Influence of post-anthesis drought stress on germination and malting quality in barley

Dissertation
zur Erlangung des Doktorgrades
der Naturwissenschaften (Dr. rer. nat.)

der
Naturwissenschaftlichen Fakultät
– Biowissenschaften –
der Martin-Luther-Universität
Halle-Wittenberg

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geb. am: 17.04.1986 in: Koper, Slowenien

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Datum der Verteidigung: 06.03.2019

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Abbreviation

AAO	Abscisic aldehyde oxidase
ABA	Abscisic acid
ABA'8-OH	Abscisic acid hydroxylase
ABA-GE	Abscisic acid glucose ester
ABF	ABA binding factor
ABI	ABA-insensitive
ABRE	ABA responsive element
AGPP	ADP-glucose pyrophosphorylase
ANOVA	Analysis of variance
BA	Brassinosteroids
BHB	Benjamini Hochberg
bZIP	Basic leucine zipper
C	Carbon
cDNA	Complementary DNA
CHX	Cycloheximide
CK	Cytokinin
CPC	Chloramphenicol
DAF	Days after flowering
DAP	Days after pollination
DAS	Days after stress
DBF	Days before flowering
DH	Double haploid
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
DPA	Dihydrophaseic acid
DTT	Dithiothreitol
EGTA	Ethylene glycol tetra acetic acid
ER	Endoplasmatic reticulum
ETH	Ethylene
EU	European Union
FLA	Final limit of attenuation
FDR	False discovery rate
GA	Gibberellic acid
GAMYB	Gibberellin and abscisic acid-regulated MYB
GBSS	Granule-bound starch synthase
GID1	GA-insensitive dwarf 1
HCL	Hierarchical clustering
HSP	Heat Shock protein
Hv	*Hordeum vulgare*
IAA	Indole-3-acetic acid (Auxin)
JA	Jasmonate
KI	Kolbach index
LC-ESI-MS/MS	Liquid chromatography tandem mass spectrometry
LEA	Late embryogenesis abundant
LoHi	Hv<u>L</u>ea::Hv8'-hydroxylase RNAi construct lines
LOX	Lipoxygenase
LTP	Lipid transfer protein
ME	Malt extract

MMC	Malt moisture content
mPa*s	Millipascal seconds
N	Nitrogen
NCED	9-cis-epoxycarotenoid dioxygenase
Os	*Oryza sativa*
P	Protein
PA	Phaseic acid
PF	Polysome fraction
PIMT	Protein L-isoaspartyl methyltransferase
PP2C	Type 2C protein phosphatase
PPP	Pentose phosphate pathway
PYL	PYR-like protein
PYL	PYR-like protein
qRT PCR	Quantitative real time polymerase chain reaction
QTL	Quantitative trait locus
RCAR	Regulatory component of ABA receptor
RGA	Repressor of *ga1-3* mutant
RGL	Root growth loss
RL	Respiration loss
RNA	Ribonucleic acid
RNase	Ribonuclease
RT	Room temperature
RWC	Relative water content
SlA	Salicylic acid
SA	Seed area
SB	Seed breadth
SBE	Starch branching enzyme
SDR	Short chain dehydrogenase
SDS	Sodium dodecyl sulphate
SL	Seed length
SLN1	Slender 1
SM200	Soil moisture sensor 200
SNP	Single nucleotide polymorphism
SnRK	SNF-related protein kinase
SSS	Soluble starch synthase
ST	Steeping degree
TCP	Teosinte branched 1
TGW	Thousand grain weight
TL	Total loss
VIS	Viscosity
VP1	Viviparous 1
WT	Wild-type (*Hordeum vulgare* cv. Golden promise)
XN	*Ta1AX1* promoter driving the expression of *AtNCED6*
Y	Yield
ZEP	Zeaxanthin epoxidase

1 Introduction

Barley (*Hordeum vulgare* L.) is the primary cereal used in the production of malt, which makes it a very important economic cereal crop. From the global barley production, 60-70% is utilized for animal feed, only 2-4% is directly consumed by humans, and 30-40% is used for malting (Newman, 2006; Baik and Ullrich, 2008). Beer and whiskey are the main beverages produced out of malt and, according to World Health Organisation, beer is the second most consumed alcoholic beverage worldwide (World Health Organisation, 2014). The European Union (EU) is positioned first in the world barley production owning 65.4% of the global production share (Faostat, 2014). After Russian Federation (20.4 million tonnes) and France (11.7 million tonnes), Germany keeps the third place in the world production of barley producing a total of 11.5 million tonnes of barley per year (Faostat, 2014). Between 2010 and 2014, 9.5 million tonnes of malting barley were produced in the EU per year, making it the first in the export of malt to non-EU countries. Of the 2.1 million tonnes of exported malt, about 750,000 tonnes were exported to Africa, 550,000 to the Americas, and 640,000 to Asia. About 200,000 tonnes are distributed to European non-EU countries (Euromalt, 2015). Today the revenue from EU malt exports reaches approximately 850,000,000 euros (Euromalt, 2015). Although the world beer consumption had grown to over 1,960 million hectolitres (equating to over 21.5 million tonnes of malt) by 2014 (E-malt, 2015a), malting had been a rather small-scale activity until the beginning of industrialisation in the 19th century, which brought up new technologies and made it possible to expand production (Briggs, 1998). However, the roots of malting date back to the ancient times (approximately 5.000 BC), and over its extensive evolution – starting with primitive malting techniques performed in wells by Sumerians and Egyptians (Singer, 1954; Wild, 1966), followed by centuries of floor malting in caves or "malt houses", until the development of pneumatic malting and until today – germination of barley grains has been and remains the core of the process and an important object of monitoring. Germination capacity is arguably one of the most vital information and resources that the mature grain keeps stored until it is ready to germinate. A major goal of barley malters is to predict the behavior of the grain during germination in order to optimise the whole malting process – and this still remains a key challenge (Edney and Mather, 2004).

1.1 Characteristics of malting barley grain

Statistical data indicate that because of the inadequate quality the barley grain becomes unuseful for industrial processings like malting, and gets utilized as animal feed instead, which

means an economic loss for the producer (E-malt, 2015a). Therefore, to act satisfactorily under the brewhouse conditions, certain quality standards need to be met. The major parts of the barley grain are the endosperm, the embryo and their covering layers of maternal origin. Each of them contributes to defining the final product of malt.

Endosperm

The barley endosperm consists of starchy endosperm surrounded by the aleurone layer. It is the major storage organ of photoassimilates which makes it the largest morphological part of the barley grain comprising approximately 75% of its weight (Evers and Millar, 2002). Its role is to nutritionally supply the embryo as it grows during germination. Starch is the most abundant photoassimilate of the endosperm (60% of the grain). It consists of two polymers, amylose and amylopectin. Amylose is a linear polymer made up of glucose molecules linked via α-(1-4*)* glucosidic bonds. Amylopectin is branch formed polymer with α-(1-4) glucosidic and α-(1-6) glucosidic linkages (Hough, 1991). The ratio of amylopectin to amylose is around 3:1 in the mature barley grains (Palmer, 1972). The large starch granules (A type), are shape round and contain 70% - 80% amylopectin. The small, spherical, B type granules, contain 40% - 80% amylose (May and Buttrose, 1959; Evers, Blakeney and O'Brien, 1999). The expansion of endosperm cells due to intracellular accumulation of storage compounds occurs during grain filling phase (Bosnes, Weideman and Olsen, 1992). Grain filling begins with the appearance of the first starch granules at approximately 10 days after pollination (DAP) or soon after cellularization phase of the endosperm is finished and the first cell walls have been formed (Bosnes, Weideman and Olsen, 1992; Duffus, 1993). Starch is synthesized from glucose-1-phosphate by the action of four classes of enzyme activity (Duffus, Cochrane and Shewry, 1992; Hannah, 2007; Hannah and James, 2008). ADP-glucose pyrophosphorylase (AGPP) catalyses the first reaction in starch synthesis (James, Denyer and Myers, 2003). Granule-bound starch synthase (GBSS) is involved in amylose synthesis and soluble starch synthase (SSS) catalyses the elongation of amylopectin chains. Amylose and amylopectin are formed simultaneously during the formation of starch (Rahman *et al.*, 2007). Starch branching enzyme (SBE) introduces branch points into the amylopectin chains. The genes responsible for the synthesis of starch are expressed shortly after anthesis (Fox, 2010). Starch branching enzymes I and II (SBEI and II) have been shown to express 12 days post anthesis (Mutisya *et al.*, 2003). The first starch granules appear in the central stroma of each amyloplast, where one single starch granule is initiated and grows steadily in size (Duffus, Cochrane and Shewry, 1992; Wei *et al.*, 2010). Grain filling ceases after the mid-stage of grain maturation. At this

point, the metabolic activities in the starchy endosperm are reduced, partly due to the induction of desiccation, but also as coordinated by ethylene-mediated programmed cell death, which doesn't involve the degradation of cell contents and cell walls (Young and Gallie, 2000; Sreenivasulu *et al.*, 2006; Sabelli, 2012). During the later stages of grain maturation, the integrity of the membranes of protein storage vacuoles as well as that of amyloplasts envelopes is lost (Miflin *et al.*, 1983; Kawakatsu and Takaiwa, 2010). As a result, starch granules are localized in a protein matrix in the mature starchy endosperm. Based on the high, low or zero amylase content in the grain, the malt gelatinisation (solubilisation) property might change (Evers, Blakeney and O'Brien, 1999). The temperature at which gelatinisation occurs varies between 55°C and 65°C (MacGregor, Bazin and Izydorczyk, 2002). High amylose and and high amylopectin (waxy starch) have gelatinisation temperatures higher than that for the normal starch. (Ellis, Swanston and Bruce, 1979). Waxy barleys generally have lower malt extract values, and higher β-glucan content and slower modification rates (Ullrich *et al.*, 1986; Swanston *et al.*, 2011). Limited starch breakdown occurs during malting (Allosio-Ouarnier *et al.*, 2000) although increased levels of maltose, maltotriose, and maltotetraose during germination are reported. Starch was degraded more in the mashing process by the hydrolytic enzymes α-amylase, β-amylase, β-glucosidase, and limit dextrinase. High temperature infusion mashes readily solubilise the starch but limit the activity of thermolabile enzymes, in particular α-glucosidase and β-amylase (Osman, 2002). Starch properties, namely granule volume and size, have been related to hot water extract (Oliveira, Rasmusson and Fulcher, 1994).

The major constituent of barley endosperm cell walls is (1→3, 1→4)-β-D-glucan (75%), with a minor component (20%) identified as arabinoxylans (or pentosane) (Fincher, 1975; Fincher and Stone, 1986; Henry, 1987). The solubility of β-glucan in beer varies according to the number and arrangement of (1→3) and (1→4) linkages (Izawa, Takashio and Tamaki, 1996) as well as the size of the molecules. β-glucan corresponds to 2% - 10% of total barley grain weight (Henry, 1987) (Henry, 1987). Cultivar and environmental influences on the content of β-glucan have been reported (Henry, 1987; Stuart, Loi and Fincher, 1988; Zhang *et al.*, 2001; Li *et al.*, 2008). Access to the reserves stored in the endosperm is only possible after cell wall breakdown. Increased level of sugars derived from β-glucan and arabinoxylan components are present during malting (Allosio-Ouarnier *et al.*, 2000), and the breakdown of β-glucan gradually decreases from 5% to around 1% by the end of malting process (Henry, 1987). Arabinoxylan survives into malt and beer, albeit in smaller oligosaccharide forms (Viëtor,

Voragen and Angelino, 1993; Han and Schwarz, 1996). Both and arabinoxylan impact on wort viscosity and beer filtration rates and critical for efficient brewing (Jin *et al.*, 2004). It was demonstrated that for an increased level of β-glucan solubilization, higher temperatures during mashing are required (Palmer and Agu, 1999). High β-glucan levels may not result in higher or lower extract but relate to other malt quality traits such as Kolbach Index (ratio of soluble to total protein), viscosity or the speed of filtration (Evans *et al.*, 1999), and foam stability (Lusk *et al.*, 2001).

Hordein storage proteins act as the reserve of nitrogen and sulfur, and they are the second major constituent of the mature starchy endosperm (Ritchie, Swanson and Gilroy, 2000). They are rich in the amino acids like proline and glutamine. In barley, the major storage protein is called hordein, and this comprises 40% - 50% of total grain protein. This component is soluble in aqueous alcohol and comprises 4 fractions designated D, C, B, and A. The accumulation of endosperm storage proteins commences several days after the formation of the first starch granules in the amyloplasts (Cameron-Mills and von Wettstein, 1980; Duffus, 1993). The first hordeins have been detected as early as 10-14 dap (Shewry *et al.*, 1978; Giese, Andersen and Doll, 1983). On a subcellular level, hordeins are synthesized in the ribosomes on the surface of the rough endoplasmic reticulum (ER) in the cytosol (Brandt and Ingversen, 1976; Matthews and Miflin, 1980). Within the ER, prolamins are assumed to aggregate as protein bodies, although it is not known how these protein bodies are initially formed (Tosi, 2012). Nevertheless, it is widely accepted that prolamins are deposited in protein storage organelles via two transport routes, one dependent and the other independent of the Golgi apparatus (Galili, Altschuler and Levanony, 1993; Herman, 1999; Shewry and Halford, 2002). They are ultimately located within large protein storage vacuoles by fusion and aggregation (Cameron-Mills and von Wettstein, 1980; Tosi, 2012). Hordein accumulation during grain filling has been reported to last until 39–45 DAP with the greatest accumulation rate from 23 to 29 DAP (Møgelsvang and Simpson, 1998; Rahman *et al.*, 2007). Barley protein accounts for 8%-13% (dry basis) of the total composition of malt barley, with a more desirable range of 10%-11% for brewers. High protein is undesirable because of the strong correlation with low carbohydrate (starch) levels and thus low extract values (Bishop, 1930). But if the protein content of malt is too low, brewing performance may be impaired through poor yeast amino acid nutrition. Of total protein content, around 50% is the prolamin (hordein) protein, with albumins, globulins, and glutelins making up the remaining protein. Each hordein group has some relationship to malt extract or final beer quality (Smith, 1990; Shewry, 1995). The A-

hordein has been investigated for its possible role as a trypsin/amylase inhibitor and a possible protein involved in beer haze formation (Salcedo *et al.*, 1980; Aragoncillo, Sanchez-monge and Salcedo, 1981; Paz-Ares *et al.*, 1983; Lazaro *et al.*, 1985; Robinson *et al.*, 2007). Additional roles have been identified for these proteins, including inhibiting serine proteinases in malting (Jones, 2005). The aggregation of the sulfur-rich Band D groups forms gels which cause filtration problems in brewing (Shewry and Halford, 2002). B hordein fraction was reported with a positive effect on malt extract, and C hordein (sulfur-poor) groups had a positive effect on water uptake during malting (Skerritt and Janes, 1992; Janes and Skerritt, 1993; Molina-Cano *et al.*, 1995). C hordeins were also related to grain hardness (Brennan *et al.*, 1998). Many of these observations may be explained by indirect effects of the levels of one group of proteins on total protein. Bishop (1930) concluded that there was a negative relationship between protein and malt extract but recent studies describe a negative correlation particularly between D hordein and malt extract (Howard *et al.*, 1996; Molina-Cano *et al.*, 2000). Several studies have examined the effect of hordein breakdown products on beer quality, where the presence of D- and B-hordein components in lager foam was identified (Kauffman *et al.*, 1994). When investigating beer and beer foam quality, the initial grain barley hordein profile, malt hordein profile, level of modification, mashing conditions, and the possible interaction of polyphenols and additional proteins such as protein Z and lipid transfer proteins (LTPs), which can survive the malting process, should be considered (Silva *et al.*, 2008).

Aleurone layer

Apart from the embryo, the aleurone layer is the only tissue containing living cells within the grain. The two to three cells thick aleurone layer encases endosperm and consists mainly of arabinoxylan, while β-glucan is a minor component (Fincher and Stone, 1986). Aleurone cells also contain protein, lipids, vitamins and minerals, starch and phenolic acids (Pomeranz, 1973; Fincher, 1976; Evers and Millar, 2002). The sub-aleurone, which is found between aleurone and starchy endosperm, also contains protein embedded in starch granules (Macewicz *et al.*, 2006). The aleurone plays a critical role in the expression of endosperm degrading enzymes during germination which include β-glucanases, proteinases and peptidases (Jones, 2005), α-amylase, limit dextrinase (Sissons, Lance and Sparrow, 1992), α-glucosidase and various proteinase-, trypsin/amylase- and limit dextrinase inhibitors (MacGregor, Bazin and Izydorczyk, 2002; Stahl *et al.*, 2007). Many important transcript factors that regulate the synthesis of α-amylase and other enzymes include gibberellin oxidase (GAox) and GAMYB

(gibberellin- and abscisic acid-regulated MYB) which are expressed in aleurone cells. Up-regulation of GAMYB results in increased expression of α-amylase and (1→3,1→4)-β-glucan endohydrolase (Murray *et al.*, 2006), while inhibition of GA-oxidase proteins can stop the expression of amylase and other enzymes. Similar responses can also be observed when transcription factors in the abscisic acid pathway are affected. The growing environment affects aleurone cell integrity. Excessive heat stress can induce the development of some proteins while having an insignificant effect on others. Heat shock proteins (HSP) are expressed at elevated temperatures and can impact on the expression of other proteins (Harju, deSouza and Brodl, 2003). In addition, important trace elements including zinc and iron, are stored in aleurone cells (Dionisio, Holm and Brinch-Pedersen, 2007).

Embryo

The barley embryo is the most important living tissue within a barley grain, it is the fertile part of the barley grain. The embryo consists of an acrospire (including coleoptile, leaf primordia, and apical meristem), a nodal region between the root and the shoot, and a primary root covered by coleorhiza. It is separated from the endosperm upon germination by scutellum, which is a modified cotyledon. The outermost layer of the scutellum, the scutellar epithelium, faces towards the outermost layer of endosperm, which in this part of the grain is the layer of crushed cells formed of compressed cell wall material (Palmer, 1998). It also contains starch, protein, and lipids. These are used during the development of the embryo after fertilization and as an initial food source when the harvested seeds commence germination. The dormancy level and viability of the embryo is critical for the next generation of seeds. Many factors can have a negative impact on dormancy and viability including environmental conditions after anthesis and during grain fill, plant moisture, harvest conditions, and storage conditions. In terms of malt quality, most of the important components produced by the embryo are related to the breakdown of endosperm reserves. Two enzymes that can have a negative impact on beer quality are lipoxygenase 1 and 2 (LOX 1 and LOX2). They have been related to beer staling with the production of trans-2-nonenal which gives cardboard flavor. Both LOX1 and LOX2 were present in germinating embryos and the specificities of these enzymes on lipid substrates were different (Holtman *et al.*, 1997).

Husk

The outermost layer of the grain is the husk. It forms 10–13% of the grain weight being thus the second largest part of the grain after the starchy endosperm (Evers and Millar, 2002). The

husk consists of two distinct overlapping structures called lemma and palea covering the ventral and dorsal side of the grain. The husk is tightly attached to the pericarp layer by a cementing layer (Olkku *et al.*, 2005). The grain outer layers are separated from each other by cutin layers. The cutin layer between testa and pericarp is the thickest of all three cutin layers present, and it is the main structure influencing water impermeability of the grain (Evers and Millar, 2002; Olkku *et al.*, 2005).

Quality requirements for malting barley are strict and directly dependent on processing efficiency which is set by the producer. However, many of the desired seed quality traits depend solely on the weather conditions during the seed filling and harvesting season. The seed lot should be pure of an acceptable malting barley variety, free of any damages (less than 5% of peeled or broken kernels), insects or diseases, or chemical residues. The kernels should be fully mature, of uniform size and with no signs of pre-harvest germination. The moisture content in the grains should not exceed 13.5% in order to inactivate the enzymes involved in seed germination and to prevent the growth of disease microorganisms (Brewing and malting barley research institute, 2017). Next, the germination of 95% or higher in a 3-day test is absolutely critical to the malting process. If barley won't germinate, it cannot be processed into malt and any factor interfering with uniformity of germination or reducing seed vigor will reduce the quality of malts produced (Brewing and malting barley research institute, 2017).

1.2 Malting process

Malting process is a seed germination process conducted under strictly controlled environmental conditions with stable humidity and temperature. During this process, the starchy endosperm is the source of action of hydrolytic enzymes which produce small raw materials which are important for brewing (McFadden *et al.*, 1988; Ranki, Sopanen and Voutilainen, 1990; Jones, 2005). Malting is a three-stage biological process which includes steeping (soaking or imbibition), germination and kilning (drying) (figure 1A).

Steeping

The first step is steeping where dry grains are submerged in water, in order to increase moisture and prepare them for germination. The steep will undergo a minimum of two or three water changes with ventilated air rests between them because of cleaning (Schwarz and Li, 2011). Uptake of water by a dry seed is triphasic with a rapid initial uptake (phase I or imbibition) followed by a plateau phase (phase II, metabolic preparation for germination). The

third phase occurs after the completion of germination, when further water uptake takes place (Figure 1B) (J. D. Bewley, 1997) . During the first immersion, the dry outer grain layers rapidly take up water but minimal metabolic activity occurs (Kunze, 2004a). The grain tends to take up water quickly while submerged, but internal distribution through the endosperm occurs during the air rests. Over the course of steeping, the grain reaches 35% moisture content, and the embryo resumes metabolic activity (Fincher, 2010; Schwarz and Li, 2011). One of the first activities upon imbibition is the resumption of respiratory activity and a steep increase in oxygen consumption (Botha, Potgieter and Botha, 1992; Bewley and Black, 1994). The glycolytic and oxidative pentose phosphate pathways both resumes during steeping, and Kerbs cycle enzyme becomes activated (Nicolas and Aldasoro, 1979; Salon, Raymond and Pradet, 1988; Kennedy, Rumpho and Fox, 1992). The activation of stored pre-existing mitochondria in the mature dry seed contain sufficient Krebs cycle enzymes and terminal oxidases to provide adequate amounts of ATP to support metabolism for several hours after imbibition (Ehrenshaft and Brambl, 1990; Attucci *et al.*, 1991). The repair and activation of stored pre-existing organelles predominate during imbibition as there is no biogenesis detected (Morohashi and Bewley, 1980; Morohashi, 1986). The first DNA synthesis takes place soon after imbibition and probably involves repair of damaged DNA because of prominent levels of oxidative stress during dehydration and rehydration (Rajjou *et al.*, 2004; J. Derek Bewley *et al.*, 2013). The repair of damaged proteins also takes place during imbibition. L-iso aspartyl methyltransferase (PIMT) catalyses the conversion of abnormal l-iso aspartyl residues to normal l-aspartyl form and its overexpression has been linked to increased seed vigor and longevity (Dinkins *et al.*, 2008; Oge *et al.*, 2008). Furthermore, during water uptake seeds generate reactive oxygen species which are scavenged by superoxide dismutase, catalase, ascorbate peroxidase, dehydroascorbate reductase and glutathione reductase (Wojtyla *et al.*, 2006). Gibberellic acid (GA) is synthesized (Fincher, 2010; Schwarz and Li, 2011) and water and GA diffuse through the seed from the proximal end of the grain toward the distal tip, gradually activating the aleurone layer. Once activated, the aleurone layer releases hydrolytic enzymes into the endosperm cavity, and modification commences. Steeping continues until the grain reaches a final moisture content of 42-48% (Schwarz and Li, 2011). This moisture level is required for uniform enzyme diffusion throughout the endosperm, promoting adequate modification of resources to support germination. Seeds exhibit size change following water uptake, and a testa rupture, which occurs only during the later stages of seed imbibition (J. D. Bewley, 1997).

Germination

During malting, germination is controlled by temperature, moisture and time to allow kernels to progress to the point where the enzymes necessary for brewing are generated but the root and shoot growth is limited. The small components such as sugars, amino- and fatty acids, which are the result of endosperm modification, are generally utilized by the developing embryo but in brewing, these components are essential to the proper nourishment of yeast during fermentation (White and Zainasheff, 2010; Shewry and Ullrich, 2014). The radicle extension through the surrounding structures is the event that terminates germination and marks the commencement of seedling growth (J. D. Bewley, 1997). This extension can or may not be accompanied by cell division and is regulated by gibberellic acid (GA), where it has a key role (Bewley 2014), based on the observation that seeds unable to produce GA are incapable of seed germination. Embryos that are constrained by a mechanical barrier such as the surrounding endosperm, require a weakening of these structures to permit radicle protrusion. This awakening involves enzymatic degradation of the cell wall (J. Bewley, 1997). The specific modifications facilitating cell wall loosening to promote embryo growth are unknown but endo-beta-mannanase, expansins, xyloglucan endotransglycosylase/hydrolase and cellulase might be involved (Nonogaki, 2006). Numerous genes have been characterized that are capable of regulating the transition from completion of germination to seedling growth in Arabidopsis seeds (Holdsworth, Bentsink and Soppe, 2008). These tend to be associated with hormonal and light responses, such as include abscisic acid, gibberellic and ethylene responses, as well as phytochrome interacting factor (PIL5). It is unknown how these regulators influence downstream targets of cell expansion, water channel proteins and cell wall modifying enzymes. Once the process of germination has commenced, utilization of stored reserves for energy production is necessary before the plant becomes autotrophic by establishing photosynthesis (Bassel *et al.*, 2008).

Kilning

Kilning reduces the moisture content of the kernels, preserves enzymes generated during germination, and generates colour and flavour compounds. The grain is dried (withering) and then cured. Withering takes place over the first 10 hours of kilning at temperatures ranging from 50-75℃ (Schwarz and Li, 2011). The purpose of withering is to slowly remove the moisture from the grain so that modification is arrested, and the enzymes stop working but are not denatured. Curing entails gradually increasing the temperature to between 80-110℃ over the remainder of the kilning time (Bokulich and Bamforth, 2013). When kilning is completed,

the dried rootlets are removed, and the malt is relocated to a storage facility. The finished malt reaches a moisture content of approximately 3-5% (Bamforth, 2000), which allows to keep dormant the enzymatic activity and reduces the likelihood of spoilage.

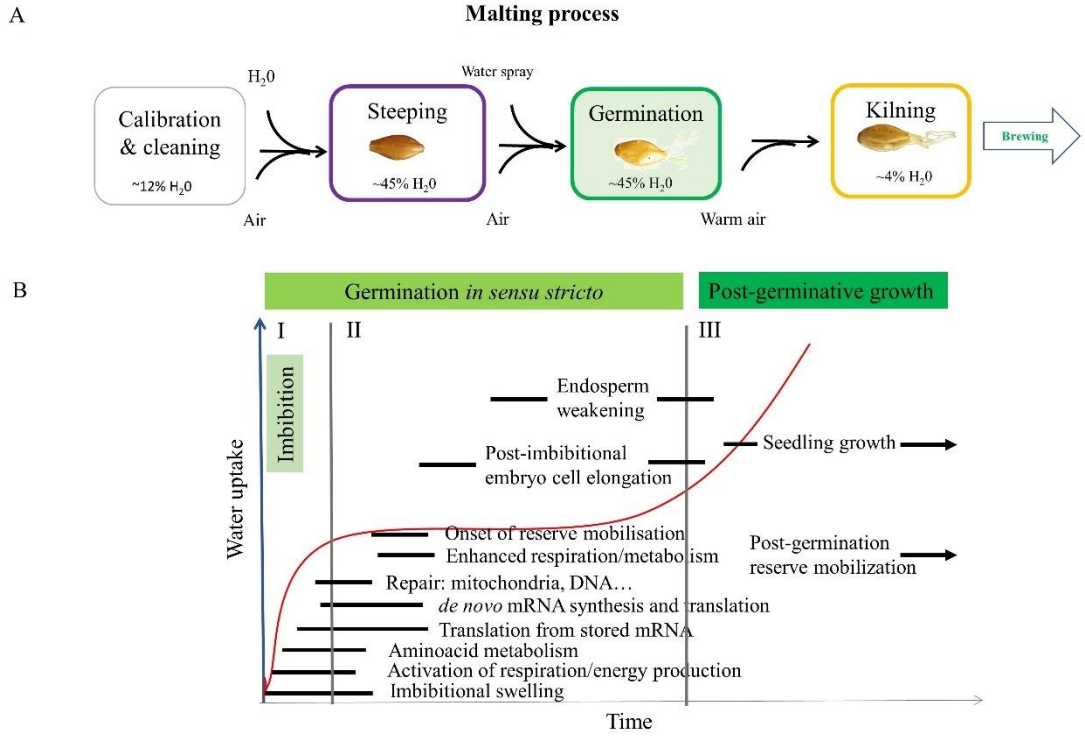


Figure 1. A General flow of the malting process. Steeping, germination and kilning are the main steps in creating malt under strictly controlled conditions. Grains are immersed in water during steeping to increase moisture content in the grain to above 40%. Growth and modification of the grain mark the germination step. Kilning is the final step where grains are dried to prevent further germination. **B** Time course of water uptake (red curve) by an orthodox seed with the main cellular and metabolic events. The three stages of water uptake are imbibition (phase I), lag phase or preparation for germination (phase II), and completion of germination where another water uptake takes place (phase III). Modified from Bewley (1997).

1.3 Malting quality parameters

To operate satisfactorily under brewhouse conditions and throughout the brewing process, an appropriate malt with desirable parameters needs to be supplied to the brewer. Malting quality parameters such as malt extract, β-glucan and protein contents, malting losses, friability, α-amylase activity, viscosity, and soluble nitrogen to total nitrogen ratio are commonly tested, as well as some physical properties like pH, odour and colour (Fox, 2010). The most important malting parameter is malt extract because it is the sum of all solids in a watery solution, such as sugar, protein, minerals. According to European Brewery Convention, malt extract is expressed as a percentage (%) of dry matter over total weight of malt (EBC, 2004). When malt extract is measured as „dry extract“ it also measured as „extract as is“ which includes the second most important parameter, the malt moisture content. Generally, the moisture content

Table 1. Desirable parameters for a lager malt according to European Brewery Convention. Modified from O'Rourke (2002).

Malt Specification	**2-row spring barley**
Moisture	<5 %
Malt extract	>80.5 %
Kolbach index	36-42 %
Viscosity	1.50-1.60 mPa*s
Friability	>80 %
Beta glucan	<300 mg/l
Protein content	9.5-11.5 %
Soluble nitrogen	650-750 mg/100 g
Limit of attenuation	77-83 %
Wort colour	3-4 EBC
Boiled wort colour	5-6 EBC
Dimethyl sulphide precursor (DMS-P)	<5 ppm

should be in the range from 2-5%. The soluble nitrogen content (expressed as mg/100 g dry matter) indicates the modification of the malt. The greater the value of soluble nitrogen, the higher the modification. Kolbach Index is a ratio of soluble nitrogen and total nitrogen, expressed as a percentage. The optimal values of this parameter should be between 38 and 44% (Verma *et al.*, 2008). The degree of modification can be also measured by physically disintegrating the malted grain with friabilimeter which measures the softness of the grain. Generally, friability should be greater than 85%. The parameter viscosity comes from the presence of thick molecules, especially beta glucans. It is a measure of a liquid's ability to resist flow through a capillary column. High (above 1.60 mPa*s (millipascal-second)) viscosity malts can cause filtration problems during the brewing process (Fox, 2010). Beta-glucan analysis is most commonly determined by a Congress Mash wort analysis in a flow analyzer it should be less than 180 mg/l (Vis and Lorenz, 1998; Kunze, 2004b). Total protein values are reported as a percentage of the overall sample and for all malts are typically <14% dry basis. The limit of attenuation is the sum of all the sugars, that the yeast is able to ferment, expressed as a percentage of the total extract content. The watering degree of the grains is measured through steeping degrees (%). To know how much of malt gets lost or consumed by respiration or root growth, these two losses are measured as the loss of the dry matter and should be kept low. The less is the loss doesn't mean that the malt will be of superior quality, the losses depend directly on the germination process progression, the more it is, the more are losses. Two types of barley are frequently used for the malting process: six- and two-row. Two-row barley produces malt with a large extract, lighter color, and less enzyme content than the 6-row type (Broderick, 1977).

1.4 Molecular mechanism behind the dormancy release

Barley seeds are dormant (orthodox) so the prerequisite for the germination process is the dormancy release (Hilhorst, 1995; J. D. Bewley, 1997). Over 50 years ago regulation of dormancy release and germination has been described by the antagonistic interaction of phytohormones abscisic acid (ABA) and gibberellic acid (GA), whereby ABA represses germination and GA promotes germination and their regulation could be highly conserved among seed plants (J. D. Bewley, 1997; Holdsworth, Bentsink and Soppe, 2008). Dormancy depends on the intrinsic balance of GA and ABA biosynthesis and degradation (Ali-Rachedi *et al.*, 2004; Cadman *et al.*, 2006) and it appears that the ABA:GA ratio, and not the absolute hormone contents, controls germination (William E Finch-Savage and Leubner-Metzger, 2006). By the end of barley seed maturation on the mother plant, seeds undergo intensive desiccation and retain their germination potential over long periods of dry storage (Rajjou *et al.*, 2004). During this after harvest storage period (after-ripening), the seeds go through chemical changes even at 7-12% water content (Rajjou *et al.*, 2004). The consequences of these changes in dry storage, result in changes in germination capacity due to dormancy release (Russell *et al.*, 2000; Holdsworth, Bentsink and Soppe, 2008) or result in deterioration and loss of viability of the seed (Bewley and Black, 1994). The functions of ABA in dormancy initiation and maintenance during the late seed maturation phase have been established and reviewed (Walker-Simmons, 1987; Jacobsen *et al.*, 2002; William E Finch-Savage and Leubner-Metzger, 2006; Bentsink and Koornneef, 2008; Finkelstein *et al.*, 2008). To induce dormancy, *de novo* synthesis of ABA within the embryo or endosperm is required (Kucera, Cohn and Leubner-Metzger, 2005; Nambara and Marion-Poll, 2005). The most critical enzyme in ABA biosynthesis is the 9-cis-epoxycarotenoid dioxygenase (NCED) that is essential for ABA synthesis in endosperm and embryo (Lefebvre *et al.*, 2006). ABA is catabolized by ABA 8'-hydroxylases (ABA'8-OH) during the transition from maturation to germination, which are encoded by cytochrome P450 *CYP707A* gene family causing a decline in dormancy (Lefebvre *et al.*, 2006). *HvABA'8-OH1* has been documented as a key gene lowering the ABA in the seeds (Millar *et al.*, 2006; Leymarie *et al.*, 2008; Chono *et al.*, 2013). ABA is catabolized to ABA8'-hydroxylase which is subsequently converted to phaseic acid (PA) (Kushiro *et al.*, 2004). PA can be further reduced to dihydro phaseic acid (DPA) (Gillard and Walton, 1976). ABA can be inactivated by conjugation with glucose by ABA glucosyltransferase to form ABA-glucose ester (ABA-GE) (Xu, 2002). It has been shown that the ABA content in barley embryos was greater in a more dormant variety than in a less dormant variety (Bradford *et al.*, 2008). During the dry storage of seeds, not only the seed

ABA content but also ABA sensitivity declines and an increase in GA sensitivity (Grappin *et al.*, 2000; Corbineau *et al.*, 2002; Koornneef, Bentsink and Hilhorst, 2002; Leubner-Metzger, 2002; Ali-Rachedi *et al.*, 2004; Chiwocha *et al.*, 2005; Lefebvre *et al.*, 2006). Integral to ABA signaling is sucrose nonfermenting-1-related protein kinase-2 (SnRK2). In the absence of ABA, SnRK2 is kept in an inactive state through the action of protein phosphatase 2C (PP2C). In the presence of ABA, ABA receptors PYR/PYL/RCAR bind to and inhibit PP2C, allowing the accumulation of active SnRK2 and subsequent phosphorylation of ABA-response element binding proteins (AREBPs) (Cutler *et al.*, 2010). AREBPs (also known as ABFs) are a family of basic leucine zipper (bZIP) transcription factors that recognize the ABA response elements (ABREs) present in some ABA-regulated genes (Cutler *et al.*, 2010). The seed phenotypes of the *A. thaliana ABA-insensitive* (*abi*) response mutants *abi1* to *abi5* demonstrate that ABI1 to ABI5 are involved in seed dormancy and/or germination (Finkelstein and Lynch, 2000; Kucera, Cohn and Leubner-Metzger, 2005). An ortholog of ABI3 in maize is Viviparous1 and it is one of the key genes in regulating embryo maturation, dormancy and desiccation as well as inhibition of germination (McCarty *et al.*, 1989, 1991; Giraudat *et al.*, 1992; Hoecker, Vasil and McCarty, 1995). It is also responsible for transcriptional control of the late embryogenesis abundant (LEA) class of proteins (Nambara, McCourt and Naito, 1995; Nambara *et al.*, 2000) and is involved in root growth-related interaction between ABA and auxin (Suzuki *et al.*, 2001). QTL analysis showed VP1 to be responsible for seed dormancy and pre-harvest sprouting (Lohwasser, Röder and Börner, 2005). Like ABI3, ABI5 and VP1 interact to regulate embryonic gene expression and sensitivity of seed to ABA (Lopez-Molina *et al.*, 2002). In barley, ABA-dependent up-regulation of ABI5 is positively regulated by a mechanism that involves ABI5 and VP1(Casaretto and Ho, 2005). An important feature of ABA and GA metabolism is their interaction. ABA down-regulates GA biosynthesis (GA 3-oxidase, which catalyses the conversion of inactive to active forms of GA) and up-regulates GA deactivation (GA 2-oxidase), thus modulating seed ABA content (Seo, 2006). GA biosynthesis is required for seed germination in Arabidopsis (Mitchum *et al.*, 2006) and components of GA signaling regulate seed germination (Peng and Harberd, 2002). Nuclear transcriptional regulators, the DELLA proteins, are negative regulators of GA signaling (Richards, Peng and Harberd, 2000; Dill, Jung and Sun, 2001; Itoh *et al.*, 2002; Wen and Chang, 2002). SLENDER1 (SLN1) is the only one DELLA protein barley (Dill, Jung and Sun, 2001; Chandler *et al.*, 2002; Itoh *et al.*, 2002; Peng and Harberd, 2002). Downstream of the DELLA proteins, GA regulates Myb-like (GAmyb) transcription factor binding to the promoter of α-amylase genes (Gubler *et al.*, 1995). The GA-signal is received by a soluble GA

receptor GA-INSENSITIVE DWARF1 (GID1) (Ueguchi-Tanaka *et al.*, 2007). The bioactive GAs bind to GID1 which in turn promotes interaction between GID1 and the DELLA-domain of DELLA protein (Itoh *et al.*, 2002; Willige *et al.*, 2007). This interaction enhances the affinity between DELLA-GID1-GA complex and a specific SCF E3 ubiquitin–ligase complex, SCFSLY1/GID2 (McGinnis *et al.*, 2003; Sasaki *et al.*, 2003; Griffiths *et al.*, 2006; Willige *et al.*, 2007). The ubiquitination and subsequent destruction of DELLAs are promoted by SCFSLY1/ GID2 through the 26S proteasome (McGinnis *et al.*, 2003; Sasaki *et al.*, 2003). DELLA degradation is GA-dependent and is inhibited by ABA in barley (Chandler *et al.*, 2002; Achard *et al.*, 2006).

1.5 Translational initiation and role of stored messenger RNA upon imbibition

Inhibition of transcription and translation has differential effects on germination potential (Nonogaki, 2014). It was shown over 40 years ago that transcription was not required for *de novo* protein synthesis in imbibed seeds, which suggested that stored endogenous mRNA was utilized in early stages of the germination process (Dure and Waters, 1965). In mature seeds of barley there is around 12.000 mRNA molecules present (Sreenivasulu *et al.*, 2008). Similar numbers have been found in Arabidopsis and rice (Nakabayashi *et al.*, 2005; Sreenivasulu *et al.*, 2008; Howell *et al.*, 2009) consisting with the role of pre-existing mRNA molecules playing a central role in germination. These mRNA molecules are called stored or residual messages (Ishibashi, Yamauchi and Minamikawa, 1990). They are carried over from seed development surviving cell desiccation. Many of the genes encoding residual mRNA contain abscisic acid responsive elements (ABRE) with the core motif ACTG in the promoter regions and are typically activated during seed maturation (Nakabayashi *et al.*, 2005). It is possible that stored mRNA support *de novo* synthesis before the transcriptional activity is resumed and new messages become available during early stages of imbibition. In the dry seed of wheat, all of the components needed for the resumption of protein synthesis are present (Bewley and Black, 1994). At the dry seed stage there are no polysome complexes but shortly after rehydration, ribosomes become recruited into polysomal protein – synthesizing complexes, utilizing extant mRNAs (Bewley and Black, 1994). This is supported by the experiments performed on Arabidopsis using transcriptional and translational inhibitors during imbibition and germination. Alpha-amanitin, an inhibitor of RNA polymerase II does not inhibit germination, while cycloheximide, a protein synthesis inhibitor clearly prevents radicle emergence. These observations led to the contention that *de novo* synthesis utilizing stored m RNA is sufficient to allow the completion of germination (Rajjou *et al.*, 2004). Transient, low-

level transcription and translation may occur in air dry, low hydrated seeds (Kucera, Cohn and Leubner-Metzger, 2005). Experiments in Arabidopsis and rice show that the stored transcripts are still abundant several hours after imbibition, therefore they may have specific roles during germination (Nakabayashi *et al.*, 2005; Preston *et al.*, 2009). These are mainly associated with primary metabolism, including those for PPP (Preston 2009), providing the evidence that respiration assumption is one among the first taking place during imbibition. Also, the immediate increase in the activity of glycolysis and the TCA cycle facilitate this energy-demanding process (Howell *et al.*, 2009). Genes encoding ribosomal proteins are also found abundant (Tatematsu *et al.*, 2008; Preston *et al.*, 2009). Down-regulation of key transcripts may also be of importance during germination. Several genes encoding proteins that inhibit germination, like ABA-responsive genes (ABI) and that of the GA receptor GID1, are downregulated in germination Arabidopsis seeds. Therefore regulation of germination may require both the selective removal of some transcripts and *de novo* synthesis of other (Nonogaki, Bassel and Bewley, 2010). The embryo and endosperm play opposing roles in seed germination (Dekkers *et al.*, 2013; He *et al.*, 2015). The embryo contains most of the genetic information that controls germination (He *et al.*, 2015) but in aleurone layer (endosperm) the initial signaling cascade starts and leads to the synthesis of hydrolytic enzymes (Bethke and Jones, 1997). Studies on endosperm have demonstrated that the endosperm can secrete signals to control embryo growth (Lee and Park, 2010) and bidirectional interactions between the embryo and endosperm are occurring (He *et al.*, 2015). At the dry seed state, these two different tissues (have a distinct pool of stored mRNA (Sreenivasulu *et al.*, 2008; He *et al.*, 2015), some of them being germination essential. Both sequential and selective mRNA translation that takes place during different stages of water uptake which emphasizes a fine regulation of the translational machinery (Galland *et al.*, 2014). The regulation of stored mRNA translation appears as an essential determinant of seed quality with regards to germination (Galland and Rajjou, 2015). During seed germination, protein synthesis is of the main importance (Rajjou *et al.*, 2004; Kimura and Nambara, 2010). In aged seeds, the protein synthesis capacity decline together with the loss of germination potential (Rajjou and Debeaujon, 2008) but how the two distinct tissues contribute with stored messages to on start of germination still needs to be studied.

1.6 Effects of terminal drought stress on dormancy and germination

Drought stress at grain filling, called also terminal drought stress, reduces crop production and alters seed morphological properties and nutritional components. Several reports have

suggested that drought stress during seed development of barley reduced leaf photosynthetic rate (Migdadi *et al.*, 2005; Samarah *et al.*, 2009), decreased grain filling duration (Sanchez *et al.*, 2002; Samarah, 2005), enhanced plant maturity, resulting in a serious reduction in grain yield and yield components (Forster *et al.*, 2004; Samarah *et al.*, 2009). Barley yield has been correlated with the starch level in grains (Schulman, Runeberg-Roos and Jääskeläinen, 2000), and drought stress affects yield by reducing the starch content in barley grains (Thitisaksakul *et al.*, 2012). Contrary to starch, grain protein content can increase in water stress at grain filling stage (Zhao *et al.*, 2008). In addition, increased grain nitrogen concentration associated with drought stress has been reported in barley (Farahani *et al.*, 2010; Afshari-Behbahanizadeh *et al.*, 2016). Less is known how is terminal drought stress affecting the duration of seed dormancy or seed germinability in the developing seed on the mother plant (Clark, Collier and Langston, 1967; Phillips N and Edwards J, 2006) reported that drought could affect vigour by reducing seed size. Smaller seeds contain fewer starch reserves and less starch means less energy for emergence, while a large seed will have a larger endosperm to drive germination and early growth (Wallwork *et al.*, 1998; Phillips N and Edwards J, 2006). Aspinall has tested the germination percentage of barley grains subjected to drought stress at different stages (pre- and post-anthesis) (Aspinall, 1966). He concluded that the drought stress applied at anthesis may increase dormancy of the grains and that dormancy depends on previous moisture-stress history. In sorghum, germinability was increased by terminal drought stress and the ABA levels were decreased in drought-stressed seeds (Benech Arnold, Fenner and Edwards, 1991). Phytohormones namely ABA, jasmonates are involved in drought resistance. But in these responses, all phytohormones have synergistic and antagonistic interactions. The endogenous concentration of auxin, gibberellin, and cytokinins decreases during the drought while ABA and ethylene tend to increase in almost all plants (Weyers and Paterson, 2001) but the crosstalk of these hormones still remains unknown.

1.7 Aim of the study

There are numerous studies about the influences of abiotic stresses directly applied to a germination test. Less is known about the germination performance of seeds developed and matured on the mother plant under drought condition. Until today only a few reports show the unclear effect of terminal drought stress on the germination performance or malting quality. With this study, two approaches have been undertaken to determine the effect of post-anthesis drought stress on barley germination behaviour and therefore malting quality.

1. The first approach is based on DH (double haploid) population of two malting barley elite varieties which undergo different mechanisms of terminal drought stress response.

- Germination and shoot emergence performance examination from seeds grown under watered and drought stress conditions and locating target genomic regions responsible for seed dormancy/germination via quantitative trait loci (QTL) mapping.
- Determining the relation of germination and shoot emergence traits with seed quality and malting quality parameters.
- Transcriptome analysis of dry seed and micromalted seedlings.
- Selection of candidate genes which are responsible for those changes.

2. The second approach is based on using the transgenic model of ABA transgenic lines to determine:

- The relation between ABA and germination rate of control and drought-stressed seeds.
- ABA and GA profiling from late seed maturation stage through different time points of germination.
- Identification of germination essential transcripts by microarray analyses of the wild-type and transgenic lines.
- Identification of *de novo* transcribed genes necessary for post-germinative growth phase.
- Analysis of embryo and endosperm tissues as storage of different mRNA species and their contribution to germination and malting quality.

2 Materials and methods

2.1 Plant material

There are two different sets of the experimental material used in this work:

1. *Hordeum vulgare* cv Golden promise and transgenic lines produced in its background. The transgenic lines were developed in previous work in the Abiotic Stress Genomic group (IPK Gatersleben). Two different transgenic alterations of ABA metabolism were followed. In the first one is the overexpression of *AtNCED6,* gene that encodes the key enzyme in ABA biosynthesis, driven by the endosperm 1AX1 promoter from wheat. These lines were referred as XN17 and XN26 for the *1AX1::AtNCED6* construct (Harshavardhan, 2012). The other approach was the RNAi suppression of the endogenous *ABA8'-OH* under control of barley *late embryogenesis abundant (Lea)* B19.3 promoter, in order to reduce the ABA degradation. LoHi236 and LoHi272 are the given names for the *HvLea*::*Hv8'-hydroxylase RNAi construct lines* (Seiler *et al.*, 2014).
2. *Hordeum vulgare* malting elite breeding lines LP104 (Sofiara) and LP106 (Victoriana) as parents of the double haploid population consisting of 100 lines developed from their F1 cross, obtained from KWS Lochow GmbH, Germany.

2.2 Plant cultivation and sampling

Barley plants (explained in 2.1) were grown under controlled conditions in the greenhouse and under the field for seed multiplication and experimental analysis.

At greenhouse conditions, plants were grown on the substrate that contains four parts of autoclaved compost, two parts of 'Rotes Substrat' (Klasmann-Deilmann GmbH, Germany), 1.6 parts of sand and 0.8 parts of peat. Seeds were planted in 96 well plastic trays and germinated in temperature controlled greenhouse for four weeks at 11 ºC day and 7 ºC night with 10-hour light. After four weeks of growth, seedlings were vernalized for two weeks in the cold chamber (4 °C, 12 hours light period), they were then transferred to pots (diameter 16 cm^2) and allowed to mature in the greenhouse. Further growth conditions were divided into four phases: first phase at 14 ºC day and 9 ºC night with 12 hour light for four weeks, second phase at 16 ºC day and 9 ºC night with 14 hour light for two weeks, third phase at 20 ºC day and 12 ºC night with 16 hour light for two weeks and final fourth phase at 20 ºC day and 14 ºC night with 16 hour light until harvest. Plants were fertilized with 'Plantacote plus' (AGLUKON GmbH, Germany) (15 g/pot) during vegetative phase and with liquid fertilizer

'Hakaphos Rot' (AGLUKON GmbH, Germany) (once a week, 2-4 %) from the start of spike development. Each line consisted in 50 plants/50 pots which were grown in the same condition until reaching the fifth days before flowering (DBF) stage where at least 15 plants were selected based on the same developmental stage and divided from the rest to impose drought stress. This stage was predicted by checking the position of the spike in relation to the first node below the flag leaf. The second time point chosen for stress imposition was the 5 days after flowering (DAF). This developmental stage was assessed by analyzing the caryopses in the mid-region of the ear as described by (Weschke *et al.*, 2000). The spikes were tagged manually with its date of flowering for all three cases (control, 5 DBF and 5 DAF drought stress) so that the sampling for further analysis consisted only of material deriving from the same developmental stage. Drought stress was imposed by maintaining at 20-25% field capacity (corresponds to 10% soil moisture content) until seed harvest. Control plants were maintained at 100% field capacity (40% soil moisture content). The soil moisture was monitored manually every day with soil moisture meter SM200 (Delta T devices Ltd, England). Seeds were harvested at 25 DAF, at harvest (pre-dormancy), dry seed (post-dormancy) and several time points during germination: 12, 24, 72 hours after imbibition (HAI). One biological sample consisted of material coming from three plants in average.

Already matured and harvested seeds of DH population and their parents LP104 and LP106 were provided by the InnoGrain Malt project (Kochevenko, personal communication, 2012). The lines were planted in the field in a randomized complete block design with 50 plants per line. After harvest, the seeds from all the plants were pooled into three replications for control and four replications for stress conditions. The plot sizes in each trial ranged from 0.4 m^2 to 5.5 m^2. Terminal drought stress was applied one week after anthesis by installing rain-out shelters to prevent natural rainfall and watering was stopped while control plants were continuously watered until maturity. Soil moisture was monitored twice a week in 10, 20, 30 and 40 cm depths by using 82 evenly distributed soil moisture sensors (SM300, PR2/4, Delta T devices Ltd., England). Additionally, data loggers DL-6 were placed at 4 different positions for continuous soil moisture measurement.

2.3 Phenotyping

2.3.1 Evaluation of yield and grain traits

When plants had reached maturity, grains were harvested, and yield was calculated per plant (greenhouse experiment) and per plot. TGW, grain number and grain morphological traits

such as seed length (SL), seed breadth (SB) and seed area (SA) were determined by counting grains using a digital seed analyzer (Marvin; GTA Sensorik GmbH, Germany).

