STRESS-INDUCED GERMINATION VIGOR AND ITS TRANSLATION TO SEEDLING VIGOR IN *BETA VULGARIS* L.

By

Rachel P. Naegele

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ABSTRACT
STRESS-INDUCED GERMINATION VIGOR AND ITS TRANSLATION TO SEEDLING VIGOR IN *BETA VULGARIS* L.

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Beta vulgaris L., sugarbeet, is an important plant for sucrose production in the U.S. and worldwide. One limitation for sugarbeet production is poor germination. Breeding for improved germination and seedling vigor has been unsuccessful due to low heritability of traits controlling germination vigor, few molecular markers and high environmental variability. Germination and seedling responses to stress in two beet varieties with differing vigor were studied in hydrogen peroxide and water. Differences in water absorption and internal hydrogen peroxide concentrations were observed between varieties. Gene expression changes were identified qualitatively (343 genes) and quantitatively (48 genes) during germination. In both varieties, germination was characterized by a rapid uptake of water and rapid gene activation within the first 24 h of imbibition. Upregulation of putative *LTP4* (lipid transfer protein), *MPK4* (MAP Kinase 4), *BRI1* (Brassinosteroid 1), and *MKK9* (MAPK Kinase 9) was associated with response to H_2O_2. The 48 genes, tested for expression in germination vigor, were evaluated for their ability to predict vigor in 3-week old seedlings of the same two varieties. Gene expression of seedlings treated with H_2O, *Aphanomyces sp.*, or *Rhizopus sp.* had different quantitative and temporal expression patterns depending on the variety and treatment. *BRI1* and *CAF1* (CCR4-NOT Associated Factor) were upregulated in both varieties in response to pathogen treatments. Of the 48 genes quantitatively tested between seeds and seedlings, varieties and the stress treatments, only *BRI1*, *LTP4* and *MKK9* were consistently upregulated. These genes may be useful as molecular markers for breeding efforts to enhance seedling vigor.

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LITERATURE REVIEW

Beta vulgaris L. belongs to the plant family Amarantheaceae, sub family Chenopodiaceae, in the order Caryophyllales (Kadereit et al. 2003). Amaranthaceae is a large (2,500 species) and diverse (180 genera) family with nutritionally important crops such as amaranth (*Amaranthus cruentus*), a high-protein grain and a calcium-rich leaf grown in the Americas, spinach (*Spinacia oleracea*), a well-known leafy vegetable used as an iron source, and quinoa *(Chenopodium quinoa*), a protein-rich staple crop of the indigenous people of southern South America (Bewley 1997). Some well-known noxious weed species including Lambsquarter (*Chenopodium album*), Redroot Pigweed (*Amaranthus retroflexus*), Tumbleweed (*Amaranthus albus*) and Kochia (*Kochia sp*.) are also members of this family (Muller 2005).

The Amarantheaceae is known for its diversity of plant species, its ability to tolerate stress, and the production of unique substances. Members produce the pigment betalain in place of anthocyanin, some produce squalene oil, an oil traditionally found in shark livers used for cancer treatment, and some members are halophytic and tolerant of suboptimal conditions when adults (Cai et al. 2005; Dini et al. 1992; Flowers 1972; Ryan et al. 2007; Strack et al. 2003). These scientifically interesting and economically useful characteristics have renewed interest in Amaranthaceae, in particular the Chenopodiaceae, for medicines, biofuels, land reclamation, and dyes, in addition to their current uses. In the Chenopodiaceae, the genus *Beta* is economically important. One of the species within the genus *Beta*, *B. vulgaris* L., can be divided into the four crop groups, sugarbeet, table beet (or red beet), swiss chard and fodder beet, and the wild and weedy relatives (*B. vulgaris* spp. *maritima* and *B. vulgaris* spp. *macrocarpa*) (Arnaud et al. 2010; Driessen et al. 2001; Fenart et al. 2008; Panella and Lewellen 2007).

Annual weedy and wild members of *Beta vulgaris* are found along coastlines throughout Italy and Greece, the Middle East and Northern Africa (Draycott 2006). *Beta vulgaris* is thought to have originated along the Mediterranean coast, and fossil records of beet roots, flowers and leaves suggest that some part of beets have been consumed since the Neolithic age. The earliest written records of consumption and possible cultivation of *Beta vulgari*s stem from Babylon (8th century) (Zohary 2000). Present day, cultivated *Beta vulgaris* L. spp. *vulgaris* is divided into three separate market classes based on their morphology: vegetable, ornamental and row crops.

Chards, also known as leaf beets, are grown for their nutrient-rich leaves and fleshy petioles. Chards have small, fleshy roots and contributed the extra alleles to give the sugarbeet its high sucrose levels (Draycott 2006; Fischer 1989). Chards are grown as both a vegetable and an ornamental crop. Table beets are harvested early during development and have small, round roots with lower sucrose content (Draycott 2006). Table beet's roots are mostly red or yellow in color; though specialty beets, e.g. Chioggia, have red roots with white rings. Table beets are primarily grown as a vegetable crop. Root shape, color and size are the main morphological differences between table, fodder, and sugar beets. Fodder beets, grown for animal feed, have a long, conical, yellow or orange root that contains low quantities of sucrose. Sugarbeet has a long, white, conical taproot that contains, on average, 18% sucrose (Lohaus 1994). Sugarbeet and fodder beets are grown as a field crops unlike red beets and chards. Seeds are planted in early spring and roots are machine-harvested late in the season to maximize sucrose accumulation (Scott et al. 1973).

Originating in Eastern Europe in the 1700s, the sugarbeet was likely the progeny of a cross between a table beet (or fodder beet) and a chard (Fischer 1989). The chemical composition of the sweetener and its identification as sucrose occurred in the 1740s, but it wasn't until the 1800s

that sugarbeets were marketed and grown as an alternative source of cane sugar in Europe (Ali 2004). Sugarbeet, a temperate annual crop, provided a local alternative to the importation of sugarcane from tropical and subtropical regions of South America and Asia. Modern sugarbeet is grown throughout the Great Lakes region and the western United States, Italy, France, Germany, Russia, Turkey, and the Ukraine as a sucrose source (FAO 2005). Beet sugar accounted for approximately 50% of the sucrose consumed in the United States in 2008 and 25% worldwide (Khan 2010). In Michigan, the sugarbeet industry harvested 149,000 acres of sugarbeets worth $140 million dollars in 2008 (National Agricultural Statistics Service).

Low germination, weeds, and an array of diseases work together to reduce the profitability of this crop. Many of the chemical inputs required to control weed species can also damage the beet crop, since many of the common weeds, e.g. Lambsquarter and pigweed, are in the same family (Chenopodiaceae) as sugarbeet (Wille and Morishita 1999). Preemergence herbicides are used to reduce weed pressure prior to germination and postemergence control requires timely micro-rate treatments. In 2008, Monsanto (St. Louis, MO, USA) released RoundUp Ready sugarbeets, which contain glyphosphate resistance. This allowed growers to control weeds by using the herbicide RoundUp, a broad-spectrum glyphosate herbicide. 80% of the sugarbeets grown in the U.S. in 2008 were RoundUp Ready, decreasing costs associated with herbicide sprays for weed control, while increasing fees associated with seed technology (Kemp et al. 2009; Khan 2008). RoundUp Ready varieties do not necessarily have the same level of seedling and adult disease resistance to abiotic and biotic stresses as the non-genetically modified varieties.

Disease resistance is important at both the seedling and adult stage. *Rhizoctonia solani, Cercospera beticola* and *Rhizopus spp.* are examples of pathogens that cause adult and post harvest diseases that can cause major economic losses if left untreated. In adult sugarbeets,

diseases are controlled through carefully timed fungicide sprays and selective breeding, though losses still occur (Campbell and Klotz 2006; Draycott 2006; Kiewnick et al. 2001; McGrann et al. 2009; Stevens 2007). *Aphanomyces cochlioides*, *Pythium spp.* and *Rhizoctonia solani.* are examples of pathogens that can cause sugarbeet seedling death, resulting in devastating stand losses if left untreated, and at times even when treated. Resistant germplasm and fungicide-treated seeds are used as preventative methods to reduce seedling damping-off disease incidence.

For the past two centuries, breeding efforts have focused on bringing disease resistance, sucrose accumulation, biennialism, and storage longevity into cultivated beet from its weedy, wild, and un-adapted relatives, but germination vigor has been neglected due to its low heritability (Sedlmayr 1960). Industry members have overcome much of the variability in germination vigor by using "primed" or "pre-germinated" fungicide-treated seeds, and other management practices (Bene and Eori 1992; Orzeszko-Rywka and Podlaski 2003). Yet, primed and treated seeds are only a small proportion of the total seed harvested. Much of the seed harvested is discarded because seeds are underdeveloped or internal disease concentrations are too high for a given seedlot (Kadereit et al. 2003). Sugarbeet seed and seedling mortality are two of the costliest, and possibly unrealized, limitations in sugar beet production (Ali 2004). Even today, germination in the field ranges from 0 to 100%, with an average of 60% depending upon field location, weather, and biotic pressures (McGrath et al. 2000). In order to improve profitability of this crop, methods to enhance germination vigor through mechanical or breeding means are necessary.

Cytogenetically, the chromosomes in sugarbeet, red beet, and chard are almost indistinguishable (Nakamura et al. 1991; Biancardi 2005). Most members of this species are diploid (though some triploid lines have been developed through crosses with artificial

tetraploids) and have a base chromosome number of 2n=2x=18. Despite morphological differences in root size, shape, and color, they are freely inter-crossable (Arnaud et al. 2010; Schondelmaier and Jung 1997). *Beta vulgaris* is an outcrossing species with high levels of self-incompatibility. Breakdown of self-incompatibility has been identified, but few inbred lines have been developed. Breeding efforts rely primarily on population improvement for incorporating advantageous traits (Biancardi 2005). Most cultivated sugarbeets are hybrids with high genetic heterogeneity within each population. Sucrose accumulation and disease resistance have been positively selected using conventional breeding techniques within the species. Breeding efforts to enhance the germination vigor of seeds have been limited and mostly unsuccessful to date, possibly due to the high population variance, low heritability, and physical germination impediments (Sadeghian and Khodaii 1998; Taylor et al. 2003, McGrath et al. 2008).

Wild beets, those found along the Mediterranean coast, are annuals, producing copious amounts of seeds, which they disperse via ocean currents (Fievet et al. 2007). The true seed of *Beta vulgaris* is an oil-based seed, similar to some members of the Brassicaceae. A maternally derived, carbohydrate-based tissue called the perisperm surrounds the beet embryo and provides a nutrition source for the emerging seedling. The perisperm takes the place of the endosperm, present in most other species' seeds. The true beet seed, consisting of the embryo, perisperm and a thin, seed coat, is encapsulated in a corky, maternally derived tissue, called the pericarp, which aids in seed dispersal via water and protects the embryo (Ware 1898, Guja et al. 2010). The presence of the pericarp makes working with beet seeds difficult, as it is a mechanical impediment for the germinating seed and a physical barrier for studying germination of the true seed. Beet propagules, or fruits, are typically multi-germ (multiple embryos or seeds per fruit), and as few as zero or as many as four or five embryos can germinate from the same propagule.

The multi-germ seed characteristic is retained for both chards and table beets. Sugarbeet production requires a level of uniformity in beet size and shape for machine harvesting that is unattainable using multi-germ propagules, without extensive thinning. Modern sugarbeet varieties have been bred to be monogerm (one seed per fruit), a single gene controlled trait discovered in the late 1940s (Savitsky 1952; Tekrony 1968). With the advent of monogerm seed, the sugarbeet industry had solved the problem of multiple beets per fruit, but was accosted by the unforeseen problem of low germination rates.

Certain varieties of sugarbeets exhibit higher levels of germination uniformity and vigor than others, indicating a genetic basis and control for differences in germination vigor. Increasing fitness by selecting and propagating vigorous seedlings has been attempted, but due to low heritability and a presumed multi-component system, the results were not effective. Genetic variance accounts for less than 30% of the total variation in germination with the remaining 70% being attributed to the environment (Sadeghian and Khodaii, 1998). Recently, several studies on the proteomics of dry and germinating seeds, transcript profiles at the same time points, and molecular assessments of varietal vigor differences have emerged (Catusse et al. 2008a; Catusse et al. 2008b; de los Reyes and McGrath 2003; de los Reyes et al. 2003; Elamrani et al. 1994; Elamrani et al. 1992; Hermann et al. 2007; McGrath et al. 2000; McGrath et al. 2008; Sadeghian and Khodaii 1998; Taylor et al. 2003; Pestsova et al. 2008). In several of these studies H_2O_2 concentrations and Reactive Oxygen Species (ROS) activated genes were found to be present at higher levels in germinating seeds than in quiescent seeds or seedlings, and others demonstrated an increased lipid mobilization and hormone activity associated with the germinating seeds. Hydrogen peroxide has been known to enhance germination vigor in sugarbeets since the 1960s, and in other non related species, but few studies have looked at the genetic changes hydrogen

peroxide induces to cause vigor (Ishibashi et al. 2010; Sedlmayr 1960; Kim et al. 2010; Sunkar et al. 2006; Wahid et al. 2007; Wang et al. 2009; Xing et al. 2009; Xing et al. 2008).

de los Reyes and McGrath (2003) demonstrated differences in gene expression between USH20 (a legacy commercial hybrid that consistently showed good emergence) and ACH185 (a legacy commercial hybrid with less vigor under similar field conditions). These two varieties had a difference in germination that could be negated with the application of dilute concentrations of hydrogen peroxide. Seedling vigor differences were attributed, in part, to differences in the expression of a putative oxalate oxidase (i.e. *Bv*GLP165, germin-like protein 165) expressed in the vigorous germinator in H_2O. In USH20, the GLP (germin-like protein) was expressed in water conditions, but was not expressed in ACH185 under the same conditions (H_2O) after 96 hours of treatment. In conjunction with the induced GLP expression, an increase in transcripts of key enzymes in lipid metabolism and the glyoxylate cycle was also observed. This initial seedling vigor study suggested a genetic basis for germination vigor when seeds were treated with hydrogen peroxide visible by increased stored lipid reserve mobilization and radical emergence. This group theorized that the germin-like protein in USH20 might function under non-stress conditions to produce hydrogen peroxide (plus carbon dioxide) and release calcium stored in seeds (as calcium oxalate), activating metabolic activity and enhancing vigor. However, the mechanism by which hydrogen peroxide activated metabolic activity and enhanced vigor was not addressed in this study.

In 2008, Catusse et al. used a proteomics approach to profile the complement and tissue specificity of proteins in mature sugar beet seeds (96 hours into germination). This study found a full complement of proteins involved in the glyoxylate cycle present in the germinating seed,

confirming previous work by de los Reyes et al. 2003. Another result of this study was the prevalence of stress-associated genes, with over 60 chaperones and heat shock proteins (HSPs) being identified, in addition to ROS induced enzymes. The authors suggested that the prevalence of ROS defense mechanisms was due to the enhanced oxidative stress during metabolic activity resumption associated with lipid catabolism in the peroxisomes. This study suggested that internal H_2O_2 might be more a byproduct of vigor and not its catalyst.

In other species it has been shown that H_2O_2 is produced in chloroplasts, mitochondria, and peroxisomes (Apel and Hirt 2004). H_2O_2 can regulate ABA catabolism and GA biosynthesis during germination, act as a signaling molecule, and play multiple other roles in plant development and stress response (Liu et al. 2010; Van Breusegem et al. 2001). It is both a byproduct and an instigator of lipid metabolism (Apel and Hirt 2004; Van Breusegem et al. 2001; Vranova et al. 2002). H_2O_2 is produced upon infection by pathogens and during abiotic stress, as a signaling molecule, to affect changes in gene expression in the nucleus (Van Breusegem et al. 2001; Vranova et al. 2002). H_2O_2 reportedly cross talks with a number of other signaling molecules and pathways including various hormones that can contribute to the stress response (Barba-Espin et al. 2010; Brock et al. 2010; Finkelstein et al. 2002; Kucera et al. 2005; Lu et al. 2002; Shinozaki and Yamaguchi-Shinozaki 2007; Xue et al. 2009). H_2O_2 can regulate mitogen-activated protein kinases (MAPKs) and other kinase activity, or can have a more direct role in regulating stress response genes (Pitzschke and Hirt 2006).

While the role of MAPKs in plant-pathogen and stress interactions are well studied, their role in activating lipid metabolism, though documented, is still unclear. Multiple studies have shown

that the induction or constitutive expression of MAPKs results in higher levels of stress-activated metabolism and resistance, but the process and pathways are still being unraveled (Brader et al. 2007; Cheong and Kim 2010; Shi et al. 2010; Zhang et al. 2007). While useful for understanding the genetic control of stress, this information has not been applied as a breeding tool for many crop species, including *Beta vulgaris*.

In *Beta vulgaris*, studies have demonstrated that germination is controlled through dynamic changes in gene expression and metabolism (Catusse et al. 2008b; de los Reyes et al. 2003; Pestsova et al. 2008). Many stress-related and metabolism transcripts are present in beets suggesting the importance of these processes for successful germination under both stress and non-stress conditions. ROS and stress related transcripts including heat shock proteins, MAP kinases, and germin-like proteins are all shown to be present during vigorous germination, yet the number, compilation and function of these genes is still unknown. The morphological and genetic process of germination, dry seeds to emerged radical, and the differences under stress and non-stress conditions is yet to be defined. Linking both morphological and genetic components of germination under multiple conditions is necessary to create an accurate view of germination and vigor that can be effectively modified and improved.

In summary, despite advances in breeding and chemical controls to enhance germination, disease control, and sucrose accumulation more work remains to be done. Information on the genetics behind germination and germination vigor is needed to successfully improve sugarbeet germination, either through improved breeding lines, marker assisted evaluation, or priming. The recent protein and transcript information on germinating seeds provides a possible window into the genetics behind germination vigor. Previous studies have mainly focused on 4 day-old seedlings, because of the ease of working with seedlings instead of seeds. However, germination

tests have shown that members within sugarbeet populations can germinate as early as twenty-four hours into imbibition. To effectively breed and screen for germination vigor, future research will need to focus on energy mobilization, water absorption, gene expression, and protein synthesis prior to radical emergence.

The research objective of this thesis was to identify and characterize morphological and molecular factors associated with vigorous germination and stress during early seedling growth.

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CHAPTER I: INDUCTION AND IDENTIFICATION OF STRESS-INDUCED GERMINATION VIGOR GENES IN *BETA VULGARIS*

ABSTRACT

The initial environmental conditions a germinating seed encounters affects the speed and success of germination, the availability of stored energy reserves to withstand future adverse environments, and the overall ability of the seedling to flourish. *Beta vulgaris* seedling and germination molecular markers to predict vigor are not currently available and little information exists on the genetics of germination and seedling vigor. Germination is a stressful period of development, and low dosages of stress can boost metabolism and increase vigor. To understand differences in seedling vigor under stress and identify markers useful for breeding, typical germination in water was compared to physiological and gene expression changes in hydrogen peroxide. Two former commercial varieties with known differences in vigor were germinated in water and hydrogen peroxide. Physical differences in the total number of seeds germinated and internal hydrogen peroxide for each variety were observed over 96 h. Rapid imbibition occurred, and gene expression was measured, between 0 and 24 h for both varieties. Expression patterns of 343 genes related to development and stress were qualified between varieties and treatments. A subset of forty-eight genes was tested using qPCR to quantify changes in gene expression between varieties and treatments over time. Lower internal hydrogen peroxide, increased water absorption, and an upregulation of the stress genes Deadbox and MBF1C were associated with reduced germination. Higher internal hydrogen peroxide concentrations and upregulation of putative genes involved in pathogen defense, hormone biosynthesis and lipid transfer were associated with radical emergence and a response to hydrogen peroxide during germination.

INTRODUCTION

Sugarbeet, *Beta vulgaris* L., is an economically important crop accounting for 50% of the sucrose consumed in the U.S. and 30% worldwide. Low germination, a production limitation since the 1940s, is a continuous source of revenue loss. In the field, emergence is highly variable and can range from 0 to 100% depending upon the variety and environmental conditions. Seed size, weight and the proportion of corky dried maternal fruit surrounding the seed are highly variable between and within populations and can contribute to varying levels of vigor and germination. In addition, processing of the fruit can have an effect on germination and vigor (Orzeszko-Rywka and Podlaski 2003). The corky fruit surrounding the embryo restricts germination by acting as a physical barrier, preventing radical emergence and water uptake, and as a chemical barrier, leaching solutes and inhibitors (Draycott 2006; Hermann et al. 2007; Taylor et al. 2003).

For sugarbeets, germination vigor is a measurable differential trait between varieties that ultimately impacts yield, sucrose accumulation, and profitability. From dry seeds, germination is broken into three physically distinct phases: Phase I is characterized by a rapid uptake in water, Phase II is a resting stage or plateau, and Phase III is an increase in weight followed by radical emergence and completion of germination (Bewley 1997). Physiologically, the three phases of germination include protein synthesis, biochemical activation, and germination. However, these are not well-defined phases. Germination vigor, or the ability to rapidly complete all three phases and successfully overcome less than ideal environmental conditions, is a complex heritable stress response. It has been shown for several plant species that H_2O_2 can enhance germination vigor (Barba-Espin et al. 2010; Liu et al. 2010; McGrath et al. 2000; Tekrony and Hardin 1969; Wahid et al. 2007). The mechanism is unclear, but one possibility is that H_2O_2 is acting as a signaling

molecule to activate energy mobilization and germination (Apel 2004; de los Reyes and McGrath 2003a).

In adult plants, abiotic and biotic stress results in the induction of a number of defense responses, including cell wall strengthening and energy mobilization (Beckers et al. 2009; Desikan et al. 2001; Hajheidari et al. 2005; Hu et al. 2006; Hyun et al. 2009; Kim et al. 2007; Kreps et al. 2002b; Mane et al. 2007; Shinozaki and Yamaguchi-Shinozaki 2007). The treatment of adult *Arabidopsis* plants with H_2O_2 resulted in the activation of a number of pathogen and drought defense mechanisms, ROS scavenging genes and lipid mobilization units, suggesting the role of this signaling molecule in stress recognition during the adult phase of development (Faurie et al. 2009; Hu et al. 2003; Kovtun et al. 2000; Orozco-Cardenas et al. 2001; Ramanjulu and Bartels 2002; Van Breusegem et al. 2001; Vranova et al. 2002; Yu et al. 2008). During germination, the exposure to low doses of H_2O_2 may result in similar responses in the seed. Defense mechanisms and the additional mobilization of reserves in response to stress would improve access to heterotrophic resources for the germinating seed, resulting in more rapid germination. Previous work in sugarbeet demonstrated the importance of H_2O_2 during germination, its role on lipid catabolism, and the mobilization of seedling reserves resulting in seedling vigor (de los Reyes and McGrath 2003; Pestsova et al. 2008). These mobilization events appear to be instigated by the activation of a germin-like protein gene (*BvGLP165*), shown to be activated in a variety with high germination vigor (referred to hereafter as a vigorous variety) but absent in the a variety with poor germination (non-vigorous variety) measured at 96 hours (Catusse et al. 2008; de los Reyes and McGrath 2003a). Germination could be induced in the non-vigorous variety with the addition of a dilute hydrogen peroxide solution, which resulted in

increased radical emergence and lipid mobilization similar to the vigorous variety (de los Reyes and McGrath 2003).

Germin Like Proteins (GLPs) are a large gene family with diverse roles in stress response and germination (Bernier and Berna 1999; Knecht et al. 2010; Manosalva et al. 2009). Many are oxalate oxidases, and one functional role is to produce the signaling molecule H_2O_2. Hydrogen peroxide accumulation and signaling is also essential for breaking seed dormancy by upregulating ABA catabolism and GA biosynthesis and inducing lipid metabolism (Liu et al. 2010; Puntarulo et al. 1988; Wahid et al. 2007).

ABA and GA have well documented roles in germination. ABA is essential for maintaining seed dormancy, is a negative regulator of germination and is also produced in response to moisture and pathogen stress (Atia et al. 2009; Finkelstein et al. 2002; Kucera et al. 2005). Components of the ABA-instigated mitogen-activated protein kinase (MAPK) cascade result in changes in gene expression leading to stomatal closure and pathogen defense. During germination, ABA-induced MAPK activation can result in decreased germination (through *AtMPK3* and *AtMPK6*) (Adie et al. 2007; Atia et al. 2009; Liu et al. 2010; Xing et al. 2009). Other pathogenesis-related hormones, e.g. jasmonic acid and salicylic acid, have been shown to have a role in germination vigor by activating pathogenesis-related proteins (PR) proteins (Rajiv and Stanisaw 1992; Seo et al. 2008).

The objectives of this study were to characterize germination through physical changes over the first 96 h of imbibition (water absorption) and gene expression changes over the first 24 h (Phase I of germination) under typical conditions (H_2O), and atypical germination (H_2O_2) conditions.

MATERIALS AND METHODS

Germination vigor testing. Two former commercial varieties, ACH185 (EL-A012206) and USH20 (EL-A012230), and a breeding germplasm, SP7622 (EL-A015030), were chosen for analyses based from results on previous studies that indicated genetic and visible differences in vigor (de los Reyes and McGrath 2003a; de Los Reyes et al. 2003b; McGrath et al. 2000). ACH185 is a triploid, multigerm, CMS (cytoplasmic male sterile) variety with low germination vigor (<60%) under stress conditions and low field emergence. SP7622 is a diploid, multigerm variety with high germination vigor (>60%) and high field emergence. Three replicates of fifty seeds each from ACH185 and SP7622 were placed into 125 mL flasks, and 25 mL of 18 MOhm H_2O or 88 mM H_2O_2 solution was added. Seeds were shaken continuously at 25 °C under constant fluorescent light. Seeds with radicals protruding from the fruit were counted as germinated. Germinated seeds were counted at 24, 48, 72 and 96 h after hydrogen peroxide or water was added. Germination percentages were calculated from the number of seeds germinated out of the total.

Water uptake (Imbibition) of *B. vulgaris* varieties during germination. Seeds from ACH185 and SP7622 were surface disinfested for 20 min in 0.015% hypochlorate, with Triton X-100 added as a surfactant, on a shaker at 150 rpm. Seeds were rinsed in 18 MOhm H_2O and allowed to air dry. Dry seeds (35 seeds) for fruited imbibition were placed onto filter paper in germination boxes with 10 mL of either 0.3% H_2O_2 or H_2O and weighed at 0, 6, 22.5, 30.5, 45.5 52, 70 and 99 h during imbibition grown at 25 °C. Average weight was calculated for each variety and treatment. Water uptake was measured by the amount of weight gain over time.

Individual germination boxes were treated as replicates and seed imbibition and weight was calculated as the average of the total number of individuals within each box. Embryos were manually extracted from the dry, fruited seeds by soaking them in H_2O for 3 hours, loosening the seed cap by applying pressure with the cap end of a 15 mL Falcon tube, and lifting out the embryos using a classic dental pick. Embryos (15 seeds) were treated with 10 mL of H_2O and weighed at 0, 6, 22.5, 30.5, 45.5 52, 70 and 99 h after the start of imbibition. Embryos were weighed to measure water absorption. Each treatment was replicated three times and the experiment was repeated twice. Data was analyzed between treatments and varieties using the NMLE ANOVA function of the R statistical software package (v 2.11.1) and the JMP statistical software package (v.7) using alpha = 0.05 (SAS Institute Inc 2007; R Development Core Team 2008; Hornik 2010; Pinheiro 2010).

An additional 30 surface disinfested seeds of each accession were plated onto cornmeal agar to determine the presence of internal fungi and fungal colonies were identified to genus after 5 days (replicated twice).

RNA extraction and cDNA synthesis. Seeds from ACH185 and SP7622 were surface disinfested as described. Seeds were rinsed in 18 MOhm H_2O and allowed to air dry. Fifty seeds of each accession were transferred to flasks and 25 mL of 18 MOhm H_2O or 88 mM H_2O_2 were added. Seeds were shaken constantly at 25 °C under constant fluorescent light. Samples were collected at 0, 1, 3, 6, 12, 18, and 24 h of imbibition (replicated 3 times). Total RNA was collected using the Macherey-Nagel NucleoSpin RNA Plant kit according to manufacturer's protocols (Duren, Germany). cDNA was synthesized using 1 µg of total RNA per reaction and

Superscript III reverse transcriptase according to the manufacturer's protocol (Invitrogen, Carlsbad, CA). cDNA was diluted to 50 ng/µl for downstream applications.

RT-PCR. Beet Expressed Sequence Tags (ESTs) with a sequence similarity ($<e^{-20}$ with the exception of 6 genes with a value $> e^{-20}$ and $< e^{-3}$) to *Arabidopsis* genes with a documented role in hydrogen peroxide response, growth or hormone biosynthesis were used for oligo synthesis (Appendix 1.A). Primers were developed using LaserGene PrimerSelect software (DNA Star, Madison, WI) and synthesized by IDT (Integrated DNA Technologies Coralville, IA). PCR was performed using the Promega Green GoTaq mastermix according to the manufacturer's protocol (Madison, WI). The PCR program had an initial denaturation at 94 °C for 90 s, followed by a cycle of 94 °C for 30 s, 58 °C for 30 s with a -0.8 °C per cycle, and 72 °C 60 s, repeated 12 times, and an additional program of 94 °C for 30 s, 47 °C for 30 s and 72 °C for 60 s, repeated 30 times and a final 72 °C for 10 m. PCR products were visualized on 2% agarose gels using .002% ethidium bromide. All 343 genes were clustered into clusters of similar expression patterns using k-means clustering. Gene expression patterns were grouped into 7 clusters for three variety and treatment combinations (ACH185 H_2O_2 and SP7622 H_2O and H_2O_2) and 6 clusters for one variety and treatment combination (ACH185 H_2O) (Cluster 3.0 Stanford University Palo Alto, CA). K-means clustering was performed using the Euclidean distance and 300,000 iterations. Clustering was visualized as a modified heat map using Java TreeView (Alok Saldanha, Stanford University Palo Alto, CA).

qPCR. Quantitative analysis of transcriptional changes in individual gene products were tested on a subset of 48 genes chosen from qualitative PCR analyses based their predicted roles

in abiotic and biotic stress response, hormone biosynthesis, and lipid mobilization in *Arabidopsis* seedlings and adult plants. Analyses were performed using quantitative PCR (qPCR) of the selected genes using the KapaSybr HotStart master mix (Kapabiosystems, Woburn, MA) according to the manufacturer's protocol on the Applied Biosystems StepOne Plus thermocycler (Carlsbad, CA). Relative expression was calculated using the delta delta C_t method (Livak and Schmittgen 2001). 18s rRNA (BQ589671) was used as the reference gene for analyses, and the experiment was replicated twice.

Direct peroxide assay of seeds. H_2O_2 extraction procedures were performed according to Warm and Laties (1982)**.** 350 seeds of USH20 (EL-A012230) and 350 seeds of ACH185 (EL-A012205) were soaked in 100 mL of 18 MOhm H_2O at room temperature shaken constantly. Seeds were started at staggering times to acquire samples at 3, 6, 12, 24, 48, 72, and 96 h simultaneously. Water was changed daily for seeds soaking for more than 24 h. The seed cap was manually removed from the seeds and individual embryos were placed in micro-titer plate wells. Sixty microliters of sterile 18 MOhm water was added into the micro-titer plate wells containing embryos, and embryos were crushed. After centrifuging (20 m at 12,000 g at 4 °C), 50 µl of the supernatant was transferred to a Victor plate (Perkin Elmer, Waltham, MA). 50 µl of an H_2O_2 concentration gradient prepared according to the Amplex Red (10-acetyl-3,7-dihydrophenoxazine) Hydrogen Peroxide/Peroxidase Assay Kit (A-22188 Molecular Probes Inc Eugene, OR) was loaded into the Victor plate. Fifty microliters of the reaction mix (Amplex Red reagent/HRP working solution) were added to each well. Plates were incubated at 25 °C for 30 m in the dark. Fluorescence was measured (using a 560 nm excitation filter) at 590 nm and

standard curve and hydrogen peroxide concentrations were calculated for each sample. The experiment was conducted three times.

RESULTS

Phenotypic evaluation of vigor and physical characterization of seed. Differences in the physical and genetic characteristics of vigor induced by hydrogen peroxide were observed between the two lines over time. The two varieties chosen for analyses (ACH185 and SP7622) showed differences in fruit size and weight (Figure 1.1, Table 1.1). True (embryo) dry seed weights were not statistically different between the two varieties in both experiments. ACH185 had more internal fungal contamination compared to SP7622 after surface disinfestation and performed poorly, in comparison to SP7622, under the water germination treatment (0 vs. 33 germinated, respectively) (Table 1.2, Table 1.3). These lines performed similarly (31 vs. 40) in hydrogen peroxide at 96 h, but not at earlier time points. Seeds within each population germinated as soon as 24 h for SP7622 and 48 h for ACH185 in hydrogen peroxide (13 and 9) and 24 h for USH20 in H_2O. ACH185 had no germination in water at 0 h.

