al.*, 2012). *spr2* mutants and 35::SlAOC RNAi have lost both OPDA and JA (Stenzel *et al.*, 2003; Dave *et al.*, 2011). These plants showed an enhancement of the speed of germination (Figures 5.7, 5.9). In both varieties, loss of OPDA improved the speed of germination without affecting the seedling establishment (Figures 5.8, 5.10). Generally hydro-priming improved speed of germination, but in the case of the Castlemart variety, hydro-priming decreased the seedling establishment rate while, as seen in chapter 3, maternal environment affects the speed of germination and hydro-priming improved the speed of germination of low temperature matured seeds (Chapter 3, Figure 3.5). As hydro-priming was more effective on Microtom variety than Castlemart variety, it was impossible to distinguish if the effect of hydro-priming on mutant was due to genetic (variety) or treatment (hydro-priming) even if primed seeds germinated faster than un-primed seeds (Figures 5.7, 5.9). In this study, the role of only OPDA as seed germination inhibitors was investigated as well as work done on Arabidopsis (Figure 5.7: *acx1a*; Dave *et al.*, 2011). In the same manner, a delay of germination is observed on the mutant that is severely compromised in peroximal ß-oxidation (*acx1a*) suggesting the biochemical process is conserved between species.

Taken together, these results in this chapter highlight that OPDA was identified as metabolite leaching from seeds during hydro-priming but experiments with lines with altered OPDA levels show weak support for the hypothesis that OPDA is important in the effectiveness of hydro-priming.

Chapter 6

Characterisation of hydro-priming mechanism(s) by transcriptomic analysis

Chapter 6: Characterisation of hydro-priming mechanism(s) by transcriptomic analysis

6.1 Introduction

Higher plants such as tomato, accumulate proteins, oils and carbohydrates in their seeds during maturation. Studies have shown that maternal environment during seed maturation affects seed quality (Hilhorst and Toorop, 1997; Baskin and Baskin, 1998). Most of the genes involved in germination have been studied (see chapter 1). Seeds have developed protective molecules to defend themselves against stress and to permit germination. These molecules such as heat shock proteins or late embryogenesis abundant proteins are associated with seed longevity and germination (Kushwaha *et al*., 2013; Kaur *et al.*, 2015). Furthermore, flavonoids and more especially PAs that are present in the testa are known to act on seed germination (Debeaujon *et al.*, 2000). Results in previous chapters showed that 24h of hydro-priming at 15°C was most effective in the enhancement of germination and hydro-priming was more effective on seed matured at low temperature (Chapter 3). Moreover, hydro-priming had stronger effect on the speed of germination than iron (Chapter 4). Metabolomic analysis revealed that OPDA, a germination inhibitor, is leaked out of seed during hydro-priming (Chapter 5).

Clusters of genes with related functions exhibit expression patterns that are correlated and co-expression networks have been proven to be very effective to identify relevant gene interactions (Li *et al*., 2016). For example, Li used transcriptome analysis to link physiological development processes of seed dormancy and germination with auxin signal and regulatory networks. Global transcriptome analysis of un-primed seeds in comparison with hydro-primed seeds can provide fundamental molecular understanding of germination processes in tomato seeds. Moreover, transcriptome analysis can bring information on specific processes in hydro-priming mechanism(s). The first step of transcriptome analysis is RNA-sequencing (RNAseq), a sensitive technique that creates short reads by deep sequencing of cDNA fragments (Wang *et al.*, 2009). The key aim of this is to quantify the changing expression levels of a transcript under different conditions. Here, I used RNAseq to generate a high-resolution map for *S. lycopersicum* cv. Kanavaro during hydro-priming to catch the moments when key events occur. I present the global analysis of gene expression during hydro-priming treatment. Utilizing gene annotation data from

the tomato assembly (Solgenomics), the transcriptome analysis of gene expression under the different hydro-priming conditions led to the identification of genes related to germination.

6.2 Results

After optimisation of hydro-priming conditions, a time-course experiment was conducted on Kanavaro seeds to confirm the effectiveness of hydro-priming (Figure 6.1). Germination speed and T50 were measured for each condition: un-primed seeds, 3h, 6h, 16h and 24h of hydro-priming. Primed seeds germinated significantly more rapidly than controls. Therefore, I concluded that increasing time of hydro-priming improved speed of germination and seeds that were primed during 16h or 24h had a higher speed of germination. I used this batch to do the RNAseq.

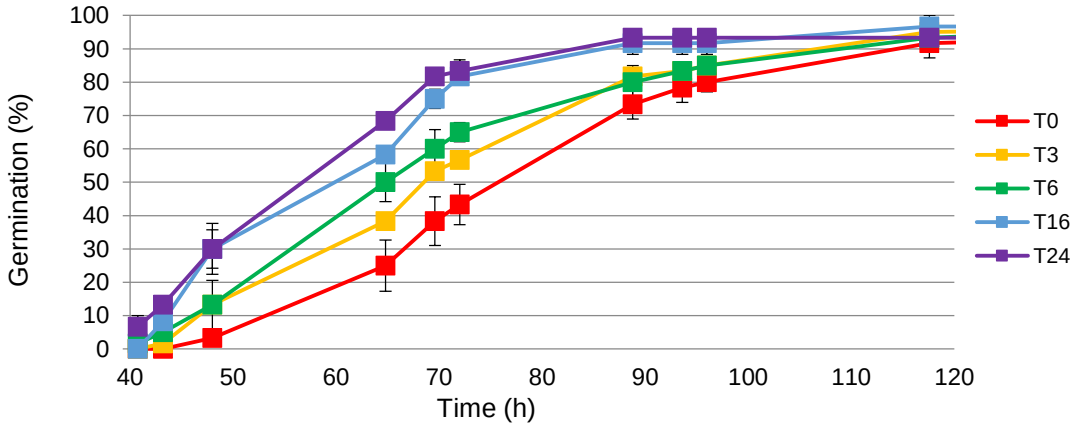

Figure 6. 1 **Effect of hydro-priming for *S. lycopersicum* cv. Kanavaro line**. Effect of hydro-priming on the duration on the speed of germination. Duration of hydro-priming is indicated on the legend: un-primed seeds (T0), seeds primed for 3h (T3), 6h (T6), 16h (T16) and 24h (T24). The data presented are mean values ± SE of 4 replicates of 50 seeds each.

Table 6. 1 **T50 of hydro-priming time-course for *S. lycopersicum* cv. Kanavaro line**. Reciprocal of time to respectively 50% of viable seeds to germinate (h) in the control (T0), 3h (T3), 6h (T6), 16h (T16) and 24h (T24) of hydro-priming treatment. F-values for Student's *t-test* and T-values for Student's *t-test*.

Treatment	T50	SD	F-Values	T-Values
T0	75	± 2,887	-	-
T3	69	± 3,215	0,8929	≥0,05
T6	64	± 1,000	0,2143	≤0,05
T16	58	± 1,732	0,5294	≤0,01
T24	55	± 1,528	0,4375	≤ 0,01

The transcriptome analysis was carried out using RNAseq and data obtained can give an indication of which genes are differentially regulated between different experimental conditions.

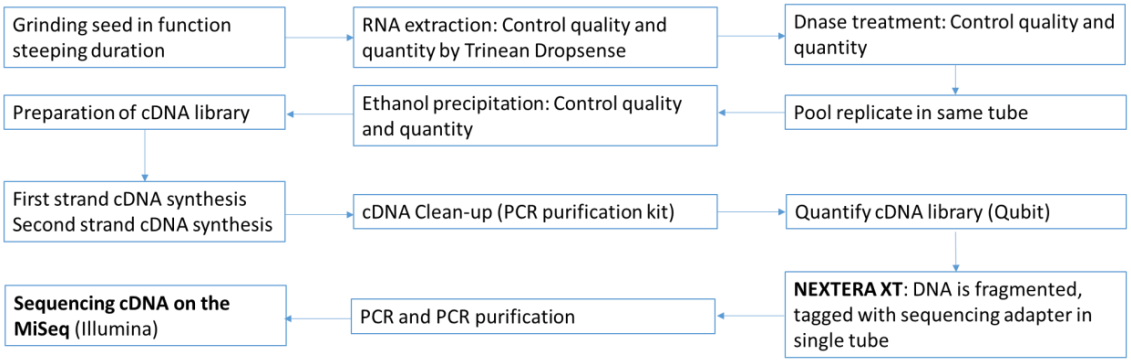

Figure 6. 2 **Summary of the RNA sequencing process**.

The transcriptome of twenty-five seeds of Kanavaro from each conditions was sequenced and compared with wild-type seeds. A summary of the RNAseq process is shown in Figure 6.2 and a summary of the data analysis is shown in Figure 6.3. Reads were aligned to the reference genome *S. lycopersicum* cv. Heinz 1706 (Solgenomics). Transcriptome sequencing was performed on a hydro-priming time-course. All seeds that I used were dried after hydro-priming during two days at 25°C. Replicates of un-primed seeds (T0) and seeds primed for 3h (T3), 6h (T6), 16h (T16) or 24h (T24) were collected, mixed and then used for RNA isolation. Afterwards, paired-end libraries were prepared and sequenced as described in Figures 6.2 and 6.3. RNAseq data was analyzed using WGCNA (Langfelder and Horvath, 2008), Java Treeview (Saldanha, 2004) and Gene Cluster 3.0 (Eisen et al., 1998) as complementary differential expression analysis methods.

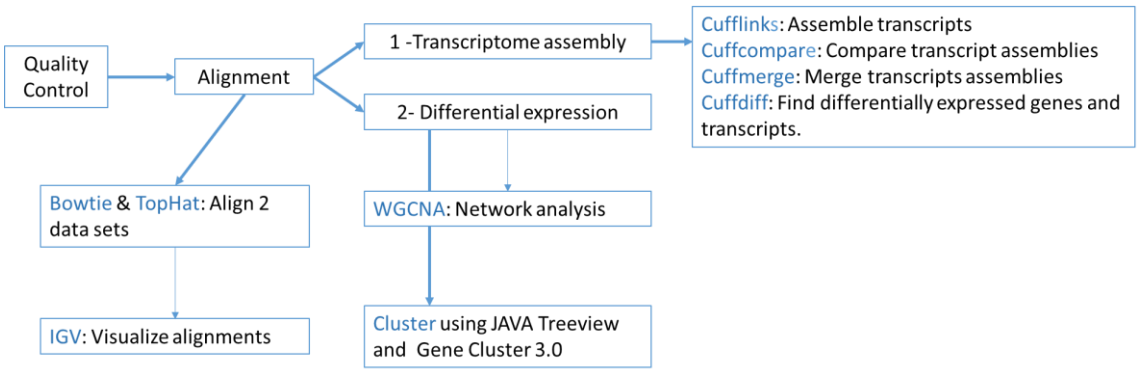

Figure 6. 3 **Summary of the transcriptomic data analysis pipline**.

6.2.1 Molecular analysis and clustering

The obtained dataset showed that, among the 34 725 annotated genes in the tomato genome, there were 25 279 expressed genes in seeds during hydro-priming (Figure 6.4 and Table 6.2). Expressed genes are described as being differentially expressed between primed and un-primed (see Chapter 2) identified as differentially expressed genes (DEGs). Integrating the reproducibility between biological replicates, genes non-expressed during time-courses are removed and the distribution of these genes is shown in a Venn diagram (Figure 6.4A). This diagram highlights that 21 198 expressed genes (87%) were commonly identified in the five treatments. These results implied that the expression pattern changed quickly after 3h of hydro-priming while, after 16h of hydro-priming, the pattern changed again and the number of expressed genes increased. It was found that 21 198 genes were expressed and showed a continuous up-regulation pattern or a continuous down-regulation pattern (Figure 6.4B). To highlight biological pathways involved during hydro-priming, I used MapMan software which classified genes and metabolites (Usadel *et al.*, 2009). Many genes changed or reacted during hydro-priming but 35% of expressed genes are of unknown function (Figure 6.4C). Among the genes identified, those involved in protein metabolism (amino acid activation, assembly and cofactor ligation, degradation, folding, glycosylation, postranslational modification, synthesis and targeting) or in RNA metabolism (processing, regulation of transcription, binding and transcription) had a majority of transcripts (10%). Then those involved in lipid metabolism, secondary metabolism, transport, enzyme, signalling and stress pathway were expressed (4-5%). A third group having 1-2% of transcript expression were involved in cell metabolism (organisation, cycle, vesicle, death), hormone metabolism, development metabolism (late embryogenesis, multi-target, squamosa, storage protein), DNA metabolism (repair, synthesis, chromatin synthesis) and cell wall metabolism (protein, synthesis, degradation, hemicellulose, modification, pectin, precursor, death). Others pathways had a low change in genes expression (≤ 1%). Most of these genes are up-regulated during hydro-priming (Table 6.2). Therefore, I concluded that hydro-priming involved various metabolic processes.

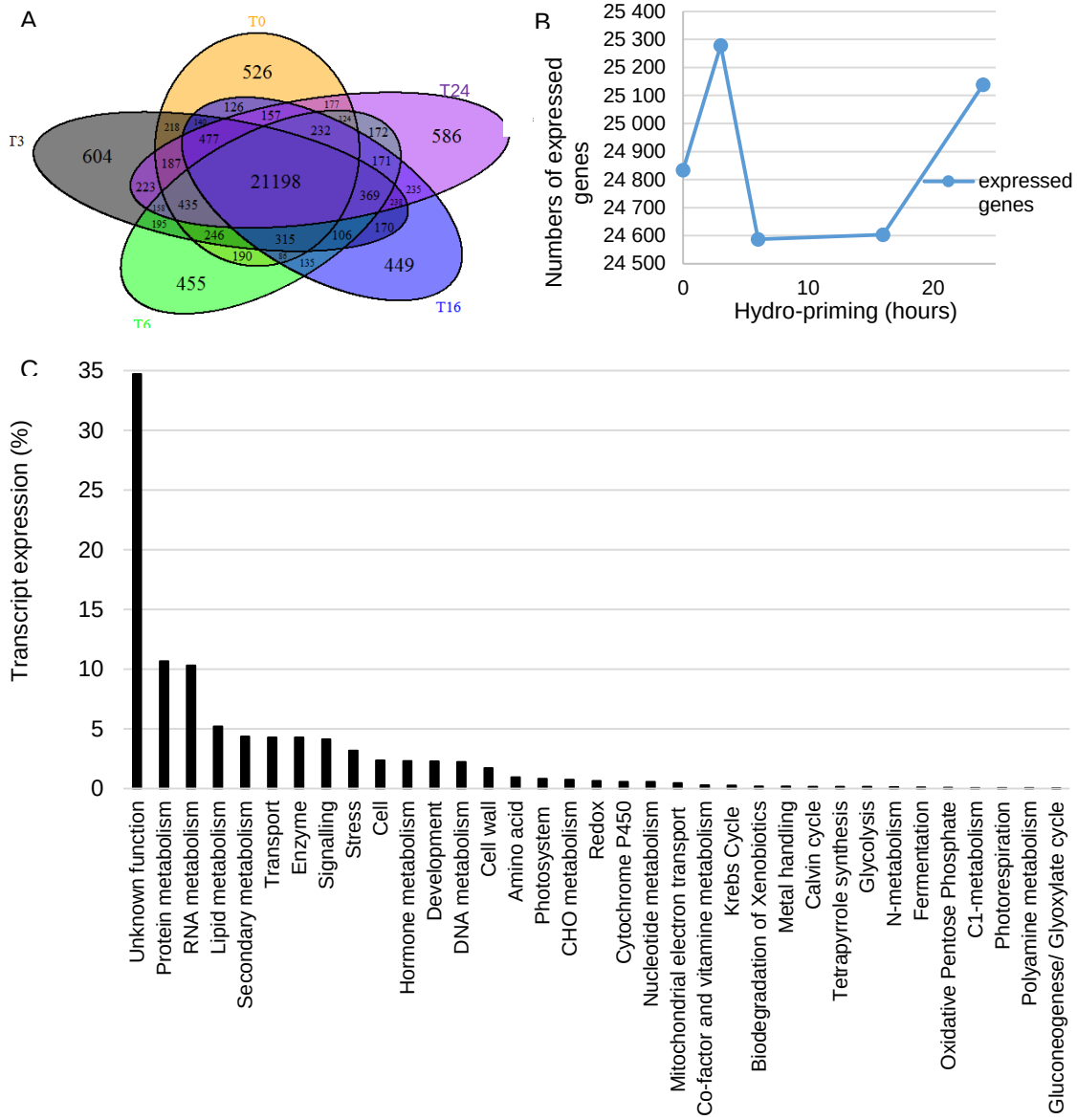

Figure 6. 4 **Differentially expressed genes (DEGs) in hydro-primed seeds and non hydro-primed seeds for *S. lycopersicum* cv. Kanavaro**. A. Venn diagram of the 25 279 DEGs in seeds primed (T24) and un-primed (T0). B. Number of DEGs in each of the samples at five time-point of the hydro-priming. C. Percentage of transcript expression in function of metabolic pathways.

Table 6. 2 **Genes regulated during hydro-priming compared to the control**.

	Up-regulated genes (%)	Down-regulated genes (%)
Amino acid	81	19
Biodegradation of Xenobiotics	70	30
C1-metabolism	72	28
Calvin cycle	72	28
Cell	76	24
Cell wall	80	20
CHO metabolism	75	25
Co-factor and vitamine metabolism	86	14
Cytochrome P450	67	33
Development	76	24
DNA metabolism	80	20
Enzyme	75	25
Fermentation	83	17
Gluconeogenese/ Glyoxylate cycle	56	44
Glycolysis	72	28
Hormone metabolism	68	32
Krebs Cycle	82	18
Lipid metabolism	75	25
Metal handling	78	22
Mitochondrial electron transport	87	13
N-metabolism	71	29
Nucleotide metabolism	83	17
Oxidative Pentose Phosphate	86	14
Photorespiration	70	30
Photosystem	78	22
Polyamine metabolism	56	44
Protein metabolism	81	19
Redox	75	25
RNA metabolism	79	21
S-assimilation	55	45
Secondary metabolism	73	27
Signalling	72	28
Stress	69	31
Tetrapyrrole synthesis	86	14
Transport	78	22
Unknown function	73	27

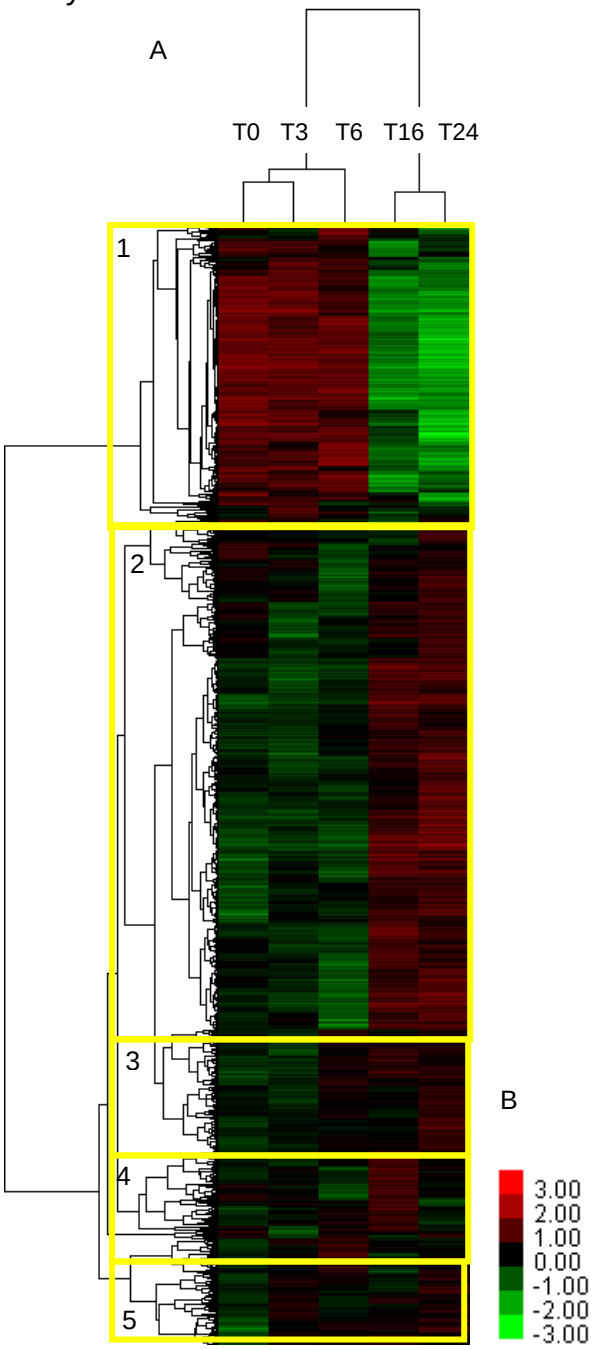

Figure 6. 5 **Clustered display of data from hydro-priming time-course for *S. lycopersicum* cv. Kanavaro**. A. Array with 5 clusters in yellow. B. Color scale, gene expression levels are shown with high expression represented in red and low expression represented in green.

Java Treeview and Gene Cluster 3.0 were used to display differential expression and revealed differences in gene expression between the five investigated time-points (Figure 6.5). A cut-off q-value ≤0.05 and fold change ≥2 was used to identify 7941 genes up and down regulated. The transcriptomes of the individual time-points clustered in a specific way, with time-points T16 and

T24 similar to each other but clearly distinct from earlier time-points. I identified five clusters:

- Cluster 1 contains 2 138 genes that are up regulated at T0, T3 and T6 then they are down regulated at T16 and T24.
- Cluster 2 contains 3 482 genes that are down regulated at T0, T3 and T6 then they are up regulated at T16 and T24.
- Cluster 3 contains 855 genes that are down regulated at T0 and T3 then they are up regulated at T6, T16 and T24.
- Cluster 4 contains 1 038 genes that are down regulated at T0, T3, T6 and T16 then they are up regulated at T24.
- Cluster 5 contains 428 genes that are down regulated at T0 then they are up regulated at T3, T6, T16 and T24.

The results obtained on DEGs coincides with the results on germination assay (Chapter 3). Previous results showed that hydro-priming increased the speed of germination after 16h of imbibition and 16h and 24h of imbibition showed similar results. Therefore, I concluded that hydro-priming is effective after 16h of imbibition and 16h corresponds to a large transcriptional change compared to 6h.

Subsequently, I analysed the expression changes for individual genes. Un-primed seeds corresponded to T0 and primed seeds corresponded with T3, T6, T16 and T24. A comparison of gene expression of two conditions: un-primed seeds (y axis: T0) vs. primed (x axis: 3h, 6h, 16h or 24h) was conducted (Figure 6.6). Selecting genes in the dendrogram were used (Figure 6.5) and gene score was used to produce the scatterplot (Saldanha, 2004). A comparison of gene expression of un-primed seeds and seeds hydro-primed during 3h (Figure 6.6 A) or 6h (Figure 6.6 B) showed a positive correlation. A comparison of gene expression of un-primed seeds and seeds hydro-primed during 16h (Figure 6.6 C) or 24h (Figure 6.6 D) showed a negative correlation, suggesting these variables (un-primed seeds and primed seeds during 16h or 24h) had a negative association. These results confirmed that 16h or 24h of hydro-priming were sufficient to observe a change in gene regulation.

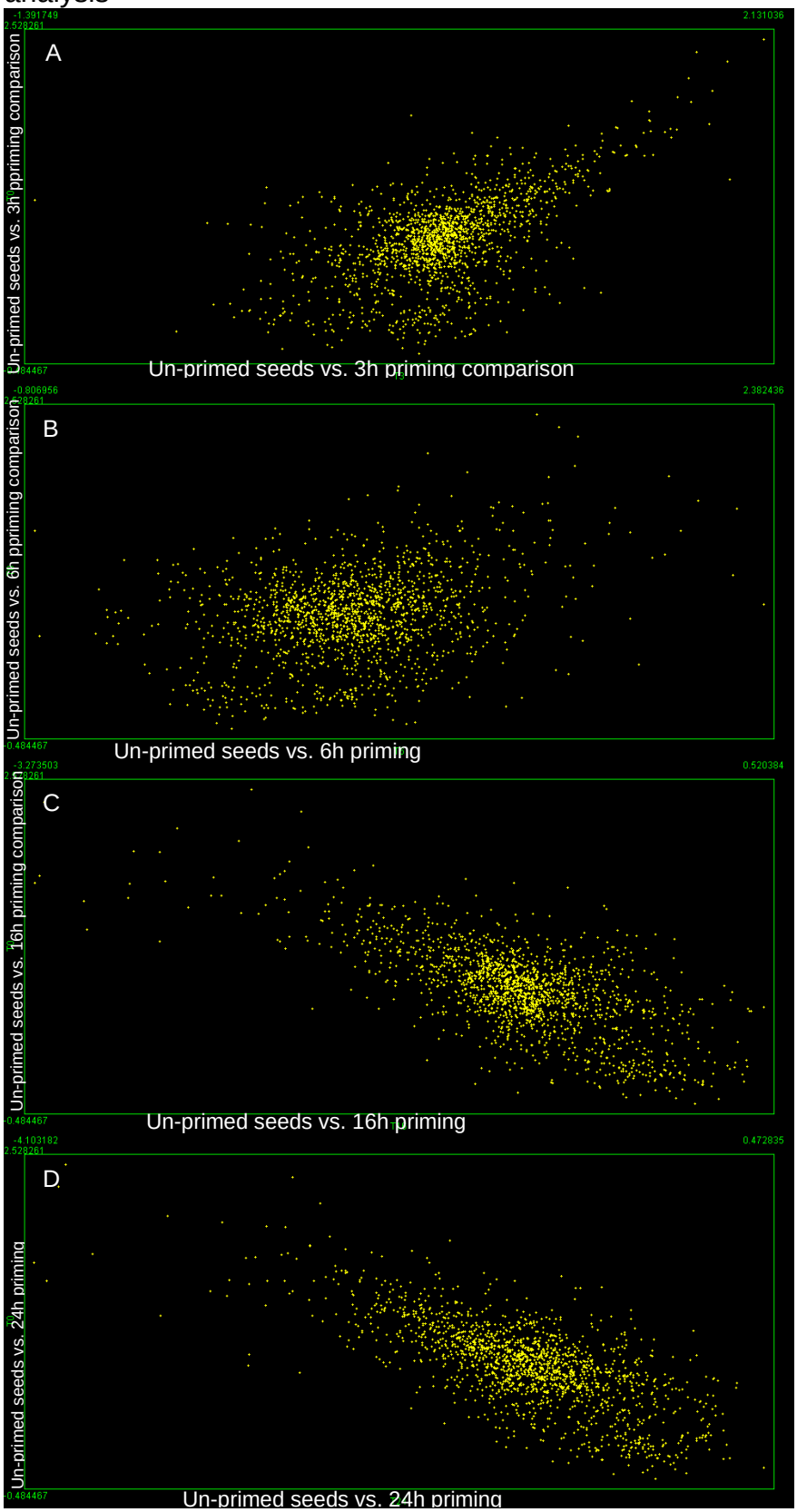

Figure 6. 6 **Global transcriptome relationship among different duration of hydro-priming of *S. lycopersicum* cv. Kanavaro.** Yellow dots are DEGs. A. Un-primed seeds vs. 3h of primed seeds. B. Un-primed seeds vs. 6h of primed seeds. C. Un-primed seeds vs. 16h of primed seeds. D. Un-primed seeds vs. 24h of primed seeds.

To find evidence about the way in which hydro-priming affects seed vigour, I observed the changes in gene expression level in individual genes in two conditions: un-primed seeds (T0) and primed seeds (T24). To reduce the list of candidate genes which have a role in hydro-priming efficiency, I selected DEGs having more than 100 hundred reads (Figure 6.7). Under these criteria 5207 genes were expressed both in the negative control and in seeds after hydro-priming, only 322 were especially expressed uniquely in the control and 2391 were specially expressed after 24h of hydro-priming. Therefore, I concluded that 24h of hydro-priming involved more DEGs than un-primed treatment.

Figure 6. 7 **DEGs having more than 100 reads in hydro-primed seeds versus un-primed seeds for *S. lycopersicum* cv. Kanavaro.** Among the DEGs, 5207 are commonly expressed in un-primed seeds and in primed seeds. 322 DEGs are only presents in un-primed seeds and 2391 DEGs are only presents in primed seeds.

To identify the genes which were differentially expressed during hydro-priming, I clustered these DEGs (Figure 6.8). I used bioconductor in R to create the clusters using RPKMs in log 2 scale as expression value. I used un-primed seed as a control then in functions of the five conditions (T0, T3, T6, T16 and T24) and of DEGs, to finally create five clusters: blue, darkgreen, magenta, purple and turquoise. In the “Blue” and “Turquoise” clusters, DEGs showed a peak in expression value after 3h of imbibition. As 3h of hydro-priming was not enough to have an effect on the germination (Chapter 3), the DEGs did not correlate gene expression with the effects of hydro-priming. The cluster “Purple” had transient DEGS, down-regulated from T0 until at T6 then up-regulated until T16 and down-regulated until T24. The change in DEGs was unlikely to be associated with hydro-priming effects. DEGs in the cluster “Darkgreen” were up-regulated until T3 then down-regulated until T24. The expression value at T24 was lower than the expression value at T0. In the cluster “Magenta”, the expression value increased from T6 until T24. Germination assays have shown that the longer it takes for the hydro-priming to process, the more satisfactory is the efficiency of

hydropriming (Chapter 3). As the cluster “Magenta” showed an increased of expression values and the cluster “Darkgreen” showed a decrease of expression values, these two clusters were used to identify the candidate genes that their expression correlates with the hydro-priming process. Consequently, the function of the genes in the three others clusters (blue, turquoise and purple) were not considered as the major ones that are causing a difference in germination mechanism through hydro-priming.

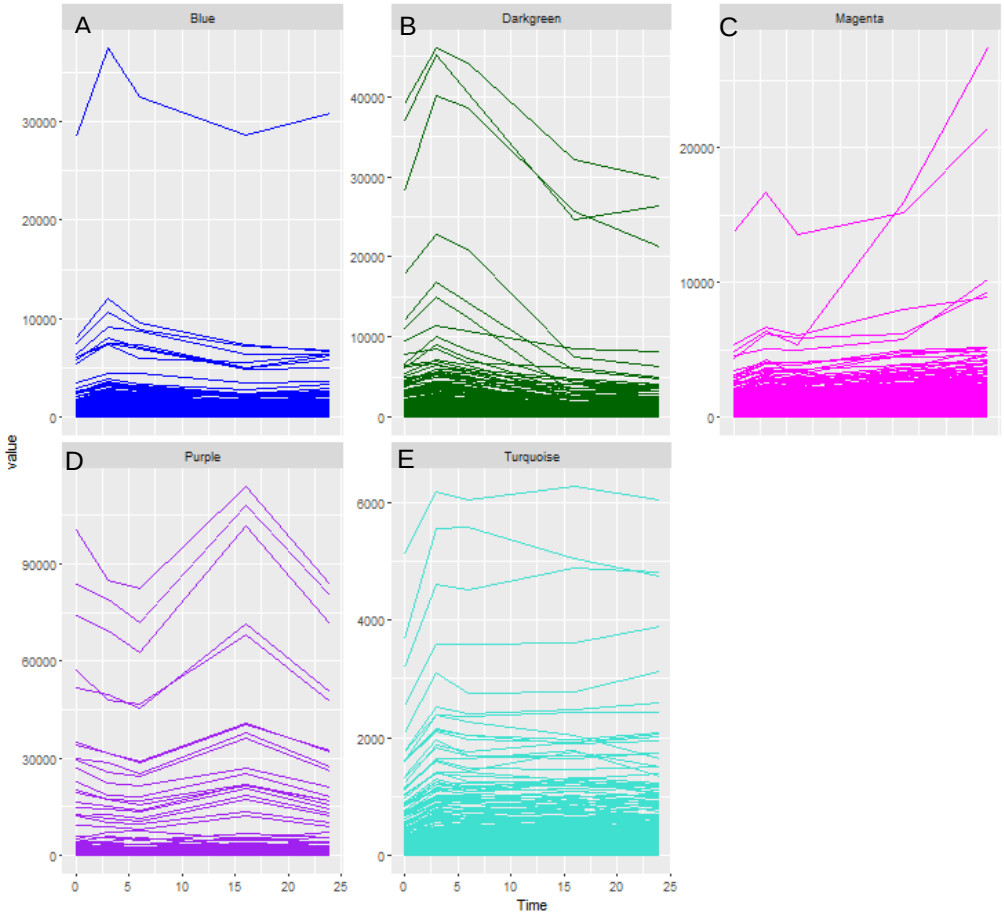

Figure 6. 8 **K means clustering for gene expression during hydro-priming in *S. lycopersicum* cv. Kanavaro**. A. Cluster blue. B. Cluster darkgreen. C. Cluster magenta. D. Cluster purple. E. Cluster turquoise.

6.3.2 Determination of hydro-priming mechanism(s)

To determine whether the OPDA found in hydro-priming flow through liquid was related to differential gene expression patterns, I compared transcript levels of genes involved in OPDA pathway between primed and un-primed seeds (Figure 6.9). The OPDA biosynthesis pathway is known and the key genes involved in its regulation are identified (Dave *et al.*, 2011; Wasternack *et al.*, 2012). OPDA is released of fatty acids from plastidial membrane lipids by lipases

such as *DEFECTIVE IN ANTHER DEHISCENCE1* (*DAD1*) (Ishiguro *et al.,* 2001; Hyun *et al*., 2008; Ellinger *et al.*, 2010) then OPDA is catalized by a lipoxygenase (LOX), the allene oxide synthase (AOS) and the allene oxydase cyclase (AOC) in plasmid to form the cis-OPDA (Weber *et al.*, 1997; Acosta and Farmer, 2010). Cis-OPDA migrates from plastid and is imported into the peroxisome by a transporter COMATOSE (CTS) and peroximal β-oxidation (CTS/PXA) (Theodoulou *et al.,* 2005). Then the 12-oxophytodienoate reductase 3 (OPR3) reduces cis-OPDA (which is active in peroxisome) into OPC-8:0 (Sanders *et al.,* 2000; Schaller *et al*., 2000; Stintzi and Browse, 2000). A coumarate-CoA ligase activates OPC-8:0 to OPC-8-CoA which undergoes three rounds of β-oxidation by acyl CoA oxidase1 (ACX1), a multifunctional protein (MFP) and L-3-ketoacyl-CoA thiolase 2 (KAT2) to form JA (Cruz Castillo *et al.*, 2004; Pinfield-Wells *et al.,* 2005; Schilmiller *et al.,* 2007; Graham, 2008). The *spr2* mutant is affected in the generation of fatty acid. The transcript levels of *AOC*, *APR3*, *AOS3*, *LOX*, *DAD1*, *ACX1A*, *MFP* and *SPR2* were increased significantly in primed seeds compared to un-primed seeds (Figure 6.9). Increasing in OPDA catabolism would be in consonance with biosynthesis activation suggesting a dynamic and active metabolism.

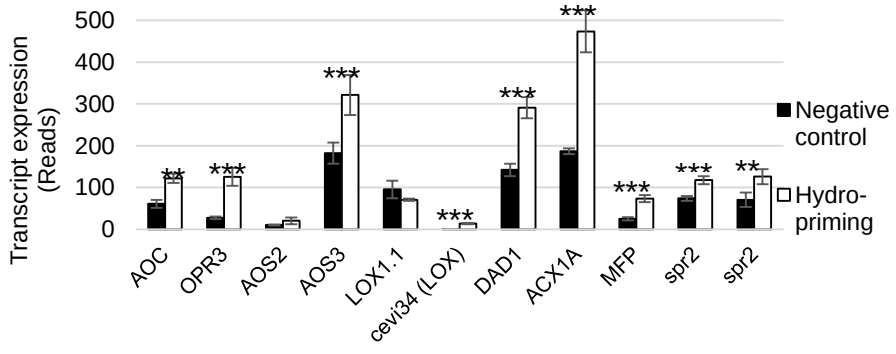

Figure 6. 9 **Transcript expression of genes involved in OPDA pathway**. Significant differences between negative control and primed seeds by a Student's *t-test*: *, P<0.01; **, P<0.001; ***, P<0.0001. Abbreviations of enzymes: AOC, allene oxide cyclase; OPR3, OPDA reductase 3; AOS, allene oxide synthase; LOX, lipoxygenase; *DAD1*, *DEFECTIVE IN ANTHER DEHISCENCE1*; ACX1A, acyl-CoA oxidase; MFP, multifunctional protein; *spr2* mutant, affected in α-LeA fatty acid.

