Potato Plants under Drought, Heat, and Combined Drought-Heat Stress: Morpho-Physiological and Molecular Responses

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ABBREVIATIONS

ABA: abscisic acid
ANOVA: analysis of variance
cDNA: complementary deoxyribonucleic acid
CMS: cell membrane stability
Ct: cycle threshold
DREB: dehydration responsive element binding
EBN: endosperm balance number
ERF: ethylene response factor
FAO: Food and Agriculture Organization
gDNA: genomic DNA
HSFs: heat shock transcription factors
HSD: honest significance difference
HSP: heat shock protein
IPCC: Intergovernmental Panel on Climate Change
LAI: leaf area index
LL: leaf length
LW: leaf width
MS: Murashige and Skoog
NASA: National Aeronautics and Space Administration
nsLTP: non-specific lipid transfer protein
PCR: polymerase chain reaction
PDM: plant dry matter
PEG: polyethylene glycol
PH: plant height
PSII: photosystem II
QY: photosystem II quantum yield
RGR$_H$: relative growth rate of plant height
RNA: ribonucleic acid
ROS: reactive oxygen species
RR: response ratio

RT-qPCR: reverse transcription-quantitative PCR

RWC: relative water content

SL: stomata length

SPAD: soil plant analysis development

SPL: stomata pore length

SPW: stomata pore width

StCEL: *Solanum tuberosum* cold responsive ERF-like factor

SW: stomata width

WUE: water use efficiency

SUMMARY

Abiotic stress is the most limiting factor in crop yields, as it interferes with crop growth and development. Drought stress and heat stress are the major abiotic stresses hampering world food production. The intensity and distribution of drought and heat stress are becoming more severe with the present climate changes. The increasing average global temperature triggers the uprise of heat stress event, and the decreasing annual mean precipitation in some mid-latitude and sub-tropical regions leads to water deficit. Even more critical is when drought and heat stress occur together in nature. Potato (*Solanum tuberosum* L.) is the third most important food crop in the world. Although potatoes are grown worldwide over wide agro-climatic zones in various environments and seasons, potato plants require specific physiological conditions for growth and tuber production. High temperatures and water deficits have become the most serious constraints for potato production. Several research groups have examined the effects of drought stress or heat damage on potato, but few investigations of the effects of combined drought-heat stress have been reported. As an addition to the cultivated potato that is sensitive to abiotic stress, the genetic potato resources comprised of landraces and wild potato relatives (which could contribute to tolerance to a range of abiotic stress) have not been well studied.

Using diploid breeding lines and a tetraploid commercial potato, I studied how these potatoes respond to drought stress, to heat stress, and to a combination of drought and heat stress. The diploid breeding lines have various landraces and wild relatives' genetic backgrounds. The morphological and physiological responses of potatoes to PEG-induced drought stress, heat stress, and combined drought-heat stress are presented in Chapter 2. Abiotic stress-related traits were evaluated in a growth-room environment under non-stress and abiotic stress treatments, and the results demonstrated that all of the potato lines responded to the drought and combined drought-heat stress by reducing their plant height. In contrast, the potato lines' responses to the effect of heat stress on plant height differed; some heights increased while others decreased. The leaf size in all of the potato lines became

smaller under drought, heat stress, and combined drought-heat stress compared to the non-stress condition. The potato plants responded to the drought stress and combined drought-heat stress by increasing their chlorophyll content and decreasing their relative water content (RWC). A decrease in water content related to wilting symptoms was observed under drought stress and under combined drought-heat stress. The potato line L1 (84.194.30) showed the lowest level of wilting in all three types of abiotic stress, supported by a small RWC change compared to the control condition; L1 is thus considered relatively tolerant to abiotic stress.

Several plant transcription factors (TFs) and genes are induced by abiotic stress and have substantial roles in improving the abiotic stress tolerance of plants. My evaluation of the expression levels of the abiotic stress-related genes *StCEL, StTAS14, StnsLTP1,* and *StHSP70* under drought, heat, and combined drought-heat stress is discussed in Chapter 3. The gene expression levels were examined by a reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Samples were taken from potato plants under polyethylene glycol (PEG)-induced drought stress, heat stress, and combined drought-heat stress in a growth room. The results showed that the abiotic stresses induced various levels of the expressions of *StCEL, StTAS14, StnsLTP1,* and *StHSP70* in the potato plants. Generally, the combined drought-heat stress induced the highest expression levels of the tested abiotic stress-related genes. Under the different abiotic stresses, the potato lines showed different patterns of *StCEL* and *StnsLTP1* expression levels.

Chapter 4 provides a general discussion. The potato lines' different responses to each type of abiotic stress indicates that the potato lines have different levels of sensitivity or tolerance to each abiotic stress. The potato lines can thus be used in other studies and in abiotic stress breeding programs. The simple abiotic stress-related traits described herein could be used to reliably differentiate stress-tolerant and stress-susceptible potato lines. The application of these traits will be helpful in screenings that involve a large number of accessions. The present findings also indicate that the expression levels of *StCEL, StTAS14, StnsLTP1,* and *StHSP70* might be associated with different sensitivity or tolerance of potato lines in response

to abiotic stress. More extensive investigations are thus necessary to further clarify the roles of these genes, particularly under combined drought-heat stress. The correlations between gene expression level and abiotic stress-related traits can be a consideration in the utilization the genes or the traits over diploid potato lines in future studies in 4x × 2x crosses to transfer genetic attributes.

Chapter 1: General introduction

1.1. Abiotic stress and climate change

The climate change that we are now experiencing threatens all aspects of human life, including food security. Climate change directly influences the increase of Earth's surface temperatures, irregular and unpredictable precipitation, and drought in arid and semi-arid areas (Andjelkovic 2018; Trenberth 2005). Compared to the middle of the 20th century, the global temperature in 2016 was warmer by 0.99°C (NASA 2018). Relative to the end of the 20th century, the global mean surface temperature is projected to increase by 1.0°–3.7°C by the late 21st century (IPCC 2014). These projections also showed that an increase will occur in the annual mean rainfall at high-latitude areas, the equatorial Pacific, and some mid-latitude wet areas, whereas the annual mean precipitation will decrease in some other mid-latitude areas and dry subtropical regions by the end of this century (IPCC 2014).

Plants suffer from abiotic stress when they are exposed to unusual or variable environmental conditions, which may negatively affect the plants' growth and yields (Cramer *et al.* 2011). An unfavorable environment could high temperature, low temperature, water deficit, salinity, or metal toxicity. High temperature (heat) and drought are the most important abiotic stresses related to climate change. An increase in the mean global temperature will trigger an increase in heat stress events and decreasing annual mean precipitation will lead to water deficits in some mid-latitude and subtropical regions (IPCC 2014).

1.1.1. Drought stress

One of the profound effects of climate change is drought. Drought is identical to a lack of water/dry conditions. There are four types of drought based on the disciplinary perspectives: meteorological, agricultural, hydrologic, and socio-economic droughts (Wilhite and Glantz 1985). Precipitation is the main factor in those drought types. The differences in the drought types are based on how their impacts are manifested and felt. The present study focused on agricultural drought, which is defined as a condition in which the availability of soil moisture to plants is below the average level and cannot support the growth of crops. This lack of soil moisture ultimately affects plant yields adversely (Mannocchi *et al.* 2004). Compared to the vegetative stage of plants, the reproductive stage is more sensitive to abiotic stress, thus resulting in yield losses (Barnabas *et al.* 2008; Sehgal *et al.* 2018). The yield decrease depends on the species. For example, a 20.7% yield decrease in wheat and a 39.3% decrease in maize were the results of a 40% water reduction (Daryanto *et al.* 2016).

1.1.2. Heat stress

Increasing global temperatures due to climate change increase the intensity and expansion of heat stress, particularly in the tropical and subtropical regions. Heat stress is a condition in which the temperature — either the air or soil temperature — rises beyond the temperature required for optimum growth, thus adversely affecting plant growth and development (Lamaoui *et al.* 2018). All tissues in a plant are affected by high temperature, but the plant's reproductive tissues are the most susceptible (Lamaoui *et al.* 2018; Zinn *et al.* 2010). Another important feature of plants that contributes to crop yields and is sensitive to heat stress is the photosynthetic apparatus (Mathur *et al.* 2014). With the use of a simulation model, Asseng *et al.* (2014) predicted that the global wheat production would decrease up to 6% for each degree (Celsius) of elevated temperature.

1.1.3. Combinations of drought and heat stress

Drought stress and heat stress often co-occur in nature. These two abiotic stresses can even induce each other. The exposure of plants to a high temperature can stimulate drought stress since it increases the plants' evapotranspiration. Drought stress can also increase a plant's temperature. The stress combination provided by drought and heat stress together must be considered, since the effect of each single stress may be different; moreover, when the combined effect occurs, it will be more detrimental (Dreesen *et al.* 2012; Sehgal *et al.* 2018). In wheat, single-stress heat and drought negatively affected the harvest index, spikelet fertility, grain number, and yield, and the effects become more severe when drought was combined with heat stress (Mahrookashani *et al.* 2017).

1.2. Plants' adaptation to abiotic stress

Three mechanisms related to water-deficit stress are commonly observed in the higher plants: escape, avoidance, and tolerance. A plant can escape from stress if it can complete its full life cycle before a water deficit occurs (De Micco and Aronne 2012). In wheat, a short vegetative stage followed by early flowering and maturing is the drought-escape strategy that is expected to improve the yield (Shavrukov *et al.* 2017). Drought avoidance is the ability of a plant to maintain a higher water content in its tissues when the soil's water level is reduced. Drought avoidance can be performed via two strategies: (1) by minimizing the water loss (water saving) by reducing transpiration, and (2) by optimizing the water uptake (water spending) by increasing the rooting system (Basu *et al.* 2016). Drought tolerance is the use of adaptive traits to endure a low tissue water content, and drought tolerance traits include the maintenance of the plant's cell turgor by adjusting the osmotic potential and increasing the protoplasmic resistance (Basu *et al.* 2016).

Avoidance and tolerance strategies are also observed in plants under heat stress. Heat stress avoidance is the ability to survive in a high-temperature condition by short-term acclimation mechanisms, i.e., leaf orientation, transpiration, and alteration of the membrane lipid composition (Hasanuzzaman *et al.* 2013). Morphological and phenological adaptations are critical aspects of the avoidance mechanisms against abiotic stress. Heat tolerance is closely related to both a plant's ability to grow and to the economic yield of the plants under heat stress (Hasanuzzaman *et al.* 2013). Plants perform a heat-tolerance adaptive strategy by expressing heat shock proteins (HSPs), which are involved in the transduction of stress signals, the protection and repair of damaged proteins and membranes, and the protection of the photosynthesis process (Asthir 2015b).

Morphological and phenological adaptations are essential aspects of the avoidance mechanisms against abiotic stress (Hasanuzzaman *et al.* 2013). Plants' adaptation to abiotic stress occurs not only at the morphological and phenological levels but also at the physiological and molecular levels. To survive, plants have developed a holistic adaptation mechanism. The plant adaptation strategies for dealing with combinations of abiotic stresses consist of shared and unique responses (Pandey *et al.* 2015). Shared responses are the responses that are common to two different abiotic stresses, whereas a unique response is specific to the stress combination and different from the response to each single stress (Rizhsky *et al.* 2002; 2004).

1.3. Potatoes and abiotic stress

1.3.1. The importance of potatoes

Potato (*Solanum tuberosum* L.) is one of the most important food crops in the world, ranked three after wheat and rice (Figure 1.1). In 2017, the harvesting area of potato exceeded 19 million hectares, with the production of >388 million tons (FAO 2019). Potatoes are consumed mainly as fresh food, but potatoes also provide raw materials for food processing (e.g., potato chips, French fries) and for specific industries (e.g., starch, ethanol) (Birch *et al.* 2012; Watanabe 2015). Potatoes contribute to many populations' food security and are ideal for the human diet because potato tubers are high-carbohydrate and low-fat, and they provide balanced nutritional value with vitamins and minerals (Birch *et al.* 2012; White *et al.* 2009).

Cultivated potatoes originated from the highlands of the Andes in South America (Hawkes 1994), which are characterized by a cool temperate climate and a short photoperiod. This crop is grown across wide agro-climatic zones around the world, in a variety of environments and seasons. Today's distribution of potato plants covers almost all of the world, from 47°S to 65°N, but 90% of the production of potatoes is concentrated between 22°N and 59°N (Figure 1.2) (Hijmans 2001). In tropical areas such as Southeast Asia, potatoes are grown in the highlands.

1.3.2. The genetic diversity of potatoes

Most of the commercial potatoes are abiotic stress-sensitive and show narrow genetic variation. Other resources must therefore be identified and explored toward the goal of determining stress-tolerance attributes that will improve the traits of cultivated potatoes against abiotic stresses; such resources include exotic cultivated potatoes, landraces, and wild relatives of potato (Figure 1.3). The wild relatives are more often exploited for biotic (pest and diseases) stress resistance than for abiotic stress (Jansky *et al.* 2013; Prohens *et al.* 2017). However, these types of genetic resources also provide abiotic tolerance (Table 1).

Solanum acaule and *S. demissum* have multiple types of tolerance that can be used for combined abiotic stress-tolerant breeding (Arvin and Donnelly 2008). However, the incorporation of desired traits from wild relatives into cultivated varieties by conventional methods is hampered by genetic barriers, i.e., differences in the ploidy level and in the endosperm balance number (EBN) (Jackson and Hanneman 1999; Johnston and Hanneman 1982; Novy and Hanneman 1991). To overcome these barriers, researchers have used ploidy manipulation, somatic fusion, and bridge-crossing strategies (Bidani *et al.* 2007; Jansky 2006; Jansky and Hamernik 2009).

As described by Bradeen and Haynes (2017), the primary genepool of potato is composed of cultivated potatoes and landraces (2n=4x=48, 4EBN), and breeders can use these resources directly. Wild relatives in the secondary genepool (2n=2x=24, 2EBN, 2n=4x=4EBN) can be crossed sexually with cultivated potato with some manipulation. The tertiary genepool consists of wild *Solanum* species (2n=2x=24, 1EBN) which are sexually isolated from cultivated potato and need a specific strategy for the incorporation of the target traits into cultivated potato (Watanabe 2015). Such a strategy could be cross-bridging (Yermishin *et al.* 2014, 2017), somatic hybridization (Symda *et al.* 2013), the use of 2n gametes (Ortiz *et al.* 1997; Watanabe *et al.* 1992), or gene transformation (Oosumi *et al.* 2009; Song *et al.* 2003).

1.3.3. The sensitivity of potatoes to abiotic stress

Although potato crops are grown under various environmental and climatic conditions, potato plants do experience environmental stresses. Water stress (drought and flooding), extreme temperature (low and high), and ion toxicity (salinity and heavy metal) are the abiotic constraints that are faced by plants (including potatoes) in their habitats (Bohnert 2007). In a potato crop, the optimum temperature for vegetative growth is 24°C, but the maximum total biomass would be produced at 20°C, as would the maximum final tuber yield (Fleisher *et al.* 2006; Timlin *et al.* 2006). Potatoes are highly sensitive to high temperatures, which is an obstacle in tropical and sub-tropical areas. Potato plants that are exposed to high temperature at an earlier time point achieve a lesser tuber yield than those with late exposure to high temperature, due to the delay in tuber initiation and the shortened bulking duration, as well as a low net assimilation rate (Aien *et al.* 2016).

Potatoes are known to be efficient water-use crops and to produce more food per unit of water than other main crops (Vos and Haverkort 2007). However, potatoes are extremely sensitive to a water deficit; this is related to the shallow and low density of the root architecture of this crop species (Wishart *et al.* 2014; Yamaguchi and Tanaka 1990). A deficit of water negatively affects plant growth and tuber yield and quality (Aliche *et al.* 2018; Soltys-Kalina *et al.* 2016). The effects of heat stress and drought stress are summarized in Figure 1.4.

As noted, drought and heat stress can occur simultaneously in nature. The combination of a drought and a heat wave creates huge losses in the agricultural sector (Mittler 2006). Globally, climate change was predicted results in 18%–32% and 9%–18% decreases in the potato yields without and with adaptation, respectively (Hijmans 2003). Due to climate change, rain-fed areas of potato cultivation in England and Wales are predicted to decline by 74%–95% by the 2050s (Daccache *et al.* 2012). The consequences are decreasing potato production or changes the potato production areas to the irrigated fields, which will compete for the water supply with other sectors such as direct human consumption.

1.4. Abiotic stress-related traits in potato

The effects of abiotic stress on plant morphology and physiology (i.e., phenotypes) can be observed visually and directly. Some of these plant features are correlated with abiotic stress tolerance (Table 2). The leaves are the part of the plant that are most directly affected by high temperature (Berry and Bjorkman 1980), and thus the photosynthesis process and some of its apparatus could be used to assist the process of heat-tolerance breeding. However, for the development of heat-tolerant potatoes, at least three physiological processes need to be considered: photosynthetic efficiency and haulm growth, tuber initiation, and photosynthate partitioning (Vayda 1993).

Three factors must also be considered when attempting to develop drought-stress tolerance in plants: the cell membrane's stability, the photosynthesis system, and the root system (Farooq *et al.* 2009). The integrity of the cell membrane ensures that cellular activities proceed optimally. Photosynthesis is correlated with a plant's growth and yield. A plant's cell membranes are involved in many important roles related to abiotic stresses, such as in the protection barrier, sensor, and transduction of various external signals, and in the activation of mechanisms to maintain cell homeostasis (Barkla and Pantoja 2011). As shown in Table 1, cell membrane stability enhances the abiotic stress tolerance as well as the photosynthetic activity in some landraces and wild types of potato. The root architecture of plants is related to drought tolerance (Khan *et al.* 2016; Koevoets *et al.* 2016) since the root depth contributes positively to drought tolerance (Lahlou and Ledent 2005; Zarzyńska *et al.* 2017). It is also known that water-deficit stress tolerance is associated with high water use efficiency (WUE), which is regulated by a low transpiration rate; this implies low stomatal conductance (Blum 2005; Levy *et al.* 2013; Li *et al.* 2017).

It is important to determine the main traits related to stress with high variability, heritability and genetic advance under stress conditions to assist the breeding steps to get the stable tolerance (Benavides *et al.* 2017; Luthra *et al.* 2013). However, because there is a genotype × environment (G×E) interaction for several desirable traits, we should consider conducting the selection steps at different times and different locations (Benites and Pinto 2011; Gautney and Haynes 1983). In chili pepper, membrane thermostability has high heritability and genetic advance values and has a positive genetic correlation with yield (Usman *et al.* 2014).

The abiotic stress-related traits can be used as criteria for selection, but only when considering a single stress. Working with combined abiotic stresses is more complex. Each response to combined abiotic stresses is unique and different from the response to each individual stress (Mittler 2006; Pandey *et al.* 2015; Shaar-

Moshe *et al.* 2017; Zandalinas *et al.* 2018). Basic information on how potato plants respond to combined stress is needed. The identification of traits related to combined drought and heat stress is the first step in decision-making on how to alleviate the effects of stress on potatoes, and this identification could be accomplished by cultivation techniques and by genetic modifications in breeding research.

1.5. General objective

The general objective of this study was to determine the responses of potato plants under abiotic stress (drought, heat, and combined drought-heat) by using several potato lines with different genetic backgrounds.

Table 1.1. Genetic resources for abiotic stress in landraces and wild species of potato

Abiotic stress	Source (Ploidy, EBN level)	Reference	Tolerance Trait(s)
Heat	*S. commersonii* (2x, 1EBN), *S. demissum* (6x, 4 EBN)	Arvin and Donnelly (2008)	Membrane stability
	S. juzepczukii (3x)	Havaux (1995)	High PS II activity
	S. gandarillasii cardenas (2x, 2 EBN)	Coleman (2008)	Membrane stability
	S. chacoense (2x, 2EBN), *S. bulbocastanum* (2x, 1 EBN), *S. demissum* (6x, 4EBN), and *S. stoloniferum* (4x, 2 EBN)	Reynolds and Ewing (1989a)	Shoot growth and tuberization ability
	S. acaule (4x, 2EBN) *and* *S. circaefolium* (2x, 1 EBN)	Midmore and Prange (1991)	High dry matter content
	S. phureja (2x, 2EBN)	Hetherington *et al.* (1983)	High chlorophyll fluorescence
Drought	*S. juzepczukii* (3x)	Vacher (1998)	Stomatal tolerance and high net photosynthesis
	S. gandarillasii cardenas (2x)	Coleman (2008)	Water use efficiency
	S. acaule (4x, 2 EBN)	Arvin and Donnelly (2008)	Membrane stability
	S. chillonanum (2x), *S. jamesii* (2x, 1 EBN), and *S. okadae* (2x)	Watanabe *et al.* (2011)	Rooting system

Table 1.2. Physiological and morphological traits associated with abiotic stress tolerance

Abiotic stress	**Target trait(s)**	**Reference(s)**
Heat	High net photosynthesis	Dou *et al.* (2014), Reynolds *et al.* (1990), Wolf *et al.* (1990)
	High stomatal conductance	Reynolds *et al.* (1990), Wolf *et al.* (1990)
Drought	Low stomatal conductance	Moon *et al.* (2015)
	Low transpiration rate High WUE	Coleman (2008)
	High cell membrane stability	Rudack *et al.* (2017)
	Stay green	Ramírez *et al.* (2014), Rolando *et al.* (2015), Schafleitner *et al.* (2007a)
	High root mass system	Ahmadi *et al.* (2017), Iwama (2008), Wishart *et al.* (2014)
	High leaf area index	Iwama (2008), Romero *et al.* (2017)
	High biomass	Schafleitner *et al.* (2007a)
	High photosynthesis per leaf area unit	Romero *et al.* (2017)

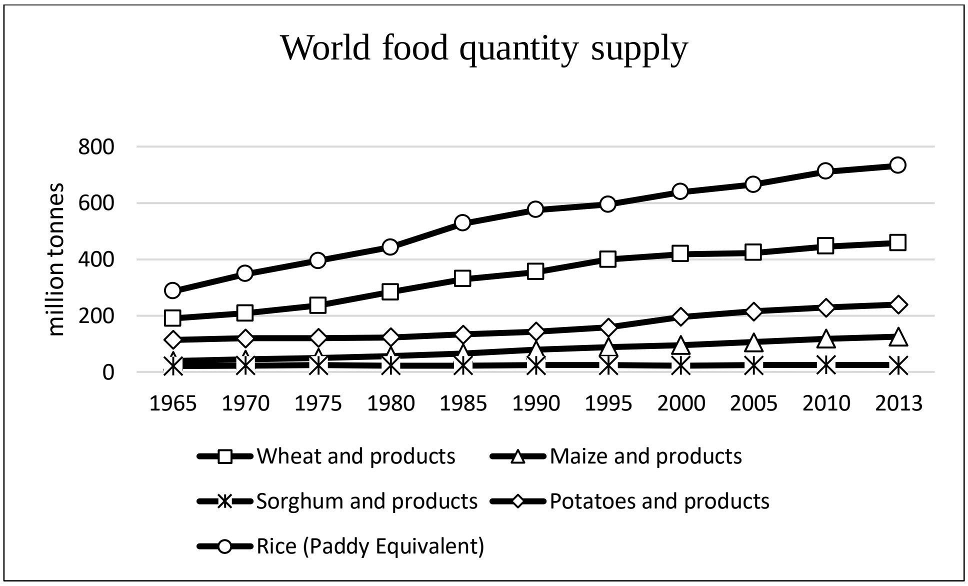


Figure 1.1. World food quantity supply.

Potatoes are the third most important food crop in the world based on food quantity supply, after rice and wheat (Data source: FAO 2019).

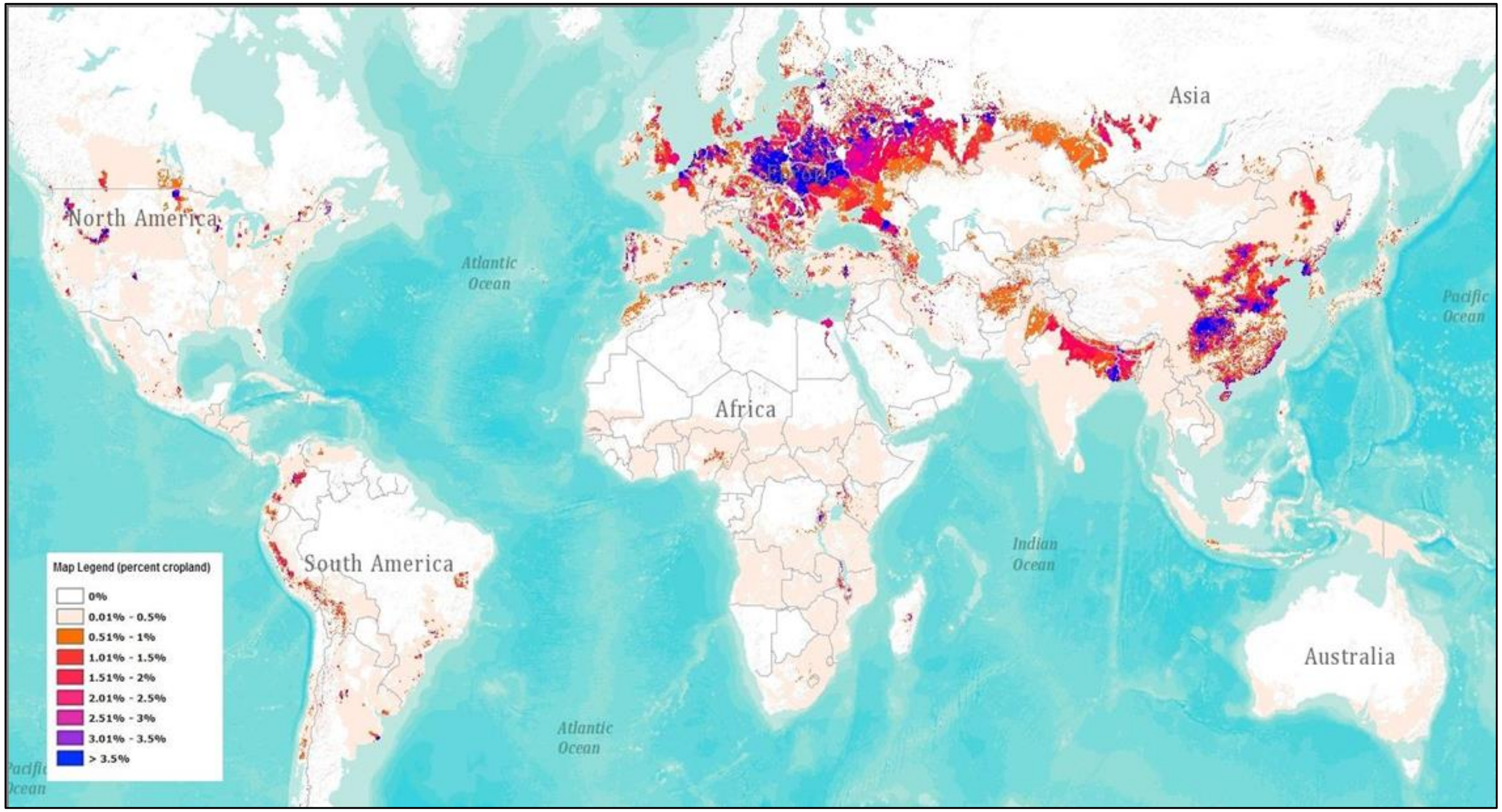


Figure 1.2. Distribution of potato harvest area.