During imbibition of fruited seeds, ACH185 had a greater weight gain on average than SP7622 (Table 1.1). Imbibition did not consistently significantly differ between treatments for SP7622 across all time points. ACH185 showed a slight decrease in water-uptake ($p = 0.06$) in H_2O_2 in comparison to the H_2O treatment. For both varieties, Phase I, the period of most rapid water uptake, of germination was completed prior to the first 30 hours measured. Phase II spanned from 30 to 80 h depending on the variety and treatment and Phase III and secondary

Figure 1.1 Single seeds of ACH185 A) fruited, unpolished seed and B) fruited seed with the seed cap removed showing the embryo inside. Single seeds of SP7622 C) fruited, semi polished seed and B) fruited seed with the seed cap removed showing the embryo inside. (Orange scale bar is in mm.)

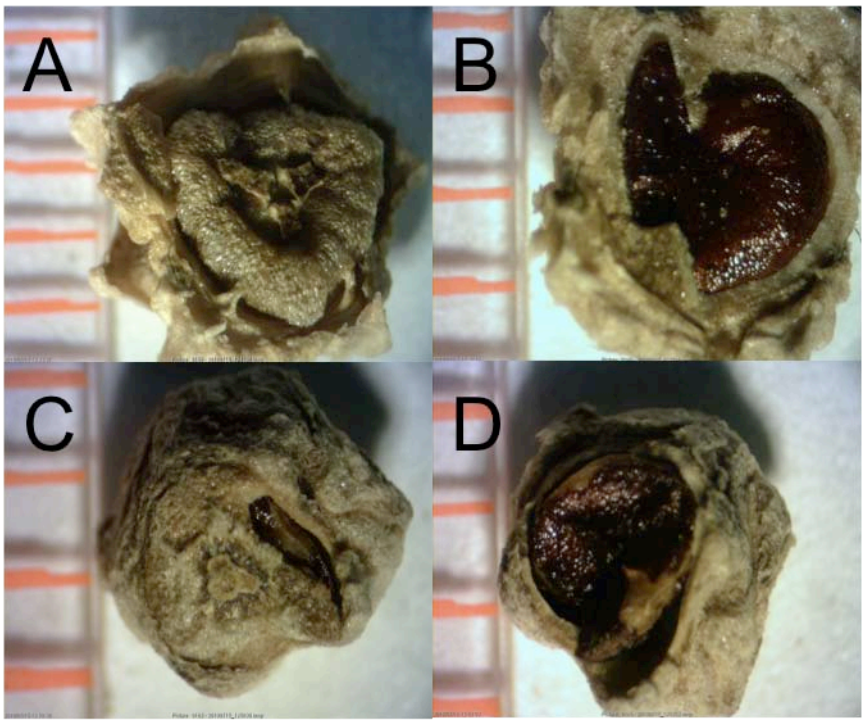


Table 1.1 Water absorption over time in fruited seeds and embryos of ACH185 and SP7622 in hydrogen peroxide and water

Imbibition Experiment 1 Individual Seed wt (mg)

			0.0 h		6.5 h		22.5 h		30.5 h		45.5 h		52.0 h		70.5 h		99.0 h	
V[1]	Frt[2]	Trt[3]	Avg[4]	SD	Avg[4]	SD	Avg[4]	SD	Avg[4]	SD	Avg[4]	SD	Avg[4]	SD	Avg[4]	SD	Avg[4]	SD
AC	Pre	H_2O_2	12^{a}	1	22^{a}	2	27^{a}	1	$28^{a\,b}$	2	$29^{a\,b}$	1	28^{ab}	1	31^{ab}	1	35^{a}	2
SP	Pre	H_2O_2	16^{b}	2	19^{a}	4	21^{a}	3	20^{b}	3	$23^{a\,b}$	3	22^{ab}	2	24^{ab}	3	32^{a}	14
AC	Pre	H_2O	12^{a}	1	26^{a}	2	30^{a}	2	32^{a}	2	33^{a}	4	3^{a}	4	36^{a}	3	38^{a}	3
SP	Pre	H_2O	16^{b}	4	24^{a}	7	27^{a}	7	25^{ab}	7	26^{b}	7	26^{b}	6	27^{b}	7	28^{a}	9
AC	Abs[5]	H_2O	4^{c}	0	6^{c}	0	7^{b}	0	7^{c}	0	7^{c}	0	7^{c}	0	7^{c}	0	7^{b}	0
SP	Abs[5]	H_2O	3^{c}	0	5^{d}	0	5^{c}	0	5^{d}	0	5^{d}	0	5^{d}	0	5^{d}	0	13^{b}	11

Imbibition Experiment 2 Individual Seed wt (mg)

			0.0 h		6.5 h		22.5 h		30.5 h		45.5 h		52.0 h		70.5 h		99.0 h	
V[1]	Frt[2]	Trt[3]	Avg[4]	SD	Avg[4]	SD	Avg[4]	SD	Avg[4]	SD	Avg[4]	SD	Avg[4]	SD	Avg[4]	SD	Avg[4]	SD
AC	Pre	H_2O_2	13^{b}	0	24^{b}	0	27^{b}	0	28^{b}	0.0	31^{b}	1	31^{b}	0	36^{b}	1	38^{b}	1
SP	Pre	H_2O_2	16^{a}	1	21^{c}	2	23^{c}	2	25^{bc}	1.9	27^{c}	2	27^{b}	2	28^{c}	2	28^{c}	2
AC	Pre	H_2O	14^{ab}	2	30^{a}	3	36^{a}	3	39^{a}	4.0	42^{a}	3	42^{a}	4	44^{a}	4	43^{a}	4
SP	Pre	H_2O	14^{b}	2	21^{c}	2	22^{c}	2	22^{c}	1.7	23^{d}	2	23^{c}	2	24^{c}	2	24^{c}	1
AC	Abs[5]	H_2O	2^{c}	0	4^{d}	1	4^{d}	1	4^{d}	0.7	5^{e}	1	5^{d}	1	5^{d}	0	5^{d}	-
SP	Abs[5]	H_2O	2^{c}	0	4^{d}	0	4^{d}	1	4^{d}	0.6	5^{e}	1	5^{d}	1	6^{d}	1	6^{d}	2

[1]Variety AC=ACH185 and SP=SP7622 [2] Fruit is Pre=Fruit present Abs=Fruit absent [3]Treatment [4] Average numbers within a column followed by the same letter are not different at p = 0.05 [5]embryos were analyzed separately. SD is the standard deviation between replications

Table 1.2 The proportion of internal fungi present after surface disinfesting seeds of ACH185 and SP7622

	Proportion of fungal contamination* (plated on cornmeal agar after 5 days)					
Variety	***Aspergillus***	***Alternaria***	***Penicillium***	***Fusarium***	***Rhizopus***	***Trichoderma***
ACH185	0.02	0.10	0.43	0.15	0.02	0.02
SP7622	-+	-	0.48	-	-	-

*proportion of seeds out of the 30 possible with the fungal contamination +indicates that the fungus was not detectable

Table 1.3 Number of ACH185 and SP7622 seeds germinated (out of 50) in water and hydrogen peroxide over time

		Sugarbeet Germination (Number germinated out of 50)							
		24 h		**48 h**		**72 h**		**96 h**	
Variety	**Treatment**	**Mean***	**SE**	**Mean***	**SE**	**Mean***	**SE**	**Mean***	**SE**
ACH185	H_2O	0.00[b]	0.00	0.00[d]	0.00	0.00[c]	0.00	0.00[b]	0.00
SP7622	H_2O	10.00[a]	2.89	24.33[b]	4.67	31.00[ab]	3.21	32.67[a]	2.91
ACH185	H_2O_2	0.00[b]	0.00	9.00[c]	0.58	27.00[b]	5.03	31.00[a]	6.11
SP7622	H_2O_2	13.33[a]	0.88	36.33[a]	2.60	39.00[a]	3.06	40.00[a]	3.00

*Numbers within a column followed by the same letter are not significantly different at p = 0.05. SE is the standard error between replications

weight gain, due to water absorption or growth, was detected at 72 through 96 h. The time of most rapid imbibition was 0 to 24 h.

Imbibition differences were compared between seeds of ACH185 and SP7622 that had the fruit removed in H_2O. ACH185 embryos did not complete imbibition significantly faster than SP7622 ($p=0.06$). However, there was a significant ($p=0.01$) increase in the total amount of H_2O absorbed over time by ACH185 embryos compared to SP7622 (7mg vs. 5mg respectively at 22 to 70.5 h). Embryos of both varieties completed Phase I of imbibition after approximately 6 h.

The internal hydrogen accumulation assay revealed differences in H_2O_2 accumulation between the two varieties in H_2O over time. Individual seeds within each population showed a range of H_2O_2 concentrations over time (Table 1.4). Concentrations of H_2O_2 were indistinguishable between the two varieties at 3 and 12 h post treatment. Visible differences were detected at 6, 24, 48, 72 and 96 h; however they were not statistically significant, except at 72 h. USH20 (the vigorous variety) seed had higher average concentrations of internal H_2O_2 than ACH185 (non vigorous variety) seed over the 96 hours monitored.

Genetic evaluation of vigor. For large scale, qualitative gene expression profiling, RT-PCR was performed on 343 primer combinations developed from sugarbeet ESTs showing sequence similarity to *Arabidopsis* stress response, hormone biosynthesis, developmentally regulated and MAP Kinase genes (Swarbreck et al. 2008) (Table A.1). Genes were scored as present or absent, and expression fingerprints were created for each variety and treatment. Large genetic differences were visible between the vigorous (SP7622) and non-vigorous (ACH185) variety and between treatments (H_2O and H_2O_2) during the first 24 h of imbibition. Out of the possible 343

Table 1.4 Internal hydrogen peroxide concentrations for embryos of ACH185 and SP7622 over time in water

			Hydrogen peroxide concentration (µM)													
			3 h		6 h		12 h		24 h		48 h		72 h		96 h	
V[1]	Frt[2]	Trt[3]	Avg[4]	SD	Avg[4]	SD	Avg[4]	SD	Avg[4]	SD	Avg[4]	SD	Avg[4]	SD	Avg[4]	SD
AC	Abs	H_2O	1.32[a]	0.22	1.19[a]	0.12	1.22[a]	0.25	1.10[a]	0.16	0.90[a]	0.00	0.59[b]	0.15	0.63[a]	0.24
SP	Abs	H_2O	1.18[a]	0.10	1.73[a]	0.36	1.41[a]	0.09	1.81[a]	0.19	1.50[a]	0.12	1.48[a]	0.45	1.25[a]	0.33

[1]Variety AC=ACH185 and SP=SP7622 [2] Fruit is Pre=Fruit present Abs=Fruit absent [3]Treatment [4] Average numbers within a column followed by the same letter are not different at p = 0.05

genes tested in dry seeds, only 39 were shared between the two varieties. Many of the 39-shared genes had sequence similarity to *Arabidopsis* genes involved in growth and response to pathogens (Table A.2). Eighteen of the genes shared at 0 h were still detectable at 1 h in at least one variety and treatment. Most of these genes were present in ACH185 and not SP7622 at 1 h (data not shown). Putative *BvGSTF8* and *GSTF7*, similar to *Arabidopsis* genes expressed during seedling growth and in response to fungi, were two examples of genes expressed in both varieties at 0 h, but only detectable in the ACH185 at 1 h. Only *ROF* and *M3Ka* were detectable in both varieties at 0 h but only in SP7622 at 1 h.

One hundred twenty transcripts were uniquely present in SP7622 dry seeds, and 21 were uniquely present in ACH185 dry seeds (Table A.3 and Table A.4). Out of the 120 unique transcripts present in SP7622 at 0 h, only eight were also detected at 1 h. Only 7 of the 21 genes uniquely present in ACH185 at 0 h were also detectable at 1 h. SP7622 had a greater number of putative hormone, growth, metabolism and signaling transcripts present in dry seeds compared to ACH185.

Post 0 h, SP7622 had fewer genes expressed in growth and stress-related than ACH185 in H_2O (data not shown). In the H_2O_2 treatment, SP7622 had a similar number of stress, growth and signaling genes detectable to ACH185, for the first 6 h followed by a rapid increase in growth and stress-related transcripts at 24 h. Between treatments, the total number of genes detectable at any one time for each variety was very similar, with the exception of SP7622 at 24 h (H_2O vs. H_2O_2). However, the functional composition of those genes differed between treatments. In SP7622, there were increased stress transcripts present at 3 (30 vs. 8), 6 (51 vs. 28), 18 (44 vs. 26) and 24 (94 vs. 34) h in H_2O_2 as compared to H_2O. Growth transcripts were

also more prevalent in the H_2O_2 treatment of SP7622 as compared to H_2O at 3 (14 vs. 2), 6 (27 vs. 17) and 24 (69 vs. 22) h. In contrast, ACH185 had a greater number of stress related transcripts detectable at 1 (26 vs. 6) and 18 (46 vs. 26) h for the H_2O treatment compared to the H_2O_2. Growth-related transcripts were also more prevalent in the ACH185 H_2O treatment compared to the H_2O_2 at 3 (17 vs. 7), 18 (26 vs. 17) and 24 (19 vs. 10) h.

Stress genes detected during the first 24 hours were grouped by whether they were abiotic, biotic, or undetermined stress genes. Similarities in the varietal and treatment specific expression patterns of genes over time were no longer visible when stress-associated genes were looked at more closely (data not shown). In SP7622, increases in the total proportion of stress genes (abiotic, biotic, undetermined and both) occurred at 6 and 24 h in the H_2O_2 treatment. In ACH185 an increase in biotic stress genes was seen at 18 h in the H_2O treatment, and not the H_2O_2.

Both SP7622 treatments and the ACH185 H_2O_2 treatment resulted in germination, but the ACH185 H_2O treatment did not. Genes common between these three variety and treatment combinations at a given time point, and not present in ACH185 H_2O, were termed germination genes (Table 1.5). Imbibition genes were those genes expressed in both treatments and varieties at a given point as the seeds were imbibing water, but not necessarily involved in the actual process of germination. When the germination genes were compared across time to the imbibition genes, few genes were unique (Table 1.6). The most notable difference between the

Table 1.5 Germination genes, shared at a specific time between the treatments that resulted in germination, SP7622 H_2O_2 and H_2O treatments and ACH185 H_2O2_2 treatment. Genes highlighted in green were shared with the imbibition list at a different time. Genes not highlighted were specific to germination using RT-PCR.

Germination genes			
Bv* Accession	***At* gene/function****	***At* Protein*****	**Time (h)**
BQ060614	MPK16	NP_197402.1	1
BQ488461	AOX1A	NP_188876.1	6
BQ586400	JAZ1	NP_973862.1	6
CK136793	HSP18.2	NP_200780.1	6
ED032901	retrotransposon	-	6
BE590397	XTR7	NP_193149.2	12
BQ584876	kinase	NP_197362.1	12
BU089558	HSL1	NP_174166.1	12
ED032901	retrotransposon	-	12
BQ489189	MPK4	NP_192046.1	18
BQ582409	kinase	NP_567574.1	18
BQ588055	kinase	NP_567072.1	18
CK136793	HSP18.2	NP_200780.1	18
CX779686		-	18
BF011227	HSP81-2	NP_200414.1	24
BI096237	EF	NP_182029.1	24
BQ583369	PLDA	NP_188194.1	24
BQ588646	AT1G03790	NP_194648.1	24
BQ594997	AML1	NP_568946.1	24
BQ595152	HSP17.8-CI	NP_172220.1	24
BU089558	HSL1	NP_174166.1	24
CK136793	HSP18.2	NP_200780.1	24

* *Beta vulgaris* ** *Arabidopsis thaliana* gene *** *Arabidopsis* thaliana NCBI protein

Table 1.6 Imbibition genes, present in both varieties and treatments at a specific time using RT-PCR. Genes in green were also present in the germination list at an earlier time point. Genes in pink were present in the hydrogen peroxide vigor gene list at an earlier time point. Genes not highlighted were specific to imbibition.

Imbibition genes			
Bv* Accession	***At* gene/function****	**At Protein*****	**Time (h)**
BI096176	GER1	NP_177405.1	1
BQ587887		NP_567072.1	1
CF543001		NP_179361.1	1
BE590444	HSP17.8	NP_172220.1	3
BF011227	HSP81-2	NP_200414.1	3
BI096176	GER1	NP_177405.1	3
BQ060614	MPK16	NP_197402.1	3
BQ587622	MYB60	NP_172358.1	3
BQ589141	MYB60	NP_172358.1	3
BQ589734	HSP18.2	NP_200780.1	3
BQ594558	MBFC1	NP_189093.1	3
BQ595152	HSP17.8-CI	NP_172220.1	3
BU089558	HSL1	NP_174166.1	3
CF543001		NP_179361.1	3
CK136658	HSP17.8-CI	NP_172220.1	3
CK136793	HSP18.2	NP_200780.1	3
CK136863	HSP18.2	NP_200780.1	3
AW063023	ACT7	NP_196543.1	6
AW697779	AT3G29970	NP_190397.1	6
BE590444	HSP17.8	NP_172220.1	6
BF010998	ACT11	NP_187818.1	6
BF011036	AT3G29970	NP_190397.1	6
BF011227	HSP81-2	NP_200414.1	6
BI073235	HSP81-3	NP_200412.1	6
BI096111	AT3G29970	NP_190397.1	6
BI543285	AT3G29970	NP_190397.1	6
BI543685	HSP70	NP_187864.1	6
BI543772	HSC70-1	NP_195870.1	6
BQ488119	IP5PII/BME3	NP_849745.1	6
BQ584136	SCL13	NP_193456.4	6
BQ592168	GRP2/GR-RBP4	NP_849377.1	6
BQ593588	HSP81-3	NP_200412.1	6
BQ595152	HSP17.8-CI	NP_172220.1	6
BU089563	HSL1	NP_174166.1	6
AW697779	AT3G29970	NP_190397.1	12
BE590444	HSP17.8	NP_172220.1	12

Table 1.6 continued

BF010998	ACT11	NP_187818.1	12
BF011036	AT3G29970	NP_190397.1	12
BF011227	HSP81-2	NP_200414.1	12
BI073235	HSP81-3	NP_200412.1	12
BI096111	AT3G29970	NP_190397.1	12
BI543285	AT3G29970	NP_190397.1	12
BI543685	HSP70	NP_187864.1	12
BI543772	HSC70-1	NP_195870.1	12
BQ488461	AOX1A	NP_188876.1	12
BQ489189	MPK4	NP_192046.1	12
BQ582382		NP_194839.2	12
BQ586930	CAT2	NP_195235.1	12
BQ593588	HSP81-3	NP_200412.1	12
CX779686		-	12
ED032482		-	12
AW697779	AT3G29970	NP_190397.1	18
BE590397	XTR7	NP_193149.2	18
BE590444	HSP17.8	NP_172220.1	18
BF011036	AT3G29970	NP_190397.1	18
BI073235	HSP81-3	NP_200412.1	18
BI543685	HSP70	NP_187864.1	18
BQ595152	HSP17.8-CI	NP_172220.1	18
BG577441	XTR6	NP_194311.1	24
BI073128	GER3	NP_197563.1	24
BI096176	GER1	NP_177405.1	24
BI543685	HSP70	NP_187864.1	24
BI543772	HSC70-1	NP_195870.1	24
BQ060614	MPK16	NP_197402.1	24
BQ489189	MPK4	NP_192046.1	24
BQ584082		NP_194839.2	24
BQ593209	OEP37	NP_973684.1	24
BQ594412	MKK3	NP_198860.1	24
CX779686		-	24

* *Beta vulgaris* ** *Arabidopsis thaliana* gene *** *Arabidopsis* thaliana NCBI protein

germination and the imbibition genes was a lag in expression at one or more time points for many of the genes observed. This indicated that many of the genes termed “germination” were actually a combination of imbibition and germination genes. Both varieties and treatments resulted in the activation of transcripts involved in the process of germination; however, the timing of activation was differential.

The few germination genes not present in the imbibition genes were a putative jasmonate biosynthesis (JAZ1), phospholipase D alpha (PLDA1), two retrotransposons, a protein kinase, an elongation factor, a negative regulator of light-dependent seed germination and a gene involved in meristem development and growth (AML1). Contrastingly, genes found to be specific to the H_2O_2 treatments in ACH185 and SP7622 had no overlap with the germination genes and little overlap with the imbibition genes (Table 1.7). Hydrogen peroxide transcripts included genes involved in metabolism, brassinosteroid signaling, heat shock proteins, stress transcription factors, and defense.

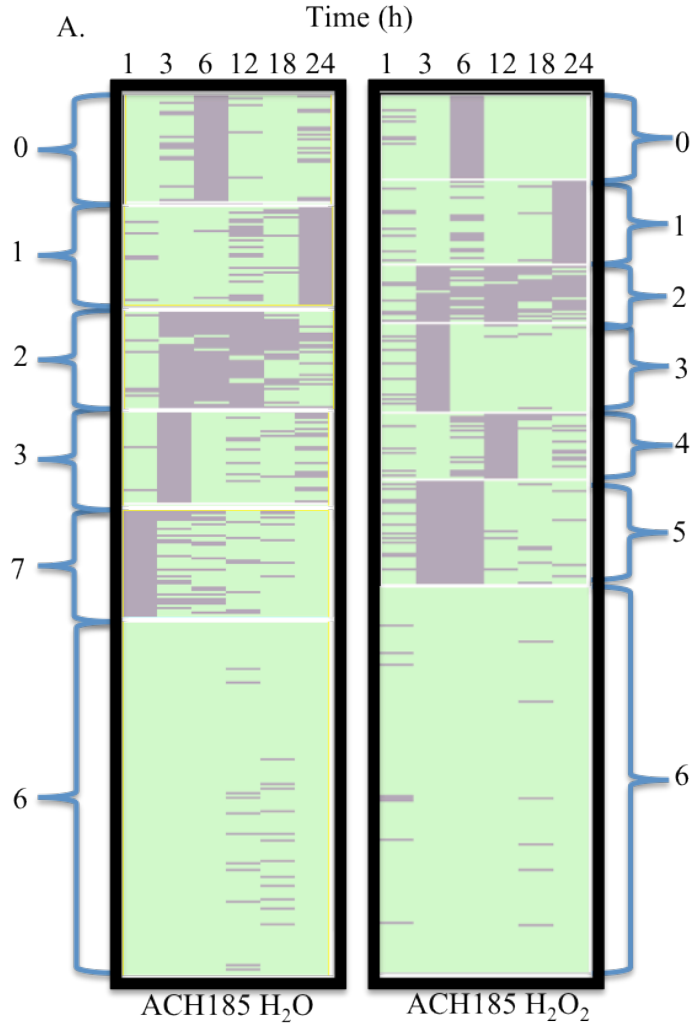
K-means clustering of gene expression revealed expression patterns common between varieties and treatments (e.g. Clusters 0, 1, 2, 3, and 6) (Figure 1.2). It should be noted that individual genes within a specific expression cluster were not typically shared between varieties and treatments and the total number of genes within each cluster varied greatly (Table A.2). In Cluster 6, a large cluster representing genes with little to no expression, only 18 genes were detected in both ACH185 and SP7622 in both the H_2O and the H_2O_2 treatments. At least one cluster was unique to the specific profile for each variety and treatment. Cluster 9 was only present in SP7622 and Cluster 10 was only present in the hydrogen peroxide treatment of SP7622 (Figure 1.2). No expression clusters were shared solely between in the two hydrogen peroxide treatments or the two water treatments. Treatment and variety specific clusters did not

Table 1.7 Genes shared between treatments and varieties at a specific time using RT-PCR. Hydrogen peroxide induced vigor genes, genes shared between both varieties in the hydrogen peroxide treatment at a specific time. Genes highlighted in pink were also present at one time point in the imbibition list.

Hydrogen peroxide specific sugarbeet gene expression			
Bv* Accession	**Gene Name****	***At* protein*****	**Time (h)**
BI096011	GER3	NP_197563.1	1
BI096145	LOX1	NP_175900.1	3
BQ583306	LOX2	NP_566875.1	3
BQ584422	LOX3	NP_564021.1	3
BQ584988	LOX2	NP_566875.1	3
BQ586920	BRH1	NP_191705.1	3
BQ591929		NP_564717.1	3
BQ592393	UCC2	NP_182006.1	3
BQ594715		NP_568046.1	3
CK136649	OSM34	NP_192902.1	3
BQ487747	XTR6	NP_194311.1	6
BQ589734	HSP18.2	NP_200780.1	6
BQ592254	DREB2C	NP_565929.1	6
BQ594875	RGA1	NP_178266.1	6
BQ595355	SSI2	NP_181899.1	6
BQ654409	XTR6	NP_194311.1	6
CK136263	NIC1	NP_565539.1	6
BF011089	DTC	NP_197477.1	12
BI096232	PLDALPHA1	NP_188194.1	18
BI543460	MTHSC70-2	NP_196521.1	18
BQ589734	HSP18.2	NP_200780.1	18
CK136863	HSP18.2	NP_200780.1	18
BI543265		NP_194648.1	24
BI543360	CAT2	NP_001031791.1	24
BQ586930	CAT2	NP_195235.1	24
BQ587848	MKK9	NP_177492.1	24
BQ587874		NP_567072.1	24
BQ587887		NP_567072.1	24
BQ589925	RPN10	NP_195575.1	24
BQ592254	DREB2A	NP_001031837.1	24
BQ595856	ATTPC1	NP_567258.1	24

* *Beta vulgaris* ** *Arabidopsis thaliana* gene *** *Arabidopsis* thaliana NCBI protein

Figure 1.2 K-means groupings of A) ACH185 and B) SP7622 in H_2O and H_2O_2 using RT-PCR over the first 24 hours of germination. Purple indicates presence of a particular transcript and green indicates absence.



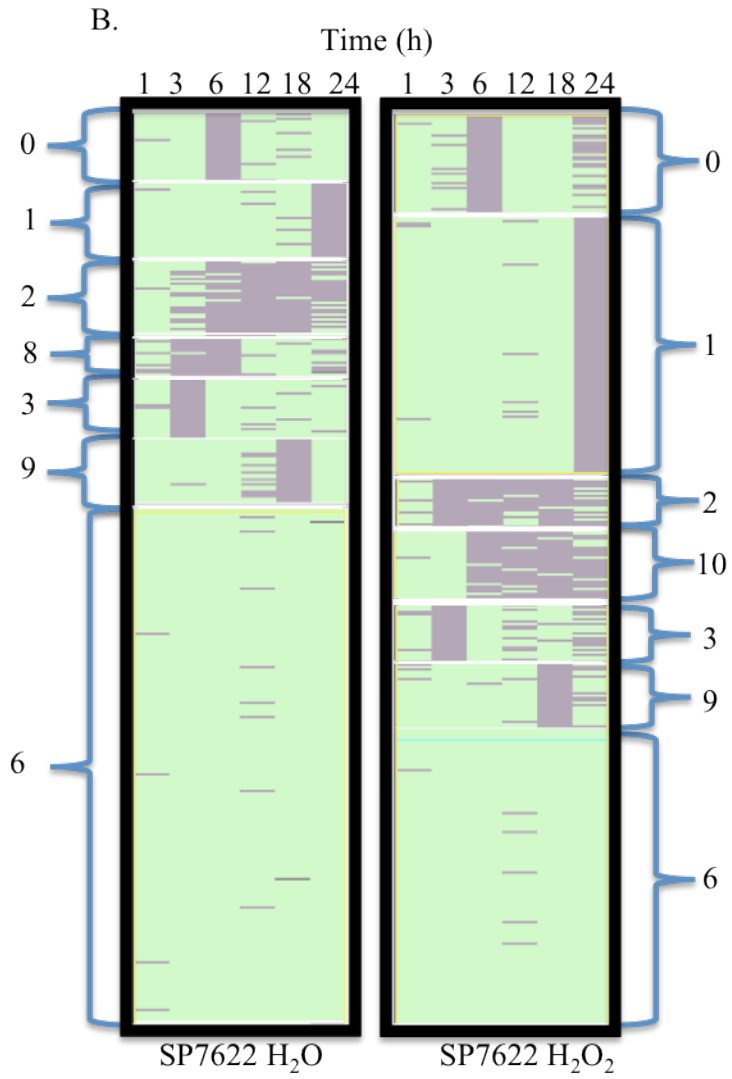
ACH185 H_2O_2 K-means Cluster

Functional Category	K0	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10
Growth and Development	17	14	8	17	7	19	64	-	-	-	-
Signaling	2	4	0	2	13	3	20	-	-	-	-
Stress	14	17	11	18	23	23	75	-	-	-	-
Abiotic	6	6	7	6	13	13	24	-	-	-	-
Biotic	2	4	4	5	5	15	12	-	-	-	-
Both	5	2	0	6	4	4	18	-	-	-	-
Undetermined	1	5	0	1	1	1	21	-	-	-	-
Undetermined	4	2	0	2	1	1	7	-	-	-	-

ACH185 H_2O K-means Cluster

Functional Category	K0	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10
Growth and Development	24	16	14	14	-	-	64	15	-	-	-
Signaling	4	3	0	4	-	-	21	2	-	-	-
Stress	13	23	19	24	-	-	67	27	-	-	-
Abiotic	8	7	12	9	-	-	21	12	-	-	-
Biotic	2	5	2	10	-	-	6	4	-	-	-
Both	2	4	5	4	-	-	19	7	-	-	-
Undetermined	1	7	0	1	-	-	21	4	-	-	-
Undetermined	6	1	2	1	-	-	5	4	-	-	-

Figure 1.2 continued



SP7622 H_2O_2 K-means Cluster

Functional Category	K0	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10
Growth and Development	14	47	3	10	-	-	50	-	-	11	11
Signaling	5	7	0	3	-	-	16	-	-	3	0
Stress	23	45	13	12	-	-	52	-	-	12	16
Abiotic	8	20	10	4	-	-	19	-	-	5	8
Biotic	4	7	2	2	-	-	6	-	-	2	2
Both	7	15		4	-	-	10	-	-	2	3
Undetermined	4	3	1	2	-	-	17	-	-	3	3
Undetermined	0	9	1	1	-	-	6	-	-	1	1

SP7622 H_2O K-means Cluster

Functional Category	K0	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10
Growth and Development	13	14	7	11	-	-	89	-	4	8	-
Signaling	3	2	1	3	-	-	20	-	1	4	-
Stress	12	15	13	14	-	-	95	-	10	15	-
Abiotic	5	7	9	5	-	-	34	-	6	4	-
Biotic	2	2	2	3	-	-	15	-	1	4	-
Both	3	3	2	4	-	-	25	-	2	2	-
Undetermined	2	3	0	2	-	-	21	-	1	5	-
Undetermined	3	0	2	0	-	-	11	-	2	1	-

typically translate into genes uniquely expressed in a variety or treatment. Cluster 7, an ACH185 H_2O specific expression cluster with 48 genes, had only five genes that were not expressed in ACH185 H_2O_2, SP7622 H_2O and H_2O_2. The five genes were glutathione ROS reductase, glutathione S transferase, *MPK20*, *LOX2* and *PAL1*.

Cluster 9, a SP7622 specific cluster, did not have many genes common between the H_2O and H_2O_2 treatments. In H_2O, 28 genes were placed into Cluster 9. Out of the 28 possible, two genes were also in Cluster 9 for the H_2O_2 treatment, while seven were in Cluster 10, and five were not expressed at all in the H_2O_2 treatment. Of the 27 total in the H_2O_2 treatment, two were shared and 14 were not expressed in H_2O.

Of the 343 genes tested, only 36 were not detectable at any time, in any treatment or variety during the first 24 hours of germination, and 27 were detected at only one time or treatment. It is unlikely that a lack of amplification was due to failed primers, as the primers were able to amplified in other sugarbeet cDNAs (data not shown.) However, many of the genes had little expression over the first 24 hours, including genes with known roles in hydrogen peroxide response in *Arabidopsis*. Non-detectable genes using RT-PCR did not segregate with a particular set of processes, such as pathogen defense, metabolism or growth. For ACH185 in H_2O and H_2O_2, 156 and 167 genes were in Cluster 6, respectively and for SP7622 H_2O and H_2O_2, 215 and 124 genes, respectively were in Cluster 6 (Table A.5 and Table A.6).

Quantitative PCR was used to evaluate the robustness of RT-PCR for detecting transcript presence from sugarbeet seeds and to evaluate quantitative differences in transcripts between the

varieties and treatments. Differences in the 48 genes, selected based on their roles in abiotic and biotic stress, growth, and hormone biosynthesis, were detectable between varieties and treatments throughout the first 24 h of germination. Varietal specific responses to hydrogen peroxide were evident (Table 1.8). Out of the 21 genes quantitatively detectable in SP7622 at 1 h H_2O_2 only four were expressed at a basal level (level similar to expression at 0 h or 1 h H_2O). Of the 25 genes detectable in ACH185 at 1 h in H_2O_2 only 10 were expressed at a basal level. By 1 h, kinase activity, calmodulin signaling, and peroxidases were all upregulated (>10 fold increase) in the H_2O_2 treatment of ACH185 (Table 1.9). In the H_2O treatment of ACH185, only a single kinase (*M3Ka*) and a respiratory burst oxidase gene were upregulated. In SP7622, none of the stress-related genes were upregulated at 1 h in the H_2O treatment. In H_2O_2, SP7622 had an array of gene upregulation involving ABA biosynthesis, lipoxygenase activity, kinase signaling, and histone deacetylation (>10 fold). A putative *GRAS1* (BQ584136), *CAF1* (BQ586375), and *CML41* (BQ587396) showed an increase (>4 fold) in expression in ACH185 in response to H_2O_2. In SP7622, a putative *PDC* (BQ490338), *BRI1* (BQ583692), *LOX3* (BQ584422), *Br6OX2* (BQ585998), *MKK3* (BQ594412), *NSP5* (BQ594578) were all upregulated (>5 fold increase) in response to H_2O_2 (Table 1.10).