In order to understand the important molecular basis of hydro-priming mechanism in tomato seeds, I examined the changes in expression abundance for gene-encoding proteins that were involved in the endosperm cap weakening.

It has previously been shown that testa rupture appeared in micopyle area and *MAN* genes were involved in the testa rupture (Dahal *et al.*, 1997). Most of the genes encoding for micropyle area breaking showed a significant difference in transcript levels between the negative control and the hydro-primed seeds (Figure 6.10). *GluB* had more transcripts in the negative control than in hydro-primed seeds, but *MAN1*, precursor of *MAN1*, *EXPA4*, *EXPA10* and *XET4* had more transcripts in primed seeds. Therefore, these results showed that hydro-priming promotes the activation of genes involved in weaking in the endosperm cap leading to radicle protrusion, with the exception of *GluB*.

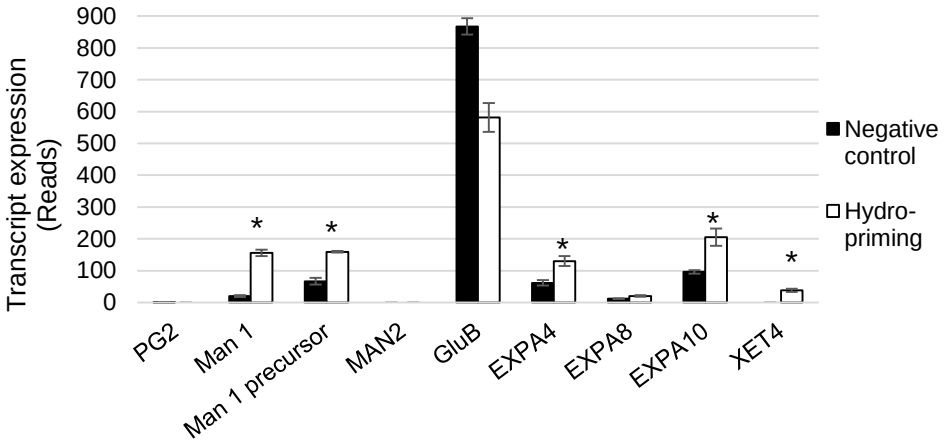

Figure 6. 10 **Transcript expression of endosperm genes for *S. lycopersicum* cv. Kanavaro**. Significant differences between negative control and primed seeds by a Student's *t-test*: *, P<0.0001. Abbreviations of genes: *PG*, polygalacturonase; *MAN,* endo-β-mannamases; *GluB*, endo-β-1,3-glucanase; *EXPA*, expansins; *XET*, xyloglucane endo-transglycosylases.

6.4 Discussion

To determine the global patterns of gene expression and highlight the involvement of the identified DEGs during hydro-priming, DEGs were annotated and those having more than 500 reads were reported in appendices table 4. A total of 24 282 genes were expressed (Figure 6.4) and I found that the majority of transcripts encoded proteins regulated various metabolic / biosynthesis processes including those involved in OPDA pathway and endosperm cap weakening (Figure 6.4). Majority of DEGs were expressed at different levels in un-primed seeds and primed seed but one third of DEGs were only expressed in primed seeds (Figure 6.7).

As shown in Figure 6.9, the majority of the genes involved in OPDA pathways (*AOC*, *OPR3*, *ACX1A* and *DAD1* genes), a precursor of jasmonate, showed an increase in gene expression after hydro-priming compared to controls. Jasmonate is known to inhibit the germination of non-dormant seeds and to stimulate the germination of dormant seeds (Yildiz *et al.*, 2008; Linkies and Leubner-Metzger; 2012). Methyl jasmonate has been reported to have various effect on seed dormancy and germination. It inhibits germination of seeds of lettuce, sunflower, amaranthus, tobacco (*Nicotiana attenuata*), oat, wheat, and rape seeds (Daletskaya and Sembdner, 1989; Krock *et al.*, 2002; Preston *et al.*, 2009) but it enhances germination of a number of dormant seeds including apple, pear, acer and ash (Daletskaya and Sembdner, 1989; Berestetsky *et al.*, 1991; Ranjan and Lewak, 1992; Jarvis *et al.*, 1997; Yildiz *et al.*, 2008). Moreover, Wasternack showed that wounding causes jasmonate synthesis as well as changes in the pattern of gene expression (Wasternack et Hause, 2002).

Expansins (*EXPA*) are large gene famillies and isoforms that have different functions in plant development such as leaf elongation and fruit development (Harrison *et al.*, 2001; Reidy *et al.*, 2001; Wu *et al.*, 2001b; Zhang *et al.*, 2014). In tomato, *EXP4* was especially expressed in the endosperm cap and the expression of mRNA was correlated with endosperm weakening (Chen *et al.*, 2001). *EXP8* was only expressed only in radicle tip and *EXP10* was expressed in seeds but both were known to have a role in seed development, germination and early seedling growth (Chen *et al.*, 2001; Brummell *et al.*, 1999). As *EXP8* was only expressed in radicle tip, the fact as *EXP8* had no significant difference in transcript expression level may indicate that this gene was not involved in the early step of germination and *EXP8* may only have a role in the elongation of radicle tip (Figure 6.10). The expression of *EXP10* was higher after 24h of hydro-priming, suggesting this gene was involved in germination and endosperm weakening but this result needs to be confirmed by quantitative RT-PCR. Tomato seeds contained >60% *MAN* whereas embryo cell walls contained it at only 30% (Dahal *et al.*, 1997). Moreover GluB was expressed in the micropylar endosperm cap of *Lycopersicon esculentum* seeds just before radicle emergence through this tissue to complete germination (Wu *et al.*, 2001a). Therefore, I concluded that hydro-priming increased the speed of germination by

activating genes that are involved in endosperm weakening but in order to confirm these results, additional information on the proteins coded by these genes and their activity on different types of cell walls were needed. Then, it would be important to confirm the gene expression differences by quantitative PCR (qPCR) before publication.

Altogether, these findings suggest that there are considerable differences between negative control and hydro-primed seeds. *MAN* can contribute with expansin to cell wall disassembly in the endosperm cap to permit radicle emergence during germination.

Chapter 7

General discussion

7.1 Summary of the thesis findings

The main aim of my thesis was to identify the mechanism by which hydro-priming improves germination of tomato seeds, and to test whether hydro-priming can overcome the effects of variation in the maternal environment. To achieve this I measured the effects of hydro-priming on the seed physiology, on the metabolome and on the transcriptome of the seeds.

In **chapter 3**, I examined interactions between maternal environment and hydro-priming effects. All types of tomatoes were selected for this study to encompass a wide range of tomatoes. Seeds from plants growing at temperatures below 22°C had a lower seed size and germination speed was slower as well. After hydro-priming, the differences in vigour between produced lots was greatly reduced. Hydro-priming reduced the time necessary for germination, as well the emergence and the effectiveness of hydro-priming was related to the seed maturation environment: hydro-priming improved the vigour of seed set matured at low temperatures (below 22°C).

In **chapter 4**, I examined ions that were leaked from seeds into the hydro-priming liquid. The ionomic analysis of hydro-priming time-course showed that iron is released from seeds and I tested whether this could play a role in the effectiveness of hydro-priming. I ruled out a role for iron in the process, but showed that the iron released from seeds was present mainly in the seed coat and correlated with seed coat colour. I also showed that there were no proanthocyanidin pigments in tomato seeds.

In **chapter 5**, I examined the metabolites that were leaked from seeds during hydro-priming and the kinetics of their release from seeds during hydro-priming. I identified the presence of an important seed germination inhibitor, OPDA, in the hydro-priming flow through liquid. I tested whether this is important in the mechanism of hydro-priming. The presence of other metabolites such as glycoalkaloids or flavonols was also found, and I suggested that plant seeds secrete metabolites to protect against pathogens to permit a better seedling establishment.

In **chapter 6**, I examined transcript levels of genes under two conditions: un-primed seeds and primed seeds during 24h to compare the reactions of seeds to hydro-priming. The transcriptomic analysis of control seeds (i.e. un-primed seeds) and treatment (hydro-primed seeds) were compared to assess if hydro-priming had an effect on the molecular pathways. The amounts of RNA expressed from control and primed seeds were compared. Genes involved in OPDA pathway were more expressed after hydro-priming, as were the genes involved in the weakening of the endosperm cap leading to radicle protrusion. One possibility is that hydro-priming reduces time of germination and improves seedling efficiency by activating the metabolic activities such as respiration, recruitment of ribosomes in polysome complexes or DNA repair.

7.2 The suitability of hydro-priming as a treatment to improve germination

The two important mechanisms in developmental processes are dormancy and germination which are regulated through environmental signals and by maternal effects. The dormancy in tomato seeds is physiological and non-deep (Bewley and Black, 1982; de Castro and Hilhorst, 2000; Hilhorst and Downie, 1995). To understand how tomato germination can be improved is an imperative factor for seed industries. It is imperative for seed industries to understand how tomato germination can be improved for economical reasons.

It is not clear how hydro-priming improves germination (Cheng and Bradford, 1999; Zulueta-Rodriguez *et al.*, 2015). In this thesis I have addressed this question by measuring the performance of seed germination and seedling establishment on different cultivar of tomatoes (Chapters 3), by determining ions leaked in hydro-priming flow through (Chapter 4), by identifying the metabolites that are present in this leachate (Chapter 5), and by characterizing the genes expressed during hydro-priming (Chapter 6).

Maternal environment is known into be an important factor which drives seed behaviour (McGregor *et al.*, 2015). On Arabidopsis, seed set matured at low temperature are more dormant than seed matured at 20°C (Penfield and Springthorpe, 2012). Dormant seeds need signals such as cold stratification to break dormancy. As tomato seeds have not the same dormancy as *Arabidopsis* seeds, cold stratification is not required to improve the speed of germination. In

this case, hydro-priming is used to improve the speed of germination of tomato seeds. In this thesis (Chapter 3), hydro-priming experiment was tested on laboratories variety of tomato, Microtom. Results showed that the speed of germination of low maternal set temperature were improved after hydro-priming treatment but hydro-priming did not change the proportion of normal seedlings. To verify the hypothesis on low set temperature, hydro-priming treatment was generalised on large sample groups of tomato cultivars (beef, cherry, plum tomatoes) grown under different temperatures and locations. Results on hydro-priming was similar as results observed on laboratory variety. However, the mechanism underlying the enhancement of speed of germination through hydro-priming treatment could be investigated further. In reality, seed set matured at low temperature are more sensitive to hydro-priming. It is likely this is because, even if hydro-priming is efficient for low set temperature, hydro-priming may act on germination process by promoting pre-germinative metabolic events. However my results in chapter 3 showed that, if poor seed vigour has been caused by suboptimal conditions during seed production, such as low temperatures, then hydro-priming is effective at increasing vigour. However, if seeds are produced in optimal environments, then hydro-priming does not further improve vigour, and may even have negative effects. These results can be used by seed companies to decide when it is worth trying hydro-priming.

The characterisation of the hydro-priming mechanism via molecular analysis was carried out by LC-IT-MS (Chapter 5). Metabolomic analysis measures thousands of metabolites, but no single metabolomic method is capable of measuring all the metabolites within a metabolome (Hall, 2006). The sample of metabolites measured is biased towards certain types of chemical compounds by the extraction and LC-MS methods used (Sanchez *et al.* 2008). I cannot assess what proportion of the metabolome the metabolic analysis represents because the total number of metabolites within a plant species is unknown, although estimates are in the region of several thousand metabolites per species (Davies *et al.* 2010). Metabolomic approaches have been already used to understand the regulation network in tomato plants (Toubiana *et al.*, 2012). Metabolites had been observed before in plants but the liquid after hydro-priming had never been observed.

For instance, I wished to examine the metabolites leaked by seeds during hydro-priming (Chapter 5), and the coverage of the metabolome was sufficiently large to detect metabolites in hydro-priming flow through liquid (744 peaks). Such metabolites would probably not be revealed in a targeted analysis that measures a much smaller number of metabolites (it usually focuses on fewer than 50 metabolites), therefore I used untargeted method to detect them. This method is able to include a wide range of metabolites because the metabolic analysis encompassed > 3000 metabolites per plant species. Moreover, METLIN database has a huge metabolite ranging analysed and provided MS/MS data. This molecule library permitted the investigation of molecules found in hydro-priming flow. A summary of the results from this work which indicates peaks and their corresponding RT is shown in Appendices: Table 4. From these results, the role of OPDA in hydro-priming was investigated. OPDA is known to inhibit seed germination (Dave *et al.,* 2011, 2012). Previous works on Arabidopsis have shown that *pxa1-1/cts* mutant seeds are more dormant due to the accumulation of OPDA into chloroplast (Dave *et al.,* 2016). ABA was quantified in the *cts-2* mutant and the levels of ABA were higher in mutant seeds than WT. OPDA represses germination by increasing *ABI5* protein. In this thesis, the role of OPDA was investigated on an overexpression line 35S::SlAOC RNAi, and two mutant loss of function: *spr2* and *acx1a*. Increases in the speed of germination of this mutant were in correlation with the work on Arabidopsis seed and the negative effect of OPDA in seed germination. Thus, I expected that adding exogenous OPDA in hydro-priming flow of these mutants could overcome OPDA effect, whereas in reality exogenous OPDA partially affects the speed of germination. The leaching of OPDA only partly explained differences in germination in this study, which suggests that there were other mechanisms determining the success of germination by hydro-priming. In the future it will be interesting to measure ABA levels in seeds after hydro-priming treatment to compare them with those on Arabidopsis seeds. Hydro-priming may down-regulate ABA through the leakage of OPDA. It may be important to know OPDA levels to induce *ABI5*. Furthermore, the active OPDA pool was identified into the peroxisome, and during the imbibition phase, OPDA is transported from peroxisome to out of the seeds, suggesting that OPDA is involved in some germination/dormancy mechanisms and OPDA may be involved in feedback loop.

The integration of RNA-seq data with other types of genome-wide data allows us to connect the regulation of gene expression with specific aspects of molecular or physiology. For example, in chapter 5 I identified OPDA as potential candidate in hydro-priming mechanism and in chapter 6, I observed higher transcript expression of genes involved in OPDA pathway after hydro-priming. Integration of RNA-seq with metabolomics data has been used to identify pathways that are regulated at both the gene expression and the metabolite levels (Low *et al*., 2013).

7.3 Future works

7.3.1 Determining the function of abundant metabolite detected in workflow

Previously, metabolites that were known to leach from the seeds such as sugars, polypeptides have been evaluated using GC-MS (Shu *et al.*, 2008; Rosental *et al.*, 2016; Han *et al.*, 2017), whereas in this thesis I showed that approximately 744 metabolites were found in hydro-priming flow through with the positive mode of LC-IT-ToF/MS. There are no previous record of compounds transferring between seeds and leachate, probably because it is rarely used as a study system. I showed that seed leachate had a role in seed germination (Chapter 5). Such chemically intact metabolites in water retained from hydro-priming are interesting because they suggest that either the metabolites are leached to protect the rhizosphere, or they inhibit the germination. The seed metabolome has already been explored in relation to germination and in developing seedling (Allen *et al.*, 2010; Bhandari *et al.*, 2015; Gorzolka *et al.*, 2016; Rosental *et al.*, 2016). In a similar manner, the metabolome could be used to investigate potential biochemical mechanisms underpinning the speed of germination and seedling establishment. For example, changes in the metabolome could hold the key to understanding the mechanisms determining an increased normal seedling rate under hydro-priming, or the ability of seed to germinate faster when hydro-priming is used. This would determine the metabolites that are increased or decreased under these different treatments. Through the use of metabolomic databases that can suggest the flavonoid pathways some metabolites are associated with the mechanisms that help the seed to germinate. Screening for such metabolites through the examination of the metabolic data, as done here, could prove a productive research strategy for the following reasons. Firstly, OPDA was identified using only the molecular

weight of the metabolite and spectrum (Chapter 5) and OPDA is known to be seed inhibitors (Dave *et al.*, 2011). Secondly, a standard of OPDA was used to confirm this identification. In research trials, I observed that exogenous OPDA was not sufficient to overcome the promoting effect of hydro-priming on the speed of germination. Therefore there is a possibility that the metabolites which are identified in this thesis are toxic for pathogens (if validated) and have the potential to play a role in the germination by protecting the rhizosphere.

7.3.2 Determining the function of expressed genes after hydro-priming treatment

Approximately 2391 genes were expressed after 24h of hydro-priming. Like metabolomic data, there are no previous record of gene expression after hydro-priming treatment, probably because hydro-priming is not used as a study system of germination. As hydro-priming promotes the speed of germination of seed matured at low temperature (Chapter 3), the transcriptomic data could highlight the metabolic pathways involved in germination (Chapter 6). Preliminary results obtained in this thesis showed that is possible to link results obtained by physiology analysis (Chapter 3) and metabolic data (Chapter 5) to have a holistic view.

7.3.3 Holistic view of hydro-priming mechanism(s)

The grouping of metabolites found in hydro-priming liquid (Chapter 5) suggested that seeds leached certain metabolites that determined germination performance, whether these metabolites are known to be beneficial chemicals promoting growth or harmful metabolites inhibiting pathogen growth. Establishing if such chemicals exist and finding out the chemical identities of such metabolites would help to answer questions surrounding the suitability of hydro-priming. Such an experiment could first establish if the apparent relationship between metabolic data and seed germination proved robust when the metabolite is included in the hydro-priming liquid to reverse the effect of this metabolite. The metabolic and transcriptomic data could be used to distinguish the metabolites or elements that seeds need to germinate faster and develop normal seedlings. Metabolites can be associated with a known biochemical pathway by determining if a metabolite is increased or decreased in abundance at the time as up or down regulated genes. Through this approach, correlation network of expressed genes and

metabolites could potentially be created, providing new knowledge of how hydro-priming improved seed performance.

Appendices

Table 1 **List of tomato seeds used.** Tomato batches information given in the table below. Seeds provided by Enza Zaden B.V. (Enkhuizen).

Batch	Temperature of seed production (average)	Type of tomatoes
Aba 490.010	28°C	Cherry
Babette 608.564		Large beef
Benthe 536.054	20°C	
Buffy 616.811	24°C	
Emma 616.831	24°C	
Emma 630.727	22°C	
Emily 596.037	30°C	
Emily 673.803	24°C	
Emily 673.829	24°C	
Fame 446.670 (Fame 2)	30°C	
Fame 478.198 (Fame 1)	24°C	
Fame 498.010 (Fame 3)	30°C	
Feline 626.590	24°C	
Feline 776.681	24°C	
Fonda 607.290	24°C	Rootstock
Kiki 543.998	22°C	Small beef
Kiki 547.392	22°C	Small beef
Kiki 776.704		Small beef
Microtom	19°C, 22°C or 25°C	Cherry
Nienke 234.419	28°C	Plum
Nienke 268.135		Plum
Nienke 272.384	28°C	Plum
Nienke 276.611	28°C	Plum
Predator 266.093 (Predator 2)	28°C	Large beef
Predator 282.234 (Predator 1)	22°C	Large beef
Predator 616.831 (Predator 3)	24°C	Large beef
Predator 630.727 (Predator 4)	22°C	Large beef
Predator 776.677 (Predator 5)	22°C	Large beef
Rootstock N403 125 E28.34679	30°C	
Rootstock N403 842 E28.34190	22°C	
Rootstock N403 843 E28.34190	22°C	
Rootstock N408 488 E28.34190	24°C	
Rootstock N408 528 E28.34679	24°C	
Rootstock N611 824 E16R.40408	24°C	
Sofia 445.811	24°C	Plum

Sofia 446.684		Plum
Sofia 446.883		Plum
Sofia 453.382		Plum
Sofia 453.399		Plum
Vayen 470.268		Plum

Table 2 **List of metabolites found in LC-IT-ToF/MS positive ionization**.

Ion m/z	Ion RT	T0 (peak area)	T3 (peak area)	T6 (peak area)	T16 (peak area)	T24 (peak area)
540,8609	0,575	0	0	0	0	21 888
545,1187	0,597	0	0	0	0	23 936
641,4907	16,64	0	0	0	0	23 979
254,1384	0,836	0	0	0	0	24 043
298,0807	0,559	0	0	0	0	24 105
406,1189	3,323	0	0	0	0	25 173
415,3228	6,765	0	0	0	0	25 175
433,3246	5,508	0	0	0	0	25 237
448,9865	0,563	0	0	0	0	25 402
464,1949	2,350	0	0	0	0	25 899
494,8394	0,574	0	0	0	0	26 069
511,4177	5,453	0	0	0	0	26 069
527,6601	5,389	0	0	0	0	26 133
575,3947	5,597	0	0	0	0	26 155
594,3582	5,072	0	0	0	0	26 196
615,4686	16,048	0	0	0	0	26 197
636,2675	6,339	0	0	0	0	26 197
434,1017	0,607	0	0	0	0	26 240
214,0011	6,600	0	0	0	0	26 261
253,1737	5,546	0	0	0	0	26 283
324,163	3,037	0	0	0	0	26 411
383,3274	6,112	0	0	0	0	26 813
384,1406	2,187	0	0	0	0	27 051
471,2285	5,682	0	0	0	0	27 115
485,1765	2,525	0	0	0	0	27 157
562,1324	5,668	0	0	0	0	27 221
564,3546	4,348	0	0	0	0	27 285
581,4265	5,366	0	0	0	0	27 307
1170,85	4,399	0	0	0	0	27 349
1175,121	4,589	0	0	0	0	27 371
261,1084	0,837	0	0	0	0	28 032
1337,802	4,579	0	0	0	0	28 043
578,4042	4,808	0	0	0	0	28 203
855,7029	17,757	0	0	0	0	28 224
314,0873	0,645	0	0	0	0	28 288
288,1715	0,637	0	0	0	0	28 331

363,9702	0,623	0	0	0	0	28 331
111,0283	5,959	0	0	0	0	28 373
634,1544	4,211	0	0	0	0	28 565
329,0535	4,323	0	0	0	0	29 291
184,0693	0,674	0	0	0	0	29 504
941,4687	6,399	0	0	0	0	29 914
432,2943	4,675	0	0	0	0	30 421
444,9949	0,588	0	0	0	0	30 464
104,1054	0,712	0	0	0	0	30 592
478,7444	4,697	0	0	0	0	31 078
1391,796	4,948	0	0	0	0	31 680
742,4439	5,619	0	0	0	0	31 781
1181,097	5,300	0	0	0	0	33 097
416,3238	5,863	0	0	0	0	33 390
232,0328	0,609	0	0	0	0	33 749
521,1289	5,287	0	0	0	0	34 880
353,2427	7,534	0	0	0	0	35 371
606,2559	6,233	0	0	0	0	35 861
323,1603	3,044	0	0	0	0	35 883
1621,068	4,262	0	0	0	0	36 075
298,0541	0,596	0	0	0	0	37 845
274,1315	5,227	0	0	0	0	39 936
1340,998	4,462	0	0	0	0	43 072
269,1468	0,887	0	0	0	0	44 843
281,0734	0,613	0	0	0	0	46 016
222,4091	0,638	0	0	0	0	47 467
588,9065	0,559	0	0	0	0	50 283
333,0225	0,510	0	0	0	0	51 968
433,3454	4,690	0	0	0	0	55 104
289,0784	0,629	0	0	0	0	55 189
916,4943	4,563	0	0	0	0	66 251
261,0392	0,667	0	0	0	0	74 368
415,3204	6,366	0	0	0	0	83 669
354,231	6,989	0	0	0	0	96 469
399,3215	9,619	0	0	0	0	98 688
331,1077	2,733	0	0	0	0	116 843
291,9776	0,526	0	0	0	0	119 199
297,2308	9,331	0	0	0	0	126 539
235,0697	0,480	0	0	0	0	224 364
281,1638	1,858	0	0	0	20 096	151 044
392,2546	3,772	0	0	0	26 027	25 131
278,1965	0,812	0	0	0	26 155	24 064
685,4184	16,636	0	0	0	26 155	29 376
814,4602	5,537	0	0	0	27 029	63 061
525,1484	0,602	0	0	0	28 267	31 232
327,0573	9,052	0	0	0	28 499	77 342

425,1413	3,341	0	0	0	29 291	27 051
367,0851	0,623	0	0	0	29 291	38 848
625,8315	3,742	0	0	0	29 617	220 908
413,3052	5,550	0	0	0	30 464	45 483
273,9767	0,624	0	0	0	30 699	34 116
297,022	0,577	0	0	0	31 539	56 405
369,289	13,876	0	0	0	31 905	58 475
418,3536	5,383	0	0	0	32 192	38 855
615,0342	0,596	0	0	0	32 640	24 204
465,3359	16,555	0	0	0	32 683	33 643
468,987	0,504	0	0	0	33 493	29 778
262,0184	0,611	0	0	0	33 749	56 469
460,1452	5,381	0	0	0	34 494	30 635
337,1091	9,678	0	0	0	38 997	152 969
576,3731	4,690	0	0	0	41 553	44 203
276,0505	0,600	0	0	0	41 941	58 112
307,1674	3,232	0	0	0	42 784	73 131
338,3413	16,061	0	0	0	44 096	102 445
653,4106	5,753	0	0	0	44 253	86 208
130,5252	18,115	0	0	0	45 956	28 203
527,1521	0,602	0	0	0	51 755	65 045
427,2643	6,567	0	0	0	53 382	116 172
307,0485	0,623	0	0	0	57 536	30 613
261,1112	6,973	0	0	0	62 477	126 805
612,4016	5,081	0	0	0	67 392	123 138
435,1081	0,599	0	0	0	71 360	32 768
900,4977	5,214	0	0	0	76 544	62 059
223,9753	0,586	0	0	0	87 040	35 968
498,9793	0,576	0	0	0	94 080	83 585
442,2405	4,505	0	0	0	96 612	251 776
122,7989	0,532	0	0	0	156 653	87 189
214,0694	0,336	0	0	0	294 489	76 992
500,121	4,712	0	0	23 893	59 551	27 200
678,6511	0,583	0	0	23 979	27 243	36 651
560,2315	5,564	0	0	26 069	30 421	26 133
645,9564	0,592	0	0	28 245	34 816	24 917
529,1277	0,592	0	0	28 288	36 992	28 559
211,271	0,828	0	0	29 440	69 376	36 096
451,066	0,593	0	0	30 891	26 112	32 896
220,7139	0,639	0	0	31 147	64 199	30 592
1050,33	4,466	0	0	32 704	39 829	44 053
339,1373	0,630	0	0	33 515	48 213	30 549
436,8797	0,600	0	0	34 816	56 171	30 421
401,0184	0,602	0	0	34 837	34 389	52 202
388,9271	0,563	0	0	36 373	34 944	54 059
303,087	6,507	0	0	37 888	45 547	84 501

94,047	0,514	0	0	37 973	116 288	275 878
417,352	5,388	0	0	39 765	29 291	86 272
415,3203	5,590	0	0	40 768	83 299	83 537
683,0958	0,629	0	0	43 243	27 093	26 240
309,1444	2,370	0	0	44 117	28 373	52 608
224,9908	0,517	0	0	46 784	45 355	62 848
662,7238	4,500	0	0	49 515	52 992	131 328
81,5209	0,511	0	0	51 883	157 240	494 586
274,0794	6,352	0	0	56 555	38 208	49 963
236,9986	0,572	0	0	64 469	32 683	86 414
925,481	9,627	0	0	64 533	102 274	211 566
462,7593	5,385	0	0	69 184	74 787	173 544
240,878	0,537	0	0	76 309	108 971	103 488
1293,596	4,505	0	0	77 675	182 889	160 725
527,7343	5,347	0	0	78 210	166 761	161 503
282,1255	4,281	0	0	80 427	76 544	149 474
247,1026	0,840	0	0	82 805	103 594	36 523
432,3474	4,692	0	0	84 032	208 417	354 268
455,0582	0,597	0	0	108 116	55 168	90 731
446,1128	0,593	0	0	125 141	267 912	37 973
362,0769	0,716	0	0	150 218	56 128	48 405
349,0043	0,499	0	0	173 316	34 859	132 991
644,7211	0,585	0	0	189 883	64 807	48 171
266,1579	2,688	0	0	270 785	174 144	87 467
309,9504	0,521	0	0	338 013	134 099	103 829
233,0781	5,307	0	0	367 200	121 515	330 297
415,3205	6,932	0	22 592	30 976	58 432	106 112
353,2169	7,549	0	23 552	24 021	31 509	42 432
524,1053	0,594	0	24 747	34 837	33 131	33 067
592,3911	4,555	0	25 856	38 699	30 400	35 797
315,1368	2,731	0	25 899	35 456	28 245	29 525
432,3254	5,757	0	26 155	47 381	65 472	106 539
225,9872	0,517	0	26 944	83 419	77 568	139 686
284,8874	0,521	0	26 987	28 331	36 565	56 128
516,7852	0,586	0	27 925	189 223	142 454	39 317
469,4562	4,672	0	29 120	33 280	27 179	38 976
535,7493	4,706	0	30 144	33 067	35 285	57 761
1180,645	8,183	0	30 165	30 336	33 045	26 197
517,5732	0,588	0	30 229	23 936	27 845	28 373
286,0965	0,665	0	30 357	60 631	49 707	35 413
412,0186	0,575	0	31 253	29 312	24 960	58 965
751,4334	6,928	0	31 851	27 285	47 573	65 685
284,1053	0,997	0	33 515	46 656	59 882	45 717
227,0814	4,333	0	33 536	37 163	30 997	26 261
189,1191	0,690	0	35 648	30 635	60 245	53 973
460,1805	0,647	0	35 776	96 619	209 118	33 389

1032,527	4,420	0	37 504	49 088	102 717	141 227
273,0675	0,620	0	38 549	44 288	78 405	96 578
383,0796	0,612	0	38 613	205 399	80 191	103 296
323,9626	0,538	0	41 237	135 622	128 056	147 670
400,2476	4,272	0	41 685	84 485	37 077	98 475
543,121	0,598	0	41 856	66 944	66 517	119 917
331,1048	2,732	0	42 091	54 293	71 275	144 384
377,2124	3,514	0	42 923	95 744	169 872	242 009
291,0985	4,332	0	42 965	46 123	128 313	221 236
345,0716	0,587	0	43 883	231 665	178 215	168 378
307,0832	0,810	0	44 288	21 717	31 616	79 360
204,1652	0,672	0	44 352	68 309	59 627	102 443
200,9184	0,524	0	46 144	155 328	44 907	108 448
303,0509	4,214	0	46 669	52 608	74 133	69 696
111,0512	0,512	0	47 317	52 864	58 560	78 464
182,0922	0,832	0	48 341	44 139	54 293	146 889
384,1192	2,178	0	50 005	120 000	116 786	87 317
592,3841	5,755	0	50 560	41 195	98 475	112 789
636,4068	5,334	0	50 688	262 784	92 473	159 773
646,9447	4,506	0	51 029	114 389	82 688	113 792
905,5312	5,353	0	51 563	30 485	31 467	38 144
241,9777	0,519	0	53 397	159 344	100 042	123 070
741,4405	5,615	0	53 803	48 192	80 960	132 907
577,3917	4,392	0	55 660	62 251	77 355	129 828
342,0855	4,577	0	59 285	82 539	88 939	244 295
369,2191	6,983	0	59 520	211 141	139 567	156 231
353,2267	6,990	0	61 269	71 080	142 768	163 925
272,0713	0,597	0	61 355	73 216	100 160	30 592
652,4044	5,754	0	61 525	115 483	165 653	406 557
442,8731	4,506	0	64 661	132 380	92 163	166 483
167,1033	1,403	0	65 945	36 480	31 531	30 592
1292,594	4,508	0	66 901	107 029	133 227	334 399
533,1525	0,623	0	68 992	48 619	81 772	70 485
416,3525	5,383	0	69 227	180 759	215 009	325 575
904,5163	5,355	0	73 383	133 163	170 211	236 145
465,1062	4,210	0	74 709	138 435	151 274	41 086
273,1247	5,226	0	77 243	147 152	229 496	403 410
341,033	0,678	0	77 648	50 411	32 597	39 659
382,0822	0,609	0	79 983	53 781	94 187	60 885
338,2368	9,334	0	85 525	63 573	59 456	136 469
576,3899	5,223	0	86 987	121 090	196 981	497 780
773,4668	4,083	0	90 263	331 846	339 543	256 831
501,0329	0,530	0	94 933	39 765	98 261	133 419
314,1865	5,362	0	99 371	174 981	36 907	48 904
104,1042	0,572	0	100 146	58 880	68 139	81 600
264,0946	0,589	0	101 056	92 459	85 293	33 365

529,4505	5,383	0	102 607	84 656	65 161	212 611
652,4077	5,193	0	105 707	108 672	150 976	219 797
291,0968	4,331	0	106 016	281 199	156 830	160 350
492,3541	6,254	0	109 602	87 403	157 811	403 862
814,4578	4,954	0	110 933	271 152	122 335	305 273
645,3689	4,482	0	120 192	164 672	129 344	236 363
143,9868	0,535	0	123 456	99 115	56 789	75 072
420,3244	9,512	0	130 155	137 598	173 824	303 761
295,2205	6,991	0	149 845	288 896	161 173	219 618
580,4119	5,367	0	176 734	213 956	152 590	278 250
1273,523	4,475	0	177 144	147 894	151 255	252 712
940,4861	4,772	0	179 221	220 821	307 320	57 451
259,0798	0,673	0	183 562	214 570	65 216	95 914
362,0965	0,622	0	245 205	34 837	76 672	45 419
152,0473	0,511	0	328 780	500 811	204 372	219 403
315,132	2,734	0	1 204 303	304 264	668 257	144 655

Table 3 **List of transiants metabolites found in LC-IT-ToF/MS in positive ionization**.