2.3.2 Germination assays

Germination assay was performed according to International Rules for Seed Testing (Ista, 2015). Each germination test consisted of 100 seeds which were germinated on 13 cm Petri plates (25 seeds X 4 Petri plates). The seeds were randomly selected and placed between two layers of filter paper and moistened with 9 ml of distilled H_2O. The germination test was carried out at 20° C under 16-hour light / 8-hour dark photoperiod with four replications. Germination percentage was scored every 24 hours. At 72 hours after imbibition, shoot and root lengths were recorded. The seed was considered as germinated if had a visible root protrusion (2 mm).

$$\frac{\text{Number of germinating seeds x 100}}{\text{Total number of seeds tested}} = \text{Percentage of germination}$$

To check the seed viability and germination speed of DH materials, fifty seeds of each genotype and their parents were germinated as described above. Germination test was performed three times for each line.

2.4 Micromalting

Grain samples, 80g per genotype were malted in the malting facilities of the Research Institute for Raw Materials (VLB, Berlin, collaboration with Prof. Dr. Rath). Micromalting conditions were the following: 5 h wet steep, 19 h air rest, 2 h wet steep, 22 h air-rest, spray steeping until reaching a final moisture content of 45%, the germination for 96 h at 14.5° C and 95 to 98% relative air humidity. Germinated samples were kilned with the following regime: 16 h at 50° C, 1 h at 60° C, 1 h at 70 °C and 5 h at 80° C.

Afer the malting process twelve important malting related parameters were measured: malt moisture content (MMC), fine-grind extract (FGE), wort viscosity (VIS), malt protein content (KOL), limit of attenuation (FLA), friability (FRI), wort β glucan content (BGL), total malting losses (TML), respiration losses (REL) and rootlet losses (RTL). Data were obtained from the VLB Research Institute for Raw Materials in collaboration with Prof. Rath, according to the methods of MEBAK (Mitteleuropäische Brautechnische Analysenkommission).

2.5 Biochemical analysis

2.5.1 Carbon and nitrogen analysis of mature seeds

Carbon and nitrogen analysis was carried out with the elemental analyzer (vario EL III; Elementar analysensysteme GmbH Hanau, Germany). About 3 to 5 mg of dried sample was weighed in an aluminum foil, folded and placed in the carousel (autosampler). The sample enclosed in the foil is lowered in the center of the combusted tube and combusted with excess oxygen kept at 900° C using tungsten oxide (WO_3) as a catalyst. Various gasses formed during combustion (CO_2, H_20, NOX) pass through a silica tube packed with copper granules held 500° C, also called the reduction tube where all the remaining oxygen is bound and nitric/nitrous oxides are reduced to N_2. The leaving gas stream includes CO_2, H_20, N_2, and SO_2. Gasses are separated and determined with a thermal conductivity detector. High purity helium (Quality 5.0) is used both as a carrier and reference gas which pushes the combustion gasses through the analyzer. Blank values are obtained from empty aluminum capsules and calibration is done by elemental analysis of standard substances supplied by the instruments' manufacturer.

2.5.2 Starch measurement

Around 10-15 mg of powdered seed was incubated in 80% ethanol for 30 min at 60° C with continuous shaking at 500 rpm in thermomixer. After 10 mins centrifugation at 13,000 rpm, the pellet was resuspended in 2N HCl (1.5 ml) and incubated for 1 h at 95 °C (Kozloski *et al.*, 1999). The supernatant obtained after centrifugation (13,000 rpm for 5 min) was used for glucose estimation, the hydrolyzed product of starch. 5 µl of the supernatant is incubated with 750 ul imidazole buffer (2 Mm NAD and 1 mM ATP, pH 6.9) and 2 µl of glucose-6-phosphate dehydrogenase (2 units) at room temperature and initial absorbance is recorded immediately at 340 nm to measure NADH. Absorbance at 340 nm is recorded after incubation with 10 µl of hexokinase (8 units) for 25 min. The difference in absorbance for the NADH is proportional to the amount of glucose formed by the hydrolysis of starch.

2.6 Phytohormone analysis

Plant material was freeze-dried and used for extraction. Auxins (AUX), abscisic acid (ABA), salicylic acid (SA) cytokinins (CK) were extracted from the same powdered material. The extraction of gibberellins (GA) was undertaken separately, due their extraction requires a

different separation column. The analysis by LC-ESI-MS/MS was performed as described in (Kojima et al. 2009; Seo et al. 2011; Urbanova et al. 2013;) with minor modifications.

2.6.1 Abscisic acid, cytokinin, auxin and salicylic acid extraction

The extraction was carried out from 50 mg of freeze-dried tissues with 1 ml extraction solvent (acetone:formic acid:water=14:1:5) as described in (Kojima *et al.*, 2009; Seo, Jikumaru and Kamiya, 2011) with minor modifications. The homogenate was sonicated and shaken for 1 h at 4 °C. After centrifugation at 14,000 rpm for 10 min, the supernatant was transferred to a new tube. The extraction steps were repeated two additional times from the same pellet. The collected supernatants were combined and evaporated with vacuum centrifuge to the desired volume. The internal standard in MeOH (OlChemIm Ltd, Czech Republic) was added to the sample. To remove interfering compounds, the extract was first passed through the Oasis HLB cartridge 30 mg (Waters, Milford, MA, USA) equilibrated with 0.1 % formic acid in water. The eluate in 0.1 % formic acid with MeOH was evaporated and then reconstructed with 0.1 % formic acid in water. The hormone-containing solution was passed through an Oasis MCX cartridge 30 mg (Waters) where the acidic and neutral hormones (ABA, AUX, SA) were eluted with 0.1 % FA in MeOH and evaporated in the vacuum centrifuge to dryness and stored at -20 °C. Basic phytohormone (CX) was eluted from the same MCX cartridge first with 5% ammonium in 60 % MeOH and then with 5% ammonium in 60 % acetonitrile and evaporated to dryness and stored at -20 °C. From each sample two tubes containing different hormones were obtained, one with ABA, AUX, and SA, in the other one CX were present. Prior measurement, the hormone-containing tubes were resolved in 0.1 % FA in 10 % MeOH and transferred to an HPLC vial.

2.6.2 Gibberellic acid extraction

For gibberellic acid extraction, a similar procedure was followed as for the other hormones (Urbanová *et al.*, 2013). The extraction was carried from 100 mg of freeze-dried tissues with extraction solvent consisting of formic acid:water:acetone (5:15:80). The extraction was performed three times from the same pellet and the extract was cleaned up through the Oasis HLB column 60 mg as described above. The extracts were eluted with methanol:acetonitrile:propanol:water (15:60:20:5) and evaporated to dryness. The dry pellets were activated with MeOH and 25 Mm of ammonium bicarbonate. Such prepared hormone samples were passed through Oasis MAX columns 60 mg (Waters) equilibrated with water and 25 mM ammonium bicarbonate and eluted with 0.8 % FA in acetonitrile and evaporated to

dryness and stored at -20 °C. The samples were activated by 0.1 % FA in 10 % MeOH and transferred to an HPLC vial before the measurement.

2.6.3 Quantification of phytohormones by Electrospray Ionization Tandem Mass Spectrometry

10 µl Samples were injected into an LC (liquid chromatography) system (ACQUITY Ultra-performance LC) coupled with Xevo TQ MS mass spectrometer from Waters, USA. The sample analytes AUX, ABA, SA, and GA were separated on an ACQUITY UPLC® BEH C18 1.7µm, 2.1 mm x 100 mm coupled with a VanGuard pre-column BEH C_{18} 1.7µm, 2.1 mm x 5 mm. The CK´s were separated on a CORTECS UPLC® C_{18+} 1.6 µm, 2.1 mm x 100 mm coupled with a VanGuard pre-column CORTECS C_{18+} 1.6µm, 2.1 mm x 5 mm. The column temperature for all methods was set to 40°C. For the LC the used mobile phases were H_2O containing 0.1 % FA (A), MeOH containing 0.1 % FA. The flow was set to 0.4 ml min-1. The gradient conditions for the auxins, abscisic acid and salicylic acid analysis were as follows: A, 90 % in 0 - 0.3 min; 90 - 80 % in 0.3 - 0.7 min; 80 - 40 % in 0.7 - 8 min; 40 - 1 % in 8 – 8.5 min; 1 % in 8.5 – 8.9 min; 1 - 90 % in 8.9 – 9.0 min; 90 % in 9.0 – 10.0 min. The gradient conditions for the cytokinins analysis were as follows: A, 95 % in 0 - 0.5 min; 95 - 90 % in 0.5 - 1.0 min; 90 - 85 % in 1.0 - 2 min; 85 - 1 % in 2 – 8.0 min; 1 % in 8.0 – 8.9 min; 1 - 95 % in 8.9 – 9.0 min; 95 % in 9.0 – 10.0 min. The gradient conditions for the gibberellic acid analysis were as follows: A, 90 % in 0 - 0.3 min; 90 - 70 % in 0.3 - 0.7 min; 70 - 50 % in 0.7 - 2 min; 50 - 40 % in 2 – 4 min; 40 - 20 % in 4.0 – 8.0 min; 20 - 1 % in 8.0 – 8.5 min; 1 % in 8.5 – 8.9 min; 1 - 90 % in 8.9 – 9.0 min; 90 % in 9.0 – 10.0 min. The Xevo TQ MS operated in both ESI+ and ESI- ion mode. The electrospray capillary voltage was 2.7 kV for auxins, abscisic acid, salicylic acid, cytokinins and gibberellins with a cone voltage of 20 V. The cone and desolvation gas flows were set to 20 and 1000 l h^{-1}. The source and desolvation temperatures were 150 and 650 °C respectively for all methods. The collision energy of the MS/MS was between 10 and 50 eV. For the quantification of the analytes we used three fragment ions one for quantification two for qualification and for the internal standards, two fragment ions were used (Tab. S1). MS data processing was done by using TargetLynx V4.1 SCN 904. The peak area of the diagnostic product ion was used for quantification.

2.7 Transcriptome analysis

2.7.1 Polysomal RNA extraction

Polysomal RNA extraction was optimized for seed material based on the protocol of Bailey-Serres (Mustroph, Juntawong and Bailey-Serres, 2009). Seedlings were ground to fine powder in liquid nitrogen and added to the extraction buffer (200mM TRIS-HCl (pH 9.0), 200 mM KCl, 25 mM EGTA, 35 mM $MgCl_2$, 1 % (w/v) Brij-35, 1 % (v/v) Triton X-100, 1 % (v/v) Tween-20, 1 % (v/v) Igepal CA-630, 1 % (v/v) deoxycholic acid, 2 % (v/v) polyethylene-10-tridecyl ether, 0.5 mg/mL^{-1} heparin, 5 mM DTT, 50 μgmL^{-1} cycloheximide, 50 $\mu g/mL^{-1}$ chloramphenicol) and let sit on ice for 10 min. The samples were spun and the supernatant was cleaned onto a QIA shredder (QIAGEN). The cleaned sample was layered onto a sucrose gradient and ultracentrifugation at 50,000 rpm in Beckman LM-90 ultracentrifuge for 2 h. This allows the separation of heavier ribosome-associated mRNAs from the translationally inactive sub-polysomal mRNAs. After ultracentrifugation, the sucrose gradient was fractionated into 11 microtubes using the ISCO fractionation system. The system was set up to sensitivity=1.0 and flow rate 1.5 mL/min. The fractions were combined 1-5 (non-polysomal) and 7-11 (polysomal) in separate tubes.

Table 2. Preparation of sucrose gradients for polysome fractionation. 1µl of each cycloheximide (CHX) and chloramphenicol (CPC) is added per 10 ml of final volume.

Layer	2M (68,5 %) sucrose (ml)	10X sucrose salts (ml)	dd H_20 (ml)	Final volume/gradient	Sucrose	Final volume
	Vol/10 ml	Vol/10 ml	Vol/10 ml	ml	% (w/v)	(ml)
1 (bottom)	8,8	1	0,2	0,80	60	10
2	13,1	2	4,9	1,60	45	20
3	8,8	2	9,2	1,60	30	20
4 (top)	2,9	1	6,1	0,80	20	10

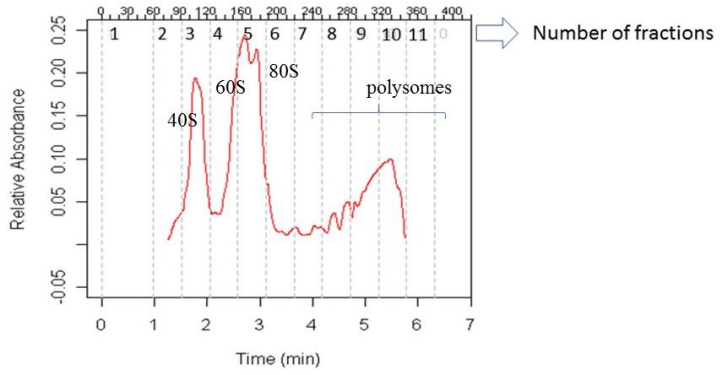


Figure 2. Polysome absorbance profile of dissected barley embryo (12 HAI) continuously recorded at 254 nm. Cell lysates were ultracentrifuged and fractionated on 15–50 % sucrose gradient to isolate polysomes. Fractions 1-5 contain the free mRNA and monomers, fractions 7-11 contain low and high weighted polysomal RNA. The positions of the 60S and 40S ribosomal subunit, 80S monosomes, and polysomes are indicated.

2.7.2 RNA extraction from sucrose fractions

The following protocol is adapted to precipitation of RNA from a gradient that was fractionated into two fractions: non-polysomal (free mRNA with monosomes) and a polysomal fraction (figure 2). Most of the procedure is aimed at removing proteins (e.g., RNases) or reagents that will inhibit the labeling step (such as heparin). To each fraction, 1 volume of 8 M guanidium-HCl and 2 volumes of 100 % ethanol were added and left overnight at -20 °C. The samples were centrifugated at >10,000 rpm for 30 min at 4 °C. The supernatant was discarded, 1 ml ice-cold 75% ethanol was added to the pellet and centrifuged at >10,000 rpm for 20 min at 4 °C. The pellets were resuspended in 400 µl TE (pH 8.0) and left for few minutes at room temperature to allow efficient dissolving. 1 volume of 3 M sodium acetate (pH 5.2) and 2.5 volumes of 100 % ethanol were added to the tube, incubated for at least 1 h at -20 °C and spun at 14,000 rpm for 30 min at 4 °C. The supernatant was discarded and 650 µl RNase-free water and an equal volume of water-saturated phenol:chloroform (5:1), pH 5.2 were added to the pellet. After vortexing and centrifugation at top speed for 5 min at room temperature, 500 µl of the aqueous phase was transferred into a new microtube tube. This step removes any residual proteins. RNase-free water was added to 1 ml and lithium chloride to a final concentration of 1.5 M (175 µl from 10 M stock), and incubated overnight at −20 °C. The LiCl precipitation is necessary to remove any residual heparin, which may interfere with the labeling reaction. After precipitation, the tubes were thawed on ice and centrifugated at 14,000 rpm for 20 min at 4 °C. The pellets were washed with 1 ml of 75 % ethanol and centrifuged at top speed for 20 min at 4 °C. The pellet is dissolved in 25 µl RNase-free water and stored at −80 °C.

2.7.3 Total RNA extraction

Total RNA was isolated from 150 mg frozen seedlings (Li and Trick, 2005). 750 µl of extraction buffer (100 mM Tris, pH 8.0, 150 mM LiCl, 50 mM EDTA, 1.5 % SDS, 1.5 % 2-mercaptoethanol) is added to the 2 ml tube containing the powdered sample. After vortexing, the sample is centrifuged at 13,000 rpm for 10 min at 4 °C. the supernatant is transferred to a new 2 ml tube and 1 ml Trizol and 200 µl chloroform is immediately added. Samples are vortexed well, incubated for 5 min at room temperature and centrifuged at 13 000 rpm for 10 min at room temperature. The supernatant is transferred to a new 1.5 ml tube, 500 µl of chloroform is added and the tube is inverted for 2 min for efficient mixing. After centrifugation at 13,000 rpm for 10 min at 4 °C, to the recovered the supernatant (around 750 ul) 600 ul of isopropanol and 400 µl 1.2 M sodium chloride are added. The tubes are mixed by

inversion and put in -20 °C for 1 h. Samples are then centrifuged at 13,000 rpm for 15 min at 4 °C followed by washing the RNA pellets with 70 % EtOH and dissolving them in 30 µl of RNase-free H_20. Finally, DNAse treatment is performed according to manufacturer´s protocol described in RNase-free DNase set kit (QIAGEN).

2.7.4 Quality checking of the RNA using Agilent 2000 Bioanalyzer

The quality of the RNA was checked using Agilent 2100 Bioanalyzer using Agilent RNA 6000 nano kit as per manufacturer's instruction and protocol. Depending on the sample the RNA 18S and 28S peaks are visible on the electropherogram (Figure B). Depending on the electrophoresis and the RNA integrity number the quality of the RNA was determined.

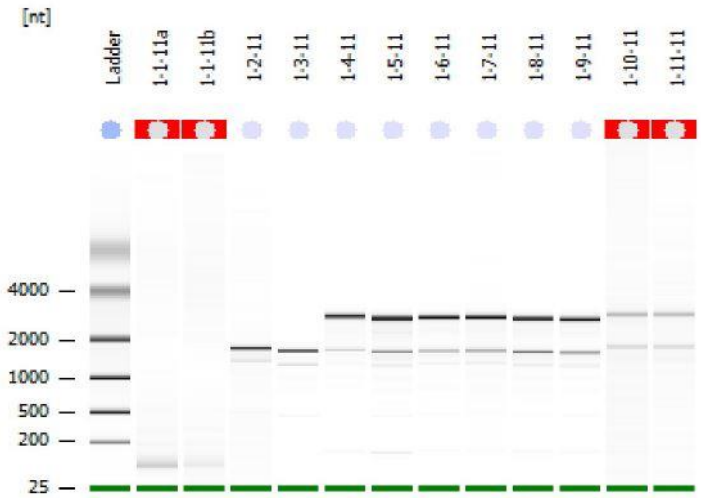


Figure 3. Bioanalyzer (Agilent technologies) Nano kit gel image depicting the RNA quality extracted from selected sucrose fractions from 1-11. Ladder: Size standard [nt] for RNA molecules, 1-1-11a, 1-1-11b: free mRNA, 1-2-11,1-3-1: mRNA bound to small ribosome subunit, 1-4-11: mRNA bound to the large ribosome subunit, 1-5-11: monosomes, 1-6-11 to 1-11-11 polysome bound-RNA, starting from light to heavy weighted.

2.7.5 Synthesis of complementary DNA

The first strand complementary DNA (cDNA) was synthesized from RNA which was free from any DNA contamination using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). 1 µg of total RNA, 1 µl of 100 Um oligo (Dt-18mer) primer and water were added to each tube to obtain a total volume of 12 µl and the mixture was incubated at 65 °C for 5 min, and then cooled on ice. 8 µl of cDNA master mix consisting of 4 µl 5XReaction buffer, 1µl of RiboLock RNase Inhibitor (20 Units/µl), 2 µl of 10mM dNTP mix and 1 µl of RevertAid M-MuLV RT (200 U/µl) was added and incubated at 42 °C for 60 min. The reaction was terminated by incubating at 70 °C for 5 min and chilled on ice. The synthesized cDNA was stored at -20 °C for further use.

2.7.6 Quantitative real-time polymerase chain reaction (qRT-PCR)

qRT-PCR reactions were carried out using power SYBR Green master mix reagent and the amplification was monitored with an ABI PRISM 7900 HT sequence detection system. The reaction was carried out in optical 384-well plates, each reaction well consists of 5 µl of Power SYBR Green master mix reagent (Applied biosystems), 1 µl of cDNA and 200 nM of each gene-specific primer in a final volume of 10 µl. The following standard thermal profile was used for all PCR reactions: 50 °C for 2 min, 95 °C for 10 min, 45 cycles of 95 °C for 15 sec and 60 °C for 1 min. Amplicon dissociation curves, i.e. melting curves, were recorded after 45^{th} cycle by heating from 60 °C to 95 °C with a ramp speed of 1.9 °C per minute. For internal control, three reference genes were checked for their expression pattern in different tissues. Three biological replicates, as well as three technical replicates per sample, were used. The expression level of the gene of interest (GOI) was calculated using comparative CT method as described by (Livak and Schmittgen, 2001). All the data were analyzed using the SDS2.2.1 software (Applied Biosystems). The expression level of the gene of interest (GOI) relative to the reference gene (RG) was calculated using the equation $2^{-\Delta CT}=2^{-(CT(GOI)-(CT(RG))}$.

2.7.7 Microarray hybridization

50 ng RNA was used for cRNA synthesis and Cy3-labelling with a Low Input Quick Amp Labelling Kit (Agilent Technologies). The quantity and quality of labeled cRNA were assured using an ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, USA) and Bioanalyzer system. 600 ng labeled cRNA was used for fragmentation and array loading (Gene Expression Hybridization Kit, Agilent Technologies).

Hybridization was done for 17h at 65°C on 60K barley custom array chip. After washing (Gene Expression Wash Buffer Kit, Agilent Technologies) and drying, arrays were laser scanned at 3 µm (double pass) resolution using an Agilent Technologies Scanner G2505C. Resulting fluorescence images were evaluated (determination of spot intensities, background correction) and used for extraction of the data with Feature Extraction V11.5 (Agilent Technologies).

2.7.8 Microarray data analysis

Data evaluation was done with Genespring V12.5 (Agilent Technologies). Raw data values were the first $\log_2$ transformed and quantile normalized. Relative expression values were calculated by baseline transformation, meaning subtracting the median expression of each probe from the other values of this specific probe. With filtering options, bad quality data and

low or absent data are filtered out before the start of analysis. Statistical analysis by ANOVA or Student t-test ($P \leq 0.005$, $FC \geq 1.5$) and FDR correction (Benjamini-Hochberg) was performed. To identify transcripts with similar expression profiles, K-means clustering (Pearson correlation) was performed. MapMan tool was used as visualization tool of differentially expressed genes (Usadel *et al.*, 2009), as well as MeV tool (Saeed *et al.*, 2003)

2.7.9 Gene ontology enrichment analysis

Gene ontology (GO) enrichment analysis was performed in collaboration with Dr. Braeutigam, workgroup Network Analysis and Modelling (IPK Gatersleben). GO term association was derived from an annotation file provided by Dr. Scholz, workgroup Bioinformatics and Information Technology (IPK Gatersleben). Briefly, the harvest35 contigs were annotated with functional terms, the oligos were mapped to the contigs of harvest35, and the matching annotation was transferred resulting in an oligo file with associated GO terms. GO term enrichment was tested for each file with oligos specified against a background of all oligos using TopGO as implemented in (Alexa, 2016). Minimum node size was thresholded at 10 nodes. Enrichment was tested using classic Fisher's Exact Test with the 100 top nodes reported. A visualization with of the top 20 nodes and their surroundings irrespective of significance was plotted. No multiple testing corrections were applied.

2.8 QTL analysis and statistics

For QTL mapping the composite interval mapping (CIM) model was applied (Jansen and Stam, 1994; Zeng, 1994). QTL analysis and the correlation between traits were carried out using WinQTL Cartographer V 2.5 and GenStat 17.0 (VSN International, 2011). QTL regions were identified based on the SNP-based linkage map of DH population designed by Kochevenko (personal communication, 2012). Only 1810 SNP markers (23%) were polymorphic and employed for the construction of the genetic map. To saturate generated a map with additional markers and enhance the ability to detect QTL, the genotyping-by-sequencing (GBS) approach was applied to genotype a mapping DH population (Kochevenko, personal communication, 2013). QTL analysis was performed separately for each environment. First, the single marker regression was applied from the initial model in which the dependent variable was the phenotypic record of each genotype in each environment. Then the CIM method was applied with a walking speed of 1cM and a window size of 5cM. To detect main effect QTL, the comparison is made of the maximum likelihood L_1 of the model containing all cofactors as well as the main effect of the putative QTL and the maximum

likelihood L_0 of the model containing cofactors only. A genome-wide threshold for p-values or LOD scores was obtained by performing a permutation test for each trait (Churchill and Doerge, 1994). QTL was considered if it had a LOD ≥ 3.0. MapChart (Voorrips, 2002) was used to graphically represent QTL regions on a consensus linkage map. Pearson correlation was applied for the correlation testing of phenotypical traits (VSN International, 2011).

3 Results

Environmental conditions during seed maturation play a key role in the determination of seed dormancy (William E Finch-Savage and Leubner-Metzger, 2006). Dealing with drought stress during seed development and maturation results in inevitably different seed quality traits which are influencing germination behavior and therefore the malting process (Barrero *et al.*, 2012). Seed quality is defined as a set of genetic, physical, physiological and health-related attributes that influence the capacity of a seed batch to produce a uniform crop consisting of vigorous plants. The physiological status of the seeds, for commercial purposes, is determined by germination testing, as described by Powell (Alison Powell and Stan Matthews, 2012). For malting barley, long dormancy increases cost and the potential damage resulting from grain storage. Varieties with inferior germination index have also inappropriate malting quality traits such as Kolbach index, low enzyme activity and producing low malt extract. To come to terms with this problem in beer production, malting additives are used to improve germination and malt quality (Hattingh *et al.*, 2014). Gibberellic acid is an additive that is often added to improve the germination for production of malt. This also means higher costs for the beer industry. Furthermore, the addition of gibberellic acid must be strictly controlled because excess leads to extensive rootlet formation and abnormal hydrolysis of macromolecules (Eßlinger, 2009). An optimized malting process requires efficient and uniform germination of the grains in order to accelerate the production of high levels of enzyme activity that are required to re-mobilize starch. Selection for low dormancy in the breeding programs has reached a point where many commercial varieties have little or no dormancy because this enhances the malting process. Two elite malting breeding lines, LP104 (Sofiara) and LP106 (Victoriana), have been selected for this study because of their distinct terminal drought tolerance mechanism. LP104 exhibits an early senescing phenotype with faster storage compound remobilization while LP106 maintains a stay-green phenotype under drought stress, where the senescence syndrome proceeds slowly (Rajesh, 2012). Harvest-ripe grains were collected from plants at physiological maturity and kept in storage capacity at 4°C to surpass the dormancy period. From the same batch, mature dry grains were used for dormancy testing by germination assays, biochemical and transcriptome analysis and micromalting quality testing.

3.1 Two contrasting barley elite lines differ in their abscisic acid content and germination behavior

The levels of abscisic acid (ABA), a key stress and germination hormone, were measured in mature grains in parental lines (figure 4). The hormone was extracted from four biological replications and it was measured by liquid chromatography-mass spectrometry. Mature grains were harvested from field-grown plants, under optimal and under imposed drought stress condition.

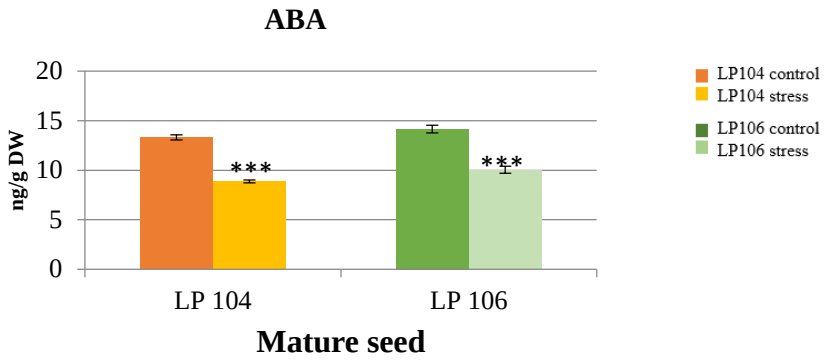


Figure 4. ABA content in mature seed measured after post-harvest storage. Seeds grown under drought-stressed condition have a lower ABA level at this stage. Values are mean +- S.D., (n=4) and expressed as ng per gram dry weight. Significant difference between the treatments are indicated by asterisks, P<0.001:***.

ABA content in mature grains of LP104 and LP106 are comparable; grains which developed under drought stress condition show a significant decrease of ~30% (P<0.001) in ABA. Comparing the lines, LP104 has lower ABA content in drought-stressed mature grains. To investigate the performance of seeds derived from field conditions, germination rates of control and stressed seeds was tested. Germination tests were performed according to International Seed Testing Association (Ista, 2015), in Petri dishes and in phytochamber with 16 hours light and 8 hours dark periods. Seed is defined as germinated when a part of the embryo, the radicle, extends to protrude the seed coat (J. D. Bewley, 1997). The second structure that emerges from the seed coat is the coleoptile or the shoot, a process that marks the post-germination phase. The results of germination test negatively correlate with the ABA content in the grains, which further confirms the significance of the role of this hormone during the switch from seed maturation to germination process. Overall seeds developed under stress condition have a faster germination, which means a higher germination rate respect to the control condition. The harvested grains of line LP104 which had the lowest ABA content (8.8 ng/g DW) developed under stress showed the fastest germination rate at 24 hours after imbibition (HAI) where it reached 48% of germinated seeds (figure 5). Line LP106 which had the highest level of ABA (14.2 ng/g DW) grown under control condition, showed the lowest germination rate at 24 HAI. This increase of germination speed of stressed seeds became even

stronger when the shoot emergence was evaluated. While germination rate accounts for root protrusion, shoot emergence data account only the visible coleoptiles (shoots). In this case stressed seeds are faster in shoot emergence to a higher significance degree.

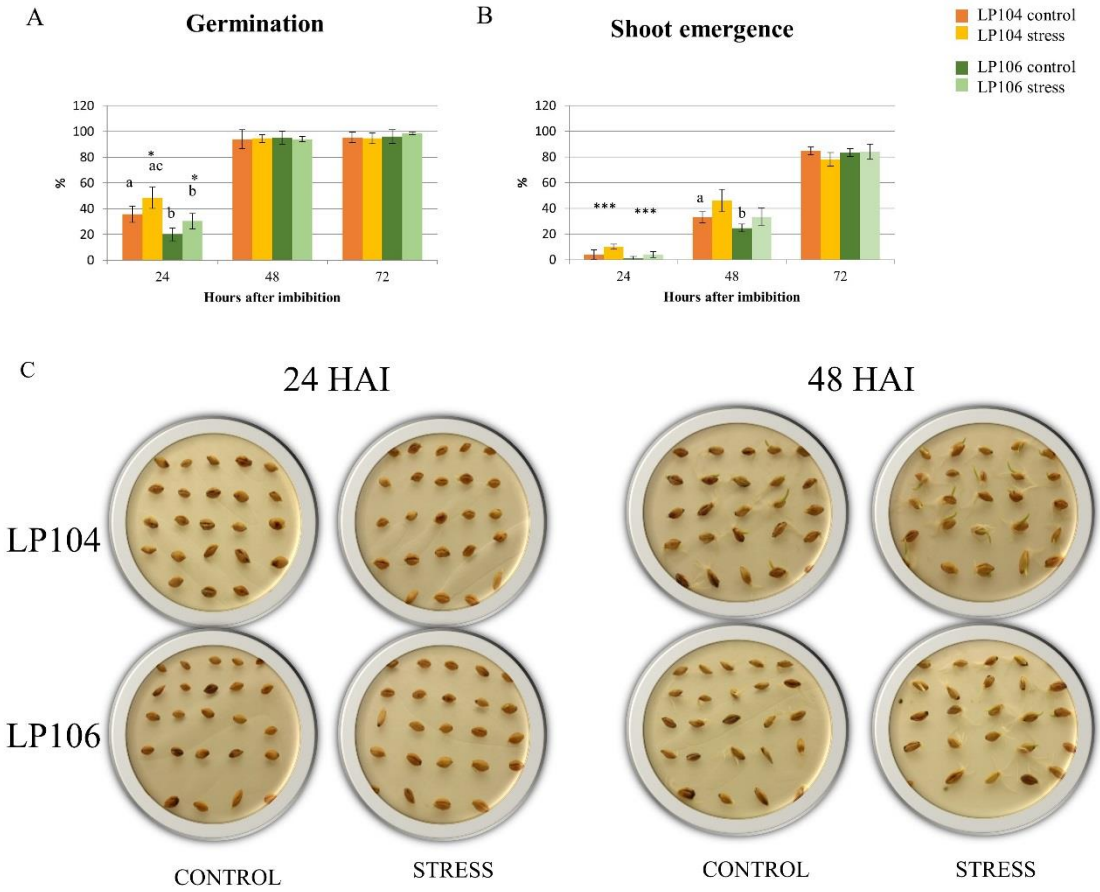


Figure 5. Germination tests show an earlier root and shoot protrusion of the drought-stressed seeds. Germination assay was performed using 4x100 seeds. **A**. Germination data where the significance between control and stress as well as between the lines is present at 24 HAI. **B**. Shoot emergence data which reflect germination percentage data, but with higher significance. At 48 HAI control seedlings of LP104 are significantly faster than the control seedlings of LP106. **C**. Images of two time points of germination, 24 HAI and 48 HAI, which show the difference in germination speed of LP104 and LP106, LP104 stress being the fastest one, and LP106 control being the slowest in on-start of germination process. a: significant difference from LP106, b: significant difference from LP104. A significant difference between the treatments is indicated by * ($P<0.05$).

At 24 HAI, shoot emergence is 10% for the stress-treated seeds of the line LP104. The control seeds have 50% less of emerged seedling at that time point. The line LP106 has less emerged shoots respect to the line LP104, as seen in the germination data (figure 5 A). Compared to seeds from the LP106 control batch (0.85% shoot emergence), 4% of the stressed seed have emerged shoots. At 48 HAI and 72 HAI, germination rate reaches the maximum and there are no more visible differences detectable. The pattern of shoot emergence is maintained at 48

HAI but shows only significant differences between LP104 and LP106 just under control condition, where LP104 has 33% of shoots emerged respect to LP106 which has only 24.6 %. At 72 HAI seedlings reach the full emergence. Seed vigor is a physiological trait that is necessary to ensure the rapid and uniform emergence of plants in the field (Ventura, 2012). Optimal germination performance such as high vigor and germination capacity or viability of barley at the time of malting process is without any doubt the most important quality criterion (Briggs, 1972; Romagosa *et al.*, 2001). These lines, having a different ability to germinate were the basis for the dormancy/germination study of the double haploid (DH) population.

3.2 Germination and shoot emergence of the DH population

The selected DH population consisted of 200 (later reduced to 100) lines, which was developed from the F_1 cross of two elite malting lines LP104 and LP106 (KWS, Einbeck, Germany). Germination rate, shoot emergence and vigor were assessed in the population, for control and drought stressed grown grains. Uniform germination, which depends on grain dormancy (J. D. Bewley, 1997), is a prerequisite for unique malting quality. Weak dormancy leads to pre-harvest sprouting, while strong dormancy leads to non-uniform germination and both bring to big agronomical losses. Addressing the mean value for the entire population it was found that at 24 hours after imbibition 34.05% of the seeds developed under stress conditions were germinated while there were only 30 % of the germinated control seeds. Shoot emergence at that time point was also delayed, with stressed ones having the mean of 3.23% and control ones 1.94%. As shown in figure 5 for the parental lines the mean for the germination rate at 48 HAI and 72 HAI, is 94 and 96 for both conditions, and thereby presents almost the germination maximum. At 48 HAI, stressed seeds show 24% increase in shoot emergence (36.3 vs 27.7 control), while at 72 HAI the mean value of emerged shoots is 90% for both conditions. The frequency distributions of the germination data are shown in figure 6. The left panel shows the frequency distributions of the germination rate, the right panel presents the frequency distributions of the shoot emergence rate of the DH population. The distribution of germination speed also indicates that the seeds derived from the stressed DH population show the same tendency as the parental lines. The variation of the stress response, represented by a more abundant germination (bins 50-70 at 24 HAI) and more abundant shoot emergence (bins 6-10 at 24 HAI and bins 60-70 at 48 HAI) make this population interesting to detect lines with a positive performance for selected malting quality traits. Stressed seeds, generally, show a tendency for a faster speed in early germination, or a lower dormancy. This fact, in turn, raised two questions:

Firstly, is the observed faster germination having a direct effect on malting quality traits? Secondly, is this faster early germination of stressed seeds directly dependent on the ABA content of the mature grain?

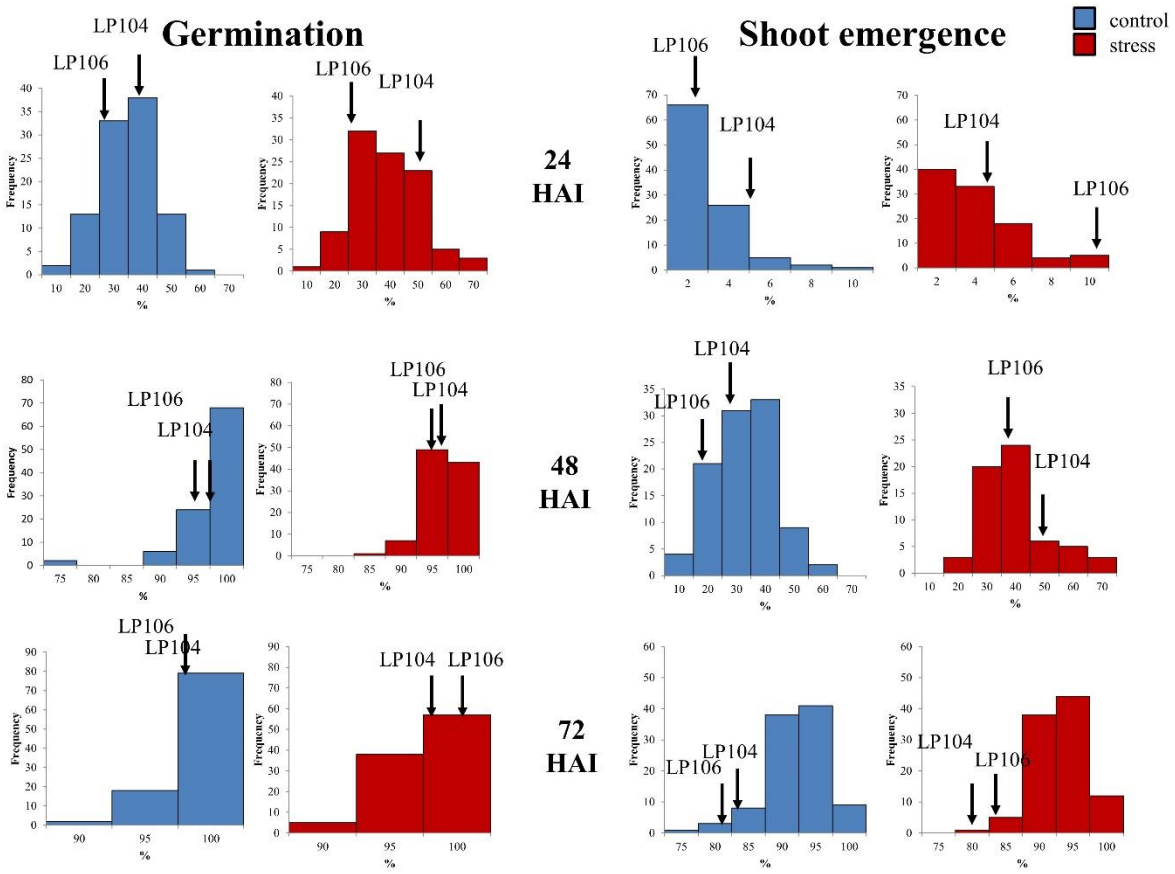


Figure 6. Frequency distributions of germination and shoot emergence data for the LP104xLP106 DH population at time points 24, 48 HAI and 72 HAI. The left panel shows the frequency distributions for germination, the right panel shows frequency distributions for shoot emergence, the blue bars represent control and the red one stress condition. Data are means of three independent germination tests consisting of total 150 seeds. Arrows indicate values for LP104 and LP106. The bins represent the value (%) of the observed frequencies.

3.3 Relationship among traits

To identify seed quality traits and malting quality parameters that correlate to germination and shoot emergence performance, Pearson correlation tests have been performed on the generated data from the contrasting lines and the DH population. The analyzed traits are listed in table 3. Yield, TGW and seed characteristics such as area, length and breadth were screened and assessed by Marvin (GTA Sensorik GmbH, Germany). Seed quality parameters such as nitrogen, carbon, and starch content were measured in mature grains of the DH population, consisting of 98 genotypes and parental lines, with three independent measurements.

Table 3. List of measured traits in mature, germinated and micromalted seedlings. Yield (orange), seed quality traits (shown in yellow), germination data (in green) and micromalted traits (in blue). ABA, PA, DPA were measured only in selected lines and parents. Mean values are shown for LP104, LP106 and the DH population, as well as range of the value for the given trait within the DH population for control and stress conditions.

	Control				**Stress**				
Biological trait	**LP104**	**LP106**	**DH mean**	**DH range**	**LP104**	**LP106**	**DH Mean**	**DH range**	
Yield	114.3	149.3	180.23	114.3 – 228.3	117	171.25	161.08	117 – 204.33	Yield
TGW	59.93	58.26	56.72	51.61 – 69.66	56.72	55.63	54.67	49.27 – 67.36	
Seed area (mm^2)	29.37	29.3	28.71	26.5 – 32.03	28.33	27.93	27.13	25.45 – 29.66	Seed quality
Seed length (mm)	9.37	9.5	9.28	8.73 – 10.06	9.1	9.34	8.99	8.55 – 9.63	
Seed breadth (mm)	4.2	4.1	4.11	3.93 – 4.4	4.05	3.95	3.98	3.83 – 4.23	
Nitrogen %	1.71	1.82	1.69	1.46-1.93	1.7	1.85	1.66	1.5 -1.86	
Protein %	10.71	11.36	10.55	9.1 – 12.04	10.63	11.53	10.8	8.51- 11.59	
Carbon %	40.99	40.63	40.9	40.4 – 41.27	40.56	40.625	40.96	40.46 – 41.31	
Carbon/Nitrogen	24.02	22.45	24.48	21.2 – 28.1	23.9	22.12	24.83	22.19 – 27.3	
Starch (mg/seed)	35.53	31.53	30.79	27.19 – 35.12	31.94	29.05	29.1	25.36 – 33.73	
Abscisic acid (ng/g DW)	13.31	14.17	14.02	10.12 – 17.75	8.89	10.05	12.86	9.99 – 16.25	
Phaseic acid (ng/g DW)	14.48	14.38	15.18	9.52 – 20.10	11.61	12.36	14.01	6.88 – 19.95	
Diphaseic acid (ng/g DW)	499.18	494.11	686.68	360.86 - 1157.12	324.65	325.15	646.92	316.94 – 1113.1	
Germination 24 HAI (%)	35.66	21.6	30.74	6.66 – 51.33	48.4	28.85	34.1	8.67 – 67.33	Germination
Shoot emer. 24 HAI (%)	4	0.85	1.94	0 – 8.66	10	4	3.22	0 – 10.00	
Germination 48 HAI (%)	94	95.33	94.23	63.33 - 100	94.66	94	94.3	82.67 – 98.67	
Shoot emer. 48 HAI (%)	33.11	24.67	27.68	8.66 – 54.00	46	33.33	36.3	15.33 – 71.33	
Germination 72 HAI (%)	95.33	96	96.33	70.00 – 100.00	95	98.67	95.3	88.67 – 100.00	
Shoot emer. 72 HAI (%)	84.66	83.33	89.78	74.66 – 94.00	78	84	90.78	80.00 – 97.33	
Vigour (mm)	214.3	213.3	237.6	197.7 – 288.0	186.0	213.6	232.1	163.0 – 278.0	
Malt water content (%)	6.0	6.2	5.6	5.3 – 6.0	6.1	6.3	6.1	5.4 – 6.3	Micromalting parameters
Malt extract (% DW)	82.2	80.0	82.1	80.5 – 83.7	83.9	80.3	83.4	80.3 – 84.1	
Viscosity (8.6 % mPa*s)	1.5	1.5	1.5	1.42 – 1.62	1.5	1.5	1.5	1.42 – 1.67	
Protein content (% DW)	12.5	12.1	11.3	9.5 – 13.6	11.0	12.7	10.9	9.4 – 12.7	
Soluble nitrogen (mg/100 g DW)	849.0	749.0	772.7	687 – 902	829.0	776.7	825.0	682.0 – 937.0	
Kolbach Index (%)	42.7	38.3	42.7	35.0 – 53.0	47.0	38.7	47.0	38.0 – 53.0	
Final Attenuation (%)	81.9	81.0	80.9	78.6 – 83.6	81.2	80.7	81.3	78.8 – 83.8	
Friability (%)	78.6	71.9	79.4	60.4 – 92.8	87.8	66.8	87.7	66.8 – 92.3	
Beta-glucan (mg/l)	185.0	290.3	273.5	80 - 707	169.7	505.7	173.8	86 - 650	
Total loss (%)	11.3	10.4	11.5	9.7 – 13.6	12.3	11.4	12.3	9.5 – 13.8	
Respiration loss (%)	5.7	5.4	5.9	5.2 – 6.9	6.1	5.7	6.2	5.2 – 8.2	
Root growth loss (%)	5.6	5.1	5.5	4.4 – 6.9	6.2	5.7	6.1	4.1 – 7.1	
Steeping degree 1 (%)	33.7	31.6	33.1	30.7 – 36.0	33.4	31.8	33.8	31.3 – 35.3	
Steeping degree 2 (%)	41.4	39.7	41.7	39.2 – 44.4	43.1	39.8	42.9	39.3 – 43.5	
Steeping degree 3 (%)	44.2	43.8	44.4	43.6 – 45.3	45.5	44.4	45.2	41.6 – 45.5	
Abscisic acid (ng/g DW)	2.9	2.7	2.4	1.39 – 3.16	1.55	2.61	2.37	1.77-3.31	
Phaseic acid (ng/g DW)	18.1	27.9	21.5	12.27 – 31.81	12.72	25.97	21.20	11.23 – 28.24	
Diphaseic acid (ng/g DW)	81.1	132.6	126.5	87.49 – 172.92	104.02	153.58	125.1	73.98 – 199.1	

Nitrogen and carbon content were measured with Elemental Analyzer (vario EL III; Elementar analysen systeme GmbH Hanau, Germany), and protein content was recalculated based on nitrogen values (Tkachuk, 1969). Starch content was enzymatically determined. Furthermore, ABA and its catabolites were determined in mature grains and micromalted material in the parental and 8 selected lines representing the population. ABA was assessed with LC-MS/MS and in collaboration with Dr. Eggert (Molecular Plant Nutrition Group, IPK Gatersleben). The selection of lines was based on contrasting yield data under terminal drought stress: lines 654, 755, 760 and 780 were selected based on their superior and lines 663, 676, 711 and 716 for inferior performance under drought stress condition. Data for germination, shoot emergence [percentage] were collected at 24, 48, 72 HAI. Malting quality parameters consist of 16 different traits: malt moisture content (MMC), malt extract (ME), viscosity (VIS), malt protein content (MPC), soluble nitrogen (SN), Kolbach index (KI), limit of attenuation (FLA), friability (FRI), β glucan content (BG), total malting losses (TL), respiration losses (RL), rootlet losses (RGL) and SD1 (steeping degree at 24 HAI), SD2 (steeping degree at 48 HAI) and SD3 (steeping degree at 72 HAI). Malting quality traits were measured in germinating grains under strictly controlled conditions (described in material and methods) in collaboration with Prof. Rath (The Versuchs- und Lehranstalt für Brauerei in Berlin, Research Institute for Raw Materials, Berlin**)**. Germination and malting quality data were collected from the entire population and for each of values listed at least three independent measurements were performed, with exception of malting parameters, where three independent measurements were applied to parental and selected lines.

Germination rate at 24 HAI of control seedlings correlates negatively (r=-0.37***) with beta-glucan content and positively with friability (r=0.39***). Another malting trait which correlates with germination rate at 24 HAI is VIS, although the correlation is low, it is highly significant. As expected, germination rate at 24 HAI positively correlates with shoot emergence [%] at 24 HAI and 48 HAI. Consistently shoot emergence rate at 24 HAI is only strongly correlated with shoot emergence rate at 48 HAI (r=54***). It has a high significance with seed breadth and friability, but the correlation is missing in this case. Germination [%] at 48 HAI has a highly significant and positive correlation with germination rate at 72 HAI (r=0.3***), shoot emergence rate at 72 HAI (r=0.27***) and vigor (r=0.3***). Shoot emergence rate at 48 HAI has a positive correlation with shoot emergence rate at 24 HAI (r=0.66***) and with SD2 (r=0.2*). It is found to be significant with malt BG, DPA dry seed, ME, FRI, VIS and seed length, but without strong correlation.