Both RT-PCR and qPCR results showed activation of genes as soon as 1 h of treatment and an increase in expression (qPCR) or preferential detection (RT-PCR) of growth, abiotic and biotic stress genes including brassinosteroid responsive, lipoxygenases and MAPKs. A more pronounced up-regulation of stress-regulated genes in response to H_2O_2 was observed in both varieties. The highest level of gene expression (both number of genes and expression quantity) in

Table 1.8 Quantitative gene expression (relative fold change) over time in ACH185 and SP7622 in H_2O and H_2O_2 treatments during the first 24 h of germination. Lt. pink denotes a fold change <3. Blue indicates a fold change >3 and <10. Purple indicates a fold change >10. Gray squares indicate the relative expression was not calculated (NC) because of a lack of expression in the control treatment and nd indicates the gene was not detected.

ACH185

		H_2O						H_2O_2					
Putative	**Genbank**	**1**	**3**	**6**	**12**	**18**	**24**	**1**	**3**	**6**	**12**	**18**	**24**
Function*	**Accession**	**FC****	**FC**	**FC**	**FC**	**FC**	**FC**	**FC**	**FC**	**FC**	**FC**	**FC**	**FC**
M3Ke1[a]	BF011062	nd	nd	0	0	nd	138	nd	nd	nd	3	nd	272
GLP165[b]	BI073128	1	nd	nd	nd	nd	nd	0	nd	nd	nd	0	0
MKK9[a]	BQ487860	1	nd	2	nd	2	56	4	nd	nd	nd	nd	nd
NCED4[b]	BQ487982	1	nd	11	nd	nd	32	nd	nd	nd	3	nd	171
ATL2[b]	BQ488179	1	nd	nd	nd	nd	nd	4	nd	nd	0	0	0
ABA2	BQ488279	nd	nd	nd	nd	nd	nd	1	nd	nd	nd	nd	nd
RH26[a]	BQ488337	1	4	nd	nd	2	32	nd	nd	nd	3	nd	nd
RBOHD[a]	BQ488466	13	80	nd	0	2	nd	10	2	0	3	0	147
ABA1[b]	BQ488795	1	4.5+09	nd	nd	0	444	4	nd	nd	nd	nd	61
MKK2[a]	BQ488850	nd	nd	nd	nd	2	32	nd	nd	nd	325	1833	nd
ERF9[a]	BQ488935	nd	nd	nd	nd	nd	742	nd	nd	nd	9	8	nd
MPK4[a]	BQ489189	1	0	100	151	5	221	12	1	nd	8	3	1149
PDC[a]	BQ490338	1	2	1	1	2	11319	7	26	0	7	30	66
WAK[b]	BQ582382	1	8	nd	0	nd	215	24	nd	nd	nd	nd	nd
PHS2[g]	BQ582629	nd	nd	nd	nd	nd	1	nd	nd	1	nd	nd	nd
CYP76C7[a]	BQ582634	5	nd	8	4	4	899	0	6	0	0	37	1106
XLG1	BQ583062	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1	1

Table 1.8 continued

PLDa[b]	BQ583369	1	nd	0	0	0	11	2	0	0	5	0	54
BRI1[b]	BQ583692	1	nd	nd	nd	4	111	2527	nd	nd	nd	nd	nd
LTP4[b]	BQ584083	1	6	8	nd	nd	nd	19	0	0	8	11	189
GRAS1[b]	BQ584136	1	nd	2	nd	2	nd	nd	nd	nd	5	nd	nd
LOX3[d]	BQ584422	nd	nd	1	1	1	1	nd	nd	1	nd	nd	nd
M3KA[a]	BQ585699	53	4	2	nd	7	16	2	5	nd	nd	83	nd
BR6OX2[a]	BQ585998	1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
CAF1[a]	BQ586375	1	nd	0	0	nd	nd	nd	2	nd	21	nd	130
Kinase[b]	BQ586464	1	nd	0	0	nd	9	1	nd	nd	1	nd	nd
PP2C[b]	BQ586635	1	nd	nd	0	nd	2	nd	nd	nd	nd	nd	nd
RD26[a]	BQ586991	2	nd	nd	nd	nd	nd	nd	0	nd	4	nd	nd
CML41[a]	BQ587396	1	0	2	nd	2	nd	24	nd	nd	nd	nd	nd
MKK9[b]	BQ587848	1	0	2	nd	nd	50	nd	2	nd	nd	nd	nd
RPN10[b]	BQ589925	1	4	0	0	2	25	9	2	nd	5	14	125
Kinase[b]	BQ590125	nd	1	nd	nd	nd	nd	nd	nd	1	nd	nd	nd
Myb[a]	BQ591669	nd	0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
GRP2[a]	BQ592168	5	18	10	8	18	3983	98	5	0	1	29	2246
DREB2C[c]	BQ592254	nd	1	1	1	nd	nd	nd	nd	nd	nd	nd	nd
MPK4[a]	BQ592267	nd	nd	0	nd	nd	nd	5	1	nd	2	3	1601
CDKC[e]	BQ592936	nd	nd	nd	1	nd	nd	1	1	nd	nd	1	nd
MKK3[b]	BQ594412	1	nd	nd	0	nd	51	nd	nd	nd	38	nd	884
MBF1C[a]	BQ594558	5	0	0	2	2	74	2	nd	nd	nd	nd	nd
NSP5[a]	BQ594578	nd	nd	nd	nd	0	0	nd	nd	nd	nd	0	nd
MPK6[a]	BQ594736	0	nd	2	0	2	nd	nd	nd	2	nd	nd	nd

Table 1.8 continued

ATLP[a]	BQ594810	nd	0	0	nd	0	nd	0	0	0	0	0	0
PXA1[b]	BQ595543	1	16	4	nd	nd	44	nd	nd	16	nd	nd	nd
MKK9[b]	BQ595738	1	7	nd	nd	3	46	15	nd	nd	4	nd	229
BAM1[a]	BU089560	1	8	1	nd	19	nd	nd	2	nd	48	61	nd
HDA1[b]	CF543165	1	nd	4	0	nd	50	0	2	0	2	15	6375
PER50[a]	CK136719	nd	23	8	0	4	219	11	15	0	42	20	0
Total		31	21	25	20	22	27	24	18	14	25	20	20

*Fold change normalized to [a] 0 h [b] 1 h H_2O [c] 3 h H_2O [d] 6 h H_2O [e] 12 h H_2O [f] 18 h H_2O [g] 24 [h] H_2O **FC is fold change

SP7622

		H_2O						**H_2O_2**					
Putative	Genbank	1	3	6	12	18	24	1	3	6	12	18	24
Function[+]	Accession	FC	FC	FC	FC	FC	FC	FC	FC	FC	FC	FC	FC
M3Ke1	BF011062	nd	nd	nd	NC	NC	nd	nd	nd	nd	nd	NC	NC
GLP165[a]	BI073128	0	2	4	0	2	210	5	0	8	46	1	nd
MKK9[a]	BQ487860	nd	17	nd	42	nd	53	nd	nd	nd	nd	nd	nd
NCED4[a]	BQ487982	nd	17	nd	nd	nd	17	10	2	nd	nd	nd	2
ATL2[a]	BQ488179	1	3	nd	0	1	6	5	8	8	nd	nd	nd
ABA2	BQ488279	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
RH26[c]	BQ488337	nd	1	0	nd	nd	nd	nd	nd	nd	nd	nd	0
RBOHD[c]	BQ488466	nd	1	0	0	0	0	nd	nd	0	nd	nd	4
ABA1[a]	BQ488795	nd	15	nd	0	nd	2	42903	9	nd	nd	nd	nd
MKK2[d]	BQ488850	nd	nd	1	0	0	1	1	nd	nd	5	nd	nd
ERF9[b]	BQ488935	1	8	8	nd	nd	nd	nd	1	nd	39	39833	2
MPK4[a]	BQ489189	1	1	nd	1	265	3	18	2	1	nd	881	0

Table 1.8 continued

PDC[b]	BQ490338	1	2	nd	0	nd	4	nd	0	4	703	2426	7
WAK	BQ582382	nd	nd	nd	NC	NC	nd	nd	NC	nd	NC	nd	NC
PHS2[e]	BQ582629	nd	nd	nd	1	0	498	nd	0	nd	658	nd	4
CYP76C7[a]	BQ582634	nd	8	nd	2	5	24	21	1	26	85	10486	3
XLG1	BQ583062	nd	nd	nd	nd	nd	NC	nd	nd	nd	nd	nd	nd
PLDa[a]	BQ583369	nd	2	nd	0	nd	3	nd	0	nd	nd	nd	1
BRI1[a]	BQ583692	nd	1	2	0	nd	nd	9	0	3	nd	2.7E+09	7
LTP4[a]	BQ584083	nd	46	0	19	8	3	nd	8	32	81	35440	2
GRAS1[a]	BQ584136	nd	nd	nd	1	nd	1	nd	1	nd	nd	nd	nd
LOX3[a]	BQ584422	nd	nd	nd	2	nd	1	30	0	6	12	18790	nd
M3KA[a]	BQ585699	1	11	16	0	nd	nd	14	2	nd	nd	nd	nd
BR6OX2[a]	BQ585998	nd	nd	nd	0	1	nd	nd	0	8	21	nd	1
CAF1[a]	BQ586375	1	0	0	0	0	nd	nd	nd	nd	nd	nd	nd
Kinase[c]	BQ586464	nd	1	3864	1	nd	1	nd	0	nd	nd	nd	nd
PP2C[a]	BQ586635	1	8	nd	1	15	nd	nd	nd	nd	nd	nd	2
RD26[a]	BQ586991	nd	2	2	0	1	1	5	1	19	nd	nd	nd
CML41[a]	BQ587396	nd	1	nd	1	12	0	nd	nd	nd	5	nd	0
MKK9[d]	BQ587848	nd	nd	1	1	1	nd	nd	nd	1	nd	nd	nd
RPN10[a]	BQ589925	1	nd	nd	nd	nd	0	nd	0	nd	nd	nd	0
Kinase	BQ590125	nd	nd	nd	nd	nd	nd	NC	nd	nd	NC	nd	NC
Myb[a]	BQ591669	nd	nd	nd	0	nd	nd	8	1	nd	nd	nd	nd
GRP2[c]	BQ592168	nd	1	0	0	0	0	2	0	6	16	198	18
DREB2C[c]	BQ592254	nd	1	nd	nd	nd	0	nd	nd	nd	nd	nd	0
MPK4[c]	BQ592267	nd	1	nd	0	0	0	1	0	nd	nd	984	0
CDKC[b]	BQ592936	1	nd	9	578	2	23	380	1	16	nd	nd	1

Table 1.8 continued

MKK3[a]	BQ594412	nd	nd	nd	1	nd	nd	11	nd	8	82	nd	2
MBF1C[c]	BQ594558	nd	1	nd	0	1	1	nd	0	1	2	233	0
NSP5[a]	BQ594578	nd	nd	nd	nd	nd	nd	4	nd	nd	nd	1647	0
MPK6[a]	BQ594736	nd	nd	nd	0	nd	nd	nd	nd	nd	nd	nd	nd
ATLP[b]	BQ594810	1	372	nd	nd	2	4	nd	nd	15	nd	nd	16
PXA1[a]	BQ595543	nd	nd	nd	nd	nd	nd	nd	6	nd	nd	nd	2
MKK9[a]	BQ595738	1	nd	nd	2	nd	nd	nd	0	nd	20	4612	1
BAM1[a]	BU089560	nd	0	nd	0	0	nd	2	nd	nd	nd	1115	0
HDA1[a]	CF543165	1	nd	nd	0	23	nd	10	1	nd	nd	nd	42
PER50[a]	CK136719	nd	nd	75	0	3	2	12	29	10	31	5538	6
Total		14	26	15	35	25	26	21	29	18	17	16	31

[+] Fold change normalized to [a] 0 h [b] 1 h H_2O [c] 3 h H_2O [d] 6 h H_2O

both varieties was seen at 24 h for most genes tested. In response to H_2O_2, both varieties upregulated a putative *MKK9, BRI1* and *MPK4*. In addition, a *LTP4* and *RD26* were upregulated in both treatments of SP7622 and the H_2O_2 treatment of ACH185 indicating a possible role in germination vigor. Several genes putatively functioning in drought and salt response as well as hydrogen peroxide signaling (BQ488279 (*ABA2*), BQ586464 (kinase), BQ591669 (Myb), BQ594736 (*MPK6*)) showed little (< 2 fold) change in expression between the two varieties and treatments during the first 24 h.

DISCUSSION

Germination is a multigenic, dynamic process, easily modified by external stresses. In sugarbeet seeds, germination was characterized by rapid water absorption during the first 24 h of imbibition and, in the vigorous variety, higher levels of internal hydrogen peroxide. Genetic characteristics of germination were a rapid degradation of endogenous mRNA over the first 3 h of imbibition and a slow concurrent activation of seed-based genes in growth, stress and signaling. Sugarbeet germination was a very dynamic process with gene expression changes, likely involved in vigor induction, occurring rapidly upon treatment.

RT-PCR analyses of the vigor-inducing hydrogen peroxide treatment revealed a number of putative metabolic, drought, and pathogen related genes between 3 and 24 h. A number of hydrogen peroxide specific genes were shared between 3 and 6 hours and again at 24 h. Shared genes at these particular times suggest a common mode of early stress response, which may lead to a common response later, e.g. growth, and may prove useful for early screening of sugarbeet germplasm. The upregulation of abiotic, water response in particular, genes was expected due to

Table 1.9 Genes in ACH185 associated with hydrogen peroxide-induced vigor over time using qPCR

ACH185		
H_2O_2	**Gene name**	**Putative Role**
Only	CML41	Stress regulated (Undetermined)
Only	MPK4	Stress signaling (Biotic)
Only	CAF1	Stress regulated (Biotic)
Only	LTP4	Stress regulated (Abiotic)
Pred	HDA1	Stress regulated (Abiotic)
Pred	BRI1	Stress and growth
Pred	MKK3	Stress signaling (Biotic)
Pred	MKK9	Stress signaling (Both)
Pred	MPK4	Stress signaling (Biotic)
Pred	PLDa	Growth
Pred	BAM1	Growth
Pred	MKK2	Stress signaling (Abiotic)

*Only indicates gene was only upregulated in H_2O_2. Pred indicates gene was predominantly upregulated in H_2O_2

Table 1.10 Genes associated with hydrogen peroxide-induced vigor in SP7622 over time using qPCR.

SP7622		
H_2O_2*	**Gene name**	**Putative Role**
Only	Myb	Stress regulated (Both)
Only	RD26	Stress regulated (Abiotic)
Only	LOX3	Stress regulated (Biotic)
Only	GRP2	Stress regulated (Abiotic)
Only	BRI1	Stress and growth
Only	MKK3	stress signaling (Biotic)
Only	BAM1	Growth
Only	KELCH	Growth
Only	MBF1C	Stress regulated (Abiotic)
Pred	ABA1	Stress regulated (Abiotic)
Pred	PDC	Growth
Pred	PER50	Stress regulated (Abiotic)
Pred	CYP76C7	Undetermined
Pred	ERF9	Stress regulated (Biotic)
Pred	MKK9	Stress signaling (Both)
Pred	MPK4	Stress signaling (Biotic)
Pred	LTP4	Stress regulated (Abiotic)

*Only indicates gene was only upregulated in H_2O_2. Pred indicates gene was predominantly upregulated in H_2O_2

the abiotic nature of the stress, but upregulation of biotic-related stress genes was not. Quantitative transcript changes of a putative lipid transfer protein (*LTP4*) showed a large increase in expression in the H_2O_2 treatment in ACH185 and SP7622 in comparison to the H_2O treatment. Expression was quantitatively high (>10 fold increase at any given time point compared to the 0 h expression) in the SP7622 H_2O treatment, though significantly less than in H_2O_2. Upregulation of this specific lipid transfer protein was positively correlated with hydrogen peroxide vigor in the two varieties tested. Lipid transfer proteins in general have been implicated largely in pathogen response, and are upregulated by ABA (Gonorazky et al. 2005; Mane et al. 2007). LTP4, in particular, has been associated with pathogen response in barley(Molina 1996).

BRI1, another gene found to be associated with vigor and hydrogen peroxide response in germinating seedlings, has no known role in germination in *Arabidopsis*. *Arabidopsis bri1* mutants are insensitive to Br (Brassinosteroids) and show a range of phenotypes including dwarfing, sensitivity to ethylene, ABA, GA and auxins, male sterility, reduced cell and leaf expansion, and a misregulation of genes regulated by GA (Swarbreck 2008). A lack of *BRI1* expression during germination could result in enhanced sensitivity to ABA and would hinder germination. Conversely it is also possible that upregulating *BRI1* expression could be instigating growth and working through GA dependent pathways. It has been shown that Br signaling provided tolerance to a range of stresses and was activated as soon as 1 h (Che et al. 2010). These two genes (*LTP4* and *BRI1*), which are associated with germination vigor in both varieties, could be potential molecular markers for screening sugarbeets.

Of the MAPKs tested, a putative beet *MPK4* showed an induction in both varieties in response to H_2O_2, but not in the H_2O treatment. A putative *BvMKK2*, had an increase in expression (quantity and duration) in the ACH185 H_2O_2 treatment compared to the H_2O treatment. *MKK2* expression levels in SP7622 were not determined, because of a lack of a suitable treatment and time for normalization. However, expression was present in both H_2O and H_2O_2 treatments and showed differential timing. Another putative *MKK9* showed a >900 fold increase of expression in the H_2O_2 treatment for both varieties in comparison to the H_2O treatment. H_2O_2 has numerous roles as a signaling molecule in abiotic and biotic stress response (Liu 2010; Orozco-Cardenas 2003). BQ594736 (a putative beet *MPK6*) showed minimal changes in expression (<2.5 fold change) in both varieties and treatments over the first 24 h measured. *ANP1* and *MPK3* were not tested quantitatively for expression changes, but were not detectable in the RT-PCR assay. Our results imply that the H_2O_2 activated *ANP1/MPK3/6* pathway does not play a role in germination vigor. *MKK2/MPK4* and *MKK9* pathways may have a positive role on improved emergence and the hydrogen peroxide response in the two beet varieties tested.

This research suggests that H_2O_2 accumulation instigates vigor prior to lipid catabolism and that this vigor induction is through the activation of signaling molecules and abiotic and pathogen-defense related genes. Differences in gene expression between the two varieties prior to treatment revealed that ACH185 had a number of stress-related transcripts present that were not detectable in SP7622, but no metabolic-related transcripts. These additional stress-related transcripts at 0 h and over time may have been present as a result of the fungal contamination of the seed, as the incidence of fungal contamination was higher for the ACH185 seed. SP7622 had

a diverse array of signaling molecules, metabolic transcripts, and transcription factors that were not present in the ACH185 seed. This suggests that natural priming, for germination and defense, may occur during seed development and maturation to dictate germination vigor (Beckers 2009; Masoudi 2010). Transcripts shared between the two varieties prior to treatment, likely to be essential for seed development and germination, included a number of MAPKs, a jasmonate inducible, and fatty acid biosynthesis genes. The array of pre-germination metabolic and signaling transcripts present in SP7622, but not in ACH185, may be important for germination vigor. RT-PCR was able to show that 1 h in H_2O_2 was sufficient to induce a genetic response to hydrogen peroxide during germination for both varieties. Germination-vigor specific RT-PCR genes, genes shared by SP7622 (H_2O and H_2O_2) and ACH185 (H_2O_2), included a large number of genes involved in cell signaling and defense (protein kinases, MAP kinases, etc). Shared genes were mainly detected between 6 and 24 hours of treatment, which indicates that different initial responses may converge to common pathways over time. The large proportion of genes that were shared between the germination and imbibition list indicated that within each variety, despite their vigor differences, steps were being taken for imbibition and successful germination. For ACH185 in the H_2O treatment, germination, though delayed, was being instigated.

BI073121, one of the five genes upregulated only in ACH185 H_2O treatment according to RT-PCR, was similar to an *Arabidopsis* gene putatively involved in the glutathione cycle to degrade toxic hydrogen peroxide (Swarbreck 2008). Another gene, BI543980, is a glutathione S transferase (Sappl 2004). While response may be mediated through the activation and repression of genes, clustering of the RT-PCR results showed that differential timing might also play a large role in moderating the hydrogen peroxide induced germination response. Many of the clusters,

even those specific to a variety or treatment, did not have genes uniquely expressed across all the time points tested. The addition of exogenous H_2O_2 activated, repressed and changed the timing of expression for many of the genes observed. qPCR further confirmed that gene expression changes were occurring in response to H_2O_2, and revealed qualitative and quantitative changes in stress, growth, and signaling genes. H_2O_2 elicited a response as soon as 1 h in the seed and continued throughout the first 24 h of germination. Whether the hydrogen peroxide signals that induce seedling vigor are activated that early is unclear, but it is certain that seeds are able to perceive and respond rapidly to this stress treatment. While a proportion of the genes found to respond to H_2O_2 in adult *Arabidopsis* plants did have altered gene expression in response to hydrogen peroxide during germination for sugarbeets, many of the stress-related transcripts showed little response to H_2O_2. Under H_2O_2 exposure, microarray data has revealed hundreds of genes activated by more than 1.5 fold increase in adult *Arabidopsis* plants (Desikan et al. 2001). Our work suggests that many of these genes (shown to be induced under H_2O_2 stress during adult stress in *Arabidopsis*) do not play a role in hydrogen peroxide induced germination vigor, at the time points examined in *Beta vulgaris*.

Internal hydrogen peroxide concentrations were inversely related to the differences in water absorption between the two varieties. ACH185, which absorbs more water than SP7622 over time, had a more rapid decrease in internal hydrogen peroxide than SP7622. This decrease in internal hydrogen peroxide was correlated with an increase in water absorption and a decrease in germination by ACH185. This possibly results in the dilution of internal H_2O_2 during imbibition, which can be restored with the exogenous application of H_2O_2. Differences in

internal hydrogen peroxide concentration were observed in the two varieties at 6 h and 24 h. Since physical differences were clear in the first 24 h of imbibition between good and poor emerging treatments (H_2O_2 and H_2O) it is likely that Phase I, or the first 24 hours of imbibition for fruits and 6 h for embryos, are when germination speed and vigor are defined. Phase I of germination and internal hydrogen peroxide concentrations may be good initial physical indicators of a variety and seedlot's germination vigor.

The complexity of the varietal gene expression response to a vigor inducing stress was clearly demonstrated in this study. ACH185 and SP7622, which both show increased vigor in H_2O_2, had very different genetic reactions during Phase I (first 24 h) of germination. Despite the very divergent profiles observed, several key genes were identified as possible components of a shared response. Within the varieties and conditions tested, it appeared that low levels of oxidative stress during sugarbeet germination activated a complex series of signaling cascades and defense responses involving the MAPK cascades (*MPK4* and *MKK9*), lipid mobilization (*LTP4*) and catabolism, jasmonate biosynthesis, and brassinosteroid signaling (*BRI1*) that resulted in enhanced germination in H_2O_2 (both varieties) and in H_2O (for the vigorous variety).

In germinating sugarbeets, hydrogen peroxide results in a large-scale activation and repression of many growth and development, hormone biosynthesis, and stress-related genes as soon as 1 h after treatment and continues through at least 24 h. *MPK4*, *MKK9*, *LTP4* and *BRI1* were genes found to be associated with hydrogen peroxide induced vigor that were shared between the two (highly genetically different) varieties. These genes, upon confirmation in other varieties, may be useful as molecular markers for screening germplasm and selective breeding to improve germination in sugarbeets. While further studies will need to be completed, this study

provides the first look at large-scale transcriptional and physical changes in sugarbeet seeds during the first 24 h of germination, and describes the possible role of stress-related transcripts in sugarbeet germination vigor.

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CHAPTER 2: EARLY SEEDLING RESPONSE TO WATER AND PATHOGEN STRESS AND THE IDENTIFICATION OF VIGOR RESPONSE GENES IN *BETA VMLGARIS*

ABSTRACT

Sugarbeet seedlings are extremely susceptible to environmental stress during the first few weeks of growth. Abiotic stresses, such as changes in water availability, are difficult to control and limit crop productivity. Soilborne pathogens such as *Aphanomyces cochlioides*, *Rhizopus sp.*, *Pythium spp.* and *Rhizoctonia* can also significantly impact the quantity and quality of beet seedlings in the field. Extensive breeding, expensive chemical control, and cultural practices are essential to combat losses due to seedling stress. Currently, molecular markers for early screening of quantitative disease and stress resistance traits are unavailable for sugarbeet breeders. Forty-eight genes, previously tested for their role in germination vigor, were quantitatively evaluated in 3-week old seedlings of two sugarbeet varieties under an oomycete, a fungal, and a water stress condition. Differences were observed between two varieties in the type of genes activated in response to each stress. The pathogen treatments, *Rhizopus sp.* and *A. cochlioides,* showed greater similarity in their gene expression to each other than with the water treatment for both varieties. Putative *PP2C* (Protein phosphatase 2C) and *GRP2* (Glycine-rich binding protein) were upregulated in both sugarbeet varieties in the water treatment suggesting a possible role in flooding stress. Genes involved in basal pathogen response in sugarbeet, specific *Rhizopus sp.* and *A. cochlioides* responses, and varietal specific pathogen responses were identified among upregulated genes. Gene expression was compared in 3-week old stressed seedlings with expression during seed germination. Genes common between varieties and developmental stages revealed overlapping expression patterns between germination and early seedling vigor.

INTRODUCTION

The first few weeks of growth are the most critical for *Beta vulgaris* (sugarbeet) germination, growth and stand establishment. During this time, the plants are highly susceptible to abiotic and biotic stress as cells are rapidly dividing and growing (Nevins et al. 1968; Sanchez-Rodriguez et al. 2010). Cold, heat and drought hinder sugarbeet germination and growth while soilborne pathogens, such as *Rhizopus sp., Pythium spp.* and *Aphanomyces cochlioides,* can infect and debilitate susceptible seedlings. Breeding efforts and fungicide applications have limited the prevalence of seedling diseases, but stand losses still occur when conditions are suitable for disease development. Breeding for tolerance to individual pathogens and abiotic stresses is time consuming, narrows the genetic base and is often only effective for a few years (Acosta-Leal et al. 2010; Kuzdowicz 2009; Sadeghian and Khodaii 1998; Taguchi and Ogata 2010). *A. cochlioides*, an oomycete, has been listed as a major cause of seedling death since the 1920s (Dyer et al. 2004; Humphries and French 1969). Partial resistance has been incorporated into most of the modern sugarbeet varieties grown in the Midwest, but damage and losses still occur annually. Breeding for improved germination, seedling vigor and durable disease resistance traits is difficult due to low heritability and significant environmental influence. In many instances, breeding efforts are limited by inconsistent lab and greenhouse results, few resistant germplasm and a lack of molecular markers.

Molecular markers, which rely on genetic variation associated with phenotypic traits, can serve as tags to rapidly evaluate germplasm and incorporate desired traits into alternative genetic backgrounds. In beets, self-incompatibility has limited the number of available populations segregating for specific traits, increasing the need for markers associated with traits of interest. Previous work (Chapter 1) identified expression differences in genes in two sugarbeet lines

germinated in hydrogen peroxide and water, with potential roles as molecular markers. High hydrogen peroxide concentrations and upregulation of specific genes involved in growth, signaling and defense were associated with increased germination vigor and growth. Upregulation of abiotic and biotic stress responses in particular were associated with successful germination and vigor in the two varieties tested. Germination markers have limited usefulness, due to the rapidity with which germination can be tested. However, germination markers that are also predictive of early seedling vigor to pathogens or abiotic stress would be invaluable.

Over the last decade, multiple studies in model organisms have shown that cellular responses to abiotic and biotic stimuli can converge through overlapping signaling cascades, such as mitogen-activated protein kinases (MAPKs), to induce changes in gene expression that can activate plant defenses (Beckers et al. 2009; Colcombet and Hirt 2008; Pitzschke and Hirt 2006). In *Arabidopsis thaliana,* the constitutive expression of defense related MAPKs results in enhanced disease and stress resistance to multiple stimuli (Brader et al. 2007; Cheong and Kim 2010; Jeong et al. 2008; Shi et al. 2010; Zhang et al. 2007). Hormone and metabolic components are also common response elements activated by multiple stresses. Abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA) and brassinosteroids (Br) are hormones produced upon infection, wounding or stress. These hormones can activate plant cell defenses such as stomatal movement, cell death, systemic acquired resistance (SAR) and reactive oxygen species (ROS) production (Adie et al. 2007; Beckers et al. 2009; Colcombet and Hirt 2008; Krishna 2003; Pitzschke and Hirt 2006; Yu et al. 2008; Zhao et al.). Metabolic-related proteins such as alternative oxidases (AOX), lipoxygenases (LOX) and phospholipase D alpha (*PLDA*) are also induced by external stresses and result in the mobilization of stored lipid reserves, similar to the lipid mobilization seen by de los Reyes et al. (2003). Information on common elements of stress

response derived from model organisms may provide potential markers or genetic indicators to assess germination and seedling vigor in less studied crop species useful for assessing tolerance to abiotic and biotic stresses.

Identification of genes involved in early stress responses has been primarily achieved by mapping genetic traits through bulk segregant analyses (BSA) or mapping populations (Bansal et al. 2008; Bariana et al. 2001; Li et al. 2010; Manangkil et al. 2008; Mano and Takeda 1997; Shankar et al. 2008; Yu et al. 2009). Expression QTLs (eQTLs) have been used to link QTLs (quantitative trait loci) with the genes for metabolite changes and certain disease resistances such as aphid and rust resistance in various plant species (Dai et al. 2009; Druka et al. 2008; Kliebenstein 2009; Michaelson et al. 2009). While effectively linking the expression of a particular gene or set of genes of a given locus with a response, an eQTL's ability to detect differential timing of gene expression as well as those genes located externally to QTL regions associated with a response were limited. The ability to combine eQTLs and association mapping to correlate gene expression changes with a trait of breeding interest (disease or insect resistance, metabolite changes, etc.) could be a more accurate and useful way to determine resistance when traits are controlled by more than a single gene and mapping populations are not available.

The ability to run diagnostic tests on sugarbeet seeds and accurately detect germination potential and early season abiotic and biotic stress resistance would greatly assist sugarbeet breeders in developing improved varieties. Identifying genetic components associated with plant stress responses at early stages of growth may also provide resources for breeders and seed companies to assess the adult vigor in addition to seedling vigor of populations. The objectives of this study were to identify genes involved in two sugarbeet varieties' seedling response to two

seedling pathogen stresses, and to identify genes associated with vigor in both germinating seeds and 3-week old seedlings.

MATERIALS AND METHODS

Inoculation. A *Rhizopus sp.* (RP08-1) and an *Aphanomyces cochlioides* isolate collected from Michigan (provided by Dr. Linda Hanson, USDA-ARS, East Lansing, MI) were individually grown on potato dextrose agar (PDA) for two weeks. An actively growing plug (7 mm diameter) of each isolate was transferred to cornmeal agar (CMA) and grown for 5 days at 25 °C under constant fluorescent light. Five milliliters of 18 MOhM H_2O were added to the *Rhizopus sp.* plates and the resulting spore suspension was diluted to 100 spores/ml with 18 MOhm water. Four 10 mm diameter disks from the *A. cochlioides* plates were transferred to an empty sterile Petri dish and flooded with sterile pond water (Horticulture Demonstration Gardens, Michigan State University), and placed in the dark according to Yu (2003). Zoospores were counted 18 h later and diluted to a suspension of 100 zoospores/ml with 18 MOhm water.

Germination and growth of seedlings. A former commercial variety, ACH185 (EL-A012206) and breeding germplasm, SP7622 (EL-A015030), were chosen for analyses. ACH185 is a triploid, cytoplasmic male sterile variety with low average germination (<60%) under stress conditions, low field emergence, partial resistance to *A. cochlioides*, and complete resistance to *Rhizopus sp.*. SP7622 is a diploid germplasm with high germination (>60%) and field emergence, partial resistance to *A. cochlioides* and resistance to *Rhizopus sp.*. 400 seeds of each accession were germinated in 88 mM H_2O_2 for 4 days shaken constantly at 25 °C under constant

light. Germinated seeds were transferred onto filter paper in germination boxes with 25 ml of 18 MOhm H_2O and grown at 25 °C under constant fluorescent light for 17 days (water levels were maintained) in a growth chamber. Fifteen seedlings were transferred to each flask containing 25 ml of H_2O, 100 spores/ml *Rhizopus sp.* or a 100 zoospores/ml *Aphanomyces cochlioides* spore suspension and shaken constantly at 25 °C. Three replicates of 15 plants were used per time point for each treatment. Inoculated seedlings were removed at 6, 12, 18 and 24 h.