Ion m/z	Ion RT	T0 (peak area)	T3 (peak area)	T6 (peak area)	T16 (peak area)	T24 (peak area)
183,0615	0,787	0	0	0	22720	0
277,151	0,792	0	0	0	23808	0
900,4959	4,354	0	0	0	28117	0
907,7695	17,78	0	0	0	28352	0
251,0182	0,570	0	0	0	32555	0
255,0798	0,595	0	0	0	32597	0
438,0346	0,564	0	0	0	33771	0
439,0168	0,600	0	0	0	33877	0
432,1552	0,615	0	0	0	34816	0
300,146	5,697	0	0	0	40981	0
1068,556	5,622	0	0	0	41641	0
1180,602	8,649	0	0	0	53568	0
109,9263	11,427	0	0	0	54116	0
265,1243	0,674	0	0	0	58496	0
463,4109	5,410	0	0	0	76145	0
391,2771	10,068	0	0	0	111680	0
274,0791	6,356	0	0	0	168068	0
358,8131	0,576	0	0	0	316245	0
261,0708	0,677	0	0	21675	0	26283
336,0922	0,667	0	0	21760	0	0
593,3958	4,559	0	0	22848	0	31445
355,0701	3,058	0	0	23765	0	0
897,4247	5,103	0	0	26027	0	30507
417,1516	3,925	0	0	26069	0	45760
441,3123	12,066	0	0	27157	0	33792
443,1255	0,613	0	0	27989	0	0

402,9957	0,512	0	0	28117	0	24107
182,9857	19,008	0	0	28309	0	29461
286,1207	0,654	0	0	29141	0	30507
273,9936	0,619	0	0	29227	0	71305
528,1603	0,595	0	0	30357	0	0
478,9429	0,632	0	0	30507	37675	0
896,4702	5,108	0	0	30549	0	0
1173,64	4,242	0	0	30613	0	0
329,0588	4,334	0	0	32555	67456	0
325,037	0,606	0	0	32640	0	0
324,8239	4,704	0	0	32939	0	0
230,1387	0,701	0	0	33109	54458	0
150,0619	0,748	0	0	34411	21717	0
577,4046	4,844	0	0	34837	0	58560
1349,132	5,827	0	0	35371	0	27541
462,5908	4,821	0	0	36843	0	0
278,1453	0,783	0	0	36971	0	40123
579,3868	9,623	0	0	37461	0	26155
472,6796	0,565	0	0	38656	0	28373
465,0958	3,791	0	0	38805	0	0
371,1575	17,466	0	0	39851	71051	0
656,8137	0,557	0	0	41195	40192	0
350,9992	0,546	0	0	41365	0	34581
271,1727	5,449	0	0	43115	0	25109
1176,657	4,341	0	0	43520	0	0
391,2843	20,213	0	0	44011	51840	0
1216,427	4,309	0	0	45696	0	0
417,3362	5,648	0	0	46485	30528	0
205,1052	2,264	0	0	46592	0	0
268,972	0,519	0	0	49600	0	25047
1573,802	4,403	0	0	50069	41963	0
290,9294	0,574	0	0	50709	0	0
310,0923	0,794	0	0	50816	0	29506
293,9697	0,523	0	0	51648	0	0
611,1657	4,212	0	0	51733	0	54016
247,1156	0,680	0	0	51880	0	34773
304,0539	3,816	0	0	53568	0	0
329,1468	3,246	0	0	54677	0	30617
541,1545	3,576	0	0	57515	0	23244
132,4504	10,731	0	0	58432	0	37184
273,0852	4,312	0	0	63718	0	83543
342,9294	0,569	0	0	64012	0	0
335,0286	0,615	0	0	65396	0	45547
625,1403	0,616	0	0	66624	0	31616
313,5023	4,630	0	0	68464	26653	0
426,0615	0,602	0	0	71595	0	0

414,0597	0,608	0	0	73152	0	30400
435,1044	3,226	0	0	74347	0	0
489,3397	6,791	0	0	116971	0	0
185,0262	0,559	0	0	122806	0	42176
546,0608	0,588	0	0	140333	0	0
611,2506	4,058	0	0	149867	0	113920
597,8923	0,597	0	0	149909	0	0
414,3323	5,891	0	0	163395	0	62357
297,2383	6,882	0	0	185564	0	74986
353,0381	0,591	0	0	188279	264947	0
284,0992	0,988	0	0	197213	0	64853
535,1258	0,579	0	21504	0	0	0
292,2602	0,630	0	21547	0	25045	0
550,8983	0,550	0	22421	30507	0	24107
442,2297	4,075	0	22592	25003	0	29291
917,4952	4,562	0	23595	45781	40939	0
448,1642	0,612	0	23616	28203	0	0
539,1183	4,913	0	23637	0	0	0
310,0507	0,492	0	23637	24917	26112	0
516,1519	0,594	0	23701	28288	0	0
559,3877	5,571	0	23723	0	0	0
207,21	0,572	0	23765	0	23616	0
605,2636	0,589	0	23765	43349	0	0
583,8477	0,590	0	24768	0	0	0
486,9744	0,529	0	24789	0	0	25941
130,0862	0,610	0	25685	0	34411	26240
399,0485	0,579	0	25771	188238	0	37973
577,3833	5,223	0	25792	0	36312	106278
404,1367	2,227	0	25813	0	26112	25131
594,1387	0,597	0	25899	0	0	0
461,7658	4,848	0	26155	0	0	25468
277,1131	0,645	0	26944	0	0	34570
269,983	0,657	0	28011	0	29419	26325
663,1541	4,116	0	28075	0	0	26197
295,1434	2,742	0	28075	31467	25045	0
610,3334	0,593	0	28117	0	0	0
404,8726	0,552	0	28160	45419	0	35755
1049,552	5,761	0	30165	32576	45184	0
901,5092	5,212	0	30251	0	56256	58880
463,0346	0,620	0	30677	38741	0	0
371,1314	0,616	0	32341	0	0	24107
443,7355	0,610	0	32597	46443	75605	0
599,3938	16,366	0	34539	0	0	48640
322,8638	0,566	0	34688	31424	33621	0
533,9313	0,603	0	34731	1093922	1392984	0
902,501	4,841	0	34944	42240	28331	0

461,7656	4,842	0	35221	0	0	71107
594,3999	4,692	0	35243	41024	0	0
740,4586	5,358	0	35520	35968	27371	0
609,3378	16,496	0	35840	0	0	0
322,9462	0,563	0	36373	0	42027	0
360,1369	0,612	0	36523	0	0	30379
319,2315	2,722	0	37589	50603	63424	0
640,9822	0,583	0	38379	0	0	0
606,1665	0,605	0	38933	153771	0	0
332,2781	9,313	0	39637	50091	0	88736
492,6031	4,728	0	44096	0	0	34937
658,3296	4,497	0	45013	217850	0	74987
319,3108	0,610	0	47360	20352	0	0
617,0038	3,974	0	47531	0	0	50773
132,1034	0,904	0	48469	0	34773	79893
304,1371	0,692	0	51648	0	0	26368
269,1057	0,914	0	52011	0	0	56405
525,8101	0,600	0	57963	89280	81643	0
371,1523	17,626	0	58005	54549	50816	0
208,1714	6,818	0	59861	135467	135637	0
1087,527	5,771	0	61598	0	0	27307
394,9986	0,618	0	64320	0	72981	0
342,139	1,393	0	64789	75925	70443	0
469,1423	3,500	0	67221	73877	77099	0
245,0655	0,570	0	67477	78165	155964	0
887,4872	5,943	0	70115	105131	96299	0
580,3931	5,616	0	72021	366319	0	140007
311,7696	0,600	0	82645	158059	207125	0
275,0621	0,652	0	85440	103063	175833	0
903,4947	5,738	0	87765	0	0	0
320,91	0,556	0	90936	0	129465	211144
429,0524	0,544	0	92821	425849	494508	0
256,1219	0,689	0	98709	30421	0	24981
275,1399	1,193	0	99157	76745	173553	0
256,1178	0,802	0	114880	49813	0	0
316,1274	2,744	0	125254	35925	51349	0
423,2583	5,006	0	132565	142891	124459	0
905,7594	15,663	0	133012	0	0	0
251,1206	1,995	0	133765	115007	0	36544
513,1614	0,614	0	135400	787216	821009	0
260,0997	0,724	0	163648	140800	165760	0
261,9944	0,603	0	193473	30507	65003	0
260,0947	0,664	0	205803	35989	64908	0
202,1075	0,629	0	250932	0	0	0
872,4886	5,487	0	268966	58069	0	24000
418,3403	5,622	0	304721	40832	0	28501

382,6975	5,34	0	448182	0	0	28331

Table 4 **Genes expressed > 500 reads.**

Gene ID	Annotation	T0	T3	T6	T16	T24
Solyc00g006470	Unknown Protein	32642	31692	29079	40858	32157
Solyc00g006670	Unknown Protein	23032	25695	24592	36196	25990
Solyc00g006680	Plant senescence-associated protein (Fragment)	2189	2415	2160	3744	2663
Solyc00g006690	Unknown Protein	921	855	975	681	610
Solyc00g007270	Translation initiation factor	1648	2126	2045	1975	2085
Solyc00g008580	Unknown Protein	1231	1310	1113	1703	1376
Solyc00g009020	Mitochondrial ATP synthase		602	572	685	862
Solyc00g009760	Cytochrome P450 monooxygenase	31966	31651	28673	40447	32191
Solyc00g009800	Unknown Protein	976	740	562	905	
Solyc00g011150	Unknown Protein	18005	17161	15448	21376	16746
Solyc00g011160	Unknown Protein	8026	8697	8007	12302	8769
Solyc00g011170	Unknown Protein	2050	2342	2151	3012	2314
Solyc00g012430	Unknown Protein	2499	2500	2295	3413	2793
Solyc00g012440	TO54-2 (Fragment)	10621	11530	10335	17095	12253
Solyc00g013180	NADH-ubiquinone oxidoreductase chain 4	1007	1525	1345	1096	1073
Solyc00g019730	Cytochrome c oxidase subunit 3		713	645	628	652
Solyc00g068970	Cytochrome P450 like_TBP	74788	79222	71862	107989	80424
Solyc00g068980	Unknown Protein	20939	22290	21533	26982	20886
Solyc00g102000	Unknown Protein	45051	47930	46697	67952	47663
Solyc01g005400	Calcium binding protein Caleosin	936	1068	1029		
Solyc01g005550	Carboxyl-terminal peptidase		541		751	757
Solyc01g005560	Isocitrate dehydrogenase					582
Solyc01g005620	Mitochondrial 2-oxoglutarate/malate carrier protein				673	855
Solyc01g005810	Mak16 protein	1243	1603	1433	1755	1504
Solyc01g005820	Splicing factor arginine/serine-rich 4					511
Solyc01g006120	Casein kinase-like protein		636	640		
Solyc01g006170	rRNA processing protein ebna1-binding protein-related		632	596	817	660
Solyc01g006280	Formate-tetrahydrofolate ligase					521
Solyc01g006430	Omega-6 fatty acid desaturase	653	752	767	552	629
Solyc01g006800	Methionine aminopeptidase					542
Solyc01g006900	Phosphatidylglycerol/phosphatidylinositol transfer protein	769	1104	846	660	698
Solyc01g006990	F-box family protein		521	579		
Solyc01g007110	NC domain-containing protein	1183	1599	1917	1497	1282
Solyc01g007250	U3 small nucleolar ribonucleoprotein protein IMP4				787	834
Solyc01g007320	ATP synthase subunit beta chloroplastic		585	539	536	696
Solyc01g007330	Ribulose bisphosphate carboxylase large chain		652	520	521	501
Solyc01g007490	ATP-dependent Clp protease proteolytic subunit	860	1181	1055	1130	1186
Solyc01g007560	DNA-directed RNA polymerase subunit alpha	1499	1850	1395	1318	1595

Solyc01g007570	30S ribosomal protein S11 chloroplastic				513	866
Solyc01g007580	30S ribosomal protein S8					585
Solyc01g007600	50S ribosomal protein L16 chloroplastic		531		510	545
Solyc01g007610	30S ribosomal protein S3 chloroplastic	1523	1833	1427	2077	2243
Solyc01g007630	50S ribosomal protein L2 chloroplastic	2998	3843	3356	2780	3293
Solyc01g007640	Ycf2	1045	1581	1147	1509	1802
Solyc01g007670	30S ribosomal protein S7 chloroplastic	1525	1992	1554	1733	1762
Solyc01g007690	Unknown Protein	1611	1527	1391	1416	891
Solyc01g007710	Unknown Protein	1462	1221	1186	1210	517
Solyc01g007720	ORF42f				503	550
Solyc01g007730	ORF91	1541	1887	1729	1986	2185
Solyc01g007940	Alanine aminotransferase 2	554	719	594	917	1142
Solyc01g008000	ADP-ribosylation factor	439	568	563		
Solyc01g008060	Phosphoglycerate mutase	510	712	611	731	738
Solyc01g008080	Ribosomal protein S27	955	1232	1211	1048	1117
Solyc01g008090	Ribosomal protein S27		521	520	562	669
Solyc01g008110	Cytochrome P450					610
Solyc01g008360	Pre-mRNA branch site p14-like protein		701	653	606	618
Solyc01g008370	26S proteasome regulatory subunit	1566	2091	1913	1719	2012
Solyc01g008550	Cinnamoyl CoA reductase-like protein		660	572	562	660
Solyc01g008780	Phospholipase A22				559	
Solyc01g008820	Signal peptide peptidase family protein				608	693
Solyc01g008840	COP9 signalosome complex subunit 2				535	560
Solyc01g008850	CBL-interacting protein kinase 18	614	780	822		
Solyc01g008910	Scarecrow transcription factor family protein		587	568		
Solyc01g008950	Calmodulin 5/6/7/8-like protein	1448	1968	1688	1694	1738
Solyc01g008960	Argonaute 4-like protein	820	1306	1025	792	1204
Solyc01g008970	Heterogeneous nuclear ribonucleoprotein A3-like protein 2	651	810	827	583	816
Solyc01g009100	Ribosomal protein L30	805	1050	1034	1471	1616
Solyc01g009170	Ethylene insensitive 3 class transcription factor	969	1180	1110	1032	1116
Solyc01g009310	Sterol reductase				507	649
Solyc01g009470	Poly(ADP-ribose) polymerase, catalytic region	505	692	648		
Solyc01g009520	Ribosomal protein	1515	2182	2081	2218	2587
Solyc01g009660	Low-temperature-induced 65 kDa protein	1705	2138	1692	983	919
Solyc01g009850	Unknown Protein				564	521
Solyc01g009990	Peptidyl-prolyl cis-trans isomerase					601
Solyc01g010270	Nitrilase associated protein-like	504	586	564	450	402
Solyc01g010440	Unknown Protein		505			
Solyc01g010540	Ribosomal protein		536	519	754	915
Solyc01g010580	Ribosomal protein	1134	1312	1310	1041	1203
Solyc01g010700	AKIN gamma	533	642	614		
Solyc01g010760	Porin/voltage-dependent anion-selective channel protein				537	726

Solyc01g011000	Eukaryotic translation initiation factor 5A	672	787	895	1019	1280
Solyc01g011040	LRR receptor-like serine/threonine-protein kinase		522			
Solyc01g014180	Zinc finger A20 and AN1 domain-containing stress-associated protein	1479	1923	1951	1177	1307
Solyc01g015020	PRLI-interacting factor A (Fragment)	631	737	709		520
Solyc01g028810	chaperonin	1251	1694	1514	1462	2050
Solyc01g028860	YTH domain family 2 (Predicted)	1099	1724	1619	1458	1929
Solyc01g044360	Importin beta-3				580	806
Solyc01g044480	Elongation factor P	963	1205	1222	1360	1525
Solyc01g049890	Unknown Protein	1075	1372	1257	1459	1574
Solyc01g057000	Universal stress protein family protein	1820	2569	1871		
Solyc01g058410	Unknown Protein	1216	1330	1345	1231	1250
Solyc01g058500	Unknown Protein	44667	49791	45352	71732	50378
Solyc01g059930	Universal stress protein	587	782	859		
Solyc01g059980	Beta-glucanase	867	1124	861	868	581
Solyc01g060030	Zinc finger CCHC domain-containing protein 10	787	927	886	582	576
Solyc01g060070	Pore protein homolog	2719	3593	3159	1891	1824
Solyc01g060130	ADP-ribosylation factor-like protein 3				800	513
Solyc01g060150	Unknown Protein		553		597	542
Solyc01g060470	Importin alpha-1b subunit					584
Solyc01g065580	Pumilio domain-containing protein KIAA0020				743	881
Solyc01g065980	Ethylene responsive transcription factor 2b					563
Solyc01g066720	Hypoxia induced protein conserved region containing protein	1273	1577	1459	985	811
Solyc01g066840	40S ribosomal protein S21	1794	2041	2116	2569	2429
Solyc01g066910	PVR3-like protein					864
Solyc01g067070	Mitochondrial deoxynucleotide carrier	500	622	596		
Solyc01g067360	CHY zinc finger family protein expressed				527	520
Solyc01g067390	Chromodomain-helicase-DNA-binding protein 2					521
Solyc01g067730	Acyl carrier protein	527	553	599	646	681
Solyc01g067740	Superoxide dismutase	775	932	985	895	1189
Solyc01g068240	Ubiquitin carboxyl-terminal hydrolase					500
Solyc01g068530	30S ribosomal protein S9	1528	2126	2066	2334	2678
Solyc01g073650	Serine/threonine-protein phosphatase	868	1244	1081	1128	1191
Solyc01g073660	Uncharacterized MFS-type transporter C19orf28				507	526
Solyc01g073740	Citrate synthase	600	839	684	654	749
Solyc01g079230	Agenet domain-containing protein					528
Solyc01g079250	mRNA splicing factor ATP-dependent RNA helicase	987	1445	1301	942	1213
Solyc01g079420	Cytochrome c oxidase subunit VC family protein				500	
Solyc01g079710	Uncharacterized conserved protein		532	512	517	593
Solyc01g079870	Nuclear transcription factor Y subunit C-2		555	598		573
Solyc01g079880	Asparagine synthetase					569
Solyc01g080500	Unknown Protein	864	1103	1021		
Solyc01g080540	Histidine-containing phosphotransfer protein				584	

Solyc01g080640	Multidrug resistance protein ABC transporter family	1024	1326	1034	1139	1250
Solyc01g080880	Receptor-like kinase					507
Solyc01g081010	Nucleolar GTP-binding protein	1180	1441	1457	1296	1927
Solyc01g081450	Unknown Protein			506		
Solyc01g081500	Histone-lysine N-methyltransferase SUV39H2					556
Solyc01g081590	Non-specific lipid-transfer protein					534
Solyc01g086750	Protein TIF31 homolog				651	842
Solyc01g086870	BHLH transcription factor		566	533		
Solyc01g086970	Zinc finger A20 and AN1 domain-containing stress-associated protein	1665	2132	1845	982	913
Solyc01g087180	Unknown Protein	1709	2135	1648	1851	2003
Solyc01g087210	Cellulose synthase					532
Solyc01g087620	Unknown Protein	1356	1627	1734	958	834
Solyc01g087730	50S ribosomal protein L1	660	794	693		503
Solyc01g087900	N-alpha-acetyltransferase 20, NatB catalytic subunit	546	714	627	839	920
Solyc01g088010	Lactoylglutathione lyase family protein	784	997	831		
Solyc01g088080	T-complex protein theta subunit		614	619	616	915
Solyc01g088100	Zinc finger CCCH domain-containing protein 22		671	530	1009	856
Solyc01g088370	Eukaryotic translation initiation factor 3 subunit B	519	676	687	928	1259
Solyc01g088700	Eukaryotic translation initiation factor 4 gamma 1				526	658
Solyc01g089970	Nucleoside diphosphate kinase	1378	1643	1626	1585	1976
Solyc01g090190	Nuclear RNA binding protein					535
Solyc01g090350	Non-specific lipid-transfer protein	40181	46012	44241	32129	29714
Solyc01g090360	Non-specific lipid-transfer protein	10173	11343	10734	8575	8241
Solyc01g090750	T-complex protein 1 subunit alpha					629
Solyc01g091060	Methionine aminopeptidase					547
Solyc01g091160	Agmatinase	605	814	796	1318	1403
Solyc01g091350	ATP-dependent DNA helicase 2 subunit KU80				562	610
Solyc01g091730	Peroxisomal membrane protein 11-5	1193	1556	1609	2343	2635
Solyc01g094120	Cytochrome P450		516			
Solyc01g094480	Pre-mRNA-splicing factor prp46		502			
Solyc01g094560	60S ribosomal protein L36	527	694	659	967	921
Solyc01g094800	Chromodomain-helicase-DNA-binding protein 1				531	598
Solyc01g094950	Protein BPS1, chloroplastic	4626	5868	4657	4134	3284
Solyc01g095050	Negatively light-regulated protein		580	606		
Solyc01g095140	Late embryogenesis abundant protein (Fragment)	710	827	682		
Solyc01g095150	Late embryogenesis abundant protein (Fragment)	1006	1021	1025		
Solyc01g095200	Reticulon family protein	525	700	693	562	748
Solyc01g095320	BCL-2-associated athanogene 6	1722	2060	1749	1011	1090
Solyc01g095410	Eukaryotic translation initiation factor 1A	1352	1855	1843	2286	1933
Solyc01g095460	G-box binding factor 3	700	881	730		
Solyc01g095790	Splicing factor 3a subunit 3	1502	1932	1636	771	892
Solyc01g096040	Aspartic proteinase nepenthesin I		533	541	555	693

Solyc01g096270	Unknown Protein	786	1018	971	1090	988
Solyc01g096290	Ubiquitin	1912	2485	2392	2098	2310
Solyc01g096340	Auxin-induced SAUR-like protein		514			
Solyc01g096580	30S ribosomal protein S10	1688	2115	1970	1919	2060
Solyc01g096590	30S ribosomal protein S10	894	1131	1070	1237	1360
Solyc01g096700	Chaperone protein dnaJ 1			550		614
Solyc01g096940	Receptor like kinase, RLK				905	1093
Solyc01g097760	Ribosomal protein L7a	1511	1859	1914	2061	2857
Solyc01g097870	40S ribosomal protein S24	2055	3043	2556	2875	3136
Solyc01g097880	Deaminase	527	846	715	538	
Solyc01g098000	Elongation factor-like protein	751	979	884	1311	1554
Solyc01g098030	RNA-binding protein		586	612		
Solyc01g098100	60S ribosome subunit biogenesis protein NIP7	503	623	562	825	769
Solyc01g098760	Heavy metal-associated domain containing protein expressed		621	684		
Solyc01g098770	Xylanase inhibitor (Fragment)					686
Solyc01g098850	Short-chain dehydrogenase/reductase family protein	4657	5680	4657	1677	1959
Solyc01g098860	Uncharacterized GPI-anchored protein		556	519		528
Solyc01g098880	Seryl-tRNA synthetase					601
Solyc01g098910	Mitochondrial carrier protein		595			
Solyc01g098920	Mitochondrial Rho GTPase 1		647	591		741
Solyc01g099100	Long-chain-fatty-acid coa ligase	549	669	715	841	1135
Solyc01g099370	SRC2 homolog (Fragment)	2714	3097	2898		
Solyc01g099670	40S ribosomal protein S10-like	1087	1278	1351	1789	2137
Solyc01g099680	REF-like stress related protein 1	831	977	1299	995	692
Solyc01g099760	26S protease regulatory subunit 6A homolog		569	510	556	695
Solyc01g099770	Translationally-controlled tumor protein homolog	30662	40109	38532	25618	21322
Solyc01g099780	Translationally-controlled tumor protein homolog	1180	1497	1645	1321	749
Solyc01g099800	Unknown Protein	560	817	717		
Solyc01g099810	Arginine/serine-rich splicing factor	526	615	565		
Solyc01g099830	60S ribosomal protein L22-2	3406	4602	4519	4879	4803
Solyc01g099900	Ribosomal protein L18	609	808	890	956	1255
Solyc01g099910	Epoxide hydrolase					642
Solyc01g099920	Photoassimilate-responsive protein PAR-1b-like protein		539			
Solyc01g100140	Protein DEHYDRATION-INDUCED 19 homolog 3	587	745	677	547	571
Solyc01g100320	Thioredoxin/protein disulfide isomerase	543	613	605	1115	1628
Solyc01g100350	ADP-ribosylation factor-like protein 3	1018	1208	1112	904	1006
Solyc01g100370	Universal stress protein		569		538	603
Solyc01g100380	Calreticulin 2 calcium-binding protein				954	1582
Solyc01g100710	Importin alpha-1b subunit					513
Solyc01g100750	Susceptibility homeodomain transcription factor (Fragment)				544	898
Solyc01g100820	Galactinol synthase	931	1046	850		
Solyc01g100960	Pyrrolidone-carboxylate peptidase	501	733	615	532	555

Solyc01g100990	Kinase family protein	691	982	1003	940	1207
Solyc01g101000	Protein of unknown function DUF408		541	538		
Solyc01g101050	S-adenosylmethionine synthase	3828	5611	5468	2573	2896
Solyc01g102320	Acetyl xylan esterase A				765	880
Solyc01g102650	Maleylacetoacetate isomerase / glutathione S-transferase	576	705	646	820	902
Solyc01g102750	PHD finger family protein				813	749
Solyc01g103000	Cullin-associated NEDD8-dissociated protein 2					528
Solyc01g103040	Auxin response factor 1		586	542	546	532
Solyc01g103210	Cytochrome c	762	997	1088	922	863
Solyc01g103400	Nucleic acid binding protein	653	854	814	630	732
Solyc01g103440	Chaperone DnaK		575		721	935
Solyc01g103530	YTH domain family 2	2395	3184	3065	2037	2544
Solyc01g103710	Ribosome biogenesis protein ERB1					625
Solyc01g103790	40S ribosomal protein S12	1020	1174	1248	952	1064
Solyc01g103930	Serine/threonine protein kinase	592	660	746	601	516
Solyc01g103960	RING finger protein 170	545	653	767	512	543
Solyc01g104120	Oxoglutarate and iron-dependent oxygenase				506	542
Solyc01g104160	Ankyrin repeat domain-containing protein 2	883	947	985	830	986
Solyc01g104360	60S acidic ribosomal protein P1	716	920	949	1132	1331
Solyc01g104460	mRNA turnover protein 4 homolog	5810	7495	6981	4791	4939
Solyc01g104550	Pinin/SDK/memA protein					526
Solyc01g104580	Ribosomal protein L3	2500	2830	2930	3107	3990
Solyc01g104670	GTP-binding nuclear protein Ran-A1	1314	1776	1590	1585	1960
Solyc01g104690	GTP-binding nuclear protein Ran-A1	1891	2356	2082	1606	2066
Solyc01g104910	26S protease regulatory subunit 8 homolog				671	691
Solyc01g104940	Alpha-L-arabinofuranosidase/beta-D-xylosidase				537	521
Solyc01g105230	Ubiquitin carboxyl-terminal hydrolase			541		
Solyc01g105330	Chaperone protein dnaJ		575	592		
Solyc01g105400	Unknown Protein				710	608
Solyc01g106200	Chaperone DnaK	1048	1293	1095	1412	1828
Solyc01g106220	Transcriptional factor B3					541
Solyc01g106270	DNA-binding bromodomain-containing protein		511			
Solyc01g106670	Unknown Protein	552	716	574	992	937
Solyc01g107120	Vacuolar sorting receptor					513
Solyc01g107160	Zinc finger protein		513	611		
Solyc01g107320	SWIB/MDM2 domain protein					510
Solyc01g107720	Cyclin					530
Solyc01g107810	UDP-glucuronosyl/UDP-glucosyltransferase	2959	3673	3790	1815	1017
Solyc01g107860	Poly(A) RNA binding protein	1623	2270	2172	2402	3239
Solyc01g108270	Serine/threonine protein kinase				675	750
Solyc01g108490	Nucleotide-binding, alpha-beta plait	6969	9063	8701	6296	6366
Solyc01g108590	Presequence protease, mitochondrial					596

Solyc01g108900	COSII_At2g15890 (Fragment)	628	754	884		527
Solyc01g109290	4-hydroxy-3-methylbut-2-enyl diphosphate reductase		511			
Solyc01g109340	WD-40 repeat-containing protein		584			566
Solyc01g109350	Tyrosyl-tRNA synthetase		710	658	1027	1194
Solyc01g109400	Dolichyl-diphosphooligosaccharide-protein glycosyltransferase		570	524	679	885
Solyc01g109530	Coatomer subunit gamma					599
Solyc01g109600	2-hydroxyacid dehydrongenase (Fragment)	543	652	531		529
Solyc01g109610	4Fe-4S ferredoxin, iron-sulphur binding, subgroup	822	1132	1074	921	866
Solyc01g109650	Glycine-rich RNA-binding protein	665	834	846	957	1082
Solyc01g109780	Glucose-1-phosphate adenylyltransferase				667	594
Solyc01g109840	Diaminopimelate decarboxylase					574
Solyc01g109870	BZIP transcription factor	1367	1714	1062	3231	2259
Solyc01g109910	Dehydrin	4424	4954	4726	3550	3880
Solyc01g109930	26S protease regulatory subunit				587	780
Solyc01g110380	NADH dehydrogenase	594	738	791	734	812
Solyc01g110440	Arginine decarboxylase				618	647
Solyc01g110510	Amidophosphoribosyl transferase				539	636
Solyc01g110530	Unknown Protein					568
Solyc01g110690	Unknown Protein	1128	1320	1427	1548	1913
Solyc01g111150	Peptidyl-prolyl cis-trans isomerase			637	1089	1219
Solyc01g111160	Unknown Protein	2629	3141	2593	2356	2928
Solyc01g111290	Cold-shock protein, DNA-binding			544	618	649
Solyc01g111410	Ribosomal protein L1	609	717	734	914	812
Solyc01g111430	Senescence-associated protein 12	1356	1509	1925	604	608
Solyc01g111510	C2 membrane targeting protein		558	546		592
Solyc01g111550	Ras-like GTP-binding protein RHO	708	867	798	970	1024
Solyc01g111590	Heavy metal transport/detoxification protein	1382	1632	1410	1478	1693
Solyc01g111630	E3 ubiquitin ligase, SCF complex, Skp subunit	581	680	787	510	
Solyc01g111640	E3 ubiquitin ligase, SCF complex, Skp subunit	2763	3447	3431	2469	2185
Solyc01g111700	26S proteasome non-ATPase regulatory subunit 3		554	542	672	853
Solyc01g111750	V-type ATP synthase beta chain					532
Solyc01g112220	EC metallothionein-like protein, family 15	1030	1373	1001	1139	1432
Solyc01g113620	Pre-mRNA processing ribonucleoprotein, binding region	5736	6627	6066	7941	8842
Solyc02g014310	Polyadenylate-binding protein	843	1000	992	1591	2038
Solyc02g014460	WD repeat-containing protein				958	965
Solyc02g014670	Genomic DNA chromosome 3 P1 clone MPN9	1551	1847	1810	796	691
Solyc02g021220	Peptidase S8, subtilisin-related	699	810	627	1176	1803
Solyc02g022870	EMB1611/MEE22		518	501	834	699
Solyc02g023970	GTP-binding protein	1116	1439	1456	1566	1746
Solyc02g062240	Small nuclear ribonucleoprotein Sm D3				543	
Solyc02g062460	Oxoglutarate and iron-dependent oxygenase	1266	1784	2579	1127	573
Solyc02g062620	Nuclear cap-binding protein subunit 2		705	589	676	775

Solyc02g062770	Late embryogenesis abundant protein	1140	1526	1300	807	717
Solyc02g062920	Splicing factor U2AF large subunit				519	595
Solyc02g062970	Xaa-Pro aminopeptidase 2	512	714	663		559
Solyc02g063070	14-3-3 protein beta/alpha-1	891	1113	1130	1253	1565
Solyc02g063280	Unknown Protein	733	890	922	1402	1112
Solyc02g063490	Malate dehydrogenase	661	851	817	956	930
Solyc02g064670	Ribosomal protein L26-like 1		637	591	773	1023
Solyc02g064730	Expressed protein having alternate splicing products	1329	1746	1592	1245	1081
Solyc02g065300	Leucyl-tRNA synthetase				670	877
Solyc02g065370	Unknown Protein	687	878	777	948	811
Solyc02g065770	COBRA-like protein 4	653	972	936	762	708
Solyc02g067440	Cupin RmlC-type					555
Solyc02g067460	Porin/voltage-dependent anion-selective channel protein	713	918	857	1387	1686
Solyc02g067580	B12D-like protein		587	543		
Solyc02g067870	Chalcone isomerase	724	803	832		
Solyc02g068130	Mitochondrial import receptor subunit TOM20		608	604	630	545
Solyc02g068150	Nuclear transport factor 2					503
Solyc02g068420	Tumor suppressor protein Gltscr2		574	517	556	598
Solyc02g068740	Glycine cleavage system H protein 1		510	603		
Solyc02g068770	50S ribosomal protein L30	576	828	803	883	866
Solyc02g069090	Cathepsin B	722	898	938	1670	1966
Solyc02g069150	Vesicle-associated membrane protein 7B	1348	1721	1693	1460	1388
Solyc02g069490	FAD linked oxidase domain protein				1018	1457
Solyc02g069590	Coatomer protein epsilon subunit family protein				500	595
Solyc02g069610	Protein transport protein GOT1				517	
Solyc02g069850	40S ribosomal protein S25-1	600	784	830	776	1035
Solyc02g069950	Ribosomal RNA assembly protein mis3		621		696	630
Solyc02g070310	Ribosomal protein L32	2542	3202	3002	3678	4216
Solyc02g070320	40S ribosomal protein S4-like protein	841	1171	1147	1056	1251
Solyc02g070330	40S ribosomal protein S4-like protein	751	943	956	1179	1526
Solyc02g070340	40S ribosomal protein S4-like protein		670	674	945	1146
Solyc02g070350	40S ribosomal protein S4-like protein	557	765	735	836	940
Solyc02g070360	40S ribosomal protein S4-like protein		589	595	835	1170
Solyc02g070500	Susceptibility homeodomain transcription factor (Fragment)	650	669	608		
Solyc02g070510	Proteasome subunit alpha type				551	704
Solyc02g070570	30S ribosomal protein S11					527
Solyc02g070640	60S ribosomal protein L18a	831	1139	1074	1386	1679
Solyc02g070650	60S ribosomal protein L18a	1079	1476	1328	1418	1537
Solyc02g071150	DNA-binding protein		501	515		511
Solyc02g071180	TA9 protein (Fragment)			501	639	858
Solyc02g071250	Protein phosphatase	729	910	877	585	
Solyc02g071320	Unknown Protein				1000	1031

Solyc02g071510	Bromodomain-containing protein		520	549		576
Solyc02g071760	DUF1264 domain protein	1119	1362	1118	649	786
Solyc02g072130	Protein transport protein SEC61 alpha subunit	812	1013	1055	1473	2181
Solyc02g073580	BZIP transcription factor	1565	1927	1216		
Solyc02g077410	Casein kinase substrate, phosphoprotein PP28	887	1211	1135	1217	1174
Solyc02g077440	Transcription elongation factor 1 homolog	1662	2182	1994	925	730
Solyc02g077530	O-methyltransferase	1980	2627	2092	3899	4162
Solyc02g077880	Auxin-repressed protein	1136	1480	1149	1072	1484
Solyc02g077970	Late embryo abundance protein (Fragment)	4936	5693	4796	3558	3623
Solyc02g077980	Unknown Protein	775	1070	735		
Solyc02g078120	Translation initiation factor 3 subunit 7	533	594	586	1006	1112
Solyc02g078150	Plant-specific domain TIGR01615 family protein	507	679	805	931	805
Solyc02g078260	DNA-directed RNA polymerase	697	912	817	645	840
Solyc02g078300	Transcription initiation factor TFIID subunit		574			
Solyc02g078380	Aluminum-induced protein-like protein		551		530	512
Solyc02g078610	Splicing factor 3b subunit 2	681	831	799	693	714
Solyc02g079060	Translation initiation factor eIF3 subunit	949	1285	1153	1280	1172
Solyc02g079290	SELF PRUNING 2G	1107	1329	1086	524	537
Solyc02g079400	Nitric oxide synthase interacting protein	684	890	942	997	1026
Solyc02g079780	Pro-resilin		566		650	595
Solyc02g080130	Heat shock protein DnaJ		564	570		506
Solyc02g080370	Tobamovirus multiplication protein (Fragment)		675	589		527
Solyc02g080530	Peroxidase	552	720	701	797	1128
Solyc02g080630	Lactoylglutathione lyase	1168	1376	1301	853	916
Solyc02g080880	Aspartic proteinase	3028	3696	3275	1882	2373
Solyc02g080970	Unknown Protein		609	611		
Solyc02g081430	Microsomal glutathione S-transferase 3				560	528
Solyc02g081590	FACT complex subunit SPT16		514			557
Solyc02g081680	Nucleolar complex protein 2 homolog				715	760
Solyc02g081700	Proteasome subunit alpha type	513	695	661	995	1043
Solyc02g081810	tRNA pseudouridine synthase B	1352	1673	1511	2496	2655
Solyc02g081880	Molybdenum cofactor sulfurase protein-like	564	729	656	520	603
Solyc02g082000	30S ribosomal protein S19	3031	3951	4083	4547	5178
Solyc02g082020	Unknown Protein				528	573
Solyc02g082130	Unknown Protein		509		652	724
Solyc02g082270	RNA recognition motif, RNP-1		599	596		
Solyc02g082340	RNA polymerase I-specific transcription initiation factor		567	511		
Solyc02g082700	High mobility group protein	2584	3596	3082	2089	2370
Solyc02g083200	Unknown Protein	567	587	785	716	848
Solyc02g083250	Cellular retinaldehyde-binding/triple function C-terminal	888	985	1045	1268	1302
Solyc02g083280	Thiosulfate sulfurtransferase	547	587	636		
Solyc02g083350	DNA-directed RNA polymerase	577	886	840	663	746