Potatoes are cultivated all over the world, with concentrations between latitude 22°N and 59°N, which covers India, China, Russia, and Europe (Map source: https://www.rtb.cgiar.org/RTPMaps/).

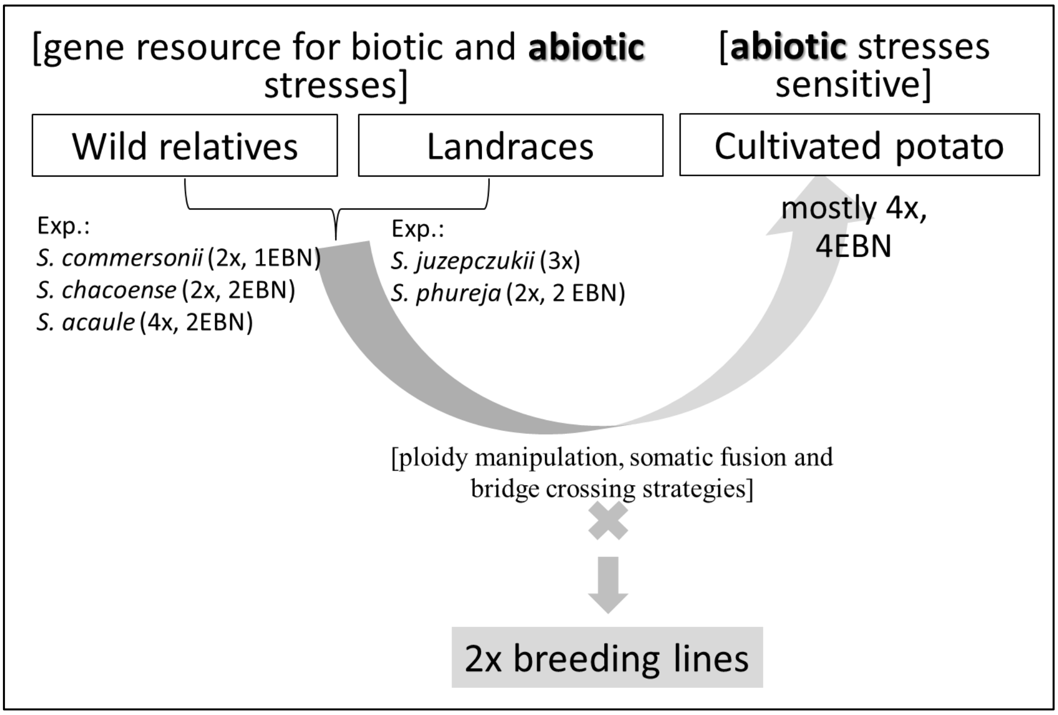
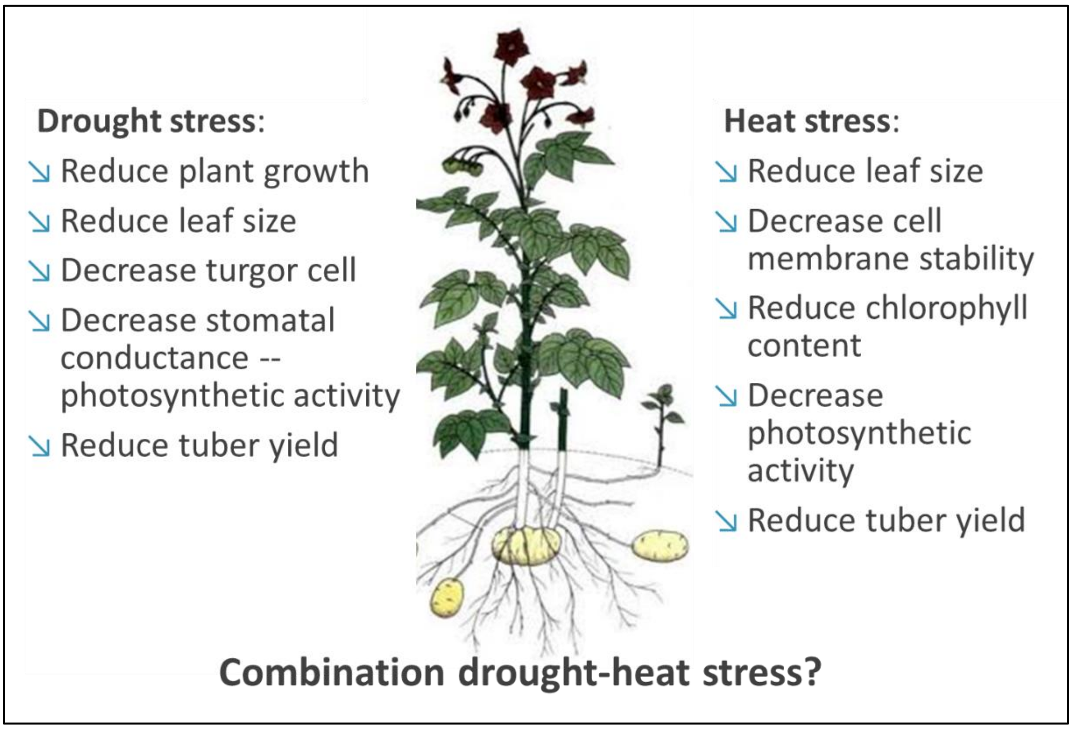


Figure 1.3. Genetic resources of potatoes and their uses in abiotic stress tolerance breeding.

Cultivated potatoes are generally sensitive to abiotic stress. Other potato genetic resources (e.g., wild relatives and landraces) are known be resistant to biotic and abiotic stressors. Since there are genetic barriers among them, the incorporation of desired traits from wild relatives and landraces into cultivated potatoes has been achieved by various techniques (e.g., ploidy manipulation, somatic fusion, and bridge-crossing strategies). The diploid (2x) breeding of lines with various genetic backgrounds thought to contain abiotic stress-tolerant traits as well as biotic stress tolerance has been conducted.



Drought stress:

- ↘ Reduce plant growth
- ↘ Reduce leaf size
- ↘ Decrease turgor cell
- ↘ Decrease stomatal conductance -- photosynthetic activity
- ↘ Reduce tuber yield

Heat stress:

- ↘ Reduce leaf size
- ↘ Decrease cell membrane stability
- ↘ Reduce chlorophyll content
- ↘ Decrease photosynthetic activity
- ↘ Reduce tuber yield

Combination drought-heat stress?

Figure 1.4. The effects of drought stress and heat stress on potato plants.

The effect of drought stress and heat stress have been studied robustly but based on the individual stresses. However, the effects of drought and heat stress in combination on potato plants have not been established (Figure source: https://cipotato.org/crops/potato/how-potato-grows/).

Chapter 2: Morphological and physiological responses of potato plants to abiotic stress: Drought, heat and combined drought-heat

2.1. Introduction

There are many definitions of abiotic stress that all of them refer to non-living factors in the environment. These factors can lead to reduced plant growth and yields if the factors' existence is below or exceeding plant requirements (Bohnert 2007; Cramer *et al.* 2011; Zhu 2016). Among the abiotic factors, a drought that brings water deficit stress and high temperatures that lead to heat stress are the most important factors related to climate change and food production (Fahad *et al.* 2017). Unfortunately, in nature, abiotic stress comes not only as a single stress but also in combinations. Such combinations can be based on the simultaneous occurrence of two or more stresses, or one stress (the primary stress) that induces another (the secondary stress). Potato plants are a food crop that requires specific environmental conditions for optimum growth, and abiotic stress exerts various negative effects on potato plants. As adaptation to environments with abiotic stress, potato plants have shown a variety of responses.

2.1.1. The effect of drought stress on potato plants

A wide range of drought stress effects on potato plants has been studied. A terminal effect of drought stress is a decrease in the tuber yield caused by a disturbance of agro-physiological mechanisms (Lahlou *et al.* 2003; Obidiegwu *et al.* 2015). Drought stress decreases the development of cells, which reduces the plant size, inhibits growth (Albiski *et al.* 2012; Deblonde and Ledent 2001), induces stomatal closure, and reduces the efficiency of photosynthesis (Schapendonk *et al.* 1989). The relative water content (RWC), an indicator of a plant's water status, is also decreased by drought stress (Anithakumari *et al.* 2012; Soltys-Kalina *et al.* 2016).

2.1.2. The effects of heat stress on potato plants

High temperatures inhibit the tuberization of potatoes (Ewing 1981; Reynolds and Ewing 1989b) and limit the translocation of carbohydrate from the source (leaf) to the sink (tuber) (Ghosh *et al.* 2000; Lafta and Lorenzen 1995), leading to both poor quality and poor quantity of the tuber yield (Rykaczewska 2015; 2017). As the does drought stress, heat stress alters some of the morphology and physiological traits of plants. Generally, plant growth is suppressed by heat stress (Lafta and Lorenzen 1995). Heat stress more negatively influences tuberization and tuber development compared to the growth of the haulm, and this is reflected by an increase in the leaf dry matter and a decrease in the tuber dry matter (Fleisher *et al.* 2006; Timlin *et al.* 2006). Chlorophyll reduction was reported in response to heat stress, along with photosynthesis inhibition (Aien *et al.* 2011; Reynolds *et al.* 1990).

2.1.3. The effects of combined drought-heat stress on potato plants

In light of plants' unique and responses to individual stresses (Mittler 2006), close attention should be paid to the combination of abiotic stress that often occur in nature (Mittler 2006; Vickers *et al.* 2009). The potato yield in Ontario was reported to be decreased by 35%–50% due to a combination of heat and drought in the summer of 2016 (Banks and VanOostrum 2016). Few studies of the combined effects of drought-heat stress on potato plants have been conducted, and the information about these effects is limited.

2.1.4. The adaptation of potato plants to abiotic stress: Morphological and physiological aspects

Potato plants develop various strategies to adapt to abiotic stress. Morphological and physiological aspects should be considered in strategies to maintain the water potential in the plant cells. Under water-deficit conditions, the water loss (by transpiration) and the leaf water status in plants can be counteracted by reducing the leaf number and leaf size and closing the stomata (Cutler *et al.* 1977). An increase in the chlorophyll content occurs on potato leaves in response to drought stress (Ramírez *et al.* 2014; Rolando *et al.* 2015; Romero *et al.* 2017; Rudack *et al.* 2017).

Variation among potato plants' responses to abiotic stress has been observed in a range of potato genotypes, including cultivars, landraces, and breeding lines (Arvin and Donnelly 2008). It brings consequences on the different strategies to help potato plants to cope with abiotic stress in order to maintain high yields under stress conditions.

2.2. Hypothesis and Objective

The hypothesis on which the following experiment was based is that combined drought-heat stress would have greater effects on potato plants in terms of morphology and physiology than the individual drought stress or heat stress. The experiment was thus conducted to evaluate the responses of potato plants' morphological and physiological traits to both individual and simultaneous heat and drought stress.

2.3. Materials and Methods

2.3.1. Genetic and plant materials

Four diploid potato breeding lines and one tetraploid potato cultivar ('Desiree') provided by the Gene Research Center (GRC), University of Tsukuba, as *in vitro* plants (plantlets) were used as the initial plant material (Table 2.1) (Watanabe *et al.* 1994). The diploid potato breeding lines used have varying genetic backgrounds that cover wild and landrace germplasm in addition to cultivated potato. These lines were developed for pest and disease resistance (Watanabe *et al.* 1996, 1999), but the wild and landrace germplasm in these lines is also known to tolerate abiotic stress (Chapter 1). The use of these lines thus provides a reasonable foundation for abiotic stress evaluations.

In order to have enough plant material for the experiment, propagation was done in two steps. First, plantlets were micro-propagated in Murashige and Skoog (MS) medium with 3% sucrose and solidified with 1% agarose. Shoots and node cuttings were transplanted into 20-ml test tubes containing 5 ml of MS medium. Incubation was performed in a cultivation room under a 16/8-hr photoperiod at 25±1°C. After 30 days, the plantlets were transferred into the growth room for the second step of propagation. Before propagation, the plantlets were acclimatized by transplanting into 10-cm-dia. plastic pots with media granulated soil : sterilized peat moss : perlite : vermiculite (9 : 1 : 1 : 1) and covered by transparent plastic bag for 1 week to maintain the moisture. The plants were then allowed to grow without the plastic cover. The plants were ready for propagation by shoot cutting within 2–3 weeks. The shoots were dipped into root growth hormone and planted in a cell tray. The medium used for shoot propagation was the same as that used for acclimatization. Shoot cutting propagation was conducted every week until a sufficient number of rooted plants for the experiment was obtained. The plants were grown at 24±1°C with a 12/12-hr photoperiod.

2.3.2. Plant growth and treatment

After propagation in the growth room, the 3-week rooted plants were transferred to quadrangular pots. The medium used in this step was the same as that used for the propagation steps. The plants were grown at 24±1°C with a 12/12-hr photoperiod. Watering with Hoagland solution was done every 2 days. After 10 days, abiotic stress was applied to ten plants of each potato line in each of the following conditions:

1. Non-stress (C): Watered with Hoagland solution
2. PEG-induced drought stress (D): Watered with Hoagland solution + PEG 8,000 0.39 g g^{-1} H_2O (Huynh 2013; Michel 1983)
3. Heat stress (H): Watered with Hoagland solution, temperature 30±1°C
4. Combined PEG-induced drought and heat stress (DH): Watered with Hoagland solution + PEG 8,000 0.39 g g^{-1} H_2O and temperature 30±1°C

The treatments were arranged in a randomized block design with a split plot arrangement in six replications. Abiotic stresses were used for the main plot, and the subplot was the potato lines. This abiotic stress treatment was performed for 21 days, followed by recovery (as a non-stress condition) for 9 days (Huynh 2013).

2.3.3. Observation and measurements

As stated in the Introduction in section 2.1, abiotic stress affects various morphological and physiological traits, and some of these traits could be used to distinguish stress-sensitive genotypes from stress-tolerant genotypes. It was possible to evaluate several traits in this experiment despite the simplicity of the methods used and the inexpensive equipment. These methods and equipment could also be used to analyze large numbers of samples.

Plant growth

The plant growth parameters examined were the plant height (PH), leaf size (leaf length-LL and leaf width-LW), and plant dry matter (DM). At the end of the treatment period, these traits were measured in three plants from each treatment from each replication. The plant height was measured from the surface of the medium to the top of the shoot. The completely developed third leaf from the top was used for the measurement of leaf size. The leaf length was measured from the attachment of the leaf to the stem to the end of the terminal leaflet, and the leaf width was assessed at the widest diameter of leaf. The relative growth rate of the plant height (RGR_H) was calculated by the formula reported by Chen *et al.* (2002):

$$RGR_H = \frac{lnH2 - lnH1}{T2 - T1}$$

where H1 is the plant height at time 1 (T1) and H2 is the plant height at time 2 (T2). The plant height measurements were done four times:

- 10 days after transplanting or at 0 days of treatment (before treatment)
- 20 days after transplanting or at 10 days of treatment (middle of treatment)
- 31 days after transplanting or at 21 days of treatment (after treatment)
- 40 days after transplanting or at 9 days after recovery (after recovery)

Wilting symptom

Plant abiotic stress injury was scored visually based on leaf wilting (middle of treatment, after treatment and after recovery) using the following scoring (Ekanayake 1989):

1: no stress or all leaves are turgescent
3: 30% of the leaves are wilted
5: 50% of the leaves are wilted
7: 80% of the leaves are wilted
9: completely wilted

Chlorophyll content

The potato plants' chlorophyll content was measured with a chlorophyll meter (model SPAD-502, Minolta Camera Co., Tokyo). At the end of the treatment period, three measurements were made for each individual plant, with three individual plants from each treatment. Only three measurements in three leaves were made for each plant, because the number of leaves was limited.

Photosystem II quantum yield (QY)

The leaf samples for the measurement of the plants' photosystem II (PSII) quantum yield (QY) were the same as those used in the chlorophyll content measurement. The QY was assessed using a portable fluorometer (model FluorPen FP100, Photon Systems Instruments, Drasov, Czech Republic).

Cell membrane stability (CMS)

Ten-millimeter leaf discs taken at the end of treatment were used for the assessment of the cell membrane stability (CMS), following the method described by Rudack *et al.* (2017). The leaf discs were washed with distilled water three times and then placed in tubes with 10 ml of distilled water. The tubes were kept at room temperature in the dark for 18 hr. The leakage of electrolytes was then measured by a conductivity meter (model B-771, Horiba, Fukuoka, Japan) to obtain the initial conductivity (C1) value. The tubes were then autoclaved at 121 psi for 15 min and allowed to cool down at room temperature. The final conductivity (C2) values were obtained by measuring the electrolyte leakage used conductivity meter. The CMS value was calculated using the formula:

$$CMS = \left(1 - \frac{C1}{C2}\right) \times 100$$

Relative water content (RWC)

The relative water content (RWC) was measured at the end of the treatment period by following the method devised by Drapal *et al.* (2017). The third leaf from the shoot was sampled and immediately weighed to obtain the fresh weight (FW). The leaf was then immersed in distilled water for 24 hr. After this immersion, the excess water was removed by gently blotting the leaf on a paper towel. The turgid weight (TW) was then measured. For the dry weight (DW) measurement, the leaf was dried in the oven at 70°C for 24 hr and then weighed. The RWC was calculated using the formula:

$$RWC\ (\%) = \left(\frac{FW - DW}{TW - DW}\right) \times 100$$

Plant dry matter (PDM)

Three samples from each treatment and each replication were harvested after abiotic stress treatment. Samples that were free of the medium were then immediately weighed for the FW. After being oven-dried at 70°C for 48 hr, the dry weight (DW) of the samples was obtained. The plant dry matter (PDM) content was then calculated using the formula:

$$PDM\ (\%) = \left(\frac{FW}{DW}\right) \times 100$$

Stomatal traits

The measurement of stomatal traits was conducted on Desiree plants at 10 days after they were subjected to various abiotic stresses. Stomatal imprints were made by applying liquid bandage (Kobayashi Sakamu Care Medi-Shield, Tokyo) on the abaxial leaf. After the liquid bandage dried, a piece of tape was stacked on the dried bandage and carefully lifted off. The tape was then stuck to a glass object. Observations and measurements were performed by light microscopy (Olympus System Microscope model BX53, Olympus, Tokyo) under 20× magnification using the cellSens imaging software platform (Olympus). The stomata length (SL), stomata width (SW), stomata pore length (SPL), and stomata pore width (SPW) were measured.

2.3.4. Data analysis

An analysis of variance (ANOVA) of the data and a post hoc test (Tukey's HSD test) were performed by using Agricolae library, R package ver. 1.3-1 (Mendiburu 2019). The correlation analysis was done by Hmisc library, R package ver. 4.2-0 (Harrell 2019).

For an illustration of the behavior of the potato lines under various abiotic stresses, the response ratio (RR) between the non-stress and abiotic stress conditions was calculated as the logarithm of the ratio of the mean value of treatment (T) to the mean value of the non-stress condition (C) by the following formula (Rymaszewski *et al.* 2017):

$$RR \; = ln\frac{mean\ value\ T}{mean\ value\ C}$$

An RR >0 indicates that a positive response occurred by increasing the values of traits from the non-stress condition, and conversely, an RR <0 indicates a negative response that occurred by decreasing the value of traits. The closer that the RR is to 0, the smaller that the decrease or increase is, or nearly the same as the non-stress condition.

2.4. Results

The ANOVA results demonstrated that independently, abiotic stress affected all of the traits measured except the QY. A significant effect on all traits by the potato line was also observed. The interaction effects of abiotic stress and potato line were significant on the plant height, chlorophyll content, QY, and RWC (Table 2.2). Stomata traits were significantly affected by abiotic stress only in L5 (Desiree) (Table 2.2).

2.4.1. The morphological responses of potato plants to abiotic stress

Variations of plant height, leaf length, leaf width, and plant dry matter were observed among the treatments (Table 2.3, Figure 2.2). All of the potato lines responded to the abiotic stress negatively by reducing their plant height, with the exception of L3 under heat stress (Figs. 2.1, 2.6). Variation of the leaf size, in terms of leaf length and leaf width, was not significantly affected by the interaction of abiotic stress and the potato line, but it was independently affected by abiotic stress and the potato line (Table 2.3). As observed for the plant height, abiotic stress also reduced the leaf size, and combined drought-heat stress caused the highest reduction in leaf size (Table 2.3, Figure 2.2), demonstrating that the greatest responses were induced by the combined drought-heat stress (Figure 2.6).

Variation was also observed in the PDM content, which was significantly affected by the potato line and abiotic stress separately (Table 2.3). Unlike the plant height, the PDM of the potato lines generally increased under abiotic stress, with the exceptions of L3 and L5 under heat stress (Figure 2.2). L1, L4, and L5 showed the same RR order as that for the PDM in response to abiotic stress, and the most positive responses were to the combined drought-heat stress (Figure 2.6). L2 had an RR order that was similar to that of L3 (Figure 2.6).

The relative growth rate was calculated based on plant height. Drought stress suppressed the plant height faster than both the heat stress and the combined drought-heat stress (Figure 2.3). However, in the extended treatment, the combined drought-heat stress resulted in the slowest plant height growth rate in all of the potato lines. Recovery for 9 days after drought and combined drought-heat stress increased the relative plant height growth rate in all potato lines except L2.

The wilting symptom that was observed at three different time points (middle of treatment, end of treatment, and after recovery) showed that L3 and L4 wilted under the drought stress mid-treatment, and all of the potato lines wilted under the combined drought-heat stress. The intensity of the wilting was increased at the end of treatment (21 days) and occurred in all of potato lines under the drought stress and the combined drought-heat stress (Figure 2.4). The recovery step by putting the plants back in the non-stress condition for 9 days helps to decrease the wilting symptom (Figure 2.4).

Stomata properties including stomata size (length and width) and stomata pore size (length and width) were measured in L5 (Desiree). The abiotic stress significantly decreased the size of the stomata (Figure 2.5). A decrease was also observed in the stomata pore length. However, a decrease in the stomata pore width was observed only after the drought stress. The RR to the abiotic stress of the stomata length was similar to that of the stomata width. A different response was observed for the stomata pore width, which responded negatively to drought stress, whereas the stomata pore width responded positively both heat stress and combined drought-heat stress by increasing the size of pore width (Figure 2.7).

2.4.2. The physiological responses of potato plants to abiotic stress

The ANOVA results revealed that the physiological traits measured in the experiment were significantly affected by the potato line, the abiotic stress (except for the QY), and the interaction of potato line and abiotic stress (with the exception of the CMS) (Table 2.2). The chlorophyll content of five potato lines measured by the soil plant analysis development (SPAD) method was varied under different abiotic stress (Table 2.4, Figure 2.2). Variation in the chlorophyll content was also observed independently among the potato lines and the abiotic stress treatments (Table 2.4). Generally, the potato plants responded positively to the abiotic stress by increasing their chlorophyll content (Figure 2.6). Three potato lines (L3, L4, and L5) responded negatively to heat stress.

The analyses also revealed that the potato line and the interaction of abiotic stress and potato line affected the QY (Table 2.2). Potato line L5 showed the highest QY under the non-stress condition, and its QY values were significantly different from those of L4 under drought stress, which showed the lowest QY (Table 2.4, Figure 2.2). Unlike the chlorophyll content's RR, the response ratio of QY was generally negative (Figure 2.6). However, L2 and L4 responded positively to the heat stress, and L1 and L2 responded positively to the combined drought-heat stress. The QY of L2 responded positively under all abiotic stress conditions.

The variability of CMS values was affected by the potato line and abiotic stress independently (Table 2.2). Even though a reduction of the CMS was observed among the potato lines under different abiotic stresses (Figure 2.2), the CMS was not affected by the interaction of abiotic stress and potato line. The decrease in the CMS under abiotic stress relative to the non-stress condition indicates that the potato plants responded negatively to the abiotic stress (Figure 2.6).

The variation in the RWC that was observed among the treatments was significantly affected by the interaction of abiotic stress and potato line, and by the abiotic stress as well as the potato line (Tables 2.2, 2.4). The RWC decreased under the drought conditions, but the RWC values were not significantly different from those under the non-stress condition. A significant decrease in the RWC was observed under heat stress and combined drought-heat stress (Figure 2.2). The potato plants responded to the abiotic stress negatively by decreasing their RWC (Figure 2.6).

The morphology and physiology traits in the general responses of the potato plants to the various abiotic stresses are summarized in Figure 2.14.

2.4.3. The relationships among variables under abiotic stress

Pearson's correlations were obtained to evaluate the relationships between the characteristics observed in each condition (non-stress, drought stress, heat stress, and combined drought-heat stress). Under the non-stress condition, there were seven significant correlations between traits (Table 2.6, Figure 2.8). Under the drought condition, a more significant correlation was found (Table 2.7, Figure 2.9). Seven significant correlations were detected under heat stress (Table 2.8, Figure 2.10); four of the correlations were the same as those observed under the drought stress, and the other three correlations were not observed under drought stress.

Nine significant correlations between traits were detected under the combined drought-heat stress (Table 2.9, Figure 2.11). Two correlations were identified only under this combined condition, i.e., between the CMS and the wilting score, and between the RWC and PDM values; both correlations were in a positive direction. Two correlations were the same as those under drought stress, i.e., between the plant height and leaf length, and between the plant height and RWC. The positive correlation between the leaf length and the wilting score and the negative correlation between the RWC and the wilting score under the combined drought-heat stress were also detected under the heat stress. Three other correlations were detected under both the drought stress and the heat stress: a negative correlation between the plant height and PDM, a positive correlation between the leaf length and leaf width, and a negative correlation between the chlorophyll content and wilting score.

2.5. Discussion

A growth room experiment was conducted to evaluate the responses of different potato lines to abiotic stress. Experiments conducted in a growth room with relatively homogeneous conditions throughout the experimental period have the advantage of eliminating the effects of factors other than those tested. The results indicated that the effects of the replication or block on the plant traits were generally not significant (Table 2.2). PEG was used to induce the osmotic stress that simulates drought conditions because this can reduce the water potential of nutrient solutions; thus, less water is absorbed by the plant roots. As an osmotic agent with high molecular weight, PEG is non-absorbable, non-metabolized, and non-toxic (Yang *et al.* 2019).