RNA extraction and cDNA synthesis. Total RNA was extracted using the Plant NucleoSpin Total RNA (Machery-Nagel) according to the manufacturer's protocol (Duren, Germany). cDNA synthesis was performed using 1 μg of the total RNA with Superscript III reverse transcriptase according to the manufacturers protocol (Invitrogen, Carlsbad, CA). cDNA was diluted to 50 ng/μl and used for qPCR analyses.

qPCR analysis of genes over time. The forty-eight primers developed from putative beet stress response genes, used previously for germination vigor quantitative PCR studies, were tested against the three-week old seedling cDNA (Table A.1). qPCR was performed using the HotStart Kapa Sybr, Sybr Green master mix (Kapabiosystems, Woburn, MA), according to the manufacturer's protocol on the ABI StepOne Plus (Applied Biosystems, Carlsbad, CA) with 50 ng/μl of cDNA per reaction and analyzed as described previously (Chapter 1). The experiment was replicated twice. qPCR results from the study on germinating seeds of ACH185 and SP7622 over time (Chapter 1 (Table 1.5)) were compared with qPCR results from 3-wk old seedlings to identify conserved modes of vigor between germination and seedling vigor.

RESULTS

Three-week old sugarbeet seedlings showed varietal and treatment differences in gene expression when exposed to two different pathogen treatments and one abiotic treatment. SP7622 displayed a rapid activation of genes involved in signaling, lipid mobilization, hormone biosynthesis and DNA transcription in response to the individual treatments (11 in H_2O, 35 in *Rhizopus sp.*, 37 in *A. cochlioides*) (Table 2.1). In contrast, ACH185 had fewer genes (4 in H_2O, 22 in *Rhizopus sp.*, 19 in *A. cochlioides*) upregulated in response to any given treatment tested (Table 2.2). Expression between varieties, within each treatment, was different in the total number of genes expressed, the quantity of expression and the time at which genes were expressed.

ACH185 showed changes in gene expression as soon as 6 h between the three treatments, and continued through the 24 h monitored. Of the 48 genes tested in ACH185, only putative *DREB2C* (BQ592254) and a protein kinase (BQ590125) were not detectable at any time or treatment. The remaining 46 were expressed intermittently across the time points tested in one or more treatment. ACH185 did not show induced expression of 17 of the 44 genes tested (at a fold change >3 from the 6 h water control), and few (9 genes) overlaps were visible between the pathogen treatments. Sixteen of the genes tested in ACH185 at 3 wks had an induction (> 3 fold increase) in either the *Rhizopus* or *Aphanomyces* treatments compared to the abiotic flooding control (H_2O) treatment at the times observed. Genes that were upregulated (>3 fold increase) in response to both *Rhizopus* and *Aphanomyces* in ACH185 were a putative *GER3* [Germin protein 3] (BI073128), *PDC* [pyruvate decarboxylase] (BQ490338), *CYP76C7* [cytochrome P450 76C7] (BQ582634), *BRI1* [Brassinosteroid insensitive 1] (BQ583692), *M3Ka* [MAPKK Kinase alpha]

Table 2.1 Quantitative gene expression (fold change) over time of 3 wk seedlings of ACH185 and SP7622 and the Aphanomyces, Rhizopus and H_2O treatments. Numbers in bold indicate these genes were associated with hydrogen peroxide induced vigor during germination. Genes highlighted in purple were upregulated in both pathogen treatments and not the water treatment.

		ACH185										
		Aphanomyces				*Rhizopus*				H_2O		
***At* gene**[a]	**Genbank**	**6**	**12**	**18**	**24**	**6**	**12**	**18**	**24**	**6**	**18**	**24**
M3Ke1	BF011062	0	0	20	nd	0	nd	0	0	1	nd	0
GER3	BI073128	2	3	3	1	0	3	250	1	1	1	2
MKK9	BQ487860	0	0	1	nd	nd	nd	0	0	nd	1	0
NCED4	BQ487982	0	0	0	nd	nd	nd	0	0	nd	1	0
ATL2	BQ488179	0	nd	0	nd	nd	nd	11	0	1	nd	0
RBOHD	BQ488466	nd	0	nd	nd	nd	nd	nd	0	1	nd	nd
ABA1	BQ488795	2	1	2	1	2	2	3	2	1	1	2
MKK2	BQ488850	nd	0	nd	nd	nd	nd	nd	nd	nd	nd	1
ERF9	BQ488935	nd	nd	nd	nd	nd	nd	nd	NC	nd	nd	nd
MPK4	BQ489189	0	0	0	0	nd	0	0	0	1	0	0
PDC	BQ490338	3	1	2	2	5	1	2	3	1	6	7
WAK	BQ582382	4	2	nd	nd	nd	nd	nd	nd	nd	nd	1
PHS2	BQ582629	nd	NC	nd	nd	nd	nd	nd	nd	nd	nd	NC
CYP76C7	BQ582634	3	1	5	3	6	2	6	5	1	1	1
PLDa	BQ583369	38	0	2	2	nd	nd	3	57	nd	1	4
BRI1	**BQ583692**	**38**	**0**	**2**	**3**	**2**	**27**	**3**	**58**	**1**	**1**	**1**
LTP4	**BQ584083**	**1**	**0**	**2**	**24**	**2**	**2**	**3**	**1**	**1**	**5**	**2**
GRAS1	BQ584136	2	1	2	nd	nd	80	1	2	1	2	1
LOX3	BQ584422	2	0	2	1	5	3	3	3	1	1	0
M3KA	BQ585699	3	0	2	2	4	3	6	5	1	1	1
BR6OX2	BQ585998	6	7	2	2	5	1	5	4	1	1	1
CAF1	BQ586375	264	1	2	nd	nd	28	1	1	1	1	1
Serine kinase	BQ586464	nd	1	2	nd	nd	nd	nd	nd	nd	nd	1
PP2C	BQ586635	3	7	3	nd	4	14	2	4	1	10	1

Table 2.1 continued

RD26	BQ586991	2	1	2	1	2	1	2	2	1	1	1
CML41	**BQ587396**	**52**	**1**	**5**	**0**	**7**	**1**	**1**	**0**	**1**	**0**	**1**
MKK9	BQ587848	0	3	1	0	0	1	0	0	1	0	0
RPN10	BQ589925	1	2	2	nd	nd	3	5	3	nd	nd	1
Myb	BQ591669	nd	NC	nd	nd	nd	nd	nd	nd	nd	nd	nd
Protein kinase	BQ590125	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
GRP2	BQ592168	nd	1	19	nd	nd	nd	nd	nd	1	nd	3
DREB2C	BQ592254	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
MPK4	**BQ592267**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**NC**
CDKC	BQ592936	nd	nd	nd	nd	nd	nd	nd	nd	1	nd	nd
MKK3	BQ594412	1	1	1	1	1	1	1	1	1	0	0
MBF1C	BQ594558	2	0	2	nd	nd	2	6	2	1	1	1
NSP5	BQ594578	3	0	1	nd	nd	1	nd	nd	nd	1	1
MPK6	BQ594736	1	0	1	nd	1	1	2	0	nd	1	0
ATLP	BQ594810	nd	nd	nd	nd	nd	nd	NC	nd	nd	nd	nd
PXA1	BQ595543	1	0	1	nd	nd	1	4	22	1	0	0
MKK9	**BQ595738**	**2**	**2**	**1**	**5**	**2**	**1**	**3**	**4**	**1**	**2**	**1**
BAM1	BU089560	4	1	2	3	4	8	2	3	1	2	2
HDA1	CF543165	2	1	2	1	2	6	4	2	1	1	1
PER50	CK136719	2	2	2	nd	3	4	2	3	1	1	49

[a] *Arabidopsis thaliana* genes

		SP7622											
		Aphanomyces				***Rhizopus***				**H_2O**			
***At* gene**[a]	**Genbank**	**6**	**12**	**18**	**24**	**6**	**12**	**18**	**24**	**6**	**12**	**18**	**24**
M3Ke1	BF011062	2	8	68	0	69	240	1	0	1	0	1	2
GER3	BI073128	1	0	25	0	10	85	0	0	1	0	0	0
MKK9	BQ487860	37	nd	39214	2	1505	681	5	21	1	nd	0	7
NCED4	BQ487982	23	3	3028	24	214	2172	27	13	1	4	23	17
ATL2	BQ488179	4	nd	1	0	1	nd	0	0	nd	nd	nd	nd

Table 2.1 continued

RBOHD	BQ488466	15	8	4157	16	4795	15376	8	117	1	9	566	55
ABA1	BQ488795	0	0	1	0	0	2	0	0	1	0	0	0
MKK2	BQ488850	nd	0	39	1	2	101	1	0	1	nd	0	0
ERF9	BQ488935	nd	nd	nd	nd	nd	nd	nd	0	1	nd	nd	nd
MPK4	BQ489189	6	0	0	2	657	4	2	74	1	1	2	1
PDC	BQ490338	0	nd	1	0	0	2	0	0	1	0	1	0
WAK	BQ582382	3	0	304	5	197	770	3	2	1	1	7	5
PHS2	BQ582629	90	0	49	1	4	185	0	0	1	0	1	0
CYP76C7	BQ582634	2	12	0	0	0	1	0	0	1	0	0	0
PLDa	BQ583369	0	0	nd	0	0	nd	0	0	1	nd	nd	nd
BRI1	**BQ583692**	**2**	**0**	**119**	**0**	**0**	**159**	**0**	**0**	**1**	**0**	**1**	**0**
LTP4	**BQ584083**	**nd**	**0**	**47**	**5**	**35**	**684**	**1**	**0**	**1**	**0**	**1**	**1**
GRAS1	BQ584136	84	nd	7731	13	31	41	258	17	1	nd	nd	nd
LOX3	BQ584422	1	0	51	0	13	64	9	0	1	0	1	0
M3KA	BQ585699	nd	0	132	1	5	371	1	0	nd	nd	1	2
BR6OX2	BQ585998	6	2	104	2	16	1886	2	1	1	1	3	2
CAF1	BQ586375	2	0	28	1	18	nd	0	0	1	nd	1	0
kinase	BQ586464	4	5	14	2	0	344	3	1	1	2	1	2
PP2C	BQ586635	1	0	284	6	42	955	4	11	1	0	64	127
RD26	BQ586991	1	0	25	0	5	143	0	0	1	0	0	0
CML41	**BQ587396**	**4**	**0**	**970**	**nd**	**26**	**586**	**1**	**1**	**1**	**0**	**6**	**3**
MKK9	BQ587848	2	0	24	0	0	185	1	0	1	0	1	0
RPN10	BQ589925	4	1	111	2	8	1020	1	1	1	123	0	1
Myb	BQ591669	0	0	nd	0	2	nd	0	0	1	nd	0	nd
kinase	BQ590125	nd	nd	19	nd	nd	nd	nd	1	nd	nd	nd	1
GRP2	BQ592168	103	12	991	27	257	3801	39	25	1	14	32	4
DREB2C	BQ592254	4	nd	nd	nd	3	nd	0	nd	nd	nd	nd	1
MPK4	**BQ592267**	**2**	**0**	**31**	**0**	**7**	**198**	**0**	**0**	**1**	**0**	**1**	**0**
CDKC	BQ592936	2	0	76	1	8	440	1	0	1	1	1	1
MKK3	BQ594412	129	0	48	1	3	447	1	8	1	0	1	1
MBF1C	BQ594558	37	3	373	2	2	28	5	4	1	nd	0	2
NSP5	BQ594578	4983	nd	nd	37	1079	nd	1083	19	1	nd	nd	nd

Table 2.1 continued

MPK6	BQ594736	nd	0	44	0	nd	464	0	nd	1	nd	0	24
ATLP	BQ594810	nd	nd	0	nd	nd	nd	0	0	nd	nd	1	nd
PXA1	BQ595543	316	0	29	1	409	nd	0	0	1	0	0	1
MKK9	**BQ595738**	**1**	**0**	**39**	**0**	**12**	**165**	**0**	**0**	**1**	**0**	**1**	**0**
BAM1	BU089560	1	0	20	0	12	19	1	0	1	nd	0	0
HDA1	CF543165	6	0	141	2	16	1039	2	1	1	1	3	2
PER50	CK136719	0	0	1	0	0	7	0	0	1	0	0	0

[a] *Arabidopsis thaliana* genes

(BQ585699), Br6OX2 [Brassinosteroid 6 oxidase] (BQ585998), *CAF1* [CCR4 Associated Factor 1] (BQ586375), *PP2C* [Protein Phosphatase 2C] (BQ586635), *CML41* [Calmodulin-like 41](BQ587396), *MKK9* [MAPK Kinase 9] (BQ595738), and *BAM1* [Barely Any Meristem 1] (BU089560) (Table 2.2). *PP2C* and *PDC* were also upregulated in the water treatment, and therefore not exclusive to the pathogen stimuli. These upregulated genes included a mixture of putative abiotic and biotic stress responses as well as growth and development regulated genes. ACH185 specific responses to the individual treatments were also identifiable. *Aphanomyces* upregulated genes (>5 fold increase compared to *Rhizopus* and H_2O treatments at any time) for ACH185 were *M3Ke1* [MAP2K Kinase epsilon 1] (BF011062), LTP4 [Lipid transfer protein 4] (BQ584083), *CML41*, *CAF1* and *GRP2* [Glycine-rich binding protein 2] (BQ592168). The *Rhizopus* treatment in ACH185 had a higher number (18) of transcripts than both the *Aphanomyces* (11) and H_2O (4) treatment with a fold change >3. A putative *GER3*, *ATL2* [*Arabidopsis* toxicos en levadura 2] (BQ488179), *BRI1*, *GRAS1* [Scarecrow-like 13] (BQ584136), and *PXA1* [peroxisomal ABC transporter] (BQ595543) were induced when treated specifically with *Rhizopus*. The transcripts detected were again a mixture of abiotic, biotic and growth related responses.

In the water treatment of ACH185, 31 of the genes detected were expressed at basal levels and 6 were upregulated ($\geq$ 3 fold change). Five of the six genes upregulated in the H_2O treatment were also upregulated in the pathogen treatments, only a putative *PER50* [peroxidase] (CK136719) had significantly higher expression in the H_2O treatment compared to the pathogen treatments in ACH185. One gene, a putative *MPK4* (BQ592267) was only detectable in the H_2O

Table 2.2 Genes upregulated and associated with response to Aphanomyces and Rhizopus treatment over time in ACH185 using qPCR.

	ACH185
Gene name	**Putative Role**
GER3	Stress regulated (Abiotic)
PDC	Growth
BRI1	Stress and Growth
Br6OX2	Stress and Growth
CYP76C7	Undetermined
M3KA	Growth
CAF1	Stress regulated (Biotic)
CML41	Stress regulated (Undetermined)
MKK9	Stress signaling (Both)
BAM1	Growth

treatment, not the pathogen treatments, but quantity of expression was not determined due to the lack of a reference.

In SP7622, differences were observed in the level and patterns of expression between the three treatments as soon as 6 h and continued until the last time point measured (24 h). All of the 48 genes tested were detectable in SP7622 in at least one time and treatment. SP7622 showed an induction of gene expression in response to the Rhizopus treatment at 6 h with maximum expression detected at 12 h. In response to the Aphanomyces treatment, maximum gene expression was observed at 18 h. Many of the genes tested were upregulated in response to both pathogens, but not the control (H_2O) treatment (Table 2.3). Of the forty-eight genes tested, only *RBHOD* [Respiratory Burst Oxidase D] (BQ488466), *PDC*, *PLDA* [Phospholipase D alpha] (BQ583369), myb (BQ591669), and *ATLP* [Thaumatin-like protein] (BQ594810) were not upregulated in either of the pathogen treatments at the times observed in SP7622. Putative *NCED4*, *RBOHD*, *WAK* [Wall Associated Kinase] (BQ582382), *PP2C*, *RPN10* [Regulatory particle non-ATPase 10], *GRP2*, and *MPK6* (BQ594736) were also upregulated in H_2O with a fold change >3. The genes also present in the H_2O treatment included putative abiotic stress response genes shown to be involved with water stress, genes involved in growth and development, and two genes putatively involved in defense responses.

In SP7622, expression differences were also observed between the *Rhizopus sp.* and *A. cochlioides* pathogen treatments. The Rhizopus and Aphanomyces treatments elicited the induction of similar genes, with 28 genes being upregulated in response to both the pathogen treatments. Differences in the timing of expression varied between the pathogen treatments in SP7622. Under Aphanomyces treatment in SP76222, plant responses were detected at 6 h,

Table 2.3 Genes upregulated and associated with pathogen response to both Aphanomyces and Rhizopus treatments in SP7622 over time using qPCR

	SP7622
Gene name	**Putative Role**
M3Ke1	Signaling
GER3	Stress regulated (Abiotic)
MKK2	Stress signaling (Abiotic)
WAK	Growth
PHS2	Growth
BRI1	Stress and Growth
LTP4	Stress regulated (Abiotic)
GRAS1	Stress regulated (Biotic)
LOX3	Stress regulated (Biotic)
MAP3KA	Growth
RD26	Stress regulated (Abiotic)
Br6OX2	Stress and Growth
CML41	Stress regulated (Undetermined)
MKK9	Stress signaling (Both)
GRP2	Stress regulated (Abiotic)
MPK4	Stress signaling (Biotic)
DREB2C	Stress regulated (Abiotic)
Kinase	Undetermined
CDKC	Growth
MKK3	Stress signaling (Biotic)
MBF1C	Stress regulated (Abiotic)
NSP5	Growth
PXA1	Growth
BAM1	Growth
HDA1	Stress regulated (Both
CAF1	Stress regulated (Biotic)

downregulated at 12 h, and reactivated at 18 hours. Stress and defense related mechanisms were evident and upregulated at 6 h and again at 18 h, before returning to basal levels at 24 h in the Aphanomyces treatment. In the Rhizopus treatment of SP7622, upregulation was seen at 6 h and 12 h followed by a return to basal levels at 18 and 24 h. In the SP7622 Aphanomyces treatment, 8 genes were upregulated with a fold change >3, compared to the Rhizopus and H_2O treatments. *CYP76C7* and a putative serine/threonine kinase (BQ590125) were only upregulated in the Aphanomyces treatment of SP7622.

In the Rhizopus treatment of SP7622 there was a greater number (26) of genes with a >3 fold increase in expression compared to the Aphanomyces and H_2O treatments. Genes solely expressed in the Rhizopus treatment of SP7622 were a *MPK4* (BQ489189) and a peroxidase. *RPN10,* a putative serine/threonine kinase, *ABA1*, *PDC*, *PER50,* and *MKK9* showed no change in expression in SP7622 in response to the Rhizopus treatment. Genes classified as growth or developmental were upregulated during the pathogen stress in both varieties in response to at least one of the pathogen treatments. Unlike ACH185, none of the genes measured had an upregulation only in the H_2O treatment. Both the varieties tested showed readily identifiable responses to the treatments tested throughout the 24 h measured.

Expression patterns common and distinct between the two varieties in a particular treatment were observed over time. In particular, ACH185 had a higher number of genes (7 to 24) not detectable at any given time compared to SP7622 (2 to 16) regardless of the treatment. An increase in gene expression in both varieties indicated that both treatments were initiating a response, in the plant, as soon as 6 h. In response to the pathogen treatments, early recognition of *Aphanomyces cochlioides* and *Rhizopus sp.* and subsequent downregulation was evident for both

varieties tested. However, in SP7622 a secondary increase in gene expression was seen in the Rhizopus (12 h) and Aphanomyces (18 h) treatments, but there was no corresponding increase in gene expression in ACH185.

Aphanomyces treatment differences between the two varieties showed 5 genes were upregulated in both varieties. *BRI1*, *LTP4*, *CAF1*, *CML41*, and *GRP2* were upregulated in common with both varieties under the *Aphanomyces* treatment. The remaining genes upregulated in either SP7622 or ACH185 showed no regulation or a decrease in expression over time in the other variety. *LOX3* (BQ584422), *PP2C*, *CDKC*, *RBOHD*, *GRP2*, *GRAS1*, *HDA1*, and *RD26* (BQ586991) all showed no activity (or a decrease) in *Aphanomyces* for ACH185, while all of these genes were upregulated in SP7622. SP7622 showed little or no activity (or a decrease) in *PDC*, *PLDA*, *ABA1*, myb, and *PER50* under *Aphanomyces* treatment while these genes were upregulated in ACH185.

Rhizopus differences between the two varieties also showed a large variation in the number (16 in ACH185 and 38 for SP7622) and types of genes being upregulated in response to the pathogen. Genes were considered upregulated if they had >3 fold increase compared to the initial control levels (6 h H_2O). Upregulated genes, in both varieties, in the *Rhizopus* treatments were *BRI1*, *GRAS1*, *CAF1*, *PP2C*, and *PXA1*. Out of the 35 genes upregulated in SP7622 in response to *Rhizopus*, *MPK4* (BQ592267), MKK2, *WAK*, *PHS2*, serine/threonine kinase, *GRP2* and a *CDKC* were not detectable in ACH185. The remaining genes upregulated in SP7622 and detectable in ACH185, at lower levels were *M3Ke1*, *MKK9*, *NCED4*, *RBOHD*, *ABA1*, *MPK4* (BQ489189), *LTP4*, *LOX3*, *Br6OX2*, *RD26*, *RPN10*, *MKK3*, *MBF1C* (BQ594558), *NSP5*, *MPK6* and *HDA1*. Of the 22 genes upregulated in ACH185 in response to Rhizopus only a putative

PDC and *CYP76C7* were not in SP7622 under the Rhizopus treatment. Many of the genes with no change in expression in ACH185 in Rhizopus stress were signal cascade components.

An upregulation of stress-related genes was also detected in the control (H_2O) treatment. Certain genes (*PER50*, *LTP*4, and *PLDA* in ACH185 and *NCED4*, *RBOHD*, *RPN10*, *Br6Ox2*, *CML41*, *HDA1*, and *MPK6*) were upregulated (3 fold and higher) in the water treatment but not in both varieties. ACH185 had high levels (10 fold increase or greater) of *PER50* and *PP2C* transcription in the water treatment over time compared to 6 h. SP7622 also had upregulation of multiple genes under the water treatment. In the ACH185 H_2O treatment, putative *ABA1*, *CYP76C7*, *MKK2*, GRP2, PDC, *PHS2*, *CAF1*, *BRI1*, *RD26*, *MKK9*, *MPK4*, *CDKC*, *PER50*, *MKK3*, *PLDA*, and *LTP4* were detectable at low levels and present, though not always upregulated, in SP7622. *ATL2*, *M3KA*, protein kinase, *ATLP*, *Br6OX2*, and *ERF9* (BQ488935) were down regulated or not present in SP7622 compared to ACH185. ACH185 and SP7622 had a basal level of expression of the genes tested in the H_2O treatment over time. Of the genes that overlapped between the two varieties with a fold increase >3, *GRP2* is a putative glycine-rich binding protein, induced by cold and a putative *PP2C* is a negative regulator of ABA. The simulated flooding stress experienced by the seedlings in the H_2O treatment did not induce the metabolic, growth and signaling genes that the pathogen treatments did.

Varietal modes of stress response conserved between germinating seeds and developing seedlings were identifiable using gene expression of the putative varietal molecular markers previously identified in Chapter 1. In SP7622, the 9 genes upregulated (>10 fold increase) during germination and correlated specifically with the SP7622 hydrogen peroxide response showed an upregulation in the pathogen treatments at 3 wks. A putative *RD26*, *MPK4*, *MKK3*, *MKK9*,

BAM1, *Br6OX2*, *LOX3*, *BRI1*, and a putative *NSP5* were upregulated in both of the pathogen treatments tested for SP7622. Those genes, *MKK9*, the putative serine/threonine kinase, *PP2C*, and *CML41*, specifically upregulated in the H_2O treatment of SP7622 during germination were also upregulated in both of the pathogen treatments. These germination molecular markers were upregulated in the pathogen treatments, but not the H_2O treatment with the exception of *PP2C*.

Of the genes found to be upregulated (>10 fold) in the H_2O_2 treatments in ACH185 during germination, *CML41*, *LTP4*, and *CAF1* were the only H_2O_2 germination specific transcripts upregulated in both pathogen treatments (Table 2.2). *MPK4* (BQ592267), also associated with H_2O_2 germination, was not detectable in either of the pathogen treatments. Those genes specifically upregulated in ACH185 in the H_2O and not the H_2O_2 treatment during germination, *MKK9*, *NSP5*, and *ERF9* had less than 3 fold changes in expression in 3 wk seedlings. *MKK9* (BQ587848) and *MBF1C* were detectable in the 3 wk seedlings, but had minimal expression.

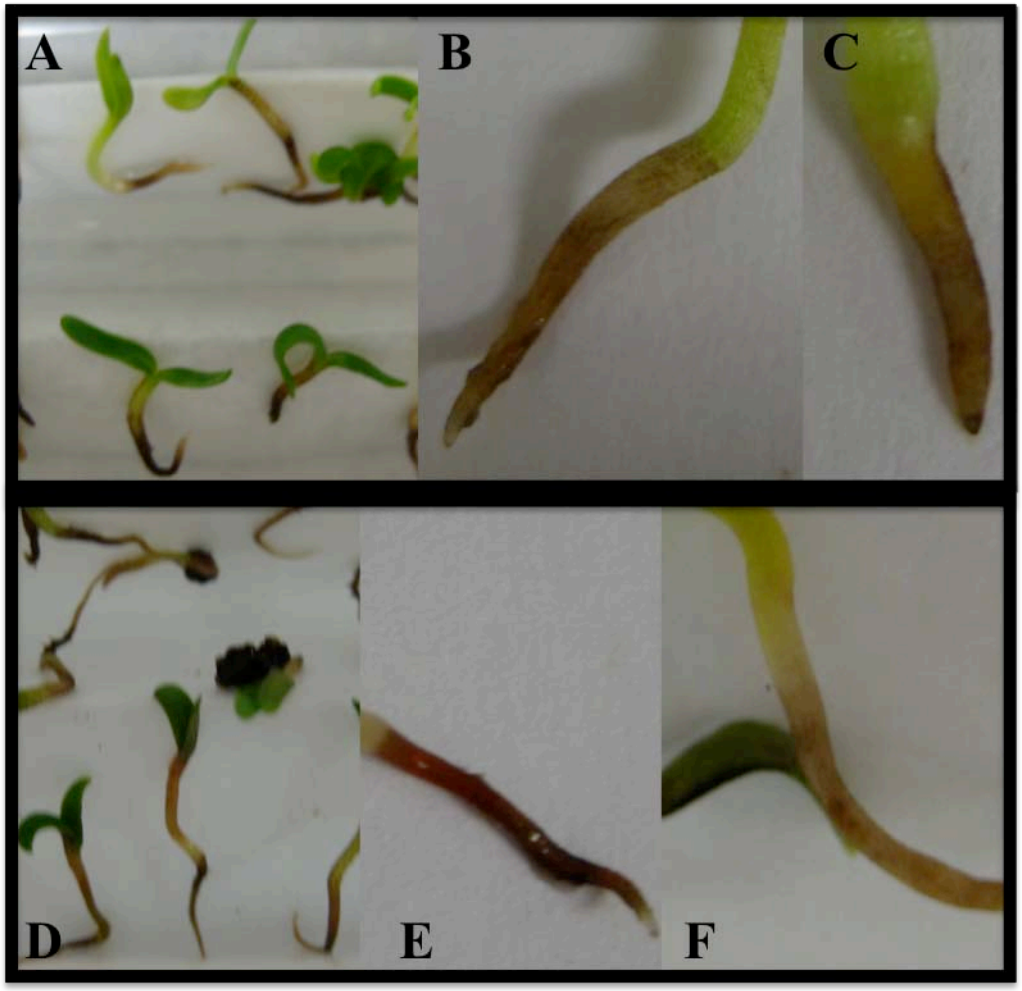
Between varieties, pathogen and H_2O_2 treatments and developmental stages (germination and 3 wk seedlings) only three genes were upregulated in common. During germination, few genes were conserved between varieties and treatments. *MKK9* (BQ487860) was the only gene, out of the 48 genes tested, upregulated exclusively in the H_2O treatments for both varieties during germination. In 3 wk seedlings, this gene had no visible shared activity. In H_2O_2, there were no genes exclusively upregulated in both varieties during germination. However, 10 genes out of the 48 tested were associated with germination vigor. Genes that were associated with germination vigor (genes with a high amount of expression over time in treatments with higher

germination) were *LTP4* (BQ584083), *MPK4* (BQ592267 and BQ489189), *PER50* (CK136719), *HDA1* (CF543165), *BAM1* (BU089560), *MKK9* (BQ595738), *MKK3* (BQ594412), *BRI1* (BQ583692), and *CYP76C7* (BQ582634). Of those 10 genes, *BAM1* (BU089560), *MKK9* (BQ595738), *BRI1* (BQ583692), and *LTP4* (BQ584083) were also upregulated (>3 fold change) across both pathogen treatments in both varieties in 3 wk old seedlings. The remaining genes were either upregulated in all but one treatment of a variety or were varietal specific.

DISCUSSION

In Michigan, continuous *Aphanomyces* pressure has resulted in a level of resistance being bred into most varieties grown, including two moderately vigorous varieties, ACH185 and SP7622, which also show resistance to *Rhizopus spp.* in the field and lab (Coe 1971; Naegele unpublished data). At 3 weeks of age, differences in gene expression between the two varieties under stress, flooding and pathogen treatments were readily detectable, despite no visible morphological differences in response to the stresses (Figure 2.1). Differences in gene expression however, displayed the large heterogeneity in gene response possible between sugarbeet varieties and may provide some insight into the genetic diversity behind pathogen and flood response in seedlings. Literature has shown that the boundary between biotic and abiotic response genes is no longer as clear as once thought (Mantri 2010; Jain 2010, Chapter 1). Crosstalk between the abiotic and biotic response pathways allows diverse signals to converge through signaling molecules, hormones, and metabolism to elicit a common response. This research showed a number of biotic, abiotic and growth-related genes being actively transcribed in 3 wk seedlings during pathogen and flooding treatments. These early seedling response mechanisms to biotic

Figure 2.1 Roots of ACH185 and SP7622 prior (0 h) and post (1 wk) treatment with *Aphanomyces cochlioides* or *Rhizopus sp.*.



and abiotic stress may have large overlaps in the genes regulated or the pathway components for abiotic and biotic stress may not be well partitioned during early development.

Quantitative analyses revealed eight genes, out of the 48 tested, shared between both varieties and pathogen treatments in 3 wk seedlings. The lack of shared genes between varieties indicates that the response to *Aphanomyces cochlioides* is highly varietal specific and may provide alternative sources of resistance for breeders. Between varieties, the plant's response to a fungal and an oomycete stress was associated with the induction of *BRI1, CAF1, GER3, MKK9, Br6OX2, M3Ka, BAM1,* and *CML41*. *CAF1* has been associated with biotic response (Sarowar 2007), *CML41*, has an undetermined stress response function (Denoux et al. 2008), and *BRI1*, is part of an abiotic and biotic signaling cascade involved in stress response and growth (Che et al. 2010). *GER3* has been implicated in cold and defense response (Swarbreck 2008; Larkindale 2007). *MKK9*, a MAPK kinase known to be involved in ethylene biosynthesis, activates *MPK3/6* under certain stress conditions (Zhou 2009; Yoo 2008). *Br6Ox2* is a component of Br biosynthesis (Nomura 2005), and *M3Ka* has no defined role, but it thought to be involved in ATP binding and development (Swarbreck 2008). *BAM1* is a receptor kinase implicated in shoot and floral development in *Arabidopsis* (Hord 2006). The genes upregulated in both pathogen treatments had putative functions involved in abiotic and biotic stimulated stress response and growth. Many of these putative genes belong to gene families and the closest *Arabidopsis* homologue may or may not indicate their true function or role. Full-length cDNA and protein characterization is needed to confirm the functionality and identity of many of these genes.

Putative *BRI1*, a component of the brassinosteroid receptor, aids in broad-spectrum resistance to drought, salt, temperature and pathogen attack in *Arabidopsis* (Che 2010). Additionally, *BRI1* may have some role in regulating a response to multiple hormones as *Arabidopsis bri1* mutants

show sensitivity to ethylene, ABA, GA and auxins (Swarbreck 2008). Some evidence also suggests that *BRI1* may modulate hypocotyl elongation (Krishna 2003; Steber and McCourt 2001). While it is known that high temperatures can induce production of Br, the role of Br in pathogen response is still unclear (Che 2010). Our data of *BRI1* and *Br6OX2* suggest that Br is being produced in response to the pathogen treatments. Upregulation of Br, during sugarbeet seedling pathogen response, may be involved in increased seedling growth, hormone regulation or pathogen defense.