Table 4. Pearson correlation coefficient between ABA, PA, DPA content, germination and shoot emergence data at 24, 48 and 72 HAI with yield, seed quality, germination, and malting quality traits coming from control condition. Red: negative correlation, blue: positive correlation, P<0.05:*

	Mature grain			Micromalted			Germination			Shoot emergence		
	ABA	PA	DPA	ABA	PA	DPA	24 HAI	48 HAI	72 HAI	24 HAI	48 HAI	72 HAI
Y (g)	0.11	0.23	0.36	0.05	0.27	0.36	0.06	0.03	0.06	-0.01	-0.11	0.07
TGW (g)	0.32	0.31	0.12	0.32	0.30	0.12	-0.02	-0.20	-0.11	0.07	-0.05	-0.40
SA (mm)	0.08	0.28	0.10	0.48	0.62	0.30	-0.09	-0.17	0.10	-0.02	-0.17	-0.27 **
SL (mm)	0.13	0.33	0.17	0.18	0.50	0.27	-0.01	-0.07	0.14	-0.01	-0.13 **	-0.24
SB (mm)	-0.10	0.03	-0.04	0.74 *	0.53	0.18	-0.14	-0.25	-0.07	-0.08 ***	-0.14	-0.16 *
N (%)	-0.27	-0.19	-0.50	-0.33	0.18	-0.16	-0.01	0.06	-0.08	0.26	0.20	-0.01
P (%)	-0.27	-0.19	-0.50	-0.33	0.18	-0.16	-0.03	0.08	-0.04	0.16	0.16	0.19
C (%)	0.08	0.64 *	0.49	-0.03	-0.06	-0.15	0.09	-0.03	-0.01	0.13	0.23	-0.08
C/N	0.23	0.20	0.46	0.27	-0.20	0.16	0.03	-0.06	0.08	-0.24	-0.16	0.00
Starch (mg/seed)	0.06	0.02	0.02	0.53	0.15	-0.07	-0.05	-0.13	-0.14	-0.16	-0.14	-0.33 *
ABA (ng/g DW)		0.60	0.55	-0.02	-0.10	0.01	-0.35	0.46	0.18	-0.35	-0.37	0.18
PA (ng/g DW)	0.60		0.57	-0.06	0.07	0.26	0.01	0.27	0.42	0.17	0.14	0.39
DPA (ng/g DW)	0.55	0.57		0.02	-0.12	0.19	-0.16	0.42	0.53	0.01	-0.29	0.44
GER 24 HAI (%)	-0.35	0.01	-0.16	-0.22	-0.31	-0.04		0.09	-0.06	0.47 ***	0.66 ***	-0.06
SE 24 HAI (%)	-0.35	0.17	0.01	0.28	-0.06	-0.04	0.47 ***	-0.14	-0.33		0.54 ***	-0.14
GER 48 HAI (%)	0.46	0.27	0.42	-0.59	-0.58	-0.22	0.09		0.30 ***	-0.14	0.00	0.27 ***
SE 48 HAI (%)	-0.37	0.14	-0.29	-0.14	0.01	-0.06	0.66 ***	0.00	-0.23	0.54 ***		0.05
GER 72 HAI (%)	0.18	0.42	0.53	-0.20	0.04	0.31	-0.06	0.30 ***		-0.33	-0.23	0.25 ***
SE 72 HAI (%)	0.18	0.39	0.44	-0.79 **	-0.61	-0.14	-0.06	0.27 ***	0.25 ***	-0.14	0.05	
Vigour (cm)	0.32	0.58	0.26	-0.71 *	-0.48	-0.04	0.36	0.30 ***	0.22**	0.06*	0.45	0.48 ***
MMC (%)	-0.09	-0.25	-0.73 *	0.07	0.22	-0.30	-0.08	-0.01	-0.04	0.03	-0.03	-0.03
ME (%)	0.02	0.20	0.52	0.16	-0.26	0.15	0.16	-0.08	0.03	-0.08	0.02*	-0.09
VIS (8.6% mPas)	-0.64 *	-0.34	-0.78 **	-0.20	0.21	-0.13	-0.14 ***	-0.08	-0.14	0.11	0.01*	0.07
MPC (%)	-0.27	-0.02	-0.54	-0.07	0.09	-0.28	0.00	0.06	-0.08	0.26	0.20	-0.01
SN (mg/100g DW)	-0.40	0.22	0.06	0.18	0.11	0.16	0.31	0.07	-0.01	0.29	0.35	-0.06
KI (%)	-0.09	0.21	0.69 *	0.26	0.00	0.43	0.29	-0.03	0.02	0.08	0.13	-0.24
FA (%)	0.37	0.00	0.45	0.30	-0.26	-0.17	0.11	-0.06	0.00	-0.11	0.05	0.02
FRI (%)	0.35	0.28	0.69 *	0.33	-0.13	0.33	0.39 ***	-0.01	0.05*	0.01	0.15**	-0.23
BG (mg/l)	-0.51	-0.25	-0.57	-0.55	0.03	-0.27	-0.37 ***	-0.06	-0.05	-0.09	-0.16 *	0.19
TL (%)	0.14	0.05	0.52	-0.43	-0.63	-0.32	-0.03	0.17	0.04	-0.04	0.01	0.27
RL (%)	0.22	0.39	0.62	-0.30	-0.41	0.08	-0.04	0.25	0.05	-0.01	0.00	0.16
RGL (%)	0.08	-0.15	0.39	-0.45	-0.66 **	-0.49	-0.01	0.07	0.03 **	-0.04	0.01	0.26 **
SD1 24 h (%)	0.38	0.32	0.48	-0.32	-0.75 *	-0.50	0.04	0.01	-0.03*	0.06	0.15	0.06
SD2 48_h (%)	0.31	0.53	0.56	-0.20	-0.48	-0.02	0.13	0.06	-0.08 **	0.06	0.20*	0.10
SD3 72 h (%)	-0.27	-0.04	0.21	-0.29	-0.36	0.02	0.06	-0.04	-0.04	-0.01	0.07	0.22
ABA (ng/g DW)	-0.02	-0.06	0.02		0.63 *	0.50	-0.22	-0.59	-0.20	0.28	-0.14	-0.79 **
DPA (ng/g DW)	0.01	0.26	0.19	0.50	0.76 *		-0.04	-0.22	0.31	-0.04	-0.06	-0.14
PA (ng/g DW)	-0.10	0.07	-0.12	0.63 *		0.76 *	-0.31	-0.58	0.04	-0.06	0.01	-0.61

Germination % at 72 HAI correlates as mentioned above with germination rate at 48 HAI, shoot emergence at 72 HAI (r=0.25***) and vigor (r=0.22**). VIS, ST1, SD2, RGL and FRI are found to be in a significant relationship with germination at 72 HAI, but without correlation. Shoot emergence at 72 HAI is positively correlating with RGL (r=0.26**) and vigor (r=0.48***). The negative correlation for this trait is the one with seed area (r=-0.27**) and a low correlation with seed breadth (r=-0.16*). The content of ABA, PA, and DPA measured in the micromalted material showed the strongest significant correlations among themselves under control condition. ABA and its degradation products PA and DPA have been found to have some significant correlations with malting quality traits. ABA content in the dry seeds has a significant negative correlation with VIS (r=-0.63*). The same correlation is found also for DPA, a catabolic product of ABA, which also negatively correlates with VIS (r=-0.78**) but it also negatively correlates with MMC (r=-0.72*). Significantly positive correlations have been found between DPA and FRI (r=0.69*) and KI (r=0.69*). PA in the dry seed is positively correlated with C% in dry seed (r=0.63*). ABA content in the micromalted material negatively correlates with shoot emergence at 72 HAI (r=-0.78**) and vigor (r=-0.736*) but correlates positively with seed breadth (r=0.736*). While DPA only correlates with PA (r=0.757*), PA in micromalted seedlings is found to have a positive correlation with ABA (micromalted) and a negative correlation with RGL (r=-0.656*) and SD (r=-0.75*).

The relationships between traits that come from drought stress conditions show a similar pattern, but also particular drought stress specific relations become apparent which are not seen under control condition. Germination percentage at 24 HAI is found to be positively correlated with FRI (r=0.44***) and shoot emergence at 24 HAI (r=0.35***) and at 48 HAI (r=0.74***), and negatively with BG content (r=-0.33***) and VIS (r=-0.33***). Shoot emergence at 24 HAI positively correlates with germination at 24 HAI (r=0.35***), shoot emergence at 48 HAI (r=0.45***) and seed breadth (r=0.38***). Germination rate at 48 HAI is correlating positively with germination at 72 HAI (r=0.65***), shoot emergence at 72 HAI (r=0.51***) and vigor (r=0.34***). For the trait shoot emergence at 48 HAI positive correlations was identified with malting quality traits, such as with ME (r=0.27**), FRI (0.3**), SD 2 (r=0.2*) but also negative ones, such as BG content (r=-0.2*) and VIS (r=-0.2*). Germination rate at 72 HAI positively correlates with RGL (r=0.32**), shoot emergence rate at 72 HAI (r=0.6***), and vigor (r=0.37***) and negatively with FRI (r=-0.21*), SD 1 (r=-0.23*) and SD 2 (r=-0.32**). Shoot emergence at 72 HAI correlates with RGL (r=0.28**), TL (r=0.16**) vigor (r=0.56***) and with seed area (r=-0.26**). ABA content determined in dry

Table 5. Pearson correlation coefficient between ABA, PA, DPA content, germination and shoot emergence data (24, 48 and 72 HAI) and yield, seed quality, germination, and malting quality traits coming from drought stress condition. Red: negative correlation, blue: positive correlation, P<0.05:*

	Mature grain			**Micromalted**			**Germination**			**Shoot emergence**		
	ABA	PA	DPA	ABA	PA	DPA	24 HAI	48 HAI	72 HAI	24 HAI	48 HAI	72 HAI
Y (g)	0.48	0.37	0.21	0.66	0.59	0.14	-0.01	0.11	0.10	-0.02	0.10	0.13
TGW (g)	0.45	0.73	0.58	-0.2	0.14	0.33	-0.04	-0.09	-0.01	0.06	-0.11	-0.27
SA (mm)	0.23	0.73 *	0.41	-0.16	0.24	0.40	-0.07	-0.06	0.07	0.05	-0.17	-0.26 **
SL (mm)	0.18	0.69 *	0.36	0.06	0.42	0.59	-0.12	0.02	0.11	-0.19	-0.31 **	-0.19
SB (mm)	0.19	0.48	0.28	-0.40	-0.14	-0.02	0.08	-0.17	-0.08	0.38 ***	0.17	-0.20
N (%)	-0.17	0.36	-0.10	0.64	0.80 *	0.54	-0.12	0.00	0.09	0.14	-0.10	-0.04
P (%)	-0.17	0.36	-0.10	0.64	0.80 *	0.54	-0.10	0.00	0.07	0.16	-0.09	-0.04
C (%)	0.21	0.42	0.02	0.14	0.36	0.14	0.00	-0.20	-0.05	0.17	-0.07	-0.04
C/N	0.07	-0.38	0.01	-0.57	-0.79 *	-0.59	0.15	-0.02	-0.08	-0.12	0.13	0.05
Starch (mg/seed)	0.78 *	0.72 *	0.66	0.50	0.63	0.66	0.03	-0.10	-0.01	-0.18	-0.12	-0.24
ABA (ng/g DW)		0.78 *	0.95 ***	0.21	0.41	0.60	-0.75 *	-0.52	-0.69	-0.57	-0.59	-0.76 *
PA (ng/g DW)	0.78 *		0.76*	0.37	0.75 *	0.73*	-0.95 ***	-0.41	-0.62	-0.83 *	-0.83 *	-0.55
DPA (ng/g DW)	0.95 ***	0.76 *		0.29	0.48	0.72*	-0.76 *	-0.65	-0.68	-0.67	-0.69	-0.81 *
GER 24 HAI (%)	-0.75 *	-0.95 ***	-0.76 *	-0.32	-0.69	-0.62		-0.18	-0.09	0.35 ***	0.74 ***	0.03
SE 24 HAI (%)	-0.57	-0.83 *	-0.67	-0.32	-0.64	-0.60	0.35 ***	-0.09	0.07		0.45 ***	-0.08
GER 48 HAI (%)	-0.52	-0.41	-0.65	-0.31	-0.38	-0.46	-0.18		0.65 ***	-0.09	-0.11	0.51 ***
SE 48 HAI (%)	-0.59	-0.83 *	-0.69	-0.61	-0.87 **	-0.79 *	0.74 ***	-0.11	0.01	0.45 **		0.13
GER 72 HAI (%)	-0.69	-0.62	-0.68	-0.57	-0.66	-0.64	-0.09	0.65 ***		0.07	0.01	0.60 ***
SE 72 HAI (%)	-0.76 *	-0.55	-0.81 *	-0.33 *	-0.46	-0.53	0.03	0.51 ***	0.60 ***	-0.08	0.13	
Vigour (cm)	-0.12	-0.37	-0.27	0.43	0.25	-0.02	0.04	0.34 ***	0.37 ***	0.04	0.13	0.56 ***
MMC (%)	-0.25	-0.20	-0.39	-0.15	-0.16	-0.57	0.01	0.04	-0.05	-0.07	0.02	-0.12
ME (%)	0.46	0.03	0.42	0.24	-0.10	-0.03	0.16	0.09	0.02	0.08	0.27 **	0.09
VIS (%)	-0.29	0.05	-0.24	-0.30	-0.01	-0.25	-0.33 ***	0.05	-0.02	-0.05	-0.20 *	-0.10
MPC (%)	-0.15	0.32	-0.11	0.07	0.48	0.32	-0.04	-0.01	-0.07	0.09	-0.01	-0.10
MSN (%)	0.33	0.54	0.43	0.79	0.89 **	0.74 *	0.17	-0.07	-0.12	0.04	0.11	-0.06
KI (%)	0.36	0.04	0.42	0.52	0.15	0.23	0.19	-0.05	-0.02	-0.05	0.11	0.04
FA (%)	0.33	0.18	0.32	0.70	0.39	0.39	0.12	-0.19	-0.13	-0.09	0.07	0.11
FRI (%)	0.29	-0.28	0.15	0.02	-0.35	-0.21	0.44 ***	-0.14	-0.21 *	0.10	0.30 **	-0.04
BG (%)	-0.55	-0.03	-0.43	-0.15	0.13	-0.04	-0.33 ***	0.12	0.16	-0.04	-0.20 *	0.01
TL (%)	0.06	-0.14	0.22	0.05	-0.06	0.34	0.07	0.06	0.18	0.15	0.12	0.16 **
RL (%)	0.64	0.37	0.63	-0.07	0.13	0.51	-0.07	-0.11	-0.16	0.10	-0.02	-0.13
RGL (%)	-0.28	-0.37	-0.08	0.10	-0.14	0.13	0.12	0.14	0.32 **	0.12	0.16	0.28 **
SD1 24 h (%)	-0.63	-0.39	-0.64	0.10	-0.02	-0.06	0.08	-0.04	-0.23 *	-0.01	0.05	-0.04
SD2 48_h (%)	-0.06	-0.44	-0.14	-0.31	-0.55	-0.36	0.10	-0.10	-0.32 **	0.19	0.20*	-0.19
SD3 72 h (%)	0.53	0.62	0.72*	0.25	0.54	0.70	0.15	-0.04	-0.13	0.01	0.06	-0.13
ABA (ng/g DW)	0.21	0.37	0.29		0.82*	0.59	-0.32	-0.31	-0.57	-0.32	-0.61	-0.33
DPA (ng/g DW)	0.60	0.73*	0.72*	0.59	0.82*		-0.62	-0.46	-0.64	-0.60	-0.79 *	-0.53
PA (ng/g DW)	0.41	0.75*	0.48	0.82*		0.82 *	-0.69	-0.38	-0.66	-0.64	-0.87 **	-0.46

seed negatively correlates with germination at 24 HAI and shoot emergence 72 HAI with r=-0.746* and -0.757*. ABA also correlate positively with high significance with its catabolic derivates, PA and DPA (r=0.78* for PA and r=0.95*** for DPA). ABA content of the seeds was also found to be positively correlated (r=0.77*) with starch mg/seed. DPA and PA contents were negatively correlated with the germination rate at 24(r=0.758*, DPA and r=-0.946*** PA). Furthermore, DPA is found to be correlating with ABA (R=0.95***) and PA (r=0.76*) content in the dry seed but also with DPA (r=0.72*) contained in the micromalted seedling. PA in the dry seed negatively correlates with shoot emergence at 24 HAI (r=-0.83**) and 48 HAI (r=-0.83**) and positively with starch mg/seed (r=0.72*). DPA positively correlating with shoot emergence at 72 HAI (r=-0.81*) and SD 3 (r=0.72*). PA and DPA content in the micromalted samples correlates with SLN content in malt (r=0.89**, r=0.74*). PA positively correlates with N content and P content in the dry seed (both cases r=0.81*), but negatively with C/N ratio (r=-0.79*) and negatively with shoot emergence percentage at 48 HAI (r=-0.87**).

3.4 Germination and shoot emergence quantitative trait loci

To identify the genomic loci associated with the analyzed traits of germination and malting quality an association analysis was performed. Quantitative trait loci (QTLs) were analyzed for two traits, germination and shoot emergence rate at three different time points, 24, 48 and 72 hours after imbibition (HAI). The quantitative trait locus analysis was carried out using QTL-Cartographer (Wang, Basten and Zeng, 2011) and composite interval mapping (CIM) method. 1782 informative SNPs were employed for the construction of the SNP Genetic map using the software package JoinMap 4.0 (Van Ooijen, 2006). Linkage groups were established using minimum LOD values of 4. Monte Carlo maximum likelihood (ML) mapping algorithm was applied to determine the orders of markers within each linkage group. Recombination frequencies were converted to centimorgans (cM) using Haldane's mapping function. The markers order of generated map was compared with already published map POPSEQ (Mascher *et al.*, 2013). The map covers 1140 cM with individual linkage groups ranging from 100 cM (chromosome 6H) to 190 cM (chromosome 1H). Mature grains of the DH population used for germination tests were harvested from the field grown plants, which were planted in a randomized block design (described in materials and methods). This permitted to analyze QTLs separately for each environment (control and stress) and search for the same QTLs for a given trait. A total of 25 QTLs were detected (table 6, figure 7) comprising both environments, 20 of them explaining more than 10% phenotypic variation. 10 detected QTLs were related to

Table 6 List of germination and shoot emergence QTLs estimated from DH population using composite interval mapping by QTL Cartographer (Wang, Basten and Zeng, 2011). Data used for the QTL calculation come from germination and shoot emergence testing (n=50 seeds x 3) and account for rates (%) at 24, 48 and 72 HAI. Control: seeds that developed under watered condition, stress: seeds that developed under terminal drought stress.

Trait	Condition	QTL Name	Chr.	Position	LR	Additive	Dom	LOD	R2 %
GERMINATION 24 HAI	control	Q-ger24-c1	3	0.6011	48.48	2.6709	0	10.52	28.75
GERMINATION 24 HAI	control	Q-ger24-c2	7	0.0201	15.49	1.4081	0	3.36	7.87
SHOOT EMERGENCE 24 HAI	control	Q-SE24-c1	3	0.6011	19.45	0.3272	0	4.22	14.44
SHOOT EMERGENCE 24 HAI	control	Q-SE24-c2	5	1.2831	14.86	-0.2746	0	3.22	10.77
SHOOT EMERGENCE 24 HAI	control	Q-SE24-c3	7	1.1811	17.37	0.3149	0	3.77	13.51
GERMINATION 48 HAI	control	Q-ger48-c1	2	0.6841	19.62	0.6762	0	4.26	6.01
GERMINATION 48 HAI	control	Q-ger48-c2	3	0.1231	9.82	-0.3461	0	2.13	2.58
SHOOT EMERGENCE 48 HAI	control	Q-SE48-c1	1	0.0381	25.95	2.2313	0	5.63	18.98
SHOOT EMERGENCE 48 HAI	control	Q-SE48-c2	7	0.0001	17.22	1.7632	0	3.73	11.95
SHOOT EMERGENCE 48 HAI	control	Q-SE48-c3	7	0.0611	16.7	1.7408	0	3.62	11.62
GERMINATION 72 HAI	control	Q-ger72-c	3	0.6011	10.34	-0.5707	0	2.24	8.73
SHOOT EMERGENCE 72 HAI	control	Q-SE72-c1	3	0.6011	16.8	-0.7824	0	3.64	11.69
SHOOT EMERGENCE 72 HAI	control	Q-SE72-c2	4	0.3291	15.43	-0.7215	0	3.35	10.65
GERMINATION 24 HAI	stress	Q-ger24-s	2	1.7721	15.91	2.0463	0	3.45	11.00
SHOOT EMERGENCE 24 HAI	stress	Q-SE24-s1	2	1.4191	16.28	0.389	0	3.53	10.19
SHOOT EMERGENCE 24 HAI	stress	Q-SE24-s2	7	1.0591	34.15	0.6121	0	7.41	23.47
SHOOT EMERGENCE 24 HAI	stress	Q-SE24-s3	7	1.1301	30.23	0.5932	0	6.56	22.46
GERMINATION 48 HAI	stress	Q-ger48-s1	2	1.3961	35.37	0.8811	0	7.68	22.77
GERMINATION 48 HAI_	stress	Q-ger48-s2	2	1.5691	25.14	-0.7785	0	5.45	15.69
GERMINATION 48 HAI	stress	Q-ger48-s3	7	1.7981	22.12	-0.5808	0	4.80	13.77
SHOOT EMERGENCE 48 HAI	stress	Q-SE48-s1	2	1.7721	16.13	1.8614	0	3.50	11.48
SHOOT EMERGENCE 48 HAI	stress	Q-SE48-s2	7	0.0381	15.8	1.9071	0	3.43	11.99
SHOOT EMERGENCE 48 HAI	stress	Q-SE48-s3	7	0.1211	14.69	1.8029	0	3.18	11.19
GERMINATION 72 HAI	stress	Q-ger72-s	3	0.6011	17.09	-0.4332	0	3.71	11.51
SHOOT EMERGENCE 72 HAI	stress	Q-SE72-s	3	0.5451	24.53	-0.8824	0	5.32	22.69

germination rate and 15 to shoot emergence. 2 QTLs were detected for germination rate at 24 HAI, one on chromosome 3 and one on chromosome 7, with a phenotypic variation of 28.7% and 7.87%. For stress condition, there was only one QTL found, on chromosome 2 with a phenotypic variation of 11%. In both cases, the additive effect was given by LP104, with the highest LOD score (10.52) for germination at 24 HAI under control condition. For shoot emergence at 24 HAI 6 QTLs were identified, 3 QTLs for control on chromosome 3, 5 and 7 and 3 for stress condition, on chromosome 2 and 7. The phenotypic variation was in the range of 10.19% to 23.47%. For all QTLs, the LP104 allele had increasing effects, except for the one QTL on chromosome 5. For germination rate at 48 HAI 5 QTLs have been detected, 2 QTLs for control condition at chromosomes 2 and 3 explaining 6.01% and 2.58% of phenotypic variation, and 3 QTLs for stress on chromosomes 2 and 7, explaining 22.77%, 15.69% and 13.77% of phenotypic variation. The trait shoot emergence at 48 HAI showed 6 QTLs in total. For control condition, 1 QTL was found on chromosome 1 with 18.98% of the phenotypic

variation and 2 on chromosome 7 with 11% of phenotypic variation, while for stress one QTL was found on chromosome 2 and 2 QTLs on chromosome 7, all with 11% of phenotypic variation. For the trait shoot emergence at 48 HAI for all 6 detected QTLs, LP104 contributed to the increased value of the trait. For germination rate at 72 HAI one QTL was detected for control condition and one for stress condition, both on the same position on chromosome 3 and with additive effects given by allele coming from LP106. Three QTLs were detected for shoot emergence at 72 HAI, two of them for control condition on chromosome 3 and 4, explaining 11.69% and 10.65% of phenotypic variation. The only one QTL for shoot emergence at 72 HAI for stress condition is detected on chromosome 3. For all three QTLs, the additive effect is contributed by LP106 allele. All the detected QTLs are mostly represented on chromosome 3, 5 and 2. The stress-related QTLs are specifically linked to the chromosome 2. These chromosomal regions have been detected previously in other studies, which further confirms these regions as hot spots for germination and shoot emergence (Mano and Takeda, 1997; Vanhala and Stam, 2006; Sato *et al.*, 2009).

3.4.1 Co-localization of quantitative trait loci related to germination and shoot emergence with malting quality traits

Co-localization between QTLs for individual traits was broadly represented. It is well known that such clustering could be a consequence of the presence of either a set of closely linked loci or a single pleiotropic locus (Cai and Morishima, 2002; Peng *et al.*, 2003; Gyenis *et al.*, 2007). The co-localization detected here is the clustering of seed quality traits or malting quality traits with already described QTL clusters of germination and shoot emergence data. The “hot spots” regions are positioned within the centromeric region of chromosome 3H and two regions on chromosome 5H. The detected QTLs on these regions are stably detected for both conditions tested. A further QTL hot spot for germination and shoot emergence which are specifically, detectable only for seeds developed under drought stress are on chromosome 2H. On the chromosome 3H there is a co-location of QTLs for traits germination rate 24 HAI_control, shoot emergence 24 HAI_control and germination rate 72 HAI_control and germination 72 HAI_stress and shoot emergence 72 HAI_stress and Kolbach Index, Friability, beta-glucan and soluble nitrogen. One of germination QTLs, associated with the telomeric region of chromosome 5H is found for both conditions and also detected as region affecting malt extract and friability traits, also under both conditions evaluated. The middle region of chromosome 5H where the shoot emergence QTL cluster (both conditions) is found it is also

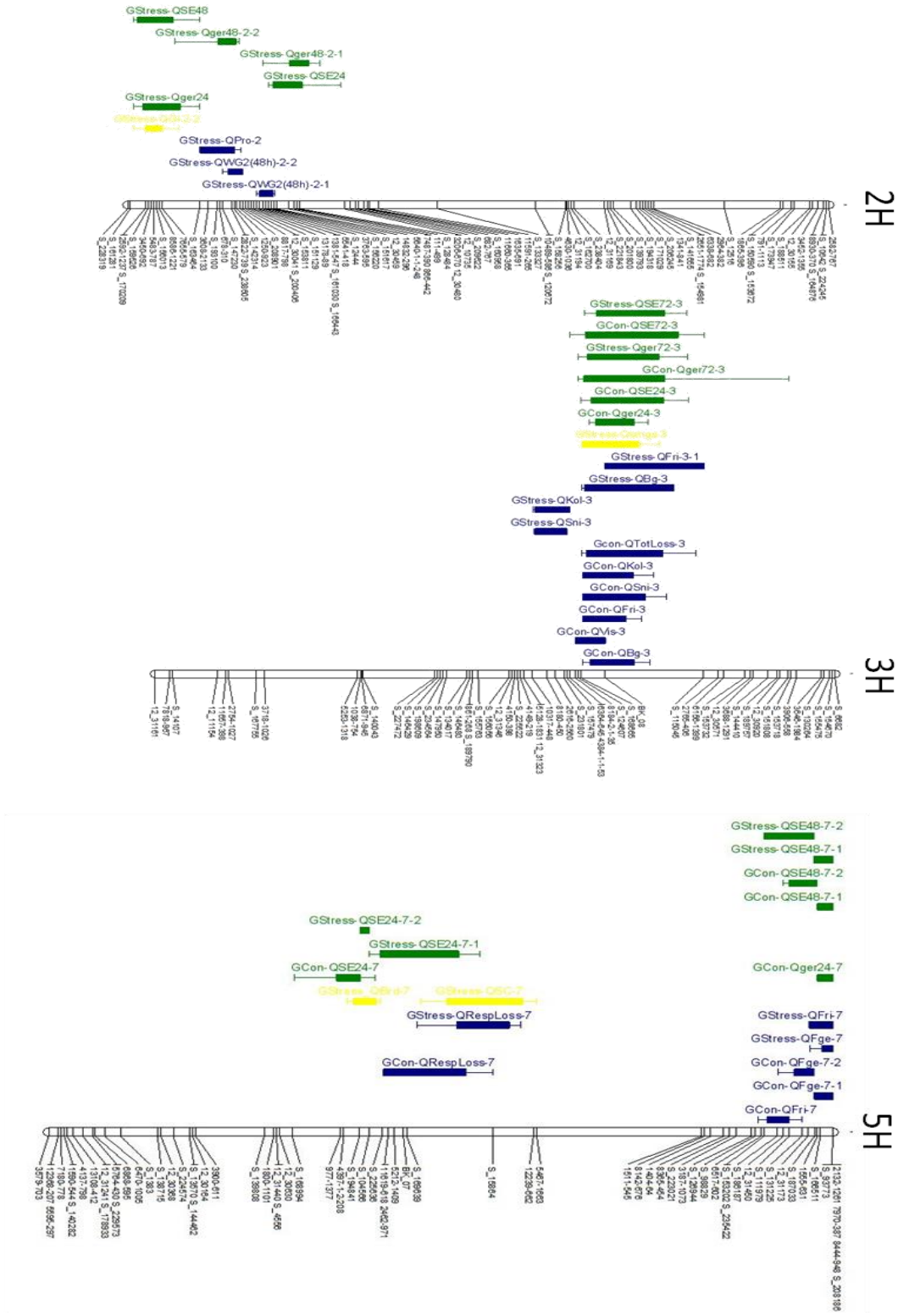


Figure 7. Chromosomal locations of major quantitative trait loci (QTL) controlling germination and shoot emergence (in green), seed quality (yellow) and malting quality traits (blue) in barley. Drawn in the software MapChart (Voorrips, 2002). QTL represented by bars were shown on the left of the linkage groups 2H, 3H and 5H, close to their corresponding markers.

affecting respiration losses and C% in dry seed. QTLs related to stress condition found on chromosome 2H (germination and shoot emergence at 24HAI and 48 HAI), co-localized with two steeping degree QTLs, malt protein content and seed length. Overall these traits demonstrated a high level of correlation of the tested traits and their relation to each other. The observed co-localizations again confirmed the fact that these traits were the interrelated components contributing to malting quality parameters.

3.5 Transcriptome analysis of LP104 and LP106

In most flowering plants, seed development and germination are separated by a period of quiescence or dormancy (J. D. Bewley, 1997; Finch-Savage and Footitt, 2012). Only after breaking dormancy, the quiescent embryo is able to germinate after imbibition (J. D. Bewley, 1997; William E Finch-Savage and Leubner-Metzger, 2006). These processes have been studied intensively at the physiological and molecular level. Seed maturation and germination are associated with coordinated changes in primary metabolism during this defined transitory period and that metabolic preparation for germination is already initiated during late stages of seed maturation. In this work, different sets of microarray analysis were performed. They have been performed using total RNA of mature grains and micromalted seedlings but also using the polysome-bound from the micromalted material of LP104 and LP106. The provided labeled RNA samples were hybridized on Agilent custom 8x60k barley chip and analyzed using the Genespring software (Agilent technologies). The pre-processing step consisted in quantile normalization across all hybridizations. The low-quality features, those with signals close or below the background levels were filtered out, according to the default settings of the Genespring software (Agilent technologies). The pre-processing resulted in 32.563 features with acceptable signal values. The use of microarray technology for accurately scoring of differential gene expression within large populations has greatly enhanced the number of potential genes that can be screened and tested for association with a specific trait of interest. The goal was to identify differentially expressed genes explaining the identified differences in germination and malting quality traits. Furthermore, to investigate the activation of stored transcripts during early seed germination and to identify gene sets under translational regulation during malting, microarray experiments have been done using the polysome-bound RNA of the micromalted seedlings.

3.5.1 Dry seed transcriptome

Mature dry seeds store various mRNAs which are accumulated during seed development (Rajjou *et al.*, 2004; Nakabayashi *et al.*, 2005; Kimura and Nambara, 2010). Their abundance and quality are affected by environmental stresses, such as drought. Seeds of LP104 and LP106 grown in the field under watered and drought stress condition were used for transcriptome analysis to find significant differences that occur under drought and that have an impact on germination behavior and therefore malting quality. For this transcriptome analysis microarray hybridization was performed with three biological replications. After readout of the data, normalization and filtering steps (explained in methods), the number of entities (or transcripts) detectable in the dry seed resulted in approximately 20.000. Most of these transcripts are pre-synthesized and stored during maturation (Ogawa, 2003; Nakabayashi *et al.*, 2005; Sreenivasulu *et al.*, 2008; J.D. Bewley *et al.*, 2013). In tables 7 and 8, 20 top abundant transcripts are shown for the lines LP104 (table 7) and LP106, (table 8). The top abundant transcripts are similar between conditions within the genotype. In control seeds of LP104 transcripts associated with transport of peptides and oligopeptides, calcium binding and calmodulin-like protein, acyl CoA synthetase, late embryogenesis proteins, cytochrome P450 superfamily protein, stigma-specific family protein, and chymotrypsin inhibitor are among the top abundant transcripts. The situation is similar in stressed seeds of LP104, but with the additional presence of transcripts associated with class I heat shock proteins, light-induced protein, nodulin-related protein, and aspartyl proteases. Heat shock proteins, calcium binding and calmodulin-like proteins have a role in stress response during seed desiccation period (Yang *et al.*, 2011). Calcium signaling proteins have also been shown to act as germination essential transcripts that promote germination in rice (Sano *et al.*, 2012). Chymotrypsin inhibitor, one of the protease inhibitors, is a storage protein that functions as regulator of endogenous proteases and protects plants from pathogen attack (Pusztai, 1972; Ryan, 1989). Long chain Acyl CoA and aspartyl protease have been defined as germination, in particular aspartic protease might be involved in ABA-dependent responsiveness and drought avoidance (Schnurr, Shockey and Browse, 2004; Yao *et al.*, 2012).

Table 7. Top abundant transcripts present in the LP104 dry seed that surpassed dormancy (0 HAI). On the left (orange) transcripts present in the seed developed in control (watered) condition, on the right (yellow), top abundant transcripts in the seed developed under water-stress condition. For each transcript normalized mean expression value (n=3) is indicated along with the annotation. Values are obtained after data processing (explained in Materials and Methods) in Genespring software (Agilent technologies). In bold: transcripts which are present in both conditions.

Harvest assembly ID	LP104 control	Annotation	Harvest assembly ID	LP104 stress	Annotation
Contig14945_at	3.096112	Transport peptides and oligopeptides	35_48001	5.557579	NA
35_31322	3.06606	Transport peptides and oligopeptides	**35_21841**	3.09292	Cell organisation
35_17779	3.066048	NA	**35_2619**	2.982884	NA
35_17480	3.063391	Calcium-binding EF-hand family protein	**Contig6268_at**	2.981806	Calcium-dependent lipid-binding (CaLB domain) family protein
Contig6268_at	3.02186	Calcium-dependent lipid-binding (CaLB domain) family protein	**35_22937**	2.940424	NA
HV_CEb0006A14f_s_at	2.969379	Calmodulin like 23 EF-hand family protein	35_4415	2.899766	High-light-induced protein, chloroplastic
Contig7016_s_at	2.902411	Calcium-binding EF-hand family protein	**35_904**	2.858447	Chymotrypsin inhibitor
35_10273	2.872014	Late embryogenesis abundant protein-related / LEA protein-related	35_14861	2.837363	17.9 kDa class I heat shock protein
35_2619	2.86211	NA	**HV_CEb0006A14f_s_at**	2.835626	Calmodulin like 23 EF-hand family protein
35_22937	2.842093	NA	**35_17779**	2.833094	NA
35_22055	2.839667	Cytochrome P450 superfamily protein	**35_17435**	2.828442	NA
35_35437	2.829097	Stigma-specific Stig1 faimily protein	35_14859	2.820501	17.9 kDa class I heat shock protein
35_17435	2.749925	NA	**35_31322**	2.772072	Transport peptides and oligopeptides
35_5826	2.686283	NA	**Contig14945_at**	2.767021	Transport peptides and oligopeptides
35_11772	2.682823	Transport p- and v-ATPases	35_37414	2.766254	Isopentenyl-diphosphate Delta isomerase II
35_20536	2.681885	Long chain acyl-CoA synthetase 9	35_3989	2.698947	Eukaryotic aspartyl protease family protein
35_21841	2.678515	Cell organisation	Contig9782_at	2.696441	Eukaryotic aspartyl protease family protein
35_31025	2.650322	NA	35_3990	2.687335	Eukaryotic aspartyl protease family protein
35_34429	2.628502	NA	Contig2006_s_at	2.686325	17.9 kDa class I heat shock protein
35_904	2.620489	Chymotrypsin inhibitor	35_647	2.67129	Early nodulin-related

Differences between the genotypes can already be seen in the list of top abundant transcripts. In control seeds of LP106, the highly abundant transcripts are associated with protein metabolism, like ubiquitin-conjugating enzyme and elongation factor 1 (only under stress). DNA synthesis related transcripts like DNA helicase and RNA processing endoribonuclease are also found. Other highly abundant transcripts are associated with succinate metabolism (succinate dehydrogenase), serine/threonine protein kinase, receptor kinase, which might have a role in abiotic stress response. The e2f transcription factor, nodulin proteins, and nudix

hydrolase are also among the top abundant transcripts and their role is related both to seed desiccation and germination (Ramirez-Parra, 2004; Wallace, Choi and Roberts, 2006; Zeng, Li and Mahalingam, 2014). These are also found in the stressed seeds of LP106, where transcripts associated with short-chain dehydrogenase, and pathogen defense and cell death related MLO protein is found.

Table 8 Top abundant transcripts in the LP106 line in the dry seed (0 HAI) which surpassed the dormancy period and it is ready for germination. Normalized expression values are indicated, obtained after data processing (explained in Materials and Methods) in Genespring software (Agilent technologies) and represent a mean value of three independent replications. In dark green normalized expression values of control, and in light green of stressed material. In bold: transcripts present in both conditions

Harvest assembly ID	LP106 control	Function	Harvest assembly ID	LP106 stress	Function
35_813	5.288672	Succinate dehydrogenase subunit 8A, mitochondrial	**35_18580**	5.519961	NA
35_2790	5.166632	NA	**35_813**	5.255563	Succinate dehydrogenase subunit 8A, mitochondrial
35_18580	5.029629	NA	**35_2790**	5.188856	NA
35_18878	4.363312	NA	**35_644**	4.332067	early nodulin-related
35_16398	3.773636	Ubiquitin-conjugating enzyme family protein	**35_630**	4.293787	early nodulin-related
35_19965	3.615437	NA	**35_18878**	4.085525	NA
35_13444	3.575743	NA	**35_16398**	3.840181	Ubiquitin-conjugating enzyme family protein
35_6030	3.387704	ATP-dependent DNA helicase 2 subunit KU70	**35_15673**	3.838828	Wound-responsive family protein
35_3778	3.308636	nudix hydrolase homolog 14	**35_814**	3.721667	Succinate dehydrogenase subunit 8A, mitochondrial
35_814	3.30292	Succinate dehydrogenase subunit 8A, mitochondrial	Contig3511_at	3.695897	Wound-responsive family protein
Contig3140_at	3.279339	Acyl-coenzyme A oxidase 3	35_4279	3.503617	Short chain dehydrogenase/reductase
Contig8092_x_at	3.279276	DNA-directed RNA polymerase family protein	**35_19965**	3.460013	NA
35_18802	3.142059	Serine/threonine-protein kinase	**35_20248**	3.448557	E2F transcription factor 1
35_24566	3.129688	Receptor kinase 2	**Contig8092_x_at**	3.348258	DNA-directed RNA polymerase family protein
35_19791	3.119378	Endoribonuclease YbeY	**35_13444**	3.329026	NA
35_630	3.07477	Early nodulin-related	**Contig3140_at**	3.27046	Acyl-coenzyme A oxidase 3
35_20248	3.033549	E2F transcription factor 1	35_42078	3.192124	Protein MLO
35_44365	3.017374	Coatomer subunit delta-3	35_13786	3.135061	Elongation factor 1-alpha
35_644	3.011943	early nodulin-related	35_36699	3.127073	NA
35_15673	2.975326	Wound-responsive family protein	**35_18802**	3.115512	Serine/threonine-protein kinase

Two-way ANOVA was used for statistical analysis of gene expression between the conditions

(control vs. stress) and the lines (LP104 vs. LP106). Taking in consideration only transcripts that have a significant p<0.05 (BHB adjusted) and fold change of at least 1.5, differentially expressed genes between control and stress were detected: in total 1286 differential expressed genes were identified, while 456 were up-regulated and 829 down-regulated in the seeds of line LP104 developed under drought stress conditions compared to its control. In the seeds of line LP106 1594 differentially expressed genes after drought stress treatment were identified, 940 up-regulated and 654 down-regulated. Both lines share 419 genes which are up- or down-regulated under stress, and they have the same regulation in both lines (figure 8).

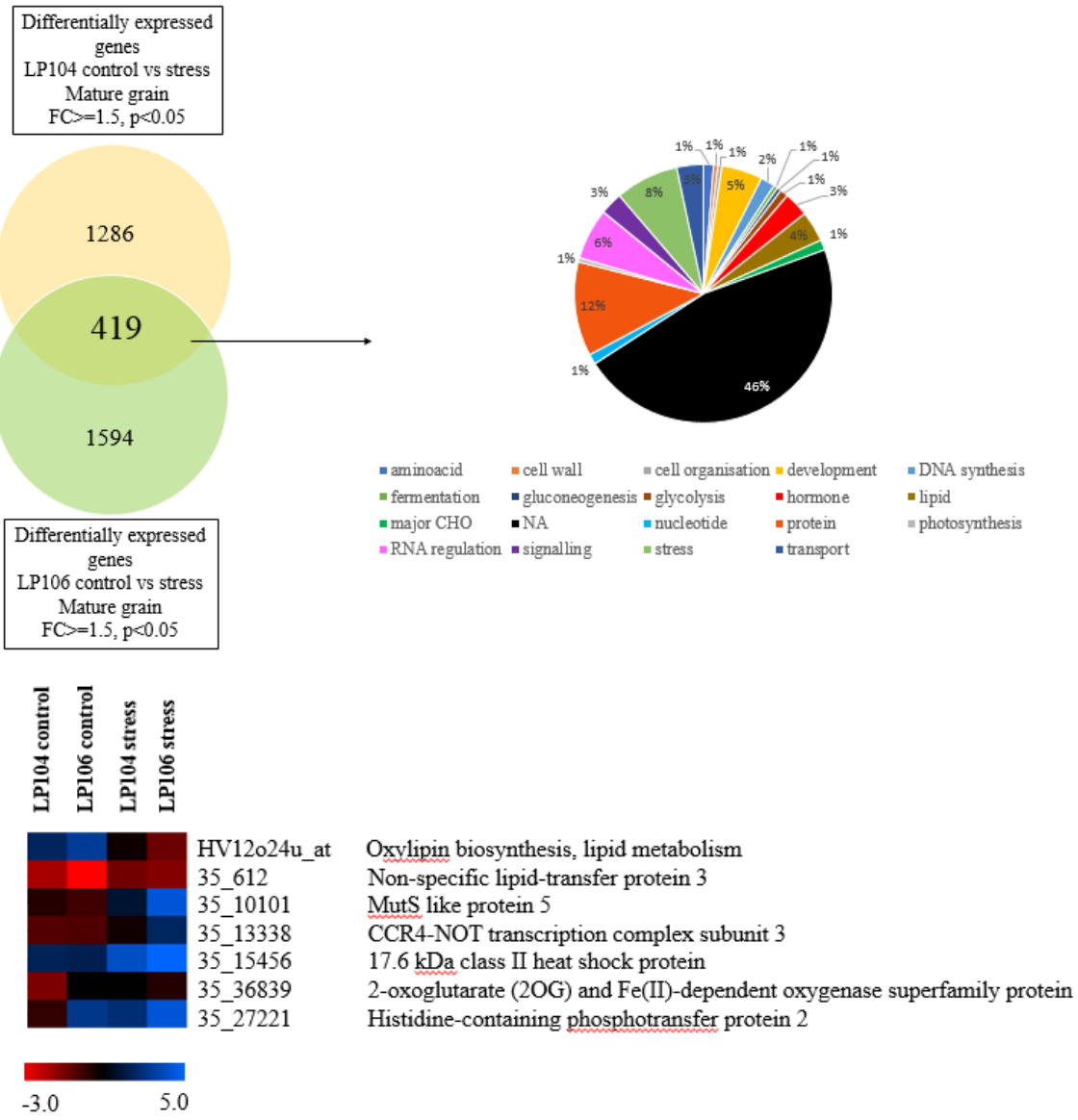


Figure 8. LP104 and LP106 share 419 genes which are differentially regulated in the stressed seed. The pie chart shows functional categories of 419 genes; protein metabolism, stress response, and development are categories that contain the major number of differentially regulated genes under stress. B: Heat map generated in MeV (TIGR, TM4) showing mutual differentially regulated genes with FC>5 within both genotypes and with annotation. Mean normalized expression value is represented (n=3).

The pie chart in the figure 8A shows mutually differentially expressed genes in both lines under stress, where they are divided according to their functional annotation. Most of the genes are categorized in protein metabolism (12%), stress response (8%), RNA regulation of transcription (6%), seed development (5%) and lipid metabolism (4%) functional groups. In the figure 8B, 7 genes with known annotation and with FC>5 in at least one comparison (control vs. stress, LP104, LP106) are shown. The two genes which are down-regulated in both lines under stress are involved in lipid metabolism. One gene encodes an oxylipin biosynthesis gene, and the other encodes a non-specific lipid transfer protein. The up-regulated ones encode a heat shock protein, a histidine-containing phosphotransfer protein 2, CCR4-NOT transcription complex subunit 3, MutS like protein. 2-oxoglutarate dependent oxygenase is down-regulated in LP106 but up-regulated in LP104 under stress.

The representation of the up (blue) or down (red) regulated genes between control and stress (BHB P correction, FC>1.5) are shown in figure 9 A for LP104 and B for LP106. Both lines under stress have a similar up-regulation of genes which fit earlier germination process and belong to light reaction processes, inhibitors of development proteins, carbohydrate degradation enzymes, various peptidases, cell division, organization and DNA repair-related genes. ABA biosynthesis genes *HvZEP1* and *HvNCED2*, are found to be down-regulated in both lines, and *HvGA20-oxidase* which is responsible for GA synthesis is been found up-regulated under stress. *HvAO7* and *HvLea19.3* are up-regulated in both lines under stress. Genes with the FC>5 between control and stress are not the same for LP104 and LP106. In LP104, genes like cellulose synthase, histone-lysine N-methyltransferase, jasmonate-induced gene, and beta-glucosidase are strongly down-regulated under stress. MATE efflux protein, myosin, transcription factor bHLH 62, anthocyanidin reductase, protein kinase superfamily protein, cyclin B, 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein are strongly up-regulated under stress. The differentially expressed genes under stress with FC>5 in LP106 are cysteine proteinases as a strongly down-regulated gene, while lipid transfer proteins and CCR4-NOT transcription complex subunit 3 are among the strongly up-regulated; others identified have no annotation (the table with genes are shown in supplementary table 11). The comparison between the lines results in a higher number of differentially expressed genes; there are 3478 differentially abundant genes comparing the line LP104 with LP106 under control condition, 1574 being under-represented and 1904 over-represented in LP106. To compare the transcriptional drought stress response of both lines differentially expressed genes detectable in the seed transcriptome after drought stress

application were tested. The analysis resulted in 2676 genes being differentially abundant in both lines after development under drought stress condition. 1360 of the detected transcripts are under-represented and 1316 over-represented in the line LP106 with the stay-green phenotype under drought stress respect to the faster senescing line LP104. To display the differentially expressed genes in their functional context a gene ontology classification was performed using the Mapman-software tool (Usadel *et al.*, 2005). Differentially abundant

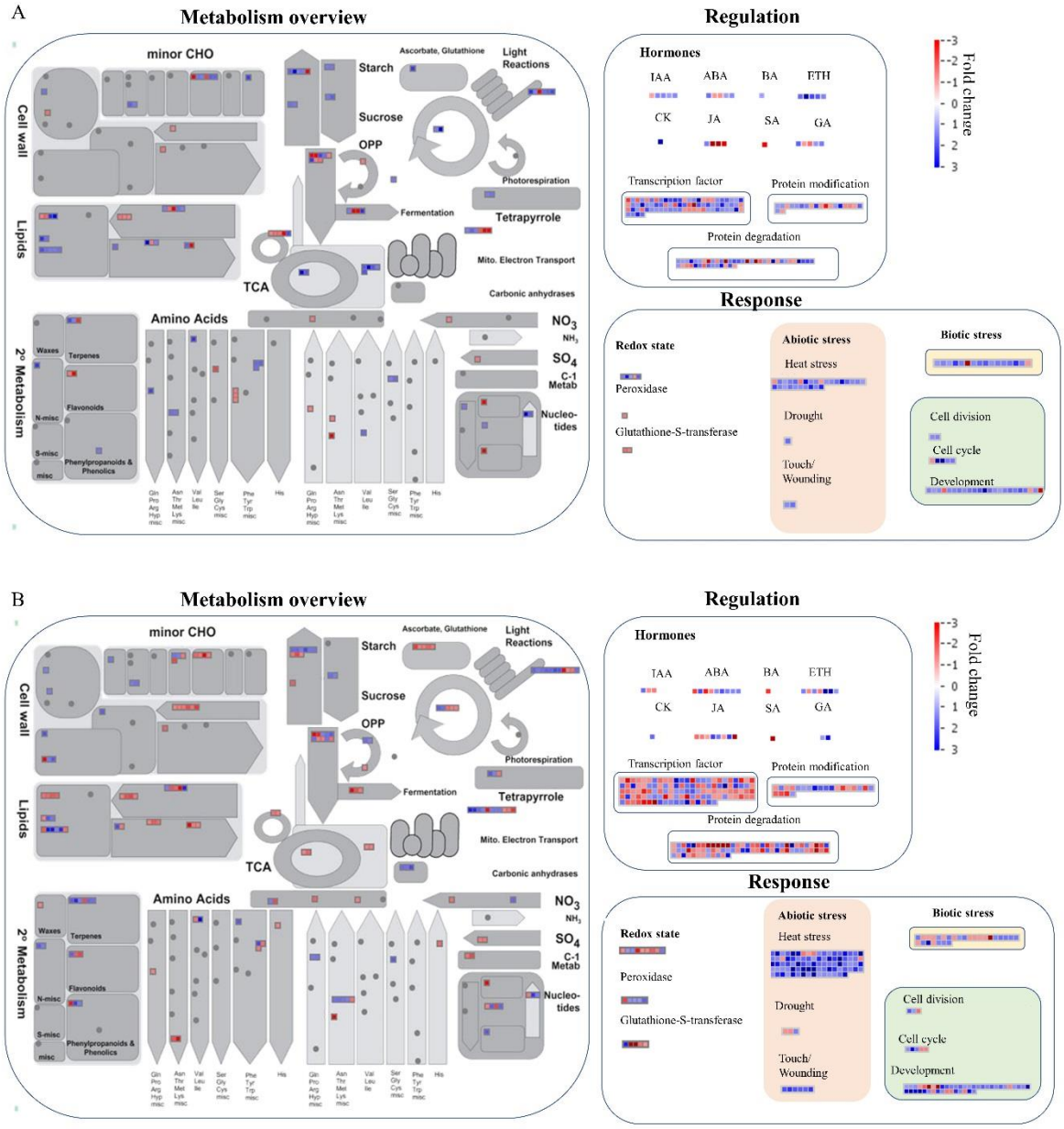


Figure 9. MapMan representation showing differences in transcript levels in seeds which grew and developed under drought stress condition. **A** LP104. **B** LP106. Fold change ratios for average transcript abundance based on three independent replicates of normalized gene expression data of control seed versus stressed seed were calculated. The resulting file was loaded into the MapMan Image Annotator module to generate the metabolism overview map. On the color scale ranging from −3 to 3, dark blue represents higher gene expression seeds which grew in drought stress in comparison to seed which grew in watered condition, and red represents down-regulated genes in the stressed seed.

genes between the lines belong to almost every functional category. In both conditions, the majority of overrepresented genes in the line LP104 belong to RNA processing and regulation, protein, starch and lipid degradation, cell wall modification. ABA-responsive GRAM containing proteins are found more abundant in LP106, while ABA'8-OH1, the key ABA enzyme is found overrepresented in LP104. This is in accordance with ABA content found in mature grains, with the stressed seeds being more prone to the faster start of germination and with LP104 line having higher germination rate at 24 HAI respect to the line LP106 under both conditions.

3.5.2 Transcriptome analysis of micromalted samples

Ripe grains grown in control and drought stressed plants were sent for micromalting to Versuchs- und Lehranstalt für Brauerei in Berlin (collaboration with Prof. Dr. Rath). For the transcriptome analysis of micromalted material three biological replications for each line (LP104, LP106) and condition (control and drought stressed seeds) were used. Transcriptome analysis was performed as explained above. An analysis of variance (ANOVA) was implemented to identify the significant differences in the transcriptome of micromalted seedlings of line LP104 and LP106. The micromalted seedling is a 72 HAI germinated seed and at the chosen time point no differences between control and stress treated seeds were detectable on the transcriptome level. This can also be deduced from the principal component analysis (figure 10) of the microarray-based transcriptome analysis.

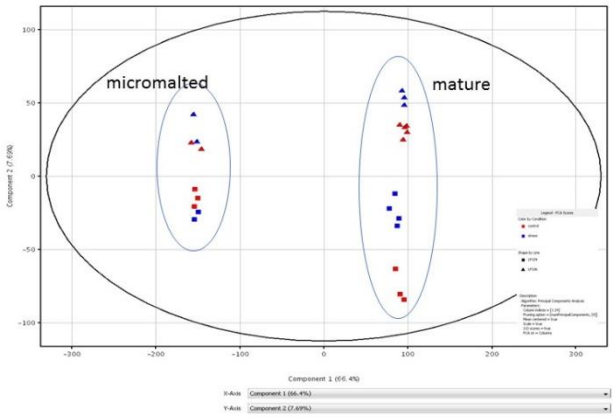


Figure 10. Principle component analysis of micromalted (left circle) and mature grain (right circle) of the lines LP104 (squares) and LP106 (triangles). Red color indicates control, blue color indicates stress condition.

2410 differentially expressed genes could be identified that vary between the two lines (FC>1.5). Mapman software was used to analyze and illustrate the categorization of differently expressed genes (figure 11) (Usadel *et al.*, 2005). A functional category related to

photosynthesis and light reactions is strongly overrepresented in the line LP104. The reason for this could be that in this line establishment of the autotrophic stage of the seedling occurs earlier. Other overrepresented functional categories include lipid metabolism, TCA cycle, cell wall modification metabolism, beta-glucan hydrolases. The under-represented categories are related to minor CHO, cell wall degradation, developmental proteins. Transcripts related to cytokinin biosynthesis and gibberellic acid biosynthesis are under-represented. Genes related to ethylene and auxin pathways are the most abundant ones, and present among the up-represented genes in the line LP104. These genes were further subjected to Gene Ontology (GO) enrichment analysis (table 13 in supplementary, Chapter 3.5.5 GO analysis) performed in collaboration with RG Network analysis (Dr. A. Bräutigam, IPK).