Solyc02g083470	Pre-rRNA-processing protein ESF1	646	687	742	708	634
Solyc02g083710	26S proteasome non-ATPase regulatory subunit 4					523
Solyc02g083860	Flavanone 3-hydroxylase	554	584	539	2264	1873
Solyc02g084240	H1 histone-like protein	542	646	512		
Solyc02g084840	Dehydrin DHN1	6019	7517	7369	4960	6159
Solyc02g084850	Unknown Protein	1777	2032	2183	1013	887
Solyc02g084920	Proteasome subunit beta type	1261	1629	1409	1582	1690
Solyc02g085150	Late embryogenesis abundant protein	714	888	721	621	604
Solyc02g085310	Unknown Protein	959	1250	1108		
Solyc02g085350	Succinate dehydrogenase flavoprotein subunit					604
Solyc02g085420	U1 small nuclear ribonucleoprotein	858	1155	940	898	1006
Solyc02g085590	Vicilin (Fragment)	3253	4048	3319	2495	2887
Solyc02g085770	ABA induced plasma membrane protein PM 19		658	518		
Solyc02g085790	T-complex protein 1 subunit zeta					574
Solyc02g085910	Unknown Protein	565	693	566		
Solyc02g086080	Mannose-6-phosphate isomerase 1.1	1436	1915	1585	1482	1424
Solyc02g086230	50S ribosomal protein L5					639
Solyc02g086360	ER Phosphatidate Phosphatase	1094	1274	1097	587	809
Solyc02g086870	Formate dehydrogenase	593	710	706	1068	1313
Solyc02g086960	Aldehyde dehydrogenase 1		561			
Solyc02g087230	NADH-quinone oxidoreductase F subunit family protein					502
Solyc02g087380	Paired amphipathic helix	506	521	653	675	676
Solyc02g087700	Structure-specific recognition protein					539
Solyc02g087920	60S ribosomal protein L34	1189	1489	1257	1508	1759
Solyc02g088810	Peptidase S10, serine carboxypeptidase					572
Solyc02g088900	Ninja-family protein 3	1326	1576	1321	940	963
Solyc02g089060	Proteasome component region PCI				562	764
Solyc02g089090	Unknown Protein	618	803	808	914	869
Solyc02g089150	Cytochrome P450		538		1181	1016
Solyc02g089250	E3 ubiquitin-protein ligase		521		542	635
Solyc02g089590	Ribosomal RNA-processing protein 7 homolog A	595	735	717	832	957
Solyc02g090360	Reticulon-like protein B13	2713	3205	2620	1148	1320
Solyc02g090380	Serine-threonine protein kinase	1481	2035	1999	1063	1015
Solyc02g092060	Growth regulating factor 1		517		625	700
Solyc02g092900	Chromatin modification-related protein EAF3		543		521	
Solyc02g093030	Cathepsin B-like cysteine proteinase	1215	1605	2059	2835	2839
Solyc02g093500	cDNA clone J023001A15 full insert sequence				716	616
Solyc02g093820	Glucose-6-phosphate dehydrogenase		589	577	570	644
Solyc02g093870	DNA-binding bromodomain-containing protein		538	515		
Solyc02g093910	Proteasome component region PCI		506		639	699
Solyc02g094030	Lipase	686	1022	927	805	537
Solyc02g094110	Sulfite oxidase	4049	5496	5150	1604	1526

Solyc02g094120	Kelch related	506	834	727		531
Solyc02g094130	Mitochondrial import receptor subunit TOM20	501	670	574	785	786
Solyc02g094460	Mitochondrial phosphate carrier protein					532
Solyc02g094860	Lateral organ boundaries, LOB				645	696
Solyc03g005230	Menaquinone biosynthesis methyltransferase ubiE		539		540	616
Solyc03g005750	Genomic DNA chromosome 5 P1 clone MPL12					558
Solyc03g005940	Unknown Protein	8720	11988	9481	7379	6644
Solyc03g006360	Auxin-repressed protein	682	875	814		
Solyc03g006490	Aluminum-induced protein-like	801	912	1268		
Solyc03g007170	Peptidyl-prolyl cis-trans isomerase, FKBP-type	759	932	899	1473	1359
Solyc03g007190	SPFH domain / Band 7 family protein	701	903	940	731	715
Solyc03g007230	Protein phosphatase 2C	906	1329	1192	535	550
Solyc03g007480	Myosin	806	1089	941	804	683
Solyc03g007600	Pentatricopeptide repeat-containing protein		628	579		
Solyc03g007740	Reticulon family protein	870	1058	1008	1521	1758
Solyc03g007890	Heat shock protein Hsp90	1743	2412	1667	753	1053
Solyc03g013460	Cytochrome c oxidase subunit 3		712	557		
Solyc03g019780	40S ribosomal protein SA	620	724	800	718	1042
Solyc03g019790	Aldolase-type TIM barrel	824	999	965	775	1026
Solyc03g019820	Aquaporin	928	1002	1077	693	873
Solyc03g025270	rRNA 2'-O-methyltransferase fibrillarin	906	1199	1143	1343	1751
Solyc03g025520	60S ribosomal protein L36	1649	2070	1996	2352	2427
Solyc03g025630	Tripeptidyl-peptidase II					608
Solyc03g025810	Low-temperature-induced 65 kDa protein		570		576	614
Solyc03g025950	Cytochrome b5	696	806	755	603	629
Solyc03g026020	Heat stress transcription factor		534			
Solyc03g031680	RNA polymerase, Rpb8		599	566	634	588
Solyc03g031690	ABC transporter FeS assembly protein SufB			541		
Solyc03g031750	Homeobox-leucine zipper protein ATHB-14					623
Solyc03g031880	Cold induced protein-like	1025	1425	1401	699	712
Solyc03g031910	Oligopeptide transporter OPT superfamily				718	1008
Solyc03g032120	High mobility group, HMG1/HMG2	1440	1931	1591	2328	2212
Solyc03g033550	Zinc finger protein	543	677	521		
Solyc03g034020	Ce-LEA	3468	4483	3689	1112	1096
Solyc03g034430	Translation initiation factor IF2/IF5		507			
Solyc03g043990	Ribosomal protein L26	921	1229	1220	1022	1341
Solyc03g044140	Subtilisin-like protease				3073	3968
Solyc03g044250	Ubiquitin-conjugating enzyme E2 I					504
Solyc03g045100	40S ribosomal protein S5	847	1151	1055	863	950
Solyc03g046340	Ribosomal RNA-processing protein		512			
Solyc03g046370	Seed maturation protein PM41	1012	1316	1230	500	524
Solyc03g046590	ORF104	964	1023	960	1173	1218

Solyc03g051890	30S ribosomal protein S1					537
Solyc03g058320	Unknown Protein	1434	1755	1484	1453	1109
Solyc03g058340	Translation initiation factor				812	820
Solyc03g058910	Porin/voltage-dependent anion-selective channel protein	890	1146	1016	1241	1538
Solyc03g062900	5'-3' exonuclease N-terminal resolvase-like domain		624		643	666
Solyc03g063470	Unknown Protein			591	530	654
Solyc03g071680	Non-symbiotic hemoglobin		549			
Solyc03g078230	UDP-glucosyltransferase	864	1023	833	600	697
Solyc03g078390	Actin	1582	2138	2216	1828	2093
Solyc03g078560	Ras-related protein Rab-6A	1296	1650	1264	1750	1894
Solyc03g079930	Mitochondrial import inner membrane translocase	1262	1544	1534	1244	1290
Solyc03g080080	No apical meristem (NAM) protein		549	660		
Solyc03g080150	Nascent polypeptide-associated complex	645	822	781	965	1200
Solyc03g082370	Arginine/serine-rich splicing factor		655	597		
Solyc03g082560	Ubiquitin	1586	1858	2098	2446	2400
Solyc03g082590	Cytochrome b5	706	945	925	741	899
Solyc03g082910	Heat shock protein 70	851	1036	993	3296	3537
Solyc03g083520	40S ribosomal protein S13	1201	1617	1550	1246	1518
Solyc03g093330	Unknown Protein		551			
Solyc03g093470	U3 small nucleolar RNA-associated protein 10		530		746	1084
Solyc03g094070	RNA recognition motif, RNP-1	597	788	705	680	741
Solyc03g095210	Translocase of chloroplast					552
Solyc03g095250	WD40 repeat-like		534	508		518
Solyc03g095710	Transcription elongation factor, TFIIS			526	604	578
Solyc03g096030	Mitochondrial peroxiredoxin with thioredoxin peroxidase activity	1659	1799	1582	1188	1621
Solyc03g096350	60S ribosomal protein	1534	1904	1896	2476	2920
Solyc03g096660	Protein phosphatase 2C	579	686	536	622	715
Solyc03g096790	Unknown Protein					529
Solyc03g096800	Transmembrane protein		599	565	533	575
Solyc03g096850	Unknown Protein	576	862	797	764	664
Solyc03g096910	Exportin-1				515	759
Solyc03g096930	NADH ubiquinone oxidoreductase subunit		661	622	767	852
Solyc03g096950	Unknown Protein	512	786	750	1086	936
Solyc03g097170	NERD domain containing protein	610	724	739		
Solyc03g097240	Unknown Protein				501	561
Solyc03g097260	Cysteine proteinase inhibitor	3916	5541	5576	5040	4748
Solyc03g097280	Alanyl-tRNA synthetase					664
Solyc03g097480	Dek protein				601	531
Solyc03g097890	40S ribosomal protein	685	891	922	959	1325
Solyc03g098040	Calmodulin 3 protein	561	766	654	736	748
Solyc03g098270	ARGONAUTE 1					576
Solyc03g111000	Glyceraldehyde-3-phosphate dehydrogenase	968	1112	1056	573	658

Solyc03g111080	Bromodomain factor	714	884	942	727	584
Solyc03g111130	Malate synthase	1949	2393	2248	2317	3773
Solyc03g111220	60S ribosomal protein	2595	3491	3380	3233	3856
Solyc03g111630	Initiation factor eIF-4 gamma, MA3	1051	1281	1387	876	1315
Solyc03g112040	Serine/threonine protein kinase	1458	1751	1789	1450	1425
Solyc03g112220	ZZ type zinc finger domain-containing protein (Fragment)	1230	1690	1649	1876	2193
Solyc03g112840	60S ribosomal protein L44	700	817	845	952	1194
Solyc03g113120	Cupin RmlC-type	518	610		590	731
Solyc03g113260	Homeobox-leucine zipper-like protein	503	620	603		
Solyc03g113790	Aldehyde dehydrogenase					515
Solyc03g113900	Gibberellin-regulated protein				679	634
Solyc03g114160	Deleted in split hand/splt foot protein 1		539		736	607
Solyc03g114350	Poly(ADP-ribose) polymerase, catalytic region			553		
Solyc03g114360	DNA/RNA helicase, DEAD/DEAH box type, N-terminal					613
Solyc03g114940	ABC transporter, transmembrane region	727	1085	1183	1228	1058
Solyc03g114960	Nitrilase associated protein-like (Fragment)					516
Solyc03g115100	ATP synthase gamma chain	531	605	713	623	722
Solyc03g115350	40S ribosomal protein S19-like	840	1139	1155	1144	1468
Solyc03g115360	Diacylglycerol kinase 1	581	721	734		
Solyc03g115620	Carbamoyl-phosphate synthase small chain	1241	1604	1507	1871	2371
Solyc03g115640	Translation elongation factor, IF5A	5751	7166	6457	2462	2024
Solyc03g116160	Nucleosome assembly protein family	678	902	925	1136	1264
Solyc03g116370	WD-40 repeat protein					536
Solyc03g116380	Late embryogenesis abundant protein	5408	6433	5835	3562	4156
Solyc03g116670	Methyl-CpG DNA binding	545	794	855	526	597
Solyc03g116740	WD-40 repeat protein-like (Fragment)		531	534		
Solyc03g117020	CCR4-NOT transcription complex subunit 7		563	501		
Solyc03g117030	Unknown Protein		580	504		570
Solyc03g117050	60S ribosomal protein	593	725	709	645	786
Solyc03g117470	Inorganic H+ pyrophosphatase	776	1021	905	1447	1788
Solyc03g117580	Chaperone protein dnaJ	764	890	1058		527
Solyc03g117670	Transcription regulatory protein SNF5	535	688	677		517
Solyc03g117760	Serine incorporator 1	1043	1456	1243	1108	1249
Solyc03g117930	U2 small nuclear ribonucleoprotein A'				524	588
Solyc03g118010	Tudor / nuclease domain-containing protein					623
Solyc03g118030	Calcium-binding protein Calnexin	553	633	572	1555	1933
Solyc03g118040	Unknown Protein	1906	2523	2118	1256	1220
Solyc03g118730	Auxin efflux carrier				510	
Solyc03g118770	Thaumatin-like protein				532	693
Solyc03g119030	Guanine nucleotide-binding protein		538	554	622	851
Solyc03g119050	Unknown Protein					1012
Solyc03g119070	Beta-glucosidase	4932	6411	5371	16012	27338

Solyc03g119120	Histone H1				531	600
Solyc03g119350	Ribosomal protein S7e	565	732	828	1045	1219
Solyc03g119410	Unknown Protein	1424	1679	1719	892	627
Solyc03g120030	Unknown Protein					1138
Solyc03g120250	G-protein beta WD-40 repeat, region				608	
Solyc03g120270	RAN binding protein	956	1167	1078	1012	970
Solyc03g120340	Tetratricopeptide-like helical				544	642
Solyc03g121080	Cold induced protein-like	2010	2339	2878	1013	934
Solyc03g121170	Lipase, GDSL	689	637		510	696
Solyc03g121260	IAA-amino acid hydrolase				712	615
Solyc03g121300	RWD domain-containing protein	665	865	805	746	789
Solyc03g121320	60S ribosomal protein	1209	1656	1730	1664	2077
Solyc03g121340	Unknown Protein	1156	1570	1288	1153	1498
Solyc03g121580	Root hair defective 3 GTP-binding	567	757	676	793	926
Solyc03g121870	Protein phosphatase 2C	735	921	810	766	663
Solyc03g122080	Protein transport protein Sec22				528	520
Solyc03g122170	mRNA 3-UTR binding protein	656	865	900	829	882
Solyc03g122370	(P)ppGpp synthetase I (GTP pyrophosphokinase) SpoT/RelA			506		
Solyc03g123490	Pathogenesis-related transcriptional factor and ERF, DNA-binding				725	889
Solyc03g123520	CCAAT-binding factor	511	660	526	706	809
Solyc03g123590	Alanine aminotransferase			514		
Solyc03g123870	FMN-binding split barrel, related		588	554		
Solyc04g005330	Periodic tryptophan protein 1 homolog	1364	1815	1550	1251	1310
Solyc04g005340	Alpha-1 4-glucan protein synthase	647	891	843	1042	1035
Solyc04g005380	Unknown Protein		631			
Solyc04g005510	Translation elongation factor, IF5A		546	510	619	663
Solyc04g005680	Ribosomal protein	1131	1441	1367	1385	1638
Solyc04g007550	ATP synthase		612	548	597	917
Solyc04g007570	Lipase, GDSL		589		1723	2268
Solyc04g007970	Ubiquitin-conjugating enzyme E2	1586	1799	1794	983	922
Solyc04g008460	Ribosomal protein L15	935	1307	1356	1556	1857
Solyc04g008500	Zinc finger, C2H2-type	683	983	751	850	654
Solyc04g008540	Unknown Protein		578	591		
Solyc04g008680	Cell division cycle and apoptosis regulator protein 1	501	560	519	684	814
Solyc04g008740	Pyruvate kinase	568	748	662	915	1252
Solyc04g008810	40S ribosomal protein S26	825	1028	1028	1309	1499
Solyc04g009410	Proteasome, subunit alpha/beta	1191	1610	1469	1468	1502
Solyc04g009770	DNAJ chaperone	2552	3129	3047	2004	2286
Solyc04g009820	Calcium-responsive transactivator	538	746	642	998	840
Solyc04g009950	Pre-mRNA splicing factor					524
Solyc04g010240	60S ribosomal protein L35			522	663	988
Solyc04g011430	Ubiquitin-conjugating enzyme, E2	6494	9080	7379	3060	2913

Solyc04g011440	Heat shock protein 70		512		596	1153
Solyc04g011510	Triosephosphate isomerase	887	1081	985	923	1069
Solyc04g012120	14-3-3 protein	878	1093	1004	1602	1532
Solyc04g014250	FIP1					507
Solyc04g014390	Ribosomal protein/NADH dehydrogenase domain	637	878	776	552	704
Solyc04g014500	Diphosphoinositol polyphosphate phosphohydrolase		516			
Solyc04g014600	Universal stress protein family protein		621			
Solyc04g014670	Glucose/ribitol dehydrogenase		605	510		530
Solyc04g015190	Glycoside hydrolase		591	595		
Solyc04g015200	6-phosphofructokinase 2	503	604			
Solyc04g015370	Acyl carrier protein	648	1000	802	601	584
Solyc04g015620	Unknown Protein		678	593	1342	1870
Solyc04g015680	Peptidase	977	1221	1152	892	1172
Solyc04g016380	Ribosome biogenesis protein	730	924	896	1469	1590
Solyc04g017670	F-box family protein	1195	1349	1067	1008	806
Solyc04g026100	30S ribosomal protein				544	690
Solyc04g039760	Chloroplast Ycf2	790	905	938	675	554
Solyc04g040180	Methyltransferase type 12		619	696	636	
Solyc04g045480	Ubiquitin thioesterase otubain-like protein	929	1247	1138	1313	1218
Solyc04g047770	VHS subgroup	536	769	726	612	651
Solyc04g049140	Transcription factor jumonji					509
Solyc04g049330	Vacuolar (H+)-ATPase G subunit		603	575		588
Solyc04g049580	Similarity the ORF shows strong similarity to EST 5419	2322	2892	2711	3084	2931
Solyc04g049960	Plasma membrane protein 3	880	1196	986	661	644
Solyc04g051280	Unknown Protein	4183	5694	5128	4670	5592
Solyc04g051350	Ribonucleoside-diphosphate reductase					513
Solyc04g051370	Proteasome component region PCI	590	882	775	935	1031
Solyc04g051510	Receptor like kinase, RLK					543
Solyc04g051670	ATPase putative					713
Solyc04g051730	Cytochrome P450	6521	7339	5934	5520	6322
Solyc04g051850	Unknown Protein		564	549		
Solyc04g053080	OTU domain-containing protein 6B	873	961	848		
Solyc04g054710	Aspartate aminotransferase				642	689
Solyc04g054890	Acyl-CoA oxidase					546
Solyc04g054910	Pathogenesis-related transcriptional factor and ERF, DNA-binding				569	562
Solyc04g055020	Iron sulfur subunit of succinate dehydrogenase	512	620			
Solyc04g055030	Iron sulfur subunit of succinate dehydrogenase		544			
Solyc04g056380	Adipose-regulatory protein, Seipin	1213	1336	1368	904	1063
Solyc04g058040	30S ribosomal protein	1649	2149	2036	1901	1938
Solyc04g058070	Phosphoribosylformylglycinamidine cyclo-ligase	1109	1398	1332	1294	1387
Solyc04g063270	30S ribosomal protein	838	1071	1221	1470	2000
Solyc04g064600	RAG1-activating protein 1 homolog		560	542	579	633

Solyc04g064680	Transcription elongation factor spt5			523		588
Solyc04g064690	Alcohol dehydrogenase	1074	1274	1249	550	630
Solyc04g064740	Serine carboxypeptidase		506		1257	2124
Solyc04g071750	Pathogenesis-related transcriptional factor and ERF, DNA-binding			533		
Solyc04g072040	Ras small GTPase, Rab type				542	542
Solyc04g072090	Microsomal signal peptidase 25 kDa subunit				529	
Solyc04g072230	Heat shock protein Hsp20	2878	3775	2517	1039	722
Solyc04g072240	Midasin				592	700
Solyc04g072420	MRNA complete cds clone RAFL23-27-B01		513		591	564
Solyc04g072430	Unknown Protein				600	698
Solyc04g072450	Defensin-like protein	500	586	511		
Solyc04g072640	Ribosomal protein L30	2200	3099	2749	2783	3117
Solyc04g072650	DNA-binding SAP				537	750
Solyc04g072870	WD-40 repeat family protein		661	528		
Solyc04g072890	Endoplasmic reticulum-Golgi intermediate compartment	968	1162	1072	889	882
Solyc04g074070	Nucleolar complex protein 3 homolog				515	636
Solyc04g074080	Senescence-associated protein	975	1279	1187	989	942
Solyc04g074210	14-3-3 protein		514	508	617	642
Solyc04g074280	30S ribosomal protein	638	757	812	950	1468
Solyc04g074560	Histone H3	864	1081	1058	618	525
Solyc04g074680	Leucine zipper, homeobox-associated				623	547
Solyc04g074890	40S ribosomal protein S21	1062	1460	1337	1258	1091
Solyc04g074970	ZF-HD homeobox protein Cys/His-rich dimerisation region				587	
Solyc04g076040	14-3-3 protein	626	745	718	709	985
Solyc04g076460	Serine/threonine protein kinase					564
Solyc04g076600	Ubiquitin-protein ligase 1		548		529	638
Solyc04g076830	AUX/IAA protein				606	839
Solyc04g076860	Phosphoenolpyruvate carboxykinase	1347	1530	1485	1276	1795
Solyc04g076870	Ubiquitin-fold modifier-conjugating enzyme 1					504
Solyc04g077000	Tubulin alpha-3 chain	999	1187	1154	1390	1750
Solyc04g077760	Zinc finger, LIM-type		640	536		
Solyc04g077950	Adenine phosphoribosyl transferase					535
Solyc04g078050	Expressed protein having alternate splicing products				658	676
Solyc04g078280	Transcription factor, MADS-box	607	733	766	840	850
Solyc04g078430	Unknown Protein	687	1031	807		
Solyc04g078620	Pathogenesis-related transcriptional factor and ERF, DNA-binding	642	865	807		
Solyc04g078900	Peroxisomal multifunctional enzyme type 2				701	743
Solyc04g079180	26S proteasome regulatory subunit			562	868	913
Solyc04g079290	RNA Binding Protein 47		514	595	635	805
Solyc04g079410	Nucleobase ascorbate transporter		527	501		
Solyc04g079590	Cytochrome P450			584		
Solyc04g079940	Ubiquitin-conjugating enzyme, E2	7123	10106	8347	4200	3841

Solyc04g080210	Unknown Protein					603
Solyc04g080250	Unknown Protein	2473	2782	2032	2230	2526
Solyc04g080270	2-hydroxyacid dehydrongenase (Fragment)		508	504		
Solyc04g080410	Maf-like protein	8555	8560	6719	6127	4986
Solyc04g080510	ST225	3233	4265	3143	4671	5166
Solyc04g080560	Proteasome, subunit alpha/beta					560
Solyc04g080570	Heat shock protein DnaJ, N-terminal					533
Solyc04g080590	Glycoside hydrolase, subgroup, catalytic core		540	526	775	1635
Solyc04g080670	Unknown Protein	584	625	741	594	842
Solyc04g080710	Basic leucine zipper		583	555		
Solyc04g080820	Thioredoxin	685	933	1065	635	578
Solyc04g081000	Low temperature viability protein				573	551
Solyc04g081060	V-type proton ATPase 16 kDa proteolipid subunit c2		527		585	590
Solyc04g081200	TLDc		531			
Solyc04g081430	Unknown Protein				668	630
Solyc04g081460	Tubulin beta-1 chain				722	1311
Solyc04g081520	Thaumatin-like protein				567	668
Solyc04g081540	Molecular chaperone, heat shock protein, endoplasmin				1392	2380
Solyc04g081550	DNA/RNA helicase, DEAD/DEAH box type, N-terminal		537	561	873	931
Solyc04g081700	Unknown Protein	1295	1750	1640	878	903
Solyc04g081850	RNA/RNA-binding protein	1142	1585	1704	1702	2022
Solyc04g082170	Dehydrin	544	691	781	613	
Solyc04g082430	Catalase	1210	1503	1748	1445	1342
Solyc04g082650	Chloroplast channel forming outer membrane protein		590	603	550	617
Solyc05g005190	ADP-ribosylation factor		548	643	849	1132
Solyc05g005510	DNA-binding related protein (Fragment)		598	537	641	637
Solyc05g005620	Splicing factor PWI				549	579
Solyc05g005690	30S ribosomal protein S9		574	564	624	639
Solyc05g005710	Spermine synthase					501
Solyc05g006070	RNA recognition motif, RNP-1				624	601
Solyc05g006160	Gibberellin regulated protein	1740	2010	1574	923	1164
Solyc05g006240	Heat shock protein DnaJ	539	652		601	566
Solyc05g006400	Unknown Protein		513			
Solyc05g006520	Pyruvate dehydrogenase E1 component					575
Solyc05g006980	Homeobox-leucine zipper-like protein				751	629
Solyc05g007120	Receptor like kinase, RLK	510	703		558	
Solyc05g007200	RNA recognition motif containing protein			569		
Solyc05g007250	Ribosomal protein			533	719	940
Solyc05g007560	60S ribosomal protein L34	1598	2256	2166	2741	2790
Solyc05g007570	Unknown Protein				627	752
Solyc05g007970	Phytanoyl-CoA dioxygenase		678	586	587	560
Solyc05g007980	Sulphate anion transporter		573		646	811

Solyc05g008010	60S ribosomal protein L31	620	785	807	755	1021
Solyc05g008440	Unknown Protein	750	898	898		
Solyc05g008460	ATP synthase subunit beta				506	763
Solyc05g008600	Fructose-bisphosphate aldolase					524
Solyc05g008630	Poly(A) polymerase					547
Solyc05g009030	Isopropylmalate dehydrogenase					533
Solyc05g009240	Unknown Protein	952	1087	990	507	660
Solyc05g009360	Unknown Protein	815	893	926	526	
Solyc05g009450	Pathogenesis-related transcriptional factor and ERF					634
Solyc05g009470	Glycoside hydrolase, family 31				1196	1658
Solyc05g009550	Unknown Protein	1835	2250	1897	539	508
Solyc05g009600	Phosphatase 2A regulatory A subunit	508	625	632	825	999
Solyc05g009730	Unknown Protein	500	681	618	848	1066
Solyc05g009840	No apical meristem (NAM) protein		516			
Solyc05g009910	Coiled-coil domain-containing protein 94	1478	1850	1925	691	555
Solyc05g010540	F-box domain containing protein	551	823	913		
Solyc05g010810	Phosphatidylinositol kinase	504	740	740		654
Solyc05g011990	Nucleolar protein				591	561
Solyc05g012370	Glucan endo-1 3-beta-glucosidase 1		564	503		
Solyc05g013680	GDSL esterase/lipase				1539	2423
Solyc05g013810	Peptidase T1A, proteasome beta-subunit				699	829
Solyc05g013910	Cathepsin B-like cysteine proteinase	4571	5022	4978	5789	10216
Solyc05g013980	T-complex protein 1 subunit epsilon	510	714	685	932	1372
Solyc05g014120	COP1-interacting protein 4	573	723	761	704	708
Solyc05g014460	Glyceraldehyde 3-phosphate dehydrogenase	580	657	640	590	910
Solyc05g015380	Rubber elongation factor	1721	2253	1917	1213	1444
Solyc05g015410	MYND finger family protein expressed	580	535	695		
Solyc05g015480	Lipid transfer protein/seed storage/trypsin-alpha amylase inhibitor	602	611	536	1079	1518
Solyc05g015490	Zinc finger, RanBP2-type		626	562		
Solyc05g015520	Ribosome associated membrane RAMP4	608	736	763	1018	943
Solyc05g016120	Photosystem I assembly protein ycf3	595	743	528		526
Solyc05g017880	THO complex subunit					598
Solyc05g018480	Early fruit mRNA	616	735	694	763	669
Solyc05g018800	Nucleosome assembly protein (NAP)	1917	2195	2042	1616	1725
Solyc05g023790	GTP-binding nuclear protein Ran-A1		628	676	831	1081
Solyc05g024150	Pyruvate dehydrogenase E1 component	788	1040	914		
Solyc05g025500	WD40 repeat, region	740	884	798	505	567
Solyc05g025560	Unknown Protein	911	707	527	888	
Solyc05g026040	Charged multivesicular body protein 4b		645	589		
Solyc05g042020	BAH-PHD domain-containing protein (Fragment)	1190	1396	1255	780	735
Solyc05g050190	Translation initiation factor 1A (eIF-1A)	1102	1404	1409	1262	1195
Solyc05g050790	Phosphoglycerate mutase family protein		555			

Solyc05g050840	Unknown Protein				547	534
Solyc05g050990	40S ribosomal protein S13				500	666
Solyc05g051040	Serine/threonine protein kinase	505	669	678	519	
Solyc05g052400	Pathogenesis-related transcriptional factor and ERF, DNA-binding	530	604	610		510
Solyc05g052780	Organic anion transporter		555	512		
Solyc05g053130	26S proteasome non-ATPase regulatory subunit				591	689
Solyc05g053150	Plasma membrane associated protein	1460	2066	1538	989	1012
Solyc05g053340	Desiccation-related protein	1229	1235	950	1036	1323
Solyc05g053430	60S ribosomal protein L29	528	668	689	870	1033
Solyc05g053460	Chaperonin Cpn60		535		519	831
Solyc05g053540	Chalcone synthase	630	643		1786	2105
Solyc05g053640	26S proteasome regulatory subunit					617
Solyc05g053660	60S ribosomal protein L13a-like protein	1240	1406	1472	1761	2261
Solyc05g053800	Serine hydroxymethyltransferase					509
Solyc05g054060	60S ribosomal protein L6		539		761	940
Solyc05g054090	Transmembrane protein	571	773	752	854	866
Solyc05g054120	Calcium-binding protein	917	1170	1072	833	834
Solyc05g054180	Trans-membrane				512	550
Solyc05g054340	Epoxide hydrolase	1088	1336	1370	519	540
Solyc05g054360	Acyl-CoA dehydrogenase	546	636	595		
Solyc05g054480	Trans-2-enoyl-CoA reductase					553
Solyc05g054490	DNA/RNA-binding protein KIN17	647	775	715	516	539
Solyc05g054570	60S acidic ribosomal protein	972	1314	1490	1737	2884
Solyc05g054630	2-oxoglutarate dehydrogenase E1 component					573
Solyc05g054720	Cation efflux protein					544
Solyc05g054800	60S ribosomal protein L35				504	690
Solyc05g054910	Splicing factor arginine/serine-rich 6			508	546	643
Solyc05g055150	Heat shock protein DnaJ	4809	5970	5768	2274	2396
Solyc05g055220	40S ribosomal protein S17-like protein	559	721	737	711	870
Solyc05g055250	Small nuclear ribonucleoprotein G				764	847
Solyc05g055430	Histone H2B	545	620	522	511	567
Solyc05g055440	PolyrC-binding protein 2				530	587
Solyc05g055630	60S ribosomal protein L38	644	754	768	980	1000
Solyc05g055760	Basic leucine zipper and W2 domain-containing protein	1406	1862	1790	2036	2256
Solyc05g055860	F-box protein PP2-B1			668	552	741
Solyc05g055870	Pre-mRNA processing ribonucleoprotein		551	517	788	869
Solyc05g056100	mRNA-decapping enzyme		576	506	511	589
Solyc05g056150	Proteasome subunit beta type	528	663	708	1086	1177
Solyc05g056230	Acyl carrier protein (ACP)	589	834	680		
Solyc05g056240	Asparaginyl-tRNA synthetase				554	754
Solyc05g056270	RNA-binding protein Luc7-like 2				539	633
Solyc05g056300	T-complex protein 1 subunit gamma	677	955	942	910	1249

Solyc05g056340	Cleft lip and palate associated transmembrane protein-like	1962	2686	2531	2146	2397
Solyc05g056460	ABC-2 type transporter					616
Solyc06g005060	Protein synthesis factor, GTP-binding	4448	6132	5811	6117	9289
Solyc06g005210	Cytochrome P450 like_TBP	10646	12605	11155	18662	13075
Solyc06g005360	Actin-binding, cofilin/tropomyosin type	1231	1692	1703	1326	1197
Solyc06g005670	mRNA binding protein	717	938	799	795	1018
Solyc06g005790	Ribosomal protein S1, RNA binding domain				521	
Solyc06g005940	Protein disulfide isomerase	599	778	712	1489	2331
Solyc06g007200	Methionine aminopeptidase					516
Solyc06g007210	Unknown Protein	549	695	659	878	942
Solyc06g007220	Unknown Protein	510	764	726	969	1196
Solyc06g007340	Gamma-interferon-inducible lysosomal thiol reductase	712	1005	880	1037	1215
Solyc06g007470	40S ribosomal protein S26	828	1028	1053	1010	1355
Solyc06g007510	Ubiquitin-conjugating enzyme, E2	4034	5278	4234	2178	2038
Solyc06g007520	Ribosomal protein L10			537	658	738
Solyc06g007540	Cytochrome b-c1 complex				689	680
Solyc06g007670	60S ribosomal protein L5	2049	2628	2669	3093	4070
Solyc06g007710	Mitochondrial import receptor subunit TOM40					524
Solyc06g008170	50S ribosomal protein L14		546	571	661	848
Solyc06g008260	60 ribosomal protein L14		558	559	680	712
Solyc06g008870	GID1-like gibberellin receptor	727	998	868		
Solyc06g009000	Mediator of aba-regulated dormancy 1	1178	1534	1145		
Solyc06g009020	Glutathione S-transferase	1569	2058	2155	1310	828
Solyc06g009050	Universal stress protein	769	933	752		
Solyc06g009140	Late embryogenesis abundant protein	553	726			
Solyc06g009210	Ribosomal protein L19	848	1040	1102	1190	1541
Solyc06g009530	Carbohydrate-binding-like fold					515
Solyc06g009960	Translation elongation factor EF1A					562
Solyc06g009970	Translation elongation factor EF1A	2363	3087	2874	3497	5118
Solyc06g011280	Translation elongation factor EF1B	849	1150	1102	1635	2184
Solyc06g011490	Unknown Protein	2393	2738	2550	3100	3299
Solyc06g016660	Unknown Protein	3236	3636	3365	5077	3717
Solyc06g017860	Peptidase S10, serine carboxypeptidase	530	614		890	1326
Solyc06g024210	TO54-2 (Fragment)	61634	69236	62547	102031	71483
Solyc06g024230	TO54-2 (Fragment)	2316	2778	2532	3987	2857
Solyc06g024350	Unknown Protein	2730	2263	1721	2836	1463
Solyc06g024370	Unknown Protein	3624	3875	3447	5663	3918
Solyc06g024380	Unknown Protein	16081	17376	16747	21921	16748
Solyc06g034040	Oleosin	1476	1915	1651	941	1102
Solyc06g035450	DNA/RNA helicase, DEAD/DEAH box type, N-terminal	805	1232	1387	964	1221
Solyc06g035460	DEAD-box ATP-dependent RNA helicase	1083	1328	1418	986	1260
Solyc06g035970	Tubulin beta chain	564	687	687	926	1029

Solyc06g036050	60S ribosomal protein L36	1091	1376	1373	1569	1824
Solyc06g036290	Heat shock protein Hsp90	772	941	599		610
Solyc06g048610	tRNA-binding region					605
Solyc06g048840	Late embryogenesis abundant protein	11392	14891	12386	2886	3264
Solyc06g050120	Ribosomal protein L7A	2274	2806	2809	2956	3298
Solyc06g050770	Alpha-soluble NSF attachment protein	618	924	897	1082	1097
Solyc06g050870	Hypoxia induced protein conserved region containing protein	797	838	857		
Solyc06g050980	Ferritin		504	603		
Solyc06g051810	Unknown Protein	598	611	577	813	1025
Solyc06g053160	Cytochrome b5	588	819	702	649	597
Solyc06g053310	DNA-directed RNA polymerase I subunit rpa49	797	1151	1076	1223	1111
Solyc06g053800	30S ribosomal protein S19		527	579	685	728
Solyc06g053820	AUX/IAA protein	700	727	1059	661	
Solyc06g059970	Undecaprenyl pyrophosphate synthase	617	771		933	1857
Solyc06g060130	Replication factor C					513
Solyc06g060170	Cytochrome P450	1286	1357	1364	1378	1810
Solyc06g060210	No apical meristem (NAM) protein		570	553		
Solyc06g060270	Protein disulphide isomerase					690
Solyc06g060380	Ribosomal protein L15	2108	2469	2375	2212	2560
Solyc06g060700	Peroxisomal biogenesis factor	707	962	921	913	966
Solyc06g060820	Oleosin	531	534			
Solyc06g060980	Casein kinase II, regulatory subunit	664	901	892	563	677
Solyc06g061090	Unknown Protein	1365	1752	1494	1008	1380
Solyc06g062330	Holliday junction ATP-dependent DNA helicase ruvB				646	644
Solyc06g062410	Inositol oxygenase				1032	1461
Solyc06g062480	60S ribosomal protein L28	519	686	693	613	690
Solyc06g062570	Transmembrane 9 superfamily protein member	645	763	784		557
Solyc06g062770	Serine/threonine protein kinase		511		511	578
Solyc06g062980	Polyadenylate-binding protein	1109	1632	1409	737	769
Solyc06g063050	Pathogenesis-related transcriptional factor and ERF, DNA-binding	1077	1320	1287	2316	2725
Solyc06g063120	26S protease regulatory subunit				608	692
Solyc06g063280	Kelch-domain-containing protein	663	846	765	587	513
Solyc06g063300	Unknown Protein			541		
Solyc06g064450	Ribosomal protein L7	2842	3596	3553	3843	3975
Solyc06g064610	Ribosomal protein L15	803	1083	1007	1419	1843
Solyc06g064630	Glucose/ribitol dehydrogenase	636	774	699		
Solyc06g064800	Lipase, GDSL					637
Solyc06g064810	WD-40 repeat protein				578	737
Solyc06g065000	TGF-beta receptor, type I/II extracellular region		631	612		604
Solyc06g065030	Transmembrane protein	1613	2122	1591	928	953
Solyc06g065210	GCN5-related N-acetyltransferase					524
Solyc06g065350	Subtilase family protein		605	631	595	537