The experiment in the growth room environment revealed that abiotic stresses (i.e., drought, heat, and combined drought-heat stress) generally affected the growth of the potato plants and some of their physiological traits (Figure 2.14). The use of different potato lines with various genetic backgrounds revealed that some of the morphological and physiological characteristics are genotype-dependent. The present results established that some traits of the different potato lines responded differently to each type of abiotic stress.

The potato plants responded to the unfavorable conditions caused by the drought stress, heat stress, and combined drought-heat stress by suppressing their growth, in this case by reducing the plant height and leaf size. The sizes of plants and their organs are based on the cell numbers, cell size, and cell elongation (Bundy *et al.* 2012). A water deficit leads to a lower cellular osmotic potential, and since plant cells need turgor pressure as a physical force for their enlargement, a water deficit suppresses the cell size (Tardieu *et al.* 2014). By reducing the size of its cells, a plant can maintain the cell turgor needed to adapt to drought stress (Cutler *et al.* 1977). High temperature induces osmotic stress in plant cells, and plants under heat stress thus also reduce their plant height and leaf size. In the present experiment however, heat stress increased the plant height of L3 (87HW13.7).

The combined drought-heat stress generally had the worst effect on plant height, but the interaction effect of abiotic stress and the potato line resulted in differing responses by the potato lines to each type of abiotic stress. Even though the potato plants responded to the abiotic stress by decreasing their plant height and leaf size, the opposite response was observed for the plant dry matter content, which generally increased with the abiotic stress. The negative correlation between PDM and plant height supports this contrasting response. The finding that abiotic stress decreased the plant height and leaf size indicates that the overall plant size is smaller than that which occurred under the non-stress condition. Abiotic stress is also known to impair the photosynthate allocation to sink organs, which in potato plants is the tuber. The total photosynthate is thus distributed mainly in the upper part of a potato plant, which was reduced by the abiotic stress effect.

Wilting is a visual condition that is easy to assess and commonly used to measure tolerance to drought stress (Engelbrecht *et al.* 2007; Pungulani *et al.* 2013). A water deficit causes decreasing leaf water potential, which leads to a decrease in the leaf turgor potential and results in leaf wilting (Jensen 1981). In the present study, the wilting symptom of the potato plants under the combined drought-heat stress was similar to that observed under the drought stress. The negative correlation between the wilting score and the RWC that reflects the leaf water potential indicates that the more severe the plant wilting is, the lower the RWC will be. Re-watering might help the plant increase its turgor potential and recover from the wilting symptom. The recovery capacity depends on the plant's genetic background, since the degree of recovery varies among potato lines. The closing of the stomata and a reduction in the stomata size are also related to the turgor potential (Jensen 1981).

In addition to the morphological features, abiotic stress affects the physiological features of plants, including photosynthesis. Chlorophyll, one of important leaf pigments involved in the photosynthetic process, becomes a parameter of interest from a physiological perspective when plants are grown under abiotic stress (Penuelas and Filella 1998). The performance of photosynthesis itself can be determined by the measurement of chlorophyll fluorescence (Maxwell and Johnson 2000). As one of the chlorophyll fluorescence parameters, the maximum photosynthetic efficiency of PSII and the maximum quantum yield (QY) are being more widely applied in physiological plant analyses. An increase in the chlorophyll content is one response mechanism under abiotic stress (Monneveux *et al.* 2014; Ramírez *et al.* 2019; Rolando *et al.* 2015). A reduction in the leaf area leads to the accumulation of chlorophyll in the leaf (Rolando *et al.* 2015). However, an increase in the chlorophyll content was not followed by high PSII QY values. Abiotic stress is well known to adversely affect the efficiency of photosynthesis by the photoinhibition phenomenon (Gururani *et al.* 2015). The photoinhibition of PSII has been described as a negative mechanism to protect PSII that limits the photosynthesis process (Guidi *et al.* 2019). The quantum yield of photosynthesis was reported to be suppressed by abiotic stress (Guidi *et al.* 2019) due to the damage of the photosynthesis apparatus. However, in the present experiment, the potato line L2 showed increased QY under the heat stress and the combined drought-heat stress, indicating that this line could adapt to the stress until the measurement time point; however, it is unknown whether the QY would remain high if the stress is extended.

Drought stress and heat stress are also known to disrupt the plasma membrane structure and composition, which consist of lipid and protein (Asthir 2015a; Bray 2007). Electrolyte leakage from the membrane injuries caused by abiotic stress reflects the level of membrane stability under abiotic stress (Arvin and Donnelly 2008). In this case, L1, L3, and L5 were more stable under drought, heat, and combined drought-heat stress since they had small decreases in their cell membrane stability.

The RWC is an indicator of a plant's water status that is widely used in abiotic stress assessments (Anithakumari *et al.* 2012; Soltys-Kalina *et al.* 2016; Tani *et al.* 2019; Zhou *et al.* 2017). Herein it was shown that different potato lines responded to the abiotic stress by decreasing their RWC to differing degrees, with the combined drought-heat stress producing the lowest RWC. This result suggested that the potato lines have different sensitivities to each type of abiotic stress, but in general, all potato lines will be the most sensitive when grown under combined drought-heat stress. Drought-tolerant genotypes of alfalfa showed small decreases of their RWC (Tani *et al.* 2019). Three of the potato lines examined here (L1, L3, and L5) showed small reduction in RWC under all types of abiotic stress, suggesting that these lines are tolerant to drought stress, heat stress, and combined drought-heat stress. These results imply the ability of these potato lines to maintain the water status that is crucial for the plants' physiological processes.

2.6. Proposed practical application

The traits examined in this experiment were simple and did not require high-tech equipment for their measurement. The traits were affected by abiotic stress, and some of the traits could be used to differentiate the stress tolerance of the potato genotypes used herein. The techniques used can thus be applied in low-budget experimental germ plasm screenings or early generation evaluations that involve many genotypes.

Information gained from this experiment can be considered in breeding programs for abiotic stress tolerance, such as the development of markers related to specific morpho-physiological traits that are affected by abiotic stress. In addition, the potato lines that are confirmed to be abiotic stress-tolerant could be used as parental lines.

Table 2.1. Genetic materials used in the experiment

Code	Potato line	Ploidy	Genetic background
L1	84.194.30	2x	adg, chc, phu, spl, stn, tbr
L2	86.61.26	2x	phu, spl, stn, sto, tbr
L3	87HW13.7	2x	adg, mlt, tbr
L4	DG81-68	2x	tbr, chc, yun
L5	Desiree	4x	–

adg: *Solanum andigena*, chc: *S. chacoense*, phu: *S. phureja*, spl: *S. sparsipilum*, stn: *S. stenotomum*, sto: *S. stoloniferum*, mlt: *S. multidissectum*, yun: *S. yungasense*, tbr: *S. tuberosum.*

Table 2.2. ANOVA results of morpho-physiological traits

Trait	**Replication**		**Abiotic stress**		**Potato line**		**Abiotic stress × potato line**	
	F	**p**	**F**	**p**	**F**	**p**	**F**	**p**
Plant height	3.8386	*	28.3349	***	8.6633	***	3.0873	**
Leaf length	2.0086		89.5960	***	10.4670	***	1.8278	.
Leaf width	0.6471		88.8644	***	5.7297	***	0.9639	
Plant dry matter	1.1938		6.4603	**	12.0842	***	1.0008	
Chlorophyll content	0.5259		47.4978	***	26.7723	***	2.5003	**
QY	1.8617		0.6074		2.6967	*	2.1005	*
CMS	1.4818		8.8627	**	7.9143	***	0.9143	
RWC	3.8447	*	21.8245	***	6.3255	***	3.2553	***
Stomata traits only observed in Desiree:								
Stomata length	0.9100		0.0022	**				
Stomata diameter	0.9270		0.0011	**				
Pore length	0.7242		0.0073	**				
Pore diameter	0.9381		0.0003	***				

***p<0.001, **p<0.01, *p<0.05. CMS: cell membrane stability, QY: photosystem II quantum yield, RWC: relative water content.

Table 2.3. Plant growth of five potato lines under various abiotic stress

Treatment	**Plant height (cm) ± SE**	**Leaf length (cm) ± SE**	**Leaf width (cm) ± SE**	**Plant dry mater (%) ± SE**
Abiotic stress				
C	19.79 ± 1.25^{a}	6.16 ± 0.25^{a}	3.83 ± 0.14^{a}	10.11 ± 0.43^{b}
D	12.06 ± 0.49^{c}	3.81 ± 0.18^{b}	2.34 ± 0.13^{b}	12.5.9 ± 0.66^{a}
H	15.12 ± 0.96^{b}	2.73 ± 0.11^{c}	1.34 ± 0.09^{c}	10.12 ± 0.45^{b}
DH	10.66 ± 0.75^{c}	2.43 ± 0.08^{c}	1.14 ± 0.07^{c}	13.81 ± 0.79^{a}
CV(a) (%)	11.60	11.50	12.80	16.10
Potato line				
L1	12.95 ± 0.98^{bc}	3.37 ± 0.29^{b}	1.94 ± 0.23^{bc}	13.74 ± 0.78^{a}
L2	15.34 ± 1.85^{ab}	3.86 ± 0.37^{b}	2.27 ± 0.23^{a-c}	12.76 ± 0.81^{a}
L3	15.81 ± 1.01^{ab}	3.56 ± 0.32^{b}	1.83 ± 0.23^{c}	9.36 ± 0.50^{b}
L4	11.13 ± 0.83^{c}	3.54 ± 0.30^{b}	2.31 ± 0.24^{ab}	12.28 ± 0.73^{a}
L5	16.83 ± 0.91^{a}	4.56 ± 0.44^{a}	2.47 ± 0.31^{a}	10.15 ± 0.47^{b}
Abiotic stress × Potato line				
CL1	18.58 ± 1.98^{a-d}	5.29 ± 0.23^{bc}	3.57 ± 0.12^{ab}	11.96 ± 1.05^{a-e}
CL2	25.00 ± 5.39^{a}	6.47 ± 0.54^{ab}	3.92 ± 0.24^{ab}	9.79 ± 1.05^{c-e}
CL3	18.21 ± 0.55^{a-e}	5.85 ± 0.56^{bc}	3.36 ± 0.33^{a-c}	9.28 ± 1.01^{de}
CL4	16.39 ± 1.42^{b-f}	5.58 ± 0.40^{bc}	3.82 ± 0.32^{ab}	10.26 ± 0.75^{c-e}
CL5	20.78 ± 1.19^{ab}	7.60 ± 0.57^{a}	4.50 ± 0.34^{a}	9.27 ± 0.83^{de}
DL1	13.41 ± 1.19^{b-g}	3.58 ± 0.41^{de}	2.12 ± 0.23^{d-g}	14.10 ± 1.32^{a-d}
DL2	12.11 ± 1.40^{c-g}	3.76 ± 0.27^{de}	2.20 ± 0.29^{d-f}	15.12 ± 2.17^{a-c}
DL3	10.28 ± 0.61^{e-g}	3.36 ± 0.22^{e}	2.10 ± 0.17^{d-g}	10.88 ± 1.09^{a-e}
DL4	10.56 ± 0.68^{d-g}	3.46 ± 0.35^{de}	2.41 ± 0.20^{c-e}	12.42 ± 1.14^{a-e}
DL5	13.96 ± 0.82^{b-g}	4.89 ± 0.43^{cd}	2.86 ± 0.47^{b-d}	10.44 ± 0.61^{b-e}
HL1	11.14 ± 0.96^{c-g}	2.28 ± 0.22^{e}	1.09 ± 0.10^{f-h}	12.79 ± 0.48^{a-d}
HL2	14.03 ± 1.86^{b-g}	2.87 ± 0.25^{e}	1.65 ± 0.20^{e-h}	11.37 ± 0.50^{a-e}
HL3	21.21 ± 1.38^{ab}	2.75 ± 0.17^{e}	1.03 ± 0.14^{gh}	7.04 ± 0.36^{e}
HL4	10.45 ± 1.03^{d-g}	2.86 ± 0.35^{e}	1.68 ± 0.32^{e-h}	10.61 ± 0.60^{b-e}
HL5	18.79 ± 1.21^{a-c}	2.87 ± 0.20^{e}	1.28 ± 0.11^{e-h}	8.80 ± 1.00^{de}
DHL1	8.66 ± 0.76^{fg}	2.32 ± 0.18^{e}	1.01 ± 0.13^{gh}	16.10 ± 2.49^{a}
DHL2	10.23 ± 1.45^{e-g}	2.32 ± 0.14^{e}	1.30 ± 0.14^{e-h}	14.75 ± 1.40^{a-c}
DHL3	13.53 ± 1.42^{b-g}	2.30 ± 0.11^{e}	0.85 ± 0.04^{h}	10.25 ± 0.73^{c-e}
DHL4	7.11 ± 0.59^{g}	2.27 ± 0.19^{e}	1.33 ± 0.21^{e-h}	15.84 ± 1.94^{ab}
DHL5	13.78 ± 2.05^{b-g}	2.90 ± 0.19^{e}	1.23 ± 0.08^{f-h}	12.12 ± 0.81^{a-e}
CV(b) (%)	12.50	9.50	10.10	10.30

Treatments with the same letter are not significantly different (Tukey's HSD test, $\alpha = 0.05$). CV(a): coefficient of variation of main plot, CV(b): CV of subplot and its interaction with the main plot. Each value is the mean value ± SE (standard error) of six samples. C: non-stress condition, D: drought stress, H: heat stress, DH: combined drought-heat stress. L1: 84.194.30, L2: 86.61.26, L3: HW13.7, L4: DG81-68, L5: Desiree.

Table 2.4. Physiological traits of five potato lines under various abiotic stress

Treatment	Chl (SPAD unit) ± SE	QY ± SE	CMS (%) ± SE	RWC (%) ± SE
Abiotic stress				
C	39.47 ± 1.09 ^{b}	0.78 ± 0.003 ^{a}	77.34 ± 1.06 ^{a}	82.11 ± 0.68 ^{a}
D	48.12 ± 0.94 ^{a}	0.76 ± 0.005 ^{a}	73.34 ± 1.68 ^{ab}	73.20 ± 1.24 ^{b}
H	39.74 ± 1.17 ^{b}	0.77 ± 0.007 ^{a}	72.39 ± 1.65 ^{b}	70.59 ± 1.31 ^{b}
DH	49.87 ± 1.49 ^{a}	0.77 ± 0.006 ^{a}	69.84 ± 1.86 ^{b}	63.08 ± 2.02 ^{c}
CV(a) (%)	9.80	5.00	7.80	12.70
Potato line				
L1	46.01 ± 0.96 ^{b}	0.77 ± 0.005 ^{ab}	77.43 ± 1.02 ^{a}	74.22 ± 1.33 ^{a}
L2	44.58 ± 1.54 ^{bc}	0.76 ± 0.004 ^{ab}	68.48 ± 1.99 ^{c}	70.69 ± 2.70 ^{ab}
L3	42.27 ± 1.56 ^{c}	0.77 ± 0.008 ^{ab}	74.89 ± 1.33 ^{ab}	74.16 ± 1.56 ^{a}
L4	37.72 ± 1.28 ^{d}	0.76 ± 0.008 ^{b}	70.12 ± 2.30 ^{bc}	68.27 ± 2.68 ^{b}
L5	50.92 ± 1.52 ^{a}	0.78 ± 0.003 ^{a}	75.22 ± 1.72 ^{ab}	73.87 ± 1.56 ^{a}
Abiotic stress × Potato line				
CL1	41.82 ± 0.85 $^{d\text{-}h}$	0.78 ± 0.005 ^{ab}	81.25 ± 1.08 ^{a}	80.17 ± 0.73 ^{ab}
CL2	36.61 ± 1.83 $^{g\text{-}i}$	0.75 ± 0.002 ^{ab}	72.92 ± 2.74 $^{a\text{-}c}$	83.74 ± 2.14 ^{a}
CL3	38.71 ± 2.14 $^{f\text{-}i}$	0.78 ± 0.004 ^{ab}	77.36 ± 1.81 ^{ab}	82.45 ± 0.93 ^{ab}
CL4	34.51 ± 2.02 ^{hi}	0.78 ± 0.002 ^{ab}	76.28 ± 2.51 ^{ab}	82.21 ± 1.65 ^{ab}
CL5	45.71 ± 2.44 $^{b\text{-}g}$	0.79 ± 0.003 ^{a}	78.89 ± 2.53 ^{a}	81.96 ± 1.79 ^{ab}
DL1	47.03 ± 1.32 $^{b\text{-}f}$	0.77 ± 0.006 ^{ab}	77.22 ± 2.16 ^{ab}	73.91 ± 2.04 $^{a\text{-}c}$
DL2	45.41 ± 1.67 $^{b\text{-}g}$	0.76 ± 0.002 ^{ab}	68.56 ± 5.03 $^{a\text{-}c}$	74.08 ± 3.95 $^{a\text{-}c}$
DL3	49.26 ± 0.70 $^{a\text{-}e}$	0.77 ± 0.003 ^{ab}	75.36 ± 3.24 ^{ab}	73.14 ± 2.84 $^{a\text{-}c}$
DL4	44.11 ± 2.00 $^{c\text{-}h}$	0.73 ± 0.023 ^{b}	69.88 ± 3.67 $^{a\text{-}c}$	71.82 ± 3.42 ^{bc}
DL5	54.80 ±1.65 ^{ab}	0.78 ± 0.005 ^{ab}	75.70 ± 3.97 ^{ab}	73.05 ± 2.08 $^{a\text{-}c}$
HL1	44.53 ± 1.07 $^{c\text{-}g}$	0.75 ± 0.015 ^{ab}	75.67 ± 2.40 ^{ab}	75.12 ± 2.06 $^{a\text{-}c}$
HL2	42.84 ± 0.73 $^{d\text{-}h}$	0.77 ± 0.007 ^{ab}	67.61 ± 4.93 $^{a\text{-}c}$	67.72 ± 3.09 ^{cd}
HL3	34.77 ± 2.48 ^{hi}	0.76 ± 0.029 ^{ab}	73.69 ± 3.23 $^{a\text{-}c}$	72.23 ± 2.20 ^{bc}
HL4	31.93 ± 1.48 ^{i}	0.78 ± 0.005 ^{ab}	73.32 ± 3.53 $^{a\text{-}c}$	66.27 ± 3.22 ^{cd}
HL5	44.61 ± 1.15 $^{c\text{-}g}$	0.78 ± 0.009 ^{ab}	71.67 ± 4.29 $^{a\text{-}c}$	71.61 ± 3.29 ^{bc}
DHL1	50.68 ± 2.22 $^{a\text{-}d}$	0.78 ± 0.011 ^{ab}	75.58 ± 1.86 ^{ab}	67.68 ± 2.81 ^{cd}
DHL2	53.45 ± 2.81 $^{a\text{-}c}$	0.76 ± 0.014 ^{ab}	64.83 ± 2.99 ^{bc}	57.22 ± 5.49 ^{de}
DHL3	46.34 ± 2.59 $^{b\text{-}f}$	0.78 ± 0.010 ^{ab}	73.17 ± 2.47 $^{a\text{-}c}$	68.84 ± 3.26 ^{c}
DHL4	40.34 ± 1.24 $^{e\text{-}i}$	0.75 ± 0.020 ^{ab}	61.00 ± 6.24 ^{c}	52.76 ± 4.17 ^{e}
DHL5	58.56 ± 2.15 ^{a}	0.78 ± 0.005 ^{ab}	74.64 ± 2.96 $^{a\text{-}c}$	68.88 ± 2.67 ^{c}
CV(b) (%)	10.40	3.10	8.90	7.20

Treatments with the same letter are not significantly different (Tukey's HSD test, α = 0.05). CV(a), coefficient of variation of main plot, CV(b), CV of subplot and its interaction with main plot. Each value shows mean value ± SE (standard error) of six samples. C: non-stress condition, D: drought stress, DH: combined drought-heat stress, H: heat stress, L1: 84.194.30, L2: 86.61.26, L3: HW13.7, L4: DG81-68, L5: Desiree.

Table 2.5. Stomata traits of Desiree under abiotic stress

Abiotic stress	Stomata length (μm)	Stomata width (μm)	Stomata pore length (μm)	Stomata pore width (μm)
C	31.69 ± 1.13 ^{a}	22.02 ± 1.31 ^{a}	19.76 ± 1.37 ^{a}	2.06 ± 0.19 ^{a}
D	26.29 ± 0.80 ^{b}	17.04 ± 0.46 ^{b}	16.06 ± 0.83 ^{ab}	1.07 ± 0.08 ^{b}
H	24.35 ± 0.74 ^{b}	16.91 ± 0.55 ^{b}	13.53 ± 0.57 ^{b}	2.79 ± 0.35 ^{a}
DH	25.09 ± 1.86 ^{b}	17.25 ± 0.82 ^{b}	13.46 ± 1.82 ^{b}	2.65 ± 0.25 ^{a}

Treatments with the same letter are not significantly different (Tukey's HSD test, $\alpha = 0.05$). Each value is the mean value ± SE (standard error) of six samples. C: non-stress condition, D: drought stress, DH: combined drought-heat stress, H: heat stress.

Table 2.6. Correlation between traits under non-stress condition

	PH	**LL**	**LW**	**Chl**	**QY**	**CMS**	**RWC**	**PDM**
PH		**0.01**	0.21	0.06	0.47	**0.02**	0.27	0.49
LL	**0.49**		**0.00**	**0.00**	0.11	0.13	0.50	0.42
LW	0.23	**0.74**		**0.00**	0.18	0.76	0.85	0.92
Chl	0.35	**0.70**	**0.59**		**0.00**	0.88	0.56	0.22
QY	−0.14	0.30	0.25	**0.51**		0.45	0.84	0.55
CMS	**−0.44**	−0.28	−0.06	0.03	0.14		**0.00**	0.06
RWC	0.21	0.13	0.04	−0.11	−0.04	**−0.50**		0.78
PDM	0.13	0.15	−0.02	0.23	0.11	−0.35	−0.05	

The lower diagonal is the correlation coefficient (R). The upper diagonal is the p-value. R values in bold are significant at $p<0.05$. Chl: chlorophyll content, CMS: cell membrane stability, LL: leaf length, LW: leaf width, PDM: plant dry matter content, PH: plant height, QY: photosystem II quantum yield, RWC: relative water content.

Table 2.7. Correlation between traits under drought stress

	PH	**LL**	**LW**	**Chl**	**QY**	**CMS**	**RWC**	**PDM**	**Wilt**
PH		**0.00**	0.08	0.16	0.15	0.81	**0.04**	**0.04**	**0.02**
LL	**0.58**		**0.00**	**0.00**	**0.01**	0.22	0.07	0.27	0.48
LW	0.33	**0.82**		**0.02**	0.11	0.17	0.09	0.42	0.70
Chl	0.27	**0.54**	**0.43**		0.07	0.62	0.61	0.05	**0.04**
QY	0.27	**0.50**	0.29	0.34		0.67	0.55	0.52	**0.04**
CMS	−0.05	−0.23	−0.26	0.09	0.08		**0.01**	0.95	0.14
RWC	**0.37**	0.34	0.31	−0.10	0.11	**−0.46**		0.65	0.48
PDM	**−0.38**	−0.21	−0.15	−0.36	0.12	−0.01	−0.08		0.91
Wilt	**−0.42**	−0.13	0.07	**−0.38**	**−0.36**	−0.28	−0.13	0.02	

The lower diagonal is the correlation coefficient (R). The upper diagonal is the p-value. R values in bold are significant at $p<0.05$. Chl: chlorophyll content, CMS: cell membrane stability, LL: leaf length, LW: leaf width, PDM: plant dry matter content, PH: plant height, QY: photosystem II quantum yield, RWC: relative water content.

Table 2.8. Correlation between traits under heat stress

	PH	**LL**	**LW**	**Chl**	**QY**	**CMS**	**RWC**	**PDM**	**Wilt**
PH		0.06	0.75	0.94	0.28	0.37	0.19	**0.00**	**0.03**
LL	0.35		**0.00**	0.73	0.86	0.37	0.44	0.75	0.38
LW	−0.06	**0.80**		0.73	0.97	0.45	0.57	0.25	**0.02**
Chl	−0.01	0.07	0.07		0.95	0.88	0.17	0.09	**0.02**
QY	−0.20	0.03	0.01	−0.01		0.43	**0.00**	0.95	0.16
CMS	−0.17	−0.17	−0.14	−0.03	0.15		0.54	0.34	0.76
RWC	0.25	0.15	0.11	0.26	**−0.52**	0.12		0.53	**0.02**
PDM	**−0.65**	−0.06	0.22	0.31	0.01	0.18	0.12		0.60
Wilt	**−0.39**	0.17	**0.42**	**−0.41**	0.26	0.06	**−0.43**	0.10	

The lower diagonal is the correlation coefficient (R). The upper diagonal is the p-value. R values in bold are significant at $p<0.05$. Chl: chlorophyll content, CMS: cell membrane stability, LL: leaf length, LW: leaf width, PDM: plant dry matter content, PH: plant height, QY: photosystem II quantum yield, RWC: relative water content.

Table 2.9. Correlation between traits under combined drought-heat stress

	PH	**LL**	**LW**	**SPAD**	**QY**	**CMS**	**RWC**	**PDM**	**Wilt**
PH		**0.04**	0.62	0.08	0.87	0.17	**0.00**	**0.00**	0.26
LL	**0.37**		**0.00**	0.22	0.74	0.09	0.12	0.42	0.73
LW	0.09	**0.54**		0.82	0.45	0.91	0.74	0.31	**0.04**
SPAD	0.32	0.23	0.04		0.19	0.09	0.32	0.52	**0.03**
QY	0.03	0.06	−0.14	0.25		0.74	0.21	0.08	0.07
CMS	0.26	0.31	0.02	0.31	0.06		0.11	0.90	**0.01**
RWC	**0.53**	0.29	0.06	0.19	0.23	0.29		0.05	**0.00**
PDM	**−0.50**	−0.15	0.19	−0.12	−0.33	0.02	**−0.36**		0.67
Wilt	−0.21	−0.07	**0.36**	**−0.41**	−0.33	**−0.48**	**−0.53**	0.08	

The lower diagonal is the correlation coefficient (R). The upper diagonal is the p-value. R values in bold are significant at $p<0.05$. Chl: chlorophyll content, CMS: cell membrane stability, LL: leaf length, LW: leaf width, PDM: plant dry matter content, PH: plant height, QY: photosystem II quantum yield, RWC: relative water content.