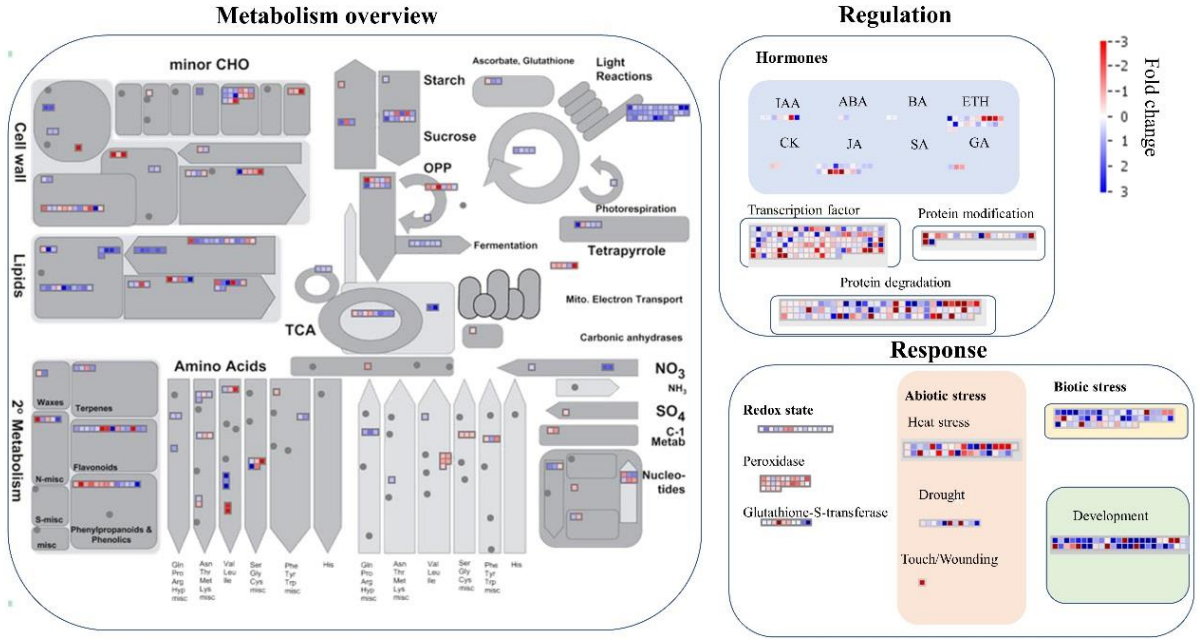


Figure 11. MapMan representation showing differences in abundance of transcript levels in micromalted seedlings of LP104. Fold change ratios for average transcript abundance based on three independent replicates of normalized gene expression data of LP104 versus LP106 were calculated. On the color scale ranging from −3 to 3, dark blue represents higher gene expression and red lower transcript abundance in LP104.

3.5.3 Identification of transcripts important for early germination processes

During seed dormancy, the majority of mRNA is stored and not used for the translation process. To identify the first important transcripts after breaking the dormancy and their contribution to the malting process ribosomal associated mRNA was analyzed and compared to the transcriptome in the aforementioned lines and conditions. Approximately 30% of dry seed transcripts are found in polysome fraction of the micromalted material. Ribosomes associated with active translated messenger RNA (mRNA), also called polysomes mediate the final step in gene expression (Davies, 2012). Polysome bound transcripts can be identified

separated from monosomes and unbound mRNAs by sucrose gradient ultracentrifugation (Figure 2). After purification, the separated polysome fraction was used for microarray hybridization and evaluated as described above using Genespring software. Comparative transcriptome profiling with total RNA is informative for determination of genes that are not only present but also actively translated. Assessment of the polysome-bound RNA is important as it reflects the proteome and it might be closer related to agronomic properties, such as seed and malting quality are concerned (Skadsen, 1993). To understand the transcriptional processes during the malting process and develop gene expression markers for malting quality, it is necessary to utilize the most appropriate mRNA (polysomal or total RNA). Interestingly no differentially abundant transcripts were detected among polysomal RNA comparing both conditions at 72 HAI. This was also found in the total RNA. Pair-wise comparison of the polysome-associated RNAs and the whole transcriptome from the lines led to the identification of 1421 genes which are found to be more or less abundant.

3.5.4 Comparison of total RNA and the polysome-bound RNA

Transcriptome profiling with total RNA is informative for determining which genes are up or down-regulated in response to a stimulus or during development. Assessment of the polysome-bound RNA is more important because it reflects the proteome and it is more important where agronomic properties, such as seed and malting quality are concerned (Skadsen, 1993). In order to utilize understand the malting process and develop markers for malting quality, it is necessary to utilize the most appropriate mRNA population. The number of differentially abundant genes between polysomal RNA shows no genes comparing control and stress condition, which is also the case for the total RNA. Between the lines, there are 1421 genes which are found to be down or up-regulated. In the figure 12 Venn diagram shows the shared differentially abundant genes between the total and polysomal RNA.

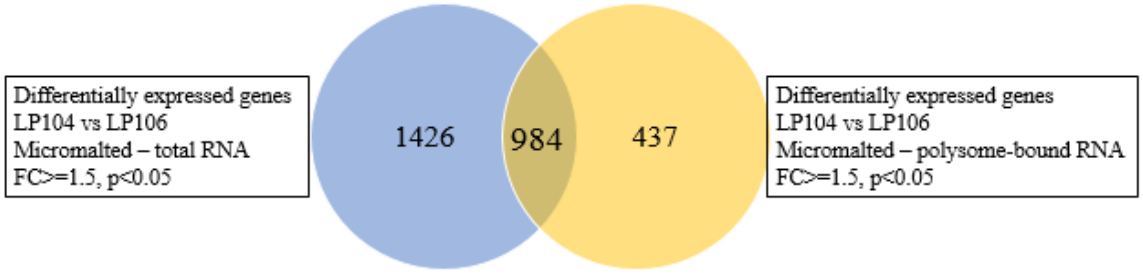


Figure 12. Venn diagram from differentially abundant transcripts in total RNA (blue) and polysomal RNA (yellow) of the micromalted material of the lines LP104 and LP106. 984 transcripts are mutual for both comparisons.

3.5.5 Gene ontology enrichment analysis

Gene ontology **(**GO) enrichment analysis of differentially expressed genes (total RNA) between the two lines in the micromalted material reveals that the biological processes categories which are particularly enriched for down-regulated genes of LP104 are regulation of post-embryonic development, regulation of shoot system development, reactive oxygen species metabolic processes, regulation of development processes. The up-regulated genes of LP104 are particularly enriched in small molecules biosynthesis and organic acid biosynthesis. Furthermore, the GO enrichment analysis was performed comparing the total RNA and polysome-bound RNA levels. The analysis was performed in collaboration with RG Network analysis (Dr. A. Bräutigam, IPK). There were no differences in abundance between polysome-bound RNA and the total RNA under stress condition. Among the up- and down-regulated genes in the polysomal fraction in the line LP104 under control condition are protein transport and localization, nitrogen mobilization, macromolecules modification enriched. In the line LP106, the up-regulated differentially abundant genes between polysome-bound and total RNA under control condition are found to be involved in phosphorous metabolism process, photosynthesis, lipid metabolism, and carbohydrate metabolism. Organic acid metabolism is the only biological process found to be enriched for the genes underrepresented in the polysome-bound RNA.

3.6 Analysis of barley seed maturation and germination process using transgenically modulated ABA levels

Drought stress during seed development alters seed quality. ABA is the key stress hormone and a key player in inducing seed dormancy (Karssen *et al.*, 1983; Frey *et al.*, 2004). ABA levels need to drop to a basal level to permit to the seed to germinate (Gubler, Millar and Jacobsen, 2005). To understand the role of ABA in the transition from seed maturation to germination, and its role in two different barley tissues embryo and endosperm (aleurone layer), two sets of transgenic plants were used. Lines XN17 and XN26 contain a copy of an *Arabidopsis thaliana AtNCED6* gene which ectopic expression is driven by the AX1 endosperm-specific promoter from wheat (Harshavardhan, 2012). Additionally, lines LoHi236 and LoHi272 were used that contain a hairpin construct triggering RNAi suppression of *ABA-8´-OH* driven by barley Lea 19.3 promoter active in the embryo (Seiler *et al.*, 2014). For each time point three biological replications were used. One biological replication consisted of grains coming from three spikes growing on different mother plants. In the previous studies, ABA content in these transgenic lines has been analyzed showing an increased accumulation during grain filling in leaf and developing seed (Harshavardhan, 2012; Seiler *et al.*, 2014).

3.6.1 Confirmation of *AtNCED6* transgene expression

The transcript level of transgene-derived *AtNCED6* mRNA was analyzed in reference cv. Golden Promise and transgenic XN17 and XN26 grains which were grown under control and stress condition by real-time qRT-PCR. Drought-stressed plants, as well as watered (control) plants were greenhouse grown. Drought stress was applied at 5 DAF by keeping the soil moisture content at 15% until harvest. *AtNCED6* transcripts were undetectable in Golden Promise. XN17 and XN26 show a similar pattern of expression through different time points; at 24 DAF the mRNA is most abundant and decreases at harvest, an up-regulation at 0 HAI is detectable. If compared to the transcript level at time point harvest, the *AtNCED6* level is doubled for XN26 and in XN17 is increased by one third at 0 HAI. At harvest, the line XN17 has a significantly higher abundance of *AtNCED6* under control, and at 0 HAI, both XN17 and XN26 under control have a significantly higher abundance of *AtNCED6* respect to the stressed material. At later time points from 12 HAI on it further, decreases. Under stress condition at 24 DAF the transcript is strongly up-regulated in both lines tested, but already at harvest time, a strong decrease is detectable.

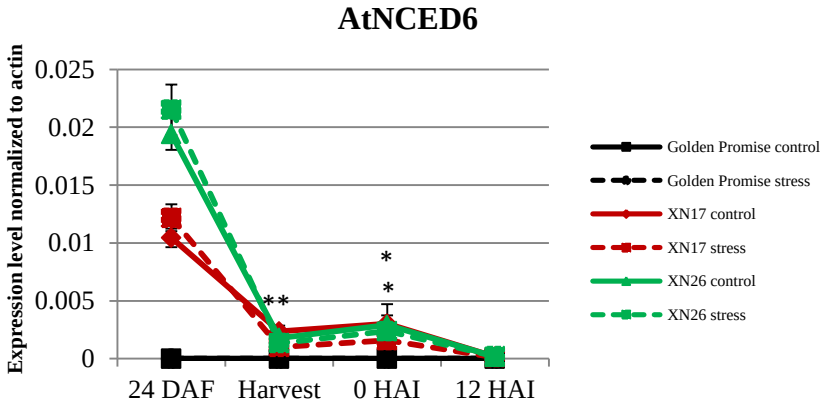


Figure 13. *AtNCED6* expression under the regulation of *Ta1AX1* promoter during late maturation phase (24 DAF), at harvest, post-dormancy (0 HAI) and at 12 hours after imbibition. Expression level of *AtNCED6* is shown in comparison to housekeeping gene (actin) resulting from three biological and two technical replications. Solid and dashed lines represent control and stress condition. Asterisks (*) represent significant differences in transcript abundance under stress within the genotype at P<0.05: *; P<0.01: **.

3.6.2 Analysis of *HvNCED and HvABA8´-OH* family in Golden Promise and transgenic lines

To investigate the transcriptional response of genes involved in ABA biosynthesis and degradation in the used transgenic lines relative mRNA levels of *HvNCED* and *HvABA8´-OH* gene families have been analyzed by real-time qRT-PCR. Starting time point of analysis was 24 days after flowering, harvest, 0 HAI, 12 HAI and 24 HAI. One biological replication

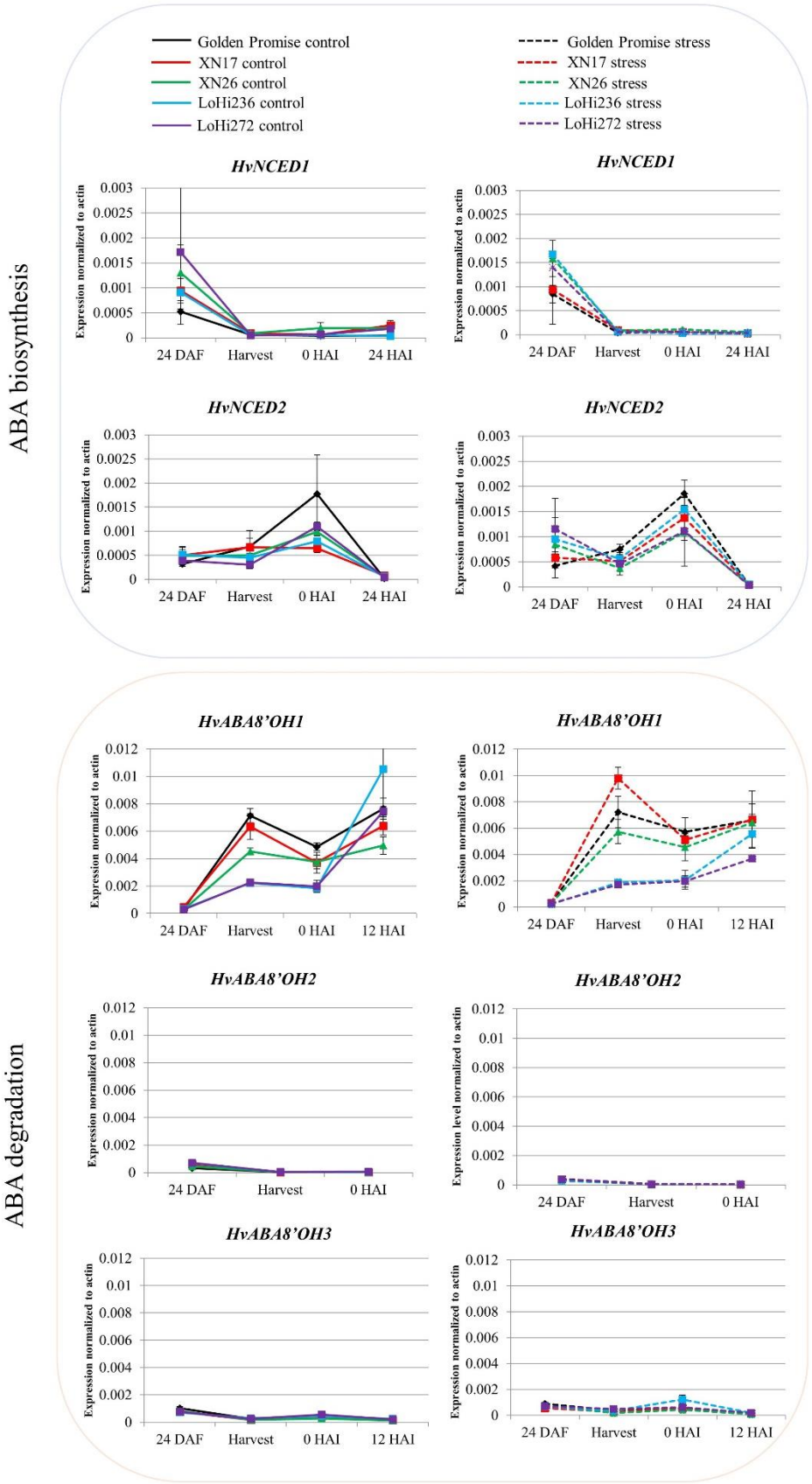


Figure 14. qRT-PCR analysis of key ABA biosynthesis genes, *HvNCED1* and *HvNCED2* and ABA degradation genes *HvABA8'OH-1*, *HvABA8'OH-2*, *HvABA8'OH-3* in Golden Promise, XN17, XN26, LoHi236, and LoHi272. The solid line represents control condition (left panel), the dashed line represents stress condition (right panel). Expression values are normalized to a housekeeping gene (actin) and are a mean value of three independent experiments and two technical replications.

consisted of grains coming from 3 different spikes grown on different mother plants. mRNA levels of *HvNCED1* and *HvNCED2* show a similar trend of expression during development under control and after drought stress treatment. *HvNCED1* is down-regulated from the time point of harvest until 24 HAI, while *HvNCED2* undergoes an up-regulation from 24 DAF and reaches a peak of expression at 0 HAI, and it is down-regulated until 24 HAI. Under control, *HvNCED1* is up-regulated in all transgenic lines at 24 DAF, but for the stress-treated grains, it is up-regulated in XN26, Lohi236, and LoHi272. In Golden Promise, XN26 and LoHi236 the mRNA levels of *HvNCED1* are more abundant in control grains. At harvest and 0 HAI XN17 and XN26 have a lower abundance of *HvNCED1* mRNA under control, while for the stressed grains this remains true only for XN26. At 24 HAI detectable *HvNCED1* mRNA is more abundant in all the lines under control, and although it is generally present at low levels, XN17, XN26, and LoHi272 have a higher abundance of this transcript present. The same is for stressed grains, where all the lines have a higher accumulation of *HvNCED1* mRNA.

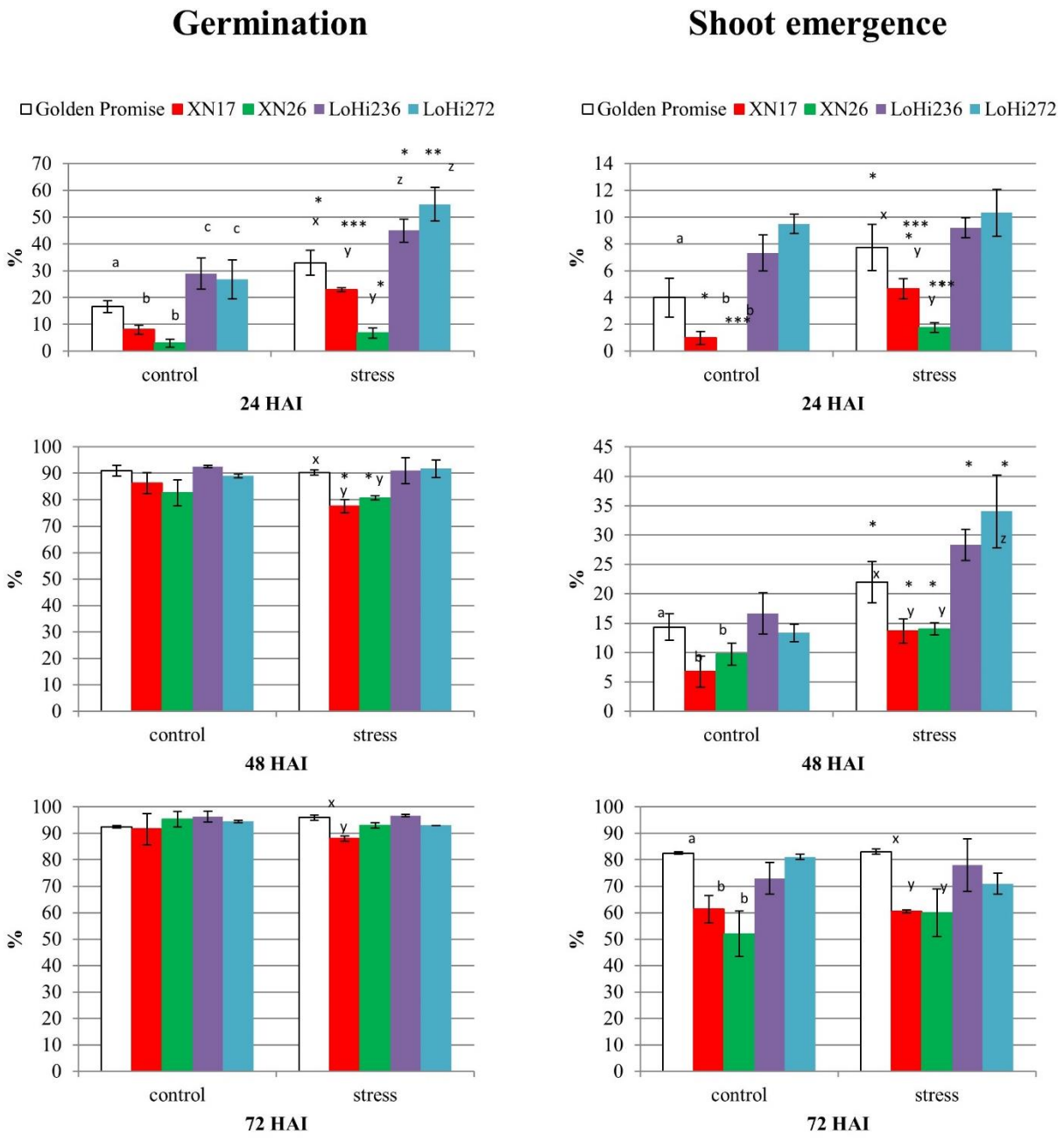
At 24 DAF, *HvNCED2* transcription is up-regulated in XN17, XN26, and LoHi236 at control, while under drought stress conditions LoHi236 and LoHi272 have the highest abundance of this mRNA. At harvest, mRNA levels of *HvNCED2* are down-regulated in transgenics and the major differences of down-regulation are seen at 0 HAI where Golden Promise has a higher abundance of this mRNA for both conditions. No difference was observed between wild-type and transgenics derived from drought condition 24 HAI. At control condition, the abundance of *HvNCED2* mRNA is still higher compared to wild-type. Based on mRNA abundance of the three *HvABA8´-hydroxylase* gene family members, *HvABA8´-OH1* is the major gene involved in the degradation of ABA during germination. Lines LoHi236 and LoHi272 show the RNAi mediated reduction of *HvABA8´-OH1* mRNA under control and stress. 12 HAI an increase in mRNA levels similar to wild-type niveau was observed.

3.7. Germination and shoot emergence of transgenic barley lines with modulated ABA content

Germination tests of seeds from the transgenic lines were performed as described before according to international seed testing association (ISTA, 2016). Randomly selected mature grains of lines Golden Promise, XN17, XN26, LoHi236, and LoHi272 were imbibed in distilled water and every 24 hours germination percentage and shoot emergence percentage were scored. Each test consisted of 100 grains and repeated four times. The grains came from the control batch, where seeds developed on the mother plant under optimal conditions, and from the drought-stressed batch. The “stress” batch contained the grains which developed and

matured on the mother plant that underwent controlled drought stress five days after flowering. In figure 15, three time points of representative germination tests are shown: 24, 48 and 72 hours after imbibition, where charts on the left show germination percentage and charts on the right show shoot emergence percentage. Seed germination commences with the uptake of water and it completes with the appearance of the radicle, through the surrounding structure. Such seed is regarded as germinated. Shoot emergence is underway after the germination has been completed and marks the emergence of the coleoptile, it is a part of post-germination (seedling establishment) phase and symbols the entrance in the phototrophic phase (Bewley, 1997; Nonogaki, 2014).

A



B

C

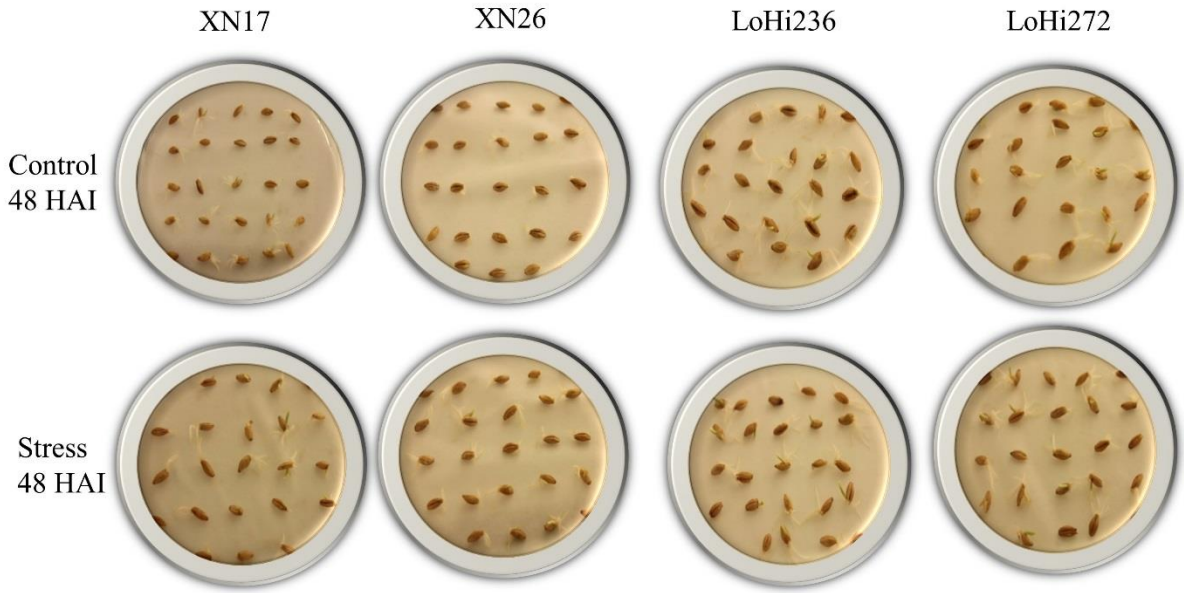


Figure 15. **A** Germination rate (left panel) and shoot emergence rate (right panel) at 24, 48, 72 HAI of Golden Promise, XN17, XN26, LoHi236, and LoHi272. The performance was scored using 100x4 control and stressed seeds. At 24 HAI, major differences are seen: XN lines have the lowest, and LoHi lines the highest rates in both conditions. For both scored traits, stressed seeds show germination and shoot emergence (only the seeds with coleoptile were scored). **B** Images taken during the germination assay of Golden Promise, showing faster germination speed of stressed seeds. **C** 48 HAI images showing the process of germination and shoot emergence of transgenic lines XN17, XN26, LoHi236, and LoHi272. XN lines show a delay in root/shoot emergence respect to the other lines.

At 24 HAI control seeds (seeds grown under normally watered condition) of Golden Promise have a germination rate of 16.6 %, while the stressed grains are significantly faster ($p<0.05$) and have a mean germination rate of 33 %. At 48 HAI and at 72 HAI germination process is already finished and differences in numbers of germinated seeds between control and stressed treatment are indistinguishable. Shoot emergence in control grains shows in average 4 % shoot protrusions, while stressed seeds have a 7.75 % ($p<0.01$) shoots protruded at 24 HAI. At 48

HAI stressed seeds have an average 22 % of shoot emergence, while the controls have 14.33 % ($p<0.05$). At 72 HAI, shoot emergence reaches more than 80 % and shows no difference between control and stress.

Analysis of the transgenic seeds generated under control condition of line XN17 revealed 8.5 % of germination and 1% of shoot emergence at 24 HAI while the seeds generated under stress conditions show a significant increase ($p<0.001$ for germination, $p<0.05$ for shoot emergence) with the mean germination rate of 25.5 % and shoot emergence rate of 4.7 %. At 48 HAI stressed seeds have significantly higher shoot emergence at 48 HAI, ($p<0.05$) with the mean 13.7 % respect to the controls which have a mean of 6.7 %. Line XN26 has a mean of 3 % of germinated control seeds and no shoot emergence at 24 HAI. Stressed grains are also significantly faster ($p<0.05$, t-test) for germination rate with the mean of 6.75 % and for shoot emergence rate with a mean of 1.75 % ($p<0.001$). At 48 HAI, a similar situation is found; control seeds have lower shoot emergence rate, with the mean of 9.75 % for control and 13.7 % for stress ($p<0.05$). Control seeds of the line LoHi236 reach a germination rate of 29 in average and a shoot emergence rate of 7.3%. Stressed seeds which are faster ($p<0.05$) at that time point with 43.2 of germination and faster ($p<0.05$) shoot emergence (9.2%). Line LoHi272 at 24 HAI has also a faster germination rate and shoot emergence rate from stressed grains, reaching an average of 50% for germination ($p<0.001$) and 10.1% for shoot emergence ($p<0.05$) respect to the control, where grains perform slower with 26.8 % germination and 9.5 % of shoot protrusion. Lines LoHi236 and LoHi272 have overall the highest number of protruded shoots of the stressed seeds at 48 HAI, having a mean of 27.3 % and 30 % and being significantly higher ($p<0.05$, $p<0.05$) compared to controls, where LoHi236 has a mean of 16.7 % and LoHi272 a mean of 13.3 %. Comparing the transgenic lines with the wild-type, transgenic XN lines show the strongest delay in germination. The delay is seen at 24 HAI in germination rate and shoot emergence in both conditions. However, at 48 and 72 HAI, the significantly reduced germination rate is still seen for stressed seeds. At 24 HAI the fastest germinating lines are LoHi236 and LoHi272 respect to Golden Promise. LoHi seeds developed under control conditions germinate 50% significantly faster than the respective wild-type seeds (Golden Promise 15% of germination rate, LoHi 30% at 24 HAI). Comparable results were obtained from the respective drought stressed seeds, where LoHi lines reach 50% of germination already at 24 HAI, while Golden Promise has 33% of germination at that time point.

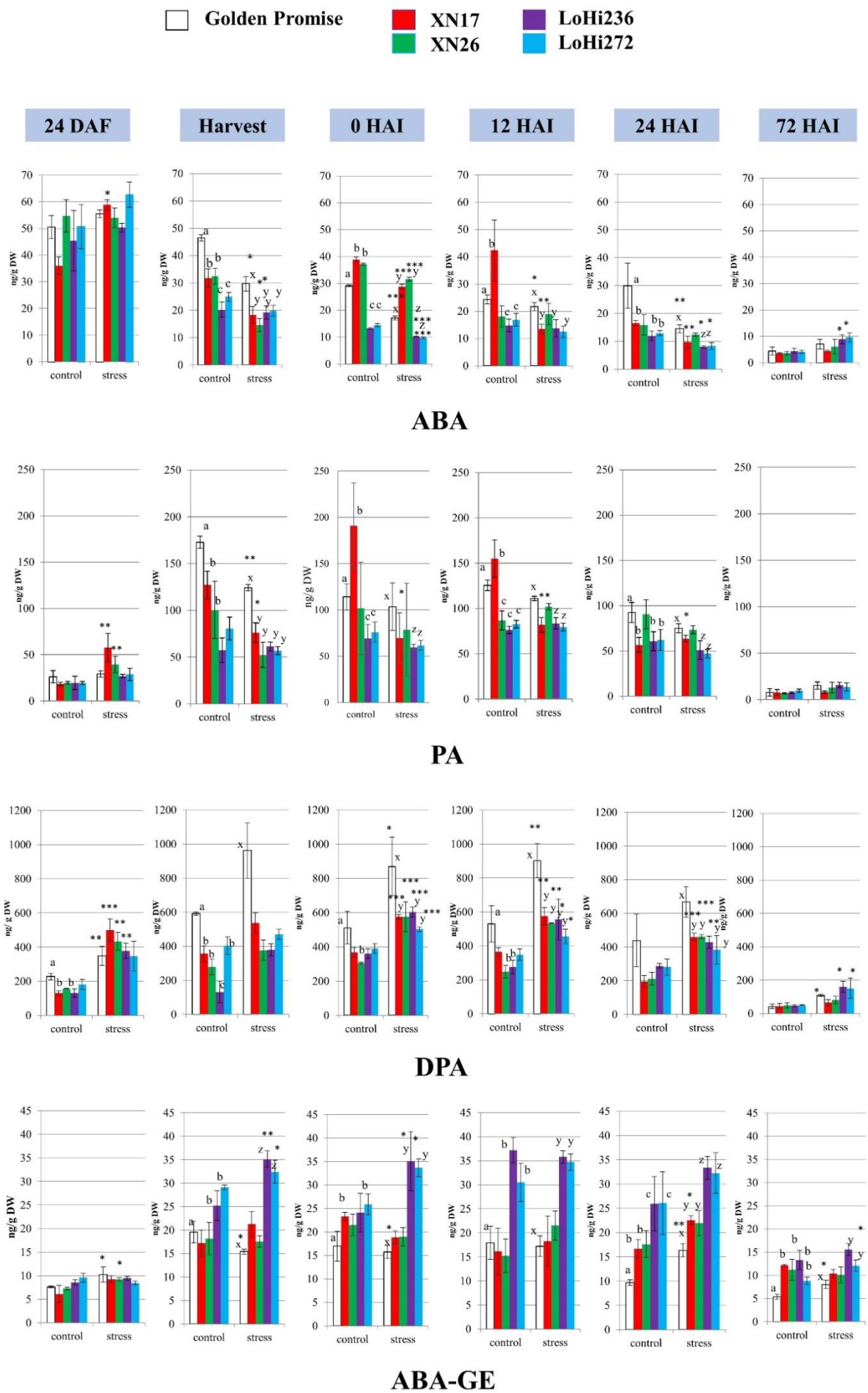
3.8 Hormone content of developing grains with transgenic modulated ABA pathway

The lines used in this study showed an alteration in ABA biosynthesis and degradation in the previous study (specify). Hormones have a profound influence on the regulation of seed germination and dormancy (Bewley, 2013). To capture the hormonal profile of the grains in the transition from late maturation, through dormancy to various time points of germination, liquid chromatography-mass spectrometry was implemented to quantify the four hormones attributed to the germination process. Abscisic acid (ABA), gibberellic acid (GA), cytokinins (CK) and auxins (AUX) with their respective precursors and degradation products were quantified. Time points that were chosen for the measurement are 24 days after flowering (DAF; late maturation phase), harvest (pre-dormancy), 0 HAI (post-dormancy), and germination time points: 24, 48 and 72 hours after imbibition. Four biological replications were used for control and for drought-stressed grains of Golden Promise, XN17, XN26, LoHi236 and LoHi272.

3.8.1 Analysis of abscisic acid and its degradation products in developing transgenic seeds

The analysis started with ABA to confirm the prior analysis. It was found that the maximum of ABA (35 to 65 ng/g DW) is detectable generally at 24 DAF decreasing throughout the developmental stages tested. From 24 DAF to 72 HAI, ABA content generally decreases in all the lines, and the decrease is seen for control and stressed grains. There is a growing scientific evidence that the ABA content in the seed must be lowered for dormancy to be broken, and that the germination potential of a given seed is determined by hormones ABA and GA. The loss of dormancy of many seeds is directly related to the increase in sensitivity to GA and the ABA/GA ratio is important in the maintenance and loss of seed dormancy (Bentsink and Koornneef, 2008; Finkelstein *et al.*, 2008; Cutler *et al.*, 2010).

The two selected, representatively chosen transgenic lines to show a similar pattern of ABA content in accordance with the expression of the transgenic target gene. While XN lines controlled by the AX1 promotor have an increase of ABA during the dormancy and imbibition period, the LoHi lines generally show a stronger degradation of ABA being indicative for activation of an alternative degradation pathway.


Golden Promise
XN17
XN26
LoHi236
LoHi272
24 DAF
Harvest
0 HAI
12 HAI
24 HAI
72 HAI
ng/g DW
control
stress
ABA
PA
DPA
ABA-GE

At 24 DAF, Golden Promise has a mean ABA content of 50 ng/g DW, and the similar amount is seen in the stressed seeds, where is about 55 ng/g DW. At this time point, also name giving for abscisic acid, the ABA content has its highest value in Golden Promise. Harvest represents the time point when the seeds are ready for the dormancy period (Bewley, 2013). Furthermore, this represents the time point where the content of stress hormone ABA between control and stress condition is getting inverted, control grains showing now significantly more ABA. Overall, Golden Promise maintains a significantly higher level of ABA under control at harvest, 0 HAI, 12 HAI and 24 HAI. 72 HAI is the last time point of the germination assay and ABA falls to minimum levels.

Lines XN17 and XN26 have a similar pattern of ABA content through time points. Line XN17 has the significantly lower amount of ABA at 24 DAF under control, being 36 ng/g DW respects to 58.9 in the stress seeds. At harvest, ABA drops to 31.8 ng/g in control, but at 0 HAI (post-storage and dormancy) it increases to 38.9 ng/g DW. A similar content is found at 12 HAI, where it is maintained at 42.3 ng/g DW for control seeds of XN17. At 24 HAI ABA finally drops to 16.5 ng/g DW in control and falls further to 3.5 ng/g DW at 72 HAI. The stressed seeds of XN17 have a similar performance to the control ones, with the switch at harvest where stressed seeds have a lower amount of ABA ($p<0.001$ at harvest, $p<0.01$ at 0HAI, $p<0.001$ at 12 HAI, $p<0.05$ at 24 HAI) and this decrease is kept until 72 HAI. At harvest also XN26 has a shift in stressed seeds having a lower ABA content (32.6 ng/g DW in control seeds respect to 14.6 ng/g DW in stresses seeds, $p<0.001$). The second shared feature is the increase of ABA during the dormancy period so that the mature dry seeds of XN lines result as lines with the highest ABA amount at this stage if compared to Golden Promise. So at 0 HAI control seeds of XN26 have 37 ng/g DW and 31.6 ng/g DW in stressed seeds ($p<0.01$). At 12 HAI, ABA levels further drop to 18 ng/g DW in both conditions, creating no difference at 12 HAI, and a slight increase is seen at 24 HAI in control seeds (no significance but 15.9 ng/g DW in control and 12.6 ng/g DW in stressed seeds).

Figure 16 ABA (abscisic acid), PA (phaseic acid), DPA (dihydrophaseic acid), ABA-GE (ABA-glucose ester) contents at six different time points: 24 DAF, harvest, 0 HAI, 12 HAI, 24 HAI, 72 HAI measured in Golden Promise, XN17, XN26, Lohi236 and LoHi272 lines in control and drought stressed seeds. ABA and the respective degradation compounds (PA, DPA) and the conjugation compound (ABA-GE) were quantified by LC-ESI-MS/MS and are here represented as means ±SD (n=4). Asterisks represent significant differences between genotypes within treatment at $p<0.05$:*, $p<0.01$:**, $p<0.001$:***. Letters a, b and c, and x, y and z represent statistical differences between genotypes under control and stress conditions, respectively. Bars with similar or no letters indicate no statistical difference among genotypes under a given treatment.

Lines LoHi236 and LoHi272 have the highest value in ABA at 24 DAF (45-62.7 ng/g DW in both conditions). The content is reduced by at least 50% at harvest, and from this time point, these two lines have the lowest ABA content under both conditions respect to all other lines. At 0 HAI the control seeds of LoHi lines have significantly higher ABA content respect to the stress condition, where the ABA content is 10 ng/g DW. At 12 and 24 HAI ABA levels remain similar and they further drop to 5 ng/g DW at 72 HAI under control, and 9 ng/g DW in stressed seeds. The inactive metabolites of ABA, conjugated or degradation products determined in this study are: phaseic acid (PA), dihydrophaseic acid (DPA), ABA-glucose ester (conjugated form of ABA, a translocation form), Neo Phaseic acid (Neo-PA), and 7´-hydroxy-ABA (Neo-PA and 7´-hydroxy-ABA data in the supplements). Degradation product contents indicate that highest degradation processes occur between harvest and 24 HAI. The main degradation form is PA. At 24 DAF, PA is about 20 ng/g DW in the wild type in both conditions. At harvest PA content increases about 8 fold in control and about 6 fold in stresses seeds ($p<0.001$) of Golden Promise. This time point represents the peak of PA for Golden Promise, after this time point PA decreases and it always significantly higher in content for control seeds. At 72 HAI PA content falls below 20 ng/g DW.

The lines XN17 and XN26 have a significantly higher content of PA under stress respect to the control at 24 HAI ($p<0.05$). At harvest, both XN17 and XN26 have a significantly higher PA content in control seeds. At 0 HAI and 12 HAI control seeds of the line, XN17 have the highest PA content (150-200 ng/g DW) among the lines, which correlates with the highest ABA contents of this line at these time points. Stressed seeds of XN17 at 0 and 12 HAI have a significantly lower PA content respect to stress ($p<0.05$), but at 24 and 72 HAI PA is found to be 60 ng/g DW and 10 ng/g DW in both conditions. Line LoHi236 and line LoHi272 also show a slight increase in PA content at harvest but are however characterized by lower amounts of PA through time points, and there are no differences between control and stress.

Dihydrophaseic acid is the final degradation product and overall the most abundant ABA degradation compound. It is not known what happens to the accumulated DPA. In control condition at 24 DAF, Golden Promise has a DPA content which is 200 ng/g DW, while it is found to be 300 ng/g DW in stressed seeds. At time point harvest DPA production is increased and DPA content in control is 600 ng/g DW and 962 ng/g DW in stressed seeds. Similar amounts are found at 0 HAI and 12 HAI. At 24 HAI DPA content drops but it is still significantly higher in stressed seeds. At 72 HAI DPA is found at a minimum level (50 ng/g DW in control in all the lines). Golden Promise has overall the highest values of DPA from

harvest until 24 HAI in both conditions. The DPA content is similar in all transgenic lines, and no line-specific pattern is visible. However, at 24 HAI the lines XN17 and XN26 have the lowest DPA content (200 ng/g DW) in control condition. At 72 HAI, LoHi236 and LoHi272 have the highest values of DPA in stressed seeds. As seen in stressed seeds of Golden Promise, the DPA content in stressed seeds of all transgenic lines is significantly higher at all time points.

ABA-glucose ester (ABA-GE) is a conjugation and translocation form of ABA. At 24 DAF in control condition, the ABA-GE content is 7.6 ng/g DW. This value is doubled at harvest and it is found the same at 0 HAI and 12 HAI. in the stressed seeds of Golden Promise, the ABA-GE content is also doubled at harvest and keeps the same level at 0 HAI and 12 HAI. At 24 HAI, in control seeds the ABA-GE content falls to 9.7 ng/g DW and 5.4 ng/g DW at 72 HAI, but in stressed seeds at 24 HAI the ABA-GE remains at 15 ng/g DW and 10.4 ng/g DW at 72 HAI, creating a significant difference between the control and stress in Golden Promise at these time points. The lines XN17 and XN26 have a similar behavior as Golden Promise. Although they have the same content of ABA-GE at 24 DAF and harvest as Golden Promise, at 0 HAI the lines XN17 and XN26 have a higher ABA-GE content in both condition respect to Golden Promise. This difference is seen at 24 HAI, where the ABA-GE is significantly higher in these lines, and their content is higher under stress. At 72 HAI ABA-GE is around 10 ng/g DW in both conditions, and the difference between Golden Promise and XN17 and XN26 is kept. Lines LoHi236 and LoHi272 have the highest amount of ABA-GE from harvest until 72 HAI. From 24 DAF to harvest ABA-GE content triples in both conditions to 25 and 29.1 ng/g DW in control, and 32.4 and 35 ng/g DW in stressed seeds; the same difference can be seen at 0 HAI. From 12 HAI until 72 HAI, there are no more differences between control and stress, but the content remains highest among the lines in both conditions, ranging from 25 ng/g DW to 37.2 ng/g DW.

3.8.2 Gibberellic acid

GA3 is the main active form of gibberellic acid produced in embryo axis during the germination process (Palmer, 1989; Palmer, 2006). It is found present at all six time points along with the GA8, the degradation form of GA. In control seeds of Golden Promise at 24 DAF, harvest and 0 HAI the content of GA3 is approximately 1 ng/g DW. At 12 HAI (imbibition phase) the content is found to be 2.5 ng/g DW, and it decreases to 1 ng/g DW again at 24 HAI and reaches 6.1 ng/g DW at 72 HAI. In contrast, the stressed seeds of Golden Promise keep the GA3 content at 1 ng/g DW until 24 HAI, where it increases to 2.4 ng/g DW.

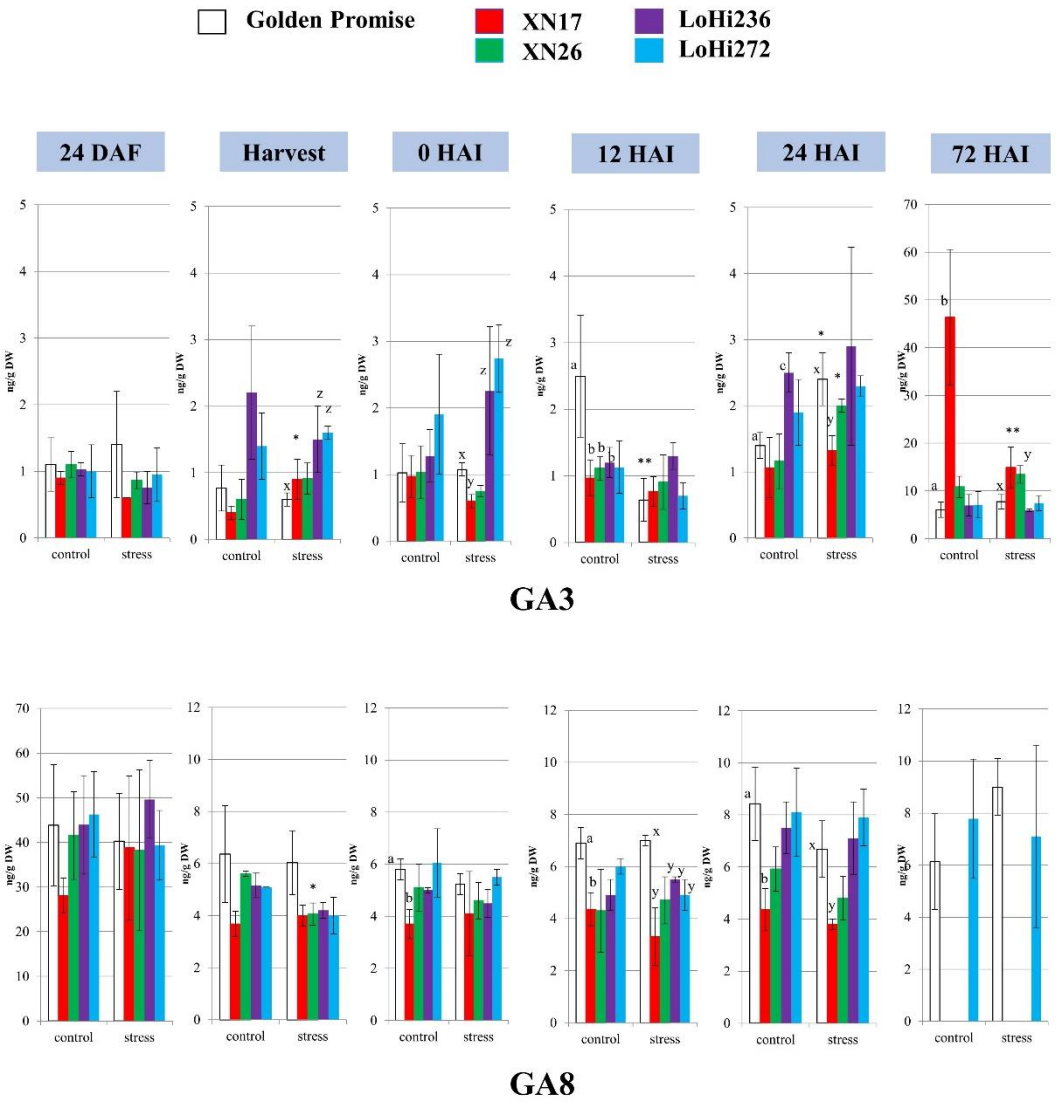


Figure 17. GA3(active GA, top) and GA8 (degradation form, bottom) content in Golden Promise, XN17, XN26, LoHi236 and LoHi272 at six time points: 24 DAF, Harvest, 0 HAI, 12 HAI, 24 HAI, 72 HAI measured with LC-ESI-MS/MS and are here represented as means ±SD (n=4). Asterisks represent significant differences between genotypes within treatment at $p<0.05$:*, $p<0.01$:**, $p<0.001$:***. Letters a, b and c, and x, y and z represent statistical differences between genotypes under control and stress conditions, respectively. Bars with similar or no letters indicate no statistical difference among genotypes under a given treatment.

At 72 HAI GA3 is 7.7 ng/g DW. 24 HAI is the time point where the stressed seeds show significantly more GA3 respect to the control condition. Control seeds of XN17 through time points show a GA3 content 0.5-1 ng/g DW at 24 DAF, harvest, 0, 12 and 24 HAI. AT 72 HAI there is a peak of GA3 of 46.4 ng/g DW. Stressed seeds of XN17 show a similar pattern to the control seeds regarding the GA3 content: The GA3 amount range from 0.5-1 ng/g DW until 72 HAI, where the highest amount of GA3 was detectable (14.9 ng/g DW). Line XN26 also shows low levels of GA3 in control and in stressed seeds. The only time point when GA3 reaches its peak is at 72 HAI, similarly to XN17. Similar GA3 pattern have also the lines

LoHi236 and LoHi272. There are no differences between control and stressed seed contents at all time points tested. But compared to other lines the GA3 peak is detectable earlier: at harvest, the amount of GA3 is doubled, and it is further increased at 0 HAI. At 12 HAI, GA3 content decreases to 1 ng/g DW but at 24 HAI GA3 has a second peak, 2.5 ng/g DW. At 72 HAI GA3 content is 6-7 ng/g DW. No significant differences for GA3 were detectable at 24 DAF, but at harvest, the line XN17 has a significantly lower content under control and LoHi236 significantly higher content under control respect to Golden Promise. As for the stress, the lines XN26, LoHi236 and LoHi272 have significantly higher content compared to Golden Promise. There are no differences between treatments. There are also no differences between the genotypes in control condition at 0 HAI, but the stressed lines XN17 and XN26 have significantly lower levels of GA3. Lines LoHi236 and LoHi272 have significantly higher levels of GA3 if compared to Golden Promise. At 12 HAI, Golden Promise has the highest GA3 content of all the lines under control, and it is also higher respect to stressed Golden Promise. GA3 levels drop down for Golden Promise control, meaning that stressed grains of Golden Promise have significantly higher GA3 content at 24 HAI. LoHi236 still maintains the highest content of GA3 under control and XN17 maintains the lowest level of GA3 under stress. At 72 HAI, the lines XN17 and XN26 reach a peak of GA3, under both conditions. GA8 is the degradation product of GA and at 24 DAF shows high variation and therefore no differences between the lines and treatments and the content are approximately 40 ng/g DW in all the lines and both conditions. At harvest, the GA8 content falls to 6 ng/g DW in Golden Promise control and stress. At 0 HAI Golden Promise stress has significantly higher GA8 content from all the other lines. Golden Promise has the highest levels of GA8 under control and stress at 12 HAI, at 0 HAI the line XN17 has still the lowest level of GA8 under control, but there are no differences between the lines under stress treatment, and there are no differences between treatments. but at 24 HAI only XN17 and XN26 maintain lower levels of GA8 respect to Golden Promise. At 72 HAI GA8 is present only in Golden Promise and LoHi272, with no differences.

3.8.3 Threshold of ABA:GA ratio as determinant for germination process

ABA and GA are the most important plant hormones in the transition from grain maturation to germination (Nonogaki, 2014; Bewley, 2013). As the biologic important active form GA3 is detectable through all time points analyzed the ABA:GA ratio was calculated relative to GA3 abundance.