CAF1 was another gene shared between the two varieties in response to the pathogens tested. Overexpression of *CAF1* in chili pepper and tomato resulted in higher growth (larger cells) and thicker cuticles, which may have been responsible for its role in successful defense against a bacterium, *Xanthomonas axonopodis*, and an oomycete, *Phytophthora infestans* (Sarowar et al. 2007). It has also been shown that *CAF1* may play a role in pathogen defense response modulation in *Arabidopsis* (Liang 2009). Other research on *CAF1* in other organisms suggests that it also has a role in mRNA turnover (in mice and yeast), which may be how it modulates defense responses (Berthet 2004; Liang 2009). In sugarbeet seedlings, *CAF1* may be contributing to both enhanced growth and cuticle development to prevent infection.

CML41, a gene shared between both varieties in both pathogen treatments, has no known biological function but is putatively involved in Ca^{2+} binding and stress response in *Arabidopsis* (Baev 2010; McCormack 2005). Recent research however, has suggested *CML41* operates in the chloroplasts and functions in defense response by suppressing or “dampening” the immune response (Baev 2010). Our data supports this role in ACH185 pathogen response, as *CML41* is the most highly expressed gene at 6 h in both the Aphanomyces and Rhizopus treatments and subsequently no large gene activation was seen in this variety. In SP7622, *CML41* expression is

moderate to low in both the pathogen treatments and high levels of expression are seen at 18 h for the Aphanomyces treatment and 12 h for the Rhizopus treatment, the time points before expression returned to basal levels. Alternatively, *CML41* may be a byproduct of enhanced growth and energy mobilization in response to pathogens and not necessarily involved in the response.

During germination, SP7622 had little to no expression of *CAF1* under either treatment, but H_2O_2 germinated ACH185 seedlings had high levels of *CAF1* induction (Chapter 1). This induction during early germination may have resulted in increased cuticle thickness, which in part may be aiding in the resistance to pathogens and drought stress during later development. Since SP7622 did not produce *CAF1* during germination, the role of *CAF1* as a marker for seedling stress tolerance during germination and 3 wk stress is limited. Also, *CAF1* expression during germination may be contributing to the later differences in pathogen response seen between these two varieties if *CAF1* was contributing to cuticle thickness. As *CAF1* was not expressed during germination in both varieties, the ability to use putative *CAF1* as a stress resistance marker to screen germinating seedlings may be limited to specific varieties. *BRI1* and *CML41*, genes found to be upregulated in response to both pathogens in both varieties, were associated with increased germination vigor in both varieties (Chapter 1). This indicates a possible conserved role in germination and seedling vigor and a potential use as a molecular marker for assessing vigor, germination and seedling, across varieties to improve the efficiency of the breeding process.

Each variety had unique patterns of gene expression in response to a pathogen treatment when ACH185 and SP7622 were compared. The difference in response could be due, in part, to the times observed; SP7622 may have a later or earlier recognition of the pathogen, or the

differences could be in the mode of pathogen recognition. Pathogens utilize different modes of infection, and different plant species or varieties can utilize different physical and genetic mechanisms, e.g. growth habit, trichomes, R genes, etc. to overcome the same stress (Torto-Alalibo 2009; Xiao 2004). Many of the genes observed in our study were common between the pathogen treatments within a variety but not between varieties. The limited number of treatment-specific genes might be a reflection of the type of genes chosen to analyze, i.e. conserved elements of stress pathways, and not similarities in the modes of defense.

In response to the Rhizopus treatment, both ACH185 and SP7622 had some level of upregulation at 6 h. SP7622 had an increase in the number of genes upregulated in stress response genes and transcription factors involved in reducing and transporting H_2O_2, increasing metabolism and growth, and signaling, indicative of recognition and response to a pathogen. Upregulation in response to the pathogen was visible at 6 h and expression peaked for most genes upregulated at 12 h before returning to basal levels. ACH185 had less genes expressed in the Rhizopus treatment, possibly due to an inability of *Rhizopus sp.* to infect the beet through physical prevention of infection, e.g. increased cuticle thickness resulting from the increased expression of *CAF1* during germination. The possibility of defense by exclusion is also consistent with *MPK4* and *MPK6* expression data, which indicated little or no expression in ACH185 under the Rhizopus treatment, but an upregulation in SP7622. While the data does not show an upregulation of MAPK expression in ACH185 under pathogen stress this may also be caused by a difference in the time of expression and not necessarily the lack of infection by the pathogens due to physical barriers. In addition there may be an alternative response pathway utilized by ACH185 in response to *Rhizopus* sp. and *A. cochlioides* not tested in this study.

In the *Aphanomyces* treatment, ACH185 had some level of pathogen detection, denoted by the upregulation of genes at 6 h post treatment, but gene activation was low in general. This again may be indicative of a lack of initial infection through increase cuticle thickness (*CAF1*), a difference in the timing of expression, or an alternative network of genes. In SP7622 the *Aphanomyces* response again had upregulation at 6 h, followed by a decrease at 12 h and a subsequent increase at 18 h before returning to basal levels. The 6 h delay in expression between the SP7622 *Rhizopus* and *Aphanomyces* treatments may be due to the nature and virulence of the pathogens. In adult beets, *Rhizopus* is a weak root rot and postharvest pathogen, while *Aphanomyces* is a devastating seedling disease and can cause a root rot in adult plants (Harveson 2009). In other species, it has been shown that *Rhizopus spp.* can cause a preemergence damping off (Howell 2002; Jackson 2004). *Rhizopus* may be a weak pathogen of sugarbeet seedlings, and the activation of most defense genes is not necessary. The differences in expression timing between the pathogen treatments for SP7622 may also be due to differences in the pathogen's mode of infection. *Rhizopus sp.* may lack the plant defense suppression machinery, present in *Aphanomyces*, necessary to cause disease without the aid of additional stress or it may be germinating and coming to contact with the host tissue more rapidly and in greater quantities.

Disease resistance depends on successful recognition of the invading pathogen and rapid induction of defense genes (Boller and Felix 2009; Faurie et al. 2009; Jones and Dangl 2006; Nimchuk et al. 2003; Park and Paek 2007). Pathogenic organisms have evolved mechanisms to suppress the natural immune system (MAPKs) of the plant and colonize the host tissue causing disease (Bent and Mackey 2007; Espinosa et al. 2003; Gudesblat et al. 2009; Houterman et al. 2008). Plants in turn have adapted elaborate systems to recognize the suppression of their basal defense and re-activate them (Lin 2007; Hardham 2010). *Rhizopus sp.* may be activating plant

defenses upon initial contact, and be unable suppress the plants basal immune system, resulting in resistance. *A. cochlioides* may be able to suppress the plant's immune system, suggested by the repression of gene expression at 12 h, however the resistant plant can overcome the repression and reactivate defenses, suggested by the upregulation of genes at 18 h in SP7622. These two pathogen treatments, despite transcript and timing differences, activated many of the same genes. Large overlaps in the genes expressed during defense can occur in *Arabidopsis* between pathogens with differing virulence and modes of infection, but transcript quantities and timing of those genes can vary dramatically (De Vos 2005). While further studies using additional times, isolates and genes are necessary to further explore the differences in response to *Aphanomyces* cochlioides and *Rhizopus sp.* between the two varieties tested; these differences in recognition and response may provide useful information for breeders looking to improve seedling resistance.

Flooding response in the two varieties tested showed unique and common genes being upregulated. Both varieties exhibited basal levels of most of the 48 genes tested and an upregulation of certain abiotic stress genes. Genes, associated with water stress response, common between the two varieties *PP2C* and *GRP2*. These two genes are both abiotic stress related and may be part of an H_2O or motion-related response that is conserved between varieties. *PP2C* is a Protein Phosphatase 2 C known to negatively regulated ABA signaling (Hubbard et al. 2010; Ma et al. 2009). During drought conditions, ABA upregulation can increase drought tolerance by closing stomata and conserving water (Chak et al. 2000; Fujita et al. 2005; Kim et al. 2010). However in flooding, ABA is downregulated to enhance growth (Chen 2010). The upregulation of *PP2C* would likely result in a downregulation of ABA, which could enable the seedlings to enhance their growth. *GRP2* is a glycine-rich binding protein

involved in cold and osmotic stress response in *Arabidopsis* (Carpenter et al. 1994; Karlson et al. 2002; Kim et al. 2007a). Functional characterization of *GRP2* in *Arabidopsis* showed that it enhanced seed germination under salt stress, but had no effect on germination or seedling growth under osmotic stress (Kim et al. 2007b; Lee et al. 2009). The slight increased expression seen in both beet varieties indicates that this gene may play a role in osmotic stress in beet seedlings.

The lack of H_2O induced transcripts was unexpected, since many of the stress genes chosen had known phenotypes under abiotic stresses. Early seedlings may be more resistant to flooding stress, or pathogen detection and defense utilizes an array of genes that differ from those utilized by adult plants. These pathogen treatments also simulated flooding and motion stress since the seedlings were submerged in water and constantly shaking. The upregulation of abiotic response genes in the pathogen treatments were likely in response to the pathogen stimuli and not flooding or motion since most of these genes were not upregulated in the water treatment.

3 wk seedlings exhibited a range of abiotic and biotic, growth and metabolic gene responses to pathogen and flooding stress. Pathogen treatments showed high similarity within a variety, and more differences between varieties. The identification of varietal specific defense responses to a pathogen provides useful information to breeders, improving disease resistance. However, those small numbers of genes shared between varieties have the potential to become molecular markers to screen resistance. Molecular markers to predict or assess the ability of a plant to withstand germination and early seedling stress would be indispensible. Of the genes upregulated in vigorous germination in both varieties, only, *BRI*, *CML41*, *LTP4,* and *MKK9*, were also in one or more of the pathogen treatments tested on the 3 wk seedlings. These particular genes may be useful as expression molecular markers for germination and early seedling vigor. Increased expression of these genes in a given variety during the first 24 h of germination under H_2O

conditions may be indicative of stress tolerance during the first few weeks of growth. More work to determine the efficacy of these genes in predicting seedling vigor is needed, but this study serves as a first approximation of the genetics behind predictive seedling vigor in sugarbeet.

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LITERATURE CITED

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APPENDIX A

SUPPLEMENTARY TABLES

Appendix Table 1.1 Sugarbeet ESTs with sequence similarity to Arabidopsis proteins involved in stress response, hormone biosynthesis and growth used for primer design. Sequences and primers in bold were used for qPCR analyses

Plant Growth and Development Regulated Genes

Bv* Accession	**Forward Primer**	**Reverse Primer**	***At* Gene****	**E value**
CF542917	ACAGAGGGACCCGACTTATTG	TCCCGCCTGTTGATTGTTAC	*CYP71A25*	1.00E-61
CF430002	GGGGCAGCTGTTGGTGAT	CATGATTCCCGATTGTGTCTG	*NAS4*	9.00E-41
BQ593316	GTCCGCTCTTTGTCTTTCTTTT	TTTCCACCAATTCCACCAT	*WOX13*	3.00E-21
BE590301	TAGGAGCAGGCATACATA	ACCGCCCAATCCCCTCAAG	*SIP1*	2.00E-09
BE590301	TAGGAGCAGGCATACATA	GAGGTGTGACAAGGGAAGT	*SIP1*	2.00E-09
BF011057	GTTCCGTGTCGAGTTTGGTGA	GGCCCCCTTCCGTAGTATTG	*SIP1*	2.00E-09
AW697779	GTTTGCGGCCGTTGGAGT	AGCAGGCCTGTGAGTGTAT	AT3G29970	9.00E-33
BF011036	TTGCGGCCGTTGGAGTTG	GCGAGTTGGATCAGCGAAAAAG	AT3G29970	1.00E-32
BI096111	GCGGCCGTTGGAGTTG	TAGAGCCTTTCAGTTACGAGTTGG	AT3G29970	1.00E-32
BI543285	TGCGGCCGTTGGAGTTG	TAGAGCCTTTCAGTTACGAGTTG	AT3G29970	1.00E-32
BQ588646	AAGATCCAACGACCCCCATTTT	ACTACCACGGCGGAACTCAG	*SOM*	3.00E-69
BQ595434	ATGCCAAGACCCACTGATGAA	TTGCCCCCACTCTAACTG	*APY2*	5.00E-57
CK136263	GTATCCATATCCTCCCCACTGCT	TTTTGCGGCGAACACATTG	*NIC1*	3.00E-87
BQ584431	CCGGAAAAGTGAAGGAAAGAAAG	AAAGGGCCAGCACCAACAGT	*NIC1*	5.00E-51
BQ594284	GAGCCCATACATTGTTGCCCACCAC	GATCCCCAACTCGCTCCCTCATT	*BGLU40*	5.00E-70
CF543263	ACATGGTTTCACTTTTGGGGTAG	TGAGGAAGATCGCCGTGTAATA	*BGLU12*	9.00E-72
BE590328	TCATTTTTGGGGCTGCTTCT	AGGGCTGTATACCGTTGTTGATTA	*BGLU12*	4.00E-62
BQ591856	GTATTGTAAAAGGGGGCAGTCATC	CATTCCGAGCATATAGTGTGGTGT	*BGLU41*	8.00E-79
BQ586318	CAGATTTGGCGGTAGTGGTGT	TCCGGCAGTGAACGAGAGG	F1N13.150	2.00E-50
BQ588744	TAAAGTGATGATTGCCGATAGG	GCTGCGTGCTTGGTTCC	AT3G51680	2.00E-46
BQ594788	GCTTGCCGTTTTGGGTTAC	CGGGTGCAAATTTGTTGATG	AT3G51680	4.00E-46
BI543526	GCACGAGGGAAAGAGAAAACT	TTGCCAAGGGATCACTAAGG	*TPS*	7.00E-38
BI096046	TCAACTATAGTCAAGCAGGAAGGA	CAGCTAACATATGGGTGACAACA	*PUMP1*	5.0E-108
BQ487636	CTTCTATGGGTGCTATGCTTC	AATGCGGCCAGGTAAAT	*ECHID*	8.00E-78
BG577441	GACCAACCCATGATGAGATAGACT	TGAGCTGCAAGATGATTTACCC	*XTR6*	2.00E-90
BQ487747	GACGCAACACCCATCAGAGA	AAGCGTTGGCATTAAAGTTCCT	*XTR6*	5.00E-86

Appendix Table 1.1 continued

BQ654409	GACCAACCCATGATGAGATAGACT	TGAGCTGCAAGATGATTTACCC	*XTR6*	2.00E-92
BQ490338	**GCCGATCGCGCCACCTGAAT**	**CGGCTCGGCTAAAAGATGAT**	***PDC***	**7.00E-70**
BQ587173	CAGCGGCATAAGTGAAATAGAGAC	CATCGCAGAACCAATCAGAACAC	*FAB1*	3.00E-55
BQ594578	**TGCGATAGCCATAGTAGGAAGGA**	**AATAGTATCAAAGGCCCAAAGGTC**	***NSP5***	**4.00E-67**
CK136733	GGCCGGGGAAACCAAGTCAC	GGGGGTCCAAGCAACAGAATAAGT	*XTH16*	4.00E-91
BQ595543	**CAATTGGGGTGAGGAACTTT**	**AACTAATGCAGGACGATGTGAT**	***PXA1***	**2.00E-73**
BQ586903	TGTTGCCCTTCTGTGTCATTCT	ACCATCGCAGCCAAAAGTATC	*LPP2*	8.0E-107
BQ582685	TTCATCATCCCAAACACAAA	TGCATACTTCTTCATCTCACATAA	*CYP707A2*	6.00E-63
BQ585998	**TAGGGTGCCCAACAATAGTG**	**GTGGGGCTGATAAGAGAAAGA**	***BR6OX2***	**4.00E-59**
BQ586518	GGACGCGTGGGGGCAAAAT	GTAAGGGGGTCGACAAGGAGAT	*BIN2*	8.0E-109
BQ586790	CCACCGACTGCGCTAAGAGAA	TAAAAGACCTGCCAAGACCAGAT	*BIN2*	3.0E-104
BQ582763	GGCTTGCCTTTGATTGGTA	CATTGATGGGAGCAGAAGTTAT	*KAO2*	3.00E-57
BQ583764	GCGTGGGCGTGAGTTGG	TAGGGTTTTAGCATCGGTGTG	*SLY1*	1.00E-10
BQ586719	AGTTCCACGCTCTTCC	TTCTTCGGGTTCAATG	*GA20OX2*	2.00E-44
CF543627	GGGAGGCCTGTGAAGAATAC	AAGAACAGTTAAGGCACCAGAGTC	*GA2OX2*	7.00E-72
BQ583692	**CTGCAAAGTGGGAGAAGAACGA**	**GACCCACCCGACAAGATTATTATG**	***BRI1***	**3.0E-101**
BQ595856	AAGCGTTACCGAGCATTTGTTG	GGCGGCGATCTGTTTTTGTT	*TPC1*	3.00E-69
AW777170	TCCCAGTGAGATTGAGAGTT	CAATCGAGGTATGGGTAGAAG	*WNK1*	1.00E-51
BF011211	TCCCAGTGAGATTGAGAGTT	CAATCGAGGTATGGGTAGAAG	*WNK1*	6.00E-53
BQ592312	GGAAGCCCCTACTGGATGG	GAGGTCGAGCGGGTGAAAC	*YDA*	3.00E-65
AW063034	CGATCACCGGGCTGCTTCTTA	CAACCCCGGGCCACTACTG	ER	3.00E-79
BQ585514	GCCTTGCCACGCTAAAT	GGTTCTCGGTCATCCTCA	ER	2.00E-71
BQ594919	GCGGACGCGTGGGTTCAT	TCGCCTTGGACAAATCAGA	ER	3.00E-88
BQ583416	GTCGCCAAACAATCAAGTA	CAACATCCGCTGCTATCT	*PERK1*	8.00E-81
BI096232	GGGGGCCTACCAACCTC	GTGTCGGGGAAGAACTCAAA	*PLDALPHA1*	2.00E-55
BQ583369	**GGGGGCCTACCAACCTC**	**GTGTCGGGAAGAACTCAAA**	***PLDALPHA1***	**9.00E-76**
BQ587272	GATTATAGCCGAGCACAGGA	TCGCGGTTTGGTTCACTT	*PLDALPHA1*	1.00E-74
BQ588870	TGGCCGTTTGACAGATT	CATTTTGAGCTTCCTTTAC	*DCL1*	7.00E-44
BQ591910	ACTAGGCGTGCCCCAACC	ATCTCTACATCCCCATCATCAGTG	*PHYB*	1.00E-81
BI543316	GCTGGTGCCATGAATGCTTTGAT	CCACTCTTCCCCTTCTTCCTTGAT	*CESA1*	4.0E-106
AW063023	CCGCCGCATATGTTGGAG	GAGAATGGCGTGGGGAA	*ACT7*	7.0E-152
BQ587329	TCCTTCCGTTGCTTCTGATG	ACTCTCCGTCTCCCCTCTAACA	AT1G56300	2.00E-46

Appendix Table 1.1 continued

BQ591929	GGGCTTTCCTCCTTTGTCCA	CAGCCGCCATTGTTTCTCAC	AT1G56300	3.00E-23
BQ582477	GAGCACGGCGATCAGAGAAAT	AGGAGTAGAGTAATGGCGAGAACC	*CDC2*	8.00E-96
BQ589455	CGTGGGGTTGCGGTTTTT	CCACTTCATTCCCTTCCTACACTC	*SPT*	2.00E-16
BQ584462	CAATGGCGTGGTGGTGTA	CTTTTTGCCTGTTTCTCCTCTCTA	*ABI8*	2.00E-82
CF543216	GACGCGTGGGCAAAAAC	AAAAATGTAGTGACCCCAATAACT	*ABI8*	2.00E-55
BQ584474	GTTCTTATGGGCAGCTTTATTCA	GACCGCTCTTCCACACTACTTC	*GCL1*	6.00E-77
BQ488119	AAGGCCGCGCATTTGAT	TACGTCTCCCCACCTT	*IP5PII*	9.00E-50
CF543420	GATTTTGCGAGACTTTCCACA	TTACATCCGGGCTTTTCTGA	*HSI2*	7.00E-26
BQ587458	ACTGATGTTGAGGGGGATGA	CTCGGCTGGCCTCTGTAAAT	*ETT*	4.00E-75
BQ595269	CCACTCGGCCTCAAACT	GAAACCGCATCCCCACAGAAAC	*ARF6*	7.00E-97
BQ595231	ATAGGCTGAAGCATAAAAAG	AATTCGGGCGTACCAACACAAC	*WNK1*	2.00E-92
BQ588748	CCACCGACTGCGCTAAGAGAA	TAAAAGACCTGCCAAGACCAGAT	*CDKB1*	2.00E-87
BI543360	CACGAGGCCTGTTTGGAAT	GTTGGGCTTGAGGGAGT	*CAT2*	3.0E-100
BQ587384	CTCCGCCACCGAACTCAAGAAC	CATCACCATCCGCATCAACAGACT	AT1G73800	2.00E-40
BQ588709	AGCTAATTTGGCAACCCTCCTT	GATTCCCCCTGCTCCTATGACT	*POT*	1.00E-67
BQ584898	CCTTCGCGACATTCTTCTTCTCT	TCACGCCGGACACCCTCTA	*POT*	1.00E-63
BQ594123	ATATGGCATCAAAGGAGGACT	GGAACAGCCAAGCCATTAGGT	AT1G63640	8.00E-83
BI073146	CAAGGGGGCCACAAGATA	ATACCCAAGCCATTCAAAG	*GER1*	3.00E-06
BI096176	GCTATGCTGCACTTTCAA	CCCGCGAGCTGGACGAC	*GER1*	2.00E-03
BQ582859	TTGCCCTTCAGCTACTCCATC	NCCTATACCCCATCCAACATTTT	*PTR2*	3.00E-94
BQ591613	AAGCCACCTGGGAAAAAGATG	GTAGACAACGGAACGACAACAAGA	*CHR5*	5.0E-107
BQ588316	TTCGGCCTCCATTCCCATCTCCT	TGCAATTCCGCCTTCTACAAAACC	*UCP5*	8.00E-33
EG551187	ATGCGGTGAATGTAGGGATAG	GAGCAATTTTAACACCAAGTAG	*BRK1*	2.00E-37
EG551187	ATGCGGTGAATGTAGGGATAG	GAGCAATTTTAACACCGAAGTAG	*BRK1*	2.00E-37
BQ590743	GACGCGTGGGCTTGTTTCTTA	AATATCCCTTGCTCTGTTTCATCC	*CPK20*	7.00E-79
BQ591253	CCATCTTACCCGCCTCAACTCTTT	CTATGCTGGGATTCGGGGTGCTAT	AT2G41380	7.00E-23
BI096237	TCACCGCCATTTCCGATTATTTTC	AGGACACGCAAGGCACGCTCTACT	*EFG*	3.00E-86
BQ488277	ACATGTGGAAGCAGCAATCAA	ACCGCCTTCACTCTTTACTGG	*SEC*	1.00E-92
BQ588706	ATGATGGGACTGAGTGGAGACAAC	TGATTAAAGCACGCAAACAAGAA	*SEC*	1.0E-114
BF010998	CTGGATTTGCCCGGAGATGATG	GAGAATGGCGTGGGGAAG	*ACT11*	2.0E-122
BQ593076	TCGGTGATCTACTGAAGGTTGTG	CAGCTCGTCGTAATTTTGTGAA	*PHS2*	1.00E-98
BQ582629	**CAGCCTAATTTTAACCCAGACC**	**TTTTGCTTCCCTCCATTCAGTT**	***PHS2***	**5.00E-93**

Appendix Table 1.1 continued

BQ489620	CGATAAAGATGGCGATGGTT	TCCGGGAAGTCAATAGTA	*CAM7*	4.00E-73
CF542908	ATGGAAATGTATAGGCCGCACAAA	GCATAGTATTCCCCTTCCCTCTCC	*HDA9*	2.0E-118
BI073176	AGCCCTCATTTCTCCCCTCTG	TTGGCCCTCCTTGGTAATCTG	*PHS2*	1.00E-76
BQ593574	ACGAGCAGCTGCCGGTGTTACT	ATGGCGAATTCTCCTTGTTAT	*CPK13*	6.00E-77
BQ595049	CATCGTATTGGTGCAGTGTTGTC	CCGAATTGGCGTATCAGC	*GTE6*	9.00E-38
BI073121	GAGCGCCTTGTTCCTGATTGGTAT	TGTTCTTTGCGTCAGCTCCTTGTA	AT3G52880	1.00E-92
BI096180	GTATGTCTAGCGCCCTGGTTGT	TGAGCGGGGAAGAAGATTTTATT	*EP3*	1.00E-41
BE590397	AACCGCTTATTATTTATCTTCA	ACGACCGCCTTGTGTAG	*XTR7*	3.00E-77
BE590397	AACCGCTTATTATTTATTTATCTTCA	ACGACCGCCTTGTGTAG	*XTR7*	3.00E-77
BQ487982	**TGAGCGCAAAGCAGGAT**	**CCAAAGAAGGCCAGGTTAGT**	***NCED4***	**5.00E-62**
BQ489959	GGTGTCCTGCCCTCCTGTCTTA	GCAAATGCCTCCCCTGTGTCTA	*NCED4*	5.00E-80
BQ582708	GAGCGCAAAGCAGGGTCTACTATC	GCAAATGCCTCCCCTGTGTCTA	*NCED4*	1.00E-63
BQ590158	GGCCTCCATTCCCATCTCCTTGTT	TGGTGGTGGTGGTGGGTTCTCC	AT4G24570	5.00E-17
BQ586930	AATGCCCCCAAGTGCTCTC	CGTCTCGATCCCCAAATAATG	*CAT2*	2.00E-89
BQ582632	TGGGCTACAGGGTCAGTCCAAATC	AGAGCGCCGGAAGGAAGCAGTA	*CSLD4*	6.0E-110
BQ583301	AGCCCCCTCGCAAGCCCAAGAAC	GCATGCATTTAAATCCCGTCAGAA	*CESA3*	4.00E-99
BQ588784	GCTTCGACATACCAATCTCTTCTA	TGCGGTCCATATCCAGTTCA	*AML4*	3.00E-47
BQ582799	CCTTGCCCGCTCTTTTTCA	CTCCCGTAGGCGTCTCTTCAT	*CDKC*	1.00E-71
BQ592936	**GCGGTTGCAGCTACAGGAC**	**GCGAGACCAGCTAAATCAT**	***CDKC***	**8.00E-82**
BQ584025	GATTTCGGTCTTTCTGTCTTTT	AACTTCTGGCCCGTATTTTCTCTT	*CPK17*	2.00E-89
BF011089	CTTCGCGATGAGGGTGTTTCTG	GGTCCGGCTCCTTTCCATAAT	*DTC*	4.00E-92
BI073250	CTTCGCGATGAGGGTCTTTCTG	GGTCCGGCTCCTTTCCATAAT	*DTC*	5.00E-88
BQ585834	CCTCCAGGATCCGGTAAAAGT	GGCCAGGAAAATCGAAAAGTAG	*QQT1*	3.00E-87
BQ585195	CACCCCCATCAGCCCTAACA	GGCGATTCTTTTGGTAACTT	AT5G27980	4.00E-27
BQ586657	CCAACTCGCGACCCAAATAGAC	AGGAACATCGGTGGGTGAAAGTAG	AT5G42830	7.00E-59
CF543447	GTTTAGCGAGGGGACAAGTG	GCAATGCCAATAGCGTGAAT	*RPT4A*	1.0E-132
BQ490300	TTGTTTATGGGCCTCTTTATTTTC	AGCGGGCCCATGACTCGTA	*CESA6*	4.00E-84
BI543415	AGATTCGGGCACTTCAGA	GGCGTCCCCTTGTTTAG	*BAM1*	3.0E-113
BU089560	CAACAAAATCGCCACCTCCAG	CTCCCCCTACCCTTTTTCATTCTT	*BAM1*	0.00E+00
BU089560	TGGGGAATTTCTTGCTTGGTC	TCGCCCGAAAACTTGTTACTACTA	*BAM1*	0.00E+00
BU089560	**TTGGGGAATTTCTTGCTTGGTC**	**TCGCCCGAAAACTTGTTACTACTA**	***BAM1***	**0.00E+00**
EG552800	GGGGTTTTATCGGAGCAG	GGTGGCACATGGAGGAAT	*FKBP*	4.00E-60

Appendix Table 1.1 continued

BQ584125	CATCAGGCCGAGCAACC	TGGGAAAATCAAGATCAACAGTC	*ARP4*	3.00E-87
BQ586159	TTGTCACCGGAAACCTAACTT	AAAAACTCCCCCACTGTAAAAT	*XCP2*	2.00E-92
BQ586036	GCTTTTCGAGAGGGGATGAG	AAGCTAAGGCCAAACACAAGAA	*SCO1*	3.00E-61
EG551101	CCACTATCGGGGACAACTCAC	TAGGATCGGGCGGAATAAAG	*FKBP15-1*	1.00E-50
BQ488890	TGCATCCGCGAATCGTCAGGTT	CCCATCCCGGCGCTAATCAAT	*ANL2*	7.00E-62
BQ488901	ATAGCTCCGTCGACCTCAACTTCC	CCATCTTTCCGGGTCAATCTTCC	*HSFA5*	2.00E-59
BI543278	GGCCAGCCAAAGCAGACTAT	CAACCCCCAGACCAGATGT	*SK11*	6.0E-126
BQ593732	AGGTGGGTAGATGATGATGATGAA	TTACGCCGGAAAATGAACGAA	*KATC*	4.00E-25
BQ594997	TGAAGGTAGAAAGTGGGAGAAGTT	ACCAGCATTAGGACCATCAGTGT	*AML1*	2.00E-38
BQ593209	GCGACCGAATCATCACCACAA	GGCTCAGCGATTTCTCCTCTTCC	*OEP37*	3.00E-31
BI543568	GCGCGTGCGGCCCAGAACAT	TGACGGAAGGGCACCACCAGGAGT	*18SrRNA*	*** -
BI543569	CTAAGAACGGCCATGCACCACCAC	CCGCCGGCACCTTATGAGAAATC	*18SrRNA*	-
BI543889	AGCGTGCGGCCCATAACATCTAA	CGGAAGGGCACCACCAGGAGT	*18SrRNA*	-
BI643062	GCCAGCACCCCCTTCCCACAGAT	CACACCGCCCGTCGCTCCTACC	*18SrRNA*	-
BI643161	CTAAGAACGGCCATGCACCACCAC	GACGGAAGGGCACCACCAGGAGT	*18SrRNA*	-
BQ589671	**GGACAGTCGGGGGCATTCGTATT**	**CCGGCGGCGTCCTAAAAGTAAC**	***18SrRNA***	-
CF543368	GCCGGCGACGCATCATTCAAA	CGCGCCTGCTGCCTTCCTT	*18SrRNA*	-

Signaling Genes

Bv* Accession	**Forward Primer**	**Reverse Primer**	***At* Gene****	**E value**
BQ489058	GTGCGCGAAATTCAGAGACAAA	CAGATCCCGGTGCAGAACATT	*MPK13*	5.00E-74
BQ584059	TTCACCGAGGCTCAGATAAA	ACAACCCGCACTCCACAT	*IBS1*	4.00E-63
BU089558	GGCGGATTTCGGAGTG	CGATGGGCGGTTGATGG	*HSL1*	0.00E+00
BU089558	GGCGGATTTCGGAGTGG	CGATGGGCGGTTGATGG	*HSL1*	0.00E+00
BU089563	GGCGGATTTCGGAGTGG	CGATGGGCGGTTGATGG	*HSL1*	0.00E+00
BU089563	GGGGTTACCGCATTTATCATTG	TCTCCCGGAAAAACCATTACTT	*HSL1*	0.00E+00
BU089563	GTCATCGCCGGTTCTTGTGG	ATTTACCGTCCCTCTTCCCTGATT	*HSL1*	0.00E+00
BF011062	**CCGGGGAGTGCAACATCGTC**	**GCTGCTTGCTCTTGCCTTCTCTT**	***M3Ke1***	**3.0E-102**
BF011062	CGGGGAGTGCAACATCGTC	CGCCATTTCGCACAACAGA	*M3Ke1*	3.0E-102
BQ588826	TTTGCGGGTCATTTTCTCTAAGT	GGATGGCATGCAGGCTCTC	*WAK1*	4.00E-57