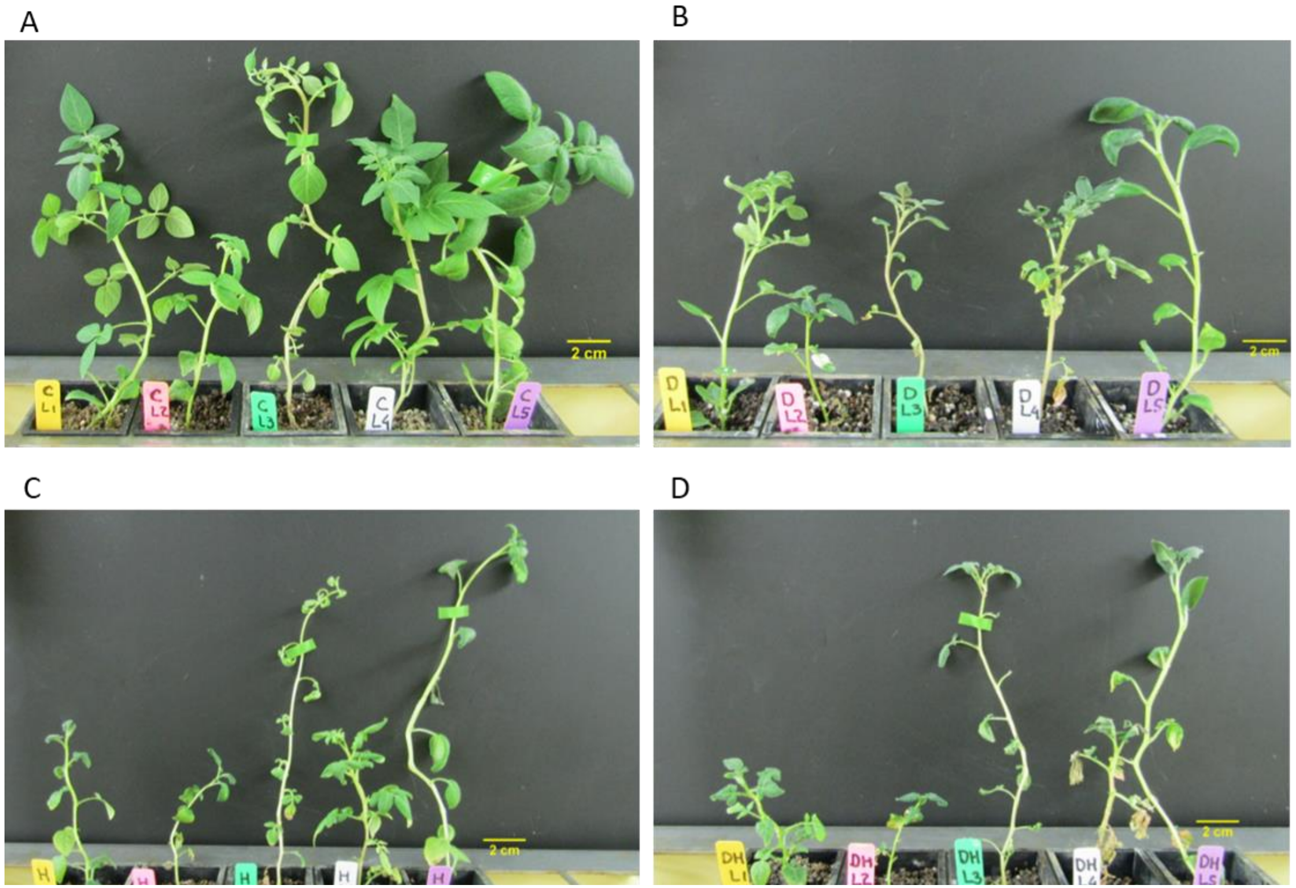


Figure 2.1. The plant performance of five potato lines under abiotic stress.
A: The plant performance at non-stress condition. **B:** The plants' performance under drought stress shows a reduction of the plant size. **C:** The plant performance under heat stress shows a reduction of the plant size. **D:** The plant performance under combined drought-heat stress shows reductions of plant size and dried leaves. Scale bar: 2 cm. C: non-stress, D: drought stress, DH: combined drought-heat stress, H: heat stress, L1: 84.194.30, L2: 86.61.26, L3: 87HW13.7, L4: DG81-68, L5: Desiree.

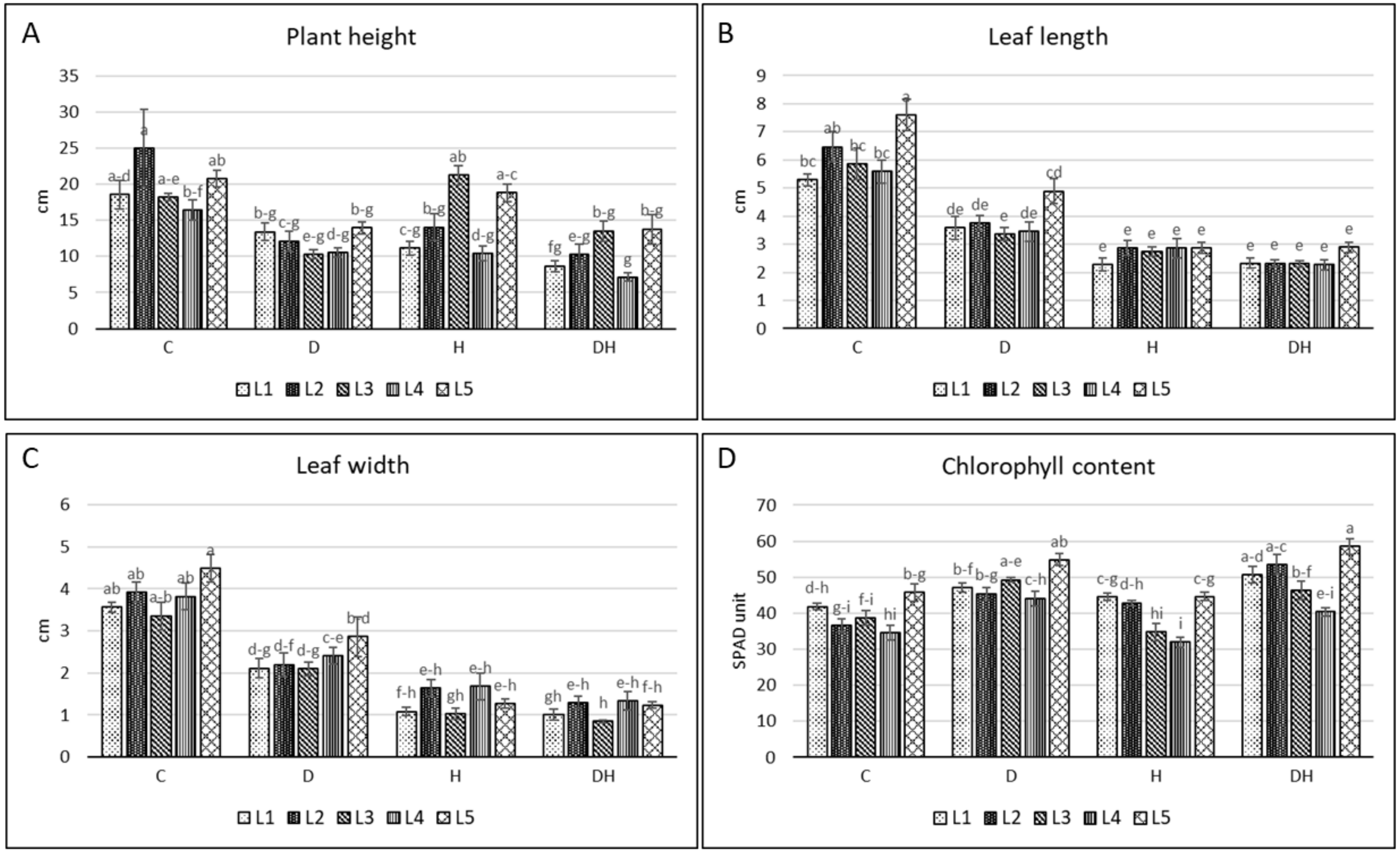


Figure 2.2. Effect of abiotic stress on five potato lines' plant growth and physiological traits.
Different letters above the error bars indicate significant differences among treatments by Tukey's HSD test at α = 0.05. Error bars: The SE of the mean from six samples. CMS: cell membrane stability, D: drought stress, DH: combined drought-heat stress, H: heat stress, QY: photosystem II quantum yield, RWC: relative water content, L1: 84.194.30, L2: 86.61.26, L3: 87HW13.7, L4: DG81-68, L5: Desiree.

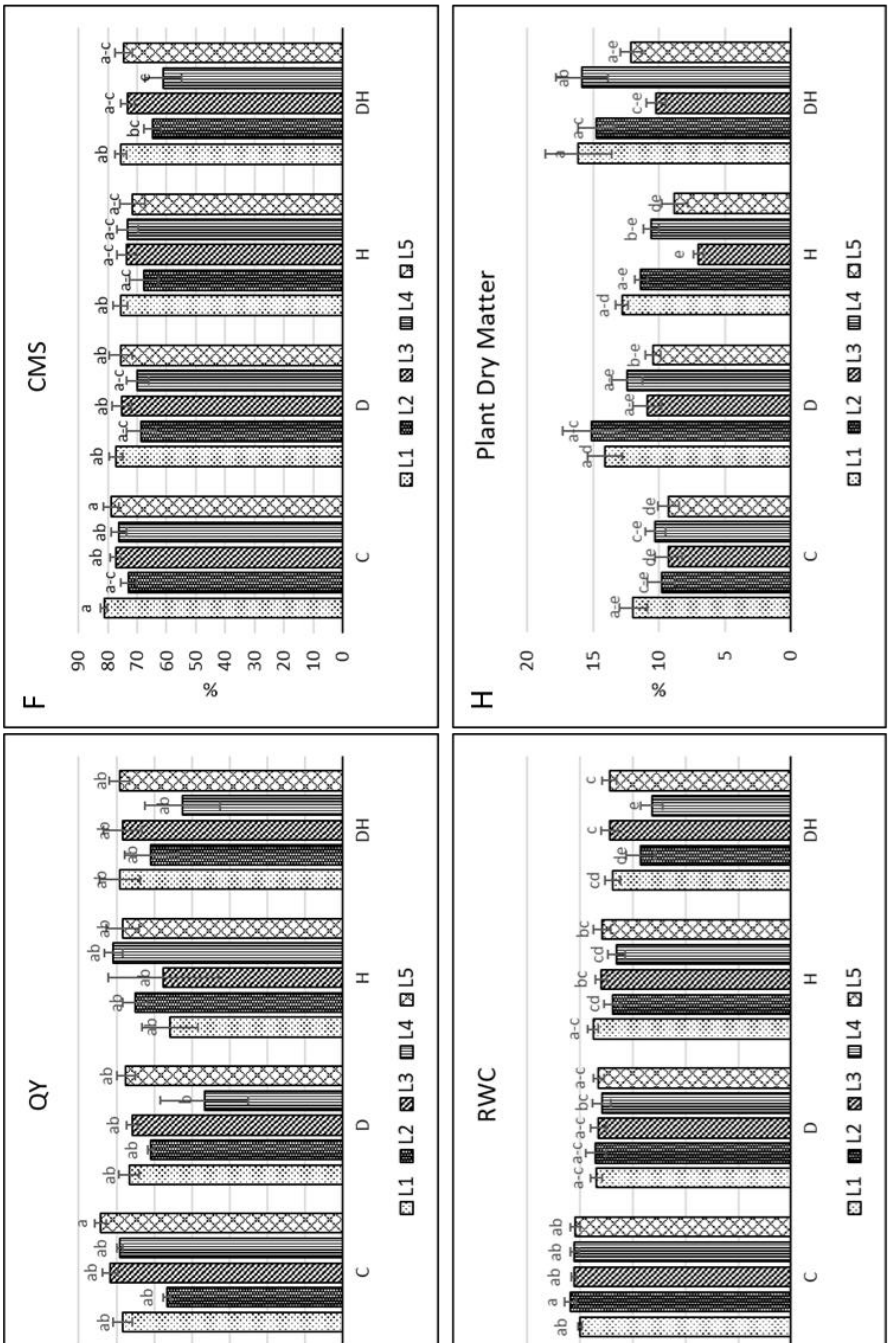


Figure 2.2. Continued.

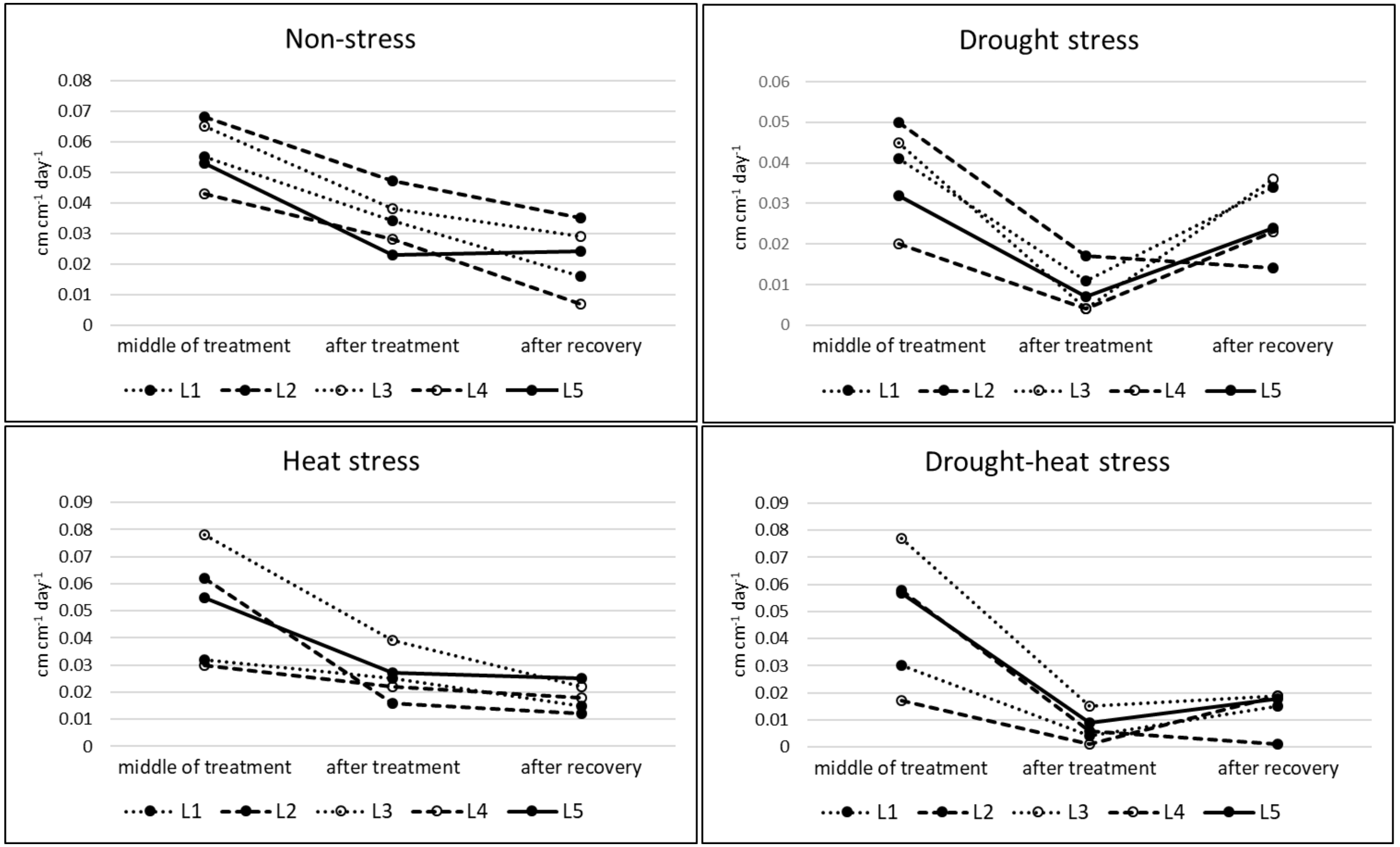


Figure 2.3. The relative growth rate of plant height (PGRPH) of the five potato lines in different abiotic stress conditions. L1: 84.194.30, L2: 86.61.26, L3: 87HW13.7, L4: DG81-68, L5: Desiree. **A:** PGRPH was decreased regularly early under the non-stress condition by drought stress. **B:** PGRPH was decreased faster at drought stress than at the other abiotic stresses, but a recovery step could increase the PGRPH. **C:** PGRPH at heat stress was slower than at the other abiotic stresses, and a recovery step could not increase the PGRPH. **D:** PGRPH under combined drought-heat stress until the middle of treatment was similar to that under heat stress and after treatment was similar to that under drought stress.

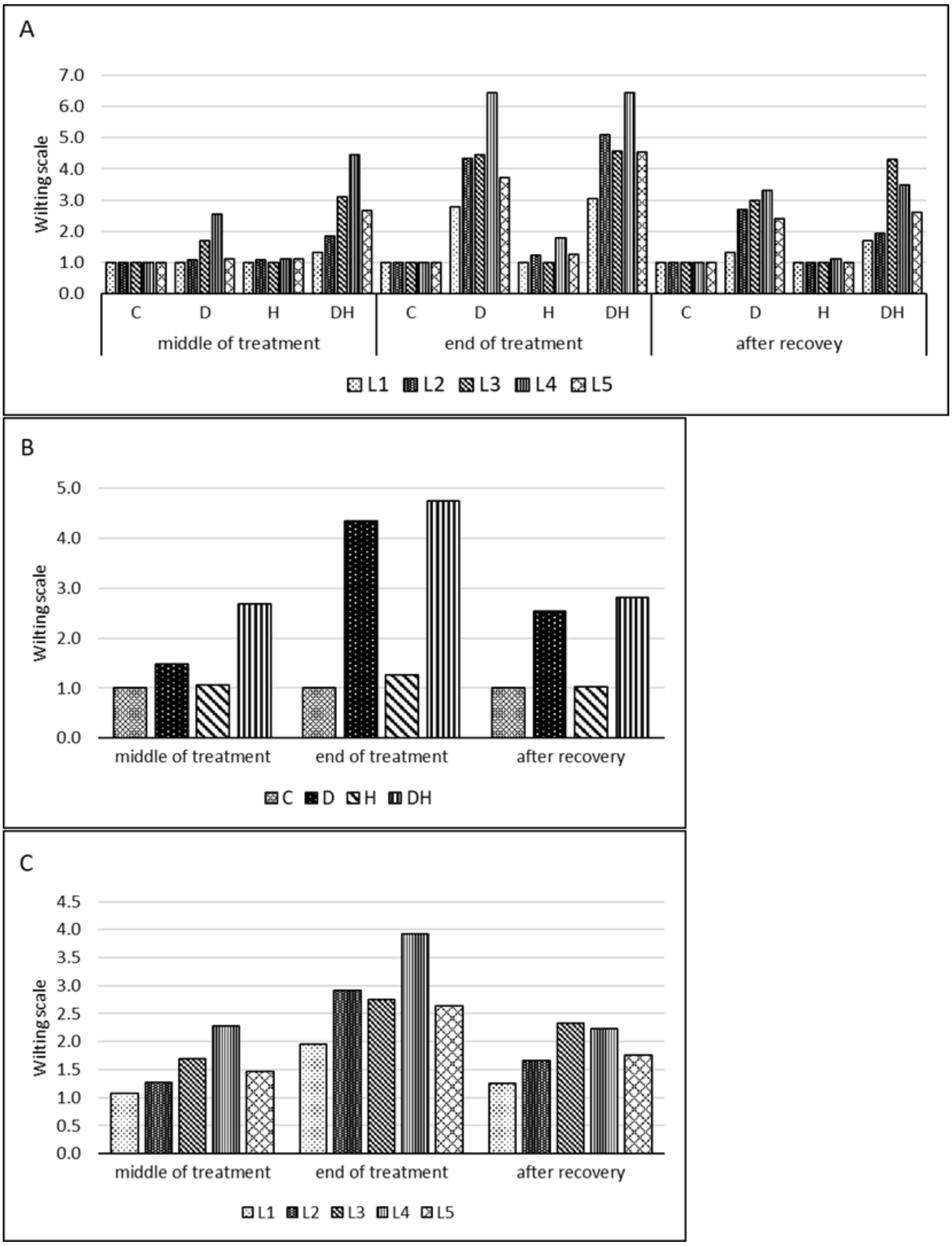


Figure 2.3. Wilting scale of potato plants.

A: The wilting scale of five potato lines under various abiotic stress at three time points. **B:** Wilting scale grouped based on abiotic stress at three time points. **C:** Wilting scale grouped based on potato lines at three time points. L1: 84.194.30, L2: 86.61.26, L3: 87HW13.7, L4: DG81-68, L5: Desiree. C: non-stress, D: drought stress, DH: combined drought-heat stress, H: heat stress. Scale: 1: All leaves turgescent, 3: 30% leaves are wilted, 5: 50% leaves are wilted, 7: 80% leaves are wilted.

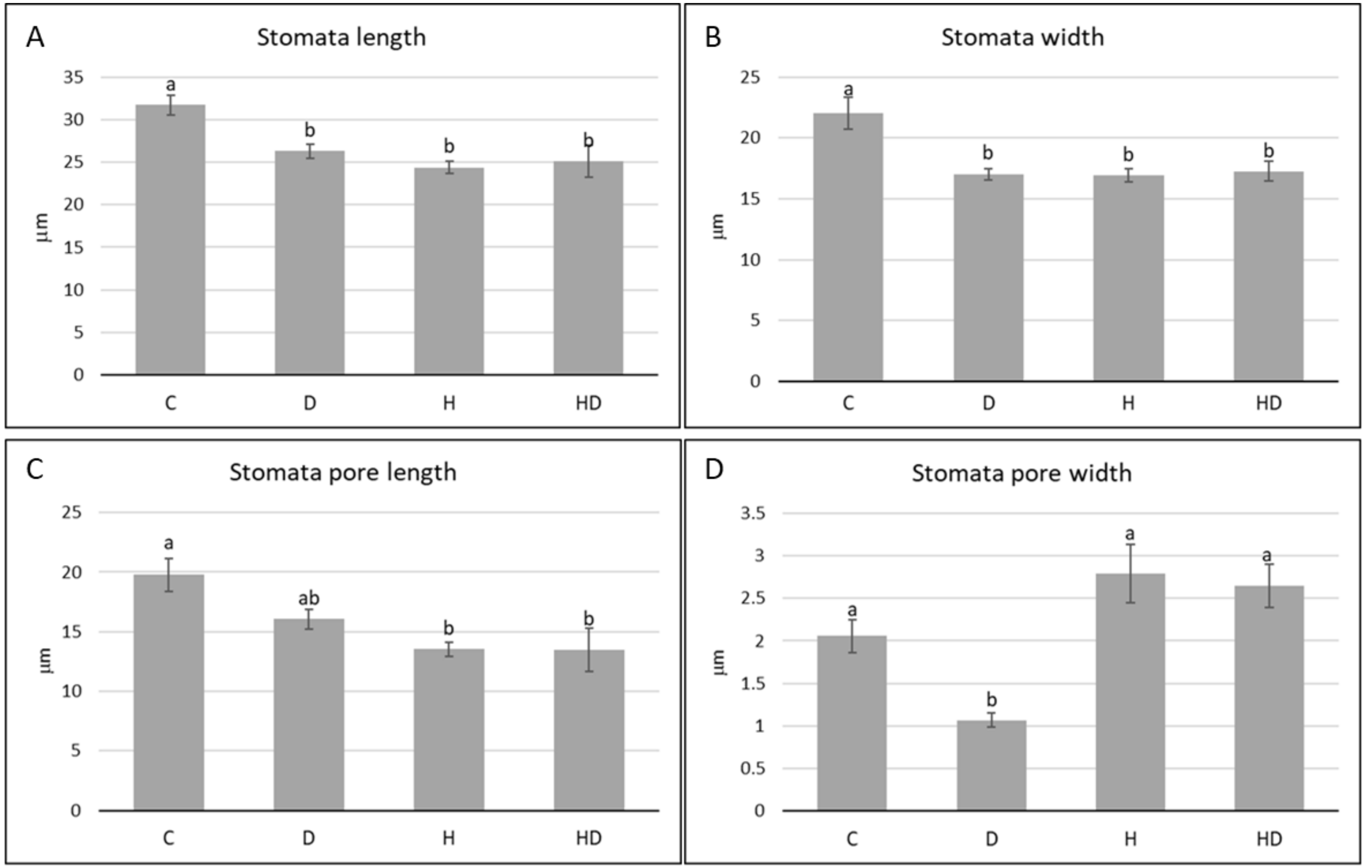


Figure 2.5. Effects of abiotic stress on stomata traits in Desiree.
A, B: Abiotic stresses decreased the stomata length and stomata width significantly. **C:** Heat and combined drought-heat stress reduced the stomata pore length significantly. **D:** Drought stress decreased the stomata pore width significantly. C: non-stress, D: drought stress, H: heat stress, HD: combined drought-heat stress. Error bars: The SE of the mean from six samples. Different letters above the error bars indicate significant differences among treatments by Tukey's HSD test at $p<0.05$.