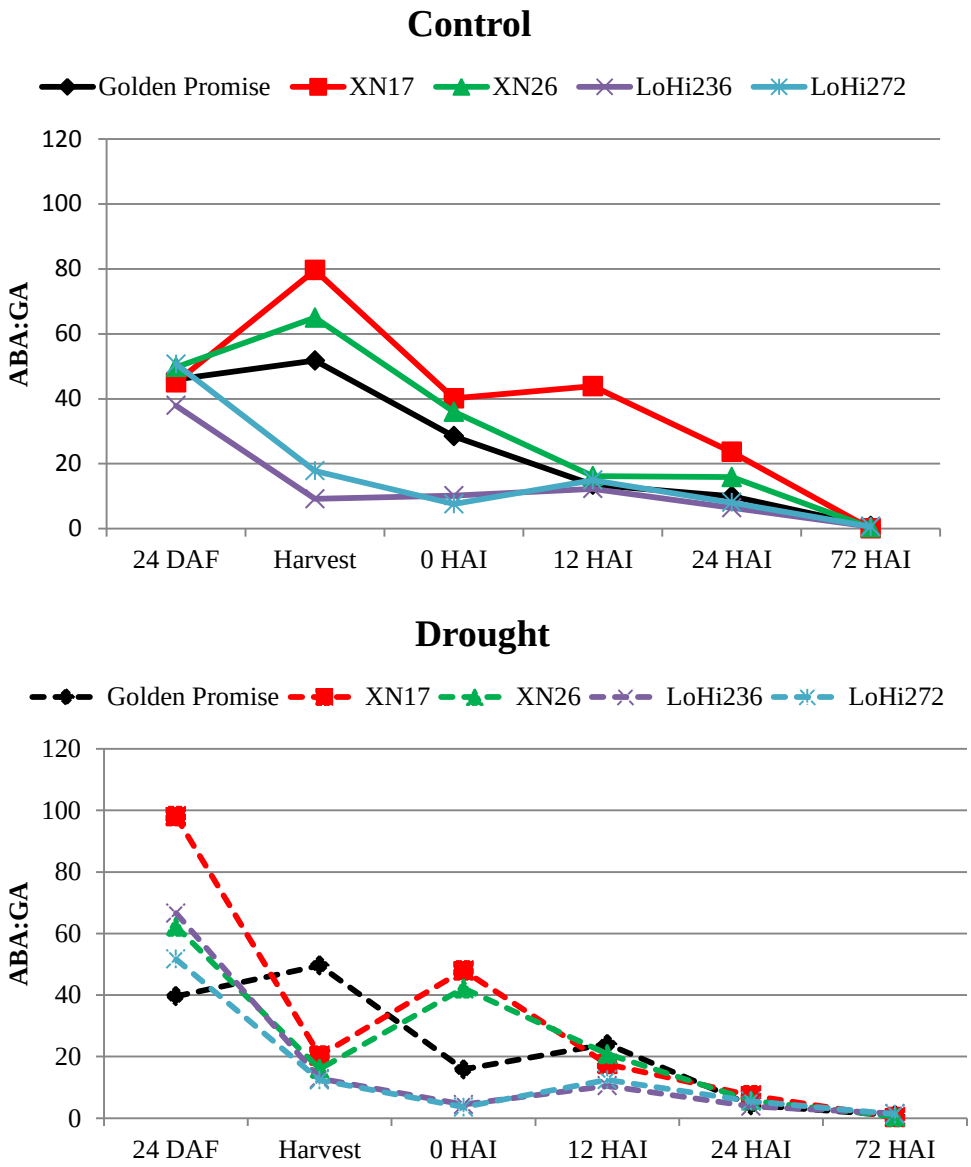


Figure 18. ABA:GA ratio starting from late maturation stage of seed development (24 HAI), harvest (pre-storage), 0 HAI (dormancy release), imbibition at 12 HAI and germination time points 24 HAI and 72 HAI. Upper chart shows the ratio pattern of control (solid lines), bottom chart (dashed lines) shows the ratio pattern of drought-stressed material. ABA:GA decrease is sharper in drought-stressed seeds.

The ratio was determined, to identify a pattern in the transition from grain maturation to germination. Developing seeds 24 DAF derived from control treatment show a similar ABA:GA ratio (~50 ABA relative units per 1 GA). Golden Promise maintains this ABA:GA till harvest followed by a slow decrease until 72 HAI where ABA equals GA. The line XN17 and XN26 have an increase of ABA:GA at harvest, which is decreasing until 72 HAI, but the ratio is higher compared to wild-type and LoHi. The lines LoHi236 and LoHi272 have a sharp decrease in ABA:GA ratio at harvest and it is kept at lowest levels, around 10, at all time points. The seeds developed under stress condition show a different ABA:GA ratio. In the

wild-type line, Golden Promise the ABA:GA ratio at 24 DAF, is at 40. The transgenic lines have all higher ratio at 24 DAF, ranging from 50 for LoHi272 to 100 for XN17. From 24 DAF to harvest, XN and LoHi lines have a strong decrease in ABA:GA, reaching 12 (LoHi236 and LoHi272) or 20 (XN17 and XN26). LoHi236 and LoHi272 keep their ratio at low levels (below 10) until 72 HAI. XN17 and XN26 reach instead another peak of ABA:GA which goes above 40 at 0 HAI, to be reduced slowly as germination proceeds. Golden Promise has a completely different ABA:GA respects to other lines, showing a slight increase in the ratio at harvest, which is reduced to 15 at 0 HAI. At 12 HAI, another ABA to GA ratio peak follows, reaching a value of 24. At 24 HAI, this peak is reduced at 5 and its further reduced until 72 HAI, just as in lines LoHi236 and LoHi272.

3.9 Transcriptome analysis during germination

The transition from seed to seedling is mediated by germination which is a complex process that starts with imbibition and completes with radicle emergence. Germination process marks the starting point of seedling establishment and the accumulated reserves during seed maturation are necessary for energy production to ensure seedling growth (Fait *et al.*, 2006; Silva *et al.*, 2016). From previous gene expression profiling studies, it is known that the transcriptome required for seed germination is already present in the dry seed that just completed development and maturation (Nakabayashi *et al.*, 2005; Kimura and Nambara, 2010; Nambara *et al.*, 2010). The main objective of this experiment was to determine which of the stored mRNA are required after seed activation by imbibition. After activation of the seed by water uptake, the stored mRNAs are loaded with ribosomes and form a polysome complex. Such polysome complex is a hallmark for active translation (Kimura and Nambara, 2010). Therefore, the identified transcripts can be called germination essential transcripts. Both embryo and endosperm accumulate transcripts during seed development. At the dry, mature state both tissues contain a pool of different transcripts (Dekkers *et al.*, 2013). The embryo and endosperm play distinct roles in barley seed germination; embryo produces the new plant and endosperm is a source of the reserves until the seed enters the autotrophic phase during germination (seedling establishment) (Dekkers *et al.*, 2013). Polysome bound RNA from embryo and endosperm at 12 HAI were used to investigate up- or down-regulated genes in these tissues during imbibition with the goal to identify germination essential transcripts needed in two different barley tissues. And how much is seed germination related to the association of specific mRNAs with polysomes during seed imbibition. Transcriptome analysis was performed in Golden Promise, XN17, XN26 and Lohi236 lines using three

independent biological replications using Agilent platform. The microarray experiment was done on 60K Barley custom array chip (Agilent technologies). The time points analyzed are a dry seed (0 HAI) and 12 HAI (imbibition) of seeds grown in optimal and drought stressed condition. From the dry (0 HAI) seed total RNA was used for hybridization, as well as from 12 HAI seed. From dissected tissues (embryo, endosperm, time point 12 HAI) polysomal RNA was used for hybridization. Here, 12 HAI was the time point chosen for this study. The arrays were scanned, and the data was extracted with Feature extraction software (Agilent technologies). Raw data were normalized, filtered and analyzed with Genespring (Agilent technologies) software.

3.9.1 Seed transcriptome of Golden Promise

Three independent biological replications were used for mature grain microarray analysis of control and stressed grains of Golden Promise and transgenic lines (see below). As explained before, grains were developed and matured under drought stress condition (15% of soil moisture content) from 5 days after flowering until harvest. After harvest, grains were kept for two months in storage capacity at 4° to surpass dormancy. The transcripts which are present in the mature after-ripened grains are called stored mRNA or long living messages (Sreenivasulu *et al.*, 2008; Kimura and Nambara, 2010; Galland *et al.*, 2014). During the post-harvest storage, the seed has minimum viability where changes such as decrease of germination inhibitors, alteration of membranes and protein degradation happen to improve germination vigor (William E Finch-Savage and Leubner-Metzger, 2006). The main goal was to understand if there was a different abundance of stored mRNA which was affected by drought stress during maturation and desiccation. After normalization and filtering of low-quality entities through filtering steps in Genespring (Agilent technologies) around 20.000 transcripts are present in the dry seed of Golden Promise (but also in transgenics, see below). A similar number was also found in the dry seed of LP104 and LP106. As seen in LP104 and LP106, the highly abundant transcripts do not differ greatly between conditions. Top 20 abundant transcripts found in seeds in Golden Promise are associated with hexosyltransferase, which is involved in grain filling process, late abundant embryogenesis proteins and heat shock proteins, related to late seed maturation and desiccation tolerance, histone 2B and ribosomal protein involved in DNA synthesis and RNA translation. Transcripts associated with germin-like protein 4, trypsin inhibitor and ectonucleoside triphosphate diphosphohydrolase 1 are also found and they are known for their role in defense of proteases or stress during seed storage (table 9). To identify the terminal drought stress-induced changes

the transcriptome of the mature grain in the wild type under both conditions was analyzed, a t-test was performed and 627 genes with FC<1.5 were found differentially expressed between the two conditions. 363 genes are found to be down-regulated and 264 up-regulated in stressed dry seed in Golden Promise. Down-regulated genes are associated auxin and cytokinin metabolism, transport, stress (heat shock proteins are all found down-regulated) and with cell wall synthesis. The up-regulated are associated with DNA synthesis, fermentation, glycolysis, major and minor CHO, lipid transfer proteins and N-metabolism and ribosomal proteins. Among the up-regulated ones are transcripts which belong to jasmonate and ethylene hormone metabolism, ethylene-responsive element. Highly diverse genes (FC>5) are shown in figure 18. Protein synthesis genes which encode ribosomal protein are found strongly up-regulated, as well as ubiquitin 5 and cytochrome P450. Down-regulated genes under stress are germin-like protein, proteases and methylesterase 1.

Table 9. Top 20 abundant transcripts in Golden Promise dry seed. Control (left) and stressed seed (right). Normalized expression values extracted from Genespring (Agilent technologies) are shown for each transcript (n=3).

Harvest assembly ID	GP control	Annotation	Harvest assembly ID	GP stress	Annotation
35_22282	7.199565	NA	**35_22282**	7.363379	NA
35_19829	6.765381	Lea 19.3	**35_19829**	7.171216	Lea 19.3
Contig3812_at	6.727867	Hexosyltransferase	**Contig3812_at**	6.862277	Hexosyltransferase
35_1096	6.611988	Hexosyltransferase	**35_1096**	6.734975	Hexosyltransferase
35_1055	6.421233	NA	**35_1055**	6.509128	NA
35_15272	5.79424	germin-like protein 4	**35_38483**	5.662075	Histone 2B
35_30848	5.666851	C type trypsin inhibitor	**35_30848**	5.646984	C type trypsin inhibitor
35_43928	5.657455	NA	**35_43928**	5.640463	NA
35_361	5.651279	Stress responsive protein	**35_23811**	5.637411	NA
35_2094	5.648502	NA	**HvLeaB19.3**	5.565728	
35_23811	5.629297	NA	**Contig1837_at**	5.550043	Lea D-19
Contig1837_at	5.502148	Lea D-19	**35_14798**	5.528833	Lea D-19
HvLeaB19.3	5.424543		**35_14796**	5.324387	Lea D-19
35_14796	5.360302	Lea D-19	**35_6738**	5.312013	NA
35_14798	5.349639	Lea D-19	35_14795	5.124234	Lea D-19
35_6738	5.132979	NA	35_48983	5.051569	NA
35_38483	5.076904	Histone 2B	Contig1834_at	5.049408	Lea D-19
35_25394	5.071641	22 kDa class IV heat shock protein	35_17096	4.99272	Trypsin inhibitor
35_25868	5.03567	NA	35_34557	4.933098	40S ribosomal protein
35_25337	4.997345	Ectonucleoside triphosphate diphosphohydrolase 1	35_41830	4.817106	NA

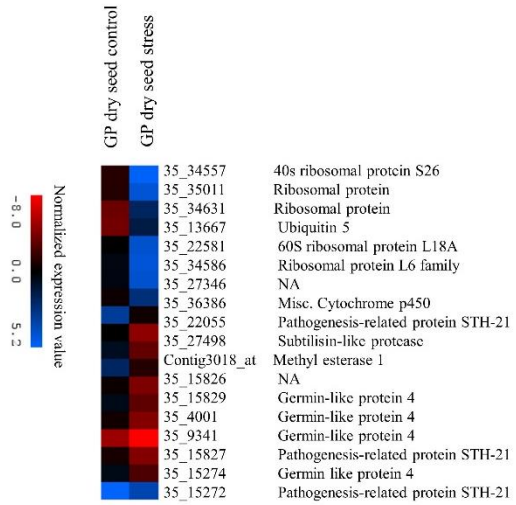


Figure 19. Top differentially expressed genes (FC>5) between control and drought stressed seed at 0 HAI. Heat map generated in Mev (TIGR, TM4) showing differential gene expression between stressed and control seeds based on microarray data (t-test, BHB adjusted P, FC>5). Each row represents 1 gene, each column 1 sample. Blue color represents a higher relative gene abundance compared with the mean, while red illustrates a relative gene expression below the mean.

3.9.2. Changes in the transcriptome of dry seed in dependence to ABA

Most abundant transcripts in transgenic lines in the after-ripened dry seed (0 HAI) are associated mostly with seed maturation process and stress response. Line XN17 has the highest ABA content at this stage. Transcripts associated with alpha-amylase/subtilisin inhibitors, aspartyl proteases, methylesterase inhibitor, lipid transfer protein and ribosomal proteins are present at high abundance in control seed. In the stress seed, the situation is different because of the annotated transcripts are only the ones associated with protein synthesis, trypsin inhibitor, and LEA. In the control seed of XN26, the most abundant transcripts are associated with ribosomal proteins and ATP/ADP transport carrier. In the stressed seed apart from ribosomal proteins, other transcripts encoding alpha-amylase and trypsin inhibitors, lipid transfer proteins and hexosyltransferase are present. The line LoHi236 is distinguished by the presence of transcript encoding ethylene receptor in control and stresses seeds (listed in table 14 in supplemental data).

To understand how is drought stress affecting seed transcriptome, microarray data were analyzed to find significant differences using Genespring (Agilent technologies). A t-test (Benjamini Hochberg adjusted P value, FC>1.5) was performed to test the significance between control and stress dry seed transcriptome of transgenic lines. In figure 19 up- and down-regulated genes under stress are shown and divided into functional groups for each line. Functional groups such as RNA regulation, stress response, transport and signaling contain the

major number of differentially expressed genes under stress. Genes annotated as development-related and genes involved in protein synthesis and degradation are also highly affected. To notice is also that in all the lines the number of down-regulated genes in the stressed dry seed is higher than the up-regulated ones. For XN17, there are 1292 differentially expressed genes between control and stress (with FC>1.5), 821 are down-regulated, and 471 are up-regulated under stress. Differentially expressed genes with FC>5 are mostly down-regulated under stress. In the line XN26, the number of differentially expressed genes is 1341, 872 are down- and 469 up-regulated and in the line LoHi236 there are 614 differentially expressed genes, 465 are down- and 150 are up-regulated.

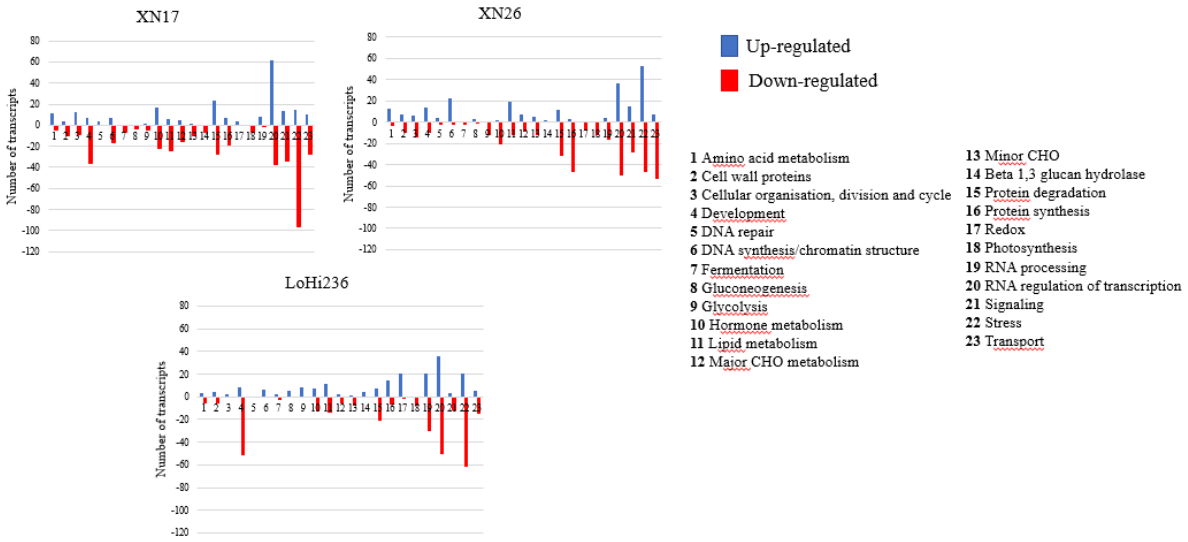


Figure 20. Microarray t-test reveals (Benjamini Hochberg adjusted P value, FC>1.5) differentially expressed in mature seeds that developed under stress condition versus control seeds within transgenic lines XN17, XN26, and LoHi236. They are divided according to their functional annotation (vertical bars). Blue: up-regulated genes, red: down-regulated genes.

Under stress, the up-regulated genes which are common for all the lines involve mostly ribosomal proteins, protease inhibitors, lipid transfer proteins, alpha-amylase, beta-glucosidase, and ethylene biosynthesis genes. Genes involved in ABA and stress response, such as dehydrins, are in all lines found down-regulated under stress. Only 20 genes are present in the comparisons between control and stress in all the lines. These are involved in lipid metabolism and transport, beta 1,3 glucan hydrolases and glycolysis, shown in figure 20. Apart from the generally similar response under stress in all transgenic lines, genes with FC>5 are mostly line specific (supplementary figure 15). Genes encoding MADS-box transcription factor 14 are the only one up-regulated in the stressed seed of XN17, while the down-regulated genes are related to storage proteins, ethylene response and starch biosynthesis. Differentially regulated genes under stress in XN26 with FC>5 are the up-regulated non-specific lipid

transfer proteins and ethylene-responsive transcription factor and down-regulated genes encoding ribosomal proteins and polyubiquitin. Line LoHi236 has the highest number of up-regulated genes when compared to other lines. They encode ribosomal proteins, hydrolases, lipoxygenases, and ethylene responsive transcription factor. Among annotated down-regulated genes encoding polyubiquitin and ribosomal protein can be found.

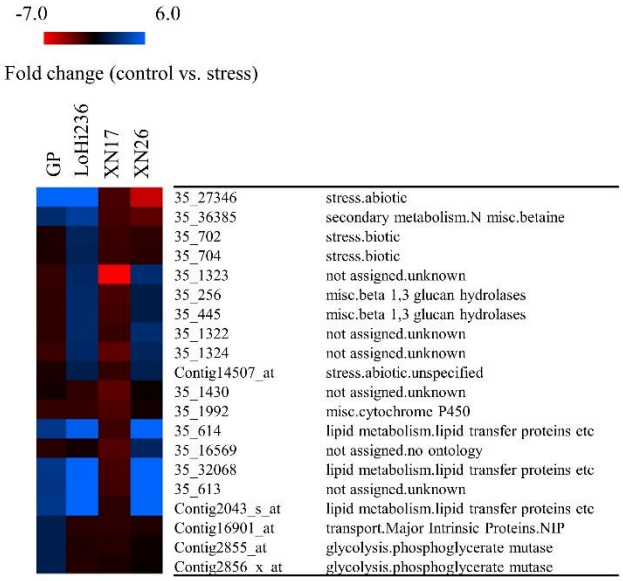


Figure 21. Heat map generated with MeV (TIGR, TM4) showing 20 mutual genes which are found differentially expressed under stress at the dry seed stage (0 HAI) in Golden Promise, XN17, XN26, and LoHi236. Color scale based on fold change (control vs. stress), blue: up-regulated under stress, red: down-regulated under stress.

One-way ANOVA was implemented to compare gene expression of Golden Promise with XN17, XN26, and LoHi236 at 0 HAI for each condition. There are 1377 differentially abundant transcripts under control, and 1632 under stress in XN17 when compared to Golden Promise. The number of differentially abundant genes between XN26 and Golden Promise under control is 1975 and 1615 under stress. Under control condition, there are 2036 differentially abundant genes between GP and LoHi236. Under stress, this number is similar, 2270, and the differences comprise genes that belong to the same groups. The difference between the lines resulted in a higher number of differentially abundant genes than between the conditions. Strikingly, most of the differentially abundant genes have the same pattern in the transgenic lines.

However, a subset of transcripts is differentially abundant in control and stress in all lines respect to Golden Promise (figure 21) and it is possible to recognize a line-based pattern. 10 different clusters are shown, based on normalized expression values of genes. Clusters 1,2,5,6 and 9 show a similar pattern, that is a higher expression of genes in transgenic lines especially

under control condition. In these clusters, most transcripts belong to protein synthesis and degradation processes, but transcripts involved in lipid metabolism and gluconeogenesis are also present, as well as some transcription regulators such as ABI3 and Argonaute. In cluster 3 and 4 under stress, Golden Promise and LoHi236 have the highest expression of all the lines. In these clusters, cell wall modification genes can be found. Cluster 7 has a peak under control in line LoHi236 and a peak in line XN17, here, only lipid transfer proteins are found. All these transcripts are also known to play a significant role in germination and malting quality.

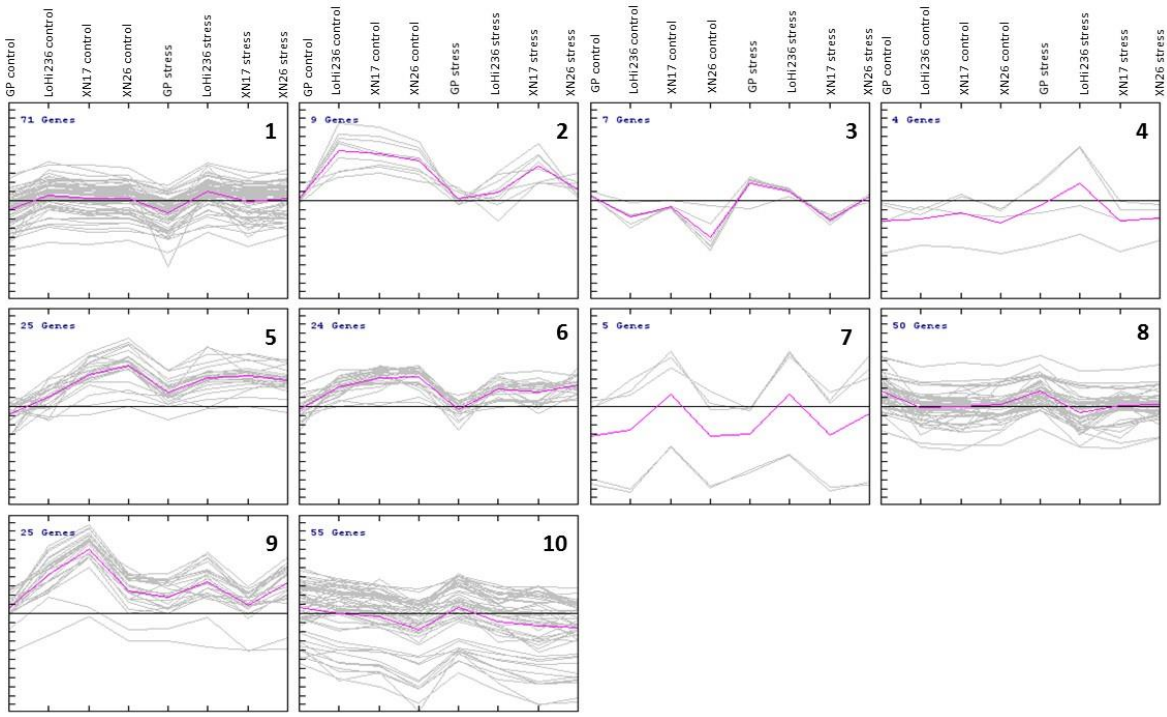


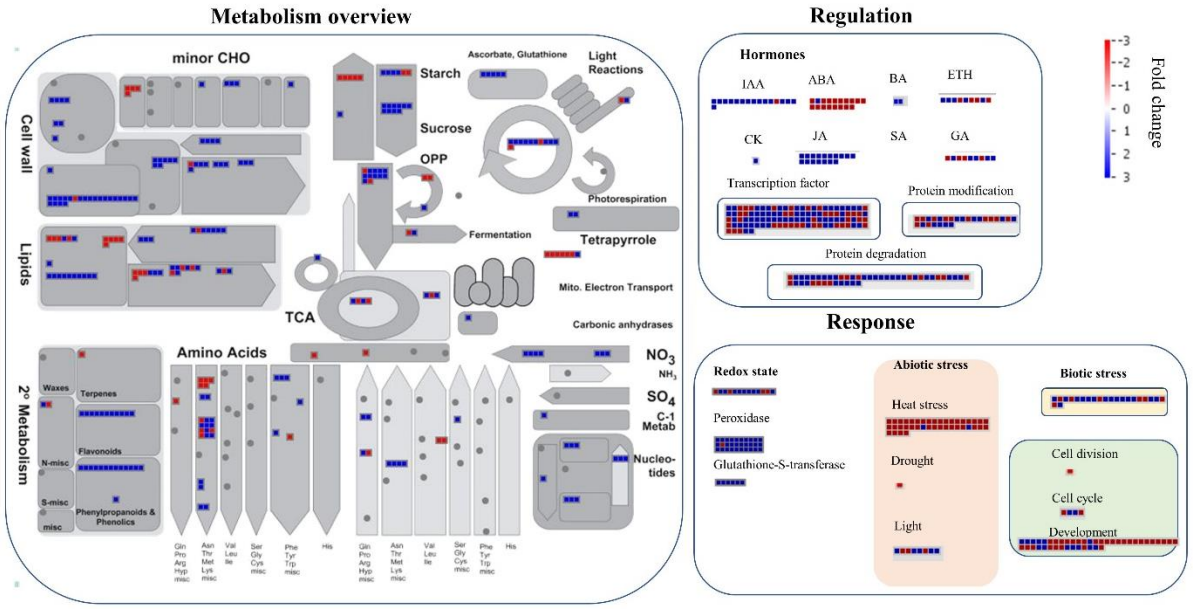
Figure 22. Gene expression data were clustered with the k-means algorithm using the MeV tool. Each gene is represented by a thin grey line which is the mean of its normalized expression value.

Under control, the highly abundant transcripts in XN17 and XN26 are annotated to protein synthesis (ribosomal proteins), and to post translational modification. Cell wall precursor proteins and proteins involved in G signalling are also highly abundant, ethylene response as well. Under stress, transcripts associated with MADS box transcription factor, and degradation of storage compounds. Photoreaction related genes and peroxidase genes are more abundant in LoHi236, as well as starch and lipid degradation but lesser are drought response genes. Under stress the highly abundant transcripts are associated with cell wall modification and cytokinin oxidase. This could be due to lower ABA content in dry seed in LoHi236 so that the seed is more "prepared" for germination.

3.9.3 Differences in the transcriptome during imbibition in Golden Promise

At 12 hours after watering germination is not completed yet and the seed is resuming metabolic activity (J. D. Bewley, 1997). The imbibition phase is a critical period for successful germination and depends on the soundness and vigor of the seed (Woodstock, 1988). The movement of water into the seed is due to diffusion and capillary action with water moving from a region of higher to lower water potential. Water is essential for cellular metabolism, needed especially for enzymatic activity, for solubilization and transport of reactants, and as a reactant itself for hydrolytic digestion of stored reserves (Woodstock, 1988). T-tests were performed on three independent biological replications to compare total RNA of the dry seed with the total RNA of the 12 hours imbibed seed from control and stressed seeds in Golden Promise. Under control condition, 1840 differentially expressed genes after 12 hours of imbibition, 724 down and 1036 up-regulated were identified. The comparison between dry stressed seed and imbibed seed (drought stress implemented during development) resulted in 2566 differentially expressed genes. 842 are down-regulated and 1724 are up-regulated. To identify pathways and mechanisms affected by imbibition, identified genes are classified and displayed based on their Gene Ontology using Mapman software (Usadel *et al.*, 2009). In figure 22, differentially regulated genes are shown for control (A) and stress condition (B). Both comparisons (dry vs imbibed; control, stress) share 954 common genes. K-means clustering (MeV, TIGR, TM4) (Saeed *et al.*, 2003) analysis divides them simply into two clusters, the up-regulated and down-regulated genes at 12 HAI imbibed seed meaning they have same regulation under both conditions. Among the down-regulated transcripts are transcripts related to seed development processes, ABA-induced late embryogenesis proteins, and stress regulation. Myb-, NAC, and APETALA2 transcription factors are also among the down-regulated transcripts. Up-regulated genes can be associated with features attributed to cell walls modification proteins like cellulases, cell organization, amino acid, starch and lipid metabolism, DNA synthesis, glycolysis, beta glucans, glucose-, galactic- mannosidases, and ribosomal proteins. Transcripts of genes associated with auxin, gibberellin, jasmonic acid hormone regulation are as well found among the up-regulated ones. The number of up-regulated genes in stress imbibed seed could suggest that the stressed seed is more “active” respect to control.

A



B

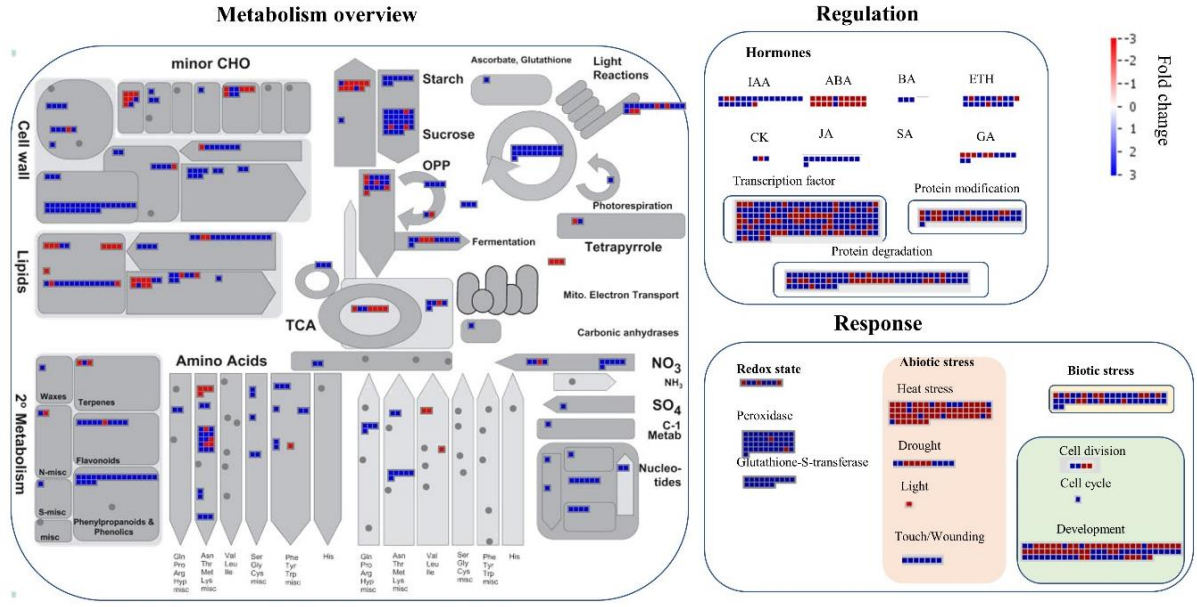


Figure 23. Mapman graphic representation of differentially expressed genes between the dry and imbibed seed. **A** control condition **B** stressed seed in Golden Promise. Metabolism overview, regulation, and response involved transcripts are shown, resulted from a t-test (BHB adjusted P, FC>1.5). Fold change scale (dry vs. imbibed) ranging from -3 to 3. Down-regulated genes are colored red, up-regulated genes are colored in blue.

Comparing the two conditions at 12 HAI, the main differences (FC>5) are seen in transcripts involved in ribosomal proteins, WRKY transcription factor, cell wall degradation enzymes which are up-regulated. The down-regulated genes belong to DNA synthesis, jasmonate response, and glycolysis functional groups.

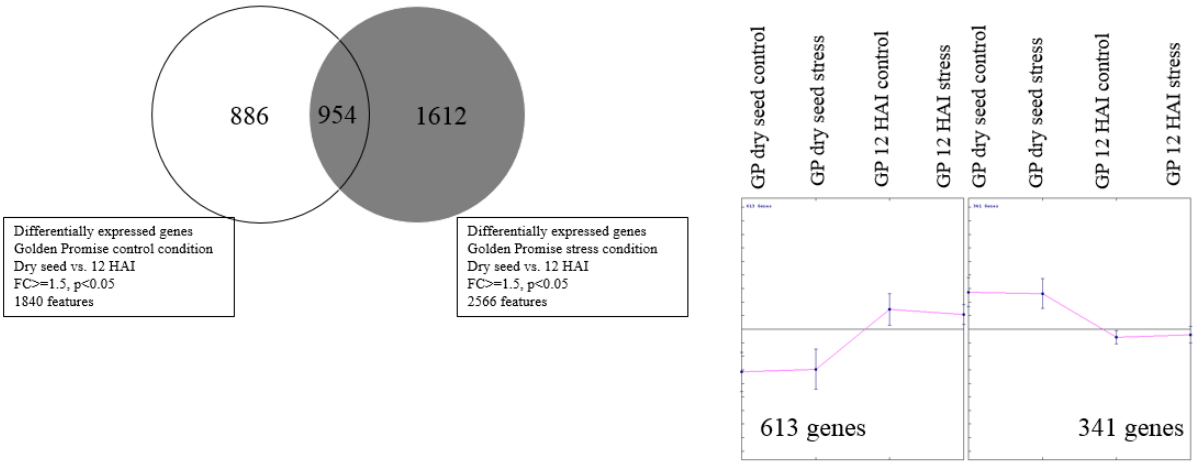


Figure 24. Venn diagram showing mutual differentially expressed genes at 12 HAI between control and stress. The mutual genes are clustered by K-mean clustering (MeV tool) showing 613 up-regulated and 341 down-regulated genes in both conditions in the imbibed seed of Golden Promise.

3.9.4 Transcriptional changes during imbibition independence to ABA

To understand which genes are up- or down-regulated during imbibition (12 hours after watering) in transgenic lines XN17, XN26, and LoHi236 microarray data analysis were performed using Genespring software (Agilent technologies) and following the steps as described for Golden Promise. Transcriptome coming from the total RNA at two different time points, 0 HAI and 12 HAI, were compared with a t-test (BHB adjusted P, FC>1.5). For each line and condition, three independent biological replications per sample were used. Under control condition in the line XN17, 3356 differentially expressed genes are found, 1036 are down-regulated and 2320 are up-regulated. Under stress condition, the number of differentially expressed genes is 5590, with 1668 down and 3922 up-regulated genes in 12 HAI stressed seed. The line XN26 has 2286 up-regulated and 685 down-regulated genes in 12 HAI control seed (2972 in total). The number of differentially expressed genes in stressed seed at 12 HAI is 6122, 3789 being up and 2333 down-regulated. For LoHi236 there are 5605 differentially expressed genes, 2021 down-, 3584 up-regulated between the control dry and control imbibed seed. In the stressed 12 hours imbibed seed of LoHi236, there are 5852 differentially expressed genes, 1896 are down and 3956 up-regulated. All these comparisons reveal a substantial number of differentially expressed genes in the imbibed seed, and accordingly, these genes belong to functional groups which indicate the reactivation of metabolism. Up-regulated genes are associated with cell wall modification, cellular organization, cell division, DNA synthesis, glycolysis, lipid metabolism, major and minor CHO, protein degradation, light reactions, transport, and signaling. The down-regulated genes are mostly associated with seed maturation and desiccation processes, and storage proteins. However, in the stressed seed of each line the presence of genes involved in degradation of

storage compound is pronounced, which fits to earlier preparation for germination in these seeds.

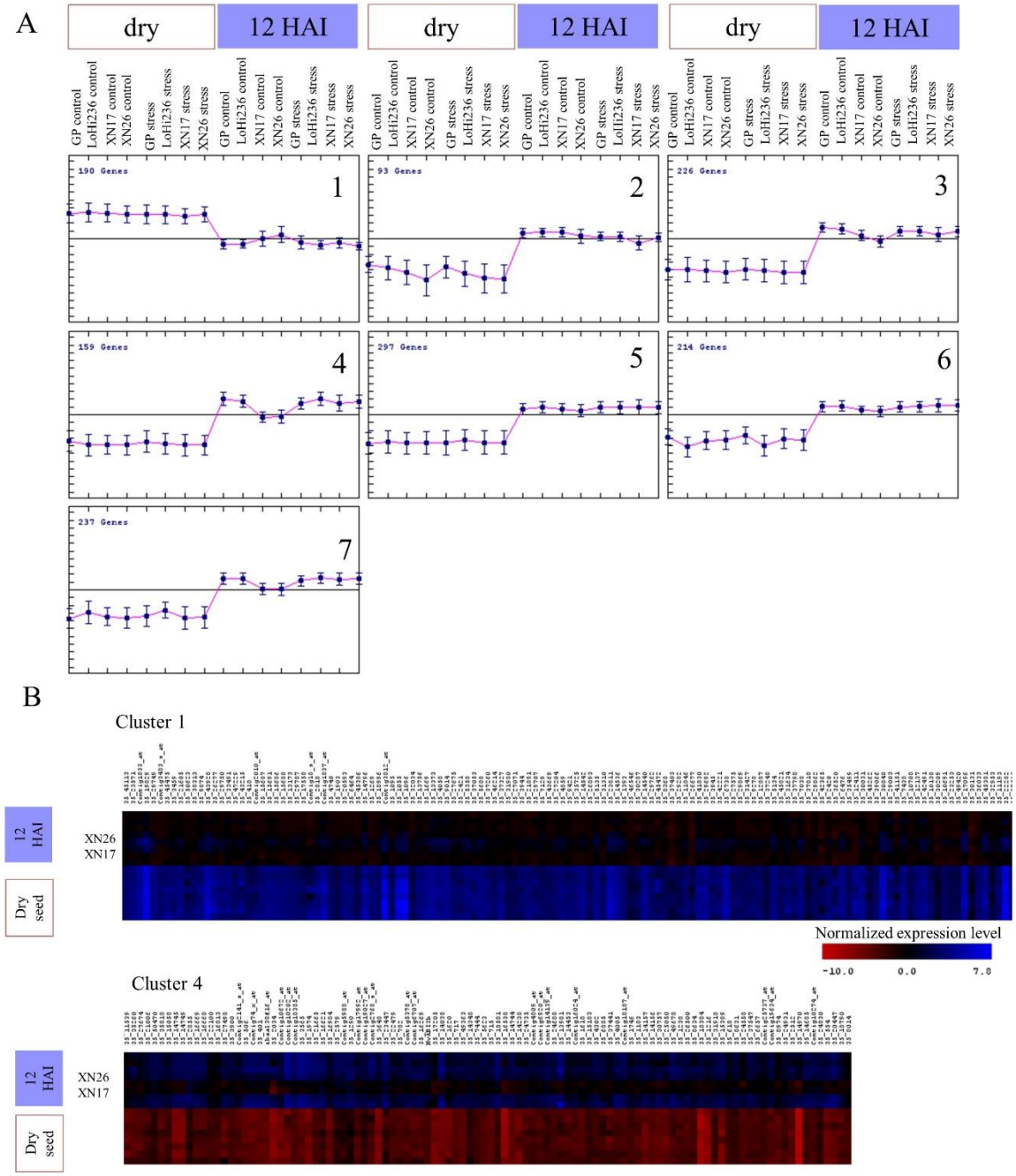


Figure 25. A1461 genes mutually up- or down regulated at 12 HAI respect to 0 HAI. They are divided into 7 clusters by K-means clustering, performed in MeV (TIGR, TM4). The line represents the mean expression value of all transcripts present in a given cluster. **B** Heat map showing cluster 1 and 4, lines XN17 (control) and XN26 (control) are indicated, which show the main difference transcript in abundance at 12 HAI.

The main differences are seen in the control seeds of the XN lines, which are characterized by a weaker up-regulation of germination associated genes. They are shown in the figure 25. In the cluster 1, genes that have high normalized expression levels in control seeds of XN lines

are present. They are mostly drought stress related, ABA-induced LEA proteins, and generally associated with mature seed metabolism. The heat map in figure 25 B, shows selected genes of the cluster 1. In all the other lines, there is a peak of expression of these genes in the dry seed, which is not the case for the imbibition phase. Trypsin inhibitors, MYB and C2H2 (zinc finger) transcription factors, DNA synthesis, cytokinin oxidase 1 and ethylene and auxin response associated transcripts can be found in the cluster 1. In the cluster 4 and 7, control imbibed seeds of XN lines have a low normalized expression levels of genes associated mainly with germination initiation process. These transcripts are associated with amino acid, lipid, starch and protein metabolism, transport of amino acid and oligopeptides, cell wall modification, DNA synthesis, receptor kinase signaling, auxin, ethylene and gibberellic acid response. The bZIP, MYB and NAC transcription factors also belong to these clusters. These result indicate that the line XN17 and XN26, which had the highest ABA content in the dry seed could have a slower down-regulation of transcripts associated with seed maturation and a slower up-regulation of transcript associated with germination.

3.9.5 Association of transcripts in the embryo and endosperm at 12 HAI with polysomes

Extraction of polysome-bound RNA enables to extract transcripts which are involved in the translation process (Chassé *et al.*, 2016). Germination process requires interactions between embryo and endosperm. They contribute as different tissue storages of important messages which are used up during germination (He *et al.*, 2015). Embryo and endosperm accumulate different transcripts during seed development which will be used when the metabolism is reactivated by water. The microarray experiment was performed to identify active genes to understand their contribution in the early germination process. Three independent experiments were used for each line. The embryo was dissected from endosperm and polysome-bound RNA was extracted. Around 31 000 transcripts in polysome complexes are present at 12 HAI in the embryo and 25 000 transcripts in polysome complexes in the endosperm. They both share a large part of transcripts (24 000), but certain transcripts are tissue specific. Both tissues share also a larg part of transcripts with the dry seed as shown in figure 26. In figure 26, a Venn diagram shows how many of the transcripts are shared between the two tissues, and it shows that in polysomal RNA in endosperm there 712 specific transcripts and in the polysomal RNA in embryo there are 7109 specific transcripts. A subgroup of the tissue specific transcripts is already present in the dry seed. The rest is present only in the polysome-bound RNA at 12 HAI, and could be that these transcripts were synthesized upon imbibition.

The mean expression value of the tissue-specific transcripts of all the lines and replicates was used to depict their presence is metabolic processes with Mapman (Usadel *et al.*, 2005).

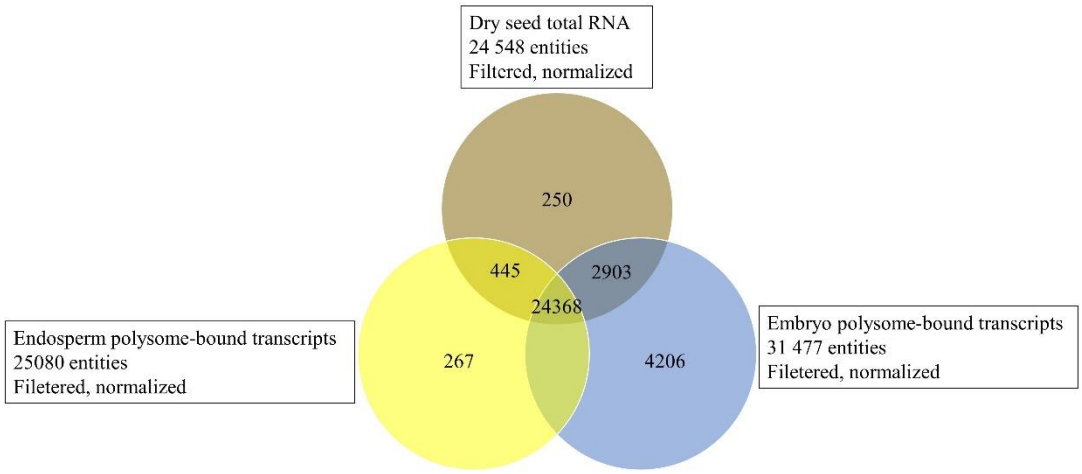


Figure 26. Venn diagram showing common and tissue-specific polysome-bound transcripts in the embryo (blue) and endosperm(red) at 12 HAI and in the dry seed. The number of entities (transcripts) are based on pre-processed and filtered data in Genespring (Agilent technologies).

In the embryo, the most of the germination-specific gene products are found and can be found in every Mapman annotation bin, like cell wall modification and cell wall degradation enzymes, cell division, protein, amino acid and nucleotide metabolism, light reactions and metabolic processes involving all hormone families (figure 27). The endosperm has been shown to affect germination even in species with a thin endosperm layer like barley. Among the endosperm-specific polysome bound-transcripts, specific leucine-rich repeat (LRR) cell wall proteins, minor CHO (raffinose family synthetases, hexokinase), major CHO (sucrose synthetase), glycolysis, gluconeogenesis, and amino acid metabolism (aspartate and glutamate family) and protein synthesis (ribosomal proteins) related transcripts can be found (figure 27). Although numerically embryo prevails, cytokinin, ethylene, and jasmonate-related transcripts, as well as GA20 oxidase and GA3 oxidase are specifically present in endosperm at 12 HAI. Hordeins and other storage proteins derived from seed development are also present. Certain endosperm specific transcripts encoding receptor kinases and transcripts involved in biotic and abiotic stresses are also specifically present in the endosperm. Transcription factor specifically present in endosperm is Apetala/ethylene-responsive element, auxin responsive factor, bHLH, NAC and MYB transcription factors.

Comparing an average value of expression of all the lines and conditions at the dry seed stage and at polysomal-bound transcripts from embryo and endosperm at 12 HAI, and considering only the high- (FC>10 or low-abundant (FC< -10), the main differences in roles during

imbibition of these tissues can be seen. Interestingly, there are certain similarities, which mostly regard hormone metabolism. In both tissues, ABA-induced genes, which had their peak at the dry stage (0 HAI) and were among the most abundant stored transcripts, are the least abundant at 12 HAI. Auxin, cytokinin, ethylene, jasmonates, gibberellic acid, and brassinosteroids have an up-regulation in both tissues. Starch degradation, cell wall modification, lipid degradation are all shared processes undergoing in both tissues at 12 HAI. The main difference comes from the fact that embryo undergoes high activity upon imbibition, and endosperm serves as a nutrition reservoir until the beginning of the autotrophic phase of the seedling. This results in higher number of transcripts being abundant in embryo only. To understand what are the differences that occur in the single lines, the t-test were performed to find differentially abundant genes in embryo and endosperm respect to the dry seed under control and stress.

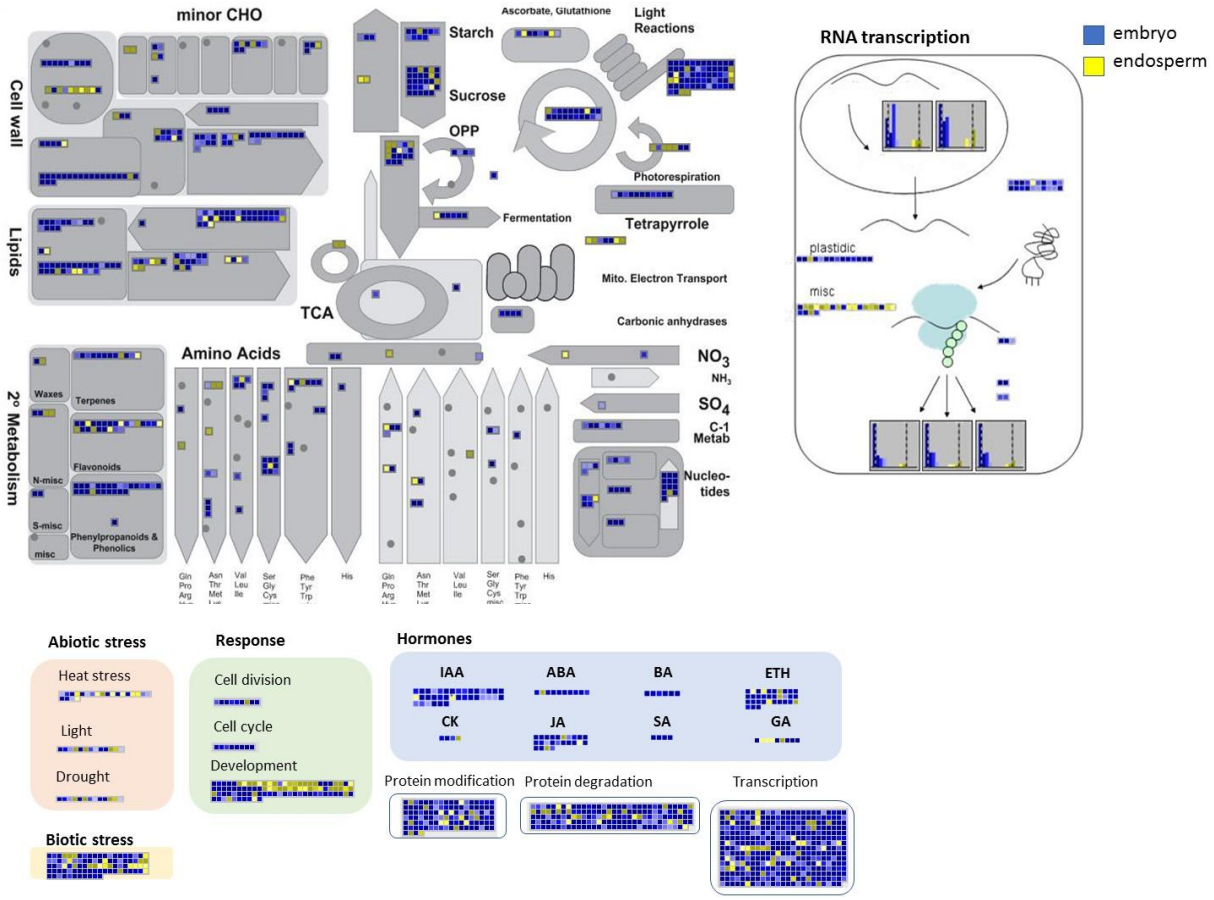


Figure 27. Mapman graphic representation of embryo- (blue) and endosperm-specific (yellow) transcripts at 12 HAI, based on the normalized mean expression value of all lines.

T-test was implemented to compare the dry seed and polysomal-RNA fraction from embryo. There are 2315 differentially abundant transcripts in the embryo polysomal RNA in Golden

Promise control condition. 1243 are less abundant and 1072 are found in higher abundance in embryo at 12 HAI. The ones which are highly abundant are the transcripts related to the DNA synthesis, lipid transfer proteins, cell proliferation, photosynthesis, chlorophyll-binding proteins, xyloglucan transferase, ethylene, gibberellic acid, cytokinin, auxin and jasmonate response. Hordeins and LEA proteins are found abundant specifically in dry seed. Under stress condition, differentially abundant transcripts are 2964. 1606 are more abundant respect to the mature grain, and 1358 are more abundant compared to the dry seed under stress. Among the most abundant up-regulated genes are associated with auxin, cell extension, lipid transfer proteins and light reactions. So, in both conditions, similar genes are up- to higher or lesser abundance. The main difference between control and stress in the embryo polysomal RNA is that under stress there is an up-regulation of ribosomal proteins, ethylene response, and MYB transcription factors. There are 25 000 transcripts present in endosperm polysomal-bound RNA. Comparing the Golden Promise dry seed with polysome-bound RNA coming from endosperm tissue, 847 are differentially abundant in the control situation when compared with the dry seed, and 114 in stress condition. The most abundant transcripts in endosperm under control condition are mainly associated with protein metabolism like prolamins, and lipid transfer proteins. Hydrolases, proteinases, and transferases are also represented as well as beta-amylases, xyloglucan transferases, involved in starch and glucan degradation. Others overrepresented involve plant hormones such as ABA-responsive proteins, GA2 oxidase, ethylene, and cytokinin metabolism. BZIP, MYBs, and auxin-responsive factors are transcription factors found in abundant in the endosperm. Senescence-related transcripts, LEA proteins, ABA, lipid transfer proteins, all transcripts which were in high abundance at the dry stage are now among the less abundant ones in the endosperm at 12 HAI. Under stress, mainly histone, dehydrin, peroxidases, and germin-like proteins are overrepresented. What is generally up between control and stress endosperm pf are ribosomal proteins- which could indicate a preparation for translation upon imbibition.