Appendix Table 1.1 continued

BQ592039	GCTTACGCTGTCGCTTGATA	TTGGCTGACTCTTTGGGAATG	*MERI5B*	7.00E-83
CK136500	GTCCAGGAGGCCAAACACTT	AACATAATTCCGCGATCTTCATAA	*MERI5B*	5.00E-91
BQ583877	GACGCGTGGGAAAAA	TTAACATCGGAAAACTCG	*MAPKKK21*	5.00E-29
BQ590382	TTATAGAGGGGCGGATTGTTGTGT	TATGAATGGGAATGCCTTGGTATC	*RABG3A*	8.00E-88
BU089551	CGGCACGAGGCTATTCTTCATC	CACCCGCGTCCCATTTGTAA	*WAKL2*	9.00E-48
BQ593921	CAAGCCGTTATCAAGTTCAAG	AGCAAGGCCAAAGTCACAT	*MPK8*	4.0E-102
BQ594267	CAAGCCGTTATCAAGTTCAAG	AGCAAGGCCAAAGTCACAT	*MPK8*	6.00E-94
BQ488487	TTATAGAGGGGCGGATTGTTGTGT	TGAATGGGAATGCCTTGGTATCTG	*RABG3B*	2.00E-73
CF543170	AAAGTTATCGTTCTCGGGGACAGT	CATGCCAATTATCAAGGGTATCAA	*RABG3B*	4.00E-95
BQ591700	GCAGCCTGTTCCTTTTTCTCA	CTCCCCGGTATATCAATTCCCTAAC	*MPK19*	9.00E-90
BQ489848	CGACAGCGGAGTGGGGAAGAC	GCACCTCGGTAGAAAGCAACAC	*RAB7B*	1.00E-71
BU089564	GGTTCCGTCGTTGCTGTGT	TCCCCGTGAGTCATCGTTGTC	*TMKL1*	0.00E+00
CF542879	TGCCGCCTAAAAGACTACGAA	TAACAGAACCGGAAGCTATCAAAG	*SRF3*	1.0E-101
BQ582382	**CGATCCCGACCTTCTTCTTG**	**AGTGCGTCGGCTATGTCAGTA**	***WAK***	**9.00E-46**
BQ585091	TTCGTCATGGAGTTCGCTAAAG	ACCTAAAATCTCCCCTGTGG	*CIPK14*	2.00E-72
BQ060547	TCCGGCTTTTGCGTCAT	TGGCTGTCGGAGTGTCATT	*MPK16*	4.0E-108
BQ060614	TCCGGCTTTTGCGTCAT	TGGCTGTCGGAGTGTCATT	*MPK16*	1.00E-86
BQ583062	**GTTGCCACCACTACATCTG**	**GGGTCCCCAACATTCA**	***XLG1***	**6.00E-66**
CF543157	TTAGGCTTGTGATATTCTGTG	ATATTTCAAGGCGGCAT	*XLG1*	8.00E-60
BQ489063	AGGCCCTTTTCAGAGA	CACCCATTGCCCACATA	*MPK20*	2.00E-87
BQ489063	TTTGCGGGTCATTTTTCTCTAAGT	GGATGGCATGCAGGCTCTC	*MPK20*	2.00E-87

Abiotic, Biotic and Undetermined Stress Genes

Bv* Accession	**Forward Primer**	**Reverse Primer**	***At* Gene****	**E value**
BQ584082	CGGGCCGATTTTTACTACTCA	CAAGCAAAAGATGGTCACGAATAA	*CYP90A*	9.00E-56
BQ586375	**TGTAATCCACCGTCCTCAAAC**	**TACCCAAAATCATACGCACTGT**	***CAF1***	**1.00E-65**
BI073128	**ACCCGGAAATAACGTAACTCTA**	**TGCGCCATTCAAACTAACA**	***GER3***	**5.00E-42**
BI095934	AGGGATCCCGCTACTCT	GCGCCATTCAAACTAACAA	*GER3*	2.00E-40
BI096011	AGGGATCCCGCTACTCT	CGCGGATTCAAACTAACAA	*GER3*	2.00E-40
BQ594432	CGGCTGCCTTTTTCTACTCCA	CTCAGCTGCCCCCATTTACATA	*GBF1*	2.00E-04
BI096145	TGGGGAGTCAGCTTTTAGTATTA	TCCCTTCTCCATTCCCTCTCAG	*LOX1*	7.00E-74

Appendix Table 1.1 continued

BQ585097	GCGGTTTGACATGAAGGCACTG	CCATCACTAGCATAAGGGTAATCT	*LOX*	9.00E-56
BQ488691	ACCGGACGGCCACCCACTGA	AATCCCGAATGTTATCTGCT	*LOX*	1.00E-49
BQ594875	TGCTTGCCGTTTTGGGTTAC	GACGGGTCGAAATTTGTTGATG	*RGA1*	5.00E-30
BQ587264	AAGCCGTGTAAAGAAAGGAAAAA	CTACGCCAAAGCCAACCAG	*MFP2*	3.0E-105
BQ589960	ATGCCAATGGGTCCCTTTAGAT	AGTAGAACCCTTTGCGTGTAT	*MFP2*	3.00E-91
BF011122	AAAAGGCCATGGTGATAAGAAGGA	GCATGGAGGGCAGAAGCAACC	*LOX5*	9.00E-73
BQ592313	AATAGAGCCCTTTTGTGATAGCAT	AGCCATTCCCCTCTTGACGA	*LOX5*	2.00E-66
BQ488466	**ATTGCGTGATTTTTGGGAACAG**	**TGGCACCTCGCTTGATGG**	***RBOHD***	**4.00E-62**
BQ584422	**AACCCCGCTAGCATTGAGAGGAT**	**AAGGGTGCCAAGTGAGGTTAGGAA**	***LOX3***	**2.00E-62**
BQ583249	AACCCTTACTTCCGCTTCAC	GTTCGCAAATGGGTAGTCCTC	*LOX2*	5.00E-55
BQ583306	CTCCGAAAGACCCGAAGAAAC	CAGGTGAATGTCCCGAAAGAAC	*LOX2*	4.00E-52
BQ584988	TAAGGGGACTAATTTGTATGGAT	TGCTGCAATTATGTATGGTTCTG	*LOX2*	9.00E-70
BQ594810	**GCACCCCAAAACTCGCAACAACAG**	**CACAAACCCGACCCACCACTTACC**	***ATLP***	**6.00E-83**
BQ586635	**GAACCGTCGACGAGAACTGCT**	**ATCCATGGCCGTCGTAAACAC**	***PP2CA***	**1.00E-39**
BQ582721	GTGGGTCGCCTGTTGTTTATGA	GTTTTCCTCCCCAGTTTGTTCC	*NCED3*	6.00E-81
BQ587840	TTCATTGCCCTTGCCTATTTT	ATGTCGCCCTGTTTCTTCTTG	*ETR1*	1.00E-97
BQ487855	ACGCCCTTCATTCCCTTTACC	TGAGATCATACGTCGGAGAAT	*MKK9*	8.00E-47
BQ487855	CGCCTCCCACTTCCAGAT	CACGGTGCACAATTTATGAG	*MKK9*	8.00E-47
BQ487860	**ACGCCCTTCATTCCCTTTACC**	**TGAGATCATACGTCGGAGAAT**	***MKK9***	**1.00E-33**
BQ487902	GCCTCCCACTTCCAGATAAAT	TAAGTGCGTAAATGGTGTTGTT	*MKK9*	6.00E-39
BQ487902	GCCTCCCACTTCCAGATAAAT	TAAGTGCGTAAATGGTGTTGTT	*MKK9*	6.00E-39
BQ587848	**AGGATTATGGGGAGAACACTTGA**	**AATCGGGTCTTTCTCCAGGTAAC**	***MKK9***	**2.00E-38**
BQ595738	CCTTCTCACTCCACCACCACTAA	TCATACGTCGGAGGATTTCTTTC	*MKK9*	3.00E-51
BQ595738	**CTTCGACGCCCTTCATTCC**	**CTTTGTAGACAGTTCCCCCATTA**	***MKK9***	**3.00E-51**
BQ583639	AGGGGCTGATGCGTGTT	TCTGGGGTGATTGTTTAT	*EIN2*	2.00E-35
BQ586400	CCGGTTGCAGTCCCAGTTT	AGCCGGTCTTTTCTCTTCT	*JAZ1*	3.00E-17
BQ588862	CCACGCGTCCGGTATTTGA	CCTAGCGCCATGCCAGTTTAT	*ABI2*	4.00E-45
BQ594725	CGCGTGGGTTTGTCCTC	CGGCGGGTTTGCTTCT	LRR PK	5.00E-89
BQ584433	ATAAGAGAAATGGCGACACAAGT	TGGATGGGCAGGCAACA	*MPK1*	3.00E-64
BQ488279	**AACGCGTTTGTCATT**	**TGCTTCATCCCATAGA**	***ABA2***	**4.00E-46**
BQ582770	GTAACCGGAGGAGCCAATG	ATATGTTCGCGTCTTCACCAA	*ABA2*	4.00E-39
BQ584498	AAATTTGGCACTCTTGATA	TAGTTCGGCTGCCACATTCTT	*ABA2*	9.00E-49

Appendix Table 1.1 continued

BQ593155	TTTGGGCCCGTGTTCA	TTGGTTGCCTTAATGTCTCTGT	LRR protein	6.00E-90
BQ593157	TTTGGGCCCGTGTTCA	TTGGTTGCCTTAATGTCTCTGT	LRR protein	4.00E-89
CF542675	CCCCGAGGAGCTTGGAGAGT	TAGGTTATTCGCCGTCAGGTCA	LRR protein	2.00E-82
BU089562	TGCCCAAACAGTCCCTTCTAATC	CGCCACCGTCGCTCCAT	LRR PK	0.00E+00
BQ488276	GTATTCCGGCGACTCTC	TCCCTGATTTGAACGAA	*MPK6*	8.00E-81
BQ488276	TCCTCTTCCCGATCATTCTCA	TCCTCTTCGCATCAACTTTATTAT	*MPK6*	8.00E-81
BQ588562	GCGGACGCGTGGGTTGC	CACGTGATCTCTGCCTGGAAATAG	*MPK6*	1.0E-110
BQ594736	**TCCTCTTCCCGATCATTCTCA**	**TCCTCTTCGCATCAACTTTATTAT**	***MPK6***	**2.00E-82**
BQ488461	AGGGCCACGCTACTTTTCCACAAC	TCCCATCGGCCCCACCACT	*AOX1A*	3.00E-19
BQ586418	GAGATGGTTGCGATGAAGAAAGATT	GGATGTATTTAAGCCCACGAAGTA	*MPK3*	3.00E-90
BQ586920	TTCCTCCGCAAGTCCGTCCTCTCC	CTCCGCACCTCGTCGTCGCTCTCG	*BRH1*	4.00E-28
BQ489189	**TTCCTACAAAGTGATAATGCTC**	**CTGTCAAAGATGGCTGCTC**	***MPK4***	**2.00E-51**
BQ592267	**GTGCGCGAAATTCAGAGACAAA**	**TCAGATCCCGGTGCAGAACATT**	***MPK4***	**3.0E-108**
BQ489814	CGGGCTGCAGGTTTCTATCTC	GCTGCACAAGGCCACCACTA	*MKK2*	4.00E-54
BQ488850	**GACGGCGATCTTTTAGTTA**	**AAGAGACCCACCATCCAT**	***MKK2***	**1.00E-51**
BQ489814	CCGGGCTGCAGGTTTCTATC	CTGCACAAGGCCACCACTACC	*MKK2*	4.00E-54
BQ593362	CAAGCTCGATGTTCCTCCTGAC	TTACCTTTGCCAATGACCTTA	*MKK2*	7.00E-39
CF543254	TGTGGAGGAAGCTGGGAAGGTGTT	GCCAATGCCGGAAGGGATAAGTC	LRR protein	8.00E-51
BU089571	TTATAGGGCGATTACACTCACTTC	TAACTTTCGGGTATCTGTCTCCA	*RLP52*	5.00E-79
BQ594412	**AATCCGTGTATCTGGCTCTTTC**	**AGGCAATTCTAACTGTTTTCTGAC**	***MKK3***	**3.00E-35**
BU089561	ATTGCCAAGGCGTTCGTAT	AATCATTCGCGGTGTAGTTAGG	LRR PK	0.00E+00
BQ488795	**CGTGCCTGCAGACATTGATT**	**GCACCACCAGCAGATTCCTT**	***ABA1***	**3.00E-49**
BQ587858	TAGTGGGCAGAAGAAGAAAGAGAA	AGGCCATTATCCGATCACCAGTA	*ABA2*	3.00E-57
BQ582800	CTGATTAAGGCGGGTGTAGTTC	CTTGGCGGCATAGGCTGGTAGG	*ABI5*	3.00E-21
BU089565	TTATGGGCCCGTTTCCTGTATC	TGGGCGAGCGTCAAGTTTC	LRR PK	0.00E+00
BU089547	AGCAGAAGGCGAGCGTGAAT	GGGGTCGAAGTAGCCAAAAGTG	PK	4.0E-149
BQ593937	TTGCCGGCTGTGAACCA	CATGTAGTCCGAAACCAGAGTAGC	PK	2.00E-57
BQ594117	TTGCCGGCTGTGAACCA	CCATGTAGTCCGAAACCAGAGTAG	PK	1.00E-51
CF543001	AGGGGCAGCTGTTGGTGA	CAGATCGCCGTGTGAGC	PK	1.00E-13
BQ595702	CCACGCGTCCGAAACTT	GTCATCCCAATACCACTGTCAAT	PK	1.00E-69
BQ593603	ATTGGGGAGAAGAGGGTCATC	AGGAGCCATATAGCCAAGTGTTC	PK	6.00E-97
BU089552	AGCGGCTGCCTTGAAAA	CAGGAGAACGACACCGAAACTAT	PK	5.0E-161

Appendix Table 1.1 continued

BQ582873	GTTAGGGGCAAGGTGGTTTT	TGGCCTCATAGTTTTCATCC	PK	9.00E-99
BI543739	TTCGCCACCGCCATCTG	CTCGGTAAGCTGTTGTCTCCTGAA	PK	2.00E-99
BI096304	GAGCGATCTATCTTCCTTAT	GTTCCCAGCCTTCACA	PK	1.0E-108
CF543002	GGGGCAGCTGTTGGTGATA	AAGGCCCGGAGGTTTTGT	PK	2.00E-73
BQ584876	TCCCCCAGGCAAAGAGC	GCCCAAGCAACGAGATTAT	PK	2.0E-112
BQ585999	GGCCGCTAATATCCTCCTC	CTGGGTCGGTTAAAGTATCAAAAT	PK	2.00E-90
BQ585841	TAAGTCGCATACACCACAGG	CGGGCTTACGTCCAGTTA	PK	3.00E-87
BQ582618	GGGCGCATCAGGGCTACA	CTCAACACGGGCAACACCAA	PK	4.00E-82
BQ593922	TTACACTGCCAATCAAAAACAACC	TGGCAAAGTAGATGGCTCACAA	PK	1.00E-81
BQ586464	**TGCTTTGCCGGACGACCTT**	**TCCCACGAACACCACCTGACA**	**PK**	**1.0E-116**
BU089554	GTTCTGCCCTTATCTTCCTTCA	ATTAACCGGCCATTCTCCA	PK	3.0E-123
BQ585434	TGGGCTGTCAAGGATAATG	TGAGCCCGGCCAAGAGGTC	PK	2.00E-52
BQ587874	TGGGCTGTCAAGGATAATG	AATGAGCCCGGCCAAGAGGT	PK	2.00E-60
BQ587887	TGGGCTGTCAAGGATAATG	TGAGCCGGCGGAAGAGGTC	PK	2.00E-60
BQ588055	TGGGCTGTCAAGGATAATG	AATGAGCCGGCCAAGAGGT	PK	2.00E-60
BQ582409	CAGCCAGAACTCTTTATCCACATC	ACTCCCACACGTACTGCTCATC	PK	6.0E-107
BQ590125	**CCTATCGCTTTTTCACCATCCTA**	**AATCCCAAATCAGCAATCTTCA**	**PK**	**7.00E-63**
BQ582687	GCGGAAAGGCAATGAAAG	CAATGATAAAGCACCCCAGTC	*AAO4*	2.00E-57
BQ488935	**GCGGCGCGTGCTTATGATGA**	**TCGGCGCCGGAAAACCAA**	***ERF9***	**6.00E-32**
BQ591669	**CTGGGAAGAAAGTGAGGAAG**	**TTTTTGGGGGTAAGGATGAG**	**myb**	**1.00E-55**
BI543377	GGCGGCTGAAGATATGATGAAC	TAGGCGGGATGGTAGGAGGTG	*ROF1*	4.00E-70
BI543386	GGCGGCTGAAGATATGATGAAC	TAGGCGGGATGGTAGGAGGTG	*ROF1*	3.00E-71
BI543980	GTTCACGGCAGCAATACCTCAA	AGCCTCGACCTCCATCCAAACT	*GSTF7*	2.00E-61
CK136876	GTTCACGGCAGCAATACCTCAA	AGCCTCGACCTCCATCCAAACT	*GSTF7*	3.00E-61
BQ592234	GATTGCTTCGTTAGGGGTTGTGAT	ATGGAGGTGGGATGTTTTGGAGT	*RCI3*	4.00E-68
BE590444	ATCCAAAACAACCGACCAACAA	TGCCAAGTATCATTTTTCTCCTC	*HSP17.8*	4.00E-57
BE590444	CCTCGACATATGGGACTCTTTCA	GCCCGTTGCCCGCTAATCTTT	*HSP17.8-CI*	9.00E-48
BQ595152	AACGCACGAATTGACTGGAAAGAA	TTGCCCGTTGCCGCTAATC	*HSP17.8-CI*	2.00E-39
BQ595152	AATCCAAAACAACCGACCAACA	TGCCAAGTATCATTTTTCTCCTC	*HSP17.8-CI*	2.00E-50
CK136617	GGCCGGGATCTCAAAC	CTCTCAACACGATGCCAAGTAT	*HSP17.8-CI*	5.00E-58
CK136658	GCCGGGGATCTCAAACA	CTCTCAACACGATGCCAAGTAT	*HSP17.8-CI*	5.00E-58
CK136658	GGCCGGGGATCTCAAACAAT	CGGAACGAGGAATGGTGGAG	*HSP17.8-CI*	6.00E-49

Appendix Table 1.1 continued

BQ587622	AACTTCCAGGGACAACATCAACA	GTCATCGTCGTGGTGCTTAGG	*MYB60*	3.00E-75
BQ587622	TTATCTTAGGCCTGGAATCAAA	GTCATCGTCGTGGTGCTTAG	*MYB60*	2.00E-75
BQ589141	TTATCTTAGGCCTGGAATCA	AGTTGCGGTTGTGTTGCTAC	*MYB60*	8.00E-73
BQ589141	TTGGACCCCTGAAGAAGAT	AGTTGCGGTTGTGTTGCTAC	*MYB60*	5.00E-73
BQ589354	ACGATAACATTAGACCAAAACAAA	ATCTCTGCAACCCGGTATTAGTAG	*MYB60*	2.00E-68
BQ586261	GTATCTTTTCTGGCCGTGTTATTG	CCGGTTACTAGGGATTTCATTGT	*GLP5*	6.00E-61
BQ592726	CAATGGCGGCGGTAGTGCTC	CTGGCCCCTTAAGAATCCCTCAT	*HSFA1D*	1.00E-55
CF542723	CGGAAAATCGGTGACTGGA	CTGGGCCCGAACATAAAAGA	*TTL1*	2.00E-77
BQ584180	ACGCCACGGCTGTCACTTTG	ATTGCCAGGTTGTAGCCATCCACT	*TLP*	3.00E-63
BQ585826	TGGGAGGCTCGCATCAAG	AAGCAAAACGCCAAAACTGTAAAT	*RFO1*	9.00E-43
BQ488894	GCTCAAAACTCCCACATCATCATT	TACTCCGCAACCATCTTCTTCACT	*PAL1*	1.00E-50
BQ582278	AAATTGCAATGGCTTCTTACTG	GGACCTTTCTGCACACCTGAC	*PAL1*	1.00E-51
BQ585675	CAGGCCTTGGCTAATGGTGATA	AGCGCGCTGCAGCCTCATACTTC	*PAL1*	2.00E-93
BQ582606	CAGGCCAAGGAATGAGA	GGGCAAGATATCCAACTGT	*RLK*	3.00E-86
BQ582606	CAGGCCAAGGAATGAGAG	GGGGCAAGATATCCAACTGT	*RLK*	3.00E-86
BQ582479	CGGCTGCCTCCCCTGCTGACA	AATGGCGTAGGGAACACTGACACC	*LTP1*	3.00E-21
BQ587197	AGAAGAGATCGCCCGCTACAT	AACTTCCCATCATAATCACCAACA	*HSPRO2*	2.00E-40
BQ595355	AAGCCATACATGCCTCCAAAAGAA	TCATATCCCCAACCAGAACAACAA	*FAB2*	3.00E-72
BQ595697	TTCCCGTCAAATGCCTGCTCT	TCATATCCCCAACCAAAACCACAT	*FAB2*	3.00E-70
BQ592393	CGTCGGTGTTTGCCTTGGAATA	GCGGTGCTGGATGGTGTAGTG	*UCC2*	2.00E-16
BI543685	CCCGATGAGGCTGTTGCTTAC	GCCTGCCCTTGTCATTTGTG	*HSP70*	3.0E-133
BQ488223	TGCGGGTCATCAATTTTCTCTTC	CGTTCGGGTATTTTCGGTCTCT	*NAC055*	6.00E-58
BQ488179	**CGTCGGCGCACTCACCTTGTATTC**	**ATGCGCCCCTGTTCTTTTTCCTCA**	***ATL2***	**9.00E-40**
BQ595328	CCGTCGGCGCACTCACCTTGTATT	ATGCGCCCCTGTTCTTTTTCCTCA	*ATL2*	9.00E-40
BQ594558	**ACAAAACCCGCCCAAAATCC**	**CTTCTTCTCAATCCTCGCCTTCTG**	***MBF1C***	**1.00E-46**
BQ587644	TAGACGCCATATGGTTAGAAGAAA	TGGCCATGTTAGTGTGATAGA	*CYP82G1*	2.00E-46
BQ582634	**ACCGGCCCCGTTTTTGATA**	**ATGAATGGAGAAGAGTGGCTAATG**	***CYP76C7***	**4.00E-72**
BQ582634	ACCGGCCCCGTTTTTGATACC	GAATGGAGAAGAGTGGCTAATGTC	*CYP76C7*	7.00E-72
BQ585612	CCCGGCCCCTATTATGAT	ATGCGGCAAGTGATTAGAACA	*CCD1*	7.00E-67
BQ592954	CGGACGCGTGGGCTAAACTC	TGGCTGCATTGGTGGGTGTG	*OSM34*	1.00E-59
CK136649	CCGACGGGTGCAAGAATCCT	TGGGCAGAAAGTAACGGTGTAATC	*OSM34*	6.00E-59
BQ584136	GTCCCCCACTTGTTCGTATCAC	AGCCGGTCTCTATGGTTTTCTGT	*SCL13*	6.00E-72

Appendix Table 1.1 continued

BQ584136	**TGTACCAGTTTTGCCCTTATTT**	**AGCCGGTCTCTATGGTTTTCTGT**	***SCL13***	**6.00E-72**
BI543265	CTTGCCGTCTTCTGAGTTTGATGA	GGAGCTGGTCGGGGGAGTG	CCH	2.00E-72
BQ593897	GCGGCAGCGAGCAGTAA	CCCCGTCGAAGGATTGTC	*TAFII15*	4.00E-58
BQ584386	TGTACCCTGTTGCTCCCATTGT	CGTTTCGCCATATTCTCACCAG	*CYP81D1*	2.00E-43
CK136719	**GCAGAAAAAGATCATCCCGACAAT**	**GAGCCCCTGAAAGAGCAACCAT**	***PER50***	**4.0E-101**
BQ589925	**AAGCCCCGTTAAATATGAAAAGA**	**CAGCAGCAGCAGCAAACC**	***RPN10***	**2.00E-68**
BQ586015	ACCCGCGTCTCAAAATA	ATACGCGGACATAATCAGG	*SNRK2-3*	2.0E-100
BQ588529	GGAATATGCTGCTGGAGGAGAG	AGACGCGGACATAATCAGGAAT	*SNRK2-3*	2.0E-107
BI543772	CAAGGGCAGGCTCTCAAAGGAA	CGGACCGGCACCACTG	*HSC70-1*	5.00E-92
BI643098	GTAACGGAAAAGGAACGAAAACT	CGGCACGAGGTCACAATCAC	*HSC70-1*	5.00E-62
BQ594995	GCCGGAAGAGAAGATTAT	GCCCCGCACGACCAC	GRBP	3.00E-40
BQ490017	CTTGCCCAAATGCTCTTAGTAT	CGAGCCGAGTTGTTATTAGGAG	peroxidase	3.00E-69
BQ488337	**ATTAAGGCCGGAGCAGCATA**	**GGTGGCGGAGAACAGAAGTG**	***RH26***	**9.00E-53**
BI543460	GGTCCCATTTCGCCTTCTTTA	GTACGGGCACTTTCAGCATTTT	*MTHSC70-2*	3.00E-65
BQ060494	CCATGGCCGCCGTTCTTC	CCTTCTGTGTTTGGGCATCATCA	*MTHSC70-2*	2.00E-81
EG552299	TCCGCCGTCCCTCTTTCTC	AGCGGCCATCCTGTAACCTG	chaperonin	9.00E-84
BQ583421	AGTGGCCTTCTAATCCTCCTTCAT	CTGGGCTTATCTGCATTCACAACT	*DMR6*	8.00E-95
BQ586894	GAAAAATCTCCGCCACCGAACTC	ACCTAATTTTGCGACGACGACGAT	*TCH2*	8.00E-37
BI073235	GTGCCAGACAAGACCAACAA	CCAAGGGGCTCACCAGT	*HSP81-3*	2.00E-90
BI096038	GCACGAGGCTGATGACGA	CAGATGGGCTTTTGCTTGTTA	*HSP81-3*	7.0E-106
BQ489704	ATCGTGCCAGACAAGACCAA	ACTCCCACACGTACTGCTCATC	*HSP81-3*	1.00E-78
BQ584196	CCACGCGTCCGAAACTT	GTCATCCCAATACCACTGTCAAT	*HSP81-3*	2.00E-90
BQ593588	CAAAGCAAATCCCCCTGTTAT	GGTGCCCGAGTGTTATCAATG	*HSP81-3*	1.00E-77
BF011227	CGTGCCAACAAGACCAACAAC	AGACTCCCACACGTACTGCTCATC	*HSP81-2*	3.0E-107
BQ589734	CGAAAACCGACGAAGTAACATCT	CACTTTGCCGTCTTCCACCTCTA	*HSP18.2*	2.00E-45
CK136793	CGACACCGCAAAAGAAACC	GTAAAAGTAAGCCCATAAAACATT	*HSP18.2*	2.00E-58
CK136793	TCAACAATCGACGCAACAACATCT	TGCCAAGTATCATTTTTCTCCTC	*HSP18.2*	2.00E-51
CK136863	CCCGGGTTAAAGAAGGAGGAAGT	TAGACGGTTTAGACACGAGCACAT	*HSP18.2*	1.00E-57
BQ490607	TGCAGCTATGGCGTTCTTCAG	ATATCCGCCGCCACCGTA	*GR-RBP3*	4.00E-58
CF543165	**ATGCGGGGCGGATTCAC**	**CTTTGGGCGAGTTCTGGTTTTC**	***HDA6***	**5.00E-83**
BQ582835	ACTCGCTGCAGCCAAGAAAG	AATAGCGGCAAGCACATCATC	*SAL1*	1.00E-74
BI543937	GGAGAAAGAATGCCCTATGACTGT	GGTTGCCCGTTTCCACTCTG	*PER72*	2.00E-98

Appendix Table 1.1 continued

BQ587584	GTACAGGCGGAGAACTTTT	AGAACGCCCTTTGTATC	*CIPK23*	4.00E-88
BQ585699	TATCTCCGGTGGGTCTATTCA	CTCAGGGGCCATCCAGTAA	*M3KA*	1.00E-77
CF542797	GCGTGGGAAGGAAGTGAGAA	CAGGGGCCATCCAGTAAGG	*M3KA*	1.0E-100
CF542821	ACGCGTGGGAAGGAAG	CAGCGTTTGGATGTGAA	*M3KA*	2.00E-20
BQ585699	**TATCTCCGGTGGGTCTATTCA**	**CAGGGGCCATCCAGTAAGG**	***MAP3KA***	**1.00E-77**
BQ593448	TATGCCGGGGAAAGGACTCTA	GCTTGCGGACATGGATACTGA	*RBOHF*	1.00E-91
BQ592254	GGGGCCAAAGAAAGGATGTA	AGTGGTGGTGGTTGCAGTAGTAG	*DREB2C*	3.00E-37
BQ592254	**TAAGGGGCCAAAGAAAGGATGTA**	**TCAAGGGCAGTGGGGAAAGTA**	***DREB2C***	**3.00E-37**
EG552103	TATACCTGCCCATGTGGATTAGAA	GTTATTGGGGGAGGATTTTAGTGA	*PLDBETA1*	7.00E-65
BI543239	AGCCCTTCACTCCATCCACCATA	ATAAACATACGGCACATTCTA	*ATL6*	7.00E-26
BQ586991	**GAGTAGCCGGGTCTGGGTATTG**	**CCGGATTTGCGAGTTGGTTC**	***RD26***	**5.00E-96**
BQ594715	GCACCCCAAAACTCGCAACAACAG	CCCGACCCACCACTTACCTCAACC	*TLP*	5.00E-72
CF543190	ACGCGTCCGAACAAGAAGAAAAT	CCGGCATCGATCCAAAAGTC	*LCL1*	9.00E-43
BQ584083	**AGGGTGGCGCTAGTCCGTCTCC**	**TTTCAGGCATCCGCAAGCAGTTTT**	***LTP4***	**6.00E-29**
BQ592168	**AACCCTAGCCGCTCCAAAACC**	**CCGCCACCACCACCGTATC**	***GRP4***	**3.00E-48**
CK136419	ACGGAAAGCAAAGGGCAGAG	CAGGGGAGGCAAGTGATGAAG	*GSTF8*	3.00E-69
CK136420	GACGGAAAGCAAAGGGCAGAG	CAGGGGAGGCAAGTGATGAAGA	*GSTF8*	5.00E-71

Genes with Undetermined Functions

Bv* Accession	**Forward Primer**	**Reverse Primer**	***At* Gene****	**E value**
BQ586553	ATGCCCTGTGATATGGTTGAG	TCCCTGTTGTTGATTGATTGTAG	AT1G30910.1	2.00E-79
BQ592405	TGCGGCAAATAGTACAAAAATCAG	GCCAGGCAAAATACCCACACT	AT1G80530	9.00E-56
BQ591201	AACATCCCGCTCACTTTCA	GGGCCCTTCCTCACGA	AT2G26270	1.00E-52
BQ583037	CCCCATTCTTTTTCCCACAA	GATATAAACATTCTTCGCAACAGC	AT2G39980	5.00E-60
BQ588349	ACTCACCCCCACATCCACA	AATTCTTCTGCTTCTTCCATCAAC	*TCP20*	9.00E-36
BQ583828	TTTTCAACCGCATTTAT	TATTGGGCAACTACAGC	*PHB1*	1.00E-65
BQ590906	CAAACATGGGGCGATCTTACGAA	ATTTTGGAACTTGCGATGGTGGAG	AT5G58020	5.00E-20
BQ587396	**TTCACTCCCGGGTCCGTTTCTAT**	**AATCACCTCTTGCGCCTCCTCCTG**	***CML41***	**4.00E-42**
BQ487898	CTACGCTTATTGGCTTGGTCTTG	AGGGCACTGCTTGGGTTCTAC	AT5G25170	1.00E-79
BI096344	CACGGTTGCGATGAAGG	TGAGACGCGATAACAA	Und.	-
BQ499841	CAGCGTCGAAAATTAGATGAT	CGAAGTGGCCCGTAGGAG	Und.	-

Appendix Table 1.1 continued

CX779686	CCACGGCTCTGATAGGGAATA	GTACGCCGGGGATGACAG	Und.	-
CX779686	CCACGGCTCTGATAGGGAATA	AGGTACGCCGGGGATGACA	Und.	-
DX811261	CCACCTCCTCCTGATGCTGACTCT	ATAATGGCGGGGGCGTTGTTG	Und.	-
ED032482	AGAGAAAAACGGCAAGAAATACA	AGGATGAATAGGCCCACAAAT	Und.	-
ED032901	TATGGCATGACCGATTAGG	ATTCCCCCAGCATTGTCT	Und.	-

* *Beta vulgaris* EST ** *Arabidopsis thaliana* gene *** No information available

Appendix Table 1.2 Genes present in both SP7622 and ACH185 in mature untreated seeds (0 h) prior to H_2O or H_2O_2 treatment using RT-PCR