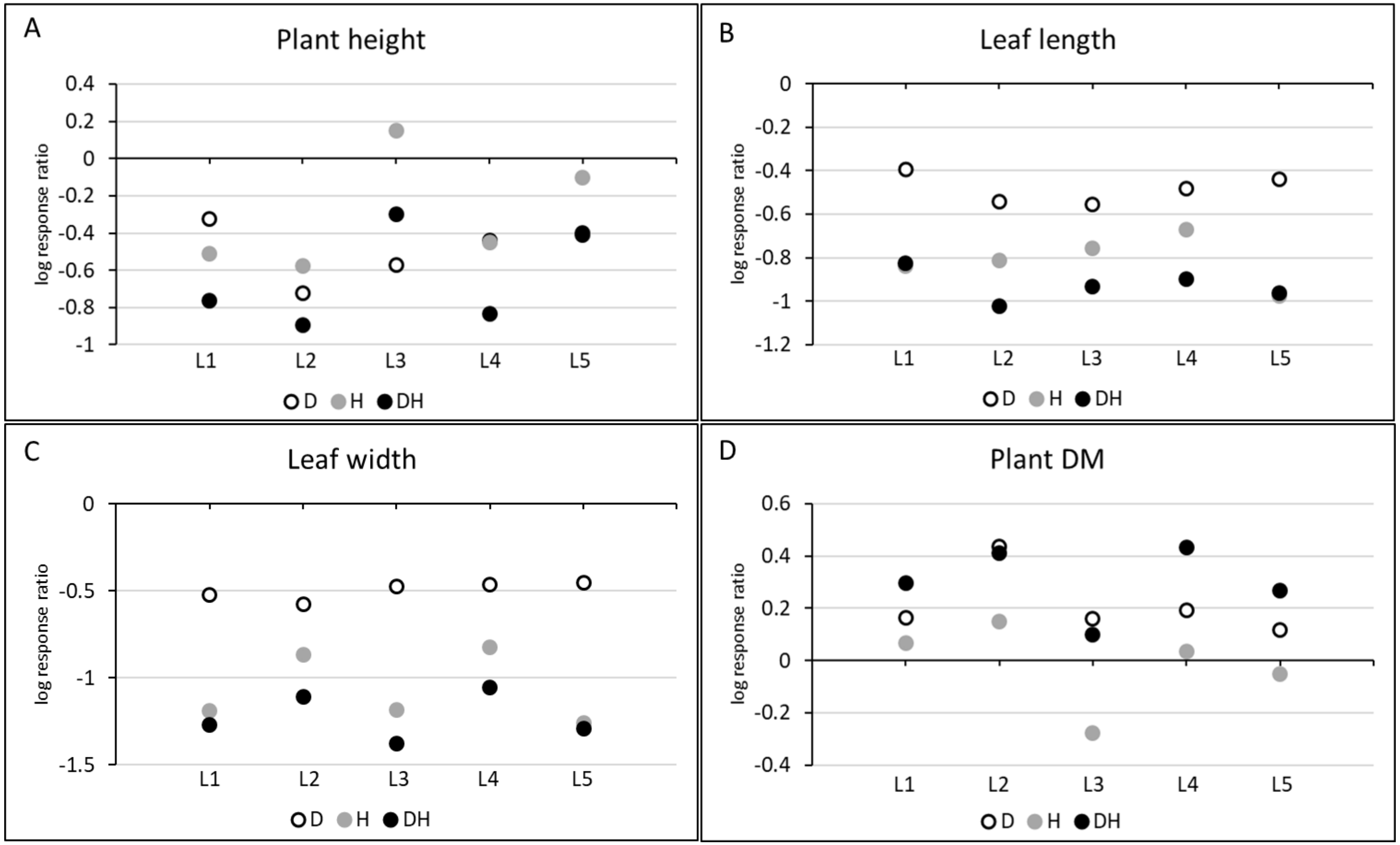
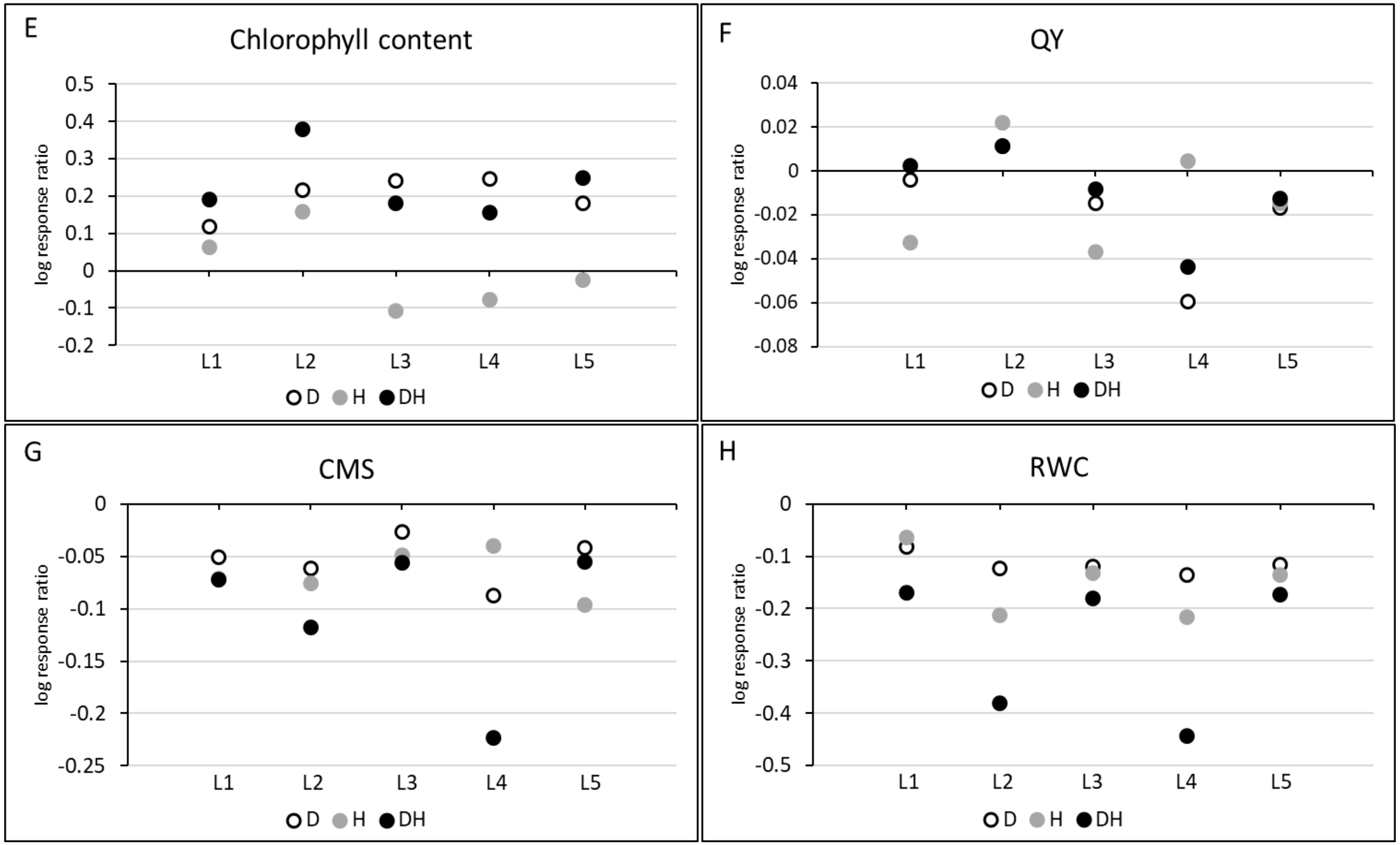


Figure 2.6. The response ratios (RRs) of the morpho-physiological traits of the potato plants subjected to various abiotic stresses. **A:** The plant height RR. **B:** The leaf length RR. **C:** The leaf width RR. **D:** The plant dry matter RR. **E:** The chlorophyll content RR. **F:** The photosystem II quantum yield (QY) RR. **G:** The cell membrane stability (CMS) RR. **H:** The relative water content (RWC) RR. L1: 84.194.30, L2: 86.61.26, L3: 87HW13.7, L4: DG81-68, L5: Desiree. The RRs were calculated relative to the non-stress condition.



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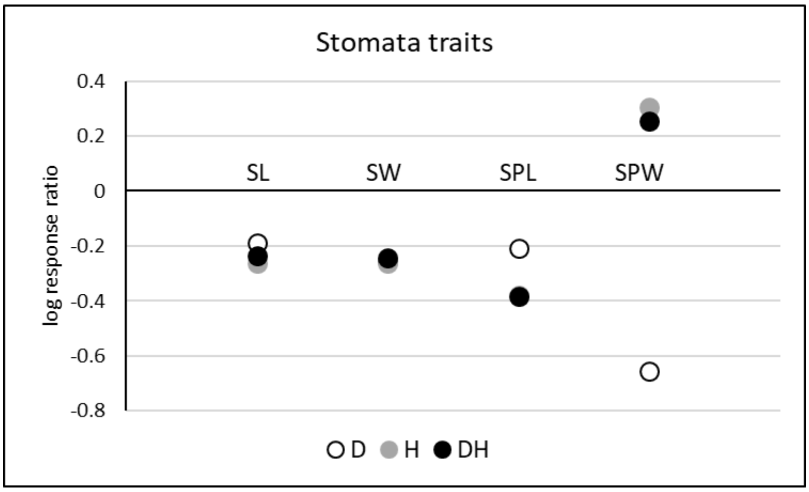


Figure 2.4. The response ratio (RR) of stomata traits of potato plants to various abiotic stresses.

The RR was calculated relative to non-stress condition. D: drought stress, DH: combined drought-heat stress, H: heat stress, SL: stomata length, SPL: stomata pore length, SPW: stomata pore width, SW: stomata width.

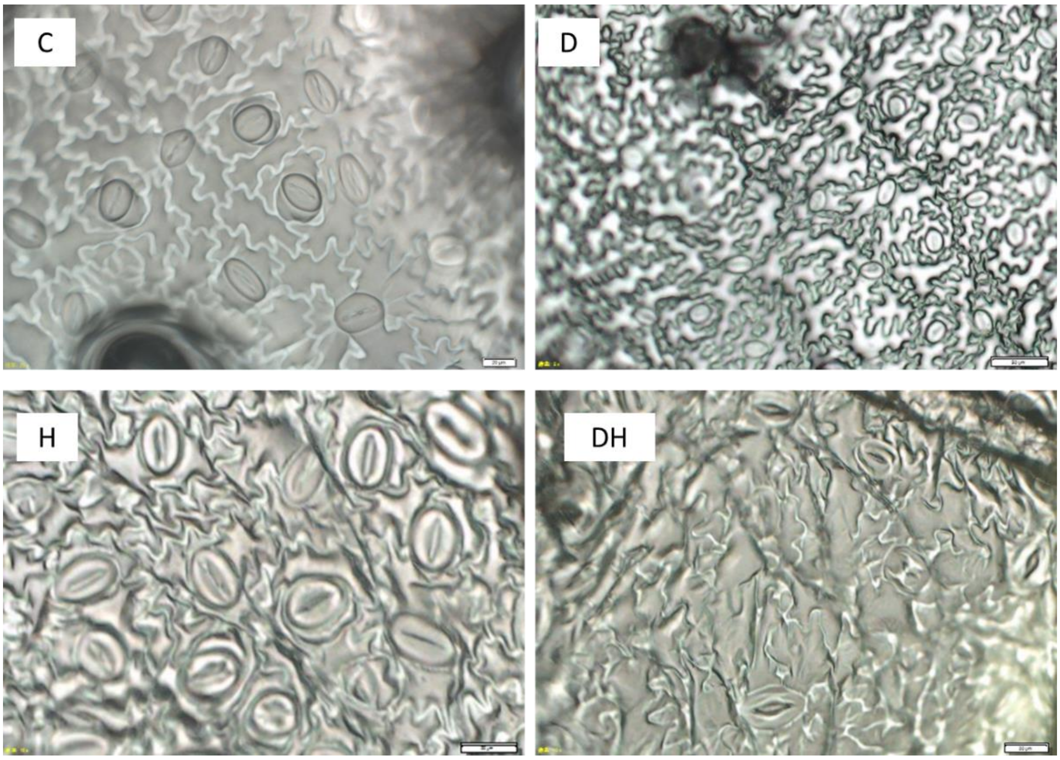


Figure 2.5. The effect of abiotic stress on the stomatal size in Desiree.

The stomatal size was reduced under drought, heat, and combined drought-heat stress. C: non-stress, D: drought stress, DH: combined drought-heat stress, H: heat stress. Scale bars: 20 μm.

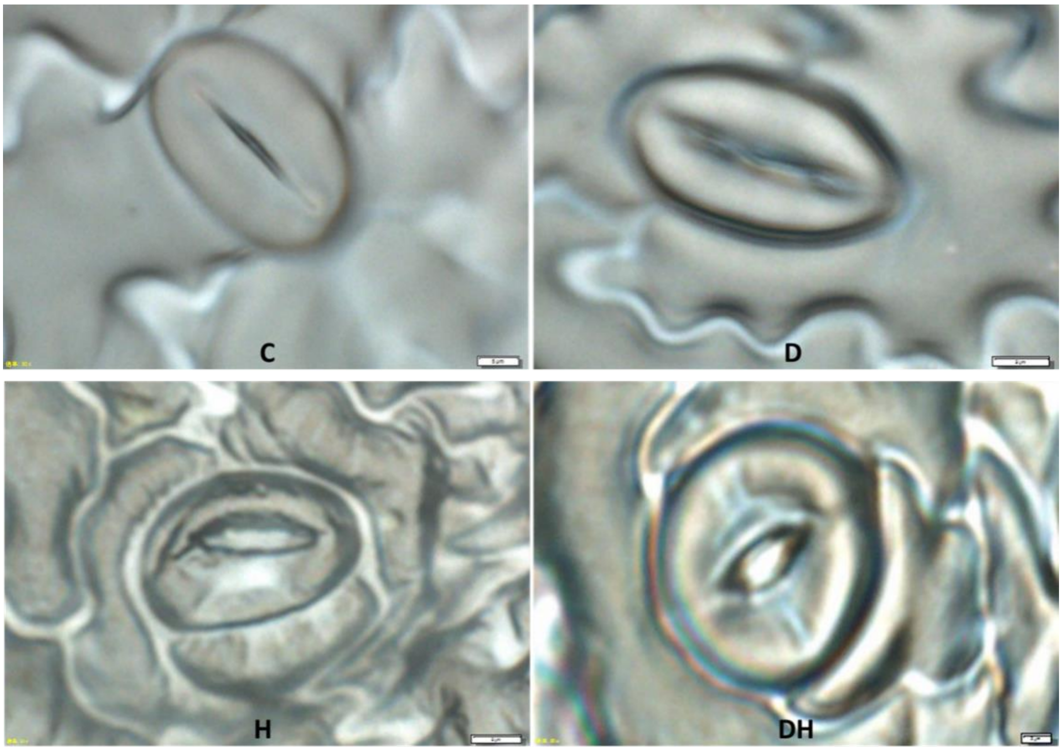


Figure 2.6. The effect of abiotic stress on the stomata pore size in Desiree.

The diameter of the stomata pore increased under heat stress and combined drought-heat stress.

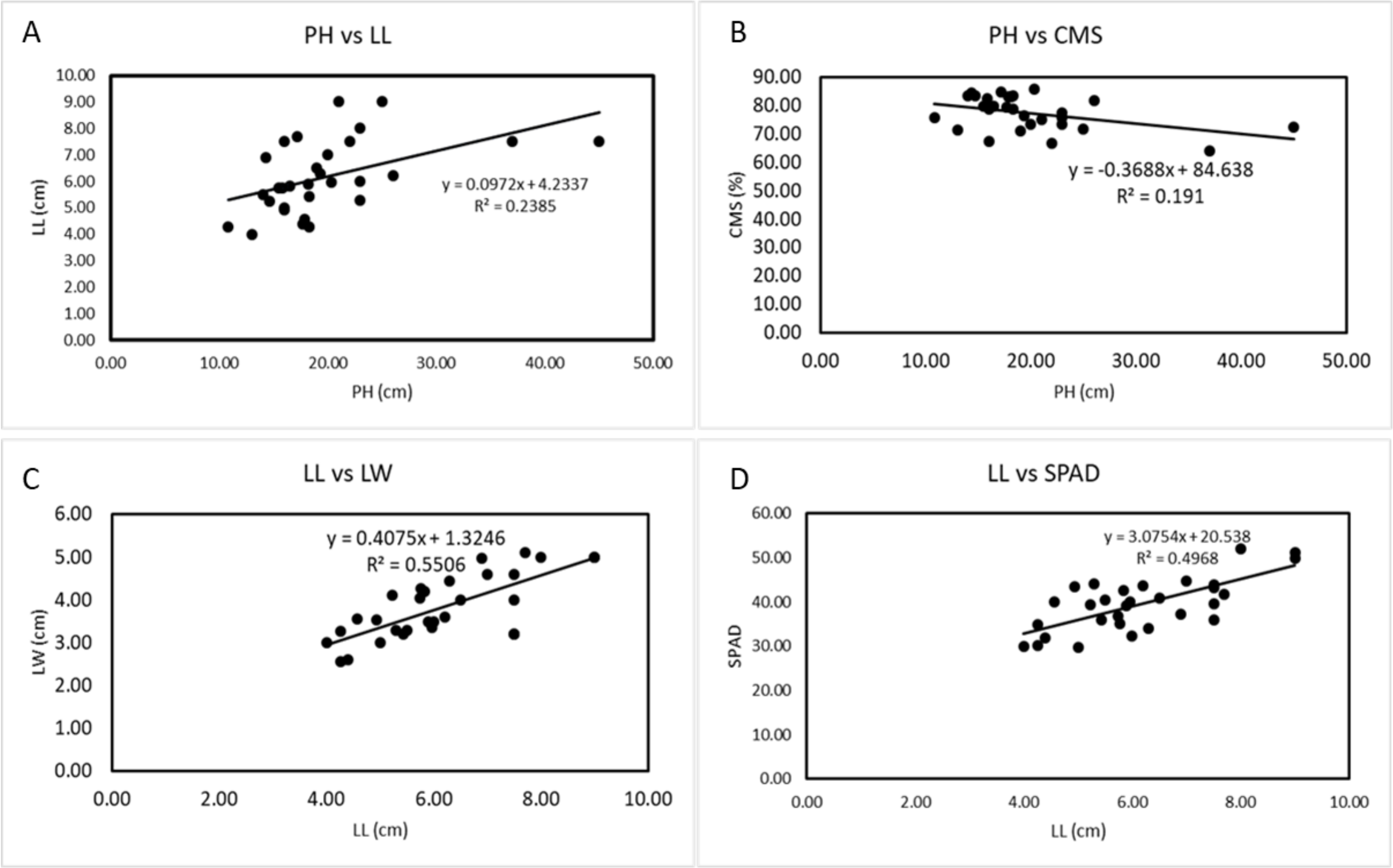
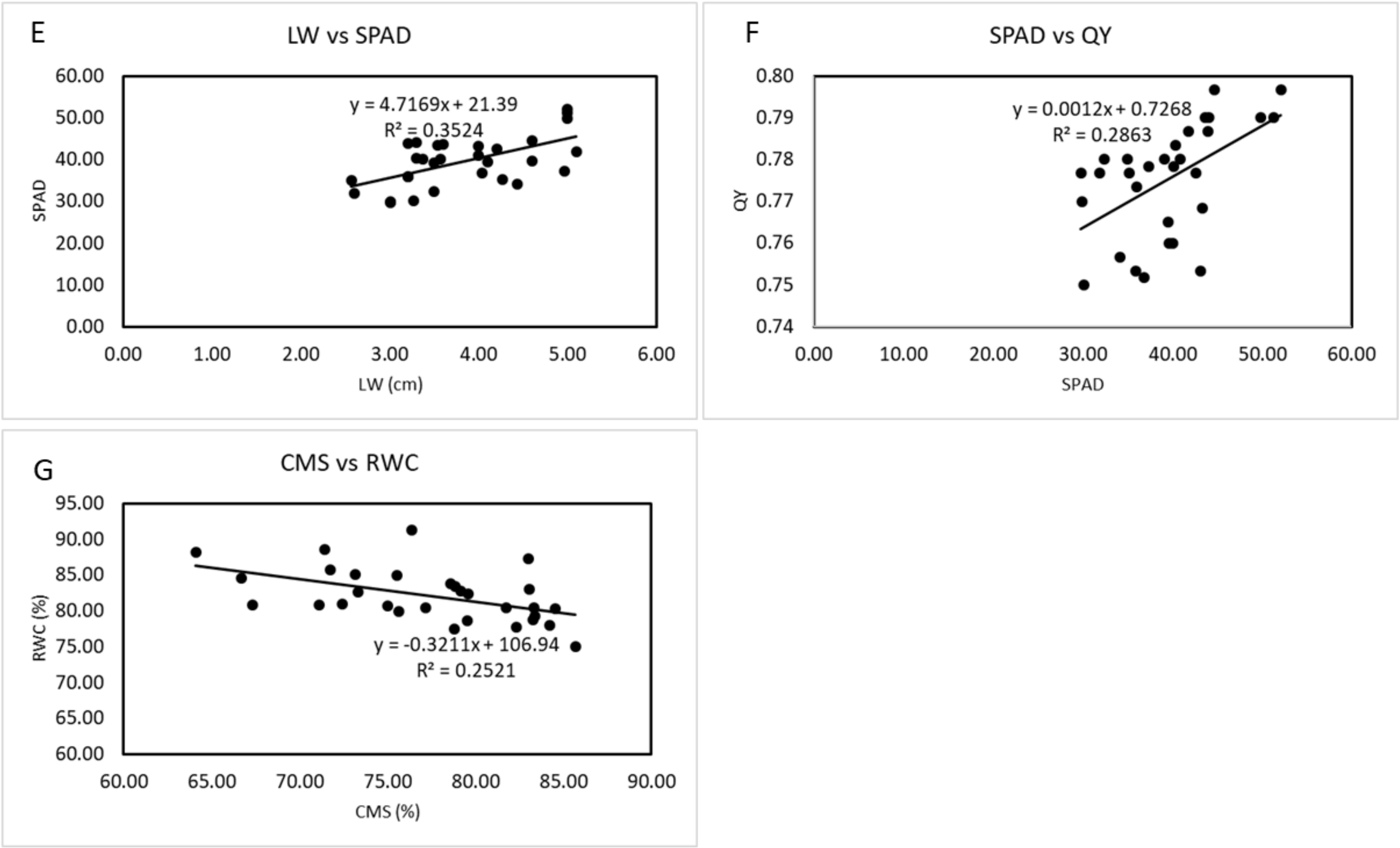


Figure 2.10. The correlations among morpho-physiological traits under the non-stress condition.
A: Positive correlation between plant height (PH) and leaf length (LL). **B:** Negative correlation between PH and cell membrane stability (CMS). **C:** Positive correlation between LL and leaf width (LW). **D:** Positive correlation between LL and chlorophyll content (SPAD). **E:** Positive correlation between LW and SPAD. **F:** Positive correlation between SPAD and photosystem II quantum yield (QY). **G:** Negative correlation between CMS and relative water content (RWC).



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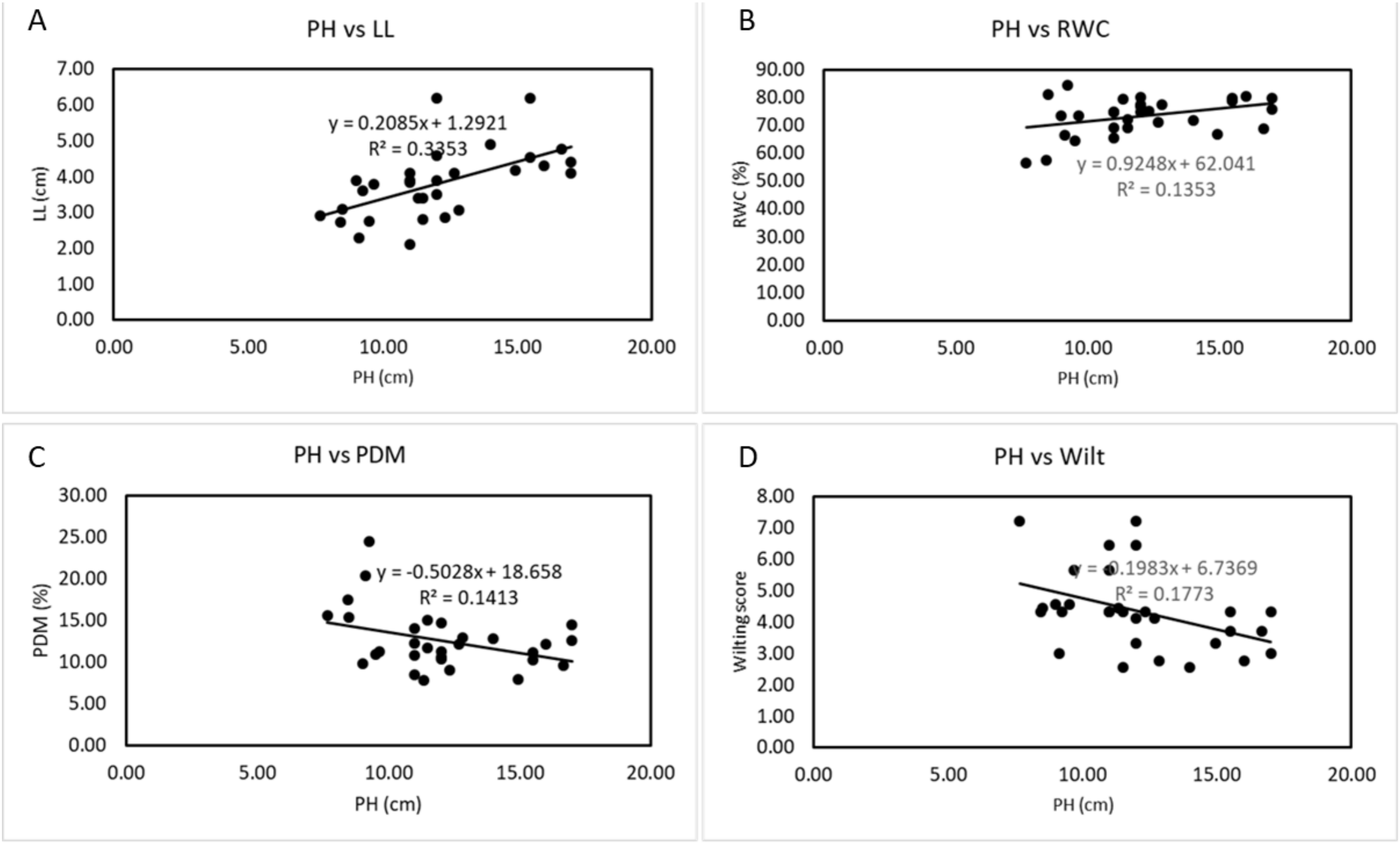
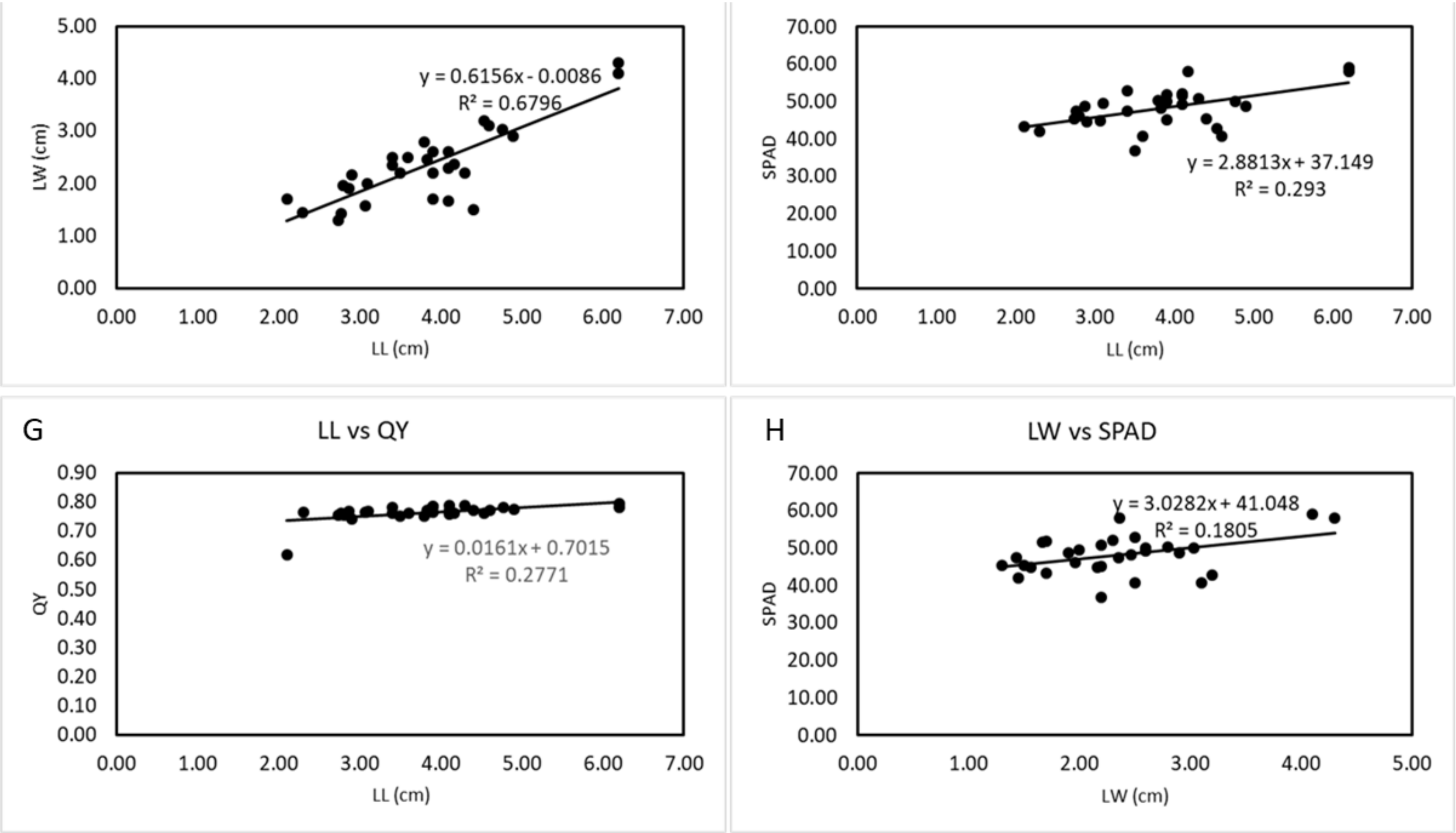


Figure 2.11. The correlations among morpho-physiological traits under the drought stress condition.