Solyc06g065500	T-complex protein eta subunit				843	1071
Solyc06g065570	60S ribosomal protein L18					671
Solyc06g065690	Sister chromatid cohesion protein PDS5 homolog B-B				600	628
Solyc06g065960	Nucleic acid binding protein				666	765
Solyc06g066040	DNA/RNA helicase, DEAD/DEAH box type, N-terminal	1212	1578	1469	1840	2145
Solyc06g066080	Nucleic acid binding protein		759	670	887	863
Solyc06g066290	Transcription factor iws1	583	702	759	651	698
Solyc06g066630	Ribosomal protein L37		601	580	562	696
Solyc06g067930	Glutaredoxin family protein		664	601	689	788
Solyc06g067950	Unknown Protein	1809	2537	2056	985	676
Solyc06g068020	Nucleic acid binding protein		693	622	613	563
Solyc06g068110	Methyl-CpG DNA binding	546	637	647		
Solyc06g068130	Cystathionine beta-synthase, core	2242	2813	2054	1071	1338
Solyc06g068240	Oxoglutarate and iron-dependent oxygenase	1374	1590	1333	1482	1608
Solyc06g068250	DNA/RNA helicase, DEAD/DEAH box type, N-terminal	528	662	598		
Solyc06g068380	CCR4-NOT transcription complex subunit	730	1009	1000	576	
Solyc06g068920	Phosphoethanolamine N-methyltransferase				505	523
Solyc06g068980	Guanine nucleotide-binding protein beta subunit-like protein		616	660	592	996
Solyc06g068990	Protein synthesis factor, GTP-binding	1692	2121	1867	1937	2098
Solyc06g069060	40S ribosomal protein S7	593	860	798	1012	1455
Solyc06g069080	Lipid transfer protein and hydrophobic protein	1315	1275	1356	1063	1734
Solyc06g069090	Unknown Protein	2256	2809	2705	1030	1188
Solyc06g069300	Serine/threonine protein kinase		583	510	553	521
Solyc06g069730	DNA-directed RNA polymerase		593	669	680	690
Solyc06g069860	Nucleosome assembly protein (NAP)				545	658
Solyc06g070950	Ubiquitin-conjugating enzyme E2	2081	2719	2521	1692	1794
Solyc06g071070	ATPase, P-type, plasma-membrane proton-efflux					623
Solyc06g071440	Peroxisome membrane anchor protein Pex14p, N-terminal					605
Solyc06g071500	60S ribosomal protein L44	1084	1438	1316	1741	1773
Solyc06g071690	60S ribosomal protein L27			538		512
Solyc06g071790	Zinc finger, TAZ-type					834
Solyc06g071840	60S ribosomal protein L17	661	761	783	988	1152
Solyc06g071850	60S ribosomal protein L17	552	587	537	713	889
Solyc06g071890	Glyceraldehyde-3-phosphate dehydrogenase	924	1063	940	783	972
Solyc06g071960	AAA-type ATPase family protein	667	924	663	588	595
Solyc06g072090	40S ribosomal protein SA	1055	1148	1280	1667	2324
Solyc06g072100	Aquaporin	549	702	664	502	700
Solyc06g072460	40S ribosomal protein S1		558	642	748	1038
Solyc06g072640	Glucose/ribitol dehydrogenase	2774	3413	3223	1146	1140
Solyc06g072650	TB2/DP1 and HVA22 related protein	906	1156	1046	687	584
Solyc06g073250	LL-diaminopimelate aminotransferase				547	695
Solyc06g073270	60S ribosomal protein L27	990	1123	1088	1086	1291

Solyc06g073280	Ribosomal protein L6	1915	2403	2267	2376	3071
Solyc06g073300	Lysyl-tRNA synthetase					595
Solyc06g073340	40S ribosomal protein S18	1858	2384	2364	2431	2421
Solyc06g073400	40S ribosomal protein S29	1533	1646	1717	1562	1735
Solyc06g073420	Unknown Protein	626	802	692		
Solyc06g073520	RNA-binding protein					578
Solyc06g073670	Small nuclear ribonucleoprotein LSM8		690	597	755	771
Solyc06g073760	40S ribosomal protein S11	1771	2262	2258	2146	2473
Solyc06g073770	40S ribosomal protein S11	1026	1377	1248	1625	1816
Solyc06g074270	Ribosomal protein L1	919	1248	1290	1750	2101
Solyc06g074360	Fatty acyl coA reductase		561		816	1282
Solyc06g074400	Ribosomal protein 60S	1171	1552	1480	1995	2081
Solyc06g074460	Unknown Protein		586	550	642	618
Solyc06g074560	Chloroplast Ycf2	859	1092	1038	1198	1236
Solyc06g074600	Glycosyl transferase, family 2					696
Solyc06g074690	Nucleotide-binding, alpha-beta plait	2602	3251	3129	2308	2564
Solyc06g074950	ATPase, AAA-type, CDC48	1293	1880	1568	1203	1565
Solyc06g075150	Ribosomal protein L12	675	959	806	941	1284
Solyc06g075490	Glutathione S-transferase, C-terminal-like	813	1103	951		
Solyc06g075960	Glyoxalase/bleomycin resistance protein/dioxygenase	919	1202	1125	789	754
Solyc06g076310	mRNA binding protein				561	815
Solyc06g076330	Chloroplast envelope protein translocase, IAP75					505
Solyc06g076460	Unknown Protein	520	582		592	
Solyc06g076490	Heat shock protein Hsp20	982	1420	1034		
Solyc06g076610	Tubulin beta chain					675
Solyc06g076960	Vitamin B6 biosynthesis protein				809	966
Solyc06g082060	Methionine aminopeptidase	1291	1636	1423	2546	2958
Solyc06g082070	Related to ATP dependent RNA helicase	529	709	683		652
Solyc06g082110	Unknown Protein	834	1195	1132	1344	1765
Solyc06g082330	U2 small nuclear ribonucleoprotein B	857	1104	1200	1115	1024
Solyc06g082360	Unknown Protein	504	616	556	523	713
Solyc06g082530	Translation initiation factor IF2/IF5					524
Solyc06g082590	RNA-binding protein PNO1-like protein	1559	2031	2001	1481	1807
Solyc06g082600	26S protease regulatory subunit					535
Solyc06g082620	60S ribosomal protein L10				627	733
Solyc06g082640	Ribosomal protein L10	1314	1707	1626	2114	2468
Solyc06g082840	60S ribosomal protein L5	1068	1304	1416	1223	1544
Solyc06g082870	Unknown Protein				514	
Solyc06g083150	40S ribosomal protein S8	557	774	733	1020	1336
Solyc06g083160	Peptidyl-prolyl cis-trans isomerase, FKBP-type	747	992	818	518	698
Solyc06g083200	GTP cyclohydrolase I		558		780	740
Solyc06g083270	Ras GTPase-activating protein-binding protein		595	607		562

Solyc06g083310	Wound-induced basic protein				604	618
Solyc06g083330	DNA-directed RNA Polymerase II subunit L	903	1199	1236	1178	1190
Solyc06g083560	B3 domain-containing transcription factor ABI3				535	579
Solyc06g083570	B3 domain-containing transcription factor ABI3	555	747	871	610	504
Solyc06g083590	26S protease regulatory subunit 4		592	570	768	1099
Solyc06g083750	50S ribosomal protein L14	1811	2518	2416	2479	2592
Solyc06g083760	Succinyl-CoA ligase		658	683	923	864
Solyc06g083790	60 ribosomal protein L14			528	662	852
Solyc06g083840	Structural maintenance of chromosomes 1 protein				688	695
Solyc06g084060	Histone H2A		525	549		
Solyc06g084200	40S ribosomal protein S24				586	879
Solyc06g084280	Small nuclear ribonucleoprotein Sm D1					531
Solyc06g084470	Unknown Protein				675	613
Solyc06g084490	Ubiquitin-protein ligase Cullin					546
Solyc07g005050	60S ribosomal protein L39			556	579	700
Solyc07g005200	Longin	567	687	666	597	538
Solyc07g005210	Outer membrane lipoprotein blc	828	1011	745	908	959
Solyc07g005230	Unknown Protein		684	538	539	
Solyc07g005300	Unknown Protein		682	597	511	
Solyc07g005530	Ubiquitin carboxyl-terminal hydrolase	632	872	883	794	873
Solyc07g005560	Translation elongation factor, IF5A	854	1089	1032	1437	1574
Solyc07g005600	Nonaspanin (TM9SF)	501	699	599	903	966
Solyc07g005810	Translation initiation factor	627	929	905	1103	1402
Solyc07g005820	Heat shock protein 70	1195	1348	1154	527	503
Solyc07g006140	Cytochrome P450			724		
Solyc07g006150	Transmembrane protein	549	750	782	899	790
Solyc07g006280	Senescence-associated protein	2767	3639	2517		558
Solyc07g006500	Glycosyl transferase			629		
Solyc07g006650	Xylose isomerase					627
Solyc07g006800	UDP-glucuronosyl/UDP-glucosyltransferase		508	835		
Solyc07g006870	Xyloglucan endotransglucosylase/hydrolase	2394	2764	2340	3383	4540
Solyc07g007210	Elongation factor like protein					587
Solyc07g007380	Glucosidase II beta subunit-like		525		719	621
Solyc07g007500	Unknown Protein				706	743
Solyc07g007600	Inorganic H+ pyrophosphatase				674	1067
Solyc07g007930	Raffinose synthase				1535	1578
Solyc07g008250	SCF E3 ubiquitin ligase complex F-box protein	2780	3746	2979	1678	1599
Solyc07g008290	Growth regulator like protein	785	1041	711		
Solyc07g008320	Calcium-transporting ATPase 1	651	979	863		529
Solyc07g008330	Nucleolar and coiled-body phosphoprotein 1-like	987	1264	1154	1832	1703
Solyc07g008340	ABC transporter, ABCE				633	640
Solyc07g008370	60S ribosomal protein L7		599	637	774	952
Solyc07g008390	Hydroxycinnamoyl CoA quinate transferase	1126	1519	964	614	852

Solyc07g008540	CONSTANS-like zinc finger protein				670	
Solyc07g008710	Major latex-like protein	894	1040	767	556	787
Solyc07g008720	Nascent polypeptide-associated complex NAC	2040	2790	2439	3532	3597
Solyc07g008750	Nuclear nucleic acid-binding protein C1D		504			
Solyc07g008880	Pre-mRNA-processing-splicing factor 8	825	1210	1229	1286	1633
Solyc07g008950	Methionyl-tRNA synthetase		611	659	879	1023
Solyc07g009140	26S proteasome non-ATPase regulatory subunit 6	524	788	759	1070	1149
Solyc07g009330	60S acidic ribosomal protein P3	600	668	705	937	1212
Solyc07g016150	Translation elongation factor EF1B, guanine nucleotide exchange	783	999	1068	915	1090
Solyc07g016200	Peptidase T1A, proteasome beta-subunit				526	626
Solyc07g017490	Red family protein		516	540	525	
Solyc07g017750	Flavin-binding kelch domain F box protein		526	569		
Solyc07g017780	ATPase, P-type, plasma-membrane proton-efflux				653	928
Solyc07g018290	Pathogenesis-related transcriptional factor and ERF, DNA-binding	852	1068	878	1700	1902
Solyc07g019460	Cytochrome P450 NADPH-reductase	969	1209	1213	1005	917
Solyc07g026660	Unknown Protein	78728	85192	82272	113827	83572
Solyc07g026770	Mitochondrial ATP synthase g subunit family protein	549	728	691	550	750
Solyc07g032260	Unknown Protein		522		941	967
Solyc07g032740	Aspartate/other aminotransferase	557	640	537	503	619
Solyc07g039200	Guanine nucleotide-binding protein subunit		593	622	740	907
Solyc07g039290	TO54-2 (Fragment)	11851	14354	13417	20554	14391
Solyc07g039330	Histone-binding protein RBBP7					526
Solyc07g040680	Heat shock factor (HSF)-type, DNA-binding	3008	3362	2924	906	645
Solyc07g040960	Unknown Protein		509			
Solyc07g040990	Protein phosphatase 2C		538			
Solyc07g041020	Cell growth-regulating nucleolar protein		562		831	823
Solyc07g041150	Myosin XI				555	551
Solyc07g041310	Ribosomal protein S3	906	962	908	1042	1260
Solyc07g041870	Tubulin--tyrosine ligase-like protein				706	766
Solyc07g041970	Subtilisin-like protease				545	750
Solyc07g042170	Jasmonate ZIM-domain protein	962	1452	1487	959	812
Solyc07g042230	Pathogenesis-related transcriptional factor and ERF, DNA-binding	762	1005	565	632	521
Solyc07g042250	Chaperonin 21, chloroplast	735	1028	897	650	932
Solyc07g042620	Prefoldin beta-like		552		609	619
Solyc07g043360	60S ribosomal protein L27	737	1032	969	1092	1126
Solyc07g043420	Oxoglutarate and iron-dependent oxygenase	3561	4227	3278	2467	2498
Solyc07g043460	Cytochrome P450				532	665
Solyc07g044760	ATP-dependent RNA helicase	557	867	799	671	788
Solyc07g044840	2 3-bisphosphoglycerate-independent phosphoglycerate mutase					617
Solyc07g045140	Uncharacterized membrane protein	589	707	599	674	712
Solyc07g045240	RNA-binding protein-like					628
Solyc07g045340	Defence response, Rin4	551	891	695	767	

Solyc07g047670	Unknown Protein	604	853	837	979	1184
Solyc07g047790	Chaperone heat shock protein Hsp90	1077	1273	1134	1392	1830
Solyc07g047800	Glucose/ribitol dehydrogenase	758	946	866	727	521
Solyc07g049360	Ubiquitin	718	855	892	1011	854
Solyc07g049450	Thioredoxin/protein disulfide isomerase				605	779
Solyc07g049720	DNA topoisomerase I	525	645	556	559	779
Solyc07g051850	Aspartic proteinase					570
Solyc07g052110	Ribosomal RNA small subunit methyltransferase F	741	948	806	1544	1709
Solyc07g052350	Aconitase/iron regulatory protein 2					756
Solyc07g052480	Isocitrate lyase	2527	2812	2741	1715	2017
Solyc07g052600	Unknown Protein	758	1141	1031	1000	920
Solyc07g052980	Xyloglucan endotransglucosylase/hydrolase				504	585
Solyc07g053260	14-3-3 protein	1477	1873	1868	1894	2196
Solyc07g053280	Ketol-acid reductoisomerase	2064	2964	2947	1973	2411
Solyc07g053360	Late embryogenesis abundant protein	2078	2733	2230	1631	1997
Solyc07g053650	26S proteasome regulatory subunit		541		915	1232
Solyc07g053750	Zinc finger, CCCH-type	1172	1572	1358	1842	2137
Solyc07g053800	E3 ubiquitin-protein ligase RING1		561	564		
Solyc07g054270	Unknown Protein					566
Solyc07g054760	Wound induced protein				621	723
Solyc07g054780	Wound induced protein				894	809
Solyc07g055210	Aspartate/other aminotransferase		528	526	575	698
Solyc07g055230	50S ribosomal protein L5		615	587	615	840
Solyc07g055840	Citrate synthase, type II		517			
Solyc07g056040	Transmembrane	1240	1359	1125	1095	1393
Solyc07g056340	RNA-binding protein				501	539
Solyc07g056370	Unknown Protein		598	607		
Solyc07g056420	Glutathione S-transferase-like protein			504		
Solyc07g056470	Glutathione S-transferase-like protein			552		
Solyc07g056480	Glutathione S-transferase-like protein		641	1192		513
Solyc07g056550	Bystin				601	721
Solyc07g061940	Acetolactate synthase		575	525		
Solyc07g062500	Cytochrome P450	3100	4298	4160	2827	1645
Solyc07g062970	Protein phosphatase 2C	1759	2346	2202	2150	2006
Solyc07g062990	Unknown Protein	675	795	646	527	
Solyc07g063100	Proton pump interactor	810	1180	1047	1302	1527
Solyc07g063270	Nucleolar GTP-binding protein	1038	1398	1337	1082	1449
Solyc07g063320	LanC-like protein	1328	1696	1367	708	943
Solyc07g063850	GH3 auxin-responsive promoter	801	927	941	502	685
Solyc07g063940	GRAS family transcription factor		545	568	607	673
Solyc07g064130	Ubiquitin	3670	4310	5264	3013	4262
Solyc07g064150	Translation initiation factor SUI1	12996	16859	14277	5744	4718

Solyc07g064520	DNA/RNA helicase, DEAD/DEAH box type, N-terminal				500	
Solyc07g064610	Calcium-dependent protein kinase 2	575	705	859	561	
Solyc07g064620	Translation initiation factor SUI1	845	1203	1406	899	
Solyc07g065170	40S ribosomal protein S8				520	516
Solyc07g065490	Dek protein	1055	1411	1241	1638	1759
Solyc07g065840	Heat shock protein Hsp90	38673	45323	40438	24697	26367
Solyc07g066080	Ubiquitin-conjugating enzyme E2	665	832	866	584	578
Solyc07g066380	Unknown Protein	538	690	699	524	489
Solyc07g066400	Seed maturation protein	504	714			
Solyc07g066600	Phosphoglycerate kinase					704
Solyc07g066650	DCN1-like protein	729	959	847	658	780
Solyc08g005020	Unknown Protein					573
Solyc08g005150	Ubiquitin ligase		539	506		
Solyc08g005270	Poly(ADP-ribose) polymerase, catalytic region	1176	1351	1309	646	751
Solyc08g005430	Growth-regulating factor				533	528
Solyc08g005470	Serine/threonine protein kinase		549	511	563	698
Solyc08g005910	Ankyrin repeat domain protein	3133	4128	4008	2952	2265
Solyc08g005960	Lipid transfer protein and hydrophobic protein		880		2076	1885
Solyc08g005970	Protein arginine N-methyltransferase	573	807	729	652	892
Solyc08g006040	40S ribosomal protein S6	779	912	842	1021	1232
Solyc08g006150	ChaC cation transport regulator-like 1	537	699	783		
Solyc08g006430	Sarcosine oxidase, monomeric				516	622
Solyc08g006800	Unknown Protein	656	868	768		
Solyc08g006890	Tubulin alpha-3 chain	1118	1394	1255	1750	2760
Solyc08g006900	Ribosomal protein L32					621
Solyc08g007140	60S ribosomal protein L37	1056	1461	1407	1705	1993
Solyc08g007220	Nuclear RNA binding protein (Fragment)				871	1017
Solyc08g007990	D-site 20S pre-rRNA nuclease		583			507
Solyc08g008160	WD40 repeat, region				555	521
Solyc08g008220	Ubiquitin-conjugating enzyme E2	1460	1684	1630	1120	1146
Solyc08g008350	Ubiquitin carrier protein			525		
Solyc08g008370	Kelch related		560	559		
Solyc08g008590	Ubiquitin		512		786	828
Solyc08g014340	Cysteine synthase				765	994
Solyc08g014550	Ribosomal L9-like protein		528	558	733	791
Solyc08g015690	Late-embryogenesis abundant protein	1264	1453	1460	826	648
Solyc08g015870	MLO-like protein	576	810	674		
Solyc08g016180	60S ribosomal protein L18	1133	1423	1304	1614	1841
Solyc08g016510	Proteasome, subunit alpha/beta		582	585	836	964
Solyc08g048500	Nucleic acid binding protein				508	584
Solyc08g060810	Ubiquitin ligase complex F-box protein GRR1	654	935	980	870	927
Solyc08g061000	DNA/RNA helicase, DEAD/DEAH box type, N-terminal					563

Solyc08g061100	Cellulose synthase					518
Solyc08g061130	bZIP transcription factor, bZIP-1				923	605
Solyc08g061320	Smr domain containing protein		510		556	606
Solyc08g061560	Receptor like kinase, RLK					500
Solyc08g061850	Ribosomal protein					539
Solyc08g061960	Ribosomal protein		546	564	685	999
Solyc08g062210	Nuclear transcription factor Y subunit A-3					522
Solyc08g062220	UDP-glucuronosyl/UDP-glucosyltransferase	581	822	675	894	595
Solyc08g062340	Class II small heat shock protein	585	678	555		
Solyc08g062450	Heat shock protein Hsp20	1230	1785	1218		
Solyc08g062660	Ran GTPase binding protein	1315	1706	1505	1698	1736
Solyc08g062800	DNA/RNA helicase, DEAD/DEAH box type, N-terminal		658	722	895	1277
Solyc08g062910	Protein synthesis factor, GTP-binding	569	771	695	930	1660
Solyc08g062920	Protein synthesis factor, GTP-binding	1299	1647	1562	1933	2944
Solyc08g063080	NAD(P)-binding domain		607	603	677	730
Solyc08g065160	Mediator of RNA polymerase II transcription subunit		585			
Solyc08g065490	Serine hydroxymethyltransferase					558
Solyc08g066110	Profilin	503	630	506	683	788
Solyc08g067090	Peptidyl-prolyl cis-trans isomerase	518	561		660	842
Solyc08g067260	Fatty acid elongase 3-ketoacyl-CoA synthase					544
Solyc08g067270	Unknown Protein	538	684	635		
Solyc08g067870	M355	511	586	716	535	
Solyc08g067950	CHY zinc finger family protein expressed	606	737	580		
Solyc08g068140	BURP domain-containing protein	3532	3713	3463	3077	4111
Solyc08g068170	Ribosomal protein L37			541	619	705
Solyc08g068300	RNA binding motif				579	602
Solyc08g068320	Aspartate/other aminotransferase					549
Solyc08g068330	RNA polymerase I-associated factor PAF67	601	690	684	887	1200
Solyc08g074230	40S ribosomal protein S6	2249	2674	2297	2303	2118
Solyc08g074280	BRI1-KD interacting protein 129 (Fragment)	801	963	1013	967	1136
Solyc08g074410	Unknown Protein	706	953	1001	960	735
Solyc08g075080	60S ribosomal protein L7	2656	3590	3575	3617	3883
Solyc08g075270	Uncharacterized membrane protein					501
Solyc08g075360	Unknown Protein	2141	2621	2834	1930	1827
Solyc08g075370	F-box protein	556	853	904	687	617
Solyc08g075530	Alternative oxidase	700	864	921		
Solyc08g075690	60S ribosomal protein L13	881	1182	1162	1139	1616
Solyc08g075720	Anamorsin homolog	557	662	676	764	707
Solyc08g075840	50S ribosomal protein L24		605	522	814	867
Solyc08g076190	DNA/RNA helicase, DEAD/DEAH box type, N-terminal	1265	1593	1645	1789	1933
Solyc08g076330	40S ribosomal protein S30-like	738	969	1066	1339	1338
Solyc08g076410	Poly(ADP-ribose) polymerase, catalytic region	584	824	903	574	657

Solyc08g076500	Unknown Protein		569			
Solyc08g076530	t-SNARE	718	804	822	639	596
Solyc08g076650	RNA recognition motif, RNP-1		542			
Solyc08g076720	TPR domain protein	557	621			
Solyc08g076850	Zinc-binding family protein	1513	1802	1811	1151	1038
Solyc08g076870	Unknown Protein		625			
Solyc08g076910	Arf GTPase activating protein		645	607	822	896
Solyc08g077090	Zinc finger, RING-type	1684	2002	2309	918	826
Solyc08g077210	Tetraspanin family protein	1110	1470	1154	1042	1027
Solyc08g077470	Unknown Protein	686	781	524	1648	1124
Solyc08g077700	60S ribosomal protein L37	626	796	745	927	1076
Solyc08g077710	60S ribosomal protein L37	674	827	822	943	1267
Solyc08g077970	Bax inhibitor					504
Solyc08g078150	Oleosin	2544	3431	3030	2187	2232
Solyc08g078420	Pre-mRNA processing ribonucleoprotein binding region	1403	1778	1723	1519	1477
Solyc08g078430	Unknown Protein		538	530		
Solyc08g078520	Agenet				657	709
Solyc08g078690	Heat shock protein Hsp20	509	609			
Solyc08g079050	Trehalose-phosphatase	1032	1352	1321		
Solyc08g079150	Vacuolar-processing enzyme	2734	3230	2731	2030	2527
Solyc08g079160	Stress-induced protein sti1-like protein	728	904	778		
Solyc08g079250	Serine/threonine-protein phosphatase (Fragment)	514	606	556		
Solyc08g079270	Cytochrome P450	688	907	811		
Solyc08g079350	Copper amine oxidase	592	786	574	933	1682
Solyc08g079560	Nucleotide excision repair, TFIIH, subunit TTDA	559	768	750	812	782
Solyc08g079570	ATP-dependent Clp protease proteolytic subunit					621
Solyc08g080430	Unknown Protein	6173	8054	7070	4984	5836
Solyc08g080440	Lipid transfer protein/seed storage/trypsin-alpha amylase inhibitor	2805	3825	3288	1128	799
Solyc08g080850	Glutathione S-transferase/chloride channel, C-terminal	501	628	629	531	
Solyc08g080890	Glutathione peroxidase	2753	3716	3710	1749	1547
Solyc08g081100	GMP synthase				653	771
Solyc08g081140	Aquaporin	502	655	700	690	858
Solyc08g081200	Aminopeptidase N				618	833
Solyc08g081290	ATP-dependent DNA helicase	519	590	557	603	561
Solyc08g081530	Pin2-interacting protein X1	759	965	973	957	1241
Solyc08g081690	ATPase, AAA-type, core	741	944	708	1317	1791
Solyc08g081900	Ubiquitin-conjugating enzyme E2	2007	2544	2285	1196	1119
Solyc08g081960	Myosin heavy chain-like protein				531	
Solyc08g082130	Abscisic acid receptor	659	915	844		
Solyc08g082210	Protein phosphatase 2C	1021	1271	925	1591	1334
Solyc08g082560	Kelch related	1416	1680	1361	804	607
Solyc08g082650	26S proteasome non-ATPase regulatory subunit		514	512	518	755

Solyc08g082770	Heat shock protein 70				584	871
Solyc08g082800	ABC transporter-like	639	877	831	629	862
Solyc08g083070	Ubiquitin	618	736	697	1023	1221
Solyc08g083270	Glycogen/starch synthases, ADP-glucose type					543
Solyc08g083280	Lipid-binding START	728	889	927		680
Solyc08g083330	Mitochondrial import inner membrane translocase subunit				549	532
Solyc09g005060	Phosphatidylethanolamine-binding protein PEBP	1922	2315	1701	776	535
Solyc09g005260	Calcium/proton exchanger	833	1203	714	992	935
Solyc09g005720	60S ribosomal protein L23	1353	1605	1639	1783	2497
Solyc09g005760	U3 small nucleolar RNA-associated protein		578	502	567	586
Solyc09g007180	Adenylate kinase	562	779	794	755	947
Solyc09g007230	Zinc finger, CCCH-type					522
Solyc09g007250	60S ribosomal protein L4/L1	688	913	826	1156	1535
Solyc09g007350	30S ribosomal protein S12	681	919	871	1311	1314
Solyc09g007850	RNA recognition motif, RNP-1				531	638
Solyc09g007920	Phenylalanine ammonia-lyase	561	563	532		872
Solyc09g008230	Zinc finger protein		666	654	575	598
Solyc09g008610	Unknown Protein					536
Solyc09g008720	Ethylene receptor				515	535
Solyc09g008770	Late embryogenesis abundant protein	3384	4014	3397	2203	2515
Solyc09g008800	60S ribosomal protein L24	595	766	733	847	1124
Solyc09g008830	Unknown Protein				977	958
Solyc09g009010	Enolase				623	936
Solyc09g009020	Histone deacetylase 2a-like	2349	2808	2528	2226	2912
Solyc09g009170	Chaperone protein dnaJ					516
Solyc09g009250	Fructose-bisphosphate aldolase					582
Solyc09g009750	bZIP transcription factor, bZIP-1					535
Solyc09g009880	Polyadenylate-binding protein					559
Solyc09g010090	30S ribosomal protein S11	933	1159	1228	1218	1302
Solyc09g010320	Nascent polypeptide-associated complex NAC		551	540	862	1045
Solyc09g010430	Actin-binding, cofilin/tropomyosin type		591			
Solyc09g010450	Proteasome component region PCI	537	747	683	1019	1407
Solyc09g010490	DNA-directed RNA polymerase	725	1015	1021	935	897
Solyc09g010500	Glucose/ribitol dehydrogenase	727	849	709		647
Solyc09g010620	Heat shock protein 70	5140	7033	7493	5232	7160
Solyc09g010790	Metallothionein	5318	6177	6027	6256	6032
Solyc09g010850	Expansin					1270
Solyc09g011460	Unknown Protein	1112	1317	1344		
Solyc09g011580	Glutathione S-transferase, C-terminal	508		830	1302	937
Solyc09g011620	Glutathione S-transferase, C-terminal				576	
Solyc09g014610	Unknown Protein		625	535		
Solyc09g014740	Late embryogenesis abundant protein	7819	10675	8785	7191	6706

Solyc09g015060	Aldo/keto reductase subgroup	2174	2594	2269	729	
Solyc09g015070	Phosphatidylinositol transfer protein SFH5				503	
Solyc09g015270	Photosystem I assembly protein ycf3	573	739	578	500	510
Solyc09g015310	Photosystem I P700 chlorophyll a apoprotein A1	666	807	617	560	616
Solyc09g015440	Unknown Protein		500	628		
Solyc09g015640	Non-green plastid inner envelope membrane protein	519	697	612		516
Solyc09g015860	Cytochrome C oxidase subunit II, transmembrane region		583		552	601
Solyc09g018440	Ubiquitin-activating enzyme E1				545	730
Solyc09g020120	60S ribosomal protein L5	1427	1825	1692	1648	1745
Solyc09g025200	Unknown Protein	710	994	792		
Solyc09g031640	Zinc finger, RING-type					509
Solyc09g050010	Cytochrome b/b6		554		556	525
Solyc09g056440	U-box domain-containing protein		577			
Solyc09g057660	Peptidyl-prolyl cis-trans isomerase, FKBP-type				501	639
Solyc09g059260	ER lumen protein retaining receptor				551	705
Solyc09g059610	SAM domain family protein		594	529	547	637
Solyc09g061380	Maturase K	624	708	590	1297	1212
Solyc09g061420	GNL3L/Grn1 putative GTPase	660	844	859	1261	1197
Solyc09g061610	Vesicle-associated membrane protein		624	564	586	515
Solyc09g064580	RNA-processing protein, HAT helix				531	729
Solyc09g064850	Ubiquitin system component Cue	1069	1288	1282	1114	1256
Solyc09g064990	Xylanase inhibitor (Fragment)	1062	1160	1262	1030	927
Solyc09g065110	Preprotein translocase secY subunit		539			
Solyc09g065320	40S ribosomal protein S24	1963	2648	2578	3338	3546
Solyc09g065450	Vicilin (Fragment)	1169	1490	1310	633	963
Solyc09g065750	Plant-specific domain TIGR01615 family protein	626	819	659		532
Solyc09g066420	60S ribosomal protein L14	1581	1952	1822	1999	2094
Solyc09g066490	Unknown Protein					532
Solyc09g072550	Unknown Protein	634	939	880		
Solyc09g072560	Pre-mRNA-splicing factor SLU7	743	839	848	531	
Solyc09g072860	50S ribosomal protein L14	943	1263	1313	1346	1637
Solyc09g072990	Elongation factor					527
Solyc09g074650	Thymidylate kinase	547	681	607		
Solyc09g074670	Cullin 1B		513			
Solyc09g074860	Ubiquinol-cytochrome C reductase hinge	966	1416	1259	1183	1118
Solyc09g074900	TspO/MBR-related protein	564	766	542		
Solyc09g075000	Prostaglandin E synthase 3		651	581	786	745
Solyc09g075010	ABC transporter, transmembrane region				778	1009
Solyc09g075110	Small nuclear ribonucleoprotein-like protein	635	805	742	815	830
Solyc09g075140	60S ribosomal protein L22		587	593	604	584
Solyc09g075200	Late embryogenesis abundant protein	531	702	727	611	605
Solyc09g075260	POT family domain containing protein expressed		669			

Solyc09g075280	Ribosomal protein L18			505	518	745
Solyc09g075420	Ribosomal protein L19				605	1023
Solyc09g075660	Gibberellin receptor GID1L2	616	759	843		
Solyc09g075820	Time for coffee	530	700	676		
Solyc09g075940	Heat shock protein 70		514			
Solyc09g082050	Cysteine synthase					566
Solyc09g082090	Seed maturation protein	2150	2389	1868	1272	1205
Solyc09g082100	Seed maturation protein	1417	1529	1174	711	794
Solyc09g082200	Unknown Protein	709	961	757	1446	1298
Solyc09g082330	Unknown Protein	14635	16632	13546	15175	21378
Solyc09g082510	40S ribosomal protein	786	953	848	816	971
Solyc09g082680	Early light-induced protein					507
Solyc09g082970	GDP-D-mannose-3â ,5â -epimerase 2	640	874	1071	1439	1514
Solyc09g083060	Pre-mRNA processing ribonucleoprotein, binding region	3334	3979	3812	4959	5192
Solyc09g083370	Unknown Protein				976	765
Solyc09g089800	Oxoglutarate and iron-dependent oxygenase	2191	2621	2073	2325	2432
Solyc09g090120	Malate dehydrogenase, NAD-dependent, cytosolic		580	605	757	1118
Solyc09g090130	Unknown Protein	1468	1865	1619	625	
Solyc09g090410	Cyanate hydratase		596	583	597	589
Solyc09g090500	Heterogeneous nuclear ribonucleoprotein	676	928	945	912	1023
Solyc09g090560	Eukaryotic translation initiation factor 4E (eIF-4E)	654	828	832	869	1087
Solyc09g090590	50S ribosomal protein L14		501	593	772	1017
Solyc09g090640	WD40 repeat, region			545		561
Solyc09g090700	26S proteasome regulatory subunit	795	971	900	788	927
Solyc09g090780	Plasma membrane associated protein	1244	1307	1540		
Solyc09g090950	Major allergen Mal d 1	30454	37416	32449	28639	30891
Solyc09g090960	Major allergen Mal d 1	2918	3529	3295	4829	4833
Solyc09g091010	Beta-amylase	946	1156	1028		
Solyc09g091160	Chaperonin Cpn60					658
Solyc09g091420	Histone deacetylase					564
Solyc09g091450	3-ketoacyl CoA thiolase	3541	4059	4073	4446	4503
Solyc09g091580	Tetratricopeptide-like helical	508	710	1113		
Solyc09g091720	60S ribosomal protein L13	852	1114	1009	781	921
Solyc09g091820	Glutathione-disulfide reductase					527
Solyc09g091920	Unknown Protein		516			506
Solyc09g092060	50S ribosomal protein L7A				552	614
Solyc09g092070	50S ribosomal protein L13				501	
Solyc09g092120	Methyltransferase					505
Solyc09g092220	Down-regulated in metastasis		575		581	682
Solyc09g092300	RNA recognition motif, glycine rich protein				823	827
Solyc09g092360	Adenosylhomocysteinase	1741	2169	2357	2066	2921
Solyc09g092370	Adenosylhomocysteinase		564	581	1134	1424