3.9.6 Differences in polysome association in relation to ABA content

One-way ANOVA (BHB adjusted P, FC<1.5) was implemented to find differentially abundant transcripts betwen the lines. Between XN17 and Golden Promise, there are 3524 differentially abundant transcripts under control, and 3285 under stress in the embryo. Comparing the XN26 with Golden Promise, there are 4995 differentially abundant transcripts under control and 3916 under stress. Most of them are shared between XN lines. The low abundant transcripts belong to light reaction, glycolysis, cell wall modification, storage compound degradation,

transport, and jasmonate metabolism functional groups. Among the highly abundant ones are those involved in abiotic stress response and storage protein synthesis. 2523 is the difference in abundance between LoHi236 and Golden Promise under control, and the same comparison gives 3605 differentially abundant transcripts under stress. The highest differences in abundance of transcription factors are found, like MYBs and all other transcription factors which are found highly abundant in LoHi236. Other highly abundant are related to starch, protein and lipid degradation. As for the hormone metabolism, cytokinin and ethylene response are more abundant in LoHi236. The stress condition is comparable to control regarding the most abundant transcripts in LoHi236. The transcripts which are found low in abundance under stress are mostly related to protein synthesis, signaling, stress, jasmonate, ethylene, and ABA metabolism. Comparing LoHi236 to Golden Promise and XN lines, transcripts that are involved in calcium signalling, light reactions, protein, lipid and degradation, are found overrepresented and indicate a possible advance in the start of germination. 789 of these transcripts are shared between the lines and conditions. One part of 789 transcripts have been selected based on their abundance and are represented in figure 28. The abundance of transcripts in the stressed tissues indicate possible stress-specific differences, like the abundance of specific transcription factors or specific metabolism pathway related transcripts. For example, while in the XN lines the up-regulated genes in embryo under stress are only involved in DNA and protein synthesis, in the LoHi236 the up-regulated genes are mostly involved in photosynthesis, cell modification, and cell organization. This is also seen in the endosperm, where the difference from Golden Promise can be explained like high or low abundance in Golden Promise respect to the transgenic lines.

In the endosperm, under control condition, the differences between Golden Promise and transgenic lines are around 300 differentially abundant transcripts, which are mostly stress related. Although these genes are mostly shared, the line specific differences are highlighted in figure 29. Glycolysis and brassinosteroids metabolism associated transcripts are more abundant in Lohi236 respect to the Golden Promise and XN lines. LEA, protein targeting and phosphatases associated transcripts are more abundant in XN lines. Under stress, the difference in abundance is, 1390 with XN26, 761 with XN17, and 1529 with LoHi236, all of which 675 transcripts are shared.

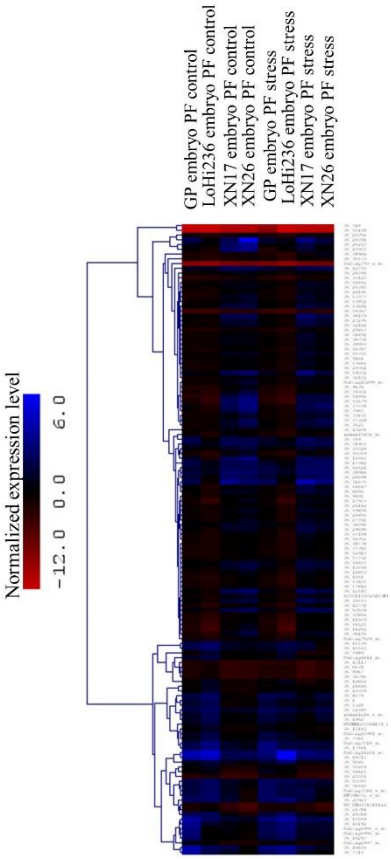


Figure 28. HCL created in MeV (TIGR, TM4) of polysome-bound transcripts from embryo. These transcripts are differentially abundant between the lines and were identified by ANOVA (BHB adjusted P, FC>1.5) and represent only a subset of transcripts selected based on their abundance. The list and FC between the lines can be found in supplementary figure 17.

The highly abundant transcripts present in all transgenic lines are mostly stress-related. Specifically to LoHi236, cell wall degradation, WRKY and MYB transcription factors, amino acid synthesis, brassinosteroids biosynthesis, photosynthesis, and glycolysis. are the highy abundant transcripts if compared to all the lines. Abiotic stress response, calcium signaling, storage protein as well as MADS box and CH3 transcription factors are those highly abundant in XN lines when compared to Golden Promise. In endosperm, transcripts associated with DNA synthesis/chromatine structure, protein degradation, Pip membrane intrinsic proteins, ABC transport protein, calcium signaling and heat shock proteins are found abundant in the XN lines. These are shown in figure 29. The list of transcripts shown in the hierarchical clustering can be found in the supplementary table 18.

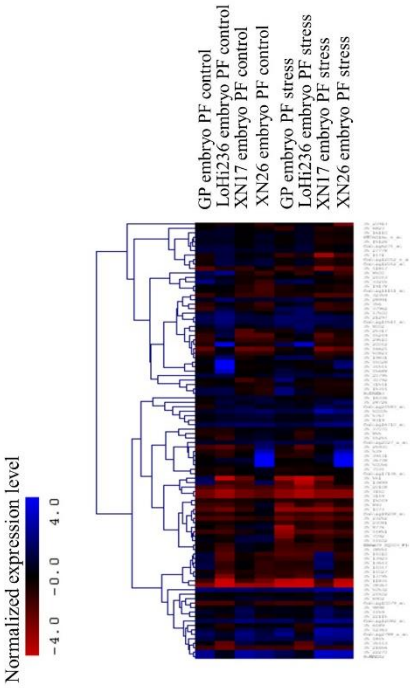


Figure 29. HCL created in MeV (TIGR, TM4) of polysome-bound transcripts from the endosperm. These transcripts are differentially abundant between the lines and were identified by ANOVA (BHB adjusted P, FC>1.5) and represent only a subset of transcripts selected based on their abundance. The list and FC between the lines can be found in supplementary figure 18.

4 Discussion

With the latest trends in beer production such as the premiumization of the beer in emerging markets and the growth of production of the craft beers, which require 4 to 7 times more malt than ordinary beers, malting barley needs are expected to grow by 14% in the next five years (Verheul, 2016; Watson, 2016). Within the global barley production, this makes no room for barley production to fail, since the demand equals the production of malting barley (Verheul, 2016). The major risks in the global malting barley production are environmental conditions, such as drought, which reduces yield and impairs seed quality (Worch *et al.*, 2011). Drought stress is a major factor compromising barley yield worldwide and that is why the irrigation management is one of the most crucial factors in barley plant development. Already in 1980 Morgan and Riggs determined that the quality of malt extracts was compromised when the plants experienced drought stress during grain development. It has been demonstrated that cereal grains that undergo drought stress during development tend to accumulate higher quantities of storage proteins during seed filling (Parchin and Shaban, 2014). Expression of dormancy is affected by genetic and environmental factors, particularly the conditions prevailing during seed development and storage after harvest (Gutterman, Corbineau and Côme, 1996; Surdonja *et al.*, 2017). Dormancy is a highly intriguing biological process also because of its practical implications in the malt production industry. Both farmers and seed companies encounter problems associated either with lack of dormancy or with profound dormancy. The process of malting relies on germination, and those genotypes that could be malted immediately after harvest are likely to be preferred because it avoids the costs and deterioration resulting from grain storage until dormancy is terminated (Rodriguez, Toorop and Benech-Arnold, 2011). Dormancy release in barley must be adjusted to occur within a narrow time window after harvest so that it keeps low dormancy but without the worry that dormancy is reduced to risk pre-harvest sprouting (Rodriguez, Toorop and Benech-Arnold, 2011). The objective of malting is to exploit the physiological processes that the germinating seed undergoes on its way to becoming a seedling (Oser, 2015). These processes liberate sugars, proteins, and amino acids, which brewers use to nourish the yeast in fermentation. Today several malting quality parameters are defined contributing to the definition of malting quality. In this study, fifteen malting quality parameters have been assessed in the (doubled haploid) DH population of two superior performing elite malting lines grown under irrigated and controlled drought stress condition. The lines LP104, described for the remobilizing drought stress phenotype and LP106, described for the stay-green drought stress phenotype,

served as parents to the 98 lines generated by DH procedure. Furthermore, germination capacity trait of these genotypes was used to discover new candidate genes that influence malting quality. Additionally, the transgenic lines modulated for their ABA homeostasis served as tools to understand the role of ABA during germination under drought stress. Further, the contribution of embryo and endosperm, during imbibition in drought stress seeds and their possible role and importance in the malting process was analyzed. Here, the molecular basis of germination and shoot emergence that involve a large number of genes and their expression patterns is discussed. Any factors (internal or external) affecting the germination capacity of seeds and ultimately their agronomical performance should be understood to improve seed quality through breeding or crop management strategies.

4.1 Post-anthesis drought stress is affecting germination and malting quality

In this study 98 DH lines and their parents, LP104 and LP106, were used to study the effect of post-anthesis drought stress on germination and therefore malting quality traits. Up to now only a limited number of studies (Morgan and Riggs, 1981; MacNicol *et al.*, 1993) combining drought stress and malting quality or heat stress and germination index were described. In this study, where drought stress is applied during seed development on the mother plant (prior dormancy and germination), results showed unexpected performance of seeds derived from stressed mother plants, showing an enhanced start of barley germination and shoot emergence. At 24 HAI, 62 % (24% of them significantly) of genotypes showed an increase in germination for stressed seeds, and 74% (16% significantly) for shoot emergence. At 48 HAI, 81% of lines show higher shoot emergence (28% significant) and at 72 HAI 62% remain higher (9% significantly) because at 72 germination and shoot emergence reach a plateau. With this finding, Pearson correlation statistics were performed to test the correlation between germination and shoot emergence rate with yield, seed quality and malting quality traits. Under both conditions a negative correlation has been found with traits beta-glucan content and viscosity, and a positive correlation with trait friability. The trait friability accounts for the level of modification of barley malt, which comes from cell wall loosening in the endosperm, making the grain softer. The malt is milled under constant pressure and the weight of the friable constituents over the hard constituents defines the modifications of the malt. On the scale of friability, more than 80% is very good, good more than 70%, from 65% to 70% medium, and those below 60% are bad malts regarding friability (more than 4% of whole grains present in malt as a result of bad germination) (E-malt, 2015). Wort viscosity measures the gumminess of wort relative to water. It is a measure of the amount of stress a plant has

undergone during grain filling. Wort viscosity is determined by measuring the amount of beta-glucan (or cell wall material) in the wort (RW Scott, 1972). Viscosity is found to be negatively correlated with germination rate or shoot emergence rate, the correlation is low (control condition) and moderate (stress condition). Barley grain with low viscosity germinates more evenly than grain with high cell wall material. This cell wall material can also restrict the conversion of starch into malt extract. Highly viscous malt slows down the separation of the sugar-rich wort from husks during brewing. This slows the amount of beer processed in a brewery each day and increases production costs (Trainor, 2017). High malt losses are supposed to occur in over modified malt, and that coincides with increased production of unfermentable soluble protein (Bathgate, Martinez-Frias and Stark, 1978). Losses are due to root growth and respiration losses due to the consumption of dry matter in growth and respiration. Malt extract has a positive correlation with shoot emergence, only under stress. Faster germination and shoot emergence bring therefore more modification, therefore extract and probably certain amount of malt losses. Malt extract measures the amount of fermentable sugars. It determines the amount of alcohol that can be made from a tonne of grain. The higher the extracts level the more alcohol that can be made. Malt extract is measured by malting the grain and measuring the amount of soluble sugar, like glucose and maltose, in wort (hot water mixed with ground malt). The export standard for malt is a minimum malt extract level of 80%. In figure 30 the frequency distribution of the trait malt extract, friability, viscosity, respiration and root growth losses and total yield are shown. The frequency of single traits is divided into bins which represent the value of the traits. The histograms of the traits show an advantage of stressed material, since the bins containing the highest values of the traits are enriched especially for malt extract (27 control against 58 stressed lines in the 85% bin), friability (the biggest bin under stress is the 85% bin, and the bin 90 where the number of individuals is more than double under stress) and respiration loss (with 6.5% and 7% bins containing one third and double more individuals under stress). Interestingly root growth losses have a similar distribution, indicating that only the respiration loss contributes to total losses. These results show that there could be a beneficial effect of drought stress applied during grain maturation on important malting quality traits such as malt extract and friability, and in turn reducing the viscosity. This is in accordance with the study found by (MacNicol *et al.*, 1993), where they suggest that a limited episode of water stress during grain filling could be of advantage to malting quality. However, the less beneficial effect of drought stress on malting quality is the inevitable loss, because of the higher activity of the stressed grains. Seed breadth is the only seed quality trait that had a negative correlation with shoot emergence

under both conditions. There were no correlations observed between yield data and malting quality. However, the lines with the best yield under stress (above 200 g) showed also good malting quality traits and the lines having superior malt extract, as well as good yield performance (above 83%), are shown in table 10.

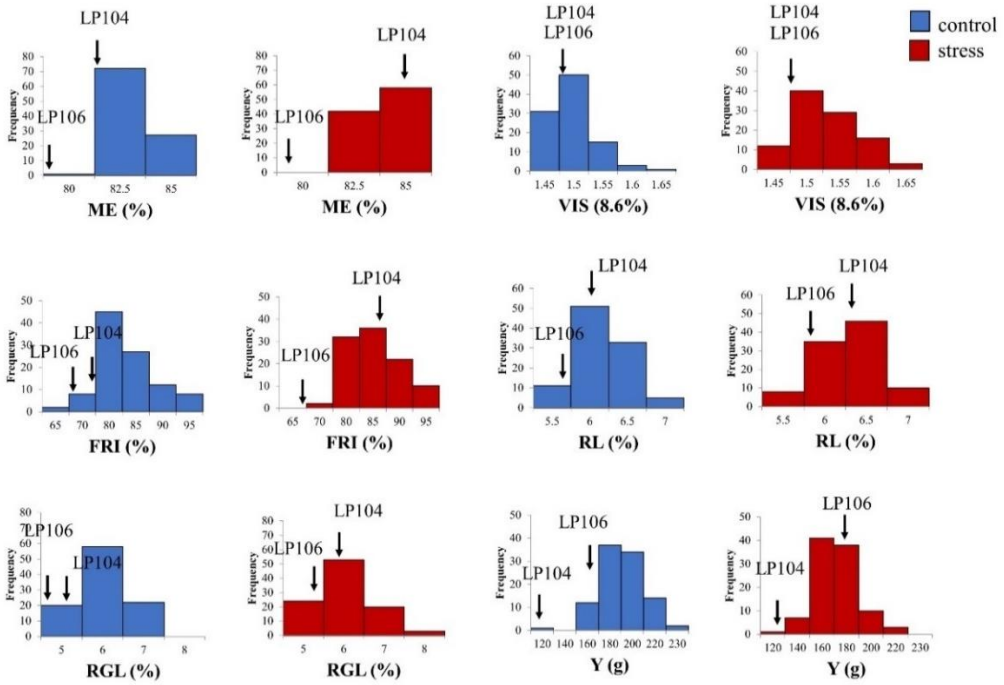


Figure 30. Frequency distribution of malt extract (ME), viscosity (VIS), friability (FRI), respiration loss (RL), root growth loss (RGL) at micromalting time point and yield data (Y) of 98 DH lines derived from LP104 x LP106. The blue histograms show control, the red ones show stress condition. Arrows indicate the mean values of the parental lines (n=3).

These lines also show a germination rate at 24 HAI from 17 to 24%, except for the line 11658 where it is 11%. Under control these lines have or lower yield (lines 11678, 11743, 11701, 11782) or they have worse malting quality traits (especially friability and malt extract).

Table 10. Eight traits of the selected lines because of their superior performance under stress condition regarding yield and malting quality traits.

Line	Malt extract (%)	Friability (%)	Viscosity (mPa*s)	Respiration losses (%)	Root growth losses (%)	Total losses (%)	Germination 24 HAI (%)	Yield (g)
11678	84.1	90.0	1.42	5.6	5.4	11.0	19.7	185.3
11684	83.7	82.9	1.48	5.6	5.8	11.5	24.3	187.0
11743	83.7	90.0	1.46	6.5	6.0	12.5	17.3	184.3
11665	83.4	84.6	1.51	5.8	5.1	10.9	18.7	193.7
11658	83.3	80.4	1.49	6.1	4.4	10.4	10.7	186.8
11677	83.3	92.2	1.44	6.3	5.7	12.0	18.3	180.3
11701	81.9	80.8	1.51	6.1	4.8	10.8	24.0	204.3
11780	83.2	85.0	1.53	5.8	4.7	10.5	18.0	204.3
11782	82.9	87.5	1.48	6.3	6.0	12.2	22.0	202.8

4.2 Early germination is dependent on abscisic acid content

Since the ABA is the key hormone in dormancy maintenance and inhibition of germination, selected lines (based on worst and best yield under stress) from 98 DH population were chosen and ABA was measured in dry mature seed (post-storage, post-dormancy) to see if the start of germination could be ABA-dependent. Interestingly, only the ABA present in the stressed seeds had a negative correlation with germination percentage at 24 HAI (figure 31).

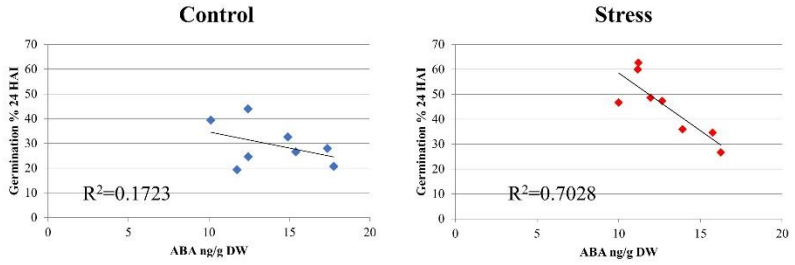


Figure 31. Graphical representation of linear regression of ABA in the dry seed (ng/g DW) on germination rate (24 HAI) of selected lines of the DH population in control (left) and stress condition (right).

ABA and its derivates were also measured in the micromalted material. The found correlation between PA and DPA with shoot emergence and nitrogen content in the malt, indicated that this hormone is of importance at the 24 HAI, and at later stages, at 72 HAI (micromalting time point, PA and DPA data in the supplementary figure 34).

The second approach in this study made use of the malting cultivar Golden Promise and derived transgenic lines XN17 and XN26 (over-expression of *AtNCED6* in endosperm) and lines LoHi236 and LoHi272 (RNAi suppression of ABA degradation enzyme ABA8'OH in embryo). Here, the greenhouse-grown plants were also subjected to drought stress 5 days after flowering, so that the seed could develop and mature under stress. The stress-grown seeds of all the lines also, like the lines LP104 and LP106, showed an enhanced germination rate at 24 HAI, as well as enhanced shoot emergence at 24 HAI and 48 HAI. Plant hormones, such as ABA, GA, CK, SA, and AUX have been measured to understand the role of these hormones in the switch from late maturation, followed by dormancy and then by germination stages. Since different tissues contribute differently to germination process, altered levels of ABA could help to shed a light on the role of ABA under stress and in the transition from grain maturation. It was described before that ABA and GA are the primary hormones that antagonistically regulate seed dormancy and germination (Gubler, Millar and Jacobsen, 2005;

Finkelstein *et al.*, 2008; Graeber *et al.*, 2012; Hoang *et al.*, 2014; Lee, Lee and Seo, 2015). The major finding here was that ABA content was lower in the stress mature dry seeds at the 0 HAI, as seen for LP104 and LP106. The lower ABA content present in the stressed seeds permits a faster start of germination, as the ABA content and germination rate at 24 HAI are negatively correlated. The lower amount of ABA in the stressed seeds is already fixed at harvest time point and it could be that the stressed seeds are in such a way prepared earlier for germination, meaning the drought stress could influence the duration of dormancy. Seeds coming from watered and drought stress development and maturation of the XN lines have an accumulation of ABA during dormancy period due to the transgene activity. In this way, the control seeds reach the highest amount of ABA and as a result, they are the slowest lines to germinate, which suggest the importance of ABA in the endosperm in this process. LoHi lines are the fastest to germinate (figure 15), correlating with the lowest ABA content (figure 16), could be because of the ABA conjugation as is shown from ABA-GE levels, which are the highest among lines. This faster germination phenotype coupled with the lower ABA content in the dry stressed seeds could be a result of transgenerational priming since the maternally derived mRNAs and proteins play a significant role in the regulation of seed dormancy and germination (Rajjou *et al.*, 2004; Donohue, 2009) and it could also be a result of enhanced degradation as seen in the figures 14 and 16. The authors suggest that hat translation of maternally derived mRNAs may facilitate adaptive growth responses for seeds germinating under stressful conditions (Rajjou *et al.*, 2004). Maternal environmental stress can also alter seed hormone content and embryonic sensitivity to hormones, as seen for ABA (Benech Arnold, Fenner and Edwards, 1991; Agarwal and Jha, 2010).

Further hormones were analyzed, such as gibberellic acid. GA3 was the only active form of gibberellic acid present at all time points analyzed, showing slight differences between time points. ABA:GA defined a pattern from 24 DAF to 72 HAI, and under control and stress the ratio between these two hormones has a different aspect. Under control at 24 HAI the ABA:GA drops to minimum levels at harvest in the lines LoHi236 and LoHi272, while the wild-type keeps a high ratio until 12 HAI. The lines XN17 and XN26 had an increase of ABA:GA, due to ABA increase during dormancy. Under stress, the decrease in ABA:GA occurs earlier respect to control condition in all the lines**.** Therefore, it might not be the hormone per se, but rather the hormone ratio that defines the germination behavior of the seed, as suggested by (William E. Finch-Savage and Leubner-Metzger, 2006). In figure 32, 5 hormonal profiling of active forms of hormones are shown in Golden Promise for control and

stressed seeds. Unfortunately, similar studies involving a complete panel of hormone measurement in drought-stressed seeds in the switch from maturation to germination have not been performed in barley or other plant species yet. Since it was shown that stressed seeds show a faster germination, in addition to ABA and GA, IAA, CK and SA were measured to get an insight into the hormonal profile of barley seeds during maturation, dormancy and germination processes. It has been reported that exogenous IAA application suppresses seed germination under high salinity (Park *et al.*, 2011), and it can also delay seed germination and inhibit pre-harvest sprouting in wheat (Ramaih, Guedira and Paulsen, 2003). One study suggested that after-ripening treatment-mediated dormancy release is associated with decreased seed sensitivity to auxin (Liu *et al.*, 2013). Emerging genetic data show that auxin protects and strictly regulates seed dormancy alongside ABA (Liu *et al.*, 2013). Evidence for this conclusion is provided by the dormancy variation among seeds with altered auxin synthesis genes. The highest auxin peak in content occurs at 24 DAF, which is a late seed maturation stage, and it could be an evidence that auxin may play a role regulating seed dormancy and germination in barley. After 24 DAF auxin content has a sharp decrease and it is always kept at basal levels, showing no other peaks. SA is a plant hormone mainly associated with various defense pathways and its role in seed dormancy and germination is not defined. In the control seeds of Golden Promise, there is a peak of SA at 24 HAI, suggesting that it might have an inhibitory role and be the reason why control seeds have a delay in root/shoot protrusion. This is further confirmed because the XN lines have a prolonged peak of SA, at 12 or 24 HAI under control. Up to now, it is known that SA can inhibit germination by inhibiting the expression of GA-induced α-amylase genes under normal growth conditions (Xie *et al.*, 2007). But under high salinity, it can promote germination via a pathway that reduces oxidative damage (Lee and Park, 2010). CKs promote seed germination by antagonizing ABA, specifically by down-regulating *ABI5* transcription (Wang *et al.*, 2011). These observations highlight the importance of *ABI5* at both mRNA and protein levels, and ABI5 is the pivot involved in CK–ABA crosstalk. It is noteworthy that although CKs have positive effects on germination, CK-receptor mutants exhibit lower dormancy levels compared with wild-type seeds (Riefler, 2006). Similarly to GA3, there is a switch of CK content that occurs from 12 HAI, where stressed seeds having an increased content of CKs. Only the cis-zeatin form could be detected at all time points. The role of CK is promotional and could play a role in shoot emergence rather than in germination in *sensu stricto* (Surdonja *et al.*, 2017). The role of CK during germination has been explored studying the promoter *HvCKX2.1* (a key cytokinin degradation enzyme), where the stressed seeds of Golden Promise showed an

increased level of DNA methylation at the *HvCKX2.1* mediated by the presence of stress-specific heterochromatic small RNA. In this way, the suppression of *HvCKX2.1* resulted in higher levels of bioactive cytokinins in stressed seeds which correlated with a faster shoot emergence behavior of stressed seeds (Surdonja *et al.*, 2017).

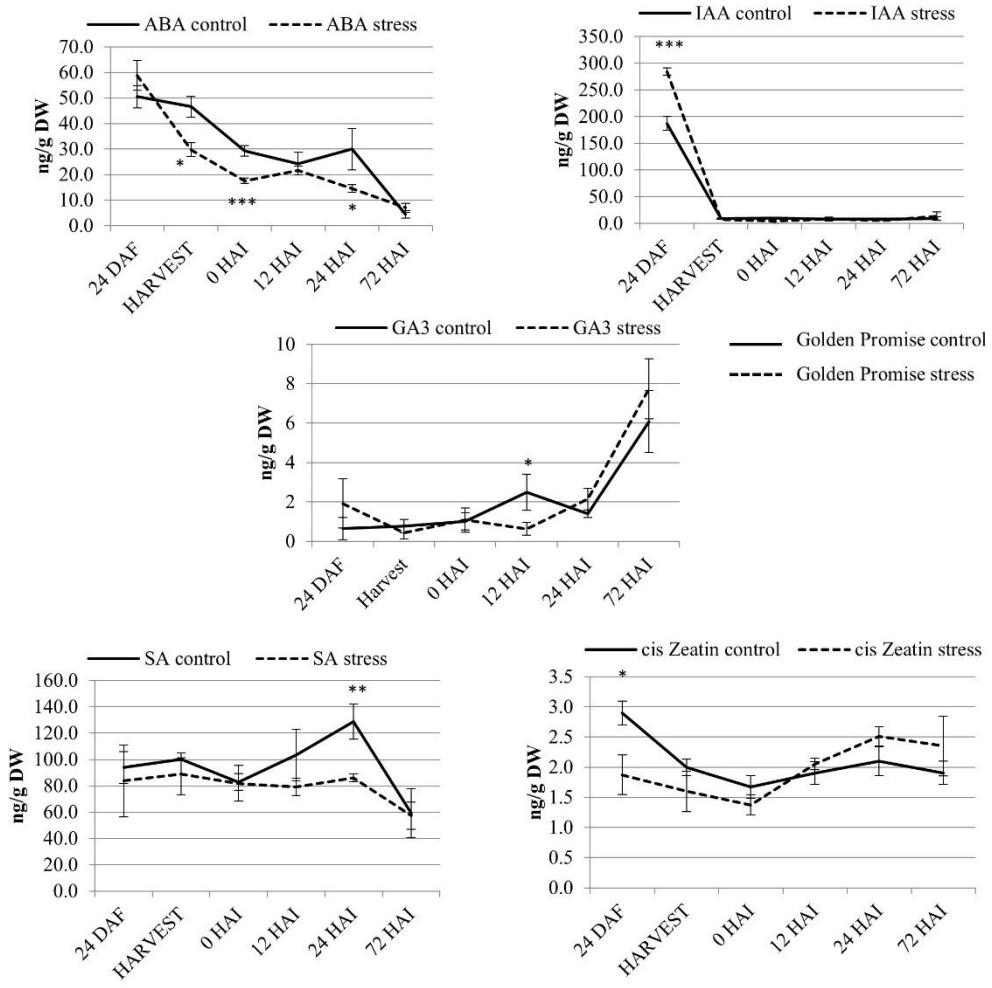


Figure 32. Phytohormone contents of abscisic acid (ABA), auxin (IAA), gibberellic acid (GA3), cytokinin (cis Zeatin) and salicylic acid (SA) in Golden Promise in control (solid line line) and stressed seeds (dashed line). Hormones were measured by LC-MS/MS (n=4) at six time points: 24 DAF, harvest, 0 HAI, 12 HAI, 24 HAI and 72 HAI. Significant differences indicated by asterisks, P<0.05:*, P<0.01:**, P<0.001:***

4.3 Confirmation of the found germination quantitative trait loci

In the present study, the estimated QTLs for germination and shoot emergence traits of the DH population was found controlled by multiple genes at different loci. QTL "hot spots" for the given traits are observed on the chromosome 3, two QTLs on the chromosome 7, and a stress "specific" one on the chromosome 2. This result supports the findings that QTLs associated with germination and shoot emergence traits are controlled by different genetic mechanisms in

barley, as mapped in other studies based on the alignment of linkage maps in Morex/Steptoe cross. Using the Steptoe/Morex doubled haploid (DH) population, (Romagosa *et al.*, 1999) reported two major QTLs on chromosome 7, one near the centromere, and one in the telomeric region of the long arm. These two QTLs were named SD1 and SD2 (Han *et al.*, 1996) and based on alignment of linkage maps, appear to be coincident with QTLs mapped in other studies (Mano and Takeda, 1997; Romagosa *et al.*, 1999; Edney and Mather, 2004; Prada *et al.*, 2004; Sato *et al.*, 2009). QTL found on chromosome 2, which was only detected in the stress condition (germination and shoot emergence of seeds which were grown under drought stress), was also detected in the study of Mano and Takeda (1997). The detected QTL by Takeda (1996) was associated with abiotic stress, with ABA response treatment at germination and seedling stage, but different since it was applied during the germination testing. The third "hot spot" on the chromosome 3 appears to be coincident with QTLs found in studies by Takeda (1996) and Prada (2004). Furthermore, these found QTLs co-localized with some of the malting and seed quality traits, such as friability and malt extract (telomeric region chromosome 7), seed starch content and malt respiration losses on the centromeric region of chromosome 7. The co-localization detected on the chromosome 3 is with most of the malting quality traits, such as Kolbach Index, malt total losses, friability, soluble nitrogen content, viscosity and beta-glucan content. On the chromosome 2, these QTLs co-localize with malt protein content, steeping degree (48 HAI) and seed starch/seed content. This QTLs co-localization further confirms the relation between these traits, as seen in the correlation tests. The putative candidate genes under the QTL peaks were searched among differentially abundant genes between the parents, and their possible role at imbibition in different tissues and during the micromalting process.

4.4 Transcriptome analysis

At the dry seed stage, there are thousands of significantly different numbers of genes that have been found comparing control and stress conditions of the single lines. The differences between control and stress disappear at micromalting time point (explained below). At the dry seed stage, across six genotypes tested (LP104, LP106, Golden Promise, XN17, XN26, and LoHi236) there are only 24 mutually differentially expressed genes. This could indicate that the drought stress response at the dry seed stage is mostly line-specific. Among the 24 differentially expressed genes there are no visible patterns that could separate one genotype from the other. They were tested to see if there was any correlation with germination or malting quality parameters, what resulted in three genes having a correlation with one or

several traits under both conditions. 35_7780 has a negative correlation with viscosity under both conditions. Additionally, only under stress, positive correlation with malt modification indicators such as malt extract, Kolbach index, final attenuation and friability are found. 35_7780 encodes a GATA zinc finger transcription factor. This transcription factor has been defined as a positive regulator of seed dormancy and a critical player during germination in Arabidopsis (Liu *et al.*, 2005). The GATA zinc finger protein was identified as another member of *sensu stricto* germination-associated genes and the expression of the GA-biosynthesis genes *GA20ox3* and *GA3ox1* was downregulated in the knockout seeds (Liu *et al.*, 2005). It is found up-regulated in all stress treated seeds, in all the lines. Interestingly, it is located at 2H chromosome which had a QTL for germination only under stress. 35_13213 encodes an ABC type CO2 transporter and correlates positively with germination, shoot emergence at 72 HAI and respiration losses under both conditions, and has a low but significant correlation with friability (positive) and viscosity (negative). These transporters have been linked to cellular uptake of ABA (Kang *et al.*, 2010). In the transgenics, the expression values are correlating with ABA content in the dry seed and germination rate at 24 HAI. 35_9897, a putative chalcone synthase, correlates negatively with root growth losses and shoot emergence under stress and shoot emergence and germination under control. Although the found differences between these genes are found at seed maturity they inevitably show a significant interaction with described parameters at the later stage (72 HAI), indicating that their expression in the dry seed might have an influence on them.

4.4.1 Identified candidate genes contributing to malting quality

At micromalting time point, which corresponds to 72 hours of germination, there are no significant differences between control and stress, but only between the lines LP104 and LP106. GO analysis resulted in showing a clear difference in the enriched different biological processes of these two lines. The up-regulated genes in LP104 are enriched in processes small molecules biosynthesis, organic acid biosynthesis, and photosynthesis. The biological processes categories which are particularly enriched for down-regulated genes of LP104 are regulation of post-embryonic development, regulation of shoot system development, reactive oxygen species metabolic processes, regulation of developmental processes. The differentially abundant transcripts between the parents, found in the germination QTL hotspots, were used for the Pearson correlation tests and revealed that there was no control condition specific correlations between the expression of these genes under control and some malting quality trait. On the other hand, there was plenty stress specific interactions. The strength of

correlation varied greatly for a given gene with a specific phenotypic trait under two different conditions. The results also revealed that certain parameters are interrelated, such as malt extract, friability, and malt losses. Viscosity, beta-glucan content and malt protein content often have a correlation with the same genes. The found genes which encode hydrolases have a negative correlation with viscosity under control condition, and in turn, under stress, this correlation is not found or is low. Often a malt loss is found to be positively correlating with some of the hydrolases under both conditions or under stress. An alpha/ beta hydrolase (35_3559) in both conditions has a negative correlation with wort viscosity and positive correlations with total and respiration losses. In the dry seed stage in XN lines has the lowest expression. At 12 HAI it is found only in embryo. An esterase (acyl-protein thioesterase, 35_18670), involved in degradation of fatty acids, is also found negatively correlated with viscosity under control, but under stress is positively correlated with steeping degree 2 (48 HAI) and shoot emergence at 48 HAI. Matrix metalloproteinase (Contig13600_at), a zinc-dependent endopeptidase, is again under control negatively correlated with viscosity, while under stress is positively correlated with C/N in the dry seed and with malt extract. 35_1915 encodes a phosphomannomutase, which plays a role in mannose metabolism, important for endomembrane system, correlates negatively with seed length under control, and under stress, it is positively correlated with steeping degree (48 HAI). It has the highest values in LP104. A sulfotransferase (Contig12910_at) has a negative correlation with shoot emergence at 48 HAI under control, but a positive correlation with shoot emergence at 72 HAI under stress. Sulfotransferase has not been fully studied in plants up to now, but they were linked to sulfate low-molecular-weight substrates such as flavonoids, coumarins, and phytohormones such as brassinosteroids, salicylic acid, and jasmonates (Hirschmann, Krause and Papenbrock, 2014). HS08H22u_s_at positively correlates with viscosity under control, under stress is negatively related to steeping degree (48 HAI) and shoot emergence at 48 HAI. It encodes an Acyl-CoA-binding domain-containing protein 1, which functions as a carrier of Acyl-Ca esters but also plays a role in fatty acid biosynthesis (Hsiao *et al.*, 2014). It is found to be highly expressed in LP106 in both conditions. Various types of transporters have also been found to have an interrelation with malting quality traits and germination, generally affecting negatively malt modification parameters (Kolbach index, friability). An ATPase ABC transporter (35_8108) correlates negatively with Kolbach Index and friability and positively with beta-glucan in both conditions. The highest expression value is found in LP106. At 12 HAI, it is expressed in embryo and in endosperm only in XN lines. Some of the ABC transporters have been proposed as transporters of ABA from endosperm to embryo(Kang *et al.*, 2010). Contig24832,

a chloroplastic Phosphoenolpyruvate/phosphate translocator 3, is positively correlated with viscosity and beta glucan, negatively with friability in both conditions. In the dry seed and imbibition there are no differences between the lines and conditions. However, it is found highly expressed in the line LP106 during micromalting. EBem05_SQ004_I01_at encodes mitochondrial electron transport / ATP synthesis. In both conditions negatively correlates with malt extract and friability. The expression of this gene is up-regulated in the XN lines in the dry seed. Hormone-related genes found to correlate with malting quality or germination are *HvABI5*, *HvCKX* 1 and *HvCKX6*. HvABI5 negatively correlates with steeping degree (72 HAI), but under stress only C% in the dry seed correlates with it. The highest expression of HvABI5 is found in the dry seed of LoHi236 in both conditions. The two CK oxidases (*HvCKX1, HvCKX6),* involved cytokinin metabolism have a positive correlation with seed quality traits such as area, length, N% and P%, and viscosity (only under stress), and a negative correlation with friability in both conditions. This could be a further evidence that CKs have a promotional role during seed germination and shoot emergence. 35_4343 encodes a ring finger E3 ligase gene. It is positively correlated with seed length, seed area, and negatively with malt extracts at 24, 48 and 72 HAI, and shoot emergence at 48 HAI. Under stress it negatively correlates only with malt extract at 48 HAI. At the dry seed stage there are no differences between genotypes and conditions, but it is found up-regulated in the XN lines and specifically in the endosperm. In embryo is down-regulated but up-regulated only in the XN lines. At micromalting time point 72 hai it is up in LP104 under stress. The overexpression of *E3 ligase* gene in rice was liked to an enhanced germination (Lee and Park, 2010). 35_4384 negatively correlates with viscosity and positively with respiration losses under control condition. Under stress it is positively related to friability and negatively to moisture content in malt. It is a putative autophagy-related protein. In Arabidopsis, autophagy-related proteins are required for degradation of peroxisomes in hypocotyls during seedling growth. Peroxisomes are required for the transition from heterotrophic to autotrophic stage in the seedlings, as they provide fixed carbons before onset of photosynthesis (Kim *et al.*, 2013). It is found highly expressed in LP104, which is a faster shoot emergence line respect to LP106. There are two MYB transcription factors (Contig16528 and Contig14119) correlating negatively with malt extract under both conditions. Contig16528 additionally correlates negatively with respiration and total losses. They are found to be highly expressed in LP106 and in XN lines, only in the dry seed. The lowest expression value of these gene was found in LoHi236 in both conditions. The third MYB transcription factor (35_9865) shows the opposite behavior respect to the latter two and negatively affects viscosity only under control and

respiration losses only under stress. The expression values are high in LP104, LoHi236 and GP (respect to XNs). A WRKY domain transcription factor (Contig7517) is negatively correlated with germination but positively with shoot emergence at 48 HAI and soluble nitrogen content in malt under control, under stress it is positively related to steeping degree at 48 HAI. It is expressed specifically in endosperm where it has the highest expression value in LoHi236 line (both conditions) during the imbibition phase and has highest expression values in stressed LP104 and LP106 lines. Other genes with no annotation or that are found only under stress can be found in the supplementary table 20 and in the figure 33. This results showed that there are dozens of possible candidate genes (taken from a QTL hotspot) that influence mostly friability, malt extract, viscosity and malt losses. Many of the found relations are only present under stress (supplementary table 18 and 19). The expression values of these genes were inspected in transgenic lines which had a different level of ABA in the dry seed and during imbibition. This genes are shown in the figure 33.

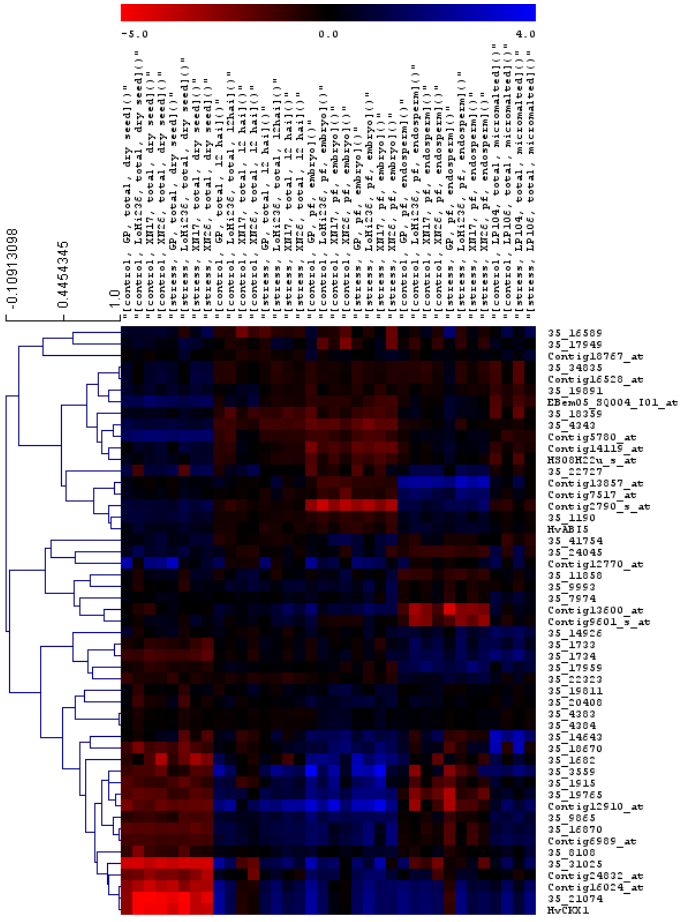


Figure 33. Hierarchical clustering of 52 transcripts that correlate with malting quality. The genes encoding these transcripts were identified from QTL "hot spots" regions associated with malting quality and germination rate. The clustering was made using MeV tool (TIGR, TM4) showing the mean normalized expression value of genes. Low expression values are shown in green, while high expression values are shown in red. Correlation of transcripts of these genes with malting quality traits can be found in the supplementary table 18 and 19. Gene names and annotation is in supplementary table 20.

The final level of dormancy is determined by the contributions of embryo and endosperm (aleurone layer) (Debeaujon, Léon-Kloosterziel and Koornneef, 2000). The results show that endosperm and embryo both are equally important players during imbibition and are harboring different candidate genes that could later affect malting quality. The embryo-specific ones are involved in transport, lipid metabolism, glycolysis, and jasmonate synthesis, while the endosperm-specific ones are related to auxin synthesis, receptor kinases and redox reactions. These results indicated also that certain candidate genes are already present in the dry seeds, like cytokinin oxidase and metabolite transporter (stored messages), while others appear during imbibition, or even later (newly synthesized genes).

4.5 Conclusion

From this work the following points can be concluded:

- Resulting interactions between germination and malting traits found under control condition suggest that it is an important agronomical trait which could be useful as a malting potential tool.
- Terminal drought stress has an impact on germination ability by accelerating it, as well as shoot emergence. The enhanced germination rates (at least 30%) in stressed material gave as consequence specific correlations with malt extract and malt losses, confirming the enhanced modification of malt due to stress and faster germination.
- Terminal drought stress significantly reduced ABA content in the dry seed by at least 20%.
- ABA to GA ratio has a sharper decrease in the stressed material. However, the ratio of 10 or lower is present in the fastest germinating lines (LoHi), and a ratio of 40 is present in the slowest lines (XNs) at the dry seed stage.
- The functional classification of the most abundant transcripts in the dry seed stage reveals transcripts associated with stress defense, translation, and storage proteins.
- The line XN17, which had the highest ABA content under control and stress at the dry seed stage, had the highest number of differentially expressed genes under stress. Differences across genotypes prevail and at micromalting, there are no differences between control and stress at the transcriptome level. During imbibition, XN lines are characterized by a low abundance of transcripts involved in modification processes like storage compounds degradation and cell wall modification. On the other side, XN lines have a higher abundance of seed maturation related transcripts.

- Polysome isolation technique was useful to detect long living germination essential transcripts and newly germination transcripts are synthesized and identified both in embryo and endosperm.
- Differentially abundant genes at a micromalting time point were tested for correlation with malting quality traits and germination and their abundance in embryo and endosperm at imbibition which resulted in 52 possible germination/malting candidate genes.

5 Summary

Drought is one of the major abiotic stresses that limits yield and impairs grain quality. Cereal grain germination is central to plant early development, and efficient germination has a key role in crop propagation and malting. To understand terminal drought stress effects on germination performance and malting, two different approaches have been implemented in this work. In the first approach, two malting elite varieties with distinct drought tolerance mechanisms and their double haploid population were tested for germination and shoot emergence rates. Grains that had developed on the mother plant under drought condition showed a significant increase in germination and shoot emergence rate, respect to the grains developed under watered condition. Relations of germination/shoot emergence rate with malting traits were identified: negative with viscosity and beta-glucan content, positive with friability and malt extract. These relations were also confirmed by QTL analysis, were germination QTL loci overlapped with these traits. ABA is a key abiotic stress and dormancy inducer/germination inhibitor hormone. In mature stressed grains ABA has a significant negative correlation with germination rate at 24 hours after imbibition. At micromalting time point, ABA content correlates negatively with shoot emergence in both conditions. There were no correlations between yield/TGW and germination or malting quality traits, however, in this work, it was possible to identify 8 superior performing lines with superior malting quality traits and stable yield under stress. On the transcriptome level, differences between the genotypes were bigger than between conditions.

The second approach in this work is based on the study of the role of ABA in the transition from seed maturation to germination using ABA manipulated transgenic lines. After terminal drought stress treatment reduced ABA content was found in the transgenic lines and wild-type at maturity. As consequence, stress treated lines were faster to germinate. Transcriptome analysis enabled to identify germination essential transcripts involved in photosynthesis, lipid metabolism, glycolysis, hormone synthesis, cell wall growth and elongation, stress response, and to demonstrate that these genes are differentially regulated within stress condition, and between the lines. Furthermore, embryo and endosperm (aleurone layer) were confirmed as important distinct messenger RNA storage tissues, where the accumulation of different mRNA species differs respect to the lines and conditions. Combining transcriptomic data with QTL mapping, candidate genes for germination and malting are proposed.

6 Zusammenfassung

Trockenheit gehört zu den wichtigsten abiotischen Stressfaktoren, die den Ertrag und die Kornqualität in der Gerste mindern können. Die Kornkeimung ist der zentrale Vorgang in der frühen Pflanzenentwicklung. Der effizienten Keimung fällt eine Schlüsselrolle zu, sowohl bei der Vermehrung, aber auch bei der Veredlung der Körner zu Malz. Ziel der vorliegenden Arbeit ist es die Vorgänge zu verstehen, die durch Trockenstress in der letzten Pflanzenentwicklungsphase ausgelöst werden und Einfluss auf die Keimvorgänge und somit auch auf den Vermälzungsvorgang haben. Hierfür wurden zwei unterschiedliche Versuchsansätze durchgeführt: Im ersten Ansatz wurden zwei Gerstenmalz-Elitesorten verwendet, die unterschiedliche Strategien zur Trockenstresstoleranz entwickelt haben. Die Keimung einer Doppelt-Haploid-Population dieser beiden Eltern wurde unter Kontroll- und Trockenstressbedingungen getestet. Die Körner die sich an einem Mutterpflanze unter Trockenstressbedingungen entwickelt hatten, zeigten eine signifikant schnellere Keimung und eine schnellere Sprossentwicklung. Für die Vermälzungseigenschaften wurden Proben zu unterschiedliche Zeitpunkten während der Vermälzung charakterisiert. Korrelationsanalysen zeigten einen möglichen negativen Zusammenhang zwischen den Vermälzungseigenschaften Viskosität und Beta-Glucan-Gehalt, sowie einen positiven Zusammenhang zwischen den Eigenschaften Friabilität und Malzextrakt. Diese Korrelationen spiegelten sich auch in der QTL Anlayse wieder, in der überlappende Loci für die Keimung und die genannten Eigenschaften identifiziert werden konnten. Dem Pflanzenhormon Abscisinsäure (ABA) kommt eine Schlüsselrolle in der Signalverarbeitung von abiotischem Stress und während der Entwicklung zu: es induziert die Dormanz und inhibiert die Keimung. In reifen, während der Reifung trocken-gestressten Körners besitzt ABA eine signifikante negative Korrelation mit der Keimrate. Zum untersuchten Vermälzungszeitpunkt konnte ein negativer Zusammenhang zwischen ABA Gehalt und der Sprosserscheinungsrate unter beiden untersuchten Bedingungen gefunden werden. Aus der analysierten Population konnten acht Linien mit herausragenden Vermälzungseigenschaften unter Trockenstress identifiziert werden. Die Transkriptomanalyse ergab einen größeren Unterschied zwischen den Genotypen als zwischen den untersuchten Bedingungen.

Der zweite Versuchsansatz untersuche die Rolle von ABA in der Phase von der Samenreifung bis zur Keimung unter Verwendung transgen modulierter Gerstenlinien mit verändertem ABA Gehalt. In den transgene Linien konnte nach terminalem Trockenstress ein verstärkter ABA Abbau nachgewiesen werde. Als Konsequenz und in Übereinstimmung mit den oben

beschriebenen Ergebnissen, zeigten die transgenen Linien eine schnellere Keimung. Die Microarray-basierte Transkriptomanalyse erlaubte die Identifikation von wichtigen transkriptionellen Regulons wie Photosynthese, Lipidmetabolismus, Glycolyse, Hormonsynthesewege, Zellwandwachstum und Zellelongation. Diese identifizierten Regulons wurden differentiell zwischen den analysierten Bedingungen reguliert oder genotypspezifisch, zwischen den untersuchten Linien. Es konnte weiterhin gezeigt werden, das im Embryo und auch im Endosperm (Aleuronschicht) die Akkumulation und Einlagerung von wichtigen Transkripten für die Keimung erfolgt. Die Verknüpfung der QTL mapping Analyse und der Transkriptdaten führte zur Identifikation von Genen mit Relevanz für die Keimung und Vermälzungsqualität.

7 Reference

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8 Supplementary figures and tables

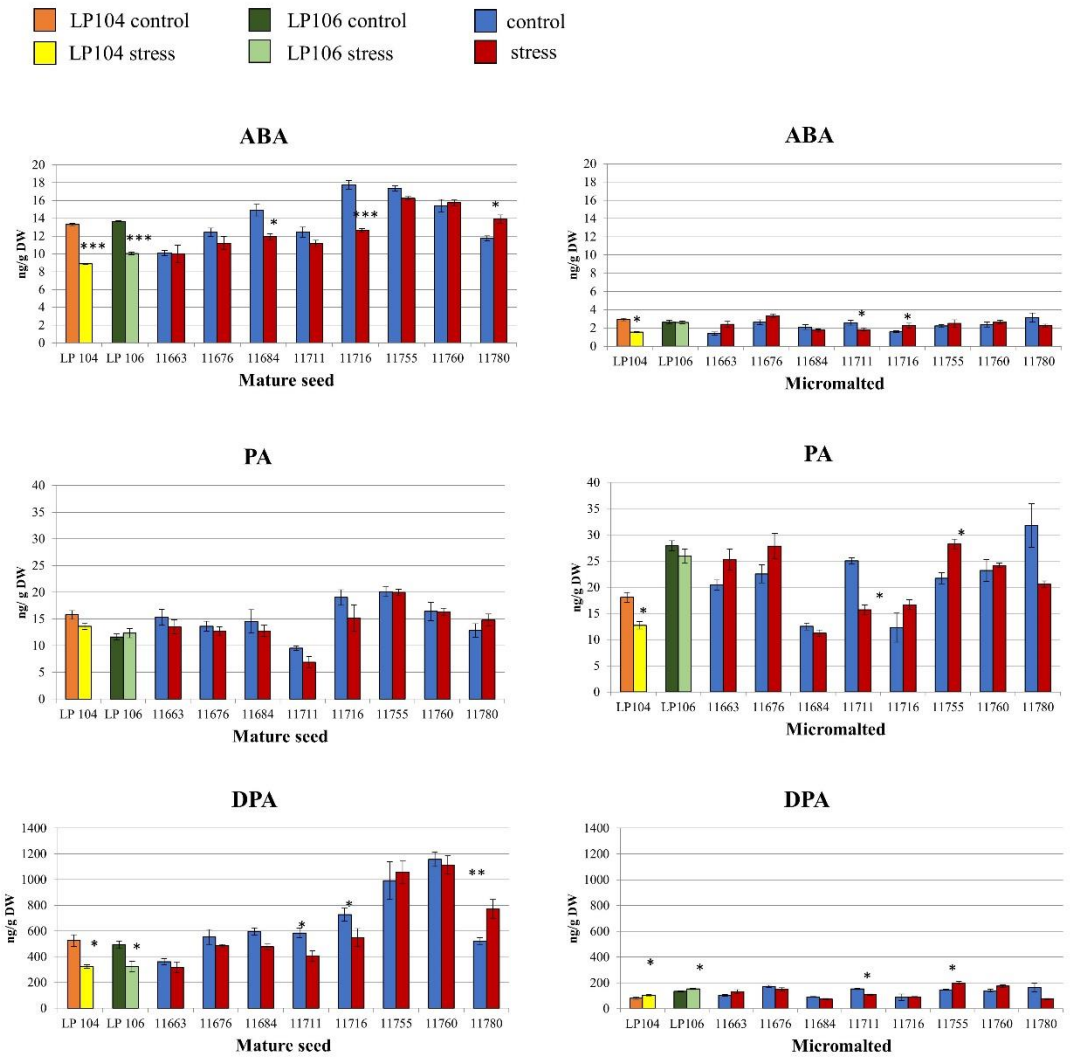

Figure 34. ABA (abscisic acid), PA (phaseic acid), and DPA (dihydrophaseic acid) contents in mature seed and micromalted time points measured in LP104, LP106 and selected lines (blue:control, red: stress). Each sample represented as mean value ± SD. Significance between condition indicated with asterisks, P<0.05:*, P<0.01:**, P<0.001:***. ABA and its derivated were assessed with LC-MS/MS (n=4).