A: Positive correlation between plant height (Song *et al.*) and leaf length (LL). **B:** Positive correlation between PH and relative water content (RWC). **C:** Negative correlation between PH and plant dry matter (PDM). **D:** Negative correlation between PH and wilting score (Wilt). **E:** Positive correlation between LL and leaf width (LW). **F:** Positive correlation between LL and chlorophyll content (SPAD). **G:** Positive correlation between LL and photosystem II quantum yield (QY). **H:** Positive correlation between LW and SPAD. **I:** Negative correlation between SPAD and Wilt. **J:** Negative correlation between QY and Wilt. **K:** Negative correlation between cell membrane stability (CMS) and RWC.



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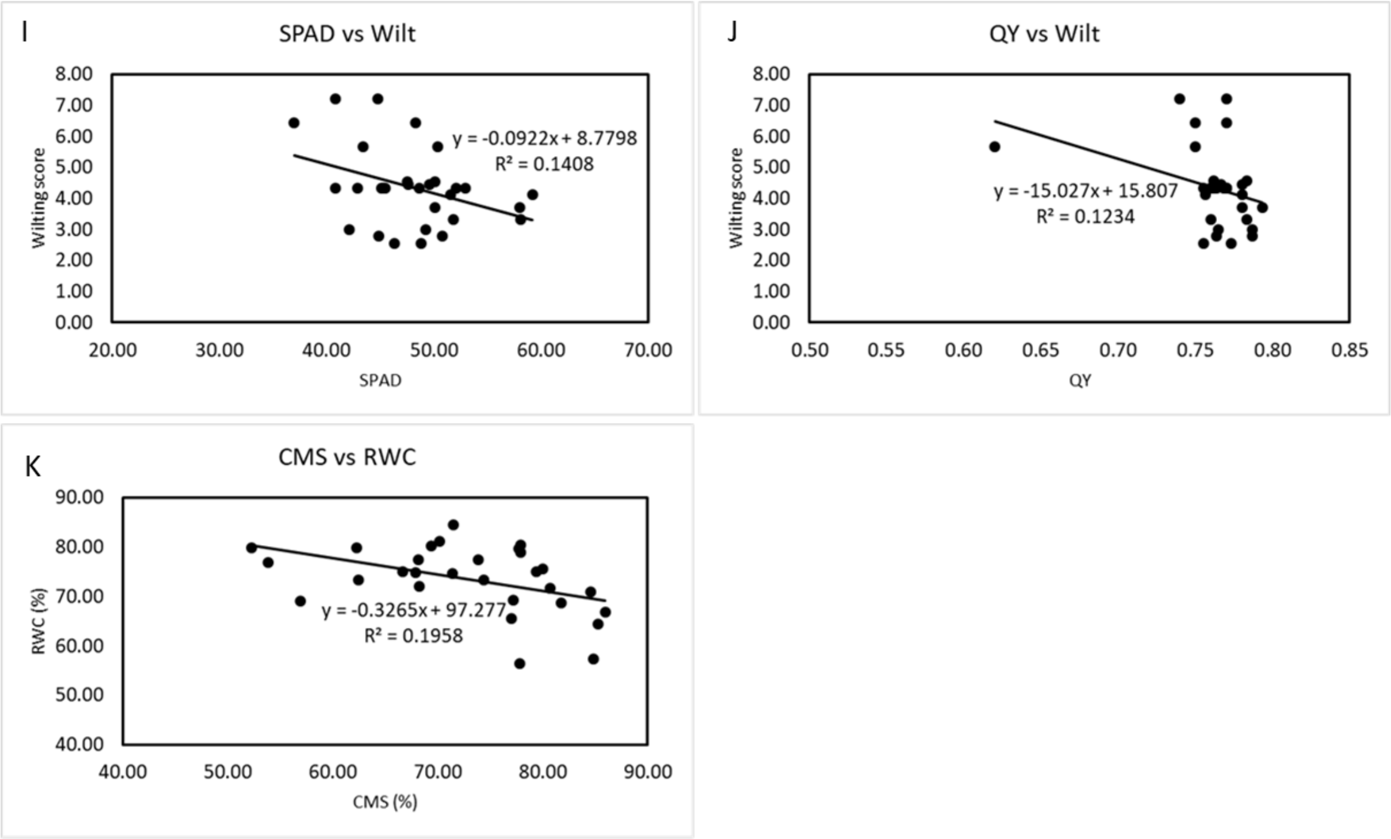

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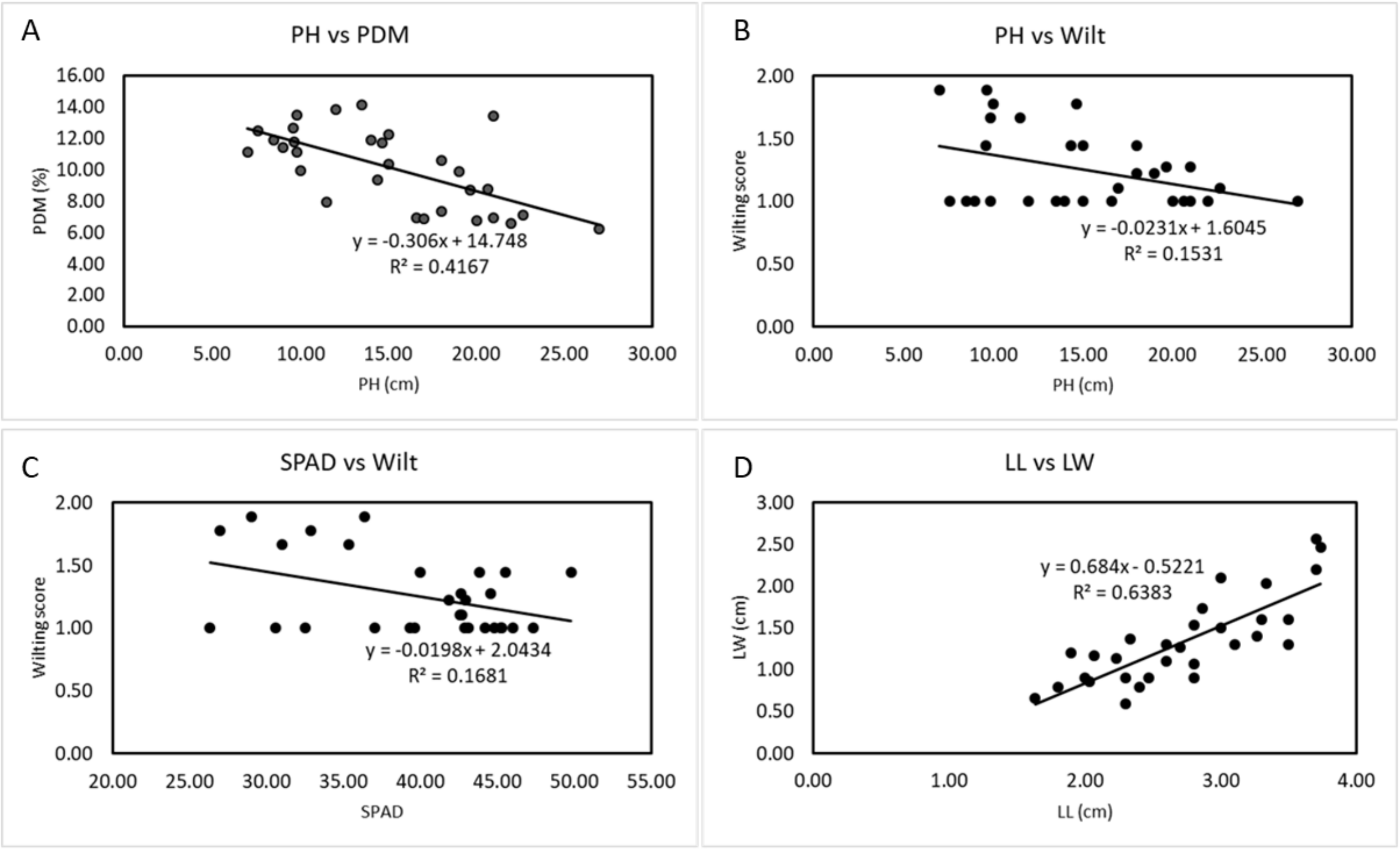
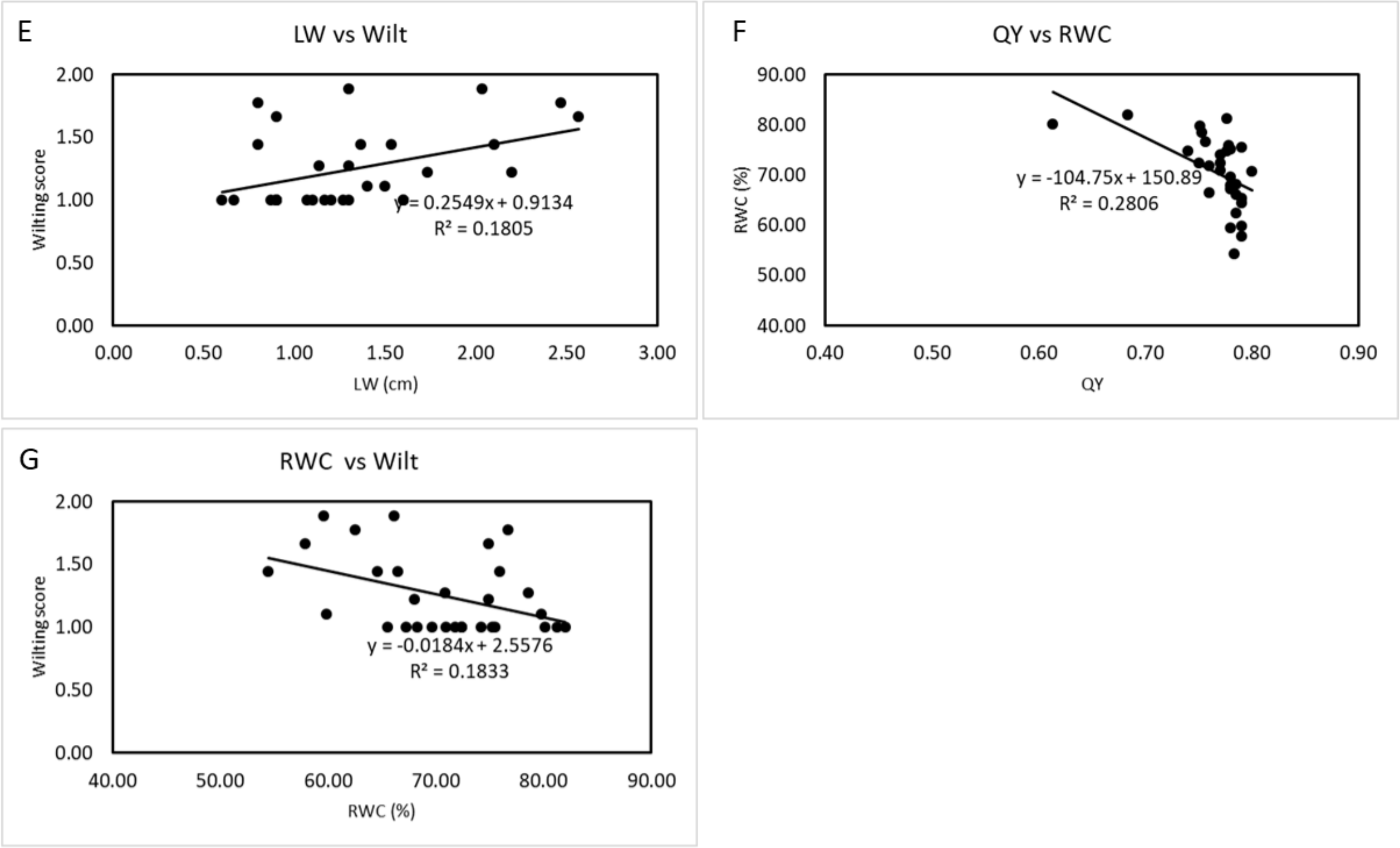


Figure 2.7. The correlations among the morpho-physiological traits under the heat stress condition.

A: Negative correlation between plant height (PH) and plant dry matter (PDM). **B:** Negative correlation between PH and wilting score (Wilt). **C:** Negative correlation between chlorophyll content (SPAD) and Wilt. **D:** Positive correlation between leaf length (LL) and leaf width (LW). **E:** Positive correlation between LW and Wilt. **F:** Negative correlation between photosystem II quantum yield (QY) and relative water content (RWC). **G:** Negative correlation between RWC and Wilt.



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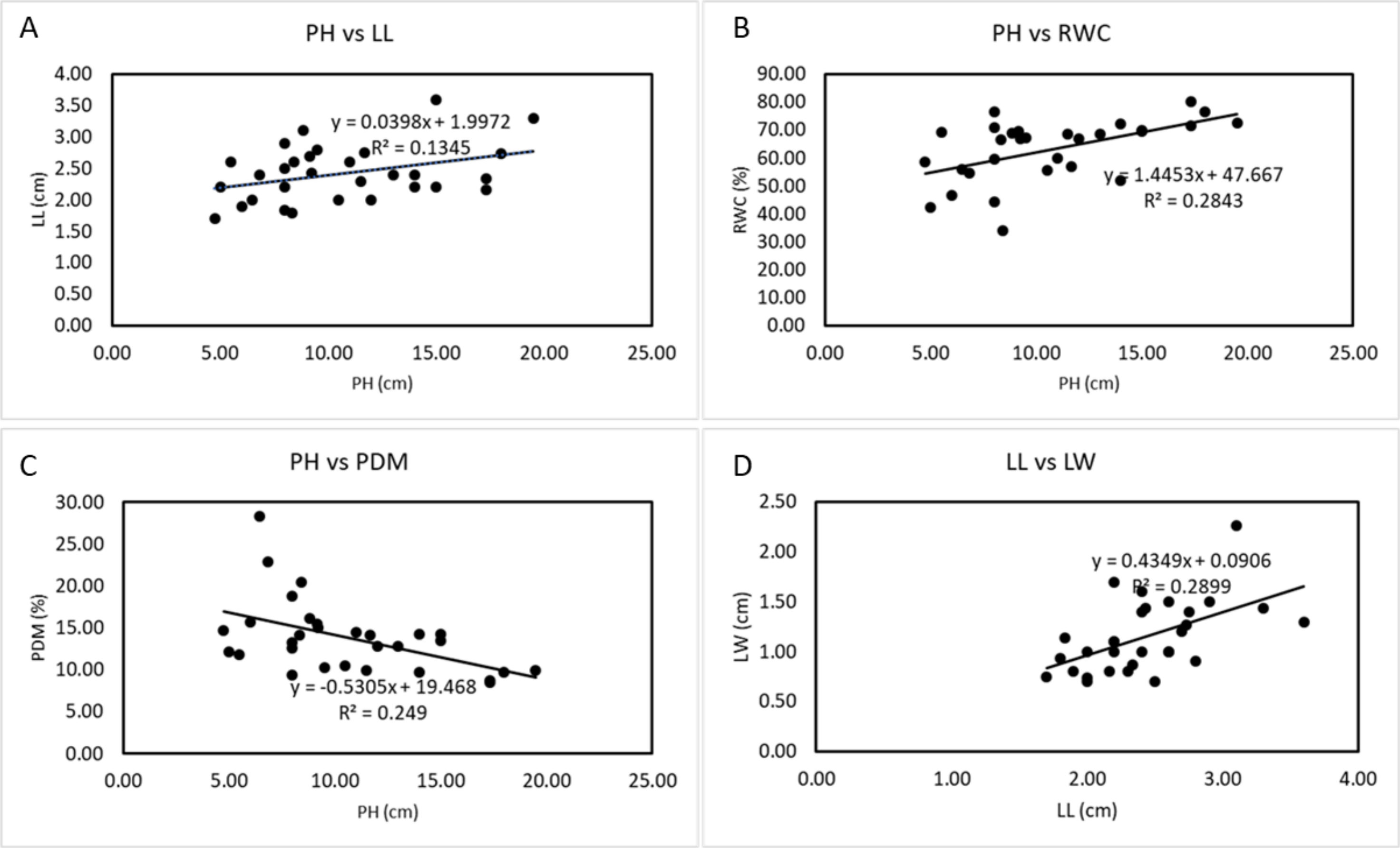

Figure 2.13. The correlations among morpho-physiological traits under the combined drought-heat stress condition. **A:** Positive correlation between plant height (PH) and leaf length (LL). **B:** Positive correlation between PH and relative water content (RWC). **C:** Negative correlation between PH and plant dry matter (PDM). **D:** Positive correlation between LL and leaf width (LW). **E:** Positive correlation between LL and wilting score (Wilt). **F:** Negative correlation between chlorophyll content (SPAD) and Wilt. **G:** Negative correlation between cell membrane stability (CMS) and SPAD. **H:** Negative correlation between RWC and PDM. **I:** Negative correlation between RWC and Wilt.

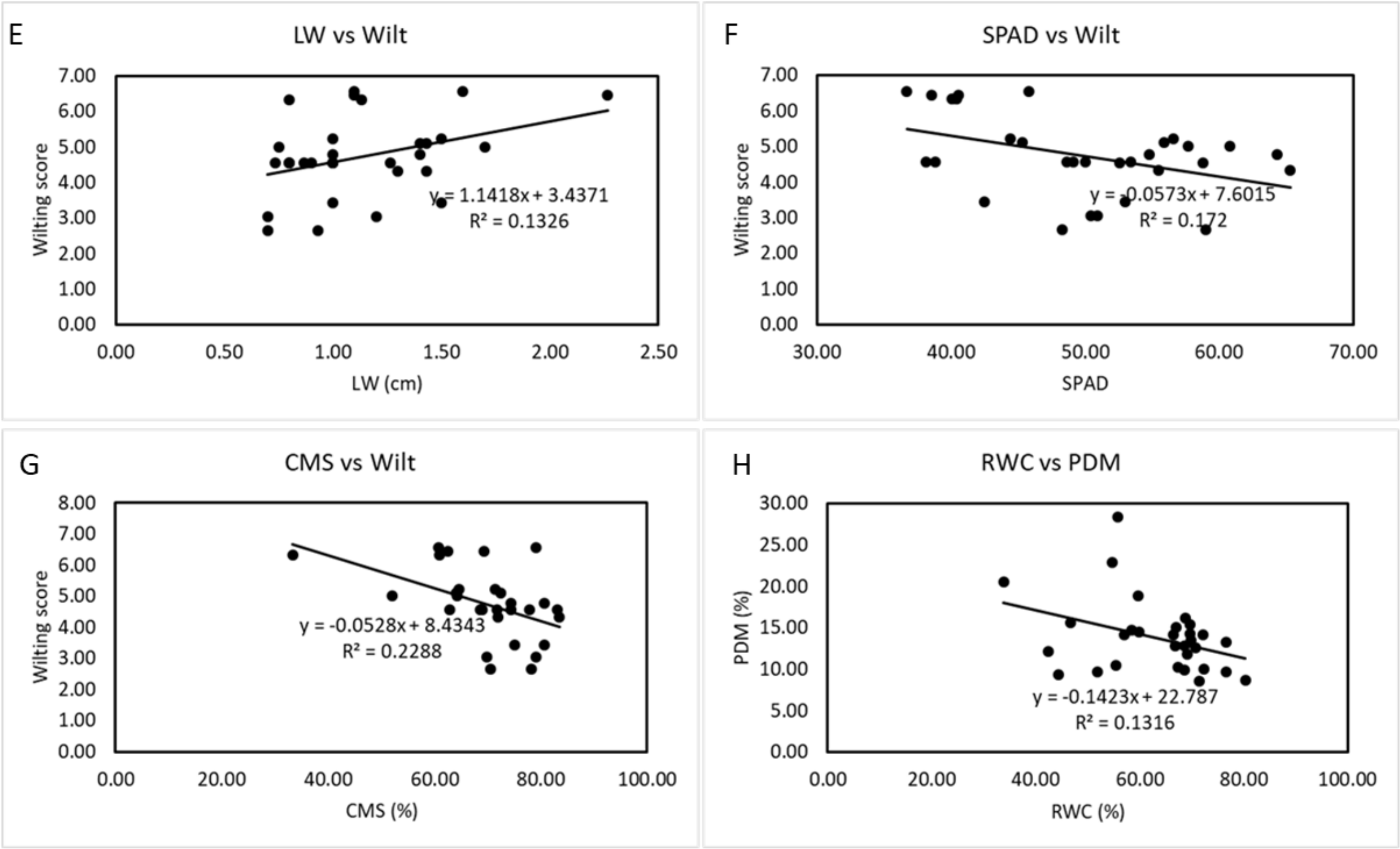

Figure 2.13. Continued

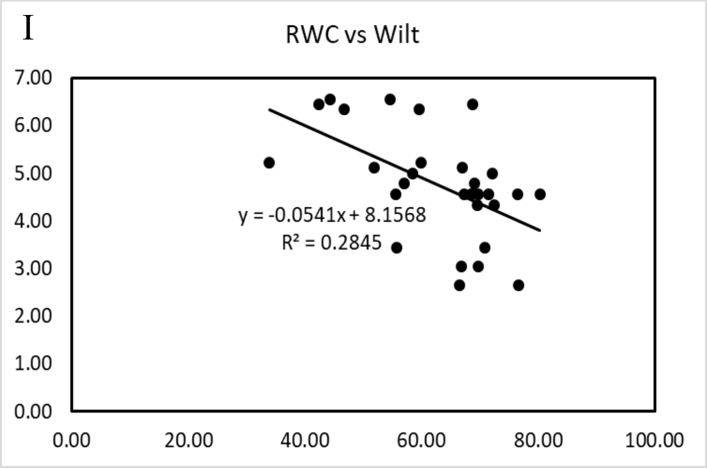

Figure 2.13. Continued.

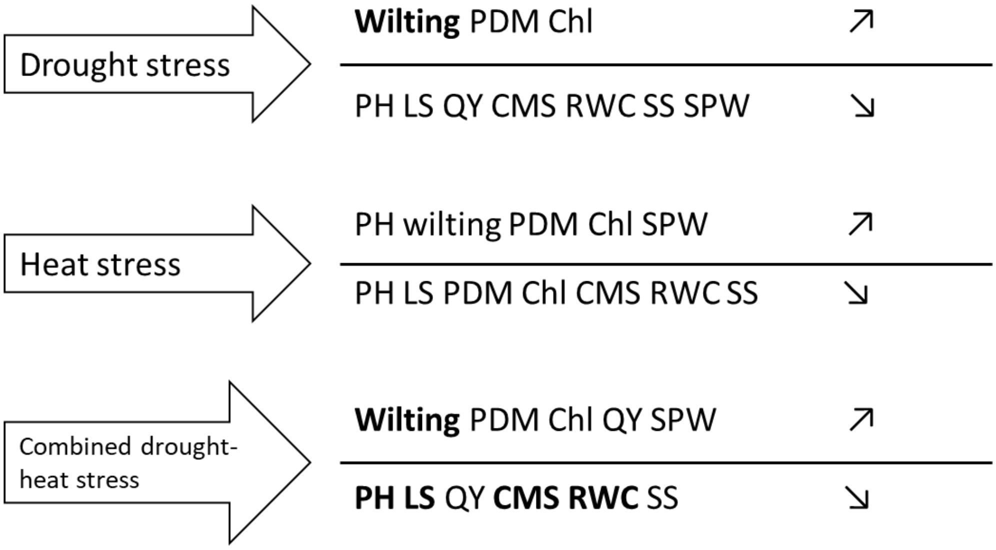

Figure 2.8. Summary of the morpho-physiological responses of potato plants to abiotic stress.

↗, increase. ↘, decrease. **Bold letter:** The most affected by the abiotic stresses. Chl: chlorophyll content, CMS: cell membrane stability, LS: leaf size, PDM: plant dry matter, PH: plant height, QY: photosystem II quantum yield, RWC: relative water content, SPW: stomata pore width, SS: stomata size, wilting: wilting scale.

Chapter 3: Potato stress-related gene expression under drought, heat, and combined drought-heat stress

3.1. Introduction

3.1.1. The molecular responses of potato plants under abiotic stress

In addition to altering the morpho-physiological characteristics of plants, abiotic stress alters plants at the molecular level. Plant cells recognize signals from the external environment via their sensing system and then respond to the abiotic stress on morphological, physiological, biochemical, and molecular levels. Once a membrane receptor receives the extracellular stress signal, it induces and activates a complex signaling cascade in the cell and generates secondary signal molecules (Huang *et al.* 2012; Trewavas and Malho 1997). As illustrated in Figure 3.1 (Huang *et al.* 2012), this signal cascade allows the expression of abiotic stress-responsive genes that enable the plant to tolerate the stress, directly or indirectly.