Solyc09g092560	Cytochrome P450	680	790	888	559	
Solyc09g097830	Cysteine proteinase inhibitor	1062	1161	1426	760	653
Solyc09g097880	Rab11-related				621	545
Solyc09g098060	Aldo/keto reductase	699	795	749	938	965
Solyc09g098090	Unknown Protein	2496	2809	2555	2423	3388
Solyc09g098200	DNA helicase, ATP-dependent, Ku70 subunit				798	1051
Solyc09g098210	Importin-7 (Imp7) (Ran-binding protein 7) (RanBP7)					586
Solyc09g098270	Unknown Protein	801	983	1225	945	804
Solyc09g098280	Unknown Protein	695	861	1026		
Solyc09g098330	Ribosome biogenesis protein NSA2	3682	4486	4423	3510	3544
Solyc09g098520	UBX domain-containing protein	639	740	607	672	789
Solyc10g005260	RNA Binding Protein 45	860	1125	1210	804	1030
Solyc10g005330	Lipid-binding START					592
Solyc10g005480	F-box family protein		609	537		
Solyc10g005510	Glyceraldehyde 3-phosphate dehydrogenase					635
Solyc10g005560	Bi-ubiquitin					554
Solyc10g005800	Cwf15/Cwc15 cell cycle control protein	900	1231	1076	850	783
Solyc10g005960	Fasciclin-like arabinogalactan protein				511	678
Solyc10g006070	40S ribosomal protein S8	818	1062	965	1065	1029
Solyc10g006080	Sodium/hydrogen exchanger		664	592	562	774
Solyc10g006130	Pathogenesis-related transcriptional factor and ERF, DNA-binding		514			
Solyc10g006260	SWAP/Surp		509	501		
Solyc10g006470	Translation initiation factor SUI1	1670	2384	2274	2039	1636
Solyc10g006480	Ubiquitin	518	619	732	562	622
Solyc10g006560	Histone H2A		585	711	609	
Solyc10g006580	50S ribosomal protein L2		554	551	717	990
Solyc10g007150	Apoptosis inhibitor	515	736	697	569	721
Solyc10g007390	Protein transport protein SEC61 alpha subunit				523	596
Solyc10g007590	Nucleotide-binding, alpha-beta plait		542		629	641
Solyc10g007650	RNA polymerase Rbp10				568	550
Solyc10g007660	Transmembrane	1529	1728	1539	803	1031
Solyc10g007760	Phosphoribosyl pyrophosphokinase	936	1227	1051	822	919
Solyc10g007980	50S ribosomal protein L5	1338	1900	1841	2079	2225
Solyc10g008040	Seed biotin-containing protein SBP65	1749	2074	1811	1187	1454
Solyc10g008130	Pre-rRNA-processing protein ESF2		522			
Solyc10g008140	Prohibitin 1-like protein		599	640	951	1254
Solyc10g008190	Nucleic acid-binding, OB-fold	568	750	724	925	932
Solyc10g008300	Glycoside hydrolase, family 5		536			
Solyc10g008870	Transcription elongation factor 1 homolog	958	1379	1364	1106	1068
Solyc10g011670	Mitochondrial carrier protein		527			605
Solyc10g011960	Unknown Protein	737	924	632	641	
Solyc10g012070	Protein BPS1, chloroplastic		578	590	696	636

Solyc10g012370	Cysteine synthase				1024	913
Solyc10g039190	Small-subunit processome, Utp12				535	631
Solyc10g044670	Phytochrome A				583	937
Solyc10g046930	Ribosomal RNA methyltransferase J					510
Solyc10g048060	Uncharacterized mitochondrial protein	1762	2174	2353	1655	1567
Solyc10g049630	Serine/threonine protein phosphatase 2C			517		
Solyc10g074980	Vacuolar sorting receptor				651	844
Solyc10g075050	Lipid transfer protein and hydrophobic protein, helical	2942	3360	3348	1799	2327
Solyc10g076510	Pyruvate decarboxylase/indolepyruvate decarboxylase	507	688	578	580	847
Solyc10g077010	EF-Hand type	609	789	765	706	663
Solyc10g078150	Nascent polypeptide-associated complex, alpha subunit	873	1038	1184	1177	1117
Solyc10g078220	Cytochrome P450	593	689		891	1019
Solyc10g078230	Cytochrome P450	701	671	630	768	775
Solyc10g078300	Single-stranded nucleic acid binding R3H		606	641	604	749
Solyc10g078620	Ribosomal protein S5	865	1130	1196	1215	1716
Solyc10g078630	40S ribosomal protein S28			514		632
Solyc10g078660	60S ribosomal protein L24			505	508	546
Solyc10g078670	BZIP transcription factor	696	773	557		551
Solyc10g078770	Late embryogenesis abundant (LEA) group 1	3305	4331	3625	1474	1544
Solyc10g078780	Late embryogenesis abundant (LEA) group 1	1839	1879	1866	1254	1392
Solyc10g078960	60S ribosomal protein L21-like protein	1711	2229	2224	2423	2859
Solyc10g079880	Translation initiation factor 3, subunit 6	896	1133	1167	1089	1161
Solyc10g080370	Unknown Protein		517	556		
Solyc10g080710	Asparaginyl-tRNA synthetase, class IIb		560	521	684	831
Solyc10g081020	Nascent polypeptide-associated complex, alpha subunit	1126	1398	1369	1630	1675
Solyc10g081110	Alpha-L-arabinofuranosidase	554	717	617		660
Solyc10g081160	EF-Hand type	658	771	757		
Solyc10g081310	MYB transcription factor				635	
Solyc10g081500	methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase				602	1044
Solyc10g081560	KED	1450	1779	1465	522	
Solyc10g081570	t-SNARE	1058	1485	1314	1127	960
Solyc10g081790	Phosphatidylglycerol/phosphatidylinositol transfer protein	1074	1287	1169	798	921
Solyc10g083370	Unknown Protein	1447	1835	1343	780	960
Solyc10g083430	UDP-glucuronosyl/UDP-glucosyltransferase				722	727
Solyc10g083590	Brix domain				575	645
Solyc10g083630	U2 auxiliary factor small subunit	536	654	623	515	
Solyc10g083710	Pyruvate kinase				695	824
Solyc10g083730	60S ribosomal protein L24					538
Solyc10g083750	Threonine dehydratase biosynthetic		524			
Solyc10g084040	26S protease regulatory subunit 6B homolog				561	701
Solyc10g084170	Zinc finger, C2H2-type		769	621	574	532
Solyc10g084300	30S ribosomal protein S12	1464	1903	1757	1429	1507

Solyc10g084990	Tubulin beta chain				587	623
Solyc10g085170	Acyl-CoA dehydrogenase/oxidase, middle and N-terminal	562	650	603	929	717
Solyc10g085180	BZIP family transcription factor				677	538
Solyc10g085390	Unknown Protein		551	566		
Solyc10g085400	Saposin (Fragment)		769	689	557	533
Solyc10g085450	60S ribosomal protein L24	1013	1408	1249	1252	1050
Solyc10g085710	GDSL esterase/lipase	1817	2369	2074	2324	3119
Solyc10g085980	60S ribosomal protein L4/L1					526
Solyc10g085990	30S ribosomal protein S12	652	805	747	797	1002
Solyc10g086110	Single-stranded DNA binding protein				686	762
Solyc10g086300	60S ribosomal protein L23				570	700
Solyc10g086380	Heat shock protein 70	1843	2222	1903	1587	2105
Solyc10g086750	Oxoglutarate and iron-dependent oxygenase				536	
Solyc11g005170	RNA splicing factor		531		565	683
Solyc11g005330	Actin		630	621	820	1249
Solyc11g005380	GT-2 factor (Fragment)		688	509		
Solyc11g005670	Ubiquitin	2720	3648	4018	1024	1114
Solyc11g005680	40S ribosomal protein S18					548
Solyc11g006070	Peptidyl-prolyl cis-trans isomerase	613	776	744	1134	1425
Solyc11g006350	Aspartate carbamoyltransferase					515
Solyc11g006460	Heat shock protein DnaJ	3044	3918	3566	1619	1644
Solyc11g006470	Unknown Protein	724	951	760	855	704
Solyc11g006720	MYB transcription factor		574	510		
Solyc11g007610	DNA/RNA helicase, DEAD/DEAH box type, N-terminal		601		581	562
Solyc11g007850	Plastid DNA-binding protein (Fragment)				555	579
Solyc11g008260	Cysteine proteinase cathepsin F	1059	1381	1432	985	1474
Solyc11g008280	Peptidase S10, serine carboxypeptidase	1144	1481	1389	1710	2157
Solyc11g008510	60S ribosomal protein L38	555	763	784	610	652
Solyc11g009080	DAHP synthetase, class II	977	1299	1099	593	676
Solyc11g010330	Zinc finger, C3HC4 RING-type	554	596	547		
Solyc11g010500	Mitochondrial substrate carrier		553			
Solyc11g010520	Unknown Protein	4111	4461	4467	3249	3159
Solyc11g010530	Unknown Protein	5124	5316	4680	4105	3736
Solyc11g010600	Unknown Protein					544
Solyc11g010960	Alcohol dehydrogenase					530
Solyc11g011220	Unknown Protein					572
Solyc11g011330	Cinnamyl alcohol dehydrogenase	675	962	1337	1091	727
Solyc11g011380	Glutamine synthetase				613	707
Solyc11g011780	Nonsense-mediated mRNA decay NMD3 family protein	1667	2103	3001	1015	607
Solyc11g012110	60S ribosomal protein L6	824	1040	1121	1411	2032
Solyc11g012190	Glycoside hydrolase, subgroup, catalytic core		529			
Solyc11g013110	Oxoglutarate and iron-dependent oxygenase	1107	1187	1113	3117	3572

Solyc11g017070	Translation initiation factor 3 subunit	716	983	951	904	840
Solyc11g020810	Ribosomal RNA methyltransferase J	1187	1437	1357	2077	1893
Solyc11g022530	Transmembrane protein	1269	1560	1373	797	891
Solyc11g027650	Unknown Protein	6629	6589	5386	4548	3797
Solyc11g027660	Unknown Protein	9559	10244	9625	13471	10131
Solyc11g027670	Cytochrome P450	2858	2833	2464	3585	2879
Solyc11g027710	Unknown Protein	26861	28531	25309	38003	27482
Solyc11g027750	Unknown Protein	17602	18556	18153	25229	18017
Solyc11g027760	Cytochrome P450	13933	14958	13999	22049	15571
Solyc11g027770	Cytochrome P450 monooxygenase (Fragment)	1431	1481	1316	1888	1480
Solyc11g027790	Unknown Protein	1213	1029	772	1230	691
Solyc11g032050	GDSL esterase/lipase	1068	1267	867	1597	1808
Solyc11g033280	DNA/RNA helicase, DEAD/DEAH box type, N-terminal					544
Solyc11g039840	Ubiquinol-cytochrome c reductase iron-sulfur subunit		513			569
Solyc11g039980	ATP synthase subunit alpha	1018	1591	1458	1431	2493
Solyc11g039990	Unknown Protein					721
Solyc11g040370	Far upstream element-binding protein				642	810
Solyc11g042610	Ribosomal protein S5	637	821	822	1088	1105
Solyc11g042930	E3 ubiquitin ligase, SCF complex, Skp subunit					536
Solyc11g043110	GDSL esterase/lipase	629	684	703	720	1443
Solyc11g044610	Cell wall-associated hydrolase	629	658	620	673	651
Solyc11g045120	Translation initiation factor SUI1	636	825	700	539	
Solyc11g045130	CCR4-NOT transcription complex subunit	525	712	743	597	612
Solyc11g045150	Uncharacterized mitochondrial protein	997	1149	1298	895	807
Solyc11g045350	Regulator RWP-RK	707	906	918	679	711
Solyc11g045440	Unknown Protein	892	885	932	864	905
Solyc11g051180	Unknown Protein	2160	2008	1608	1871	1340
Solyc11g051190	Unknown Protein	1393	1134	847	1323	739
Solyc11g051200	Cytochrome P450	5518	5449	4788	6834	5596
Solyc11g051210	Unknown Protein	6788	6947	5991	4710	4150
Solyc11g051230	Unknown Protein	578	516		640	
Solyc11g062130	Mitochondrial ADP/ATP carrier proteins	660	830	886	991	1446
Solyc11g062190	Mitochondrial ADP/ATP carrier proteins					717
Solyc11g062270	Signal recognition particle, SRP72 subunit, RNA-binding					510
Solyc11g063510	Unknown Protein	855	919	1111	1129	1283
Solyc11g063520	Unknown Protein	612	671	861	789	967
Solyc11g065180	THUMP domain-containing protein		525			
Solyc11g065600	Xyloglucan endotransglucosylase/hydrolase					505
Solyc11g065670	Ribosomal protein	517	698	680	847	843
Solyc11g066060	Heat shock protein 70					861
Solyc11g066280	U3 small nucleolar ribonucleoprotein protein	774	915	840	603	543
Solyc11g066840	Histone deacetylase-like protein	1806	2431	2456	2216	2781

Solyc11g067010	G10 protein		534			
Solyc11g067100	Ribosomal protein 60S	878	1103	1049	995	1038
Solyc11g067250	Poly(ADP-ribose) polymerase, catalytic region	997	1280	1089	649	1002
Solyc11g068420	Ribosomal protein L1	1222	1608	1529	1540	1746
Solyc11g068430	Ferredoxin [2Fe-2S]					623
Solyc11g068440	Glycoside hydrolase	777	1064	967		
Solyc11g068510	F1F0-ATPase inhibitor protein				593	531
Solyc11g069000	T-complex protein 1 subunit beta					658
Solyc11g069040	Lactoylglutathione lyase					530
Solyc11g069090	ATP-binding cassette protein	579	797	880	659	916
Solyc11g069150	Proteasome endopeptidase complex, beta subunit	542	787	692	1114	1438
Solyc11g069270	Glycoside hydrolase					716
Solyc11g069440	Ribosomal protein L12	557	693	552	788	878
Solyc11g069700	Translation elongation factor EF1A	866	1023	1094	1341	1918
Solyc11g069720	26S protease regulatory subunit 6B homolog				586	743
Solyc11g069780	2-phosphoglycerate kinase		694	578	566	726
Solyc11g069790	Chaperonin Cpn60	1192	1608	1280	886	1205
Solyc11g071490	Ribosomal protein L30	564	764	710	793	872
Solyc11g071690	Cellular nucleic acid binding protein		603	592		535
Solyc11g071870	Ubiquitin-conjugating enzyme	506	671	694		
Solyc11g072190	Translation elongation factor EF1B, guanine nucleotide exchange	1330	1848	1784	1817	2054
Solyc11g072240	EF-Hand type		569	562	713	799
Solyc11g072380	Vicilin-like protein (Fragment)	3300	3417	3421	2152	2792
Solyc11g072450	Mitochondrial F0 ATP synthase D chain		573	625	751	763
Solyc12g005270	Histone H2A	576	760	816		
Solyc12g005330	50S ribosomal protein L2		560	552	632	1005
Solyc12g005860	Aconitase/iron regulatory protein 2	565	853	739	561	761
Solyc12g006460	Cytochrome P450				1209	1803
Solyc12g006470	Aminotransferase class-III	621	793	737	1652	2356
Solyc12g006550	Ribosomal biogenesis regulatory protein				543	628
Solyc12g006680	Early nodulin 93 protein	1209	1630	1453	1018	1375
Solyc12g007030	Aldehyde dehydrogenase	1136	1167	955		522
Solyc12g008360	Oligosaccharyl transferase, STT3 subunit					554
Solyc12g008570	Arginyl-tRNA synthetase				708	831
Solyc12g008700	40S ribosomal protein S30		654	637	739	695
Solyc12g008720	60S ribosomal protein L31	1352	1748	1680	1661	1932
Solyc12g008760	Protein arginine N-methyltransferase	557	786	749	1032	966
Solyc12g008940	Nucleosome assembly protein (NAP)	570	744	734	986	1073
Solyc12g009140	Proteasome subunit alpha type				595	658
Solyc12g009560	F-box/LRR-repeat protein	860	1386	1342	783	682
Solyc12g009860	Unknown Protein		527	525	620	
Solyc12g009870	Leucine-rich repeat-like protein	978	1342	970		

Solyc12g009990	Signal recognition particle protein	533	711	614	644	677
Solyc12g010040	Leucyl aminopeptidase				591	719
Solyc12g010060	Translation elongation factor, IF5A, hypusine site	1249	1675	1418	1271	1396
Solyc12g010130	Serine/threonine protein kinase	1195	1640	1650	1786	1349
Solyc12g010350	60S ribosomal protein L39	565	654	731	741	902
Solyc12g010640	Unknown Protein	599	709	716		
Solyc12g010820	Late embryogenesis abundant protein	718	927	726	567	889
Solyc12g010920	Oleosin	4314	5370	4965	3151	3601
Solyc12g010930	50S ribosomal protein L5		501		519	656
Solyc12g011310	Glutathione S-transferase	817	998	742	671	761
Solyc12g013690	Monooxygenase FAD-binding protein	546	770	933		
Solyc12g013700	Aluminum-induced protein-like protein	2112	2634	2718	1484	1751
Solyc12g014230	DAG protein					523
Solyc12g014400	Cell differentiation protein rcd1	530	702	661	546	682
Solyc12g015770	Cellulose synthase	531	730	524	559	762
Solyc12g015880	Heat shock protein Hsp90	1919	2061	1749	2815	4743
Solyc12g017570	Rab GDP dissociation inhibitor	949	1197	1136	1235	1512
Solyc12g020000	Arginine/serine-rich coiled-coil protein 2	536	728	592	664	670
Solyc12g021320	Unknown Protein					565
Solyc12g035130	ATP dependent RNA helicase	1098	1563	1652	864	1114
Solyc12g038430	Kinesin-like calmodulin binding protein		591		643	675
Solyc12g038980	50S ribosomal protein L7A	816	1183	1000	1677	1586
Solyc12g039120	40S ribosomal protein S19	560	749	675	977	1018
Solyc12g042060	ATP-dependent clp protease ATP-binding subunit		585	505	524	812
Solyc12g042080	40S ribosomal protein S11	1337	1589	1759	1216	1549
Solyc12g042600	UDP-glucuronosyl/UDP-glucosyltransferase		660	652	881	521
Solyc12g042650	40S ribosomal protein S12	542	706	739	948	1270
Solyc12g042950	ADP/ATP carrier protein	821	1313	1286	1446	2120
Solyc12g043120	Heat shock protein 70		549			540
Solyc12g043160	Unknown Protein		649	608	582	504
Solyc12g044720	60S ribosomal protein L28	543	655	631	529	587
Solyc12g055870	RAG1-activating protein 1 homolog	682	864	647		
Solyc12g056590	Pathogenesis-related transcriptional factor and ERF, DNA-binding	750	937	882		
Solyc12g057060	UDP-glucuronosyl/UDP-glucosyltransferase			962	1222	801
Solyc12g087940	Aspartic proteinase nepenthesin				574	1254
Solyc12g088290	U3 small nucleolar RNA-associated protein				539	867
Solyc12g088720	Polyadenylate-binding protein 2	1848	2446	2347	3063	3914
Solyc12g094620	Catalase				789	1724
Solyc12g095930	Serine hydroxymethyltransferase				579	681
Solyc12g095990	DNA/RNA helicase, DEAD/DEAH box type, N-terminal		546	615	689	1097
Solyc12g096000	DNA/RNA helicase, DEAD/DEAH box type, N-terminal		542	529	735	889
Solyc12g096150	60S ribosomal protein L13				595	738

Solyc12g096220	60S ribosomal protein L7	1009	1256	1295	1248	1637
Solyc12g096300	40S ribosomal protein S6	995	1133	1122	1407	1753
Solyc12g096450	Zinc finger family protein	1755	1965	2143	1427	1283
Solyc12g096690	Cytosolic Fe-S cluster assembly factor nbp35			565		
Solyc12g096700	Ribosomal L9-like protein	1302	1863	1759	1913	2003
Solyc12g096930	Caleosin	1578	1856	1746	911	913
Solyc12g098130	Unknown Protein	1051	1090	1056		570
Solyc12g098150	Potassium channel, voltage-dependent, beta subunit, KCNAB-related	778	996	858	624	683
Solyc12g098330	60S ribosomal protein L13a-like protein					513
Solyc12g098490	Serine hydroxymethyltransferase					769
Solyc12g098500	Adenosylhomocysteinase					649
Solyc12g098850	Soluble diacylglycerol acyltransferase		605	505		
Solyc12g098900	Late embryogenesis abundant protein	19199	22861	20966	7558	6315
Solyc12g098940	Ubiquitin					572
Solyc12g099000	S-adenosylmethionine synthase	562	793	913	637	538
Solyc12g099030	Ubiquitin					559
Solyc12g099370	GATA transcription factor	506	680	641		
Solyc12g099390	Protein DEHYDRATION-INDUCED 19 homolog	574	820	709		
Solyc12g099440	3-hydroxyacyl-CoA dehydrogenase, NAD binding	892	1230	1190	1137	1464
Solyc12g099900.	GRAS transcription factor		617	749	540	

Bibliography

Acosta IF and Farmer EE. (2010). Jasmonates. *Arabidopsis Book*. 2010; 8:e0129

Adams SR, Cockshull KE and Cave RJ. (2001). Effect of temperature on tomato growth and development of tomato fruits. *Annals of Botany*. 2001 Nov;**88**(5):869-877

Afify AMR, El-Beltagi HS, El-Salam SM and Omran AA. (2011). Bioavailability of iron, zinc, phytate and phytase activity during soaking and germination of white sorghum varieties. *PLoS One*. 2011 Oct;**6**(10):e25512

Ahmadi A, Mardeh AS, Poustini K and Esmailpour Jahromi M. (2007). Influence of osmo and hydropriming on seed germination and seedling growth in wheat (*Triticum aestivum* L.) cultivars under different moisture and temperature conditions. *Pakistan Journal of Biological Sciences*. 2007 Nov;**10**(22):4043-4049

Alboresi A, Gestin C, Leydecker MT, Bedu M, Meyer C and Truong HN. (2005). Nitrate, a signal relieving seed dormancy in Arabidopsis. *Plant Cell & Environment*. 2005 Apr;**28**(4):500-512

Allen E, Moing A, Ebbels TM, Maucourt M, Tomos AD, Rolin D, Hooks MA. (2010). Correlation Network Analysis reveals a sequential reorganization of metabolic and transcriptional states during germination and gene-metabolite relationships in developing seedlings of Arabidopsis. *BMC System Biology*. 2010 May;**4:**62

Alonso-Blanco C, Bentsink L, Hanhart CJ, Blankestijn-de Vries H and Koorneef M. (2003). Analysis of natural variation at seed dormancy loci of *Arabidopsis thaliana*. Genetics. 2003 Jun;**164**(2):711-729

Aparicio-Fernandez X, Youssef GG, Loarca-Pina G, de Mejia A and Lila MA. (2005). Characterization of polyphenolics in the seed coat of black jamapa bean (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry*. 2005 Jun;**53**(11):4615-4622

APHA, AWWA and WEF. (1999). Standard method for the examination of water and waste water. Washington DC, 20th edition

Arc E, Sechet J, Corbineau F, Rajjou L and Marion-Poll A. (2013). ABA crosstalk with ethylene and nitric oxide in seed dormancy and germination. *Frontiers in Plant Science*. 2013;**4**:63

Arosio P, Ingrassia R and Cavadini P. (2009). Ferritins: a family of molecules for iron storage, antioxidation and more. *Biochimica et Biophysica Acta*. 2009 Jul;**1790**(7):589-599

Ashraf M and Bray CM. (1993). DNA synthesis in osmoprimed leek (*Allium porrum* L.) seeds and evidence for repair and replication. *Seed Science Research*. **3**(1):15

Bailly C. (2004). Active oxygen species and antioxidants in seed biology. *Seed Science Research*. 2004 May;**14**(2):93-107

Balk J and Schaedler TA. (2014). Iron cofactor assembly in plants. *Annual Review of Plant Biology*. 2014 Apr;**65**:152-153

Barrero JM, Talbot MJ, White RG, Jacobsen JV and Gubler F. (2009). Anatomical and transcriptomic studies of the coleorhiza reveal the importance of this tissue regulating dormancy in barley. Plant Physiology. 2009 Jun;**150**(2):1006-1021

Barrero JM, Millar AA, Griffiths J, Czechowski T, Scheible WR, Udvardi M, Reid JB, Ross JJ, Jacobsen JV and Gubler F. (2010). Gene expression profiling identifies two regulatory genes controlling dormancy and ABA sensitivity in Arabidopsis seeds. *The Plant Journal: for Cell and Molecular Biology*. 2010 Feb;**61**(4):611-622

Barvkar VT, Pardeshi VC, Kale SM, Kadoo NY, Giri AP and Gupta VS. (2012). Proteome profiling of flax (*Linum usitatissimum*) seed: characterization of functional metabolic pathways operating during seed development. *Journal of Proteome Research*. 2012 Dec;**11**(12):6264-6276

Baskin CC and Baskin JM. (1998). Seeds. Ecology, biogeography, and evolution of dormancy and germination. *Academic Press, San Diego*

Baskin JM and Baskin CC. (2004). A classification system for seed dormancy. *Seed Science Research*. 2004 Mar;**14**(1):1-16

Basra SMA, Farooq M and Khaliq A. (2003). Comparative study of pre-sowing seed enhancement treatments in indica rice (*Oryza sativa* L.). *Pakistan Journal of Life Social Sciences.* **1**(1): 5-9

Bastow R, Mylne JS, Lister C, Lippman Z, Martienssen RA and Dean C. (2004). Vernalization requires epigenetic silencing of FLC by histone methylation. *Nature*. 2004 Jan;**427**(6970):164-167

Benech-Arnold RL. (2004). Inception, maintenance, and termination of dormancy in grain crops: Physiology, genetics, and environmental control. In: Benech-Arnold R.L. and Sanchez R.A., eds. *Handbook of seed physiology: Applications to agriculture.* New-York, Food Product Press and the Haworth Reference Press, pp169-198

Benjamin LR. (1990). Variation in time of seedling emergence within populations. A feature that determines individual growth and development. *Advances in Agronomy*. 1990;**44**:1-25

Bentsink L, Jowett J, Hanhart CJ and Koorneef M. (2006). Cloning of *DOG1*, a quantitative trait locus controlling seed dormancy in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America*. 2006 Nov;**103**(45):17042-17047

Bentsink L and Koornneef M. (2008). Seed dormancy and germination. *Arabidopsis Book*. 2008 Dec;**6**:e0119

Berestetsky V, Dathe W, Daletskaya T, Musatenko L and Sembdner G. (1991). Jasmonic acid in seed dormancy of Acer tataricum. Biochemie und Physiologie der Pflanzen. 1991 Jan;**187**(1):13-19

Berrie AMM and Drennan DSH. (1971). The effect of hydratation-dehydration on seed germination. *New Phytologist*. 1971 Jan;**70**(1):135-142

Berry T and Bewley JD. (1992). A role for the surrounding fruit tissues in preventing the germination of tomato (*Lycopersicum esculentum*) seeds. *Plant physiologist*. 1992 Oct;**100**(2):951-957

Bewley JD and Black M. (1982). Physiology and biochemistry of seeds in relation to germination. Vol. 2. *Viability, dormancy and environmental control*. Springer-Verlag, Berlin

Bewley JD and Black M. (1986). Seeds: Physiology of development and germination. *Plenum press. N. Y. and London*

Bewley JD. (1997). Seed germination and dormancy. *Plant Cell*. 1997 Jul;**9**(7):1055-1066

Bewley JD, Bradford KJ, Hilhorst HWM and Nonogaki H. (2013). Seeds: Physiology of development, germination and dormancy. New York, NY: Springer. Doi:10.1007/978-1-4614-4693-4

Bhandari DR, Wang Q, Friedt W, Spengler B, Gottwald S, Römpp A. (2015). High resolution mass spectrometry imaging of plant tissues: towards a plant metabolite atlas. *The Analyst*. 2015 Nov;**140**(22):7696-7709

Biere A. (1991). Parental effects in *Lynchis flos-cuculi* I: seed size, germination and seedling performance in a controlled environment. *Journal of Evolutionary Biology*. 1991;**3**:447-465

Bleasdale JKA. (1967). Systematics design for spacing experiments. *Experimental Agriculture*. 1967 Jan;**3**(1):73-86

Bolger AM, Lohse M and Usadel B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 2014 Aug;30(15):2114-2120

Boter M, Ruíz-Rivero O, Abdeen A and Prat S. (2004). Conserved MYC transcription factors play a key role in jasmonate signalling both in tomato and Arabidopsis. *Genes & Development.* 2004 Jul;**18**(13):1577-1591

Botto JM, Sanchez RA, Whitelam GC and Casal JJ. (1996). Phytochrome A mediates the promotion of seed germination by very low influences of light and canopy shade light in Arabidopsis. *Plant Physiology*. 1996 Feb;**110**(2):439-444

Bradford JK. (1986). Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. *Hortscience*. 1986;**21**:1105-1112

Bradford KJ, Downie AB, Gee OH, Alvarado V, Yang H and Dahal P. (2003). Abscissic acid and gibberellin differentially regulate expression of gene of the SNF1-related kinase complex in tomato seeds. *Plant Physiology*. 2003 Jul;**133**(3):1560-1576

Bray CM, Davison PA, Ashraf M and Taylor RM. (1989). Biochemical changes during osmopriming of leek seeds. *Annals of Botany*. **63**(1): 185-193

Brummell DA, Harpster MH and Dunsmuir P. (1999). Differential expression of expansin gene family members during growth and ripening of tomato fruit. *Plant Molecular Biology*. 1999 Jan;**39**(1):161-169

Burcu P and Peksen E. (2008). Effect of seed storage periods on electrical conductivity of seed leakage, germination and field emergence percentage in common bean (*Phaseolus vulgaris L.*). *Asian Journal of Chemistry*. April 2008;**20**(4):3033-3041

Buttrose MS. (1973). Rapid water uptake and structural changes in imbibing seed tissues. *Protoplasma*. 1973 Mar;**77**(1):111-122

Cadman CS, Toorop PE, Hilhorst HW and Finch-Savage WE. (2006). Gene expression profiles of *Arabidopsis Cvi* seeds during dormancy cycling indicate a common underlying dormancy control mechanism. The *Plant Journal: for Cell & Molecular Biology*. 2006 Jun;**46**(5):805–822

Cantliffe DJ, Fischer JM and Nell TA. (1984). Mechanism of seed priming in circumventing thermodormancy in lettuce. Plant Physiology. 1984 Jun;75(2):290

Carrera E, Holman T, Medhurst A, Dietrich D, Footitt S, Theodoulou FL, Holdsworth MJ. (2008). Seed after-ripening is a discrete developmental pathway associated with specific gene networks in Arabidopsis. *The Plant Journal: for Cell & Molecular Biology*. 2008 Jan;**53**(2):214-224

Castillo MC, Martinez C, Buchala A, Métraux JP and León J. (2004). Gene-specific involvement of ß-oxidation in wound-activated responses in Arabidopsis. *Plant Physiology.* 2004 May;**135**(1):85-94

de Castro RD and Hilhorst HWM. (2000). Dormancy, germination and the cell cycle in developing and imbibing tomato seeds. *Revista Brasileira de Fisiologia Vegetal*. **12**(Edição Especial):105-136

Castro J, Hódar JA and Gómez JM. (2006). Seed size. In: *Handbook of seed science and technology*. Ed. Basra A., Haworth's Food Products Press, New York:397-427

Chasteen N.D. and Harrison P.M. (1999). Mineralization in ferritin: an efficient means of iron storage. *Journal of Structural Biology*. 1999 Jun;**126**(3):182-194

Cheminant S, Wild M, Bouvier F, Pelletier S, Renou JP, Erhardt M, Hayes S, Terry MJ, Genschik P and Achard P. (2011). DELLAs regulate chlorophyll and carotenoid biosynthesis to prevent photooxidative damage during seedling deetiolation in Arabidopsis. *Plant Cell*. 2011 May;**23**(5):1849-1860

Chen F and Bradford KJ. (2000). Expression of an expansin is associated with endosperm weakening during seed germination. *Plant Physiology*. 2000 Nov;**124**(3):1265-1274

Chen F, Dahal P and Bradford KJ. (2001). Two tomato expansin genes show divergent expression and localization in embryos during seed development and germination. *Plant Physiology*. 2001 Nov;**127**(3):928-936

Chen F, Nonogaki H and Bradford KJ. (2002). A gibberellin-regulated xyloglucan endotransglycosylase gene is expressed in the endosperm cap during tomato seed germination. *Journal of Experimental Botany*. 2002 Feb;**53**(367):215-23

Chen M, MacGregor DR, Dave A, Florance H, Moore K, Paszkiewicz K, Smirnoff N, Graham IA and Penfield S. (2014). Maternal temperature history activates Flowering Locus T in fruits to control progeny dormancy according to time of year. *Proc Natl Acad Sci U S A*. 2014 Dec 30;**111**(52):18787-92

Cheng Z and Bradford KJ. (1999). Hydrothermal time analysis of tomato seed germination responses to priming treatments. *Journal of Experimental Botany*. 1999 Jan;**50**(300):89-99

Cheng WH, Endo A, Zhou L, Penney J, Chen HC, Arroyo A, Leon P, Nambara E, Asami T, Seo M, Koshiba T, Sheen J. (2002). A unique short-chain dehydrogenase/reductase in Arabidopsis glucose signalling and abscisic acid biosynthesis and functions. *Plant Cell*. 2002 Nov;**14**(11):2723

Chini A, Fonseca S, Fernández G, Adie B, Chico JM, Lorenzo O, García-Casado G, López-Vidriero I, Lozano FM, Ponce MR, Micol JL and Solano R. (2007). The JAZ family of repressors is the missing link in jasmonate signalling. *Nature*. 2007 Aug;**448**(7154):666-671

Chiwocha SD, Cutler AJ, Abrams SR, Ambrose SJ, Yang J, Ross AR and Kermode AR. (2005). The *etr1-2* mutation in *Arabidopsis thaliana* affects the abscisic acid, auxin, cytokinin and gibberellin metabolic pathways during maintenance of seed dormancy, moist-chilling and germination. *The Plant Journal: for Cell and Molecular Biology*. 2005 Apr;**42**(1):35-48

Chopin F, Orsel M, Dorbe MF, Chardon F, Truong HN, Miller AJ, Krapp A and Daniel-Vedele F. (2007). The Arabidopsis ATNRT2.7 nitrate transporter controls nitrate content in seeds. *Plant Cell*. 2007 May;**19**(5):1590-1602

Conte SS and Walker EL. (2011). Transporters contributing to iron trafficking in plants. *Molecular Plant*. 2011 May;**4**(3):464-476

Coolbear P and Grierson D. (1979). Studies on the changes in the major nucleic acid components of tomato seeds (*Lycopersicon esculentum* Mill.) resulting from osmotic presowing treatment. *Journal of Experimental Botany*. 1979 Dec;**30**(6):1153–1162

Coolbear P, Slater RJ and Bryant JA. (1990). Changes in nucleic acid levels associated with improved germination performance of tomato seeds after low temperature presowing treatment. *Annals of Botany*. 1990 Feb;**65**(2):187–195

Cruz Castillo M, Martínez C, Buchala A, Métraux JP and León J. (2004). Gene-specific involvement of beta-oxidation in wound-activated responses in Arabidopsis. *Plant Physiology*. 2004 May;**135(**1):85-94

Cushnie TP and Lamb AJ. (2011). Recent advances in understanding the antibacterial properties of flavonoids. *International Journal of Antimicrobial Agents.* 2011 Aug;**38**(2):99-107

Custodio CC and Marcos-Filho J. (1997). Potassium leakage test for the evaluation of soybean seed physiological quality. Seed Science and Technology. **25**:549-564

Cvitanich C, Przybylowicz WJ, Urbanski DF, Jurkiewicz A, Mesjasz-Przbylowicz J, Blair MW, Astudillo C, Jensen EO and Stougaard J. (2010). Iron and ferritins accumulate in separate cellular locations in *Phaseolus* seeds. *BMC Plant Biology*. 2010 Feb;**10**:26

Cyrek M, Fedak H, Ciesielski A, Guo Y, Sliwa A, Brzezniak L, Krzyczmonik K, Pietras Z, Kaczanowski S, Liu F, Swiezewski S. (2016). Seed dormancy in arabidopsis is controlled by alternative polyadenylation of DOG1. *Plant Physiology*. 2016 Feb;**170**(2):947-955

Dahal P and Bradford KJ. (1994). Hydrothermal time analysis of tomato seed germination at suboptimal temperature and reduced water potential. *Seed Science Research*. 1994 Jun;**4**(2):71-80

Dahal P, Nevins DJ and Bradford KJ. (1997). Relationship of endo-β-mannanase activity and cell wall hydrolysis in tomato endosperm to germination rates. *Plant Physiology*. 1997 Apr;**113**(4):1245-1252

Daletskaya T and Sembdner G. (1989). Effect of jasmonic acid on germination of nondormant and dormant seeds. (In Russian). *Fiziolagia Rastenii*. 1989;**36**:1118

Dastanpoor N, Fahimi H, Shariati M, Davazdahemami S and Hashemi SMM. (2013). Effects of hydropriming on seed germination and seedling growth in sage (*Salvia officinalis* L.). *African Journal of Biotechnology*. 2013 Mar;**12**(11):1223-1228

Dave A, Hernández ML, He Z, Andriotis VME, Vaistij FE, Larson TR and Graham IA. (2011). 12-oxo-phytodienoic acid accumulation during seed development represses seed germination in Arabidopsis. *Plant Cell.* 2011; **23**:583-99

Dave A and Graham IA. (2012). Oxylipin signalling: a distinct role for the jasmonic acid precursor 12-Oxo-Phytodienoic Acid (OPDA). *Frontiers Plant Science*. 2012;**3**:42

Dave A, Vaistij FE, Gilday AD, Penfield SD and Graham IA. (2016). Regulation of *Arabidopsis thaliana* seed dormancy and germination by 12-oxo-phytodienoic acid. *Journal of Experimental Botany*. 2016 Apr;**67**(8):2277-2284

David LC, Dechorgnat J, Berquin P, Routaboul JM, Debeaujon I, Daniel-Vedele F and Ferrario-Méry S. (2014). Proanthocyanidin oxidation of Arabidopsis seeds is

altered in mutant of the high-affinity nitrate transporter NRT2.7. *Journal of Experimental Botany*. 2014 Mar;**65**(3):885

Davies HV, Shepherd LVT, Stewart D, Frank T, Rohlig RM and Engel KH. (2010). Metabolome variability in crop plant species - When, where, how much and so what? *Regulatory Toxicology and Pharmacology*. 2010 Dec;**58**(3 Suppl):S54-S61.