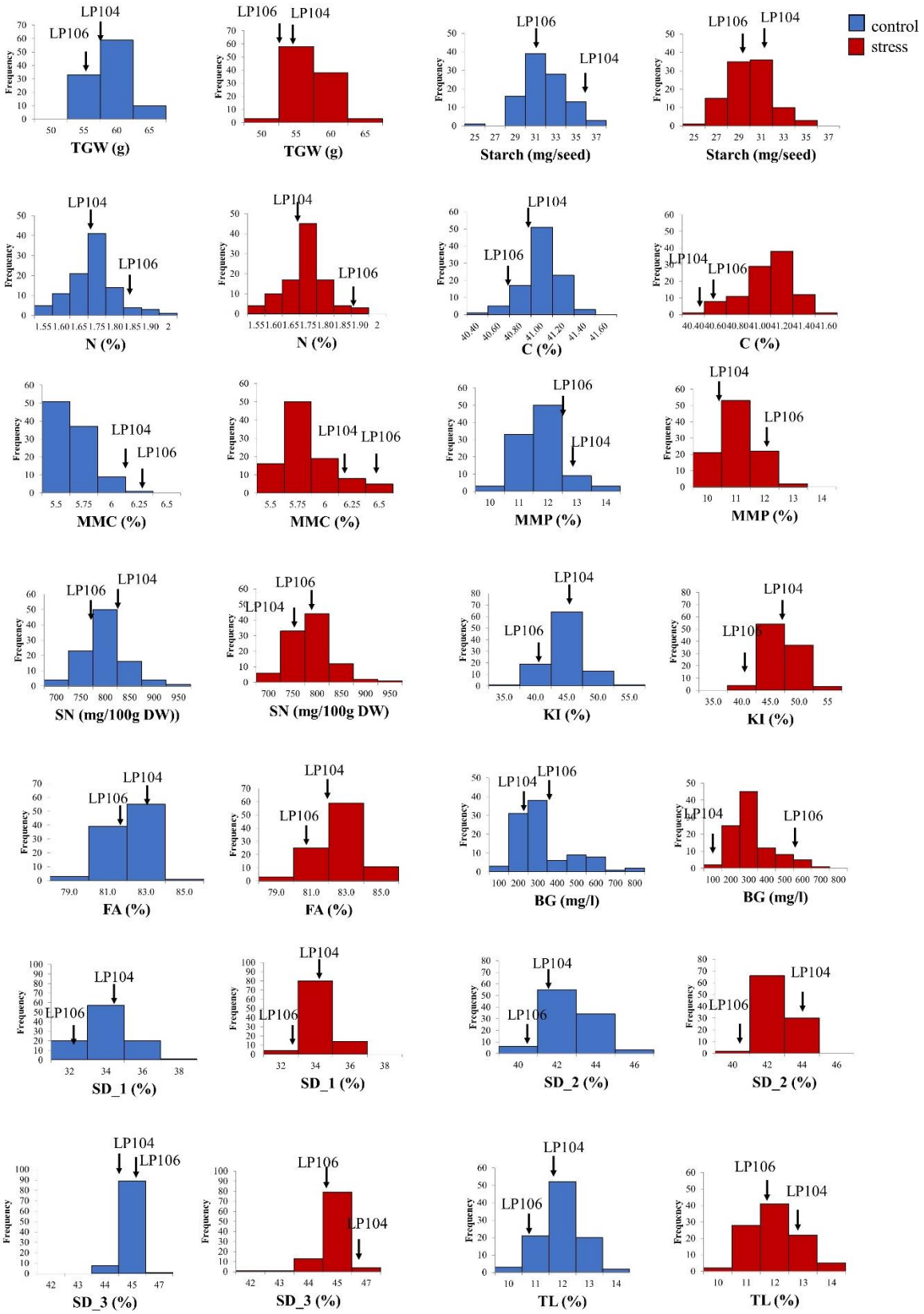

Figure 35. Frequency distributions of TGW, seed quality traits: N (nitrogen) and C (carbon), starch; and malting quality traits: malt moisture content (MMC), malt protein content (MMP), soluble nitrogen (SN), Kolbach index (KI), final attenuation (FA), beta-glucan (BG); steeping degree (SD_1,2,3) and total losses (TL). Arrows indicate the mean values for LP104 and LP106 (n=3). Blue: control condition, red: stress condition.

Table 11. Differentially expressed genes in the dry seed which deeveloped and matured in the drought stress condition. The list of genes is a result of a t-test (BHB adjusted P, FC>5).

FC ([stress-LP104-total-mature] vs [control-LP104-total-mature])	Harvest assembly ID	Annotation	FC ([stress-LP106-total-mature] vs [control-LP106-total-mature])	Harvest assembly ID	Annotation
10.770663	35_22174	NA	14.371429	35_10101	NA
-205.47531	35_45711	NA	-22.077066	35_42998	NA
-103.44735	35_38331	NA	-15.023499	HV12O24u_at	hormone metabolism.jasmonate.synthesis-degradation.12-Oxo-PDA-reductase
-97.71298	35_42664	NA	-14.239591	35_24198	glycolysis.G6PIsomerase
-44.782978	35_14315	NA	10.982305	35_25337	NA
-38.228977	35_14321	NA	8.983368	35_33885	NA
-28.418428	35_36897	NA	6.5974426	35_2258	NA
-25.503057	35_2898	NA	6.4970164	35_13338	amino acid metabolism.synthesis.branched chain group.common
-24.615314	35_29596	NA	-6.391897	35_16090	protein.degradation.cysteine protease
-23.162806	35_36357	NA	6.362739	35_38686	NA
-22.581558	35_30107	NA	-6.156848	35_16089	protein.degradation.cysteine protease
-18.214783	35_9233	NA	6.110908	35_612	lipid metabolism.lipid transfer proteins etc
-16.004347	35_42647	NA	-6.0037994	35_8597	NA
-15.959337	35_2895	NA	5.826586	35_26696	NA
-13.213269	35_39306	NA	5.7378006	35_45472	NA
-11.463409	35_42153	NA	5.636229	35_37586	NA
-10.752365	35_50082	NA	5.39691	35_22305	NA
-10.62707	35_2560	NA	5.3062816	35_15456	stress.abiotic.heat
-10.356257	35_39310	NA	5.263403	Contig2043_s_at	lipid metabolism.lipid transfer proteins etc
10.072424	35_23366	NA	5.1813574	35_613	NA
9.891101	35_19386	NA	5.129591	Contig23909_at	transport.peptides and oligopeptides
-9.77324	35_27984	NA	-5.092946	HT01G04w_at	lipid metabolism.FA synthesis and FA elongation.acyl coa ligase
-9.659113	35_26238	NA			
-9.235127	35_39369	NA			
-8.674911	35_42998	NA			
-8.44996	35_37735	NA			
-8.347745	35_42875	NA			
8.287907	35_12306	NA			
8.2769375	35_34050	NA			
8.186904	35_4800	NA			
-8.089769	35_12943	NA			
-7.9291863	35_2896	NA			
-7.6779165	35_39271	NA			
7.3733287	35_26383	signalling.receptor kinases.DUF 26			
-7.2790804	35_7913	secondary metabolism.flavonoids.d			

		ihydroflavonols
-7.2539234	35_46461	NA
-6.706096	35_43155	NA
-6.5713005	35_42585	protein.degradation.metalloprotease
-6.5365677	35_14323	NA
6.4601703	35_19871	NA
-6.4518313	35_5231	NA
-6.41734	35_37818	NA
-6.3629713	35_50340	nucleotide metabolism.deoxynucleotide metabolism.cytosine deaminase
6.357944	35_25337	NA
6.1860266	35_22168	NA
-6.137404	35_28721	RNA.processing.RNA helicase
-6.093559	35_13551	NA
-5.949637	35_50859	signalling.calcium
5.8968244	35_21610	NA
5.77399	35_36073	NA
-5.727581	35_10362	fermentation.ADH
5.719722	35_22305	NA
-5.6638103	35_27805	protein.folding
-5.562242	35_41061	NA
5.2862897	35_36839	hormone metabolism.ethylene.synthesis-degradation
5.223338	35_22219	NA
5.207748	35_27221	hormone metabolism.cytokinin.signal transduction
-5.1583853	35_50119	NA
-5.1401763	HT06N06u_at	lipid metabolism.lipid degradation.beta-oxidation.acyl CoA DH
-5.09545	35_40975	NA
-5.0843205	35_42359	PS.lightreaction.ATP synthase
-5.059922	35_25951	NA
-5.0457335	35_45047	NA

Table 12. Differentially abundant genes at dry seed stage and micromalting between LP104 and LP106 in stressed material. The list of transcript is a result of the ANOVA (BHB adjusted P, FC>5).

FC ([stress-LP104-total-mature] vs [stress-LP106-total-mature])	Harvest assembly ID	Annotation	FC ([stress-LP104-total-micromalted] vs [stress-LP106-total-micromalted])	Harvest assembly ID	Annotation
13884.068	35_14859	stress.abiotic.heat	-1435.1892	Contig3140_at	lipid metabolism.lipid degradation.beta-oxidation.acyl CoA DH
8617.789	35_14861	stress.abiotic.heat	-1182.9081	35_813	NA
8584.283	Contig2006_s_at	stress.abiotic.heat	-1159.7084	35_13988	DNA.synthesis/chromatin structure.histone
-4645.726	35_813	NA	-923.469	35_14705	amino acid metabolism.synthesis.serine-glycine-cysteine group.cysteine.OASTL
-4593.4043	35_2790	NA	-874.07434	35_14512	misc.acid and other phosphatases
-2589.7554	35_18580	NA	-863.7077	35_4279	misc.short chain dehydrogenase/reductase (SDR)
1763.988	35_647	development.unspecified	529.675	35_14859	stress.abiotic.heat
-831.13135	35_644	development.unspecified	-454.22067	35_18878	NA
749.50696	HV_CEb0006A14f_s_at	signalling.calcium	-445.50485	35_19965	NA
538.4003	35_37414	NA	-425.5213	35_16398	protein.degradation.ubiquitin.E2
449.2797	35_17435	NA	-386.3502	35_18802	NA
446.4128	35_22937	NA	369.0047	35_13711	protein.degradation.ubiquitin.ubiquitin
-430.7761	35_16398	protein.degradation.ubiquitin.E2	288.0247	35_24359	NA
-385.0565	35_1445	protein.degradation.ubiquitin.proteasom	-267.42618	35_3593	secondary metabolism.N misc.alkaloid-like
345.87842	Contig5555_at	amino acid metabolism.synthesis.branched chain group.leucine specific	249.06989	Contig2006_s_at	stress.abiotic.heat
334.73822	35_6370	NA	243.98882	35_2925	misc.cytochrome P450
-323.0768	35_18878	NA	-235.602	35_18906	signalling.receptor kinases.DUF26
-319.6693	35_814	NA	228.85315	35_14861	stress.abiotic.heat
-317.94797	35_630	development.unspecified	-219.30942	35_7523	NA
307.41257	Contig6268_at	signalling.calcium	219.28651	Contig7016_s_at	signalling.calcium
-288.64224	35_19965	NA	-215.21455	Contig8092_x_at	RNA.transcription
-285.77942	35_18802	NA	202.86234	35_17480	signalling.calcium
281.47253	35_5281	signalling.receptor kinases.wheat LRK10 like	199.2992	HT09B07u_s_at	signalling.calcium
263.55734	35_16335	not assigned.no ontology	195.00446	Contig6268_at	signalling.calcium
263.3423	35_9697	NA	169.7194	35_2125	protein.degradation.subtilases
-252.17392	35_42142	development.unspecified	166.79698	35_6632	NA
-248.94421	Contig8092_x_at	RNA.transcription	164.64621	35_2111	NA
239.48135	35_16091	protein.degradation.cysteine protease	162.0408	35_647	development.unspecified
-231.50505	35_4279	misc.short chain dehydrogenase/reductase (SDR)	153.9311	35_16335	not assigned.no ontology
-231.21799	35_2869	not assigned.no ontology	-152.47124	35_22291	Co-factor and vitamine metabolism.ubiquinone.hexaprenyldihydroxybenzoate methyltransferase
222.97931	35_13711	protein.degradation.ubiquitin.ubiquitin	149.38785	35_1370	development.unspecified
-211.84549	35_20248	RNA.regulation of transcription.E2F/DP	-149.17165	35_30791	NA

		transcription factor family			
-190.77098	35_13444	NA	-136.51346	35_4665	hormone metabolism.ethylene.synthesis-degradation
189.05711	35_25170	NA	-134.93483	35_6030	DNA.repair
-180.79015	Contig3140_at	lipid metabolism.lipid degradation.beta-oxidation.acyl CoA DH	-128.66747	35_30190	NA
167.5315	35_4386	NA	-125.90681	35_2382	signalling.calcium
-162.62256	35_5859	protein.degradation.ubiquitin.E3.RING	-124.60712	35_2869	not assigned.no ontology
158.32828	35_904	stress.biotic	-118.63423	35_3778	not assigned.no ontology
153.79684	Contig13693_at	signalling.receptor kinases.thaumatin like	-116.62715	35_47494	NA
148.79332	Contig13693_x_at	signalling.receptor kinases.thaumatin like	115.84404	35_17435	NA
140.23878	35_8758	NA	115.558495	35_6370	NA
-135.43741	35_36699	NA	-112.64441	35_50119	NA
129.1964	35_16090	protein.degradation.cysteine protease	-106.105865	35_18803	NA
127.28382	35_1130	major CHO metabolism.degradation.starch.starch cleavage	-104.15864	35_20791	protein.postranslational modification.kinase.receptor like cytoplasmatic kinase VII
127.12469	35_17480	signalling.calcium	-101.35385	35_3328	misc.cytochrome P450
126.24104	35_21841	cell.organisation	-101.160576	35_2790	NA
125.65546	35_6030	DNA.repair	-99.09918	35_644	development.unspecified
123.66092	Contig7016_s_at	signalling.calcium	-96.91302	Contig22848_at	signalling.receptor kinases.wheat LRK10 like
-121.40095	35_22291	Co-factor and vitamine metabolism.ubiquinone.hexaprenyldihydroxybenzoate methyltransferase	94.52906	35_25170	NA
120.57515	35_17779	NA	88.756935	35_4415	PS.lightreaction.photosystem II.PSII polypeptide subunits
120.006966	35_37610	NA	82.17812	Contig5555_at	amino acid metabolism.synthesis.branched chain group.leucine specific
-117.39786	35_13786	protein.synthesis.elongation	-80.32687	35_24566	NA
115.54906	35_15828	NA	79.8382	35_34429	NA
114.896164	35_31322	transport.peptides and oligopeptides	-77.6548	35_49488	NA
113.9421	35_15352	misc.oxidases - copper, flavone etc.	-76.73588	35_2687	protein.degradation.ubiquitin.E3.RING
113.74561	35_18670	lipid metabolism.lipid degradation.lysophospholipases.carboxylesterase	73.85121	Contig13429_at	signalling.receptor kinases.thaumatin like
-111.02536	35_3778	not assigned.no ontology	73.79468	35_2619	NA
109.63058	35_2619	NA	-72.713394	Contig13654_at	signalling.receptor kinases.crinkly like
109.12648	Contig14945_at	transport.peptides and oligopeptides	-71.014046	35_814	NA

Table 13. Gene ontology enrichment analysis using differentially expressed genes at micromalting time point between LP104 and LP106, as well as differences in abundance between total and polysome-bound RNA within lines.

GO ID	Term	Annotated	Significant	Expected	classicFisher
ANOVA_ UP_LP104 VS LP106_micromalted					
GO:0006633	fatty acid biosynthetic process	106	13	4.68	0.00074
GO:0072330	monocarboxylic acid biosynthetic process	195	25	8.61	1.30E-06
GO:0015994	chlorophyll metabolic process	87	10	3.84	0.00478
GO:1901698	response to nitrogen compound	72	9	3.18	0.00417
ANOVA_ DOWN_LP104 vs LP106_micromalted					
GO:0048580	regulation of post-embryonic development	111	12	4.52	0.0018
GO:0048831	regulation of shoot system development	101	11	4.11	0.0026
GO:0009909	regulation of flower development	94	11	3.82	0.0014
GO:0072329	monocarboxylic acid catabolic process	47	7	1.91	0.0027
GO:0006635	fatty acid beta-oxidation	40	6	1.63	0.0052
GO:0042744	hydrogen peroxide catabolic process	111	12	4.52	0.0018
GO:0071705	nitrogen compound transport	164	15	6.67	0.0027
GO:0006865	amino acid transport	59	8	2.4	0.0025
t-test UP_LP104 poly RNA vs total micromalted_control					
GO:0006366	transcription from RNA polymerase II pro...	152	22	9.57	0.0002
GO:0006406	mRNA export from nucleus	40	10	2.52	0.00014
GO:0071427	mRNA-containing ribonucleoprotein comple...	40	10	2.52	0.00014
GO:0051028	mRNA transport	43	10	2.71	0.00026
GO:0043412	macromolecule modification	1062	94	66.84	0.00026
GO:0009070	serine family amino acid biosynthetic pr...	44	10	2.77	0.00032
GO:0009630	gravitropism	41	11	2.58	3.10E-05
GO:0015031	protein transport	380	44	23.92	5.20E-05
GO:0016050	vesicle organization	44	11	2.77	6.30E-05

t-test DOWN_LP104 poly RNA vs total micromalted_control

GO:0015931	nucleobase-containing compound transport	68	17	8.32	0.00277
GO:0009069	serine family amino acid metabolic proce...	68	18	8.32	0.00104
GO:0009070	serine family amino acid biosynthetic pr...	44	12	5.39	0.00532
GO:0071166	ribonucleoprotein complex localization	50	14	6.12	0.00206

t-test UP_LP106 poly RNA vs total micromalted_control

GO:0019288	isopentenyl diphosphate biosynthetic pro...	82	27	8.15	9.10E-09
GO:0015995	chlorophyll biosynthetic process	66	21	6.56	7.90E-07
GO:0008654	phospholipid biosynthetic process	135	37	13.42	5.40E-09
GO:0009657	plastid organization	153	41	15.21	1.70E-09

t-test DOWN_LP106 poly RNA vs total micromalted_control

GO:0009800	cinnamic acid biosynthetic process	10	6	0.44	1.30E-06
GO:0031408	oxylipin biosynthetic process	29	8	1.28	2.50E-05

GO:1902222	erythrose 4-phosphate/phosphoenolpyruvat...	18	10	0.79	7.90E-10
GO:0006559	L-phenylalanine catabolic process	18	10	0.79	7.90E-10

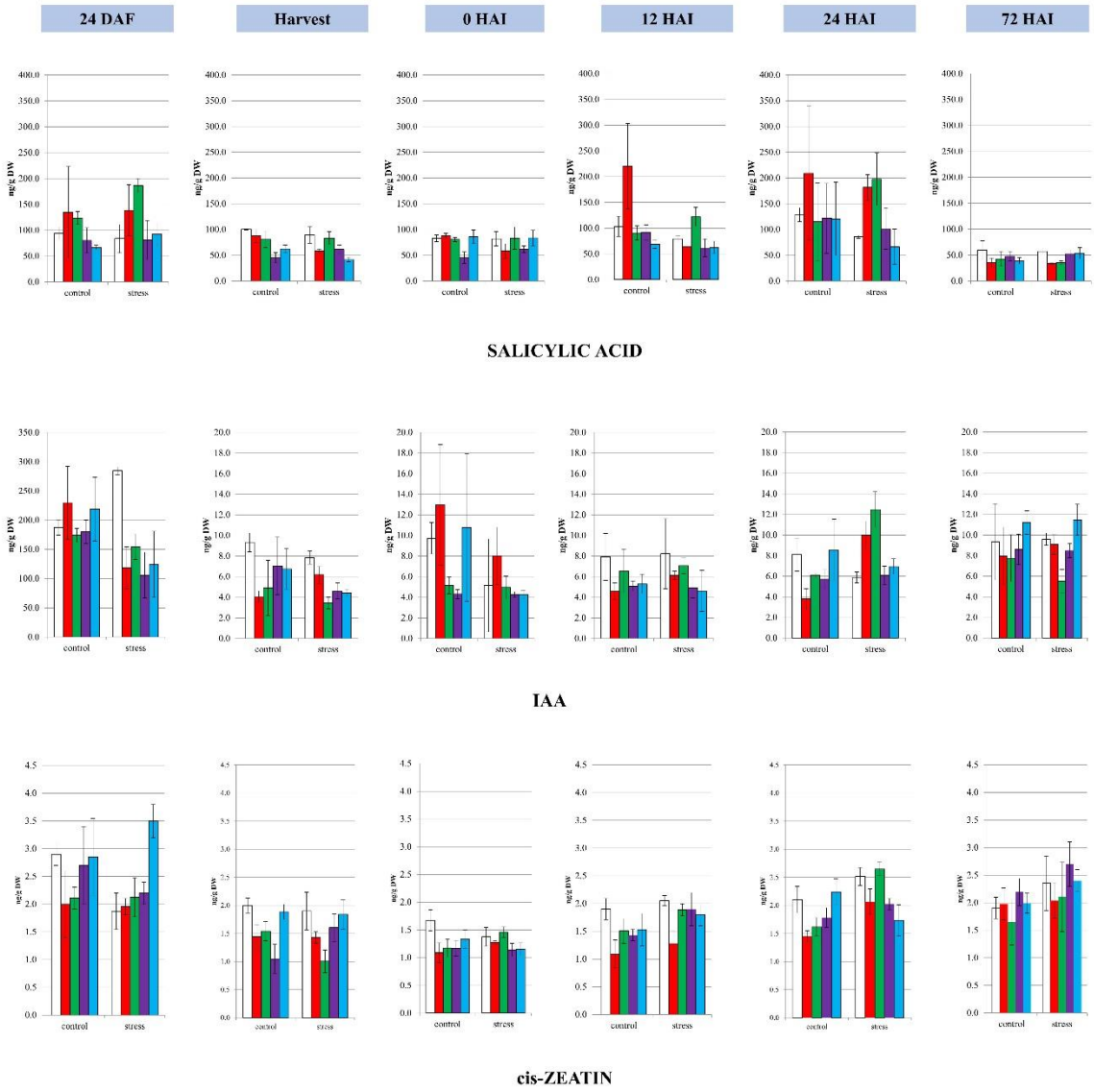

Figure 36. Salicylic acid, auxin (IAA), cytokinin (ciz-zeatin) content in Golden Promise, XN17, XN26, LoHi236 and LoHi272 measured at 24 DAF, harvest, 0 HAI, 12 HAI, 24 HAI, and 72 HAI. Hormones measured with LC-MS/MS (n=4±SD).

Table 14. Top 20 abundant transcripts in XN17 (A), XN26 (B) and LoHi236 line (C). Normalized expression values are indicated, obtained after data processing (explained in Materials and Methods) in Genespring software (Agilent technologies) and represent a mean value of three independent replications.

A

Harvest assembly ID	**XN17 control**	**Annotation**	**Harvest assembly ID**	**XN17 stress**	**Annotation**
Contig4058_s_at	9.721592	Alpha-amylase/subtilisin inhibitor	**35_27720**	6.669416	Ribosomal protein
35_1578	9.347009	Alpha-amylase/subtilisin inhibitor	**35_42283**	6.416479	Ribosomal protein
Contig9782_at	9.303329	Eukaryotic aspartyl protease family protein	35_34557	6.396267	Ribosomal protein
35_3989	8.87109	Eukaryotic aspartyl protease family protein	**35_27981**	6.385822	NA
35_3990	8.692047	Eukaryotic aspartyl protease family protein	35_22282	6.291917	NA
35_27720	8.674705	Ribosomal protein	35_19829	6.273009	Lea 19.4
35_15733	8.518165	Invertase/pectin methylesterase inhibitor superfamily protein	35_34586	6.063368	Ribosomal protein
35_36637	8.435571	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily	35_35011	6.014227	NA
35_31344	8.415921	Ribosomal protein	35_22581	5.922451	Ribosomal protein
35_42283	8.341801	Ribosomal protein	35_13462	5.867252	mito/plastid ribosomal protein
35_1552	8.222915	NA	35_42164	5.645049	Ribosomal protein
35_50459	8.197522	NA	35_30848	5.585323	trypsin inhibitor
35_27981	8.196338	NA	Contig21303_at	5.567715	NA
35_26582	8.13509	NA	35_41981	5.514956	NA
35_14586	8.078205	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily	Contig19261_at	5.453466	NA
35_4282	8.062646	RNA regulation of transcription	35_17096	5.241375	trypsin inhibitor
35_14585	8.060039	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily	35_11133	5.206975	Ribosomal protein
35_14583	8.022055	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily	35_27346	5.122916	NA
35_6241	7.944398	NA	35_27013	4.995933	Polyubiquitin 3
35_2643	7.936284	NA	35_21402	4.908339	NA

Table 15 B

Harvest assembly ID	**XN26 control**	**Annotation**	**Harvest assembly ID**	**XN26 stress**	**Annotation**
35_34632	7.662455	NA	**35_22282**	7.04829	NA
35_27346	7.482937	NA	**35_19829**	6.703047	Lea 19.4
35_34586	7.438557	ribosomal protein	Contig4058_s_at	6.081408	Alpha-amylase/subtilisin inhibitor
35_34557	7.298251	ribososomal protein	35_34637	5.603782	NA
35_22581	7.149207	ribosomal protein	Contig9782_at	5.589779	Eukaryotic aspartyl protease family protein
35_27720	7.077674	NA	35_1578	5.576635	Alpha-amylase/subtilisin inhibitor
35_35011	7.068446	NA	35_1055	5.537467	NA
35_42283	7.041049	ribosomal protein	Contig3812_at	5.496658	Hexosyltransferase
35_41981	6.946911	NA	35_30848	5.452919	C type trypsin inhibitor
35_22282	6.917408	NA	35_50219	5.448404	NA
35_34583	6.764807	NA	35_34557	5.431791	Ribosomal protein

35_19829	6.647467	Lea 19.4	35_3989	5.296876	NA
35_27981	6.637906	NA	35_1096	5.214742	Hexosyltransferase
35_21402	6.47139	NA	35_36637	5.204674	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily
35_34517	6.395091	mitochondrial electron transport / ATP synthesis cytochrome c oxidase	35_22581	5.185175	ribosomal protein
35_13462	6.316611	mito/plastid ribosomal protein	35_17096	5.179688	Trypsin inhibitor
35_11133	6.292371	ribosomal protein	35_27346	5.179672	NA
35_34567	6.187609	ribosomal protein	35_3990	5.176359	Eukaryotic aspartyl protease family protein
35_42164	6.063852	ribosomal protein	35_14583	5.12682	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily
HU05N23u_at	6.025815	ADP,ATP carrier protein, mitochondrial	**35_34586**	5.102349	Ribosomal protein

Table 15 C

Harvest assembly ID	**LoHi236 control**	**FUNCAT**	**Harvest assembly ID**	**LoHi236 stress**	**FUNCAT**
35_27720	9.13696	NA	**35_13190**	8.179425	Ethylene receptor
35_42283	8.816214	ribosomal protein	**35_22282**	7.003603	NA
35_27981	8.621514	NA	35_10232	6.935484	NA
Contig21303_at	8.205827	NA	Contig4058_s_at	6.662351	Alpha-amylase/subtilisin inhibitor
35_21402	8.203218	NA	35_9564	6.614117	ribososomal protein
35_13190	8.202891	Ethylene receptor	**Contig3812_at**	6.570155	Hexosyltransferase
35_13462	8.200961	mito/plastid ribosomal protein	Contig9782_at	6.499485	Eukaryotic aspartyl protease family protein
35_42164	7.996594	ribosomal protein	35_27346	6.486422	NA
35_22282	7.801704	NA	**35_1096**	6.456382	Hexosyltransferase
35_41981	7.716171	NA	**35_19829**	6.436821	Lea 19.4
35_27600	7.43141	NA	**35_1055**	6.425737	NA
Contig19261_at	7.403067	glycolysis.G6PIsomerase	35_34557	6.192291	ribosomal protein
Contig3812_at	7.397213	Hexosyltransferase	**35_1578**	6.11989	Alpha-amylase/subtilisin inhibitor
Contig4058_s_at	7.370022	Alpha-amylase/subtilisin inhibitor	35_30848	6.078011	NA
35_27013	7.327769	Polyubiquitin	35_3989	6.045386	NA
35_1096	7.302939	Hexosyltransferase	35_34503	6.039346	Peptidylprolyl isomerase
35_7468	7.257332	ribosomal protein	35_35011	5.957291	NA
35_1055	7.087971	NA	35_5362	5.904951	alpha/beta-Hydrolases superfamily protein
35_1578	7.035197	Alpha-amylase/subtilisin inhibitor	HvHK14	5.855549	
35_19829	6.89204	Lea 19.4	35_3990	5.847849	Eukaryotic aspartyl protease family protein

Table 15. Differentially regulated genes under stress at the dry seed stage. T-test were performed (BHB adjusted P, FC>5) between stressed and control seed. Four comparisons for four lines are shown: GP, XN17, XN26 and LoHi236.

Golden Promise			XN17			XN26			LoHi236		
FC (c vs. S)	Harvest assembly ID	Annotation	FC (c vs. S)	Harvest assembly ID	Annotation	FC (c vs. S)	Harvest assembly ID	Annotation	FC (c vs. S)	Harvest assembly ID	Annotation
82.99449	35_34557	protein.synthesis.misc ribososomal protein	9.23819	35_30190	NA	63.30105	35_614	Non-specific lipid-transfer protein 3	172.1387	HvHK14	
46.32544	35_35011	NA	5.49703	35_687	MADS-box transcription factor 14	59.37082	35_34637	Ethylene-responsive transcription facotr 1	116.7775	35_5362	not assigned.no ontology
28.77461	35_34631	protein.synthesis.misc ribososomal protein	5.029539	rbaal14f06_at	MADS-box transcription factor 14	45.02227	35_612	Non-specific lipid-transfer protein 3	72.8956	35_10232	NA
21.96627	35_13667	protein.degradation.ubiquitin.ubiquitin	-5.09636	35_36760	Eukaryotic aspartyl protease family protein	41.42907	35_613	Non-specific lipid-transfer protein 3	71.84303	35_22581	protein.synthesis.misc ribososomal protein
21.56298	35_22581	protein.synthesis.misc ribososomal protein	-5.11016	35_15414	Early light-induced protein, chloroplastic	40.82768	Contig2043_s_at	Non-specific lipid-transfer protein 3	61.449	35_34557	protein.synthesis.misc ribososomal protein
18.81052	35_34586	protein.synthesis.misc ribososomal protein	-5.14522	Contig15799_at	Ozone-responsive stress related protein	33.78107	35_32068	Non-specific lipid-transfer protein 3	45.90485	35_34589	NA
16.79951	35_27346	stress.abiotic.cold	-5.35374	35_13797	ubiquitin 11	14.6641	35_34503	NA	31.93944	35_34586	protein.synthesis.misc ribososomal protein
-13.5271	35_22055	misc.cytochrome P450	-5.42063	35_7468	NA	5.340446	35_22809	60S ribosomal protein L15	30.92848	35_35011	NA
-12.2666	35_27498	protein.degradation.subtilases	-5.48728	35_1832	Cathepsin B-like cysteine proteinase	-5.042	35_34501	protein.synthesis.misc ribososomal protein	29.33627	35_9564	protein.synthesis.misc ribososomal protein
-9.5738	Contig3018_at	stress.abiotic.unspecified	-5.54871	35_4903	Cysteine-rich SECRETORY PROTEIN 3	-5.05038	35_34586	protein.synthesis.misc ribososomal protein	24.08145	35_34505	NA
-8.39447	35_15828	NA	-5.78898	HVSMEi0004L20r2_at	Aldehyde dehydrogenase family 3member H1	-5.08407	35_34620	protein.synthesis.misc ribososomal protein	20.66712	35_11133	protein.synthesis.misc ribososomal protein
8.146218	35_36386	NA	-5.79079	35_13096	Transposon Ty1-OR Gag-Pol polyprotein	-5.16916	35_13495	protein.synthesis.misc ribososomal protein	19.02203	Contig5533_at	fermentation.PDC
-7.62362	35_15826	NA	-5.8686	35_21466	Protein kinase superfamily protein	-5.17151	35_34545	NA	18.60966	35_34633	protein.synthesis.misc ribososomal protein
-7.55815	35_15829	NA	-5.89684	35_31299	Non-specific LIPID TRANSFER PROTEIN 2	-5.19353	35_45670	NA	17.82685	35_34637	NA
-7.52096	35_4001	misc.nitrilases, *nitrile lyases, berberine bridge enzymes, reticuline oxidases, troponine reductases	-5.91434	35_14925	60S ribosomal protein L26-1	-5.19409	35_23503	|Protein translation factor SUI1 homolog	16.90083	35_24773	NA
-7.27452	35_9341	NA	-5.9437	35_10839	NA	-5.22068	35_34517	NA	15.77712	35_612	lipid metabolism.lipid transfer proteins etc
-7.02947	35_15827	stress.abiotic.unspecified	-6.18917	35_21402	NA	-5.29731	35_11380	60S ribosomal protein L31	11.15953	35_784	fermentation.PDC

-5.54699	35_15274	stress.abiotic.unspecified	-6.21255	35_42513	NA	-5.37508	35_28662	NA	10.20438	35_27346	stress.abiotic.cold
-5.40779	35_15272	stress.abiotic.unspecified	-6.29377	35_1055	alpha-mannosidase 2	-6.05954	35_43155	NA	9.333791	35_14192	hormone metabolism.jasmonate.synthesis-degradation.lipoxygenase
			-6.35612	35_8224	Nucleoporin NUP188 homolog	-6.20994	35_34613	NA	8.49124	35_34503	cell.cycle.peptidylprolyl isomerase
			-6.36927	35_50252	NA	-6.24003	35_34567	40 s	7.647749	35_613	NA
			-6.40584	35_702	Cysteine-rich venom protein	-6.3022	35_24449	Histone H2B.2	7.337174	Contig2043_s_at	lipid metabolism.lipid transfer proteins etc
			-6.44136	35_34633	NA	-9.05748	35_34583	NA	7.052747	35_34534	protein.synthesis.misc ribososomal protein
			-6.50355	HT12H02r_at	Low molecular weight glutenin subunit	-9.11089	35_34632	NA	6.98203	35_32068	lipid metabolism.lipid transfer proteins etc
			-6.63452	35_704	Cysteine-rich venom protein	-10.164	35_42359	NA	6.521044	35_614	lipid metabolism.lipid transfer proteins etc
			-6.68613	35_15829	Pathogenesis-related protein STH-21	-10.6628	35_12765	NA	5.546726	35_11380	protein.synthesis.misc ribososomal protein
			-6.96074	Contig2210_at	Cysteine-rich venom protein	-11.8464	35_23856	40S ribosomal protein s13	-5.02595	35_14440	PS.lightreaction.photosystem II.PSII polypeptide subunits
			-7.10008	35_43233	NA	-13.6683	35_24667	NA	-5.35754	35_27221	hormone metabolism.cytokinin.signal transduction
			-7.42157	Contig3017_at	germin like protein 4	-15.9712	35_27584	NA	-6.2311	35_16798	misc.protease inhibitor/seed storage/lipid transfer protein (LTP) family protein
			-7.67244	35_1096	Hexosyltransferase	-19.8264	35_12423	NA	-7.23161	35_30625	NA
			-7.67374	35_8678	12-oxophytodienoate reductase 1	-21.3087	35_25951	NA	-7.27514	35_30941	NA
			-7.86232	Contig3812_at	Hexosyltransferase	-22.353	35_42368	NA	-24.2064	35_27584	NA
			-7.92727	35_19553	xyloglucan endotransglucosylase/hydrolase 13	-27.7093	35_11528	NA	-24.3564	35_42359	PS.lightreaction.ATP synthase
			-8.00894	35_705	Cysteine-rich venom protein	-28.783	HT06N06u_at	NA	-31.3625	35_31344	protein.synthesis.misc ribososomal protein
			-8.23286	35_703	Cysteine-rich venom protein	-31.2516	35_27600	NA	-36.037	35_27013	protein.degradation.ubiquitin.ubiquitin
			-8.38726	35_15826	Pathogenesis-related protein STH-21	-34.1408	35_10357	NA	-42.5696	35_27720	protein.synthesis.misc ribososomal protein

			-8.45719	35_32130	UDP-Glycosyltransferase superfamily protein	-34.8287	35_10459	NA	-43.3287	35_27981	mitochondrial electron transport / ATP synthesis.F1-ATPase
			-8.59451	35_22862	trypsin inhibitor	-35.2498	35_27013	polyubiquitin 3	-44.5768	35_42368	protein.synthesis.misc ribososomal protein
			-8.65252	35_34611	NA	-40.8097	35_7468	NA	-48.8111	HT06N06u_at	lipid metabolism.lipid degradation.beta-oxidation.acyl CoA DH
			-8.91677	35_11555	Protein kinase superfamily protein	-44.0014	Contig19261_at	NA	-58.5422	35_10357	mitochondrial electron transport / ATP synthesis.cytochrome c oxidase
			-9.10623	35_15827	Major pollen allergen Bet v-1	-56.0854	Contig21303_at	NA	-63.0208	Contig19261_at	glycolysis.G6PIsomerase
			-9.28071	35_39074	LEA related	-59.635	35_13462	NA	-66.5564	35_25951	NA
			-9.30393	Contig1528_s_at	germin-like protein 2	-62.0781	35_27981	NA	-69.2933	35_11528	NA
			-9.45236	35_19340	NA	-66.2376	35_27720	NA	-77.7202	35_12423	NA
			-9.93135	35_19198	Phytosulfokine-alpha 2	-79.7646	35_21402	NA	-82.2707	35_27600	NA
			-9.93622	35_28027	Pathogenesis-related thaumatin superfamily protein	-84.631	35_42164	NA	-84.4964	35_42283	protein.synthesis.misc ribososomal protein
			-9.9363	35_18102	Harpin induced gene 1 homolog	-108.542	35_42283	60S ribosomal protein L1-A	-87.3397	35_42164	protein.synthesis.misc ribososomal protein
			-10.6973	35_39119	NA	-125.489	35_41981	NA	-95.0288	35_7468	protein.synthesis.misc ribososomal protein
			-11.2485	35_14439	photosystem II 10 kDa polypeptide				-112.353	35_10459	transporter.sugars
			-11.5575	35_15828	NA				-148.875	Contig21303_at	transport.porins
			-11.588	35_632	NA				-157.892	35_13462	protein.synthesis.mito/plastid ribosomal protein.plastid
			-11.811	35_48	germin-like protein 2				-189.176	35_41981	NA
			-12.1292	35_7220	calcium-binding EF-hand family protein				-289.646	35_21402	NA
			-13.0787	35_19187	B3 domain-containing protein						
			-13.7116	Contig15981_at	Calcium-binding EF-hand family protein						
			-13.8836	35_25682	NA						
			-13.912	Contig13056_at	germin-like protein 5						

			-13.997	35_15274	germin-like protein 4					
			-14.1441	35_27861	beta-galactosidase 11					
			-14.2823	Contig1419_s_at	Photosystem II 10 kDa polypeptide					
			-15.0851	35_20041	NA					
			-15.2927	35_6760	L-gulonolactone oxidase 2					
			-15.9737	35_11993	Magnesium transporter NIPA2					
			-16.2028	Contig3952_at	alpha amylase like					
			-16.3733	35_8397	NA					
			-16.4177	35_9951	NA					
			-16.6331	35_36756	Subtilisin-like PROTEASE					
			-16.7724	35_44426	LEA					
			-17.9923	35_31793	LEA					
			-18.595	35_36803	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily					
			-19.4535	35_30281	NA					
			-20.4986	35_1136	alpha amylase like					
			-20.6146	35_6726	NA					
			-20.9061	35_8714	NA					
			-21.0643	35_35664	NA					
			-21.2419	35_3681	NA					
			-21.3327	Contig3953_s_at	alpha amylase like					
			-21.796	35_25069	Heat Shock 70kda					
			-22.8764	35_45773	bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily					
			-23.0642	35_1577	Alpha-amylase/subtilisin inhibitor					
			-24.7851	35_24773	NA					
			-24.8369	35_3579	expanson B2					
			-26.0718	35_31344	NA					
			-26.25	35_19263	NA					
			-26.5895	35_24122	endonuclease 2					

			-27.3677	35_877	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily					
			-27.5048	35_10688	Retrotransposon protein					
			-27.9176	35_24647	Chromosome 3B, genomic scaffold					
			-29.4139	35_13394	NA					
			-29.6188	35_1131	alpha-amylase like					
			-29.7774	35_881	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily					
			-29.8859	35_50219	NA					
			-31.1863	35_267	RECA homolog 3					
			-31.3733	35_487	NA					
			-31.4399	35_10486	NA					
			-32.3174	35_2002	Beta-1,3-N-Acetylglucosaminyltransferase family protein					
			-32.7129	Contig10728_at	2- oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein					
			-33.3356	35_3577	NA					
			-33.561	35_19082	NA					
			-34.6444	35_1130	alpha-amylase like					
			-34.6546	35_34503	NA					
			-35.7693	35_1482	NA					
			-35.9709	35_45380	NA					
			-36.9756	35_35750	endonuclease 2					
			-37.7098	35_50399	NA					
			-38.2368	35_17524	NA					
			-38.5451	HY03I16T_at	NA					
			-38.8084	35_34637	Ethylene-responsive transcription facotr 1					
			-39.5242	35_1250	NA					
			-41.2234	35_4001	methyl estsrase 1					
			-43.0838	35_19355	2- oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily					

					protein						
			-43.1893	35_14586	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily						
			-44.6906	35_36925	pectinesterase 11						
			-46.255	35_10232	NA						
			-48.7413	35_12603	DNA biding protein lik6e						
			-50.101	35_50586	Cysteine proteinase inhibitor 8						
			-51.1251	35_13639	Protein phosphatase 2C protein						
			-51.912	35_15841	Cysteine proteinase inhibitor 8						
			-54.3735	35_37233	Cathepsin B-like cysteine proteinase 2						
			-54.8719	35_29558	early nodulin like protein 15						
			-54.9674	35_6140	NA						
			-56.5728	35_14583	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily						
			-56.8055	35_15671	NA						
			-56.9411	35_14587	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily						
			-57.5559	35_7631	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily						
			-58.5136	35_50459	FAD-binding Berberine FAMILY PROTEIN						
			-58.9186	35_3216	LEA						
			-59.982	35_22474	germin-like protein 2						
			-60.3681	35_876	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily						
			-61.964	35_14585	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily						
			-62.6688	35_1564	early noduline like 15						
			-62.8935	35_31175	NA						
			-64.8727	35_15670	NA						
			-65.5288	35_1565	early nodulin like protein 15						
			-66.3324	35_30941	NA						
			-67.7333	35_37305	Pectin lyase-like superfamily protein						
			-71.9439	35_875	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily						
			-72.8792	35_874	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily						

			-72.9453	35_15649	response regulator 24					
			-75.586	35_36637	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily					
			-76.2012	35_7410	NA					
			-80.3881	Contig22065_at	Protein kinase superfamily protein					
			-81.4143	Contig9236_at	NA					
			-82.8816	35_1566	early nodulin like protein 14					
			-83.1248	35_6241	Chromosome 3B, genomic scaffold					
			-84.7334	35_1483	NA					
			-87.1292	35_20043	NA					
			-87.692	35_23740	NA					
			-88.4854	35_2106	NA					
			-92.7077	35_5401	Chromosome 3B, genomic scaffold					
			-100.354	35_878	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily					
			-101.814	35_15648	hybrid signal transduction histidine kinase I					
			-107.472	35_37164	response regulator 24					
			-108.253	35_21108	NA					
			-109.744	Contig17901_at	Mitochondrial import inner membrane translocase subunit					
			-113.109	Contig9020_at	|Somatic embryogenesis receptor kinase 1					
			-113.412	35_14584	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily					
			-114.894	35_2643	na					
			-115.047	Contig4058_s_at	Alpha-amylase/subtilisin inhibitor					
			-117.013	35_4175	Plant invertase/pectin methylesterase inhibitor family protein					
			-117.154	35_15733	Defensin-like protein 6					
			-117.629	35_879	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily					
			-120.447	35_3990	Eukaryotic aspartyl protease family protein					
			-120.863	35_1567	early nodulin like protein 14					
			-124.33	35_4282	NA					
			-132.736	35_25410	Defensin-like protein 6					

			-134	35_880	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily						
			-136.403	35_26582	Chromosome 3B, genomic scaffold						
			-139.309	35_1578	|Alpha-amylase/subtilisin inhibitor						
			-141.791	35_3989	Eukaryotic aspartyl protease family protein						
			-143.027	35_6725	NA						
			-143.716	35_20042	NA						
			-152.132	35_1552	NA						
			-188.771	Contig9782_at	Eukaryotic aspartyl protease family protein						
			-259.85	35_2105	actin depolymerizing facotr 5						

Table 16. Differentially abundant polysome-bound transcripts in embryo between Golden Promise and transgenics. This list of transcripts is the result of ANOVA (with FC>1.5 in at least one comparison). On the left one-way ANOVA for control, on the right for stress condition. Transcripts shown in figure 28.