The signal transduction pathway under osmotic stress has commonly been differentiated into two courses: abscisic acid (ABA)-dependent and ABA-independent. ABA, a plant phytohormone that is highly accumulated under abiotic stress, induces the expression of some abiotic stress-responsive genes, which means that an ABA-dependent regulatory system takes part in the expression of abiotic stress-responsive genes (Lata and Prasad 2011). The genes under control of ABA have ABA-responsive elements (ABREs) in their promoter regions, which function as a *cis*-acting DNA element that is involved in ABA-regulated gene expression (Nakashima *et al.* 2009). The presence of ABREs differentiates the genes in the ABA-dependent pathway from the genes in the ABA-independent pathway, which contain dehydration-responsive elements (DREs) in their promoter regions. The courses of the ABA-dependent and ABA-independent systems in the signal transduction pathway between stress signal perception and gene expression are described in Figure 3.2 (Shinozaki and Yamaguchi-Shinozaki 2007).

Drought stress induces various stress-related genes' encoding proteins such as transcription factors (TFs) and enzymes that contribute to tolerance to drought stress (Shinozaki and Yamaguchi-Shinozaki 2007). A transcription analysis using native Andean potato clones revealed that 99 genes are up- or down-regulated under water deficit conditions, including photosynthetic-related genes, metabolism-related genes, secondary metabolism-related genes, transporter genes, and more (Schafleitner *et al.* 2007b). The drought-tolerant potato genotypes show less accumulations of reactive oxygen species (ROS), high mitochondrial activity, and an active chloroplast defense, which is correlates with high expressions of antioxidant-related genes and high levels of chaperone genes (Vasquez-Robinet *et al.* 2008).

Heat stress also alters the expression pattern of stress-related genes, and potato heat-sensitive and heat-tolerant genotypes show different expression patterns — particularly regarding the photosynthesis-related genes, hormonal activity-related genes, sugar transporter genes, and TFs (Singh *et al.* 2015). The transcriptional profiling of potato under heat stress identified 14 candidate potato heat tolerance genes that are involved in diverse tolerance mechanisms (Gangadhar *et al.* 2014). Associated with the cell membrane as signal perception receptors, non-specific lipid transfer proteins (*nsLTPs*) are abundant in plants that bind or transfer various types of lipid between cells (Carvalho and Gomes 2007). This type of protein is known to be induced by high temperature. Heat shock transcription factors (HSFs) are important transcription regulators that mediate the activation of abiotic stress (including heat stress)-responsive genes (Baniwal *et al.* 2004). The major role of HSFs in potato plants is to regulate heat-responsive genes in the acclimation phase; however, drought and cold stress also induce the expression of *Hsf* genes in expression patterns that differ from those observed under heat stress (Tang *et al.* 2016). For example, heat stress induces a high increase of *Hsf1* that binds to heat shock protein (HSP) promoters and results in an increase in the expression of HSP genes (Zhang *et al.* 2011).

3.1.2. The roles of abiotic stress-related genes in potato plants against abiotic stress

Various TFs have been shown to be involved in abiotic stress tolerance responses. However, dehydration-responsive element binding (DREB) TFs comprise the most well-known TF subfamily; these TFs regulate the activation of the expression of various targeted genes that are associated with osmo-protection and metabolism (Hussain *et al.* 2011) by binding to a DRE *cis*-acting element in ABA-independent gene expression (Shinozaki and Yamaguchi-Shinozaki 1997). Endo (2016) successfully isolated an abiotic stress-related candidate gene, *DREB1A*-like gene, from potato plant. Cold stress induces this gene candidate, which belongs to a family of *DREBs/*ethylene response factors (*ERFs*), and it was thus named *cold responsive ERF-like factor* (*StCEL*) (Endo 2016). *DREBs/ERFs* are members of the *AP2/ERF* superfamily that plays significant roles in transcriptional regulation in most biological processes (including plant growth and development) and responses to various types of environment stimulation (Riechmann and Meyerowitz 1998).

As transcriptional factors, *ERFs* act in both domains as a transcriptional activator and repressor (Mitsuda and Ohme-Takagi 2009). In light of the high similarity between *StCEL* and other class II *ERFs* that have repression activity, Endo (2016) suggested that *StCEL* may be a transcriptional repressor in the class II *ERF* in potato. *StCEL* was highly induced by cold stress and by wound stress, and the translated product will bound to the DRE of the promoter region in target genes (e.g., *TAS14*), which activates the transcription of that gene, followed by the initiation of abiotic stress tolerance (Endo 2016). Gene *TAS14* itself was induced by ABA in response to drought stress, and *TAS14* has roles in membrane stabilization and radical oxygen scavenging (van Muijen 2016) and in reducing the osmotic potential and accumulating solute (Munoz-Mayor *et al.* 2012).

High temperature also induces the expression of genes, some of which are known to contribute to drought stress tolerance. Gangadhar *et al.* (2014) identified non-specific lipid transfer protein in potato (*StnsLTP1*) as a heat stress-induced gene. A transgenic potato plant was developed by using *StnsLTP1,* and the results demonstrated an increase in the plants' tolerance to heat stress as well as to drought and salinity (Gangadhar *et al.* 2016). This gene also induced other heat stress-related genes such as *HSP70*, which functions as a molecular chaperone. Combining the information gained from the previous and present morpho-physiological assessments and the findings regarding abiotic stress-related genes, Figure 3.3 illustrates the potential responses of potato plants to abiotic stress.

3.2. Hypothesis and objective

The hypothesis proposed in this experiment was that (1) combined drought-heat stress will result in the expression of the abiotic stress-related genes *StCEL*, *StTAS14*, *StnsLTP1*, and *StHSP70* at different levels with each single stress, i.e., drought and heat; and (2) the different potato lines will show different gene expression patterns. The objective was to determine the expressions of *StCEL*, *StTAS14*, *StnsLTP1*, and *StHSP70* in the different potato lines under drought, heat, and combined drought-heat stress.

3.3. Materials and methods

3.3.1. Materials

The materials used in this second experiment were taken from the potato plants in the growth room that were treated under the same conditions as the first experiment's plants (Chapter 2). The young leaves were sampled at the end of a 21-day treatment period from each treatment (drought, heat, and combined drought-heat stress) in three replications (Table 3.1). The leaf samples were flash-frozen in liquid nitrogen and kept in the dark at -70°C until extracted.

3.3.2. RNA extraction and cDNA synthesis

RNA extraction was done by using RNeasy® Plant Mini Kit (Qiagen, Hilden, Germany). The extraction followed the manufacturer's protocol. A spectrophotometer (Nanodrop™, Thermo Fisher Scientific, Waltham, MA) was used to quantify and assess the purity of RNA. Electrophoresis was used for checking the integrity of the RNA. RNA was loaded in a 1% agarose gel and run in an electrophoresis system at 100 V for 25 min. Gel visualization was achieved by using a Gel Doc XR+ Gel Documentation System (Bio-Rad, Hercules, CA). Then, cDNA was synthesized by using ReverTraAce® qPCR RT Master Mix with gDNA remover (Toyobo, Osaka, Japan). The procedure followed the manufacturer's protocol.

3.3.3. Gene expression analysis

A gene expression analysis by RT-qPCR was performed using Thunderbird™ SYBR® qPCR Mix. Four microliters of cDNA template was mixed in 36 µl of SYBR master mix containing 8 µM each forward and reverse primer of the target gene. Amplification was done in three technical replications, including a negative control. The four abiotic stress-related genes analyzed were *StCEL, StTAS14, StnsLTP1*, and *StHSP70. Ef1a* was used as the reference gene because it is well-known to be stable under various abiotic stresses (Nicot *et al.* 2005). The primer sequences of the targeted genes and reference gene are provided in Table 3.2. The expression analysis was conducted using relative quantification by the comparative Ct ($2^{-\Delta\Delta CT}$) method (Schmittgen and Livak 2008). A standard curve was created for all genes in order to check the efficiency of the qPCR amplification for each gene. An ANOVA was performed using the $2^{-\Delta\Delta CT}$ value to check the effect of the source of variance on the gene expressions.

3.4. Results

The standard curves developed from each target gene and reference gene were acceptable and resulted in acceptable PCR amplification efficiency (E) values. The PCR amplification efficiency values from the reference gene and each targeted gene were relatively equal, ranging from 1.92 to 2.12. This indicates that the assumption of the comparative Ct ($2^{-\Delta\Delta CT}$) method was acceptable, and that this method can be used for quantifying gene expressions (Schmittgen and Livak 2008).

The ANOVA results revealed that the expressions of the abiotic stress-related genes (except for *StTAS14*) were significantly affected by the abiotic stress treatment and by the potato line (Table 3.3). In addition, the interaction of abiotic stress and potato line had a significant effect on the expressions of *StCEL* and *StnsLTP1*.

In each potato line, the expressions of *StCEL, StTAS14*, *StnsLTP1*, and *StHSP70* were increased by the drought stress, heat stress, and combined drought-heat stress (Table 3.4). The potato plants responded to the different types of abiotic stress by increasing the expressions of the tested abiotic stress-related genes in different fold changes (Figure 3.4). Combined drought-heat stress induced the highest fold change of *StCEL* expression, although this fold change was not significantly different from those brought about by drought and heat stress. The highest expression of *StTAS14* was induced by combined drought-heat stress, and this expression was significantly different from those induced by the other types of abiotic stress. Regarding *StnsLTP1*, drought and combined drought-heat stress induced the gene's expression at the highest fold change. The highest fold changes of *StHSP70* expression occurred due to heat stress and combined drought-heat stress.

The potato line also affected the expressions of the abiotic stress-related genes *StCEL, StnsLTP1,* and *StHSP70* (Figure 3.5). Potato L1 had the biggest fold change of *StCEL* gen, and together with L5 had the highest level of *StnsLTP1* expression. The abiotic stress induced the highest level of *StHSP70* in L5, and this level was not significantly different from those observed in L1 and L2.

The interaction of abiotic stress and potato line affected only the expressions of *StCEL* and *StnsLTP* (Figure 3.6). This interaction effect created different patterns of the expressions of genes *StCEL* and *StnsLTP1* in each potato line (Figure 3.7). No interaction effect of abiotic stress and potato line made the expression level of genes *StTAS14* and *StHSP70* had a similar pattern among the potato lines under different abiotic stresses (Figure 3.7). This similar pattern showed the highest fold change under combined drought-heat stress, with the exception of *StHSP70* in potato L5, which showed the highest fold change under heat stress.

3.5. Discussion

In addition to morpho-physiological responses, potato plants exhibit molecular responses to abiotic stress. These molecular responses are the result of stress perception that occurs on the cell surface or at the cell membrane; such signals are then passed to various subcellular locations and regulate the expressions of stress-responsive genes (Zhu 2016). The results of the gene expression analysis conducted herein demonstrated that all four of the tested genes were abiotic stress-inducible, and that due to the abiotic stress treatments' effects, abiotic stress-related genes were expressed at different levels under the various types of abiotic stresses. In general, the combined drought-heat stress induced the highest expressions of the abiotic stress-related genes. In dessert grass, combined drought-heat stress also induced higher expressions of abiotic stress-related genes compared to drought or heat alone (Alhaithloul 2019). These past findings and the present results indicate that compared to drought and heat stress, combined drought-heat stress induces a stronger effect on the signaling cascade in the cells, which then develop higher levels of gene expression.

The present findings regarding the effect of the potato line on the expression of the abiotic stress genes revealed that the different potato lines expressed the genes at different levels. For example, potato L1 was more responsive to abiotic stress compared to the other lines by increasing its expressions of *StCEL*, *StnsLTP1*, and *StHSP70*, whereas L5 responded to abiotic stress by increasing its expressions of *StnsLTP1* and *StHSP70*. This study also demonstrated different patterns of the expressions of *StCEL* and *StnsLTP1* in the various potato lines under different types of abiotic stress. For example, regarding the *StCEL* expression, combined drought-heat stress induced the largest fold change at L1, but in L2, drought stress induced the largest fold change. These results differ from those obtained for *StTAS14* and *StHSP70*; all of the potato lines showed a similar pattern for these genes, in which the combined drought-heat stress increased the expression of these genes in all lines at the largest fold change.

StCEL was first isolated as a *DREB1A*-like gene in potato, with the rationale that the incorporation of *AtDREB1A* into potato could improve the plant's tolerance to abiotic stress, based on the supposition that potato plants also develop an abiotic stress-responsive system via DREB1A (Endo 2016). The overexpression of *AtDREB1A* in transgenic potato was reported to enhance the tolerance of the potato plants to salinity and drought stress (Huynh *et al.* 2014; Shimazaki *et al.* 2016; Watanabe *et al.* 2011). The vital role of *DREBs* in the plants' tolerance to abiotic stress was suggested to attributable to this TFs binding to the *cis*-acting elements of abiotic stress-responsive genes and induce their expression in response to abiotic stress (Hussain *et al.* 2011). As a *DREB1A*-like gene, *StCEL* was also induced by abiotic stress, confirming that *StCEL* is an abiotic stress-responsive gene in potato plant. As reported by Endo (2016), *StCEL* is a transcriptional repressor of the downstream gene. It was also reported that the expression of *StCEL* increased after stress was applied, and then gradually decreased as the stress continued for a long period.

One of the downstream genes of *StCEL* is *StTAS14*. In this study, *StTAS14* was also responsive to the combined drought-heat stress. Since *TAS14* is an ABA-induced gene, in addition to *StCEL*, other factors are also likely to affect the expression of this gene in the ABA-dependent pathway. *TAS14* enhances the osmotic stress tolerance of tomato plants by reducing the osmotic potential and accumulating solute, thus maintaining the cell membrane's stability (Munoz-Mayor *et al.* 2012). *StnsLTP1* gene expression was highly inducible by drought and heat stress in the present study and was expressed at an even higher level under the combined drought-heat stress. Gangadhar *et al.* (2016) reported that potato plants with the overexpression of *StnsLTP1* showed high survival ability, chlorophyll content, and CMS under drought, heat, and salinity stresses. As a heat-responsive gene, *StHSP70* was increased by the heat stress, at a higher level than that increased by the drought stress. However, the combined drought-heat stress had the greatest effect on the expression of this gene by inducing the largest fold change.

3.6. Conclusion and future perspectives

The results of this second experiment demonstrated that all of the genes tested in the experiment are abiotic stress-inducible. Abiotic stress, i.e., drought stress, heat stress, and combined drought-heat stress, significantly affected the abiotic stress-related genes *StCEL*, *StTAS14*, *StnsLTP1*, and *StHSP70* in potato plants by increasing their expression relative to the non-stress condition. Interestingly, the combined drought-heat stress generally induced the highest expressions of these genes in all of the potato lines, suggesting that under combined drought-heat stress, potato plants develop a specific pathway which is more complex than the pathways used in response to a single type of stress.

The leaf samples used for the RNA extraction in this experiment were taken from 21-day-old plants raised in the growth room. In a future experiment, it would be of interest to take samples periodically to investigates the dynamics of gene expression over the duration of the abiotic stress. The stability of these genes might be associated with the amount of time that the stress continues.

It was revealed that *AtDREB1A* enhances abiotic stress tolerance in potato (Watanabe *et al.* 2011). Since *StCEL* is a *DREBa*-like gene, it can be speculated that the accumulation of *StCEL* also supports the tolerance of potato plants to abiotic stress, perhaps by activating the downstream genes. The *StCEL* gene was identified as a transcriptional repressor of the downstream gene, i.e., *TAS14* (Endo 2016). Dehydrin (or *TAS14*) is one of the abiotic stress-related genes that regulate the potential osmotic reduction and solute accumulation and are observed at high expression levels in potato plants under drought stress (van Muijen *et al.* 2016). The overexpression of *TAS14* increased the tolerance of tomato plants to drought stress and salinity (Munoz-Mayor *et al.* 2012).

In addition to being directly involved in plants' responses to abiotic stress by maintaining the cell membrane integrity, *StnsLTP1* also induces another heat stress-responsive gene, *StHSP70* (Gangadhar *et al.* 2016). Associations between *StCEL* and *StTAS14* and between *StnsLTP1* and *StHSP70* may help explain the mechanisms underlying potato plants' adaptation and tolerance to abiotic stress. For the clarification of such associations, advanced molecular studies using potato genotypes that are sensitive versus tolerant to each type of abiotic stress are needed.

Table 3.1. Sample materials

Sample no.	Treatment	Sample no.	Treatment	Sample no.	Treatment	Sample no.	Treatment
1	CL1.1	16	DL1.1	31	HL1.1	46	DHL1.1
2	CL1.2	17	DL1.2	32	HL1.2	47	DHL1.2
3	CL1.3	18	DL1.3	33	HL1.3	48	DHL1.3
4	CL2.1	19	DL2.1	34	HL2.1	49	DHL2.1
5	CL2.2	20	DL2.2	35	HL2.2	50	DHL2.2
6	CL2.3	21	DL2.3	36	HL2.3	51	DHL2.3
7	CL3.1	22	DL3.1	37	HL3.1	52	DHL3.1
8	CL3.2	23	DL3.2	38	HL3.2	53	DHL3.2
9	CL3.3	24	DL3.3	39	HL3.3	54	DHL3.3
10	CL4.1	25	DL4.1	40	HL4.1	55	DHL4.1
11	CL4.2	26	DL4.2	41	HL4.2	56	DHL4.2
12	CL4.3	27	DL4.3	42	HL4.3	57	DHL4.3
13	CL5.1	28	DL5.1	43	HL5.1	58	DHL5.1
14	CL5.2	29	DL5.2	44	HL5.2	59	DHL5.2
15	CL5.3	30	DL5.3	45	HL5.3	60	DHL5.3

C: non-stress condition, D: PEG-induced drought stress, DH: combined drought-heat stress, H: heat stress. L1: 84.194.30, L2: 86.61.26, L3: 87HW13.7, L4: DG81-68; L5: Desiree. The treatment codes are followed by the replication no. (1, 2, and 3).

Table 3.2. Primers used in the experiment

No.	Gene/TFs	Primer sequence	Reference
1.	*StCEL*	F: TACCCGATTTTCCCTCAGCAGC R: TCCGACACACTAGAAACAACTCC	Endo (2016)
2.	*StTAS14*	F: CAACAGCAGCTTCGTCGATC R: CATGTCCTCCTCCTGGCATC	van Muijen *et al.* (2016)
3.	*StnsLTP1*	F: CCTGAAATCGGCAGCTAATTC R: GTGGAAGGGCTGATCTTGTA	Gangadhar *et al.* (2014, 2016)
4.	*StHSP70*	F: CATTGATCTTGGTACAACTTAT R: GGAGTTGTTCTGTTGCCTTG	Gangadhar *et al.* (2016)
5.	*Ef1a*	F: ATTGGAAACGGATATGCTCCA R: TCCTTACCTGAACGCCTGTCA	Nicot *et al.* (2005)

Table 3.3. ANOVA results of gene expressions

Gene	**Replication**		**Abiotic stress**		**Potato line**		**Abiotic stress × Potato line**	
	F	**p**	**F**	**p**	**F**	**p**	**F**	**p**
StCEL	1.8090	ns	12.155	***	6.051	***	2.854	**
StTAS14	1.2003	ns	99.4699	***	0.5447	ns	0.6531	ns
StnsLTP1	5.0437	ns	22.403	***	9.476	***	4.38	***
StHSP70	0.3095	ns	30.0824	***	3.621	*	1.3812	ns

***p<0.001, **p<0.01, *p<0.05, ns: non-significant.

Table 3.4. The expressions of abiotic stress-related genes in five potato lines under various types of abiotic stress

Treatment	*StCEL*	*StTAS14*	*StnsLTP1*	*StHSP70*
Abiotic stress				
C	1.00 ± 0.00 ^{b}	1.00 ± 0.00 ^{c}	1.00 ± 0.00 ^{c}	1.00 ± 0.00 ^{b}
D	2.41 ± 0.36 ^{ab}	39.06 ± 6.33 ^{b}	13.72 ± 3.50 ^{a}	2.49 ± 0.63 ^{b}
H	2.12 ± 0.29 ^{ab}	27.60 ± 6.39 ^{b}	5.07 ± 0.76 ^{b}	17.30 ± 4.67 ^{a}
DH	2.69 ± 0.36 ^{a}	232.27 ± 33.64 ^{a}	15.78 ±2.95 ^{a}	18.31 ± 3.77 ^{a}
Potato line				
L1	2.73 ± 0.46 ^{a}	67.05 ± 32.29 ^{a}	13.95 ± 4.93 ^{a}	11.39 ± 4.91 ^{ab}
L2	1.91 ± 0.32 ^{ab}	99.00 ± 41.16 ^{a}	4.68 ± 0.96 ^{b}	8.62 ± 2.98 ^{ab}
L3	2.12 ± 0.45 ^{ab}	63.34 ± 20.27 ^{a}	6.28 ± 1.46 ^{ab}	6.65 ± 2.10 ^{b}
L4	1.99 ± 0.34 ^{ab}	84.14 ± 32.76 ^{a}	5.64 ± 1.87 ^{b}	4.86 ± 1.59 ^{b}
L5	1.64 ± 0.20 ^{b}	61.39 ± 38.89 ^{a}	13.92 ± 3.53 ^{a}	17.34 ± 6.26 ^{a}
Abiotic stress × Potato line				
CL1	1.00 ± 0.00 ^{b}	1.00 ± 0.00 ^{c}	1.00 ± 0.00 d	1.00 ± 0.00 ^{c}
CL2	1.00 ± 0.00 ^{b}	1.00 ± 0.00 ^{c}	1.00 ± 0.00 d	1.00 ± 0.00 ^{c}
CL3	1.00 ± 0.00 ^{b}	1.00 ± 0.00 ^{c}	1.00 ± 0.00 d	1.00 ± 0.00 ^{c}
CL4	1.00 ± 0.00 ^{b}	1.00 ± 0.00 ^{c}	1.00 ± 0.00 d	1.00 ± 0.00 ^{c}
CL5	1.00 ± 0.00 ^{b}	1.00 ± 0.00 ^{c}	1.00 ± 0.00 d	1.00 ± 0.00 ^{c}
DL1	2.12 ± 0.35 ^{ab}	29.99 ± 8.75 ^{bc}	26.15 ± 15.53 ^{ab}	1.86 ± 0.59 ^{c}
DL2	2.67 ± 0.92 ^{ab}	56.73 ± 13.08 ^{bc}	5.53 ± 1.89 $^{b\text{-}d}$	2.00 ± 0.59 ^{c}
DL3	2.63 ± 1.55 ^{ab}	46.12 ± 16.78 ^{bc}	13.65 ± 1.86 ^{a}-d	1.44 ± 1.07 ^{c}
DL4	2.86 ± 0.76 ^{ab}	44.61 ± 19.29 ^{bc}	4.67 ± 2.38 ^{cd}	1.06 ± 0.19 ^{c}
DL5	1.77 ± 0.33 ^{ab}	17.85 ± 6.36 ^{c}	18.60 ± 3.83 $^{a\text{-}d}$	6.09 ± 1.86 ^{bc}
HL1	2.95 ± 0.24 ^{ab}	28.44 ± 12.20 ^{bc}	6.21 ± 1.89 $^{b\text{-}d}$	11.87 ± 1.46 $^{a\text{-}c}$
HL2	1.80 ± 0.64 ^{ab}	14.82 ± 6.75 ^{c}	3.71 ± 1.41 ^{cd}	14.84 ± 8.38 $^{a\text{-}c}$
HL3	2.63 ± 0.94 ^{ab}	56.51 ± 15.23 ^{bc}	4.62 ± 0.97 ^{cd}	10.71 ± 5.20 $^{a\text{-}c}$
HL4	1.57 ± 0.79 ^{ab}	26.57 ± 17.17 ^{bc}	4.68 ± 2.99 ^{cd}	6.64 ± 2.25 ^{bc}
HL5	1.66 ± 0.37 ^{ab}	11.65 ± 8.91 ^{c}	6.12 ± 1.55 $^{b\text{-}d}$	42.43 ± 15.99 ^{a}
DHL1	4.87 ± 0.58 ^{a}	208.75 ± 87.51 $^{a\text{-}c}$	22.43 ± 8.09 $^{a\text{-}c}$	30.84 ± 15.50 ^{ab}
DHL2	2.15 ± 0.54 ^{ab}	323.45 ± 51.32 ^{a}	8.46 ± 1.48 $^{b\text{-}d}$	16.64 ± 4.79 $^{a\text{-}c}$
DHL3	1.79 ± 0.51 ^{ab}	149.73 ± 51.78 $^{a\text{-}c}$	5.83 ± 0.31 $^{b\text{-}d}$	13.44 ± 2.81 $^{a\text{-}c}$
DHL4	2.53 ± 0.52 ^{ab}	264.36 ± 28.39 ^{ab}	12.21 ± 5.38 $^{a\text{-}d}$	10.76 ± 4.15 $^{a\text{-}c}$
DHL5	2.11 ± 0.52 ^{ab}	215.08 ± 132.64 $^{a\text{-}c}$	29.96 ± 1.86 ^{a}	19.87 ± 9.38 $^{a\text{-}c}$

The gene expression values are presented as the fold change of the gene expression normalized to *Ef1a*, and relative to the non-stress condition. Treatments with the same letter are not significantly different (Tukey's HSD test, α= 0.05). Each value is the mean value ± SE (standard error) of 12, 15, and 3 samples for Abiotic stress, Potato line, and Abiotic stress × Potato line, respectively. C: non-stress condition, D: drought stress, H: heat stress, DH: combined drought-heat stress. L1: 84.194.30, L2: 86.61.26, L3: HW13.7, L4: DG81-68, L5: Desiree.