Davison PA and Bray CM. (1991). Protein synthesis during osmo-priming of leek (*Allium porrum L*.) seeds. *Seed Science Research*. 1991 Mar;**1**(1): 29-35

De Giorgi J, Piskurewicz U, Loubery S, Utz-Pugin A, Bailly C, Mène-Saffrané L and Lopez-Molina L. (2015). An endosperm-associated cuticle is required for Arabidopsis seed viability, dormancy and early control of germination. *PLOS Genetics*. 2015 Dec;**11**(12):e1005708

Debeaujon I and Koorneef M. (2000). Gibberellin requirement for Arabidopsis seed germination is determined both by testa characteristics and embryonic abscisic acid. *Plant Physiology*. 2000 Feb;**122**(2):415-424

Debeaujon I, Léon-Kloosterziel KM and Koorneef M. (2000). Influence of testa on seed dormancy, germination, and longevity in Arabidopsis. *Plant physiology*. 2000 Feb;**122**(2):403-414

Dekkers BJ, Pearce S, Van Bolderen-Veldkamp RP, Marshall A, Widera P, Gilbert J, Drost HG, Bassel GW, Müller K, King JR, Wood AT, Grosse I, Quint M, Krasnogor N, Leubner-Metzger G, Holdsworth MJ and Bentsink L. (2013). Transcriptional dynamics of two seed compartments with opposing roles in Arabidopsis seed germination. *Plant Physiology*. 2013 Sep;**163**(1):205-215

Dell'Aquila A and Bewley JD. (1989). Protein synthesis in the axes of polyethylene glycol-treated pea seed and during subsequent germination. *Journal of Experimental Botany*. **40**(9): 1001-1007

Delouche JC. (1976). Standardization of vigour tests. *Journal of Seed Technology*. 1976;**1**(2):75-85

Deng J, Cheng J, Liao X, Leng X and Zhao G. (2010). Comparative study on iron release from soybean (*Glycine max*) seed ferritin induced by anthocyanins and ascorbate. *Journal of Agricultural and Food Chemistry*. 2010 Jan;**58**(1):635-641

Desikan R, Griffiths R, Hancock J and Neill S. (2002). A new role for an old enzyme: nitrate reductase-mediated nitric oxide generation is required for abscisic acid-induced stomatal closure in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the United States of America*. 2002 Dec;**99**(25):16314

Ding ZJ, Yan JY, Li GX, Wu ZC, Zhang SQ and Zheng SJ. (2014). WRKY41 controls Arabidopsis seed dormancy via direct regulation of ABI3 transcript levels not downstream of ABA. *The Plant Journal: for Cell and Molecular Biology*. 2014 Sep;**79**(5):810-823

Dixon RA, Gang DR, Charlton AJ, Fiehn O, Kuiper HA, Reynolds TL, Tjeerdema RS, Jeffery EH, German JB, Ridley WP and Seiber JN. (2006). Application of metabolomics in agriculture. *Journal of Agricultural and Food Chemistry*. 2006 Nov;**54**(24):8984-8994

Dobrovolna J and Celt I. (1966). An increase of germination of dormant seeds by pricking. *Arabidopsis Information Service*. **3**:33

Donohue K and Schmitt J. (1998). Donohue K, Schmitt J. 1998. Maternal environmental effects in plants: adaptive plasticity? In: Mousseau TA, Fox CW, Eds. *Maternal Effects as Adaptations*. Oxford, UK: Oxford University Press, 137–158

Donohue K, Heschel MS, Chiang GC, Butler CM and Barua D. (2007). Phytochrome mediates germination responses to multiple seasonal cues. *Plant Cell & Environment*. 2007 Feb;**30**(2):202-212

Donohue K. (2009). Completing the cycle: maternal effects as the missing link in plant life histories. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*. 2009 Apr;**364**(1520):1059-1074

Doria E, Galleschi L, Calucci L, Pinzino C, Pilu R, Cassani E and Nielson E. (2009). Phytic acid prevents oxidative stress in seeds: evidence from a maize (Zea mays L.) *low phytic acid* mutant. *Journal of Experimental Botany*. 2009 Mar;**60**(3):967

Duke SH and Kakefuda G. (1981). Role of the testa in preventing cellular rupture during imbibition of legume seeds*. Plant Physiology.* 1981 Mar;**67**(3):449-456

Duke SH, Kakefuda G and Harvey TM. (1983). Differential leakage of intracellular substances from imbibing soybean seeds. *Plant Physiology.* 1983 Aug;**72**(4):919

Duran JM and Retamal N. (1993). Coat structure and regulation of dormancy in *Sinapis arvensis* L. seeds. *Journal of Plant Physiology*. 1989 Oct;**135**(2):218–222

Eggert K and von Wiren N. (2013). Dynamics and partitioning of the ionome in seeds and germinating seedlings of winter oilseed rape. *Metallomics*. 2013;**5**:1316-1325

Eich E. (2008). Solanaceae and Convolvulaceae: Secondary metabolites: Biosynthesis, chemotaxonomy, biologival and economic significance. *A Handbook* (Berlin: Springer)

Eisen MB, Spellman P, Brown PO and Botstein D. (1998). Cluster analysis and display of genome-wide expression patterns. *Proceedings of the National Academy of Sciences of the United States of America*. 1998 Dec;**95**(25):14863-14868

Elder RH and Osborne DJ. (1993). Function of DNA synthesis and DNA repair in the survival of embryos during early germination and in dormancy. *Seed Science Research*. 1993 Mar;**3**(1):43-53

Ellinger D, Stingl N, Kubigsteltig II, Bals T, Juenger M, Pollmann S, Berger S, Schuenemann D and Mueller MJ. (2010). DONGLE and DEFECTIVE IN ANTHER DEHISCENCE lipases are not essential for wound- and pathogen-

induced jasmonate biosynthesis: redundant lipases contribute to jasmonate production. *Plant Physiologist*. 2010 May;**153**(1):114–127

Ellis C and Turner JG. (2002). A conditionally fertile coi1 allele indicates cross-talk between plant hormone signalling pathways in *Arabidopsis thaliana* seeds and young seedlings. *Planta*. 2002 Aug;**215**(4):549-556

Ellis RH. (1992). Seed and seedling vigour in relation to crop growth and yield. *Plant Growth Regulation*. 1992 Aug;**11**(3):249-255

Etterson JR and Galloway LF. (2002). The influence of light on paternal plants in *Campanula americana* (Campanulaceae): pollen characteristics and offspring traits. *American Journal of Botany*. 2002 Dec;**89**(12):1899-1906

Fait A, Angelovici R, Less H, Ohad I, Urbanczyk-Wochniak E, Fernie AR and Galili G. (2006). Arabidospis seed development and germination is associated with temporally distinct metabolic switches. *Plant Physiology*. 2006 Nov;**142**(3):839

Farooq M, Basra SMA, Tabassum R and Afzal I. (2006). Enhancing the performance of direct seeded fine rice by seed priming. *Plant Production Science*. 2006 Apr;**9**(4):446–456

Fiehn O. (2002). Metabolomics—the link between genotypes and phenotypes. *Plant Molecular Biology*. 2002 Jan;**48**(1-2):155-171

Finch-Savage WE. (1995). Influence of seed quality on crop establishment, growth and yield. In: Basra S. (Ed.). *Seed Quality. Basic Mechanisms and Agricultural Implications*. Haworth Press, New York:361

Finch-Savage WE and Leubner-Metzger G. (2006). Seed dormancy and the control of germination. *New Physiologist*. 2006;**171**(3):501-523

Finch-Savage WE, Cadman CS, Toorop PE, Lynn JR and Hilhorst HW. (2007). Seed dormancy release in Arabidopsis Cvi by dry after-ripening, low temperature, nitrate and light shows common quantitative patterns of gene expression directed by environmentally specific sensing. *The Plant Journal: for Cell and Molecular Biology*. 2007 Jul;**51**(1):60-78

Finkelstein RR. (1994). Maternal effects govern variable dominance of two abscisic acid response mutations in *Arabidopsis thaliana*. *Plant Physiology*. 1994 Aug;**105**(4):1203-1208

Finkelstein RR and Lynch TJ. (2000). The Arabidopsis abscisic acid response gene ABI5 encodes a basic leucine zipper transcription factor. *Plant Cell*. 2000 Apr;**12**(4):599

Foley ME. (2001). Seed dormancy: an update on terminology, physiological genetics, and quantitative trait loci regulating germinability. *Weed Science*. 2001;**49**(3):305-317

Fonseca S, Chico JM and Solano R. (2009). The jasmonate pathway: the ligand, the receptor and the core signalling module. *Current Opinion in Plant Biology*. 2009 Oct;**12**(5):539-547

Foolad MR, Subbiah P and Zhang LP. (2007). Common QTL affect the rate of tomato seed germination under different stress and nonstress conditions. *International Journal of Plant Genomics*. 2007;**2007**:97386

Footitt S, Huang Z, Clay HA, Mead A and Finch-Savage WE. (2013). Temperature, light and nitrate sensing coordinate Arabidopsis seed dormancy cycling, resulting in winter and summer annual phenotypes. *The Plant Journal: for Cell and Molecular Biology*. 2013 Jun;**74**(6):1003-1015

Footitt S, Müller K, Kermode AR and Finch-Savage WE. (2015). Seed dormancy cycling in Arabidopsis: chromatin remodelling and regulation of DOG1 in response to seasonal environmental signals. *The Plant Journal: for Cell and Molecular Biology*. 2015 Feb;**81**(3):413-425

Footitt S, Ölçer-Footitt H, Hambidge AJ, Finch-Savage WE. (2017). A laboratory simulation of *Arabidopsis* seed dormancy cycling provides new insight into its regulation by clock genes and the dormancy-related genes *DOG1*, *MFT*, *CIPK23* and *PHYA*. *Plant Cell & Environment*. 2017 Aug;**40**(8):1474-1486

Frank T, Röhlig RM, Davies HV, Barros E and Engel KH. (2012). Metabolite profiling of maize kernels – genetic modification versus environmental influence. *Journal of Agricultural and Food Chemistry*. 2012 Mar;**60**(12):3005-3012

Galloway LF. (2005). Maternal effects provide phenotypic adaptation to local environmental conditions. *New phytologist*. 2005 Apr;**166**(1):93-99

Gfeller F and Svejda F. (1960). Inheritance of post-harvest seed dormancy and kernel color in spring wheat lines. *Canadian Journal of Plant Science*. 1960Jan;**40**(1):1-6

Gholami M, Mokhtarian F and Baninasab B. (2015). Seed halo-priming improves the germination performance of black seed (*Nigella sativa*) under salinity stress conditions. *Journal of Crop Science and Biotechnology*. 2015 Mar;**18**(1):21-26

Gonzalez-Guzman M, Apostolova N, Belles JM, Barrera JM, Piqueras P, Ponce MR, Micol JL, Serrano R and Rodriguez PL. (2002). The short-chain alcohol dehydrogenase ABA2 catalyzes the conversion of xanthoxin to abscisic aldehyde. *Plant Cell*. 2002 Aug;**14**(8):1833-1846

Górnik K, de Castro RD, Liu Y, Bino RJ and Groot PC. (1997). Inhibition of cell division during cabbage (*Brassica oleracea* L.) seed germination. *Seed Science Research*. **7**(4):333–34

Gorzolka K, Kölling J, Nattkemper TW, Niehaus K. (2016). Spatio-Temporal Metabolite Profiling of the Barley Germination Process by MALDI MS Imaging. *PLos One*. 2016 Mar;**11**(3):e0150208

Goto N. (1982). The relationship between characteristics of seed coat and dark-germination by gibberellins. *Arabidospsis information Service*. 1982;**19**:29-38

Graeber K, Linkies A, Steinbrecher T, Mummenhoff K, Tarkowská D, Turečková V, Ignatz M, Sperber K, Voegele A, de Jong H, Urbanová T, Strnad M and Leubner-Metzger G. (2014). DELAY OF GERMINATION 1 mediates a conserved coat-dormancy mechanism for the temperature- and gibberellin-dependent control of seed germination. *Proceedings of the National Academy of Sciences of the United States of America*. 2014 Aug;**111**(34):e3571-80

Graham IA. (2008). Seed storage oil mobilization. Annual Reviews of Plant Biology. 2008;**59**:115-142

Grasser M, Kane CM, Merkle T, Melzer M, Emmersen J and Grasser KD. (2009). Transcript elongation factor TFIIS is involved in *Arabidopsis* seed dormancy. *Journal of Molecular Biology*. 2009 Feb;**386**(3):598–611

Grillet L, Mari S and Schmidt W. (2014). Iron in seeds – loading pathways and subcellular localization. *Frontiers in Plant Science*. 2014 Jan;**4**:535

Groot SP and Karssen CM. (1992). Dormancy and germination of abscisic acid-deficient tomato seeds: studies with the sitiens mutant. *Plant Physiologist*. 1992 Jul;**99**(3):952-958

Gutterman Y. (1992). Influences of daylength and red or far red light during the storage of post harvested ripe Cucumis prophetarum fruit, on the light germination of the seeds. *Journal of Arid Environments*. 1992;**23**:443–449

Haigh AM. (1988). Why do tomato seeds prime?: physiological investigations into the control of tomato seed germination and priming. *PhD dissertation, Macquarie University*, Sydney, Australia

Hakala M, Rantamäki S, Puputti EM, Tyystjärvi T and Tyystjärvi E. (2006). Photoinhibition of manganese enzymes: insights into the mechanism of photosystem II photoinhibition. *Journal of Experimental Botany.* 2006 May;**57**(8):1809-1816

Hall RD. (2006). Plant metabolomics: from holistic hope, to hype, to hot topic. *New Phytologist*. 2006;**169**(3):453-468

Halloin JM. (1975). Solute loss from deteriorated cotton seed: relationship between deterioration, seed moisture, and solute loss. *Crop Science*. 1975;**15**(1):11-15

Halmer P. (2004). Methods to improve seed performance in the field. In: Benech-Arnold R.L. and Sanchez R.A., eds. *Handbook of seed physiology: Applications to agriculture.* New-York, Food Product Press and the Haworth Reference Press, pp125-166

Hampton JG, Tekrony DM and the ISTA Vigour Test Committee. (1995). Handbook of vigour test methods. *The International Seed Testing Application*

Han C, Zhen S, Zhu G, Bian Y and Yan Y. (2017). Comparative metabolome analysis of wheat embryo and endosperm reveals the dynamic changes of metabolites during seed germination. *Plant Physiology and Biochemistry: PPB*. 2017 Jun;**115:**320

Hands P, Rabiger DS and Koltunow A. (2016). Mechanisms of endosperm initiation. *Plant Reproduction*. 2016 Sep;**29**(3):215-225

Hansch R and Mendel RR. (2009). Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). *Current Opinion in Plant Biology*. 2009 Jun;**12**(3):259

Harper JL. (1957). The ecological significance of dormancy and its importance in weed control. *Proceedings of the international congress on crop protection* (Hamburg). **4**:415–420.

Harris D. (1996). The effect of manure, genotype, seed priming, depth and date of sowing on the emergence and early growth of *Sorghum bicolor* (L.) Moench in semi-arid Botswana. *Soil & Tillage Research*. **40**:73-88

Harris D, Tripathi RS and Joshi A. (2002). On-farm seed priming to improve crop establishment and yield in dry direct-seeded rice, in: Pandey S., Mortimer M., Wade L., Tuong T.P., Lopes K. and Hardy B. (Eds). *Direct sending: Research Strategies and Opportunities, International Research Institue, Manilla, Philippines, Philippines*. pp. 231-240

Harrison MH and Arosio P. (1996). The ferritins: molecular properties, iron storage function and cellular regulation. *Biochimica et Biophysica Acta*. 1996 Jul;**1275**(3):161-203

Harrison EP, McQueen-Mason SJ and Manning K. (2001). Expression of six expansin genes in relation to extension activity in developing strawberry fruit. *Journal of Experimental Botany*. 2001 Jul;**52**(360):1437-1446

Hassanpouraghdam MB, Pardaz JE and Akhtar NF. (2009). Effect of osmo-priming on germination and seedling growth of *Brassica napus L.* under salinity conditions. *Journal fo Food, Agriculture & Environment*. 2009;**7**(2):620-622

Hehenberger E, Kradolfer D and Köhler C. (2012). Endosperm cellularization defines an important developmental transition for embryo development. *Development*. 2012 Jun;**139**(11):2031-2039

Helliwell CA, Wood CC, Robertson M, Peacock JW and Dennis ES. (2006). The Arabidopsis FLC protein interacts directly *in vivo* with SOC1 and FT chromatin and is part of a high-molecular-weight protein complex. *The Plant Journal: for Cell & Molecular Biology*. 2006 Apr;**46**(2):183-192

Hepher A and Roberts JA. (1985). The control of seed germination in *Trollius ledebouri*: the breaking of dormancy. *Planta*. 1985 Nov;**166**(3):314-320

Hepler PK. (2005). Calcium: a central regulator of plant growth and development. *Plant Cell*. 2005 Aug;**17**(8):2142-2155

Hernández-Nistal J, Martín I, Labrador E and Dopico B. (2010). The immunolocation of XTH1 in embryonic axes during chickpea germination and seedling growth confirms its function in cell elongation and vascular differentiation. *Journal of Experimental Botany*. 2010 Oct;**61**(15):4231-4238

Heydecker W. (1973). Germination of an idea: the priming of seeds. *School of Agriculture Research*, University of Nottingham, 1973, pp. 50-67

Heydecker W and Coolbear P. (1977). Seed treatments for improved performance-survey and attempted prognosis. *Seed Science and Technology.* **13**:299-355

Hilhorst HWM. (1990). Dose-response analysis of factors involved in germination and secondary dormancy of seeds of *Sisymbrium officinale*: II. Nitrate. *Plant Physiology*. 1990 Nov;**94**(3):1096-1102

Hilhorst HWM. (1995). A critical update on seed dormancy. I. Primary dormancy. *Seed Science Research*. 1995 Jun;**5**(2):61-73

Hilhorst HWM and Downie B. (1995). Primary dormancy in tomato (*Lycopersicon esculentum* cv. Moneymaker): studies with the *sitiens* mutant. *Journal of Experimental Biology*. 1995 Jan;**47**(294):89-97

Hilhorst HWM and Toorop PE. (1997). Review on dormancy, germinability, and germination in crop and weed seeds. *Advances in Agronomy*. 1997;**61**:112–165.

Himi E, Mares DJ, Yanagisawa A and Noda K. (2002). Effect of grain colour gene (R) on grain dormancy and sensitivity of the embryo to abscissic acid (ABA) in wheat. *Journal of Experimental Botany*. 2002 Jul;**53**(374):1569-1574

Ho LC and Hewitt JD. (1986). Fruit development. ln Atherton J.G. and Rudich J. (Eds). *The Tomato Crop. A Scientific Basis for lmprovement*. Chapman and Hall Ltd:201

Ho CH, Lin SH, Hu HC and Tsay YF. (2009). CHL1 functions as a nitrate sensor in plants. *Cell*. 2009 Sep;**138**(6):1184-1194

Hoekstra FA, Crowe JH and Crowe LM. (1992). Germination and ion leakage are linked with phase transitions of membrane lipids during imbibition of *Typha latifolia* pollen. *Physiologia Plantarum.* 1992 Jan;**84**(1):29-34

Hoekstra FA, Golovina EA, Van Aelst AC and Hemminga MA. (1999). Imbibitional leakage from anhydrobiotes revisited. *Plant, Cell & Environment*. 1999 Sep;**22**(9):1121-1131

Holdsworth MJ, Finch-Savage WE, Grappin P and Job D. (2008a). Post-genomics dissection of seed dormancy and germination. *Trends in Plant Science*. 2008 Jan;**13**(1):7-13

Holdsworth MJ, Bentsink L and Soppe WJJ. (2008b). Molecular network regulating Arabidopsis seed maturation, after-ripening, dormancy and germination. *New Phytologist*. 2008 Apr;**179**(1):33-54

Horii A, McCue P and Shetty K. (2007). Seed vigour studies in corn, soybean and tomato in response to fish protein hydrolysates and consequences on phenolic-linked responses. *Bioresource Technology*. 2007 Aug;**98**(11):2170-2177

Hrydziusko O and Viant M. (2012). Analytical and statistical approaches to metabolomics research. Journal of Separation Science. 2009 Jul;**32**(13):2183-2189

Husted S, Persson DP, Laursen KH, Hansen TH, Pedas P, Schiller M, Hegel JN and Schjoerring JK. (2011). The role of atomic spectrometry in plant science. *Journal of Analytical Atomic Spectrometry*. 2011;**26**:52-79

Hyun Y, Choi S, Hwang HJ, Yu J, Nam SJ, Ko J, Park JY, Seo YS, Kim EY, Ryu SB, Kim WT, Lee YH, Kang H and Lee I.(2008). Cooperation and functional diversification of two closely related galactolipase genes for jasmonate biosynthesis. *Developmental Cell*. 2008 Feb;**14**(2):183-192

Ishiguro S, Kawai-Oda A, Ueda J, Nishida I and Okada K. (2001). The DEFECTIVE IN ANTHER DEHISCENCE1 gene encodes a novel phospholipase A1 catalyzing the initial step of jasmonic acid biosynthesis, which synchronizes pollen maturation, anther dehiscence, and flower opening in Arabidopsis. *Plant Cell*. 2001 Oct;**13**(10):2191–2209

ISTA. (2006). International Rules for Seed Testing. Edition 2006. International Seed Testing Association, Switzerland

Itkin M, Rogatchev I, Alkan N, Rosenberg T, Malitsky S, Masini L, Lijima Y, Aoki K, de Vos R, Prusky D, Burdman S, Beekwilder J and Aharoni A. (2011). GLYCOALKALOID METABOLISM1 is required for steroidal alkaloid glycosylationa and prevention of phytotoxicity in tomato. *Plant Cell*. 2011 Dec;**23**(12):4507-4525

Jackson J, Lindroth AM, Cao X and Jacobsen SE. (2002). Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. *Nature*. 2002 Apr;**416**(6880):556–560

Jarvis SB, Taylor MA, Bianco J, Corbineau F and Davies HV. (1997). Dormancy-breakage in seeds of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco). Support for the hypothesis that LEA gene expression is essential for this purpose. *Journal of Plant Physiology*. 1997 Apr;**151**(4):457–464

Jia L, Wu Q, Ye N, Liu R, Shi L, Xu W, Zhi H, Rahman AN, Xia Y and Zhang J. (2012). Proanthocyanidins inhibit seed germination by maintaining a high level of abscisic acid in Arabidopsis thaliana. *Journal of Integrative Plant Biology*. 2012 Sep;**54**(9):663-673

Jia H, Suzuki M and McCarty DR. (2014). Regulation of the seed to seedling developmental phase transition by the LAFL and VAL transcription factor networks. *Wiley Interdisciplinary reviews. Developmental Biology*. 2014 Jan-Feb;**3**(1):135

Johanson U, West J, Lister C, Michaels S, Amasino R and Dean C. (2000). Molecular analysis of FRIGIDA, a major determinant of natural variation in Arabidopsis flowering time. *Science*. 2000 Oct;**290**(5490):344-347

Kallenbach M, Alagna F, Baldwin IT and Bonaventure G. (2010). *Nicotiana attenuata SIPK, WIPK, NPR1*, and fatty acid-amino acid conjugates participate in the induction of jasmonic acid biosynthesis by affecting early enzymatic steps in the pathway. *Plant Physiology*. 2010 Jan;**152**(1):96–106

Kanai M, Nishimura M and Hayashi M. (2010). A peroxisomal ABC transporter promotes seed germination by inducing pectin degradation under the control of ABI5. *The Plant Journal: for Cell and Molecular Biology*. 2010 Jun;**62**(6):936-947

Kanno Y, Jikumaru Y, Hanada A, Nambara E, Abrams SR, Kamiya Y and Seo M. (2010). Comprehensive hormone profiling in developing Arabidopsis seeds: examination of the site of ABA biosynthesis, ABA transport and hormone interactions. *Plant and Cell Physiology*. 2010 Dec;**51**(12):1988-2001

Karssen CM, Brinkhorst-van der Swan DL, Breekland AE and Koornneef M. (1983). Induction of dormancy during seed development by endogenous abscisic acid: studies on abscisic acid deficient genotypes of *Arabidopsis thaliana* (L.) Heynh. *Planta*. 1983 Mar;**157**(2):158-165

Kasahara H, Hanada A, Kuzuyama T, Takagi M, Kamiya Y and Yamaguchi S. (2002). Contribution of the mevalonate and methylerythritol phosphate pathways to the biosynthesis of gibberellins in Arabidopsis. *The Journal of Biological Chemistry*. 2002 Nov;**277**(47):45188-45194

Kaur H, Petla BP, Kamble NU, Singh A, Rao V, Salvi P, Gosh S and Majee M. (2015). Differentially expressed seed aging responsive heat shock protein OsHSP18.2 implicates in seed vigor, longevity and improves germination and seedling establishment under abiotic stress. *Frontiers in Plant Science*. 2015 Sep;**6**:713

Kaya MD, Okçu G, Atak M, Çikili Y, Kolsarici Ö. (2006). Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus L*.). *European Journal of Agronomy*. 2006 May;**24**(4):291–295

Kendall SL, Hellwege A, Marriot P, Whalley C, Graham IA and Penfield S. (2011). Induction of dormancy in Arabidopsis summer annuals requires parallel regulation of DOG1 and hormone metabolism by low temperature and CBF transcription factors. *Plant Cell*. 2011 Jul;**23**(7):2568-2580

Kepczynski J and Kepczinska E. (1997). Ethylene in seed dormancy and germination. *Physiologia Plantarum*. 1997;**101**:720-726

Keukens EA, de Vrije T, van den Boom C, de Waard P, Plasman HH, Thiel F, Chupin V, Jongen WM and de Kruijff B. (1995). Molecular basis of glycoalkaloid induced membrane disruption. *Biochimica et Biophysica Acta*. 1995 Dec;**1240**(2):216-228

Kim J, Guermah M and Roeder RG. (2010). The human PAF1 complex acts in chromatin transcription elongation both independently and cooperatively with SII/TFIIS. *Cell*. 2010 Feb;**140**(4):491–503

Kim JM, To TK and Seki M. (2012). An epigenetic integrator: new insights into genome regulation, environmental stress responses and developmental controls by HISTONE DEACETYLASE 6. *Plant & Cell Physiology*. 2012 May;**53**(5):794–800

Kirkbride RC, Fischer RL and Harada JJ. (2013). LEAFY COTYLEDON1, a key regulator of seed development, is expressed in vegetative and sexual propagules of *Selaginella moellendorffii*. *PLos One*. 2013 Jun;**8**(6):e67971

Khan AA. (1992). Preplant physiological seed conditioning. *Horticultural Reviews*. **14**:131

Khan N, Kazmi RH, Willems LAJ, van Heusden AW, Ligterink W, Hilhorst HWM. (2012). Exploring the natural variation for seedling traits and their link with seed dimensions in tomato. *PLoS One*. 2012 Aug;**7**(8):e43991

Ko JH, Yang SH and Han KH. (2006). Upregulation of an Arabidopsis RING-H2 gene, XERICO, confers drought tolerance through increased abscisic acid biosynthesis. The *Plant Journal*. 2006 Aug;**47**(3):343–35

Köhler C and Makarevich G. (2006). Epigenetic mechanisms governing seed development in plants. *EMBO reports*. 2006 Dec;**7**(12):1223-1227

Koornneef M and van der Veen J.H. (1980). Induction and analysis of gibberellin sensitive mutants in *Arabidopsis thaliana* (L.) Heynh. *TAG: Theoretical and Applied Genetics*. 1980 Nov;**58**(6):257–263

Koornneef M, Reuling G and Karssen CM. (1984). The isolation and characterization of ABA-insensitive mutants of *Arabidopsis thaliana*. *Physiologia Plantarum*. 1984 Jul;**61**(3):377-383

Koornneef M. (1990). Mutations affecting the testa colour in *Arabidopsis. Arabidopsis Information Service*. 1990 Feb;**27**:1–4

Koornneef M, Bentsink L and Hilhorst H. (2002). Seed dormancy and germination. *Current Opinion in Plant Biology*. 2002 Feb;**5**(1):33-36

Kong D, Ju C, Parihar A, Kim S, Cho D and Kwak JM. (2015). Arabidopsis glutamate receptor homolog3.5 modulates cytosolic Ca^{2+} level to counteract effect of abscisic acid in seed germination. *Plant Physiology*. 2015 Apr;**167**(4):1630-1642

Krock B, Schmidt B, Hertweck C and Baldwin IT. (2002). Vegetation-derived abscissic acid and four terpenes enforce dormancy in seed of the post-fire annual *Nicotiana attenuata*. *Seed Science Research*. 2002 Dec;**12**(4):239-252

Kugler I. (1951). Untersuchungen über das Keimverhalten einiger Rassen von *Arabidopsis thaliana* (L.) Heynh. Ein Beitrag zum Problem der Lichtkeimung. *Beitr. Biol. Pflanzen*. 1951;**286**(1):211–243

Kushiro T, Okamoto M, Nakabayashi K, Yamagishi K, Kitamura S, Asami T, Hirai N, Koshiba T, Kamiya Y and Nambara E. (2004). The Arabidopsis cytochrome P450 CYP707A encodes ABA 8'-hydroxylases: key enzymes in ABA catabolism. *EMBO Journal*. 2004 Apr;**23**(7):1647-1656

Kushwaha R, Lloyd TD, Schäfermeyer KR, Kumar S and Downie AB. (2012). Identification of late embryogenesis abundant (LEA) protein putative interactors using phage display. *International Journal of Molecular Sciences*. 2012 May;**13**(6):6582-6603

Lacey EP. (1996). Parental effects in *Plantago lanceolata* L.I. A growth chamber experiment to examine pre – and postzygotic temperature effects. *Evolution*. **50**:865-878

Lacey EP, Smith S and Case L. (1997). Parental effects on seed mass: seed coat but not embryo / endosperm effect. *American Journal of Botany*. 1997 Nov;**84**(11):1617-1620

Langfelder P and Horvath S. (2008). WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics*. 2008;**9**:559

Lanteri S, Saracco F, Kraak HL and Bino RJ. (1994). The effect of priming on nuclear replication activity and germination of pepper (*Capsicum annuum*) and tomato (*Lycopersicon esculentum*) seeds. *Seed Science Research*. **4**(2):81–87

Lanteri S, Nada E, Belleti P, Quagliotti L and Bino RJ. (1996). Effects of controlled deterioration and osmoconditioning on germination and nuclear replication in seeds of pepper (*Capsicum annuum* L.). *Annals of Botany*. **77**(6):591-597

Lee S, Cheng H, King KE, Wang W, He Y, Hussain A, Lo J, Harberd NP and Peng J. (2002). Gibberellin regulates Arabidopsis seed germination via RGL2, a GAI/RGA-like gene whose expression is up-regulated following imbibition. *Genes Development*. 2002 Mar;**16**(5):646–658

Lee HG, Lee K and Seo PJ. (2015a). The Arabidopsis MYB96 transcription factor plays a role in seed dormancy. *Plant Molecular Biology*. 2015 Mar;**87**(4-5):371-381

Lee K, Lee HG, Yoon S, Kim HU and Seo PJ. (2015b). The Arabidopsis MYB96 transcription factor is a positive regulator of ABSCISIC ACID-INSENTIVE4 in the control of seed germination. *Plant Physiology*. 2015 Jun;**168**(2):677-689

Lee SJ, Lee MH, Kim JI and Kim SY. (2015c). Arabidopsis putative MAP kinase kinase kinases Raf10 and Raf11 are positive regulators of seed dormancy and ABA response. *Plant & Cell Physiology*. 2015 Jan;**56**(1):84-97

Léon-Kloosterziel KM, Keuzer CJ and Koorneed M. (1994). A seed shape mutant of Arabidopsis that is affected in integument development. *Plant Cell*. 1994 Mar;**6**(3):385-392

Leubner-Metzger G. (2003). Functions and regulation of β-1,3-glucanases during seed germination, dormancy release and after-ripening. *Seed Science Research*. 2003 Mar;**13**(1):17-34

Li B and Foley ME. (1997). Genetic and molecular control of seed dormancy. *Trends in Plant Science*. 1997 Oct;**2**(10):384-389

Li J, Yin LY, Jongsma MA and Wang CY. (2011). Effects of light, hydropriming and abiotic stress on seed germination, and shoot and root growth of pyrethrum (*Tanacetum cinerariifolium*). *Industrial Crops Products*. 2011 Nov;**34**(3):1543-1549

Li Z., Zhang J., Liu Y., Zhao J., Fu J., Ren X., Wang G. and Wang J. (2016). Exogenous auxin regulates multi-metabolic network and embryo development, controlling seed secondary dormancy and germination in *Nicotiana tabacum* L. *BMC Plant Biology*. 2016 Feb;**16**:41

Linkies A, Müller K, Morris K, Tureckova V, Wenk M, Cadman CSC, Corbineau F, Strnad M, Lynn JR, Finch-Savage WE and Leubner-Metzger G. (2009). Ethylene interacts with abscisic acid to regulate endosperm rupture during germination: a comparative approach using *Lepidium sativum* and *Arabidopsis thaliana*. Plant Cell. 2009 Dec;**21**(12):3803-3822

Linkies A and Leubner-Metzger G. (2012). Beyond gibberellins and abscissic acid: how ethylene and jasmonates control seed germination. *Plant Cell Reports*. 2012 Feb;**31**(2):253-270

Liptay A and Schopfer P. (1983). Effect of water stress, seed coat restraint, and abscisic acid upon different germination capabilities of two tomato lines at low temperature. *Plant physiology*. 1983 Dec;**73**(4):935-938

Liu Y, Geyer R, van Zanten M, Carles A, Li Y, Hörold A, van Nocker S and Soppe WJJ. (2011). Identification of the Arabidopsis *REDUCED DORMANCY 2* gene uncovers a role for the polymerase associated factor 1 complex in seed dormancy. *PLoS ONE*. 2011; 6:e22241

Lobréaux S and Briat JF. (1991). Ferritin accumulation and degradation in different organs of pea (*Pisum sativum*) during development. *Biochemical Journal*. 1991 Mar;**274**(Pt 2):601-606

Loewus FA and Murthy PPN. (2000). *myo*-Inositol metabolism in platns. *Plant Science*. 2000 Jan;**150**(1):1-19

Lopez-Molina L, Mongrand S and Chua NH. (2001). A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in *Arabidopsis*. *Proceedings of the National of Sciences of the United States of America*. 2001 Apr;**98**(8):4782-4787

Low TY, van Heesch S, van den Toorn H, Giansanti P, Cristobal A, Toonen P, Schafer S, Hübner N, van Breukelen B, Mohammed S, Cuppen E, Heck AJ, Guryev V.