Accession	*At* Gene/Function*	*At* protein**	E value
BI073128	*GER3*	NP_197563.1	5.00E-42
BI073235	*HSP81-3*	NP_200412.1	2.00E-90
BI096046	*PUMP1*	NP_190979.1	5.00E-108
BI096176	*GER1*	NP_177405.1	2.00E-03
BI096232	*PLDALPHA1*	NP_188194.1	2.00E-55
BI543386	*ROF1*	NP_001118695.1	3.00E-71
BI543568	*18S rRNA*	-***	-
BI543569	*18S rRNA*	-	-
BI543772	*HSC70-1*	NP_195870.1	5.00E-92
BI543980	*GSTF7*	NP_171791.1	2.00E-61
BI643062	*18S rRNA*	-	-
BI643161	*18S rRNA*	-	-
BQ060614	*MPK16*	NP_197402.1	1.00E-86
BQ487902	*MKK9*	NP_177492.1	6.00E-39
BQ488276	*MPK6*	NP_181907.1	8.00E-81
BQ489063	*MPK20*	NP_565989.1	2.00E-87
BQ489189	*MPK4*	NP_192046.1	2.00E-51
BQ582618	protein kinase	NP_199811.1	4.00E-82
BQ582859	*PTR2*	NP_178313.1	3.00E-94
BQ584082	*CPD*	NP_001031838.1	9.00E-56
BQ584876	protein kinase	NP_197362.1	2.00E-112
BQ585841	protein kinase	NP_198672.1	3.00E-87
BQ586400	*JAZ1*	NP_973862.1	3.00E-17
BQ587173	*FAB1*	NP_565097.1	3.00E-55
BQ587272	*PLDALPHA1*	NP_188194.1	1.00E-74
BQ587848	*MKK9*	NP_177492.1	2.00E-38
BQ587887	protein kinase	NP_567072.1	2.00E-60
BQ588562	*MPK6*	NP_181907.1	1.00E-110
BQ589671	*18S rRNA*	-	-
BQ593588	*HSP81-3*	NP_200412.1	1.00E-77
BU089563	*HSL1*	NP_174166.1	0.00E+00
CF542821	*M3KA*	NP_564635.1	2.00E-20
CF542917	*CYP71A25*	NP_680107.1	1.00E-61
CF543368	*18S rRNA*	-	-
CF543447	*RPT4A*	NP_199115.1	1.00E-132
CK136420	*GSTF8*	NP_850479.1	5.00E-71
CX779686	Undetermined	-	-
ED032482	Undetermined	-	-

Appendix Table 1.2 continued

EG551101	*FKBP15-1*	NP_566762.1	1.00E-50

* *Arabidopsis thaliana* gene ** *Arabidopsis thaliana* protein *** no information available

Appendix Table 1.3 Genes present in SP7622 and not ACH185 in mature seeds (0 h) prior to treatment using RT-PCR

	SP7622 (0h)		
Bv **Accession***	***At* gene/function****	***At* protein*****	**E value**
AW063023	*ACT7*	NP_196543.1	7.00E-152
AW697779	AT3G29970	NP_190397.1	9.00E-33
AW777170	*WNK1*	NP_001118576.1	1.00E-51
BE590444	*HSP17.8*	NP_172220.1	4.00E-57
BF010998	*ACT11*	NP_187818.1	2.00E-122
BF011036	AT3G29970	NP_190397.1	1.00E-32
BF011057	*SIP1*	NP_175970.1	2.00E-09
BF011062	*MAPKKK7*	NP_187962.1	3.00E-102
BG577441	*XTR6*	NP_194311.1	2.00E-90
BI073176	*PHS2*	NP_190281.1	1.00E-76
BI095934	*GER3*	NP_197563.1	2.00E-40
BI096011	*GER3*	NP_197563.1	2.00E-40
BI096180	*EP3*	NP_191010.1	1.00E-41
BI096237	mitochondrial elongation factor	NP_182029.1	3.00E-86
BI096344	Undetermined	****-	-
BI543265	zinc finger (CCCH-type) protein	NP_194648.1	2.00E-72
BI543285	AT3G29970	NP_190397.1	1.00E-32
BI543360	*CAT2*	NP_001031791.1	3.00E-100
BI543377	*ROF1*	NP_001118695.1	4.00E-70
BI543415	*BAM1*	NP_201371.1	3.00E-113
BI543460	*MTHSC70-2*	NP_196521.1	3.00E-65
BI543685	*HSP70*	NP_187864.1	3.00E-133
BI543739	Protein kinase	NP_190214.1	2.00E-99
BI643098	*HSC70-1*	NP_195870.1	5.00E-62
BQ060494	*MTHSC70-2*	NP_196521.1	2.00E-81
BQ060547	*MPK16*	NP_197402.1	4.00E-108
BQ487636	enoyl-CoA hydratase protein	NP_191610.3	8.00E-78
BQ487747	*XTR6*	NP_194311.1	5.00E-86
BQ487855	*MKK9*	NP_177492.1	8.00E-47
BQ487860	*MKK9*	NP_177492.1	1.00E-33
BQ487982	*NCED4*	NP_193652.1	5.00E-62
BQ488119	*IP5PII/BME3*	NP_849745.1	9.00E-50
BQ488279	*ABA2*	NP_175644.1	4.00E-46
BQ488487	*RABG3B*	NP_173688.1	2.00E-73
BQ488795	*ABA1*	NP_201504.2	3.00E-49
BQ488894	*PAL1*	NP_181241.1	1.00E-50
BQ488935	*ERF9*	NP_199234.1	6.00E-32
BQ489620	*CAM7*	NP_189967.1	4.00E-73
BQ489814	*MKK2*	NP_194710.1	4.00E-54

Appendix Table 1.3 continued

BQ489848	*RAB7B*	NP_188512.1	1.00E-71
BQ490607	*GRP3*	NP_200911.1	4.00E-58
BQ499841	Undetermined	-	-
BQ582382	WAK	NP_194839.2	9.00E-46
BQ582477	*CDC2*	NP_566911.1	8.00E-96
BQ582479	*LP1*	NP_181388.1	3.00E-21
BQ582606	*RLK*	NP_181307.1	3.00E-86
BQ582634	*CYP76C7*	NP_191663.1	4.00E-72
BQ582708	*NCED4*	NP_193652.1	1.00E-63
BQ582770	*ABA2*	NP_175644.1	4.00E-39
BQ582799	*CDKC*	NP_196589.1	1.00E-71
BQ582800	*ABI5*	NP_565840.1	3.00E-21
BQ582873	protein kinase	NP_188511.1	9.00E-99
BQ583306	*LOX2*	NP_566875.1	4.00E-52
BQ583369	*PLDALPHA1*	NP_188194.1	9.00E-76
BQ583421	*DMR6*	NP_197841.1	8.00E-95
BQ583639	*EIN2*	NP_195948.1	2.00E-35
BQ583692	*BRI1*	NP_195650.1	3.00E-101
BQ584083	*LTP4*	NP_568904.1	**6.00E-29**
BQ584136	*SCL13*	NP_193456.4	6.00E-72
BQ584196	*HSP81-3*	NP_200412.1	2.00E-90
BQ584386	*CYP81D2*	NP_195452.1	2.00E-43
BQ584433	*MPK1*	NP_172492.1	3.00E-64
BQ584498	*ABA2*	NP_175644.1	9.00E-49
BQ584988	*LOX2*	NP_566875.1	9.00E-70
BQ585195	seed maturation protein	NP_198150.1	4.00E-27
BQ585514	ER	NP_180201.1	2.00E-71
BQ585675	*PAL1*	NP_181241.1	2.00E-93
BQ585699	*M3KA*	NP_564635.1	1.00E-77
BQ585826	*RFO1*	NP_178085.1	9.00E-43
BQ585998	*BR6OX2*	NP_566852.1	4.00E-59
BQ585999	protein kinase	NP_197362.1	2.00E-90
BQ586015	*SNRK2-3 /SNRK2.2*	NP_195711.1	2.00E-100
BQ586635	*AtPP2CA*	NP_187748.1	1.00E-39
BQ586657	transferase family protein	NP_199097.1	7.00E-59
BQ586719	*GA20OX2*	NP_199994.1	2.00E-44
BQ586920	*BRH1*	NP_191705.1	4.00E-28
BQ586930	*CAT2*	NP_195235.1	2.00E-89
BQ587264	*MPF2*	NP_187342.1	3.00E-105
BQ587622	*MYB60*	NP_172358.1	2.00E-75
BQ587840	*ETR1*	NP_176808.3	1.00E-97
BQ587858	*ABA2*	NP_201504.2	3.00E-57
BQ588316	*UCP5*	NP_179836.1	8.00E-33
BQ588349	*TCP20*	NP_189337.1	9.00E-36
BQ588529	*SNRK2-3 /SNRK2.2*	NP_195711.1	2.00E-107

Appendix Table 1.3 continued

BQ589141	*MYB60*	NP_172358.1	5.00E-73
BQ589455	*SPT*	NP_568010.1	2.00E-16
BQ589925	*RPN10*	NP_195575.1	2.00E-68
BQ590158	mitochondrial substrate carrier	NP_194188.1	5.00E-17
BQ590906	Undetermined	NP_200610.1	5.00E-20
BQ591669	myb transcription factor	NP_001030662.1	1.00E-55
BQ592254	*DRE2B*	NP_565929.1	3.00E-37
BQ593448	*RBOH F*	NP_564821.1	1.00E-91
BQ593732	*KATC*	NP_568811.1	4.00E-25
BQ593897	*TAFII15*	NP_194900.1	4.00E-58
BQ594117	protein kinase	NP_177507.1	1.00E-51
BQ594412	*MKK3*	NP_198860.1	3.00E-35
BQ594558	*MBFC1*	NP_189093.1	1.00E-46
BQ594736	*MPK6*	NP_181907.1	2.00E-82
BQ594788	SDR protein	NP_190736.1	4.00E-46
BQ594875	*RGA1*	NP_178266.1	5.00E-30
BQ594995	*GRBP*	NP_196048.1	3.00E-40
BQ595049	*GTE6*	NP_190796.1	9.00E-38
BQ595152	*HSP17.8-CI*	NP_172220.1	2.00E-39
BQ595355	*SSI2*	NP_181899.1	3.00E-72
BQ595434	*APY2*	NP_197329.4	5.00E-57
BQ595738	*MKK9*	NP_177492.1	3.00E-51
BQ595856	*ATTPC1*	NP_567258.1	3.00E-69
BQ654409	*XTR6*	NP_194311.1	2.00E-92
BU089561	*LRR Kinase*	NP_199948.1	0.00E+00
CF542675	*LRR Kinase*	NP_176918.1	2.00E-82
CF542797	*M3KA*	NP_564635.1	1.00E-100
CF542908	*HDA9*	NP_190054.2	2.00E-118
CF543165	*HDA6*	NP_201116.1	5.00E-83
CF543254	LRR protein	NP_197731.1	8.00E-51
CF543420	*HSI2*	NP_850146.1	7.00E-26
CF543627	2OG-Fe(II) oxygenase protein	NP_566623.1	7.00E-72
CK136419	*GSTF8*	NP_850479.1	3.00E-69
CK136658	*HSP17.8-CI*	NP_172220.1	5.00E-58
CK136876	*GSTF7*	NP_171791.1	3.00E-61
EG551187	*BRK1*	NP_179849.2	2.00E-37

* *Beta vuglaris* EST ** *Arabidopsis thaliana* gene *** *Arabidopsis thaliana* protein **** No information available

Appendix Table 1.4 Genes present in ACH185 and not SP7622 in mature seeds (0 h) prior to treatment using RT-PCR

ACH185 (0h)

Bv **Accession***	***At* gene/function****	***At* protein*****	**E value**
BF011227	*HSP81-2*	NP_200414.1	3.00E-107
BI073146	*GER1*	NP_177405.1	3.00E-06
BI543889	*18S rRNA*	-******	-
BQ488461	*AOX1A*	NP_188876.1	3.00E-19
BQ489058	*MPK13*	NP_172266.2	5.00E-74
BQ490017	peroxidase, putative	NP_196153.1	3.00E-69
BQ490300	*CESA6*	NP_201279.1	4.00E-84
BQ582721	*NCED3*	NP_188062.1	6.00E-81
BQ584898	*POT*	NP_175630.1	1.00E-63
BQ586318	steroid 5-alpha-reductase protein	NP_197105.1	2.00E-50
BQ586991	***RD26***	**NP_567773.1**	**5.00E-96**
BQ587584	*CIPK23*	NP_564353.1	4.00E-88
BQ588055	protein kinase	NP_567072.1	2.00E-60
BQ588646	AT1G03790	NP_194648.1	3.00E-69
BQ588709	*POT*	NP_173322.1	1.00E-67
BQ588784	*AML4*	NP_196346.1	3.00E-47
BQ593316	*WOX13*	NP_195280.1	3.00E-21
BQ595269	*ARF6*	NP_001031115.1	7.00E-97
BQ595543	*PXA1*	NP_568072.1	2.00E-73
CF543001	protein kinase	NP_179361.1	1.00E-13
CK136793	*HSP18.2*	NP_200780.1	2.00E-58
EG552299	chaperonin, putative	NP_197589.1	9.00E-84

* *Beta vuglaris* EST ** *Arabidopsis thaliana* gene *** *Arabidopsis thaliana* protein **** No information available

Appendix Table 1.5 K-means clustering of 343 putative stress (abiotic, biotic and both abiotic and biotic), growth and hormone related genes in ACH185 cDNA over the first 24 h of germination time points in H_2O and H_2O_2. Accessions in bold were also used for qPCR analyses.

ACH185							
H_2O				H_2O_2			
***Bv* Accession** *	**Role 1** **	**Role 2** *+	**Cluster**	***Bv* Accession** *	**Role 1** **	**Role 2** *+	**Cluster**
AW063023	Growth		2	AW063023	Growth		5
AW063034	Growth		6	AW063034	Growth		6
AW697779	Growth		2	AW697779	Growth		2
AW777170	Growth		6	AW777170	Growth		6
BE590301	Growth		6	BE590301	Growth		6
BE590301	Growth		6	BE590301	Growth		6
BE590328	Growth		3	BE590328	Growth		6
BE590397	Growth		6	BE590397	Growth		2
BE590397	Growth		1	BE590397	Growth		4
BE590397	Growth		1	BE590397	Growth		6
BE590444	Stress	Abiotic	7	BE590444	Stress	Abiotic	4
BE590444	Stress	Abiotic	2	BE590444	Stress	Abiotic	5
BF010998	Growth		2	BF010998	Growth		2
BF011036	Growth		2	BF011036	Growth		2
BF011057	Growth		6	BF011057	Growth		6
BF011057	Growth		6	BF011057	Growth		6
BF011062	Signaling		6	BF011062	Signaling		6
BF011062	**Signaling**		6	**BF011062**	**Signaling**		6
BF011089	Growth		7	BF011089	Growth		3
BF011122	Stress	Both	6	BF011122	Stress	Both	6
BF011211	Growth		6	BF011211	Growth		6
BF011227	Stress	Abiotic	2	BF011227	Stress	Abiotic	2
BG577441	Growth		1	BG577441	Growth		3
BI073121	Growth		7	BI073121	Growth		6
BI073128	**Stress**	Abiotic	3	**BI073128**	**Stress**	Abiotic	2
BI073146	Growth		6	BI073146	Growth		6
BI073146	Growth		6	BI073146	Growth		6
BI073176	Growth		1	BI073176	Growth		5
BI073235	Stress	Abiotic	2	BI073235	Stress	Abiotic	2
BI073250	Growth		7	BI073250	Growth		3
BI095934	Stress	Abiotic	3	BI095934	Stress	Abiotic	6
BI096011	Stress	Abiotic	6	BI096011	Stress	Abiotic	6
BI096038	Stress	Abiotic	0	BI096038	Stress	Abiotic	0
BI096046	Growth		3	BI096046	Growth		6

Appendix Table 1.5 continued

BI096111	Growth		2	BI096111	Growth		4
BI096145	Stress	Biotic	1	BI096145	Stress	Biotic	3
BI096176	Growth		1	BI096176	Growth		5
BI096176	Growth		7	BI096176	Growth		2
BI096180	Growth		6	BI096180	Growth		6
BI096232	Growth		1	BI096232	Growth		2
BI096237	Growth		7	BI096237	Growth		1
BI096304	Stress	Und.	6	BI096304	Stress	Und.	6
BI096344	Und.***		6	BI096344	Und.***		6
BI543239	Stress	Biotic	1	BI543239	Stress	Biotic	6
BI543265	Stress	Abiotic	0	BI543265	Stress	Abiotic	5
BI543278	Growth		6	BI543278	Growth		6
BI543285	Growth		2	BI543285	Growth		4
BI543316	Growth		7	BI543316	Growth		0
BI543360	Growth		0	BI543360	Growth		1
BI543377	Stress	Biotic	3	BI543377	Stress	Biotic	3
BI543386	Stress	Biotic	3	BI543386	Stress	Biotic	6
BI543415	Growth		6	BI543415	Growth		6
BI543460	Stress	Abiotic	1	BI543460	Stress	Abiotic	1
BI543526	Growth		6	BI543526	Growth		6
BI543568	-***+		2	BI543568	-***+		2
BI543569	-		2	BI543569	-		2
BI543685	Stress	Both	2	BI543685	Stress	Both	2
BI543739	Stress	Und.	1	BI543739	Stress	Und.	4
BI543772	Stress	Abiotic	2	BI543772	Stress	Abiotic	4
BI543889	-		2	BI543889	-		4
BI543937	Stress	Abiotic	7	BI543937	Stress	Abiotic	6
BI543980	Stress	Abiotic	7	BI543980	Stress	Abiotic	5
BI643062	-		2	BI643062	-		2
BI643098	Stress	Abiotic	6	BI643098	Stress	Abiotic	6
BI643161	-		2	BI643161	-		2
BQ060494	Stress	Abiotic	1	BQ060494	Stress	Abiotic	4
BQ060547	Signaling		1	BQ060547	Signaling		6
BQ060547	Signaling		6	BQ060547	Signaling		4
BQ060614	Signaling		6	BQ060614	Signaling		3
BQ060614	Signaling		3	BQ060614	Signaling		1
BQ487636	Growth		0	BQ487636	Growth		3
BQ487747	Growth		1	BQ487747	Growth		5
BQ487855	Stress	Both	6	BQ487855	Stress	Both	6
BQ487855	Stress	Both	6	BQ487855	Stress	Both	6
BQ487860	**Stress**	Both	1	**BQ487860**	**Stress**	Both	6
BQ487898	Und.		6	BQ487898	Und.		6
BQ487902	Stress	Both	6	BQ487902	Stress	Both	1
BQ487902	Stress	Both	1	BQ487902	Stress	Both	4
BQ487902	Stress	Both	1	BQ487902	Stress	Both	6

Appendix Table 1.5 continued

BQ487982	**Growth**		6	**BQ487982**	**Growth**		5
BQ488119	Growth		0	BQ488119	Growth		0
BQ488179	**Stress**	Biotic	3	**BQ488179**	**Stress**	Biotic	6
BQ488223	Stress	Abiotic	6	BQ488223	Stress	Abiotic	5
BQ488276	Stress	Both	1	BQ488276	Stress	Both	6
BQ488276	Stress	Both	2	BQ488276	Stress	Both	2
BQ488277	Growth		0	BQ488277	Growth		0
BQ488279	**Stress**	Abiotic	6	**BQ488279**	**Stress**	Abiotic	6
BQ488337	**Stress**	Both	6	**BQ488337**	**Stress**	Both	1
BQ488461	Stress	Abiotic	1	BQ488461	Stress	Abiotic	4
BQ488466	**Stress**	Abiotic	0	**BQ488466**	**Stress**	Abiotic	4
BQ488487	Signaling		6	BQ488487	Signaling		6
BQ488691	Stress	Biotic	6	BQ488691	Stress	Biotic	6
BQ488795	**Stress**	Abiotic	3	**BQ488795**	**Stress**	Abiotic	3
BQ488850	**Stress**	Abiotic	1	**BQ488850**	**Stress**	Abiotic	3
BQ488890	Growth		6	BQ488890	Growth		0
BQ488894	Stress	Both	3	BQ488894	Stress	Both	0
BQ488901	Growth		3	BQ488901	Growth		3
BQ488935	**Stress**	Biotic	7	**BQ488935**	**Stress**	Biotic	0
BQ489058	Signaling		6	BQ489058	Signaling		6
BQ489063	Signaling		6	BQ489063	Signaling		6
BQ489063	Signaling		7	BQ489063	Signaling		6
BQ489189	**Stress**	Both	2	**BQ489189**	**Stress**	Both	2
BQ489620	Growth		7	BQ489620	Growth		5
BQ489704	Stress	Abiotic	7	BQ489704	Stress	Abiotic	6
BQ489814	Stress	Abiotic	6	BQ489814	Stress	Abiotic	6
BQ489814	Stress	Abiotic	1	BQ489814	Stress	Abiotic	6
BQ489848	Signaling		7	BQ489848	Signaling		5
BQ489959	Growth		3	BQ489959	Growth		6
BQ490017	Stress	Abiotic	6	BQ490017	Stress	Abiotic	6
BQ490300	Growth		7	BQ490300	Growth		6
BQ490338	**Growth**		0	**BQ490338**	**Growth**		3
BQ490607	Stress	Abiotic	2	BQ490607	Stress	Abiotic	0
BQ499841	Und.		6	BQ499841	Und.		6
BQ582278	Stress	Both	0	BQ582278	Stress	Both	5
BQ582382	**Signaling**		1	**BQ582382**	**Signaling**		4
BQ582409	Stress	Und.	1	BQ582409	Stress	Und.	6
BQ582477	Growth		6	BQ582477	Growth		0
BQ582479	Stress	Biotic	3	BQ582479	Stress	Biotic	6
BQ582606	Stress	Biotic	0	BQ582606	Stress	Biotic	1
BQ582606	Stress	Biotic	1	BQ582606	Stress	Biotic	6
BQ582618	Stress	Und.	1	BQ582618	Stress	Und.	4
BQ582629	**Growth**		6	**BQ582629**	**Growth**		6
BQ582632	Growth		6	BQ582632	Growth		4
BQ582634	Und.		2	BQ582634	Und.		3

Appendix Table 1.5 continued

BQ582634	**Und.**		0	**BQ582634**	**Und.**		0
BQ582685	Growth		6	BQ582685	Growth		6
BQ582687	Stress	Abiotic	0	BQ582687	Stress	Abiotic	5
BQ582708	Growth		6	BQ582708	Growth		5
BQ582721	Stress	Abiotic	6	BQ582721	Stress	Abiotic	6
BQ582763	Growth		0	BQ582763	Growth		0
BQ582770	Stress	Abiotic	6	BQ582770	Stress	Abiotic	6
BQ582799	Growth		6	BQ582799	Growth		6
BQ582800	Stress	Both	7	BQ582800	Stress	Both	0
BQ582835	Stress	Abiotic	6	BQ582835	Stress	Abiotic	6
BQ582859	Growth		3	BQ582859	Growth		1
BQ582873	Stress	Und.	6	BQ582873	Stress	Und.	6
BQ583037	Und.		6	BQ583037	Und.		6
BQ583062	**Signaling**		1	**BQ583062**	**Signaling**		1
BQ583249	Stress	Both	7	BQ583249	Stress	Both	6
BQ583301	Growth		2	BQ583301	Growth		3
BQ583306	Stress	Both	6	BQ583306	Stress	Both	3
BQ583369	**Growth**		2	**BQ583369**	**Growth**		2
BQ583421	Stress	Biotic	2	BQ583421	Stress	Biotic	5
BQ583639	Stress	Both	2	BQ583639	Stress	Both	5
BQ583692	**Growth**		6	**BQ583692**	**Growth**		5
BQ583764	Growth		0	BQ583764	Growth		6
BQ583828	Und.		0	BQ583828	Und.		1
BQ583877	Signaling		6	BQ583877	Signaling		6
BQ584025	Growth		6	BQ584025	Growth		3
BQ584082	Stress	Abiotic	1	BQ584082	Stress	Abiotic	5
BQ584083	**Stress**	Abiotic	3	**BQ584083**	**Stress**	Abiotic	6
BQ584125	Growth		3	BQ584125	Growth		6
BQ584136	Stress	Biotic	0	BQ584136	Stress	Biotic	5
BQ584136	**Stress**	Biotic	2	**BQ584136**	**Stress**	Biotic	5
BQ584180	Growth		6	BQ584180	Growth		6
BQ584196	Stress	Abiotic	1	BQ584196	Stress	Abiotic	6
BQ584386	Stress	Biotic	3	BQ584386	Stress	Biotic	5
BQ584422	**Stress**	Biotic	7	**BQ584422**	**Stress**	Biotic	3
BQ584431	Growth		3	BQ584431	Growth		6
BQ584433	Stress	Biotic	1	BQ584433	Stress	Biotic	1
BQ584462	Growth		6	BQ584462	Growth		6
BQ584474	Growth		0	BQ584474	Growth		0
BQ584498	Stress	Abiotic	6	BQ584498	Stress	Abiotic	0
BQ584876	Stress	Und.	1	BQ584876	Stress	Und.	4
BQ584898	Growth		6	BQ584898	Growth		6
BQ584988	Stress	Both	6	BQ584988	Stress	Both	3
BQ585091	Signaling		6	BQ585091	Signaling		6
BQ585097	Stress	Biotic	6	BQ585097	Stress	Biotic	6
BQ585195	Growth		6	BQ585195	Growth		6

Appendix Table 1.5 continued

BQ585514	Growth		1	BQ585514	Growth		4
BQ585612	Stress	Abiotic	0	BQ585612	Stress	Abiotic	5
BQ585675	Stress	Both	7	BQ585675	Stress	Both	6
BQ585699	**Growth**		1	**BQ585699**	**Growth**		6
BQ585699	Growth		6	BQ585699	Growth		3
BQ585826	Stress	Biotic	1	BQ585826	Stress	Biotic	1
BQ585834	Growth		3	BQ585834	Growth		3
BQ585841	Stress	Und.	1	BQ585841	Stress	Und.	4
BQ585998	**Growth**		0	**BQ585998**	**Growth**		5
BQ585999	Stress	Und.	1	BQ585999	Stress	Und.	6
BQ586015	Stress	Abiotic	2	BQ586015	Stress	Abiotic	5
BQ586036	Growth		2	BQ586036	Growth		5
BQ586159	Growth		6	BQ586159	Growth		0
BQ586261	Stress	Abiotic	6	BQ586261	Stress	Abiotic	6
BQ586318	Growth		2	BQ586318	Growth		5
BQ586375	**Stress**	Biotic	3	**BQ586375**	**Stress**	Biotic	6
BQ586400	Stress	Biotic	3	BQ586400	Stress	Biotic	0
BQ586464	**Stress**	Und.	6	**BQ586464**	**Stress**	Und.	6
BQ586518	Growth		6	BQ586518	Growth		6
BQ586553	Und.		0	BQ586553	Und.		3
BQ586635	**Stress**	Abiotic	0	**BQ586635**	**Stress**	Abiotic	0
BQ586657	Growth		6	BQ586657	Growth		6
BQ586719	Growth		6	BQ586719	Growth		3
BQ586790	Growth		6	BQ586790	Growth		6
BQ586894	Stress	Abiotic	0	BQ586894	Stress	Abiotic	0
BQ586903	Growth		6	BQ586903	Growth		6
BQ586920	Stress	Biotic	7	BQ586920	Stress	Biotic	3
BQ586930	Growth		2	BQ586930	Growth		2
BQ586991	**Stress**	Abiotic	7	**BQ586991**	**Stress**	Abiotic	4
BQ587173	Growth		1	BQ587173	Growth		1
BQ587197	Stress	Both	6	BQ587197	Stress	Both	6
BQ587264	Growth		0	BQ587264	Growth		5
BQ587272	Growth		6	BQ587272	Growth		6
BQ587329	Growth		3	BQ587329	Growth		6
BQ587384	Growth		2	BQ587384	Growth		5
BQ587396	**Growth**		6	**BQ587396**	**Growth**		6
BQ587458	Growth		6	BQ587458	Growth		6
BQ587584	Stress	Abiotic	0	BQ587584	Stress	Abiotic	6
BQ587622	Stress	Both	7	BQ587622	Stress	Both	0
BQ587622	Stress	Both	3	BQ587622	Stress	Both	3
BQ587644	Growth		0	BQ587644	Growth		6
BQ587840	Stress	Both	0	BQ587840	Stress	Both	5
BQ587848	**Stress**	Both	6	**BQ587848**	**Stress**	Both	2
BQ587858	Stress	Abiotic	6	BQ587858	Stress	Abiotic	6
BQ587874	Stress	Und.	7	BQ587874	Stress	Und.	1

Appendix Table 1.5 continued

BQ587887	Stress	Und.	7	BQ587887	Stress	Und.	1
BQ588055	Stress	Und.	6	BQ588055	Stress	Und.	6
BQ588316	Growth		2	BQ588316	Growth		3
BQ588349	Und.		0	BQ588349	Und.		0
BQ588529	Stress	Abiotic	7	BQ588529	Stress	Abiotic	6
BQ588562	Stress	Both	7	BQ588562	Stress	Both	3
BQ588646	Growth		7	BQ588646	Growth		1
BQ588706	Growth		0	BQ588706	Growth		0
BQ588709	Growth		1	BQ588709	Growth		6
BQ588744	Growth		6	BQ588744	Growth		6
BQ588748	Growth		6	BQ588748	Growth		6
BQ588784	Growth		0	BQ588784	Growth		1
BQ588826	Signaling		6	BQ588826	Signaling		6
BQ588862	Stress	Abiotic	6	BQ588862	Stress	Abiotic	6
BQ588870	Growth		0	BQ588870	Growth		1
BQ589141	Stress	Both	3	BQ589141	Stress	Both	3
BQ589141	Stress	Both	7	BQ589141	Stress	Both	0
BQ589354	Stress	Both	6	BQ589354	Stress	Both	6
BQ589455	Growth		7	BQ589455	Growth		6
BQ589671	**-**		2	**BQ589671**	**-**		2
BQ589734	Stress	Abiotic	3	BQ589734	Stress	Abiotic	5
BQ589925	**Stress**	Abiotic	2	**BQ589925**	**Stress**	Abiotic	4
BQ589960	Growth		0	BQ589960	Growth		0
BQ590125	**Stress**	Und.	6	**BQ590125**	**Stress**	Und.	6
BQ590158	Growth		6	BQ590158	Growth		5
BQ590382	Signaling		6	BQ590382	Signaling		6
BQ590743	Growth		0	BQ590743	Growth		6
BQ590906	Und.		7	BQ590906	Und.		0
BQ591201	Und.		0	BQ591201	Und.		6
BQ591253	Growth		6	BQ591253	Growth		6
BQ591613	Growth		0	BQ591613	Growth		0
BQ591669	Stress	Both	6	BQ591669	Stress	Both	6
BQ591669	**Stress**	Both	3	**BQ591669**	**Stress**	Both	6
BQ591700	Signaling		3	BQ591700	Signaling		3
BQ591856	Growth		6	BQ591856	Growth		6
BQ591910	Growth		0	BQ591910	Growth		0
BQ591929	Growth		6	BQ591929	Growth		3
BQ592039	Signaling		6	BQ592039	Signaling		6
BQ592168	**Stress**	Abiotic	2	**BQ592168**	**Stress**	Abiotic	2
BQ592234	Stress	Abiotic	7	BQ592234	Stress	Abiotic	6
BQ592254	Stress	Abiotic	3	BQ592254	Stress	Abiotic	0
BQ592254	**Stress**	Abiotic	6	**BQ592254**	**Stress**	Abiotic	4
BQ592267	Stress	Both	6	BQ592267	Stress	Both	6
BQ592267	**Stress**	Both	6	**BQ592267**	**Stress**	Both	6
BQ592312	Growth		6	BQ592312	Growth		1

Appendix Table 1.5 continued

BQ592313	Stress	Both	6	BQ592313	Stress	Both	6
BQ592393	Growth		0	BQ592393	Growth		3
BQ592405	Und.		3	BQ592405	Und.		6
BQ592726	Stress	Abiotic	6	BQ592726	Stress	Abiotic	6
BQ592936	**Growth**		6	**BQ592936**	**Growth**		6
BQ592954	Stress	Both	6	BQ592954	Stress	Both	6
BQ593076	Growth		6	BQ593076	Growth		0
BQ593155	Stress	Und.	6	BQ593155	Stress	Und.	6
BQ593157	Stress	Und.	6	BQ593157	Stress	Und.	6
BQ593209	Growth		1	BQ593209	Growth		1
BQ593316	Growth		6	BQ593316	Growth		1
BQ593362	Stress	Abiotic	6	BQ593362	Stress	Abiotic	6
BQ593448	Stress	Both	6	BQ593448	Stress	Both	0
BQ593574	Growth		6	BQ593574	Growth		6
BQ593588	Stress	Abiotic	2	BQ593588	Stress	Abiotic	2
BQ593603	Stress	Und.	6	BQ593603	Stress	Und.	6
BQ593732	Growth		3	BQ593732	Growth		0
BQ593897	Stress	Abiotic	2	BQ593897	Stress	Abiotic	5
BQ593921	Signaling		6	BQ593921	Signaling		6
BQ593922	Stress	Und.	6	BQ593922	Stress	Und.	6
BQ593937	Stress	Und.	6	BQ593937	Stress	Und.	6
BQ594117	Stress	Und.	6	BQ594117	Stress	Und.	1
BQ594123	Growth		6	BQ594123	Growth		6
BQ594267	Signaling		6	BQ594267	Signaling		6
BQ594284	Growth		6	BQ594284	Growth		6
BQ594412	**Stress**	Biotic	3	**BQ594412**	**Stress**	Biotic	1
BQ594432	Stress	Abiotic	6	BQ594432	Stress	Abiotic	6
BQ594558	**Stress**	Abiotic	7	**BQ594558**	**Stress**	Abiotic	3
BQ594578	**Growth**		7	**BQ594578**	**Growth**		6
BQ594715	Stress	Biotic	7	BQ594715	Stress	Biotic	3
BQ594725	Stress	Und.	7	BQ594725	Stress	Und.	0
BQ594736	**Stress**	Both	2	**BQ594736**	**Stress**	Both	5
BQ594788	Growth		7	BQ594788	Growth		3
BQ594810	**Stress**	Biotic	6	**BQ594810**	**Stress**	Biotic	6
BQ594875	Stress	Abiotic	3	BQ594875	Stress	Abiotic	5
BQ594919	Growth		6	BQ594919	Growth		6
BQ594995	Und.		2	BQ594995	Und.		0
BQ594997	Growth		0	BQ594997	Growth		1
BQ595049	Growth		0	BQ595049	Growth		0
BQ595152	Stress	Abiotic	2	BQ595152	Stress	Abiotic	1
BQ595152	Stress	Abiotic	2	BQ595152	Stress	Abiotic	2
BQ595231	Growth		6	BQ595231	Growth		6
BQ595269	Growth		1	BQ595269	Growth		1
BQ595328	Stress	Biotic	3	BQ595328	Stress	Biotic	4
BQ595355	Stress	Biotic	6	BQ595355	Stress	Biotic	5