Harvest assembly ID	FC ([control-LoHi236-embryo_pf] vs [control-GP-embryo_pf])	FC ([control-XN17-embryo_pf] vs [control-GP-embryo_pf])	FC ([control-XN26-embryo_pf] vs [control-GP-embryo_pf])	Annotation	Harvest assembly ID	FC ([stress-LoHi236-embryo_pf] vs [stress-GP-embryo_pf])	FC ([stress-XN17-embryo_pf] vs [stress-GP-embryo_pf])	FC ([stress-XN26-embryo_pf] vs [stress-GP-embryo_pf])	Annotation
Contig7018_at	2.591159	-1.5615	-2.16202	signalling.calcium	35_14559	1.900355	-1.58732	-2.20864	amino acid metabolism.degradation.aspartate family.methionine
35_19309	2.31422	-1.55313	-1.71802	protein.degradation.aspartate protease	Contig5994_s_at	1.579125	-2.34636	-2.21239	amino acid metabolism.degradation.glutamate family.arginine
35_26616	2.237164	-1.5541	-1.60973	NA	Contig5994_at	1.523246	-1.96827	-2.15937	amino acid metabolism.degradation.glutamate family.arginine
35_17481	2.135076	-1.7311	-2.31324	signalling.calcium	35_17943	-8.48807	2.176066	1.803368	glycolysis.PEPCase
35_7364	2.124908	-1.593	-1.59657	RNA.regulation of transcription.C2H2 zinc finger family	35_9690	-2.80393	1.718787	1.562003	hormone metabolism.auxin.induced-regulated-responsive-activated
35_21781	2.101466	-2.09125	-4.9409	signalling.G-proteins	Contig10299_at	-1.7887	1.543276	2.041503	hormone metabolism.gibberelin.synthesis-degradation.GA2 oxidase
rbaal1n16_s_at	2.066312	-1.7142	-1.59215	PS.calvin cyle.GAP	35_14192	1.792013	-1.52746	-1.74819	hormone metabolism.jasmonate.synthesis-degradation.lipoxygenase

Contig20981_at	1.975563	-1.83944	-1.75072	RNA.regulation of transcription.C2H2 zinc finger family	35_28391	-1.57995	1.506654	1.556468	lipid metabolism.lipid degradation.beta-oxidation.acyl CoA DH
35_1962	1.920293	-1.7045	-1.61884	PS.calvin cyle.GAP	35_17382	-1.77171	1.861023	1.789458	misc.alcohol dehydrogenases
HVSMEb0006N17f_at	1.893452	-1.54566	-1.60626	lipid metabolism.Phospholipid synthesis	35_11949	-3.0576	2.168836	2.34854	misc.cytochrome P450
35_6578	1.803756	-2.30613	-1.80228	misc.protease inhibitor/seed storage/lipid transfer protein (LTP) family protein	35_20923	1.66088	-1.96316	-4.01357	misc.cytochrome P450
35_20041	1.78799	-1.87889	-1.81486	NA	35_30856	-2.55304	2.219163	1.681506	misc.GDSL-motif lipase
35_10150	1.770228	-1.61821	-1.70797	NA	35_38109	-1.82666	5.979492	3.96778	misc.peroxidases
35_46624	1.757491	-3.55884	-3.09654	NA	35_9161	-1.79467	1.973334	1.640754	not assigned.no ontology
35_8079	1.751278	-3.91963	-5.77011	NA	35_12480	-2.59266	3.925147	4.135264	not assigned.no ontology.pentatricopeptide (PPR) repeat-containing protein
35_36765	1.738499	-2.56645	-2.10448	NA	35_1551	-2.33575	2.266776	2.192606	NA
35_19710	1.73802	-4.14622	-3.79067	major CHO metabolism.degradation.sucrose.invertases.cell wall	35_30120	-2.04139	1.623667	1.944392	NA
35_50397	1.736451	-1.55332	-2.22584	NA	35_46424	-5.07681	2.921209	3.010595	NA
35_14440	1.733763	-1.8007	-2.15048	PS.lightreaction.photosystem II.PSII polypeptide subunits	35_10206	-2.45544	1.851708	1.681424	NA
Contig7016_s_at	1.727268	-1.67583	-5.30799	signalling.calcium	35_33830	-1.5206	2.397215	2.662852	NA
35_14439	1.700772	-1.74339	-2.17991	PS.lightreaction.photosystem II.PSII polypeptide subunits	35_43604	-2.48347	2.261272	1.990048	NA
35_1	1.698072	-2.87376	-2.31695	NA	35_11252	-6.79807	2.925844	3.627436	NA
Contig6611_at	1.687578	-1.74599	-1.77488	lipid metabolism.lipid degradation.lipases	35_35473	-2.52321	2.020258	1.505814	NA
35_45249	1.645512	-2.23096	-2.31434	NA	35_43664	-2.01044	1.983007	1.62985	NA
HV_CEb0010I11r2_at	1.614287	-2.72532	-6.70758	signalling.G-proteins	35_41992	-1.71182	11.11266	11.79882	NA
35_42484	1.610225	-1.7701	-1.67647	protein.degradation.ubiquitin.E3.RING	35_25390	-1.86238	1.855921	1.777496	NA
35_9967	1.595088	-1.83243	-1.65526	NA	35_20056	-1.51316	1.644211	2.163944	NA
35_4328	1.594666	-4.32437	-3.1446	NA	35_46587	-2.19177	3.557306	2.018364	NA
35_36460	1.567769	-1.6601	-3.16307	NA	35_9676	-1.66641	2.265369	2.110639	NA

Contig11241_at	1.548592	-5.36533	-4.89848	major CHO metabolism.degradation.sucrose.invertases.cell wall	35_38778	-2.03508	1.820413	1.701387	NA
HT09B07u_s_at	1.548244	-1.93772	-6.6272	signalling.calcium	35_43691	-1.94081	3.020693	2.918865	NA
35_10117	1.534305	-2.11223	-1.59456	NA	35_49808	-3.21768	1.863117	1.722799	NA
S0001100024A03F1_x_at	-1.52805	1.827089	2.381566	RNA.processing	35_27235	-2.05393	3.179308	2.499433	NA
35_38109	-1.53708	3.712779	3.969876	misc.peroxidases	35_43377	-1.7566	3.471564	3.075457	NA
35_1551	-1.54439	1.53925	2.623267	NA	35_45067	-1.73354	2.487794	1.548991	NA
35_31903	-1.58336	1.524465	1.524298	protein.synthesis.misc ribososomal protein	35_44158	-2.63619	2.283042	1.874832	NA
35_318	-1.61585	3.440237	2.880624	NA	35_46552	-1.62169	1.925415	1.740555	NA
35_38986	-1.61664	1.985572	1.685898	NA	35_35443	-2.44214	4.462597	2.732245	NA
35_10456	-1.61991	1.834274	2.990993	NA	35_38993	-1.51085	1.926431	1.634811	NA
35_46421	-1.62224	1.743395	1.655615	cell. vesicle transport	35_37338	-1.80654	5.356241	7.116139	NA
35_45148	-1.66953	1.866635	1.728457	NA	35_25855	-2.51549	1.525336	1.659653	NA
35_49808	-1.67732	1.705769	2.304317	NA	35_37784	-1.92881	2.023878	2.03884	NA
Contig793_x_at	-1.69082	1.566152	2.525395	development.storage proteins	35_42910	-1.6515	1.815566	1.691678	NA
35_14900	-1.7431	7.720354	12.71926	NA	35_26162	-2.29743	1.682947	1.564723	NA
35_30856	-1.74904	1.803451	2.649387	misc.GDSL-motif lipase	35_32162	-1.92992	2.667123	2.169222	NA
35_7960	-1.79443	3.923094	8.610768	protein.degradation.ubiquitin.E3.RING	35_47732	-1.95924	1.864992	1.639337	NA
35_39301	-1.80061	3.201137	6.249243	NA	35_36095	-2.01457	1.738038	1.557054	NA
35_29695	-1.90182	2.058502	2.311841	NA	35_12731	-2.54363	2.92532	2.328754	NA
35_37338	-1.94446	5.944008	13.33226	NA	35_30028	-1.56017	1.772964	1.694487	NA
35_25240	-1.95997	2.661846	6.030191	NA	35_39301	-2.44287	2.258335	2.827275	NA
35_12480	-1.97356	3.440247	5.229048	not assigned.no ontology.pentatricopeptide (PPR) repeat-containing protein	35_31691	-1.68091	2.547802	1.872839	NA
35_50548	-2.30789	2.237986	3.187262	protein.synthesis.misc ribososomal protein	35_26194	-1.92196	2.21686	2.160648	NA
35_38105	-2.33387	2.773057	5.394299	RNA.regulation of transcription.putative transcription regulator	35_31674	-5.08868	4.812192	1.907465	NA
35_11949	-2.43664	1.951139	2.697523	misc.cytochrome P450	35_46387	-1.84484	2.788234	2.629869	NA

35_44679	-2.51121	12.38378	21.05268	NA	35_27392	-3.35182	1.658052	1.581762	NA
35_6692	-2.69384	1.670739	1.544286	protein.targeting.chloroplast	35_44679	-2.53736	11.03919	13.17616	NA
35_28598	-2.74224	2.223299	1.694101	NA	35_45704	-1.57969	2.169216	1.804922	NA
35_35443	-2.85762	1.87259	2.631293	NA	35_43802	-1.5628	1.845381	1.987075	NA
35_46424	-3.06108	2.851042	4.283278	NA	35_10456	-3.01969	2.114426	2.070903	NA
35_11252	-3.97316	2.581514	6.005994	NA	35_29695	-2.61568	3.507798	2.399044	NA
35_17943	-4.65852	2.002573	2.905219	glycolysis.PEPCase	35_46352	-2.24675	2.082881	1.672791	NA
35_15460	-5.31804	2.181283	4.249921	stress.biotic	35_38708	-1.90058	2.226548	1.604925	NA
35_31674	-5.68175	3.160551	1.571024	NA	35_29657	-1.67018	2.313715	1.993664	NA
					35_46604	-1.96392	1.766004	1.794413	NA
					35_22803	-2.29717	1.936339	1.885798	NA
					35_25091	-1.75008	4.081624	1.525119	NA
					35_12745	-1.54491	1.507576	1.616272	NA
					35_29761	-1.94824	1.991068	1.706016	NA
					35_38966	-2.23533	1.564715	3.746946	NA
					35_16297	1.551059	-1.96773	-2.16869	polyamine metabolism.synthesis.arginine decarboxylase
					35_9065	2.067338	-1.78808	-1.86736	protein.degradation.AAA type
					35_3989	3.060388	-2.06285	-1.62041	protein.degradation.aspartate protease
					35_47816	-2.14574	2.202354	2.372231	protein.degradation.ubiquitin.E3.RING
					35_7960	-1.95721	4.024964	4.22799	protein.degradation.ubiquitin.E3.RING
					35_49002	-2.34375	5.429188	2.153001	protein.degradation.ubiquitin.E3.RING
					35_50548	-2.27573	3.356975	2.544114	protein.synthesis.misc ribososomal protein
					35_6692	-1.98915	3.491265	1.850339	protein.targeting.chloroplast
					S0001100024A03F1_x_at	-2.00135	1.863284	1.686338	RNA.processing
					35_37328	-1.59102	2.648743	3.812437	RNA.regulation of transcription.C2H2 zinc finger family
					rbaal14f06_at	-1.85306	1.919377	1.698862	RNA.regulation of transcription.MADS box transcription factor family
					35_45059	-2.2563	2.073416	2.188999	RNA.regulation of transcription.putative transcription regulator

					35_38105	-2.71037	2.976298	3.540983	RNA.regulation of transcription.putative transcription regulator
					35_32142	-1.58591	2.253833	2.02397	RNA.regulation of transcription.SET-domain transcriptional regulator family
					35_3520	-2.19638	2.120579	3.655742	RNA.transcription
					35_15645	1.572367	-2.12404	-3.00816	secondary metabolism.flavonoids.chalcones
					35_12652	1.669487	-2.73349	-1.6478	signalling.calcium
					35_25069	2.131231	-1.82873	-2.18924	stress.abiotic.heat
					35_359	-2.14556	2.262927	2.015215	stress.biotic
					Contig2997_at	1.539335	-1.69733	-1.51644	transport.amino acids
					Contig7529_at	-1.65548	1.844066	1.816236	transport.metabolite transporters at the envelope membrane
					35_7714	1.84156	-2.76444	-4.56346	transport.metal

Table 17. Differentially abundant polysome-bound transcripts in endosperm between Golden Promise and transgenics. This list of transcripts is the result of ANOVA (with FC>1.5 in at least one comparison). On the left one-way ANOVA for control, on the right for stress condition. Transcripts shown in figure 29.

Harvest assembly ID	FC ([control-LoHi236-endosperm_pf] vs [control-GP-endosperm_pf])	FC ([control-XN17-endosperm_pf] vs [control-GP-endosperm_pf])	FC ([control-XN26-endosperm_pf] vs [control-GP-endosperm_pf])	Annotation	Harvest assembly ID	FC ([stress-LoHi236-endosperm_pf] vs [stress-GP-endosperm_pf])	FC ([stress-XN17-endosperm_pf] vs [stress-GP-endosperm_pf])	FC ([stress-XN26-endosperm_pf] vs [stress-GP-endosperm_pf])	Annotation
35_45028	18.45852	-1.36086	-1.11376	NA	35_20042	6.411954	-2.63591	-2.47908	NA
35_34544	17.60014	-1.00168	-1.02041	RNA.processing.splicing	35_25317	2.663969	-1.16556	-1.01874	NA
35_45688	9.624597	-1.01497	-1.02218	secondary metabolism.N misc.alkaloid-like	35_46624	1.917786	-3.36643	-2.20762	NA
35_45249	4.454663	-2.48161	-1.71019	NA	35_47500	1.701053	-1.25743	-1.59137	NA
35_8500	2.112233	-4.65206	-5.60292	DNA.synthesis/chromatin structure.histone	35_41847	1.638854	-2.28021	-1.08637	secondary metabolism.isoprenoids.carotenoids
35_21297	1.651779	-1.43859	-1.30981	NA	35_29510	1.604377	-1.61196	-1.21118	NA
Contig14514_at	1.631246	-1.39984	-1.24683	RNA.regulation of transcription.bZIP transcription factor family	Contig12042_at	1.581643	-1.60598	-1.24564	hormone metabolism.brassinosteroid.synthesis-degradation.BRs.other

35_6823	1.528625	-1.05924	-1.22027	protein.degradation.ubiquitin.E3.RING	HT06O16u_s_at	1.577746	-1.36197	-1.20691	glycolysis.PK
35_31541	1.302069	-1.03543	-1.2173	NA	35_15126	1.553248	-1.34353	-1.21977	glycolysis.PK
35_30792	1.181563	-2.42589	-1.41544	NA	35_8002	1.542635	-1.47047	-1.03187	NA
35_20795	1.073664	-2.23169	-1.49016	NA	Contig6274_at	1.525271	-1.35064	-1.05547	glycolysis.PPFK
HvDREB3	1.063901	-3.0248	-3.2879		Contig12042_s_at	1.476616	-1.69005	-1.26086	hormone metabolism.brassinosteroid.synthesis-degradation.BRs.other
35_15344	1.046765	-2.96907	-3.3495	RNA.regulation of transcription.AP2/EREBP, APETALA2/Ethylene-responsive element binding protein family	35_37962	1.452834	-4.1081	-3.55806	NA
Contig11141_at	1.018846	-2.02995	-2.61719	stress.abiotic.heat	35_356	1.4194	-2.02634	-1.52657	NA
35_19178	1.01783	-2.1965	-2.73682	stress.abiotic.heat	35_28991	1.410009	-1.62854	-1.43262	NA
35_15049	-1.02022	1.302106	1.461703	protein.synthesis.misc ribososomal protein	35_50823	1.28278	-1.97691	-1.61038	NA
35_893	-1.02405	1.74251	3.688141	stress.biotic	35_27778	1.28194	-1.67149	-1.1894	protein.targeting.chloroplast
Contig10079_at	-1.03174	2.320722	1.301325	amino acid metabolism.synthesis.aspartate family.lysine	35_19831	1.271953	-2.96816	-1.67407	RNA.regulation of transcription.G2-like transcription factor family, GARP
35_23081	-1.08148	1.118053	1.503216	signalling.receptor kinases.leucine rich repeat III	35_33245	1.112313	-2.20627	-2.8234	NA
35_37070	-1.10526	1.457272	1.555349	NA	35_16110	1.106751	-1.54999	-1.40513	NA
Contig17136_at	-1.12374	1.190297	1.511416	transport.ABC transporters and multidrug resistance systems	35_1471	1.084789	-6.16914	-1.55023	not assigned.no ontology
35_44851	-1.13601	1.72654	2.309701	NA	35_24043	1.012026	-1.2536	-1.62235	misc.acid and other phosphatases
35_23252	-1.15093	1.430971	1.696569	NA	35_20913	1.010382	-1.0584	-5.26618	not assigned.no ontology
Contig18208_at	-1.1661	1.317884	1.827007	signalling.receptor kinases.leucine rich repeat III	35_32359	1.009167	-1.94244	-1.88268	NA
35_7034	-1.34197	1.342946	1.74995	protein.postranslational modification	35_4459	-1.00608	2.659884	1.489117	protein.degradation.ubiquitin.E3.RING
35_25930	-1.45219	1.023083	7.998184	NA	35_50005	-1.00816	3.601962	1.987163	NA

35_7092	-1.46199	1.946924	1.872081	secondary metabolism.isoprenoids.non-mevalonate pathway.DXS	35_5767	-1.02669	1.581641	1.549007	NA
35_44502	-1.57126	1.700197	1.455687	transport.Major Intrinsic Proteins.PIP	35_36738	-1.02708	1.600209	29.3127	NA
35_1073	-1.59808	1.197322	3.428739	cell wall.modification	35_28726	-1.02883	1.314463	2.428432	NA
35_8776	-1.80739	1.407163	1.765566	RNA.regulation of transcription.GRAS transcription factor family	Contig16710_at	-1.02979	1.832246	1.95792	signalling.receptor kinases.legume-lectin
EBem09_SQ003_F16_s_at	-1.86546	1.55801	1.145062	transport.Major Intrinsic Proteins.PIP	35_50056	-1.03896	1.148355	18.58699	NA
35_38554	-2.28755	1.657259	1.305276	transport.Major Intrinsic Proteins.PIP	Contig2027_x_at	-1.04823	1.005129	1.832714	development.storage proteins
					35_6902	-1.04832	1.675681	1.139118	NA
					35_50532	-1.05247	1.743249	1.682311	misc.GDSL-motif lipase
					35_22115	-1.05591	2.294109	1.372691	transport.amino acids
					35_8319	-1.06221	1.207034	1.59964	NA
					35_11834	-1.07277	7.099486	1.155776	NA
					35_9898	-1.07667	2.147916	1.011609	NA
					35_13643	-1.09579	4.330543	1.123955	DNA.synthesis/chromatin structure.histone
					35_16336	-1.10132	1.545789	1.242365	NA
					35_24502	-1.12095	1.609896	1.053274	NA
					35_14010	-1.20544	8.393437	1.205154	DNA.synthesis/chromatin structure.histone
					35_14027	-1.21514	2.087557	1.272995	DNA.synthesis/chromatin structure.histone
					35_14047	-1.2285	4.051972	1.127223	DNA.synthesis/chromatin structure.histone
					Contig24583_at	-1.2407	1.582603	1.119491	minor CHO metabolism.trehalose.TPP
					35_13923	-1.25335	4.958468	1.068878	DNA.synthesis/chromatin structure.histone
					35_39431	-1.43857	1.789734	15.87444	misc.protease inhibitor/seed storage/lipid transfer protein (LTP) family protein

				35_539	-1.4465	1.319108	8.675263	development.unspecified
				35_13795	-1.45209	5.900064	1.117277	DNA.synthesis/chromatin structure.histone
				35_4845	-1.47422	1.493295	1.538464	misc.acid and other phosphatases
				35_38367	-1.52851	6.871588	1.117823	NA
				35_3149	-1.58375	1.083689	1.065876	signalling.calcium
				35_3150	-1.58505	1.092751	1.08421	signalling.calcium
				35_45254	-1.63641	1.417072	1.199124	NA
				Contig12082_at	-1.71667	1.797949	1.488448	signalling.G-proteins
				35_855	-1.92844	1.22204	1.088177	NA
				35_21656	-1.96619	1.187684	1.024638	RNA.processing.ribonucleases
				35_20108	-1.98697	1.139213	1.49503	development.unspecified
				35_6489	-2.01604	1.260498	1.237614	protein.postranslational modification
				HvMADS2	-2.10506	2.806322	2.318698	
				35_551	-2.23644	1.304532	6.865903	misc.acid and other phosphatases
				35_22270	-2.51615	2.806733	2.457364	RNA.regulation of transcription.MADS box transcription factor family
				35_13899	-3.25526	1.448751	3.446721	PS.calvin cyle.rubisco interacting
				Contig2788_x_at	-3.43745	1.282223	1.081907	stress.abiotic
				35_35443	-3.98704	2.244312	1.322297	NA
				35_42363	-4.83673	1.888754	1.499352	NA

Table 18. Correlation values (p<0.05) between transcripts that are differentially abundant between LP104 and LP106 at micromalting, and malting, seed quality and germination traits in control condition. TGW (thousand grain weight), SA (seed area), SB (seed breadth), SL (seed length), SN (seed nitrogen), SP (seed protein), MMC (malt moisture content), ME (malt extract), VIS (viscosity), MPC (malt protein content), SLN (soluble nitrogen), KI (Kolbach index), FLA (final limit of attenutation), FRI (friability), BG (beta-glucan), ST (steeping degree), TL (total losses), RES (respiration loss), g (germination), se (shoot emergence). These genes were selected based on their mapping regions that

Harvest ID	*TGW*	*SA*	*SB*	*SL*	*SN*	*SP*	*MMC*	*ME*	*VIS*	*MPC*	*SLN*	*KI*	*FLA*	*FRI*	*BG*	*ST1*	*ST2*	*ST3*	*TL*	*RES*	*g_24*	*se_24*	*g_48*	*se_48*	*g_72*	*se_72*

35_11858	-0.1	0.0	0.0	0.0	0.1	0.1	0.2	-0.1	0.2	0.1	-0.1	-0.1	-0.2	-0.3	0.2	0.0	-0.1	0.1	-0.2	-0.2	-0.2	0.0	0.0	0.0	0.1	0.1
35_1190	-0.1	0.0	0.0	-0.1	0.0	0.0	-0.1	0.0	-0.3	0.1	0.0	-0.1	0.2	0.1	-0.1	0.0	0.1	0.0	0.2	0.2	0.1	0.1	0.2	0.1	0.1	0.2
35_14643	0.2	0.2	0.3	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.2	0.2	0.0	0.2	-0.2	-0.1	0.0	0.1	0.0	0.0	0.2	0.1	0.0	0.2	-0.1	0.0
35_14926	0.0	0.0	0.1	-0.1	-0.1	-0.1	-0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.2	0.2	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.1
35_16589	-0.2	-0.1	-0.3	0.0	0.0	0.0	0.1	-0.3	0.2	0.1	-0.1	-0.2	0.0	-0.4	0.3	0.0	0.0	0.1	0.3	0.1	0.0	0.0	0.2	0.0	0.0	0.2
35_1682	-0.1	-0.1	0.1	-0.2	-0.1	-0.1	-0.1	0.2	-0.2	0.0	0.1	0.1	0.1	0.2	-0.1	0.1	0.2	0.1	0.3	0.2	0.2	0.2	0.0	0.3	0.0	0.1
35_16870	-0.1	0.0	0.0	-0.1	0.1	0.1	0.2	-0.1	0.2	0.1	-0.1	-0.1	-0.1	-0.3	0.2	0.1	0.0	0.1	0.0	-0.1	-0.1	0.0	-0.1	-0.1	-0.1	0.0
35_1733	0.3	0.3	0.2	0.2	0.0	0.0	0.0	0.1	0.0	0.0	0.2	0.1	0.0	0.2	-0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.1	0.0	0.0	-0.1
35_1734	0.2	0.2	0.2	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.2	0.2	0.0	0.1	-0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.1	-0.1	-0.1
35_17949	0.1	0.0	0.3	-0.2	0.0	0.1	0.0	0.3	-0.2	0.0	0.1	0.1	0.1	0.1	-0.2	0.1	0.1	0.0	0.1	0.3	0.0	0.2	0.0	0.0	-0.1	0.0
35_17959	-0.2	-0.1	-0.1	-0.1	0.1	0.0	0.2	0.0	0.1	-0.2	-0.1	0.0	-0.1	-0.2	0.1	0.1	0.0	0.0	0.0	0.0	-0.2	-0.1	0.1	-0.3	0.0	0.0
35_18359	0.2	0.2	-0.1	0.4	0.0	0.0	0.1	-0.3	0.2	0.0	-0.1	-0.1	-0.1	-0.1	0.2	-0.1	-0.2	-0.1	-0.3	-0.3	-0.1	-0.2	0.0	-0.2	0.0	-0.1
35_18670	-0.1	-0.1	0.2	-0.3	0.0	0.0	-0.1	0.3	-0.3	-0.1	0.1	0.1	0.1	0.2	-0.2	0.1	0.2	0.1	0.2	0.2	0.1	0.2	0.0	0.1	0.0	0.1
35_1915	-0.2	-0.2	0.1	-0.3	0.0	0.0	0.0	0.2	-0.3	0.0	0.1	0.0	0.1	0.1	-0.2	0.2	0.3	0.2	0.2	0.2	0.1	0.2	0.0	0.2	-0.1	0.1
35_19765	0.1	0.1	-0.1	0.2	0.0	0.0	0.0	-0.2	0.2	-0.1	-0.1	0.0	-0.1	0.0	0.1	0.0	-0.1	-0.1	-0.3	-0.3	-0.1	-0.1	-0.1	-0.1	0.0	-0.1
35_19891	-0.2	-0.2	-0.2	-0.2	0.1	0.1	0.1	-0.2	0.2	0.0	-0.1	-0.1	0.0	-0.3	0.2	0.0	0.0	-0.1	-0.2	-0.3	0.0	-0.1	-0.1	0.0	-0.1	0.2
35_20408	0.1	0.0	-0.1	0.0	0.3	0.3	0.1	-0.2	0.2	0.1	0.1	0.0	0.0	-0.2	0.1	0.0	0.0	-0.2	-0.2	-0.2	0.0	0.2	-0.1	0.1	-0.3	-0.1
35_21074	0.4	0.3	0.1	0.4	0.3	0.3	0.2	-0.2	0.3	0.2	0.3	0.1	-0.2	-0.3	0.2	-0.1	-0.2	-0.1	-0.2	-0.1	-0.3	0.0	-0.1	-0.1	0.0	-0.2
35_22323	0.2	0.3	0.4	0.1	0.0	0.0	0.0	0.2	-0.2	0.0	0.0	0.0	0.1	0.3	-0.2	0.0	0.0	-0.1	-0.2	0.0	0.1	-0.1	-0.1	0.0	0.2	0.0
35_24045	-0.1	-0.1	0.1	-0.2	0.0	0.0	-0.1	0.3	-0.3	0.0	0.1	0.1	0.1	0.2	-0.2	0.1	0.2	0.1	0.3	0.3	0.2	0.2	0.0	0.2	0.0	0.0
35_31025	0.0	0.1	-0.1	0.2	0.0	0.0	0.1	-0.3	0.2	0.1	-0.2	-0.2	-0.1	-0.2	0.2	-0.1	-0.2	-0.1	-0.2	-0.3	-0.2	-0.3	0.0	-0.2	0.1	0.0
35_34835	0.1	0.2	-0.1	0.3	0.0	0.0	0.2	-0.3	0.3	0.1	-0.1	-0.1	-0.1	-0.2	0.2	-0.1	-0.2	-0.1	-0.3	-0.3	-0.2	-0.2	0.0	-0.2	0.1	-0.1
35_3559	0.0	0.0	0.0	0.0	-0.1	-0.1	0.0	0.1	-0.3	0.1	0.1	0.0	0.1	0.1	-0.1	-0.1	-0.1	0.1	0.3	0.3	0.1	-0.1	0.1	0.0	0.1	0.1
35_4343	0.2	0.3	0.0	0.4	-0.3	-0.3	0.0	0.0	0.0	-0.1	-0.3	-0.1	0.0	0.0	0.1	-0.4	-0.4	-0.3	-0.3	-0.2	-0.2	-0.3	-0.2	-0.4	0.0	-0.2
35_4383	0.0	-0.1	0.1	-0.2	-0.1	-0.1	-0.2	0.2	-0.4	-0.1	-0.1	0.0	0.3	0.2	-0.3	0.1	0.1	-0.1	0.3	0.4	0.0	0.0	0.1	0.0	0.0	0.1
35_4384	0.0	-0.1	0.1	-0.2	-0.2	-0.2	-0.1	0.2	-0.4	-0.1	-0.1	0.0	0.2	0.2	-0.3	0.0	0.1	-0.1	0.2	0.4	0.0	0.0	0.1	0.0	0.0	0.1
35_6360	0.1	0.1	0.3	0.0	-0.1	-0.1	-0.2	0.2	-0.2	0.0	0.0	0.0	0.1	0.2	-0.2	0.0	-0.1	0.0	0.0	0.2	-0.1	-0.1	0.0	-0.2	0.2	0.0
35_7974	0.0	0.0	0.2	-0.2	0.1	0.1	0.0	0.2	-0.2	0.0	0.1	0.0	0.0	0.1	-0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.3	0.0	0.1	-0.1	0.0
35_8108	-0.2	0.0	-0.1	-0.1	0.1	0.1	0.0	-0.2	0.3	0.1	-0.3	-0.4	-0.1	-0.5	0.5	-0.1	-0.1	0.1	0.2	0.0	-0.3	-0.2	-0.1	-0.1	0.3	0.3

35_9257	-0.2	-0.2	-0.2	-0.1	-0.2	-0.2	-0.1	0.0	-0.3	-0.1	-0.1	0.1	0.1	0.2	-0.2	0.0	0.1	0.1	0.3	0.1	0.3	0.1	0.1	0.1	0.0	0.0
35_9865	0.0	0.0	0.1	0.0	0.0	0.0	-0.1	0.1	-0.4	0.0	0.1	0.1	0.2	0.3	-0.3	0.0	0.1	0.2	0.2	0.2	0.1	0.0	0.1	0.0	0.2	0.2
35_9993	0.0	-0.1	0.0	-0.1	0.2	0.2	0.0	0.0	0.0	0.1	0.0	0.0	-0.1	-0.1	0.0	0.0	0.0	0.2	-0.1	-0.2	0.0	-0.1	0.0	0.0	0.0	0.2
Contig12910_at	-0.2	-0.1	-0.2	-0.1	0.0	0.0	0.1	-0.2	0.2	0.0	-0.2	-0.2	-0.2	-0.3	0.3	0.1	0.0	0.1	0.0	-0.2	-0.3	-0.3	0.0	-0.3	0.1	0.2
Contig13600_at	-0.1	-0.1	0.0	-0.1	-0.1	-0.1	0.0	0.1	-0.3	0.0	0.0	-0.1	0.1	0.2	-0.2	0.0	0.1	0.2	0.2	0.1	0.2	-0.1	0.2	0.1	0.2	0.2
Contig13857_at	0.0	0.0	0.0	0.0	-0.1	-0.1	-0.1	0.2	0.0	0.0	0.1	0.1	0.0	0.2	-0.2	0.2	0.2	0.1	0.2	0.3	0.1	0.1	0.1	0.0	-0.1	-0.1
Contig14119_at	0.2	0.2	-0.1	0.4	0.1	0.1	0.1	-0.3	0.2	0.1	0.0	-0.1	0.0	-0.1	0.1	-0.2	-0.2	-0.2	-0.3	-0.2	-0.1	-0.2	0.0	-0.1	0.0	-0.1
Contig16024_at	0.3	0.3	0.1	0.4	0.3	0.3	0.2	-0.3	0.3	0.2	0.3	0.1	-0.2	-0.3	0.2	-0.1	-0.2	-0.1	-0.2	0.0	-0.2	0.0	-0.1	-0.1	0.0	-0.2
Contig16528_at	0.1	0.2	-0.1	0.3	0.0	0.0	0.2	-0.3	0.3	0.1	-0.1	-0.1	-0.1	-0.2	0.2	-0.1	-0.2	-0.1	-0.3	-0.3	-0.1	-0.2	0.0	-0.2	0.1	-0.1
Contig18767_at	-0.2	-0.1	-0.4	0.0	0.1	0.1	0.0	-0.1	0.1	0.1	0.1	0.0	0.0	-0.2	0.1	0.0	0.0	0.0	0.2	0.2	0.1	0.1	0.1	0.2	0.1	0.1
Contig24832_at	0.0	0.0	0.1	-0.1	0.2	0.2	0.2	-0.2	0.4	0.2	0.0	-0.2	-0.1	-0.4	0.4	0.1	0.1	0.1	-0.1	0.0	-0.2	0.0	0.1	0.0	0.1	0.2
Contig2790_s_at	-0.1	-0.1	-0.1	-0.1	0.0	0.0	-0.1	0.0	0.0	0.1	0.1	-0.1	0.0	0.1	0.0	0.2	0.2	0.0	0.2	0.3	0.2	0.2	0.2	0.3	0.0	0.1
Contig7517_at	0.1	-0.1	0.0	-0.1	0.0	0.0	-0.2	0.0	0.2	0.1	0.3	0.2	0.0	0.1	0.0	0.1	0.2	0.0	0.0	0.1	0.3	0.2	-0.1	0.4	-0.3	-0.1
Contig9601_s_at	-0.2	-0.1	0.0	-0.1	-0.2	-0.2	-0.1	0.1	-0.3	0.0	0.0	0.0	0.1	0.1	-0.1	0.0	0.1	0.2	0.2	0.2	0.2	0.0	0.1	0.2	0.1	0.2
EBem05_SQ004_I01_at	0.1	0.1	-0.1	0.2	0.3	0.3	0.1	-0.3	0.2	0.1	0.2	0.1	0.1	-0.3	0.2	-0.1	-0.1	-0.2	-0.2	-0.1	0.0	0.0	0.0	0.1	-0.1	0.0
HS08H22u_s_at	0.1	0.2	-0.1	0.3	0.0	0.0	0.1	-0.3	0.3	0.1	-0.1	-0.1	-0.1	-0.2	0.2	-0.1	-0.2	-0.1	-0.2	-0.3	-0.1	-0.2	0.0	-0.1	0.0	-0.1
HvABI5	0.0	0.1	0.1	0.2	-0.1	-0.1	0.0	0.0	-0.1	0.0	0.1	0.1	0.1	0.2	-0.1	-0.2	-0.1	-0.3	-0.2	-0.1	0.1	0.0	0.0	0.1	0.0	-0.2
HvCKX1	0.4	0.3	0.1	0.4	0.3	0.3	0.1	-0.2	0.3	0.2	0.3	0.1	-0.2	-0.3	0.2	-0.2	-0.2	-0.1	-0.2	-0.1	-0.2	0.0	-0.1	-0.1	0.0	-0.2

Table 19. Correlation values (p<0.05) between transcripts that are differentially abundant between LP104 and LP106 at micromalting, and malting, seed quality and germination traits in stress condition. TGW (thousand grain weight), SA (seed area), SB (seed breadth), SL (seed length), SN (seed nitrogen), SP (seed protein), MMC (malt moisture content), ME (malt extract), VIS (viscosity), MPC (malt protein content), SLN (soluble nitrogen), KI (Kolbach index), FLA (final limit of attenutation), FRI (friability), BG (beta-glucan), ST (steeping degree), TL (total losses), RES (respiration loss), g (germination), se (shoot emergence).

Harvest ID	*TGW*	*SB*	*SL*	*C*	*C/N*	*SSC*	*MMC*	*ME*	*VIS*	*MPC*	*FLA*	*FRI*	*BG*	*SD1*	*SD2*	*SD3*	*TL*	*REL*	*RGL*	*g_24*	*se_24*	*g_48*	*se_48*	*g_72*	*se_72*
35_11858	0.0	0.0	0.2	-0.4	0.0	-0.1	0.3	-0.1	0.3	0.2	-0.2	-0.4	0.3	-0.1	-0.1	0.1	0.0	-0.1	0.1	-0.2	-0.3	0.1	-0.2	0.0	0.1
35_1190	-0.1	0.0	-0.3	0.2	-0.1	-0.2	0.0	0.0	-0.1	0.1	0.0	0.1	-0.1	0.0	0.2	0.0	0.2	0.1	0.2	0.0	0.3	0.0	0.2	0.0	0.0
35_14926	-0.1	-0.1	-0.2	0.3	0.0	-0.1	-0.3	0.2	-0.1	-0.2	0.0	0.2	0.0	-0.1	-0.1	-0.2	0.0	0.0	0.0	0.1	0.2	0.0	0.2	0.2	0.1

35_16589	-0.1	-0.3	0.1	-0.1	0.0	0.0	0.0	-0.1	-0.2	-0.2	0.1	-0.1	0.0	0.0	-0.1	0.0	0.2	0.0	0.3	0.0	-0.1	0.1	0.0	0.3	0.2
35_1682	0.0	0.2	-0.3	0.1	-0.1	-0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.3	0.0	0.2	0.2	0.2	0.1	0.3	0.0	0.2	-0.1	-0.1
35_16870	0.1	0.1	-0.1	-0.1	-0.2	-0.1	0.2	-0.1	0.2	0.1	0.1	-0.3	0.2	-0.1	-0.1	0.1	0.3	0.1	0.3	-0.2	-0.1	0.1	-0.1	0.1	0.1
35_1733	0.0	0.0	-0.2	0.3	-0.1	0.0	-0.4	-0.1	0.1	0.0	0.0	0.0	0.0	0.0	-0.1	-0.1	0.0	0.2	-0.1	0.1	0.1	-0.1	0.1	0.0	-0.1
35_1734	0.0	0.1	-0.2	0.4	0.0	0.0	-0.4	-0.1	0.1	0.0	0.0	0.0	0.1	-0.1	-0.1	-0.1	0.0	0.2	0.0	0.1	0.1	-0.1	0.1	0.1	-0.1
35_17949	0.1	0.1	-0.2	0.1	0.0	0.1	0.0	-0.1	0.1	0.2	0.0	0.0	-0.1	0.0	0.2	0.0	0.2	0.4	0.1	-0.1	0.1	-0.1	0.1	-0.2	-0.2
35_17959	-0.1	-0.1	0.0	-0.1	-0.1	0.0	-0.2	0.0	-0.2	-0.2	0.2	-0.1	0.0	0.0	-0.2	0.1	0.1	-0.1	0.1	-0.1	-0.1	0.0	-0.1	0.2	0.3
35_18359	0.0	-0.1	0.3	-0.1	0.1	0.1	0.0	-0.1	0.1	-0.1	-0.1	-0.1	0.1	-0.1	-0.3	-0.2	-0.3	-0.3	-0.2	-0.1	-0.2	0.0	-0.2	0.2	0.1
35_18670	-0.2	0.1	-0.3	0.1	-0.1	-0.1	-0.1	0.1	-0.1	0.1	0.1	0.1	-0.1	0.1	0.3	0.1	0.3	0.1	0.2	0.1	0.3	0.0	0.3	-0.1	0.0
35_1915	-0.2	0.1	-0.3	0.0	-0.1	-0.2	0.0	0.1	0.0	0.2	0.0	0.0	0.0	0.1	0.3	0.2	0.2	0.1	0.3	0.1	0.1	0.0	0.3	-0.1	0.0
35_19765	0.2	0.0	0.3	-0.1	0.0	0.1	0.0	0.0	0.1	-0.2	-0.1	-0.1	0.1	0.0	-0.2	-0.2	-0.3	-0.2	-0.3	-0.3	-0.1	0.0	-0.3	0.0	0.0
35_19811	0.0	0.0	0.1	-0.4	0.0	-0.1	0.3	-0.1	0.3	0.2	-0.3	-0.4	0.3	0.0	0.0	0.1	0.0	-0.1	0.1	-0.2	-0.2	0.1	-0.1	-0.1	0.0
35_19891	0.1	-0.1	0.3	-0.4	0.1	0.1	0.3	-0.1	0.1	0.0	-0.1	-0.2	0.1	-0.1	-0.1	0.1	0.0	-0.1	0.0	-0.3	-0.3	0.1	-0.3	0.1	0.1
35_20408	0.3	0.2	0.1	-0.1	-0.2	0.1	0.3	-0.4	0.3	0.4	-0.2	-0.3	0.2	-0.2	0.0	0.1	0.1	0.1	0.1	-0.2	-0.2	0.0	-0.1	-0.1	-0.2
35_21074	0.3	0.2	0.1	0.1	-0.2	0.1	0.0	-0.2	0.3	0.2	-0.1	-0.4	0.3	-0.2	-0.2	0.0	0.1	0.2	0.0	-0.2	-0.1	-0.1	-0.2	0.0	-0.1
35_22323	0.0	0.2	-0.1	0.1	0.0	0.0	0.1	0.2	-0.1	0.0	0.1	0.3	-0.2	-0.1	0.0	0.0	-0.1	-0.1	0.0	0.2	0.2	-0.1	0.1	-0.2	-0.1
35_22727	0.2	0.2	0.0	-0.1	-0.2	0.1	0.3	-0.3	0.3	0.3	-0.2	-0.2	0.1	-0.2	0.0	0.1	0.1	0.2	0.1	-0.1	0.0	0.0	-0.1	0.0	-0.1
35_24045	0.0	0.1	-0.3	0.2	-0.1	-0.1	-0.1	0.1	-0.1	0.1	0.1	0.2	-0.2	0.1	0.3	0.1	0.3	0.3	0.2	0.1	0.2	-0.1	0.3	-0.1	-0.1
35_31025	0.0	-0.2	0.2	-0.2	0.1	0.0	0.1	-0.1	0.2	0.0	-0.1	-0.2	0.2	-0.1	-0.3	-0.2	-0.2	-0.3	-0.1	-0.2	-0.2	0.1	-0.3	0.2	0.1
35_34835	0.0	-0.1	0.2	-0.2	0.1	0.0	0.1	-0.1	0.2	-0.1	-0.1	-0.2	0.2	-0.1	-0.3	-0.2	-0.3	-0.3	-0.2	-0.1	-0.2	0.1	-0.3	0.2	0.1
35_3559	0.2	0.0	0.1	0.0	0.0	0.1	0.1	0.2	-0.1	-0.1	0.1	0.0	-0.1	-0.2	-0.1	0.0	0.4	0.1	0.4	0.1	0.1	0.0	0.1	0.2	0.1
35_41754	0.0	0.2	-0.3	0.1	-0.1	-0.1	-0.1	0.1	-0.1	0.2	0.0	0.1	-0.1	0.1	0.2	0.2	0.3	0.3	0.3	0.1	0.2	0.0	0.2	-0.1	-0.1
35_4343	0.1	0.0	0.3	0.1	0.2	0.1	-0.1	-0.1	0.1	-0.2	0.0	-0.1	0.1	-0.2	-0.4	-0.3	-0.3	-0.2	-0.2	0.0	-0.2	0.0	-0.2	0.2	0.0
35_4383	-0.1	0.0	-0.2	0.2	0.2	0.0	-0.3	0.1	-0.2	-0.1	0.2	0.3	-0.3	0.1	0.2	0.0	0.1	0.2	0.0	0.2	0.2	-0.1	0.2	-0.2	-0.2
35_4384	-0.1	0.0	-0.2	0.2	0.2	0.1	-0.3	0.1	-0.3	-0.1	0.2	0.3	-0.3	0.2	0.2	0.0	0.1	0.2	0.0	0.2	0.2	-0.2	0.3	-0.2	-0.1
35_6360	0.1	0.2	-0.2	0.3	-0.1	-0.2	-0.1	0.1	0.0	0.0	0.1	0.1	-0.1	-0.2	-0.1	-0.1	0.2	0.1	0.2	0.0	0.2	0.0	0.0	0.0	0.1
35_7974	0.0	0.1	-0.3	0.2	-0.1	-0.1	-0.1	0.0	0.0	0.2	0.0	0.0	0.0	0.1	0.3	0.1	0.4	0.3	0.3	0.1	0.2	-0.1	0.3	-0.2	-0.1
35_8108	-0.2	-0.1	-0.1	-0.2	0.0	-0.3	0.1	0.0	0.1	0.0	-0.2	-0.3	0.3	-0.1	-0.2	-0.1	0.2	-0.1	0.3	-0.1	0.0	0.2	0.1	0.3	0.2
35_9257	-0.1	-0.1	0.0	0.1	0.2	0.0	-0.1	0.2	-0.4	-0.3	0.2	0.4	-0.3	0.1	0.0	0.0	0.0	-0.1	0.1	0.3	0.2	-0.1	0.1	-0.1	0.0

35_9865	-0.1	0.0	-0.2	0.2	0.0	-0.2	-0.1	0.2	-0.2	0.0	0.1	0.1	-0.2	0.0	0.0	0.0	0.3	0.0	0.3	0.1	0.2	0.0	0.2	0.1	0.1
35_9993	-0.1	-0.1	0.0	-0.4	0.1	-0.1	0.4	0.0	0.1	0.2	-0.2	-0.2	0.1	-0.1	0.0	0.2	0.0	-0.1	0.1	-0.1	-0.2	0.0	0.0	-0.1	0.0
Contig12770_at	0.0	0.2	-0.2	0.2	-0.1	-0.3	-0.1	0.2	0.1	0.0	0.0	0.1	0.0	0.0	0.1	-0.2	0.0	-0.1	0.0	0.1	0.4	0.2	0.1	0.0	0.1
Contig12910_at	-0.1	-0.2	0.1	-0.2	0.0	0.0	0.0	0.0	0.0	-0.1	0.1	-0.2	0.2	-0.1	-0.3	-0.1	-0.1	-0.2	0.0	-0.2	-0.3	0.2	-0.3	0.2	0.3
Contig13600_at	-0.2	-0.2	0.0	-0.2	0.3	0.0	0.1	0.3	-0.2	-0.1	0.0	0.2	-0.1	0.0	0.1	0.0	-0.1	-0.2	0.1	0.2	0.0	0.0	0.2	0.0	0.0
Contig13857_at	0.0	0.1	-0.2	0.4	-0.1	0.0	-0.3	0.0	-0.1	-0.1	0.1	0.2	-0.1	0.1	0.1	-0.1	0.0	0.1	-0.1	0.2	0.3	-0.1	0.2	0.0	-0.1
Contig14119_at	0.0	-0.1	0.2	0.1	0.0	0.1	-0.1	-0.2	0.1	-0.1	0.0	-0.1	0.1	-0.1	-0.3	-0.2	-0.3	-0.2	-0.3	-0.2	-0.1	0.0	-0.3	0.2	0.1
Contig16024_at	0.3	0.2	0.1	0.0	-0.1	0.2	0.0	-0.2	0.3	0.2	-0.1	-0.4	0.3	-0.1	-0.2	0.0	0.1	0.2	0.0	-0.3	-0.1	-0.1	-0.2	0.0	-0.2
Contig16528_at	0.0	-0.1	0.2	-0.2	0.1	0.0	0.1	-0.1	0.2	-0.1	-0.1	-0.2	0.2	-0.1	-0.3	-0.2	-0.3	-0.3	-0.2	-0.1	-0.3	0.1	-0.3	0.2	0.1
Contig18767_at	-0.2	-0.3	0.1	-0.2	0.0	-0.1	0.0	0.0	0.0	-0.1	-0.1	-0.1	0.0	0.3	0.2	0.0	-0.1	-0.1	0.0	-0.2	-0.2	0.0	-0.2	-0.1	0.0
Contig24832_at	0.1	0.1	0.0	0.0	-0.1	0.0	-0.1	-0.3	0.4	0.2	-0.2	-0.5	0.4	0.2	0.1	0.0	0.1	0.3	0.0	-0.2	0.0	0.0	-0.1	-0.1	-0.1
Contig2790_s_at	0.0	0.2	-0.2	0.1	-0.1	-0.1	-0.1	0.0	0.2	0.1	-0.3	0.0	0.2	0.3	0.4	0.0	-0.1	0.0	-0.1	0.0	0.3	-0.1	0.2	-0.1	-0.1
Contig5780_at	-0.2	-0.1	-0.1	0.1	0.2	-0.1	-0.1	0.2	-0.2	-0.2	0.0	0.4	-0.2	0.1	0.1	-0.2	-0.2	-0.1	-0.2	0.2	0.2	0.0	0.2	-0.1	-0.1
Contig6989_at	0.1	0.1	-0.1	0.0	-0.3	-0.1	0.1	-0.2	0.3	0.2	0.0	-0.3	0.2	-0.1	-0.1	0.0	0.3	0.1	0.3	-0.2	-0.1	0.0	-0.1	0.1	0.1
Contig7517_at	0.2	0.3	-0.1	0.1	-0.2	0.2	-0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.2	0.3	0.0	-0.1	0.2	-0.2	0.0	0.2	0.0	0.1	-0.2	-0.2
Contig9601_s_at	-0.1	-0.1	0.1	-0.1	0.3	0.1	0.1	0.3	-0.2	-0.2	0.0	0.2	-0.2	0.0	0.1	0.1	0.0	-0.1	0.1	0.2	0.0	0.1	0.2	0.1	0.0
EBem05_SQ004_I01_at	0.1	0.1	0.2	0.0	-0.1	0.2	0.1	-0.4	0.2	0.2	-0.2	-0.3	0.2	0.1	0.0	0.0	0.0	0.1	0.0	-0.1	-0.1	0.1	-0.2	0.1	0.0
HS08H22u_s_at	0.1	-0.1	0.3	-0.2	0.1	0.1	0.1	-0.1	0.1	-0.1	-0.1	-0.2	0.1	-0.1	-0.3	-0.1	-0.2	-0.1	-0.2	-0.2	-0.2	0.1	-0.3	0.1	0.0

Table 20. List of possible malting quality candidates, with their annotation.

Harvest assembly ID	Annotation
35_11858	not assigned.unknown
35_1190	redox.dismutases and catalases
35_14643	hormone metabolism.jasmonate.synthesis-degradation.lipoxygenase
35_14926	C1-metabolism
35_16589	not assigned.unknown
35_1682	not assigned.unknown
35_16870	glycolysis.PK
35_1733	not assigned.no ontology
35_1734	not assigned.no ontology
35_17949	DNA.synthesis/chromatin structure
35_17959	hormone metabolism.cytokinin.signal transduction
35_18359	not assigned.unknown
35_18670	lipid metabolism.lipid degradation.lysophospholipases.carboxylesterase
35_1915	cell wall.precursor synthesis.phosphomannomutase
35_19765	not assigned.no ontology
35_19811	RNA.regulation of transcription.unclassified
35_19891	protein.postranslational modification
35_20408	not assigned.no ontology
35_21074	hormone metabolism.cytokinin.synthesis-degradation
35_22323	not assigned.unknown
35_22727	RNA.regulation of transcription.MYB-related transcription factor family
35_24045	redox.thioredoxin
35_31025	not assigned.unknown
35_34835	not assigned.unknown
35_3559	not assigned.no ontology
35_41754	not assigned.unknown
35_4343	protein.degradation.ubiquitin.E3.RING
35_4383	not assigned.unknown
35_4384	not assigned.unknown
35_6360	not assigned.unknown
35_7974	nucleotide metabolism.synthesis.pyrimidine.dihydroorotate dehydrogenase
35_8108	transport.ABC transporters and multidrug resistance systems
35_9257	not assigned.unknown
35_9865	RNA.regulation of transcription.SET-domain transcriptional regulator family
35_9993	not assigned.unknown
Contig12770_at	signalling.receptor kinases.wall associated kinase
Contig12910_at	lipid metabolism.'exotics' (steroids, squalene etc)
Contig13600_at	hormone metabolism.auxin.synthesis-degradation
Contig13857_at	transporter.sugars
Contig14119_at	RNA.regulation of transcription.MYB-related transcription factor family
Contig16024_at	hormone metabolism.cytokinin.synthesis-degradation
Contig16528_at	RNA.regulation of transcription.General Transcription
Contig18767_at	transport.unspecified cations

Contig24832_at	transport.metabolite transporters at the envelope membrane
Contig2790_s_at	stress.abiotic
Contig5780_at	PS.lightreaction.cyclic electron flow-chlororespiration
Contig6989_at	glycolysis.PK
Contig7517_at	RNA.regulation of transcription.WRKY domain transcription factor family
Contig9601_s_at	lipid metabolism.'exotics' (steroids, squalene etc)
EBem05_SQ004_I01_at	mitochondrial electron transport / ATP synthesis.NADH-DH.localisation not clear
HS08H22u_s_at	lipid metabolism.FA synthesis and FA elongation.acyl-CoA binding protein

9 Acknowledgement

First and foremost, I would like to express my gratitude to Dr. Nese Sreenivasulu for the given opportunity to start my journey as a PhD student at IPK. I thank him for the introduction into the Abiotic Stress Genomics group and for the provided help and knowledge.

Special thanks go to my supervisor Dr. Markus Kuhlmann for supporting me and encouraging my research. His guidance helped me throughout the research and in writing this thesis. I will always be grateful to him for his patient support and valuable advice.

Furthermore, I wish to thank Prof. Dr. Altmann for his support as a mentor, and Prof. Dr. Humbeck and Prof. Dr. Dröge-Laser for agreeing to evaluate this thesis.

I thank Dr. Christiane Seiler for being a steady point of reference and her efforts in troubleshooting in the lab, greenhouse and data analysis. I appreciate all her contributions of time and ideas.

I thank the members of the Inno Grain Malt project Prof. Dr. Andreas Graner and Dr. Viktor Korzun for fruitful discussions, as well as Dr. Andriy Kochevenko for precious pieces of advice regarding data analysis.

I am grateful for the financial support received from the Inno Grain Malt project founded by BMBF.

There are many persons who had offered me technical help and valuable teaching moments that allowed me to master various techniques. Many thanks go to Gabi Einert for the immense help in the greenhouse and seed dissections; Jana Lorenz and Mandy Püffeld for reagent preparations, support with molecular biology techniques and their help in the greenhouse; Ilka Schmelling for the time dedicated for ultracentrifugation, Heiko Weichert for the help with freeze-drying and Katrin Blaschek for precise starch measurements.

I thank all the past members of the Abiotic Stress Genomics group: Hongwen, Chandar, Harsha, Rajesh for constructive and inspiring discussions and lab tips, but, even more important, for all the friendly and fun moments shared in the office and group events.

I would like to thank Dr. Hirofumi Ishihara for the collaboration and for teaching me the polysome extraction procedure, as well as for his hospitality during my stay at the Max Planck Institute in Golm.

Hormone analysis would not be possible without Dr. Kai Eggert. I thank him for the technical support, his time, and data analysis.

My thanks go to Dr. Andrea Bräutigam for gene ontology analysis.

Many thanks to Dr. Britt Leps, who assisted me in administrative matters and simplified my stay at IPK.

Finally, I am grateful to my family for being always there for me and my parents for being cool and inspiring souls, my friends for the laughter, my partner for the love that we share, and Tom Petty for his tunes.

10 Appendix

Curriculum vitae

Personal data

Name: Korana
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Education:

October 2012 – PhD
Martin Luther University, Halle, Germany
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September 2008 – September 2011: Master's Degree in Functional Genomics
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September 2004 – September 2008: Bachelor's Degree in Biology
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October 2012 – January 2017: Graduate research assistant
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October 2010 – October 2011: Undergraduate research assistant
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January 2008 – July 2008: Undergraduate research assistant
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Publication:

Surdonja, K.; Eggert, K.; Hajirezaei, M.-R.; Harshavardhan, V.T.; Seiler, C.; von Wirén, N.; Sreenivasulu, N.; Kuhlmann, M. Increase of DNA Methylation at the *HvCKX2.1* Promoter by Terminal Drought Stress in Barley. *Epigenomes* **2017**, *1*, 9

Andriy Kochevenko, Yong Jiang, Christiane Seiler, Korana Surdonja, Sonja Kollers, Jochen Christoph Reif , Viktor, Korzun and Andreas Graner. Identification of QTLs for malting quality and yield using DH population derived from two elite breeding lines with distinct tolerance to abiotic stress. Submitted

Eidesstattliche Erklärung / *Declaration under Oath*

Ich erkläre an Eides statt, dass ich die Arbeit selbstständig und ohne fremde Hilfe verfasst, keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

I declare under penalty of perjury that this thesis is my own work entirely and has been written without any help from other people. I used only the sources mentioned and included all the citations correctly both in word or content.

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