(2013). Quantitative and qualitative proteome characteristics extracted from in-depth integrated genomics and proteomics analysis. *Cell Reports*. 2013 Dec;**5**(5):1469-1478

Lu W, Bennett BD and Rabinowitz JD. (2008). Analytical strategies for LC-MS-based targeted metabolomics. *Journal of Chromatography B*. 2008 Aug;**871**(2):236-242

Luerssen H, Kirik V, Herrmann P and Miséra S. (1998). FUSCA3 encodes a protein with a conserved VP1/AB13-like B3 domain which is of functional importance for the regulation of seed maturation in *Arabidopsis thaliana*. *The Plant Journal: for Cell and Molecular Biology*. 1998 Sep;**15**(6):755-764

MacGregor DR, Kendall SL, Florance H, Fedi F, Moore K, Paszkiewicz K, Smirnoff N and Penfield S. (2015). Seed production temperature regulation of primary dormancy occurs through control of seed coat phenylpropanoid metabolism. *New Phytologist.* 2015 Jan;**205**(2): 642-652

Makkar HPS, Gamble G and Becker K. (1999). Limitation of the butanol-hydrochloridric acid-iron assay for bound condensed tannins. *Food Chemistry*. 1999;**66**:129-133

Malamy JE and Ryan KS. (2001). Environmental regulation of lateral root initiation in Arabidopsis. *Plant Physiology*. 2001 Nov;**127**(3):899-909

Mancinelli AL, Borthwick HA and Hendricks SB. (1966). Phytochrome action in tomato-seed germination. *International Journal of Plant Sciences*. 1966 Mar;**127**(1):1-5

Marin E, Nussaume L, Quesada A, Gonneau M, Sotta B, Hugueney P, Frey A and Marion-Poll A. (1996). Molecular identification of zeaxanthin epoxidase of *Nicotiana plumbaginifolia*, a gene involved in abscisic acid biosynthesis and corresponding to the ABA locus of *Arabidopsis thaliana*. *EMBO Journal*. 1996 May;**15**(10):2331-2342

Marschner H. (1995). Mineral nutrition of higher plants. *Academic Press* (Second Edition), London

Matsushima K and Sakagami J. (2013). Effects of seed hydropriming on germination and seedling vigor during emergence of rice under different soil moisture conditions. *American Journal of Plant Sciences*. 2013 Aug;**4**(8):1584-1593

Matthews S and Powell AA. (1981). Electrical conductivity test. In *Vigour Test Handbook* (Edition D.A. Perry), International Seed Testing Association. 1981;37-41

Matthews S and Powell AA. (2006). Electrical conductivity vigour test: physiological basis and use. *Seed Science*

McDonald MB. (1999). Seed deterioration: physiology repair and assessment. *Seed Sciences Technology*. 1999;**27**(1):177-213

McDonald MB. (2000). Seed priming. Black M, Bewley JD (eds), seed technology and its biological basis. Sheffield Academic Press Ltd., Sheffield, UK PP: 287-325

Michaels SD and Amasino RM. (1999). FLOWERING LOCUS C encodes a novel MADS domain protein that acts as a repressor of flowering. *Plant Cell*. 1999 May;**11**(5):949-956

Millaleo R, Reyes-Diaz M, Ivanov AG, Mora ML and Alberdi M. (2010). Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. *Journal of Soil Science and Plant Nutrition*. 2010;**10**(4):470-481

Morrison-Baird LA, Leopold AC, Bramlage WJ and Webster BD. (1979). Ultrastructural modifications associated with imbibition of the soybean radicle. *Botanical Gazette.* 1979 Dec;**140**(4):371-377

Müller K, Tintelnot S and Leubner-Metzger G. (2006). Endosperm-limited Brassicaceae seed germination: abscisic acid inhibits embryo-induced endosperm weakening of *Lepidium sativum* (cress) and endosperm rupture of cress and *Arabidopsis thaliana*. *Plant & Cell Physiology*. 2006 Jul;**47**(7):864-877

Murphy JB and Noland TL. (1982). Temperature effects on seed imbibition and leakage mediated by viscosity and mambranes. *Plant Physiology*. 1982 Feb;**69**(2):428-431

Nakabayashi K, Bartsch M, Xiang Y, Miatton E, Pellengahr S, Yano R, Seo M and Soppe WJ. (2012). The time required for dormancy release in Arabidopsis is determined by DELAY OF GERMINATION1 protein levels in freshly harvested seeds. *Plant Cell*. 2012 Jul;**24**(7):2826-2838

Nakabayashi K, Bartsch M, Ding J and Soppe WJ. (2015). Seed dormancy in arabidopsis requires self-binding ability of DOG1 protein and the presence of multiple isoforms generated by alternative splicing. *PLos Genetics*. 2015 Dec;**11**(12):e1005737

Ni BR and Bradford KJ. (1993). Germination and dormancy of abscisic acid- and gibberellin-deficient mutant tomato (*Lycopersicon esculentum*) seeds (sensitivity of germination to abscisic acid, gibberellin, and water potential). *Plant Physiology*. 1993 Feb;**101**(2):607-617

Nonogaki H, Gee OH and Bradford KJ. (2000). A germination-specific endo-beta-mannanase gene is expressed in the micropylar endosperm cap of tomato seeds. *Plant Physiology*. 2000 Aug;**123**(4):1235-1246

Nonogaki H. (2014). Seed dormancy and germination – emerging mechanisms and new hypotheses. *Frontiers in Plant Science*. 2014 May;**5**:233

Ogawa M, Hanada A, Yamauchi Y, Kuwahara A, Kamiya Y and Yamaguchi S. (2003). Gibberellin biosynthesis and response during Arabidopsis seed germination. *The Plant Cell*. 2003 Jul;**15**(7):1591-1604

Oh E, Yamaguchi S, Kamiya Y, Bae G, Chung WI and Choi G. (2006). Light activates the degradation of PIL5 protein to promote seed germination through gibberellin in Arabidopsis. *The Plant Journal*. 2006 Jul;**47**(1):124-139

Oh E, Yamaguchi S, Hu J, Yusuke J, Jung B, Paik I, Lee HS, Sun TP, Kamiya Y and Choi G. (2007). PIL5, a phytochrome-interacting bHLH protein, regulates gibberellin responsiveness by binding directly to the GAI and RGA promoters in Arabidopsis seeds. *Plant Cell*. 2007 Apr;**19**(4):1192–1208

Otegui MS, Capp R and Staehelin LA. (2002). Developing seeds of Arabidopsis store different minerals in two types of vacuoles and in the endoplasmic reticulum. *Plant Cell*. 2002 Jun;**14**(6):1311-1327

Pandita VK and Nagarajan S. (2002). Germination behaviour and field performance of garden pea (Pisum sativum) in relation to seed ageing. *Indian Journal of Agricultural Sciences*. 2008 Apr;**72**(4):213-215

Panobianco M and Marcos-Filho J. (2001). Evaluation of the physiological potential of tomato seeds by germination and vigor tests. *Seed Technology*. 2001;**23**(2):151

Passam HC and Kakouriotis D. (1994). The effect of osmoconditioning on the germination, emergence and early plant growth of cucumber under saline stress. *Scientia Horticulturae*. 1994 Apr;**57**(3):233-240

Parera CA and Cantliffe DJ. (1994). Presowing seed priming. *Horticultural Reviews*. 1994;**16**(16):109-141

Penfield S, Josse EM, Kannangara R, Gilday AD, Halliday KJ and Graham IA. (2005). Cold and light control seed germination through the bHLH transcription factor SPATULA. *Current Biology: CB*. 2005 Nov;**15**(22):1998-2006

Penfield S, Li Y, Gilday AD, Graham S and Graham IA. (2006). Arabidopsis ABA INSENSITIVE4 regulates lipid mobilization in the embryo and reveals repression of seed germination by the endosperm. *The Plant Cell*. 2006 Aug;**18**(8):1887-1899

Penfield S and Springthorpe V. (2012). Understanding chilling responses in Arabidopsis seeds and their contribution to life history. *Philosophical transaction of the Royal Society B: Biological Sciences*. 2012 Jan;**367**(1586):291-297

Phillips J, Artsaenko O, Fiedler U, Horstmann C, Mock HP, Muntz K and Conrad U. (1997). Seed-specific immunomodulation of abscisic acid activity induces a developmental switch. *EMBO Journal*. 1997 Aug;**16**(15):4489-4496

Pinfield-Wells H, Rylott E, Gilday A, Graham S, Job K, Larson TR and Graham IA. (2005). Sucrose rescues seedling establishment but not germination of Arabidopsis mutants disrupted in peroxisomal fatty acid catabolism. *The Plant Journal: for Cell & Molecular Biology*. 2005 Sep;43(6):861–872

Piskurewicz U, Jikumaru Y, Kinoshita N, Nambara E, Kamiya Y and Lopez-Molina L. (2008). The gibberellic acid signaling repressor RGL2 inhibits *Arabidopsis* seed germination by stimulating abscisic acid synthesis and ABI5 activity. *Plant Cell.* 2008 Oct;**20**(10):2729–2745

Platenkamp GAJ and Shaw RG. (1993). Environmental and genetic maternal effects on seed characters in *Nemophila-menziesii*. *Evolution* **47**(2): 540-555

Porri A, Torti S, Romera-Branchat M and Coupland G. (2012). Spatially distinct regulatory roles for gibberellins in the promotion of flowering of Arabidopsis under long photoperiods. *Development*. 2012 Jun;**139**(12):2198-2209

Porter LJ, Hrstich LN and Chan BG. (1985). The conversion of proanthocyanidins and prodelphinidins to cyanidins and delphinidins. *Phytochemistry*. 1985 Dec;**25**(1):223-230

Postma FM and Agren J. (2015). Maternal environment affects the genetic basis of seed dormancy in *Arabidopsis thaliana*. *Molecular Ecology*. 2015 Feb;**24**(4):785-797

Powell AA and Matthews S. (1978). The damaging effect of water on dry pea embryos during imbibition. *Journal of Experimental Botany*. 1978 Oct;**29**(5):1215-1229

Powell AA and Matthews S. (1981). A physical explanation for solute leakage from dry pea embryos during imbibition. *Journal of Experimental Botany*. 1981 Oct;**32**(5):1045-1050

Powell AA, Matthews S. (2012). Seed aging/repair hypothesis leads to new testing methods. *Seed Technology*. 2012;**34**(1):15–25

Preston J, Tatematsu K, Kanno Y, Hobo T, Kimura M, Jikumaru Y, Yano R, Kamiya Y and Nambara E. (2009). Temporal expression patterns of hormone metabolism gene during imbibition of *Arabidopsis thaliana* seeds: a comparative study on dormant and non-dormant accessions. *Plant & Cell Physiology*. 2009 Aug;**50**(10):1786-1800

Prieto-Dapena P, Castano R, Almoguera C and Jordano J. (2006). Improved resistance to controlled deterioration in transgenic seeds. *Plant Physiology*. 2006 Nov;**142**(3):1102-1112

Rajjou L, Lovigny Y, Groot SPC, Belghazi M, Job C and Job D. (2008). (2008). Proteome-wild characterization of seed aging in *Arabidopsis*: a comparison between artificial and natural aging protocols. *Plant Physiology*. 2008 Sep;**148**(1):620-641

Rajjou L, Duval M, Gallardo K, Catusse J, Bally J, Job C and Job D. (2012). Seed germination and vigor. *Annual Review of Plant Biology*. 2012 Jun;**63**:507-533

Ranjan R and Lewak S. (1992). Jasmonic acid promotes germination and lipase activity in non-stratified apple embryos. *Physiologia Plantarum*. 1992 Oct;**86**(2):335-339

Rao NK, Hanson J, Dulloo ME, Ghosh K, Nowell D and Larinde M. (2006). Manual of seed handling in genebanks*. Handbook for Genebanks No. 8*. Bioversity International, Rome, Italy

Ravet K, Touraine B, Boucherez J, Briat JF, Gaymard F and Cellier F. (2009). Ferritins control interaction between iron homeostasis and oxidative stress in Arabidopsis. *The Plant Journal: for cell and molecular biology*. 2009 Feb;**57**(3):400-412

Reidy B, McQueen-Mason S, Nösberger J and Fleming A. (2001). Differential expression of α- and β-expansin genes in the elongating leaf of *Festuca pratensis*. *Plant Molecular Biology*. 2001 Jul;**46**(4):491-504

Roach DA and Wulff RD. (1987). Maternal effects in plants. *Annual Review of Ecology and Systematics*. 18:209-235

Robert C, Noriega A, Tocino A and Cervantes E. (2008). Morphological analysis of seed shape in *Arabidopsis thaliana* reveals altered polarity in mutants of the ethylene signalling pathway. *Journal of Plant Physiology*. 2008 Jun;**165**(9):911-919

Roff DA. (1998). The detection and measurement of maternal effects. In: Mousseau T., Fox C., editors. Maternal effects as adaptations. Oxford University Press; Oxford, UK: 1998, pp. 11–83

Rook F, Corke F, Card R, Munz G, Smith C and Bevan MW. (2001). Impaired sucrose-induction mutants reveal the modulation of sugar-induced starch biosynthetic gene expression by abscisic acid signalling. *The Plant Journal: for cell and molecular biology*. 2001 May;**26**(4):421-433

Rosental L, Perelman A, Nevo N, Toubiana D, Samani T, Batushansky A, Sikron N, Saranga Y and Fait A. (2016). Environmental and genetic effects on tomato seed metabolic balance and its association with germination vigor. *BMC Genomics*. 2016 Dec;**17**(1):1047

Roschzttardtz H, Grillet L, Isaure MP, Conéjéro G, Ortega R, Curie C and Mari S. (2011). Plant cell nucleolus as a hot spot for iron. *The Journal of Biological Chemistry*. 2011 Aug;**286**(32):27863-27866

Routaboul JM, Dubos C, Marquis C, Bidzinski P, Loudet O and Lepiniec L. (2012). Metabolite profiling and quantitative genetics of natural variation for flavonoids in Arabidopsis. *Journal of Experimental Botany*. 2012 Jun;**63**(10):3749-3764

Rudell DR, Mattheis JP, Fan X and Fellman JK. (2002). Methyl jasmonate enhances anthocyaninaccumulation and modifies production of phenolics and pigments in Fuji apples. *Journal of the American Society for Horticultural Science*. 2002;**127**:435–41

Saito S, Hirai N, Matsumoto C, Ohigashi H, Ohta D, Sakata K and Mizutani M. (2004). Arabidopsis CYP707As encode (+)-abscisic acid 8'-hydroxylase, a key enzyme in the oxidative catabolism of abscisic acid. *Plant Physiology*. 2004 Apr;**134**(4):1439

Saldanha AJ. (2004). Java Treeview--extensible visualization of microarray data. *Bioinformatics*. 2004 Nov;**20**(17):3246-3248

Sanchez DH, Siahpoosh MR, Roessner U, Udvardi M and Kopka J. (2008). Plant metabolomics reveals conserved and divergent metabolic responses to salinity. *Physiologia Plantarum*. 2008 Feb;**132**(2):209-219.

Sanders PM, Lee PY, Biesgen C, Boone JD, Beals TP, Weiler EW and Goldberg RB. (2000). The Arabidopsis DELAYED DEHISCENCE1 gene encodes an enzyme in the jasmonic acid synthesis pathway. *Plant Cell*. 2000 Jul;**12**(7):1041–1061

Sano N, Rajjou L, North HM, Deabeaujon I, Marion-Poll and Seo M. (2016). Staying alive: molecular aspects of seed longevity. *Plant & Cell Physiology*. 2016 Apr;**57**(4):660-674

Sato D, Awad AA, Chae SH, Yokota T, Sugimoto Y, Takeuchi Y and Yoneyama K. (2003). Analysis of strigolactones, germination stimulants for *Striga* and *Orobranche*, by high-performance liquid chromatography/tandem mass spectrometry. *Journal of Agricultural and Food Chemistry*. 2003 Feb;**51**(5):1162

Schaller F, Biesgen C, Müssig C, Altmann T and Weiler EW. (2000). 12-Oxophytodienoate reductase 3 (OPR3) is the isoenzyme involved in jasmonate biosynthesis. *Planta*. 2000 May;**210**(6):979-984

Schauer N, Zamir D and Fernie AR. (2005). Metabolic profiling of leaves and fruit of wild species tomato: a survey of the *Solanum lycopersicum* complex. *Journal of Experimental Botany*. 2005 Jan;**56**(410):297-307

Schilmiller AL, Koo AJ and Howe GA. (2007). Functional diversification of acyl-coenzyme A oxidases in jasmonic acid biosynthesis and action. *Plant Physiology*. 2007 Feb;**143**(2):812–82

Schmid B and Dolt C. (1994). Effects of maternal and paternal environment and genotype on offspring phenotype in *Solidago altissima* L. *Evolution*. **48**:1525–1549

Schmitt J, Niles J and Wulff RD. (1992). Norms of reaction of seed traits to maternal environments in *Plantago lanceolata*. *The American Naturalist*. 1992 Mar;**139**(3):451-466

Schmuths H, Bachmann K, Weber EW, Horres R and Hoffmann MH. (2006). Effects of preconditioning and temperature during germination of 73 natural accessions of *Arabidopsis thaliana*. *Annals of Botany*. 2006 Apr;**97**(4):623-634

Schopfer P and Plachy C. (1984). Control of seed germination by abscisic acid: II. Effect on embryo water uptake in *Brassica napus L*. *Plant Physiology*. 1984 Sep;**76**(1):155-160

Schwartz SH, Tan BC, Gage DA, Zeevaart JA and McCarty DR. (1997). Specific oxidative cleavage of carotenoids by VP14 of maize. *Science*. 1997 Jun;**276**(5320):1872-1874

Schwartz SH, Qin X and Zeevaart JA. (2003). Elucidation of the indirect pathway of abscisic acid biosynthesis by mutants, genes and enzymes. *Plant Physiology*. 2003 Apr;**131**(4):1591-1601

Searle I, He Y, Turck F, Vincent C, Fornara F, Kröber S, Amasino RA and Coupland G. (2006). The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in Arabidopsis. *Genes & Development*. 2006 Apr;**20**(7):898-912

Senaratna T and McKersie BD. (1983). Characterization of solute efflux from dehydration injured soybean (*Glycine max* L. Merr) seeds. *Plant Physiology*. 1983 Aug;**72**(4):911-914

Seo M, Nambara E, Choi G and Yamaguchi S. (2009). Interaction of light and hormone signals in germinating seeds. *Plant Molecular Biology*. 2009 Mar;**69**(4):463–472

Sethy SK and Ghosh S. (2013). Effect of heavy metals on germination of seeds. *Journal of Natural Science, Biology and Medicine*. 2013 Jul-Dec;**4**(2):272-275

Shao S, Meyer CJ, MA F, Peterson CA and Bernards MA. (2007). The outermost cuticle of soybean seeds: chemical composition and function during imbibition. *Journal of experimental Botany*. 2007 Jan;**58**(5):1071-1082

Sheldon CC, Rouse DT, Finnegan EJ, Peacock WJ and Dennis ES. (2000). The molecular basis of vernalization: the central role of FLOWERING LOCUS C (FLC). *Proceedings of the National Academy of Sciences of the United States of America*. 2000 Mar;**97**(7):3753-3758

Sheoran IS, Olson DJ, Ross AR and Sawhney VK. (2005). Proteome analysis of embryo and endosperm from germinating tomato seeds. *Proteomics*. 2005 Sep;**5**(14):3752-3764

Shichijo C, Katada K, Tanaka O and Hashimoto T. (2001). Phytochrome A-mediated inhibition of seed germination in tomato. *Planta*. 2001 Sept; **213**(5):764-769

Shivankar RS, Deore DB and Zode NG. (2003). Effect of pre-sowing seed treatment on establishment and seed yield of sunflower. *Journal of Oilseeds Research*. 20:299

Shu XL, Frank T, Shu QY and Engel KH. (2008). Metabolite profiling in germinating rice seeds. *Journal of Agricultural and Food Chemistry*. 2008 Dec;**56**(24):11612-11620

Shu K, Zhang H, Wang S, Chen M, Wu Y, Tang S, Liu C, Feng Y, Cao X and Xie Q. (2013). ABI4 regulates primary seed dormancy by regulating the biogenesis of abscisic acid and gibberellins in arabidopsis. *PLoS Genetics*. 2013 Jun;**9**(6):e1003577

Shu K, Chen Q, Wu Y, Liu R, Zhang H, Wang S, Tang S, Yang W and Xie Q. (2016). ABSCISIC ACID-INSENSITIVE 4 negatively regulates flowering through directly promoting Arabidopsis FLOWERING LOCUS C transcription. *Journal of Experimental Biology*. 2016 Jan;**67**(1):195-205

Sidaway-Lee K, Josse EM, Brown A, Gan Y, Halliday KJ, Graham IA and Penfield S. (2010). SPATULA links daytime temperature and plant growth rate. *Current Biology: CB*. 2010 Aug;**20**(16):1493-1497

da Silva EA, Toorop PE, van Aelst AC and Hilhorst HW. (2004). Abscisic acid controls embryo growth potential and endosperm cap weakening during coffee (*Coffea arabica* cv. Rubi) seed germination. *Planta*. 2004 Dec;**220**(2):251-261

Simon EW and Raja-Harun RM. (1972). Leakage during seed imbibition. *Journal of Experimental Botany*. 1972;**23**:1076-1085

Simon EW. (1984). Early events in germination. In: Seed Physiology. Volume 2: Germination and Reserve Mobilization, Murray, D.R. (Ed.). Academic Press, New York, USA

Singh DK and Bharti S. (1985). Seed manganese content and its relationship with the growth characteristic of wheat cultivars. *New Phytologist.* 1985 Nov;**101**(3):387

Singh BG. (1995). Effect of hydration-dehydration seed treatments on vigour and yield of sunflower. *Indian Journal of Plant physiology*. **38**:66-68

Singletary GW and Below FE. (1989). Growth and composition of maize kernels cultured *in vitro* with varying supplies of carbon and nitrogen. *Plant Physiology*. 1989 Jan;**89**(1):341-346

Smith JE, Moore K and Schoneweis D. (1981). Coulometric technique for iron determination. *American Journal of Veterinary Research*. 1981 Jun;**42**(6):1084

Smith FE, Herbert J, Gaudin J, Hennessy DJ and Reid GR. (1984). Serum iron determination using ferene triazine. *Clinical Biochemistry*. 1984 Oct;**17**(5):306-310

Solgenomics. ftp.solgenomics.net/tomato_genome

de Souza MO, Pelacani CR, Willems LAJ, de Castro RD, Hilhorst HWM and Ligterink W. (2016). Effect of osmopriming on germination and initial growth of *Physalis angulata* L. under salt stress and expression of associated genes. *Annuals of the Brazilian Academy of Sciences*. 2016 Apr;**88**(Suppl. 1):503-516

Stenzel I, Hause B, Maucher H, Pitzschke A, Miersch O, Ziegler J, Ryan CA and Wasternack C. (2003). Allene oxide cyclase dependence of the wound response and vascular bundle-specific generation of jasmonates in tomato-amplification in wound signalling. *The Plant Journal*. 2003 Feb;**33**(3):577-589

Stintzi A and Browse J. (2000). The Arabidopsis male-sterile mutant, opr3, lacks the 12-oxophytodienoic acid reductase required for jasmonate synthesis. *Proceedings of the National Academy of Science of the United States of America*. 2000 Sep;**97**(19):10625-10630

Sung S and Amasino RM. (2004). Vernalization in Arabidopsis thaliana is mediated by the PHD finger protein VIN3. *Nature*. 2004 Jan;**427**(6970):159-164

Tanaka M and Fujiwara T. (2008). Physiological roles and transport mechanisms of boron: perspectives from plants. *Pflugers Archiv: European Journal of Physiology*. 2008 Jul;**456**(4):671-677

Tarquis AM and Bradford JK. (1992). Prehydration and priming treatments that advance germination also increase the rate of deterioration of lettuce seeds. *Journal of Experimental Botany*. 1992;**43**(3):307-317

Taylor AG, Allen PS, Bennett MA, Bradford KJ, Burris JS and Misra MK. (1998). Seed enhancements. *Seed Science Research*. 1998 Jun;**8**(2):245-256

Tetteroo FAA, De Bruijn AY, Henselmans RNM, Wolkers WF, Van Aelst AC and Hoekstra FA. (1996). Characterization of membrane properties in desiccation-tolerant and -intolerant carrot somatic embryos. *Plant Physiology.* 1996 Jun;**111**(2):403-412

Theodoulou FL, Job K, Slocombe SP, Footitt S, Holdsworth M, Baker A, Larson TR and Graham IA. (2005). Jasmonic acid levels are reduced in COMATOSE ATP-Binding cassette transporter mutants. Implications for transport of jasmonate precursors into peroxisomes. *Plant Physiology*. 2005 Mar;**137**(3):835-840

Toh S, Imamura A, Watanabe A, Nakabayashi K, Okamoto M, Jikumaru Y, Hanada A, Aso Y, Ishiyama K, Tamura N, Iuchi S, Kobayashi M, Yamaguchi S, Kamiya Y, Nambara E and Kawakami N. (2008). High temperature-induced abscisic acid biosynthesis and its role in the inhibition of gibberellin action in Arabidopsis seeds. *Plant Physiology*. 2008 Mar;**146**(3):1368-1385

Toorop PE, van Aelst AC and Hilhorst HW. (2000). The second step of the biphasic endosperm cap weakening that mediates tomato (*Lycopersicon esculentum*) seed germination is under control of ABA. *Journal of Experimental Botany*. 2000 Aug;**51**(341):1371-1379

Torres CA, Davies NM, Yañes JA and Andrews PK. (2005). Disposition of selected flavonoids in fruit tissues of various tomato (lycopersicon esculentum mill.) genotypes. *Journal of Agricultural and Food Chemistry*. 2005 Nov;**53**(24):9536

Toubiana D, Semel Y, Tohge T, Beleggia R, Cattivelli L, Rosental L, Nikoloski Z, Zamir D, Fernie AR and Fait A. (2012). Metabolic profiling of a mapping populations exposes new insights in the regulation of seed metabolism and seed, fruit, and plant relations. *PloS Genetics*. 2012 Mar;**8**(3):e1002612

Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, Pimentel H, Salzberg SL, Rinn JL and Pachter L. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nature Protocols*. 2012 Mar;**7**(3):562-578

Tyler L, Thomas SG, Hu J, Dill A, Alonso JM, Ecker JR and Sun TP. (2004). Della proteins and gibberellin-regulated seed germination and floral development in Arabidopsis. *Plant Physiology*. 2004 Jun;**135**(2):1008–1019

Usadel B, Poree F, Nagel A, Lohse M, Czedik-Eysenberg A and Stitt M. (2009). A guide to using MapMan to visualize and compare Omics data in plants: a case study in the crop species, Maize. *Plant, Cell & Environment*. 2009 Sep;**32**(9):1211

Vick BA and Zimmerman DC. (1983). The biosynthesis of jasmonic acid: a physiological role for plant lipoxygenase. *Biochemical and Biophysical Research Communications*. 1983 Mar;**111**(2):470-477

Vidal EA and Gutiérrez RA. (2008). A systems view of nitrogen nutrient and metabolite responses in Arabidopsis. *Current Opinion in Plant Biology*. 2008 Oct;**11**(5):521

Vleeshouwers LM, Bouwmeester HJ and Karssen CM. (1995). Redefining seed dormancy: an attempt to integrate physiology and ecology. *Journal of Ecology*. 1995 Dec;**83**(6):1031-1037

Vuckovic D. (2012). Current trends and challenges in sample preparation for global metabolomics using liquid chromatography-mass spectrometry. *Analytical and Bioanalytical Chemistry*. 2012 Jun;**403**(6):1523-1548

Wada T and Lott NA. (1997). Light and electron microscopic and energy dispersive X-ray microanalysis studies of globoids in protein bodies of embryo tissues and the aleurone layer of rice (*Otyza sativa* L.) grains. *Canadian Journal of Botany*. 1997;**75**(7):1137-1147

Waldo GS, Wright E, Whang ZH, Briat JF, Theil EC and Sayers DE. (1995). Formation of the ferritin iron mineral occurs in plastids. *Plant Physiology*. 1995 Nov;**109**(3):797-802

Walker EL and Waters BM. (2011). The role of transition metal homeostasis in plant seed development. *Current Opinion in Plant Biology*. 2011 Jun;**14**(3):318-324

Wang Y, Liu C, Li K, Sun F, Hu H, Li X, Zhao Y, Han C, Zhang W, Duan Y, Liu M and Li X. (2007). *Arabidopsis EIN2* modulates stress response through abscisic acid response pathway. *Plant Molecular Biology*. 2007 Aug;**64**(6):633-644

Wang Z, Gerstein M and Snyder M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. *Nature Reviews. Genetics*. 2009 Jan;**10**(1):57-63

Wang YY, Hsu PK and Tsay YF. (2012). Uptake, allocation and signalling of nitrate. *Trends in Plant Science*. 2012 Aug,**17**(8):458-467

Wang Z, Cao H, Sun Y, Li X, Chen F, Carles A, Li Y, Ding M, Zhang C, Deng X, Soppe WJ and Liu YX. (2013). Arabidopsis paired amphipathic helix proteins SNL1 and SNL2 redundantly regulate primary seed dormancy via abscisic acid-ethylene antagonism mediated by histone deacetylation. *Plant Cell*. 2013 Jan;**25**(1):149

Wang X, Zhang L, Xu X, Qu W, Li J, Xu X and Wang A. (2016). Seed development and viviparous germination in one accession of a tomato rin mutant. *Breeding Science*. 2016 Jun;**66**(3):372-380

Wasternack C and Hause B. (2002). Jasmonate and octadecanoids: signals in plant stress responses and development. *Progress in Nucleic Acid Research and Molecular Biology*. 2002;**72**:165-221

Wasternack C. (2007). Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. *Annals of Botany*. 2007 Oct;**100**(4):681-697

Wasternack C, Goetz S, Hellwege A, Forner S, Strnad M and Hause B. (2012). Another JA/COI1-independent role of OPDA detected in tomato embryo development. *Plant Signaling & Behavior*. 2012 Oct;**7**(10):1349-1353

Weber H, Borisjuk L and Wobus U. (1996). Controlling seed development and seed size in *Vicia faba*: a role for seed coat-associated invertases and carbohydrate state. *The Plant Journal*. 1996;**10**(5):823-834

Weber H, Vick BA and Farmer EE. (1997). Dinor-oxo-phytodienoic acid: a new hexadecanoid signal in the jasmonate family. *Proceedings of the National Academy of Sciences of the United States of America*. 1997 Sep;**94**(19):10473

Webster BD and Leopold AC. (1977). The ultrastructure of dry and imbibed cotyledons of soybean. *American Journal of Botany*. 1977 Dec;**64**(10):1286- 1293

Weges R and Karssen CM. (1990). The influence of redesiccation on dormancy and K^+ leakage of primed lettuce seeds. *Israel Journal of Botany*. 1990;**39**(4-6):327-336

Wei Y, Shohag MJI, Ying F, Yang X, Wu C and Wang Y. (2013). Effect of ferrous sulfate fortification in germinated brown rice on seed iron concentration and bioavaibility. *Food Chemistry*. 2013 Jun;**138**(2-3):1952-1958

Weitbrecht K, Müller K and Leubner-Metzger G. (2011). Fisrt off the mark: early seed germination. *Journal of Experimental Botany*. 2011 Mar;**62**(10):3289-3309

Welbaum GE and Bradford KJ. (1990). Water relations of seed development and germination in muskmelon (*Cucumis melo L*.) : IV. Characteristics of the perisperm during seed development. *Plant Physiology*. 1990 Apr;**92**(4):1038-1045

Wen PF, Chen JY, Kong WF, Pan QH, Wan SB and Huang WD. (2005). Salicylic acid induced the expression of phenylalanine ammonia-lyase gene in grape berry. *Plant Sci*. 2005;**169**:928–34

Westoby M, Jurado E and Leishman M. (1992). Comparative evolutionary ecology of seed size. *Trends in Ecology and Evolution*. 1992 Nov;**7**(11):368-372

Winkel-Shirley B. (2001). Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology and biotechnology. *Plant Physiology*. 2001 Jun;**126**(2):485-493

Wobus U and Weber H. (1999). Sugar as signal molecules in plant seed development. *Biological Chemistry*. 1999 Jul-Aug;**380**(7-8):937-944

Wu CT, Leubner-Metzger G, Meins FJ and Bradford KJ. (2001a). Class I beta-1,3-glucanase and chitinase are expressed in the micropylar endosperm of tomato seeds prior to radical emergence. *Plant Physiology*. 2001 Jul;**126**(3):1299-1313

Wu Y, Meeley RB and Cosgrove DJ. (2001b). Analysis and expression of the α-expansin and β-expansin gene families in maize. *Plant Physiology*. 2001 May;**126**(1):222

Wu J and Baldwin IT. (2010). New insights into plant responses to the attack from insect herbivores. *Annual Review of Genetics*. 2010 Dec;**44**:1-24

Yamagushi S. (2008). Gibberellin metabolism and its regulation. *Annual Review of Plant Biology*. 2008;**59**:225-251

Yamauchi Y, Ogawa M, Kuwahara A, Hanada A, Kamiya Y and Yamaguchi S. (2004). Activation of gibberellin biosynthesis and response pathways by low temperature during imbibition of *Arabidopsis thaliana* seeds. *Plant Cell*. 2004 Feb;**16**(2):367

Yang SF and Hoffman NE. (1984). Ethylene biosynthesis and its regulation in higher plants. *Annual Review of Plant Physiology*. 1984 Jun;**35**:155-189

Yano R, Takebayashi Y, Nambara E, Kamiya Y and Seo M. (2013). Combining association mapping and transcriptomics identify *HD2B* histone deacetylase as a genetic factor associated with seed dormancy in *Arabidopsis thaliana*. *The Plant Journal*. 2013 Jun;**74**(5):815–828

Yildiz K, Muradoglu F and Yilmaz H. (2008). The effect of jasmonic acid on germination of dormant and nondormant pear (*Pyrus communis* L.) seeds. *Seed Science and Technology*. 2008 Oct;**36**(3):569-574

Zhang T, Liao X, Yang R, Xu C and Zhao G. (2013). Different effects of iron uptake and release by phytoferritin on starch granules. *Journal of Agricultural and Food Chemistry*. 2013 Aug;**61**(34):8215-8223

Zhang W, Yan H, Chen W, Liu J, Jiang H, Zhu S and Cheng B. (2014). Genome-wide identification and characterization of maize expansin genes expressed in endosperm. *Molecular Genetics and Genomics: MGG*. 2014 Dec;**289**(6):1061

Zhao M, Yang S, Liu X and Wu K. (2015). Arabidopsis histone demethylases LDL1 and LDL2 control primary seed dormancy by regulating DELAY OF GERMINATION 1 and ABA signaling-related genes. *Frontiers in Plant Science*. 2015 Mar;**6**:159

Zheng J, Chen F, Wang Z, Cao H, Li X, Deng X, Soppe WJ, Li Y and Liu Y. (2012). A novel role for histone methyltransferase KYP/SUVH4 in the control of Arabidopsis primary seed dormancy. *The New Phytologist*. 2012 Feb;**193**(3):605-616

Zhou C, Zhang L, Duan J, Miki B and Wu K. (2005). HISTONE DEACETYLASE19 is involved in jasmonic acid and ethylene signaling of pathogen response in Arabidopsis. *Plant Cell*. 2005 Apr;**17**(4):1196-1204

Zhou Y, Tan B, Luo M, Li Y, Liu C, Chen C, Yu CW, Yang S, Dong S, Ruan J, Yuan L, Zhang Z, Zhao L, Li C, Chen H, Cui Y, Wu K and Huang S. (2013). HISTONE DEACETYLASE19 interacts with HSL1 and participates in the repression of seed maturation genes in Arabidopsis seedlings. *Plant Cell*. 2013 Jan;**25**(1):134-148

Zielińska-Dawidziak M and Siger A. (2012). Effect of elevated accumulation of iron in ferritin on the antioxidants content in soybean sprouts. *European Food Research and Technology*. 2012 Jun;**234**(6):1005-1012

Zulueta-Rodriguez R, Hernández-Montiel LG, Murillo-Amador B, Rueda-Puente EO, Capistrán LL, Troyo-Diéguez E and Córdoba-Matson MV. (2015). Effect of Hydropriming and Biopriming on Seed Germination and Growth of Two Mexican Fir Tree Species in Danger of Extinction. *Forests*. 2015;**6**:3109-3122