Appendix Table 1.5 continued

BQ595434	Growth		0	BQ595434	Growth		5
BQ595543	**Growth**		0	**BQ595543**	**Growth**		0
BQ595697	Stress	Biotic	3	BQ595697	Stress	Biotic	6
BQ595702	Stress	Und.	6	BQ595702	Stress	Und.	6
BQ595738	Stress	Both	6	BQ595738	Stress	Both	6
BQ595738	**Stress**	Both	6	**BQ595738**	**Stress**	Both	6
BQ595856	Growth		3	BQ595856	Growth		1
BQ654409	Growth		1	BQ654409	Growth		5
BU089547	Stress	Und.	0	BU089547	Stress	Und.	5
BU089551	Signaling		6	BU089551	Signaling		6
BU089552	Stress	Und.	6	BU089552	Stress	Und.	6
BU089554	Stress	Und.	7	BU089554	Stress	Und.	1
BU089558	Signaling		3	BU089558	Signaling		1
BU089558	Signaling		0	BU089558	Signaling		5
BU089558	Signaling		6	BU089558	Signaling		5
BU089560	Growth		2	BU089560	Growth		6
BU089560	Growth		6	BU089560	Growth		4
BU089560	Growth		6	BU089560	Growth		5
BU089560	**Growth**		0	**BU089560**	**Growth**		6
BU089561	Stress	Und.	6	BU089561	Stress	Und.	6
BU089561	Stress	Und.	6	BU089561	Stress	Und.	6
BU089562	Stress	Und.	6	BU089562	Stress	Und.	6
BU089563	Signaling		6	BU089563	Signaling		0
BU089563	Signaling		0	BU089563	Signaling		6
BU089563	Signaling		6	BU089563	Signaling		0
BU089563	Signaling		0	BU089563	Signaling		4
BU089564	Signaling		6	BU089564	Signaling		6
BU089565	Stress	Und.	6	BU089565	Stress	Und.	6
BU089571	Stress	Biotic	6	BU089571	Stress	Biotic	6
CF430002	Growth		3	CF430002	Growth		6
CF542675	Stress	Und.	6	CF542675	Stress	Und.	4
CF542723	Growth		3	CF542723	Growth		1
CF542797	Growth		6	CF542797	Growth		6
CF542821	Growth		6	CF542821	Growth		3
CF542879	Signaling		6	CF542879	Signaling		6
CF542908	Growth		6	CF542908	Growth		6
CF542917	Und.		0	CF542917	Und.		5
CF543001	Stress	Und.	3	CF543001	Stress	Und.	3
CF543002	Stress	Und.	6	CF543002	Stress	Und.	6
CF543157	Signaling		0	CF543157	Signaling		1
CF543165	**Stress**	Abiotic	7	**CF543165**	**Stress**	Abiotic	1
CF543170	Signaling		3	CF543170	Signaling		6
CF543190	Stress	Both	6	CF543190	Stress	Both	6
CF543216	Growth		7	CF543216	Growth		3
CF543254	Stress	Und.	6	CF543254	Stress	Und.	6

Appendix Table 1.5 continued

CF543263	Growth		6	CF543263	Growth		6
CF543368	-		2	CF543368	-		2
CF543420	Growth		6	CF543420	Growth		6
CF543447	Growth		1	CF543447	Growth		5
CF543627	Growth		7	CF543627	Growth		5
CK136263	Growth		6	CK136263	Growth		0
CK136419	Stress	Abiotic	7	CK136419	Stress	Abiotic	5
CK136420	Stress	Abiotic	7	CK136420	Stress	Abiotic	3
CK136500	Signaling		6	CK136500	Signaling		6
CK136617	Stress	Abiotic	6	CK136617	Stress	Abiotic	1
CK136649	Stress	Both	7	CK136649	Stress	Both	3
CK136658	Stress	Abiotic	6	CK136658	Stress	Abiotic	6
CK136658	Stress	Abiotic	3	CK136658	Stress	Abiotic	5
CK136719	**Stress**	Abiotic	6	**CK136719**	**Stress**	Abiotic	1
CK136733	Growth		6	CK136733	Growth		6
CK136793	Stress	Abiotic	7	CK136793	Stress	Abiotic	1
CK136793	Stress	Abiotic	6	CK136793	Stress	Abiotic	2
CK136863	Stress	Abiotic	3	CK136863	Stress	Abiotic	3
CK136876	Stress	Abiotic	3	CK136876	Stress	Abiotic	3
CX779686	Und.		7	CX779686	Und.		1
CX779686	Und.		1	CX779686	Und.		4
DX811261	Und.		6	DX811261	Und.		6
ED032482	Und.		7	ED032482	Und.		4
ED032901	Und.		7	ED032901	Und.		4
EG551101	Growth		7	EG551101	Growth		6
EG551187	Growth		3	EG551187	Growth		4
EG551187	Growth		6	EG551187	Growth		6
EG552103	Stress	Biotic	6	EG552103	Stress	Biotic	6
EG552299	Stress	Und.	6	EG552299	Stress	Und.	1
EG552299	Stress	Und.	1	EG552299	Stress	Und.	6
EG552800	Growth		1	EG552800	Growth		6

* *Beta vulgaris* accession ** Putative function *+ Putative secondary function *** Undetermined function ***+ 18s rRNA

Appendix Table 1.6 K-means clustering of 343 putative stress (abiotic, biotic and both abiotic and biotic), growth and hormone related genes in SP7622 cDNA over the first 24 h of germination time points in H_2O and H_2O_2. Accessions in bold were also used for qPCR analyses.

SP7622							
H_2O				H_2O_2			
Bv* Accession	**Role 1****	**Role 2*+**	**Cluster**	***Bv* Accession***	**Role 1****	**Role 2*+**	**Cluster**
AW063023	Growth		2	AW063023	Growth		10
AW063034	Growth		1	AW063034	Growth		6
AW697779	Growth		2	AW697779	Growth		10
AW777170	Growth		6	AW777170	Growth		1
BE590301	Growth		6	BE590301	Growth		6
BE590301	Growth		6	BE590301	Growth		6
BE590328	Growth		3	BE590328	Growth		6
BE590397	Growth		6	BE590397	Growth		0
BE590397	Growth		6	BE590397	Growth		10
BE590397	Growth		9	BE590397	Growth		10
BE590444	Stress	Abiotic	2	BE590444	Stress	Abiotic	2
BE590444	Stress	Abiotic	8	BE590444	Stress	Abiotic	10
BF010998	Growth		0	BF010998	Growth		10
BF011036	Growth		2	BF011036	Growth		10
BF011057	Growth		6	BF011057	Growth		6
BF011057	Growth		6	BF011057	Growth		9
BF011062	Signaling		6	BF011062	Signaling		0
BF011062	**Signaling**		9	**BF011062**	**Signaling**		6
BF011089	Growth		0	BF011089	Growth		3
BF011122	Stress	Both	0	BF011122	Stress	Both	10
BF011211	Growth		6	BF011211	Growth		6
BF011227	Stress	Abiotic	2	BF011227	Stress	Abiotic	2
BG577441	Growth		2	BG577441	Growth		0
BI073121	Growth		6	BI073121	Growth		6
BI073128	**Stress**	Abiotic	1	**BI073128**	**Stress**	Abiotic	9
BI073146	Growth		6	BI073146	Growth		1
BI073146	Growth		6	BI073146	Growth		6
BI073176	Growth		6	BI073176	Growth		1
BI073235	Stress	Abiotic	2	BI073235	Stress	Abiotic	10
BI073250	Growth		0	BI073250	Growth		1
BI095934	Stress	Abiotic	6	BI095934	Stress	Abiotic	1
BI096011	Stress	Abiotic	6	BI096011	Stress	Abiotic	1
BI096038	Stress	Abiotic	6	BI096038	Stress	Abiotic	1
BI096046	Growth		6	BI096046	Growth		3
BI096111	Growth		0	BI096111	Growth		2
BI096145	Stress	Biotic	6	BI096145	Stress	Biotic	3

Appendix Table 1.6 continued

BI096176	Growth		8	BI096176	Growth		1
BI096176	Growth		1	BI096176	Growth		3
BI096180	Growth		6	BI096180	Growth		1
BI096232	Growth		0	BI096232	Growth		9
BI096237	Growth		3	BI096237	Growth		1
BI096304	Stress	Und.***	0	BI096304	Stress	Und.***	6
BI096344	Und.***		6	BI096344	Und.***		1
BI543239	Stress	Biotic	6	BI543239	Stress	Biotic	6
BI543265	Stress	Abiotic	6	BI543265	Stress	Abiotic	1
BI543278	Growth		6	BI543278	Growth		6
BI543285	Growth		2	BI543285	Growth		2
BI543316	Growth		1	BI543316	Growth		6
BI543360	Growth		6	BI543360	Growth		1
BI543377	Stress	Biotic	6	BI543377	Stress	Biotic	1
BI543386	Stress	Biotic	0	BI543386	Stress	Biotic	0
BI543415	Growth		6	BI543415	Growth		0
BI543460	Stress	Abiotic	0	BI543460	Stress	Abiotic	9
BI543526	Growth		3	BI543526	Growth		6
BI543568	-***+		2	BI543568	-***+		2
BI543569	-		2	BI543569	-		2
BI543685	Stress	Both	2	BI543685	Stress	Both	10
BI543739	Stress	Und.	6	BI543739	Stress	Und.	0
BI543772	Stress	Abiotic	2	BI543772	Stress	Abiotic	10
BI543889	-		2	BI543889	-		10
BI543937	Stress	Abiotic	6	BI543937	Stress	Abiotic	6
BI543980	Stress	Abiotic	0	BI543980	Stress	Abiotic	1
BI643062	-		2	BI643062	-		2
BI643098	Stress	Abiotic	6	BI643098	Stress	Abiotic	6
BI643161	-		2	BI643161	-		2
BQ060494	Stress	Abiotic	9	BQ060494	Stress	Abiotic	10
BQ060547	Signaling		9	BQ060547	Signaling		1
BQ060547	Signaling		6	BQ060547	Signaling		6
BQ060614	Signaling		8	BQ060614	Signaling		3
BQ060614	Signaling		1	BQ060614	Signaling		6
BQ487636	Growth		6	BQ487636	Growth		1
BQ487747	Growth		9	BQ487747	Growth		10
BQ487855	Stress	Both	3	BQ487855	Stress	Both	1
BQ487855	Stress	Both	6	BQ487855	Stress	Both	0
BQ487860	**Stress**	Both	1	**BQ487860**	**Stress**	Both	1
BQ487898	Und.		6	BQ487898	Und.		6
BQ487902	Stress	Both	6	BQ487902	Stress	Both	0
BQ487902	Stress	Both	0	BQ487902	Stress	Both	1
BQ487902	Stress	Both	6	BQ487902	Stress	Both	9
BQ487982	**Growth**		0	**BQ487982**	**Growth**		1

Appendix Table 1.6 continued

BQ488119	Growth		8	BQ488119	Growth		0
BQ488179	**Stress**	Biotic	3	**BQ488179**	**Stress**	Biotic	10
BQ488223	Stress	Abiotic	6	BQ488223	Stress	Abiotic	6
BQ488276	Stress	Both	6	BQ488276	Stress	Both	1
BQ488276	Stress	Both	8	BQ488276	Stress	Both	1
BQ488277	Growth		6	BQ488277	Growth		1
BQ488279	**Stress**	Abiotic	6	**BQ488279**	**Stress**	Abiotic	1
BQ488337	**Stress**	Both	6	**BQ488337**	**Stress**	Both	6
BQ488461	Stress	Abiotic	2	BQ488461	Stress	Abiotic	2
BQ488466	**Stress**	Abiotic	9	**BQ488466**	**Stress**	Abiotic	6
BQ488487	Signaling		6	BQ488487	Signaling		6
BQ488691	Stress	Biotic	6	BQ488691	Stress	Biotic	6
BQ488795	**Stress**	Abiotic	6	**BQ488795**	**Stress**	Abiotic	1
BQ488850	**Stress**	Abiotic	6	**BQ488850**	**Stress**	Abiotic	1
BQ488890	Growth		6	BQ488890	Growth		1
BQ488894	Stress	Both	9	BQ488894	Stress	Both	1
BQ488901	Growth		3	BQ488901	Growth		6
BQ488935	**Stress**	Biotic	6	**BQ488935**	**Stress**	Biotic	1
BQ489058	Signaling		6	BQ489058	Signaling		0
BQ489063	Signaling		0	BQ489063	Signaling		1
BQ489063	Signaling		6	BQ489063	Signaling		6
BQ489189	**Stress**	Both	2	**BQ489189**	**Stress**	Both	10
BQ489620	Growth		0	BQ489620	Growth		3
BQ489704	Stress	Abiotic	1	BQ489704	Stress	Abiotic	6
BQ489814	Stress	Abiotic	6	BQ489814	Stress	Abiotic	1
BQ489814	Stress	Abiotic	1	BQ489814	Stress	Abiotic	1
BQ489848	Signaling		2	BQ489848	Signaling		1
BQ489959	Growth		6	BQ489959	Growth		3
BQ490017	Stress	Abiotic	6	BQ490017	Stress	Abiotic	6
BQ490300	Growth		1	BQ490300	Growth		3
BQ490338	**Growth**		2	**BQ490338**	**Growth**		9
BQ490607	Stress	Abiotic	6	BQ490607	Stress	Abiotic	3
BQ499841	Und.		6	BQ499841	Und.		1
BQ582278	Stress	Both	9	BQ582278	Stress	Both	1
BQ582382	**Signaling**		9	**BQ582382**	**Signaling**		9
BQ582409	Stress	Und.	9	BQ582409	Stress	Und.	10
BQ582477	Growth		6	BQ582477	Growth		6
BQ582479	Stress	Biotic	9	BQ582479	Stress	Biotic	10
BQ582606	Stress	Biotic	6	BQ582606	Stress	Biotic	6
BQ582606	Stress	Biotic	6	BQ582606	Stress	Biotic	9
BQ582618	Stress	Und.	1	BQ582618	Stress	Und.	6
BQ582629	**Growth**		6	**BQ582629**	**Growth**		1
BQ582632	Growth		6	BQ582632	Growth		6
BQ582634	Und.		0	BQ582634	Und.		1
BQ582634	**Und.**		6	**BQ582634**	**Und.**		1

Appendix Table 1.6 continued

BQ582685	Growth		6	BQ582685	Growth		1
BQ582687	Stress	Abiotic	6	BQ582687	Stress	Abiotic	1
BQ582708	Growth		6	BQ582708	Growth		1
BQ582721	Stress	Abiotic	6	BQ582721	Stress	Abiotic	0
BQ582763	Growth		6	BQ582763	Growth		6
BQ582770	Stress	Abiotic	6	BQ582770	Stress	Abiotic	1
BQ582799	Growth		6	BQ582799	Growth		6
BQ582800	Stress	Both	6	BQ582800	Stress	Both	0
BQ582835	Stress	Abiotic	6	BQ582835	Stress	Abiotic	6
BQ582859	Growth		6	BQ582859	Growth		6
BQ582873	Stress	Und.	6	BQ582873	Stress	Und.	6
BQ583037	Und.		6	BQ583037	Und.		6
BQ583062	**Signaling**		6	**BQ583062**	**Signaling**		6
BQ583249	Stress	Both	6	BQ583249	Stress	Both	6
BQ583301	Growth		6	BQ583301	Growth		6
BQ583306	Stress	Both	6	BQ583306	Stress	Both	0
BQ583369	**Growth**		1	**BQ583369**	**Growth**		10
BQ583421	Stress	Biotic	9	BQ583421	Stress	Biotic	1
BQ583639	Stress	Both	0	BQ583639	Stress	Both	3
BQ583692	**Growth**		6	**BQ583692**	**Growth**		1
BQ583764	Growth		3	BQ583764	Growth		6
BQ583828	Und.		6	BQ583828	Und.		6
BQ583877	Signaling		6	BQ583877	Signaling		1
BQ584025	Growth		6	BQ584025	Growth		6
BQ584082	Stress	Abiotic	1	BQ584082	Stress	Abiotic	1
BQ584083	**Stress**	Abiotic	6	**BQ584083**	**Stress**	Abiotic	0
BQ584125	Growth		1	BQ584125	Growth		1
BQ584136	**Stress**	Biotic	2	BQ584136	Stress	Biotic	0
BQ584136	Stress	Biotic	9	**BQ584136**	**Stress**	Biotic	0
BQ584180	Growth		6	BQ584180	Growth		0
BQ584196	Stress	Abiotic	6	BQ584196	Stress	Abiotic	9
BQ584386	Stress	Biotic	9	BQ584386	Stress	Biotic	1
BQ584422	**Stress**	Biotic	1	**BQ584422**	**Stress**	Biotic	2
BQ584431	Growth		6	BQ584431	Growth		0
BQ584433	Stress	Biotic	6	BQ584433	Stress	Biotic	9
BQ584462	Growth		6	BQ584462	Growth		1
BQ584474	Growth		6	BQ584474	Growth		0
BQ584498	Stress	Abiotic	6	BQ584498	Stress	Abiotic	1
BQ584876	Stress	Und.	6	BQ584876	Stress	Und.	10
BQ584898	Growth		6	BQ584898	Growth		1
BQ584988	Stress	Both	6	BQ584988	Stress	Both	3
BQ585091	Signaling		6	BQ585091	Signaling		9
BQ585097	Stress	Biotic	6	BQ585097	Stress	Biotic	6
BQ585195	Growth		6	BQ585195	Growth		1
BQ585514	Growth		6	BQ585514	Growth		0

Appendix Table 1.6 continued

BQ585612	Stress	Abiotic	6	BQ585612	Stress	Abiotic	1
BQ585675	Stress	Both	6	BQ585675	Stress	Both	6
BQ585699	Growth		6	BQ585699	Growth		1
BQ585699	**Growth**		6	**BQ585699**	**Growth**		6
BQ585826	Stress	Biotic	6	BQ585826	Stress	Biotic	6
BQ585834	Growth		6	BQ585834	Growth		1
BQ585841	Stress	Und.	9	BQ585841	Stress	Und.	6
BQ585998	**Growth**		1	**BQ585998**	**Growth**		0
BQ585999	Stress	Und.	6	BQ585999	Stress	Und.	9
BQ586015	Stress	Abiotic	1	BQ586015	Stress	Abiotic	6
BQ586036	Growth		1	BQ586036	Growth		10
BQ586159	Growth		6	BQ586159	Growth		6
BQ586261	Stress	Abiotic	6	BQ586261	Stress	Abiotic	6
BQ586318	Growth		3	BQ586318	Growth		0
BQ586375	**Stress**	Biotic	3	**BQ586375**	**Stress**	Biotic	1
BQ586400	Stress	Biotic	2	BQ586400	Stress	Biotic	2
BQ586464	**Stress**	Und.	6	**BQ586464**	**Stress**	Und.	6
BQ586518	Growth		6	BQ586518	Growth		6
BQ586553	Und.		6	BQ586553	Und.		6
BQ586635	**Stress**	Abiotic	6	**BQ586635**	**Stress**	Abiotic	6
BQ586657	Growth		6	BQ586657	Growth		3
BQ586719	Growth		6	BQ586719	Growth		1
BQ586790	Growth		6	BQ586790	Growth		6
BQ586894	Stress	Abiotic	0	BQ586894	Stress	Abiotic	1
BQ586903	Growth		6	BQ586903	Growth		1
BQ586920	Stress	Biotic	0	BQ586920	Stress	Biotic	0
BQ586930	Growth		9	BQ586930	Growth		10
BQ586991	**Stress**	Abiotic	3	**BQ586991**	**Stress**	Abiotic	3
BQ587173	Growth		0	BQ587173	Growth		0
BQ587197	Stress	Both	6	BQ587197	Stress	Both	6
BQ587264	Growth		1	BQ587264	Growth		1
BQ587272	Growth		6	BQ587272	Growth		6
BQ587329	Growth		3	BQ587329	Growth		6
BQ587384	Growth		0	BQ587384	Growth		6
BQ587396	**Growth**		6	**BQ587396**	**Growth**		6
BQ587458	Growth		6	BQ587458	Growth		6
BQ587584	Stress	Abiotic	6	BQ587584	Stress	Abiotic	1
BQ587622	Stress	Both	1	BQ587622	Stress	Both	0
BQ587622	Stress	Both	3	BQ587622	Stress	Both	1
BQ587644	Growth		6	BQ587644	Growth		6
BQ587840	Stress	Both	6	BQ587840	Stress	Both	6
BQ587848	**Stress**	Both	6	**BQ587848**	**Stress**	Both	9
BQ587858	Stress	Abiotic	8	BQ587858	Stress	Abiotic	1
BQ587874	Stress	Und.	9	BQ587874	Stress	Und.	10
BQ587887	Stress	Und.	3	BQ587887	Stress	Und.	2

Appendix Table 1.6 continued

BQ588055	Stress	Und.	1	BQ588055	Stress	Und.	10
BQ588316	Growth		9	BQ588316	Growth		1
BQ588349	Und.		6	BQ588349	Und.		1
BQ588529	Stress	Abiotic	1	BQ588529	Stress	Abiotic	6
BQ588562	Stress	Both	3	BQ588562	Stress	Both	1
BQ588646	Growth		1	BQ588646	Growth		1
BQ588706	Growth		6	BQ588706	Growth		1
BQ588709	Growth		6	BQ588709	Growth		1
BQ588744	Growth		6	BQ588744	Growth		6
BQ588748	Growth		1	BQ588748	Growth		1
BQ588784	Growth		6	BQ588784	Growth		1
BQ588826	Signaling		6	BQ588826	Signaling		6
BQ588862	Stress	Abiotic	6	BQ588862	Stress	Abiotic	6
BQ588870	Growth		6	BQ588870	Growth		1
BQ589141	Stress	Both	3	BQ589141	Stress	Both	3
BQ589141	Stress	Both	1	BQ589141	Stress	Both	6
BQ589354	Stress	Both	6	BQ589354	Stress	Both	6
BQ589455	Growth		3	BQ589455	Growth		9
BQ589671	-		2	BQ589671	-		2
BQ589734	Stress	Abiotic	3	BQ589734	Stress	Abiotic	2
BQ589925	**Stress**	Abiotic	6	**BQ589925**	**Stress**	Abiotic	9
BQ589960	Growth		0	BQ589960	Growth		1
BQ590125	**Stress**	Und.	1	**BQ590125**	**Stress**	Und.	1
BQ590158	Growth		6	BQ590158	Growth		1
BQ590382	Signaling		6	BQ590382	Signaling		6
BQ590743	Growth		6	BQ590743	Growth		6
BQ590906	Und.		2	BQ590906	Und.		1
BQ591201	Und.		6	BQ591201	Und.		1
BQ591253	Growth		9	BQ591253	Growth		1
BQ591613	Growth		6	BQ591613	Growth		6
BQ591669	Stress	Both	6	BQ591669	Stress	Both	1
BQ591669	**Stress**	Both	6	**BQ591669**	**Stress**	Both	6
BQ591700	Signaling		6	BQ591700	Signaling		6
BQ591856	Growth		9	BQ591856	Growth		6
BQ591910	Growth		6	BQ591910	Growth		1
BQ591929	Growth		6	BQ591929	Growth		2
BQ592039	Signaling		6	BQ592039	Signaling		6
BQ592168	**Stress**	Abiotic	0	**BQ592168**	**Stress**	Abiotic	2
BQ592234	Stress	Abiotic	0	BQ592234	Stress	Abiotic	6
BQ592254	Stress	Abiotic	6	BQ592254	Stress	Abiotic	0
BQ592254	**Stress**	Abiotic	3	**BQ592254**	**Stress**	Abiotic	6
BQ592267	Stress	Both	6	BQ592267	Stress	Both	1
BQ592267	**Stress**	Both	6	**BQ592267**	**Stress**	Both	6
BQ592312	Growth		6	BQ592312	Growth		6
BQ592313	Stress	Both	6	BQ592313	Stress	Both	6

Appendix Table 1.6 continued

BQ592393	Growth		2	BQ592393	Growth		3
BQ592405	Und.		9	BQ592405	Und.		1
BQ592726	Stress	Abiotic	6	BQ592726	Stress	Abiotic	6
BQ592936	**Growth**		6	**BQ592936**	**Growth**		6
BQ592954	Stress	Both	6	BQ592954	Stress	Both	0
BQ593076	Growth		6	BQ593076	Growth		6
BQ593155	Stress	Und.	6	BQ593155	Stress	Und.	6
BQ593157	Stress	Und.	6	BQ593157	Stress	Und.	0
BQ593209	Growth		1	BQ593209	Growth		9
BQ593316	Growth		6	BQ593316	Growth		6
BQ593362	Stress	Abiotic	6	BQ593362	Stress	Abiotic	6
BQ593448	Stress	Both	6	BQ593448	Stress	Both	1
BQ593574	Growth		6	BQ593574	Growth		1
BQ593588	Stress	Abiotic	2	BQ593588	Stress	Abiotic	10
BQ593603	Stress	Und.	0	BQ593603	Stress	Und.	9
BQ593732	Growth		8	BQ593732	Growth		9
BQ593897	Stress	Abiotic	6	BQ593897	Stress	Abiotic	3
BQ593921	Signaling		6	BQ593921	Signaling		0
BQ593922	Stress	Und.	6	BQ593922	Stress	Und.	6
BQ593937	Stress	Und.	9	BQ593937	Stress	Und.	6
BQ594117	Stress	Und.	3	BQ594117	Stress	Und.	6
BQ594123	Growth		6	BQ594123	Growth		6
BQ594267	Signaling		1	BQ594267	Signaling		6
BQ594284	Growth		6	BQ594284	Growth		6
BQ594412	**Stress**	Biotic	8	**BQ594412**	**Stress**	Biotic	0
BQ594432	Stress	Abiotic	6	BQ594432	Stress	Abiotic	6
BQ594558	**Stress**	Abiotic	8	**BQ594558**	**Stress**	Abiotic	2
BQ594578	**Growth**		3	**BQ594578**	**Growth**		6
BQ594715	Stress	Biotic	6	BQ594715	Stress	Biotic	3
BQ594725	Stress	Und.	6	BQ594725	Stress	Und.	6
BQ594736	**Stress**	Both	8	**BQ594736**	**Stress**	Both	1
BQ594788	Growth		8	BQ594788	Growth		1
BQ594810	**Stress**	Biotic	1	**BQ594810**	**Stress**	Biotic	1
BQ594875	Stress	Abiotic	3	BQ594875	Stress	Abiotic	10
BQ594919	Growth		6	BQ594919	Growth		6
BQ594995	Und.		0	BQ594995	Und.		6
BQ594997	Growth		1	BQ594997	Growth		1
BQ595049	Growth		6	BQ595049	Growth		1
BQ595152	Stress	Abiotic	8	BQ595152	Stress	Abiotic	0
BQ595152	Stress	Abiotic	9	BQ595152	Stress	Abiotic	2
BQ595231	Growth		6	BQ595231	Growth		6
BQ595269	Growth		6	BQ595269	Growth		6
BQ595328	Stress	Biotic	3	BQ595328	Stress	Biotic	6
BQ595355	Stress	Biotic	6	BQ595355	Stress	Biotic	0
BQ595434	Growth		9	BQ595434	Growth		9

Appendix Table 1.6 continued

BQ595543	**Growth**		3	**BQ595543**	**Growth**		3
BQ595697	Stress	Biotic	6	BQ595697	Stress	Biotic	1
BQ595702	Stress	Und.	6	BQ595702	Stress	Und.	9
BQ595738	Stress	Both	6	BQ595738	Stress	Both	0
BQ595738	**Stress**	Both	6	**BQ595738**	**Stress**	Both	1
BQ595856	Growth		6	BQ595856	Growth		1
BQ654409	Growth		9	BQ654409	Growth		0
BU089547	Stress	Und.	6	BU089547	Stress	Und.	6
BU089551	Signaling		6	BU089551	Signaling		6
BU089552	Stress	Und.	6	BU089552	Stress	Und.	6
BU089554	Stress	Und.	6	BU089554	Stress	Und.	0
BU089558	Signaling		3	BU089558	Signaling		0
BU089558	Signaling		9	BU089558	Signaling		1
BU089558	Signaling		3	BU089558	Signaling		3
BU089560	Growth		0	BU089560	Growth		1
BU089560	Growth		6	BU089560	Growth		6
BU089560	Growth		6	BU089560	Growth		6
BU089560	**Growth**		6	**BU089560**	**Growth**		9
BU089561	Stress	Und.	6	BU089561	Stress	Und.	1
BU089561	Stress	Und.	6	BU089561	Stress	Und.	6
BU089562	Stress	Und.	6	BU089562	Stress	Und.	6
BU089563	Signaling		6	BU089563	Signaling		0
BU089563	Signaling		3	BU089563	Signaling		1
BU089563	Signaling		0	BU089563	Signaling		6
BU089563	Signaling		0	BU089563	Signaling		9
BU089564	Signaling		6	BU089564	Signaling		6
BU089565	Stress	Und.	6	BU089565	Stress	Und.	1
BU089571	Stress	Biotic	6	BU089571	Stress	Biotic	0
CF430002	Growth		6	CF430002	Growth		1
CF542675	Stress	Und.	6	CF542675	Stress	Und.	6
CF542723	Growth		1	CF542723	Growth		6
CF542797	Growth		6	CF542797	Growth		1
CF542821	Growth		6	CF542821	Growth		1
CF542879	Signaling		6	CF542879	Signaling		6
CF542908	Growth		6	CF542908	Growth		1
CF542917	Und.		6	CF542917	Und.		1
CF543001	Stress	Und.	8	CF543001	Stress	Und.	3
CF543002	Stress	Und.	6	CF543002	Stress	Und.	6
CF543157	Signaling		6	CF543157	Signaling		1
CF543165	**Stress**	Abiotic	1	**CF543165**	**Stress**	Abiotic	9
CF543170	Signaling		6	CF543170	Signaling		6
CF543190	Stress	Both	6	CF543190	Stress	Both	1
CF543216	Growth		0	CF543216	Growth		6
CF543254	Stress	Und.	6	CF543254	Stress	Und.	6
CF543263	Growth		6	CF543263	Growth		6

Appendix Table 1.6 continued

CF543368	-		2	CF543368	-		2
CF543420	Growth		6	CF543420	Growth		1
CF543447	Growth		6	CF543447	Growth		0
CF543627	Growth		6	CF543627	Growth		9
CK136263	Growth		6	CK136263	Growth		0
CK136419	Stress	Abiotic	2	CK136419	Stress	Abiotic	10
CK136420	Stress	Abiotic	2	CK136420	Stress	Abiotic	10
CK136500	Signaling		6	CK136500	Signaling		3
CK136617	Stress	Abiotic	6	CK136617	Stress	Abiotic	6
CK136649	Stress	Both	6	CK136649	Stress	Both	3
CK136658	Stress	Abiotic	3	CK136658	Stress	Abiotic	1
CK136658	Stress	Abiotic	6	CK136658	Stress	Abiotic	3
CK136719	**Stress**	Abiotic	6	**CK136719**	**Stress**	Abiotic	6
CK136733	Growth		3	CK136733	Growth		6
CK136793	Stress	Abiotic	2	CK136793	Stress	Abiotic	2
CK136793	Stress	Abiotic	8	CK136793	Stress	Abiotic	2
CK136863	Stress	Abiotic	8	CK136863	Stress	Abiotic	2
CK136876	Stress	Abiotic	9	CK136876	Stress	Abiotic	1
CX779686	Und.		2	CX779686	Und.		2
CX779686	Und.		8	CX779686	Und.		9
DX811261	Und.		6	DX811261	Und.		6
ED032482	Und.		8	ED032482	Und.		3
ED032901	Und.		0	ED032901	Und.		10
EG551101	Growth		6	EG551101	Growth		3
EG551187	Growth		6	EG551187	Growth		9
EG551187	Growth		0	EG551187	Growth		10
EG552103	Stress	Biotic	6	EG552103	Stress	Biotic	0
EG552299	Stress	Und.	6	EG552299	Stress	Und.	0
EG552299	Stress	Und.	9	EG552299	Stress	Und.	3
EG552800	Growth		6	EG552800	Growth		9

* *Beta vulgaris* accession ** Putative function *+ Putative secondary function *** Undetermined function ***+ 18s rRNA