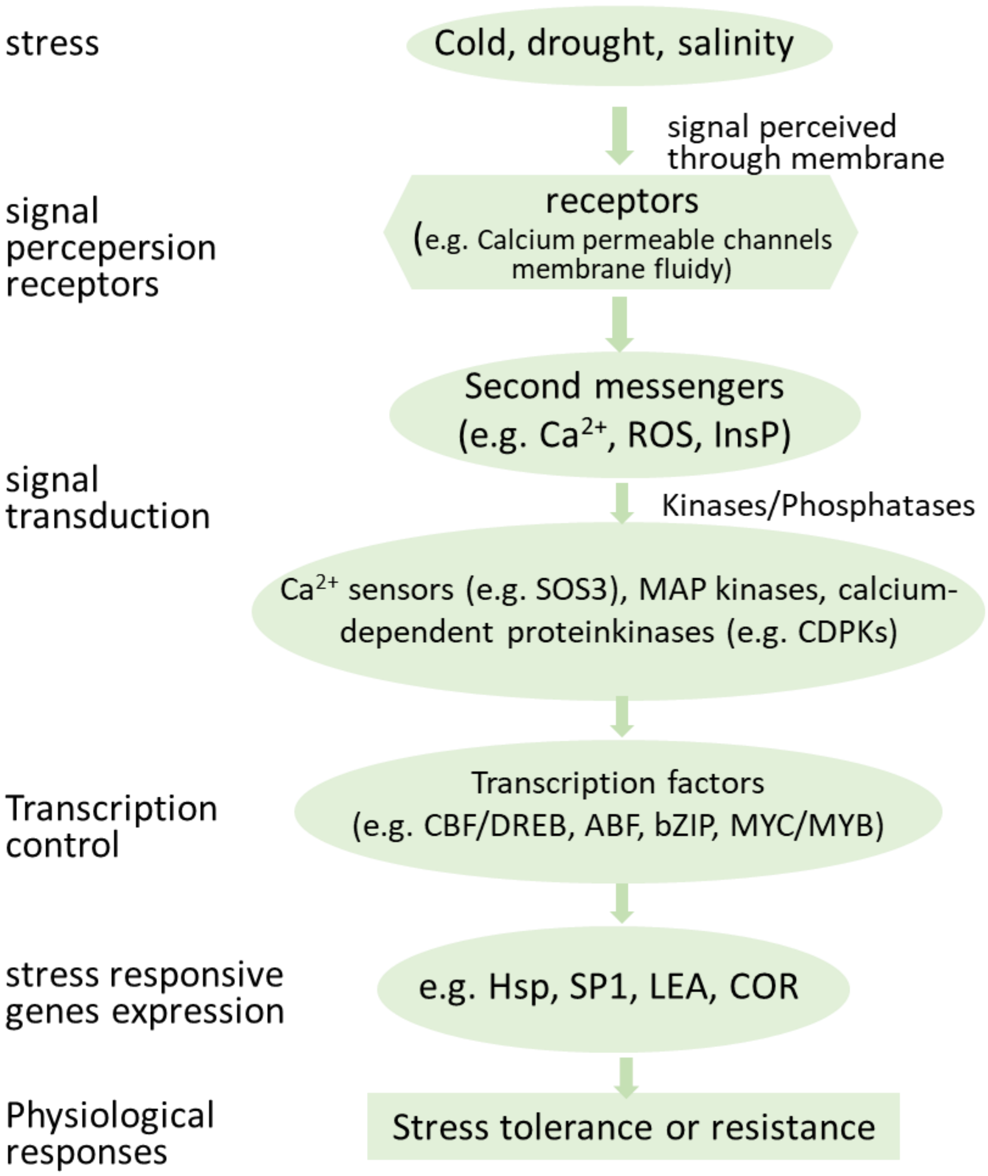

Figure 3.1. A generic pathway for plants' responses to stress.

First, the membrane receptors perceive the extracellular stress signal. A large and complex intracellular signaling cascade (including secondary signal molecules) is then activated. As a result, multiple stress-responsive genes will be expressed; these genes can develop stress tolerance, directly or indirectly. Available at: https://www.researchgate.net/publication/51128272_Signal_transduction_during_cold_salt_and_drought_stresses_in_plants [accessed Sep 30, 2019].

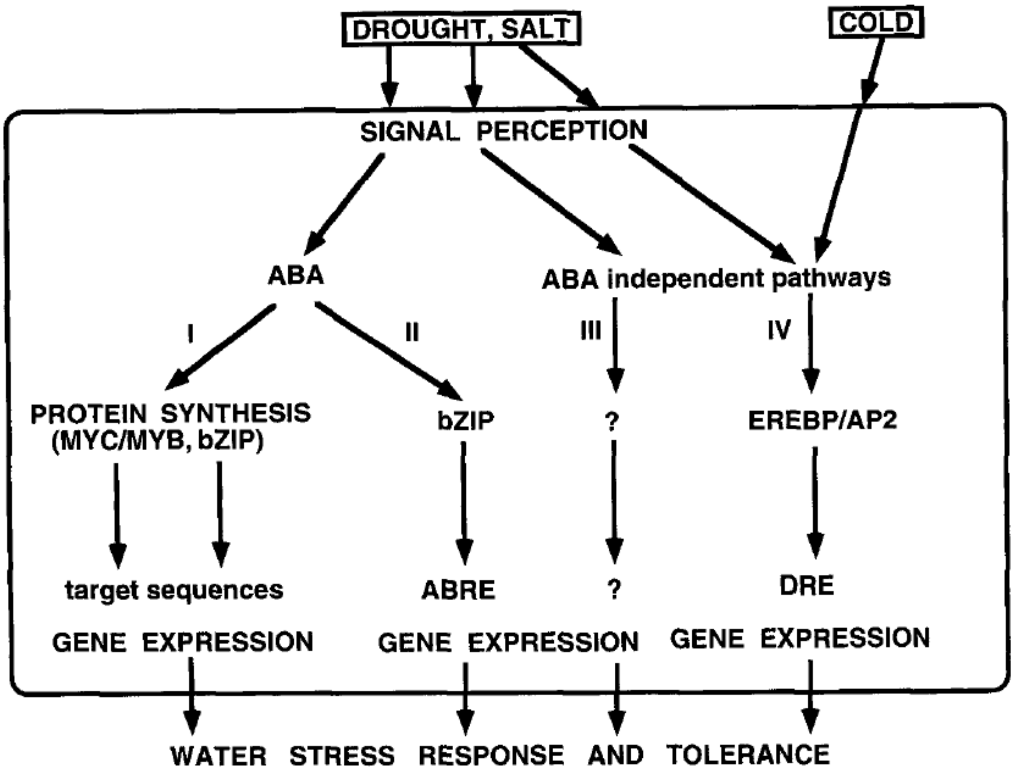

Figure 3.2. Signal transduction pathways between the perception of a water-stress signal and gene expression.

At least four signal transduction pathways exist (I–IV): two are ABA-dependent (I and II) and two are ABA-independent (III and IV). Protein biosynthesis is required in one of the ABA-dependent pathways (I). In another ABA-dependent pathway, the ABA-responsive element (ABRE) does not require protein biosynthesis (II). In one of the ABA-independent pathways, a dehydration-responsive element (DRE) is involved in the regulation of genes not only by drought and salt exposure but also by cold stress (IV). Another ABA-independent pathway is controlled by drought and salt exposure but not by cold (III) (adopted from Shinozaki and Yamaguchi-Shinozaki 1997).

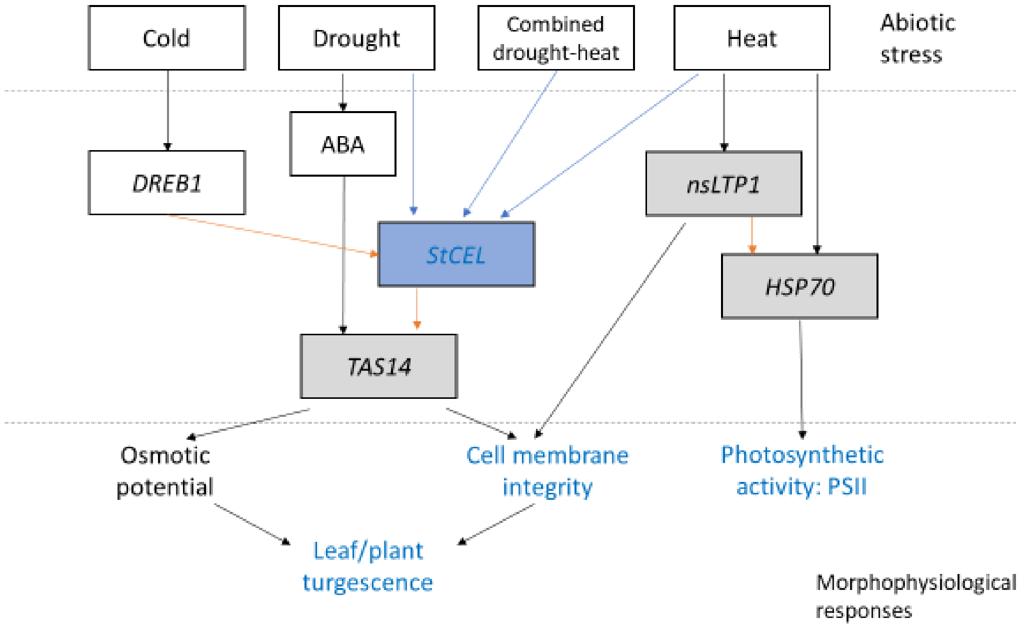

Figure 3.3. The possible potato plant responses to abiotic stress.

Abiotic stresses will activate some transcription factors (TFs) and genes in each pathway. The TFs and genes are involved in plant morpho-physiological processes that define plant responses to abiotic stresses. *Blue arrows:* Based on this study. *Orange arrows:* Downstream role (based on the relevant literature). *Blue letters:* Morpho-physiological traits that were measured in this study (photosynthetic activity is represented by the PSII quantum yield or QY).

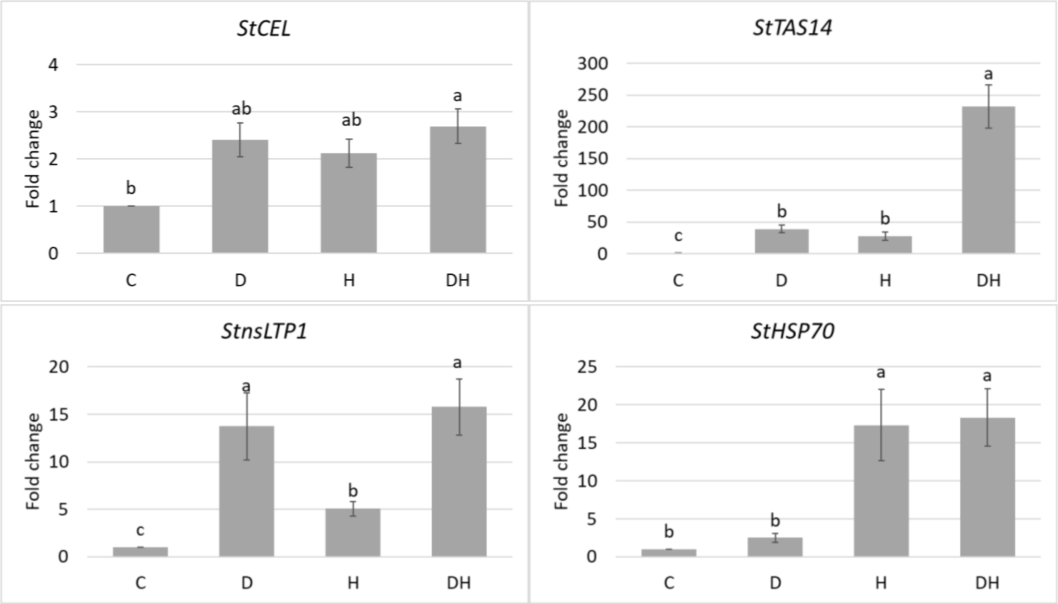

Figure 3.4. The expression of abiotic stress-related genes of potato plants under different abiotic stresses.

The gene expression values are presented as the fold change of gene expression normalized to *Ef1a*, and relative to the non-stress condition. Abiotic stress treatment significantly affected the expression of all of the abiotic stress-related genes. Generally, combined drought-heat stress induced the highest levels of the abiotic stress-related genes. C: non-stress, D: drought stress, DH: combined drought-heat stress, H: heat stress. Error bar: the SE of the mean from 15 samples. Different letters above the error bars indicate significant differences among treatments by Tukey's HSD test at $\alpha = 0.05$.

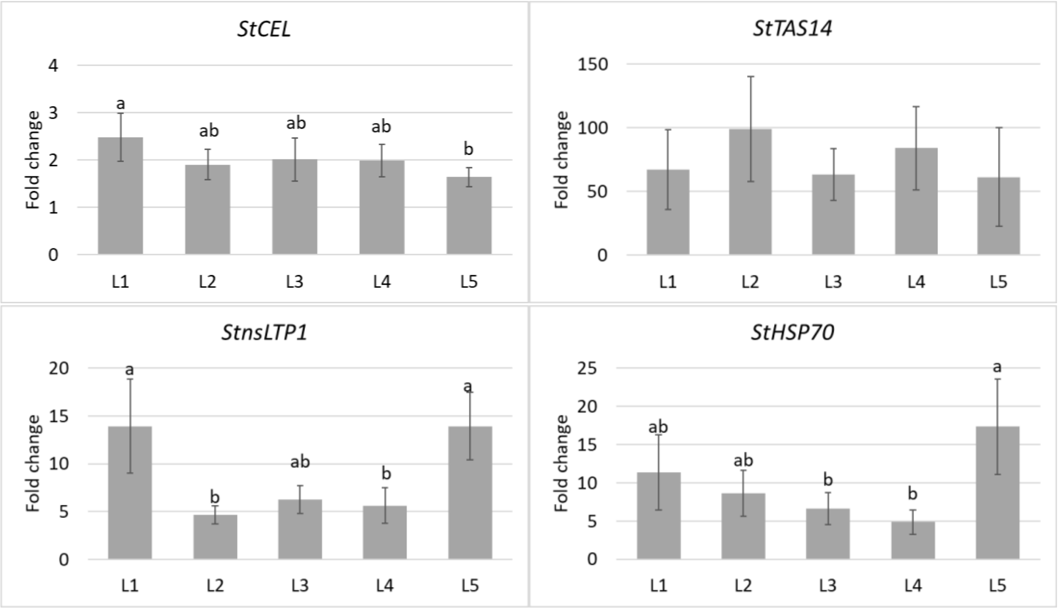

Figure 3.5. Abiotic stress-related gene-expression fold changes due to abiotic stress in the different potato lines.

The gene expression values are presented as the fold change of the gene expression normalized to *Ef1a*, and relative to the non-stress condition. Potato line treatment significantly affected the expressions of *StCEL*, *StnsLTP1*, and *StHSP70*. L1: 84.194.30, L2: 86.61.26, L3: 87HW13.7, L4: DG81-68, L5: Desiree. Error bar: The SE of mean from 15 samples. Different letters above the error bars indicate significant differences among treatments by Tukey's HSD test at α = 0.05.

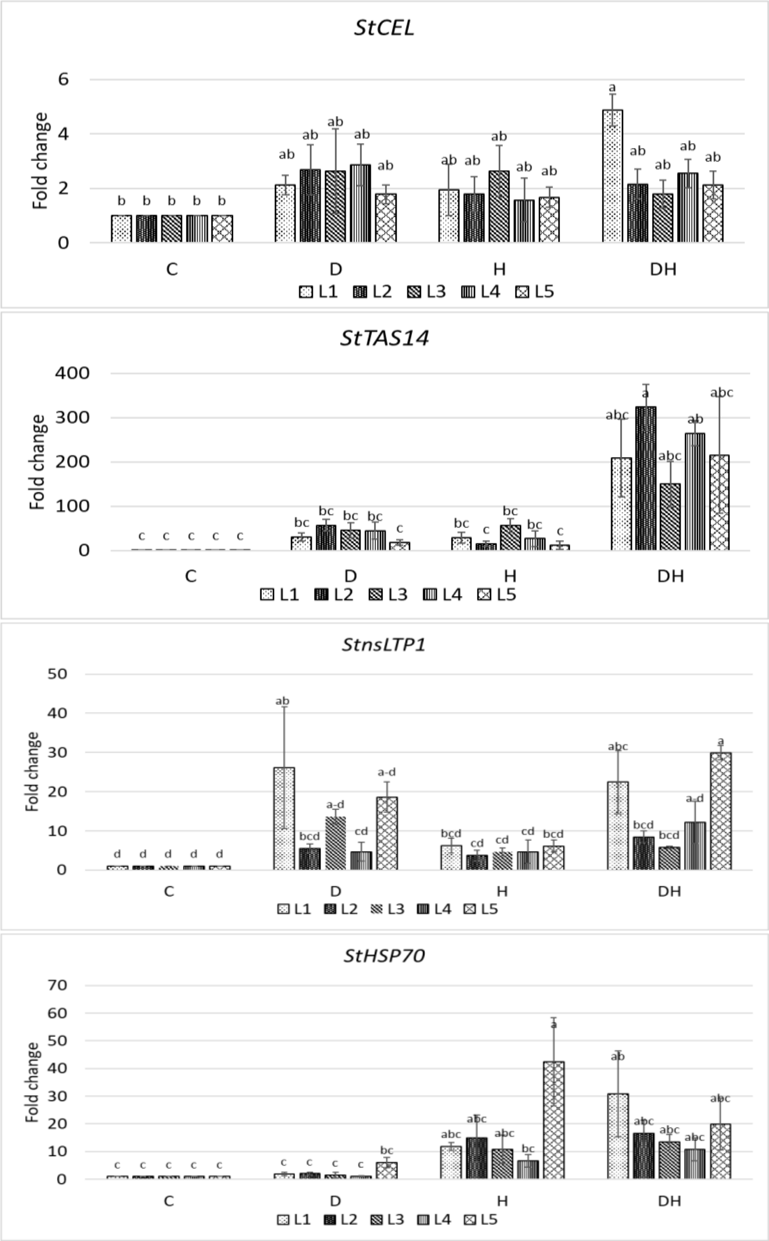

Figure 3.6. The abiotic stress-related gene expressions of five potato lines under different abiotic stresses.

The gene expression values are presented as the fold change of the gene expression normalized to *Ef1a*, and relative to the non-stress condition. The expressions of *StCEL* and *StnsLTP1* were affected by the interaction of abiotic stress and potato line. L1: 84.194.30, L2: 86.61.26, L3: 87HW13.7, L4: DG81-68, L5: Desiree. Error bar: The SE of mean from 15 samples. Different letters above the error bars indicate significant differences among treatments by Tukey's HSD test at $\alpha = 0.05$.

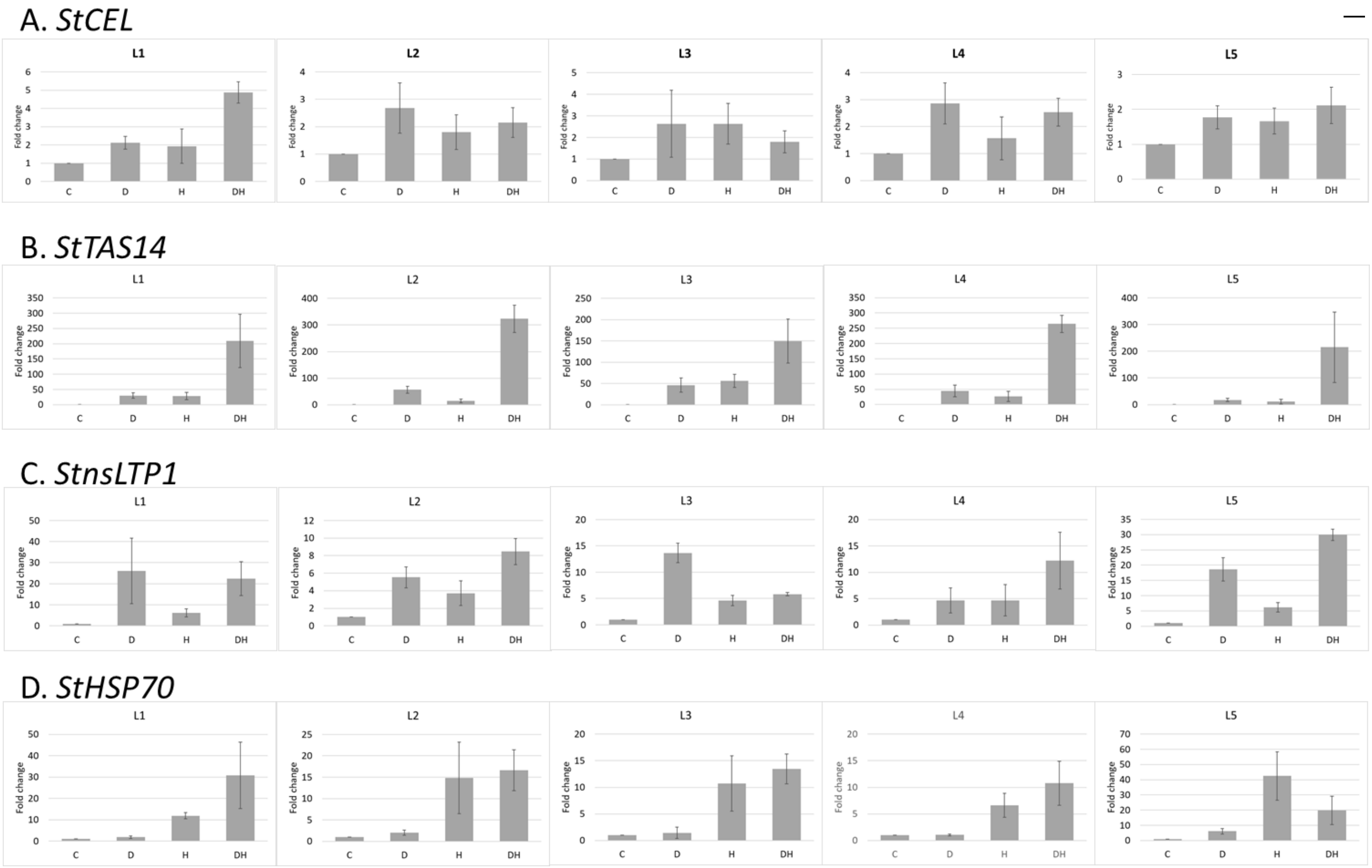

Figure 3.7. Pattern of gene expression in every potato line due to abiotic stresses.

A: Different potato lines showing different patterns of gene *StCEL* expression due to abiotic stresses. **B:** The same pattern of gene *StTAS14* in every potato line. **C:** Different potato lines showing different patterns of gene *StnsLTP1* expression due to abiotic stresses. **D:** The same pattern of gene *StHSP70* in every potato line.

Chapter 4: General Discussion

4.1. Discussion

Food consumption around the world relies on major food crops such as rice, wheat, and potato. The continued increase in the world's population must be met by increasing the food supply, in quantity and/or quality. Environmental conditions affect the production and quality of our food, and sub-optimal environments lead to losses of crop productivity. The most important abiotic stresses that adversely affect crop production are drought stress caused by less precipitation and low soil humidity, and heat stress that induces high air and soil temperatures. Abiotic stress thus hampers the world's food security. Climate change is triggering increases in the intensity and distribution of abiotic stressors — particularly drought and heat stress — because along with climate change, the temperature of the earth's surface is increasing and precipitation levels have fallen in some regions. The simultaneous occurrences of drought stress and heat stress in nature increase the risk of worse effects on global food security.

As potatoes are originally from the cold Andes mountainous area in South America, potato plants require low temperatures for optimum growth and tuber production. The appropriate temperature range for potato plants is 18°–22°C. When the temperature exceeds the optimum temperature required by a plant, the plant is subjected to heat stress. In addition, potato plants are characterized by low and small root architecture, which limits the plant's ability to reach and absorb adequate water when the soil water availability is low; the plant with thus suffer from drought stress.

Cultivated potatoes, which are dominantly tetraploid and part of the primary genepool, are susceptible to both drought stress and heat stress. Fortunately, the genetic resources of potato are not limited to cultivated tetraploid potato; the resources also include landraces and wild potato relatives that are present in the secondary and tertiary genepools. Landraces and wild potato relatives are known as gene resources for biotic and abiotic stresses since they have adapted to their harsh environments (Dwivedi *et al.* 2016). We can take advantage of them by incorporating their good traits into tetraploid cultivated potatoes. Doing so will present challenges because there are genetic barriers between the potatoes in the primary genepool and the potatoes in the secondary and tertiary genepools. In the 1980s, the International Potato Center (the CIP; Centro Internacional de la Papa) developed a range of diploid breeding lines with various landraces and wild relatives with the primary objective of obtaining biotic stress resistance (Watanabe *et al.* 1994). The landraces and wild potato relatives' backgrounds also have abiotic stress-tolerant traits (Arvin and Donnelly 2008; Hetherington *et al.* 1983; Reynolds and Ewing 1989b). These diploid breeding lines may thus be tolerant to abiotic stress, i.e., drought and heat stress. By using some diploid breeding lines and a tetraploid commercial cultivar, the present investigation clarified the responses of potato plants to abiotic stress (drought, heat, and combined drought-heat stress) in a growth room environment in terms of morpho-physiological and molecular changes.

Generally, drought stress, heat stress, and combined drought-heat stress decrease the sizes of plants (which are comprised of the plant height, leaf length, and leaf size), and it was observed herein that the combined drought-heat stress had the greatest negative effects. However, the plant heights of the potato lines L3 and L5 increased but showed different responses under heat stress versus combined drought-heat stress. A reduction of a plant's size, including its leaf size, is a result of a reduction in the size of the plant's cells, since plant cells lose the turgor that is required as a physical force for cell enlargement (Tardieu *et al.* 2014). As a drought stress adaptation strategy, plants must reduce the sizes of their cells in order to maintain cell turgor (Cutler *et al.* 1977). In this case, a potato plant develops a stress tolerance strategy by maintaining cell turgor to reduce the osmotic potential (Zhang 2007). The plant height is also correlated with plant wilting in a negative direction under abiotic stress, which shortens the plant height and increases the wilting symptom. Leaf wilting itself is a result of low cell turgor induced by low leaf water potential due to drought stress (Jensen 1981). In this case, potato plants' response to combined drought-heat stress was similar to their response to drought stress, but even stronger.

A reduction in the leaf size is also necessary when a plant must maintain its water content by minimizing water loss via transpiration, along with stomatal closing; this is a drought stress avoidance strategy (Zhang 2007). However, adverse effects on the tuber yield might arise from reductions in the plant height and leaf size if these are not accompanied by high photosynthetic performance and photosynthate transport, since the potato tuber yield is a result of coordination between the source (leaves) and sink (tuber).

Regarding stomatal closing as one response to abiotic stress (especially drought stress), ABA plays a key role in stomatal closing by altering the guard cells' ion transport (Kim *et al.* 2010) and by enhancing ROS in the production of guard cells (Mittler and Blumwald 2015). In the potato line L5 examined herein, the drought stress induced stomatal closure. The ABA status under abiotic stress was not assessed.

Some abiotic stress-related traits responded differently in each potato line tested in this study. For instance, L1 exhibited low wilting symptoms under the drought stress and combined drought-heat stress; even did not wilt under heat stress, accompanied by profound changes in the plants' values of QY, CMS, and RWC relative to the non-stress condition. In contrast, L4 showed high wilting under both drought and combined drought-heat stress, with relatively significant changes of QY, CMS, and RWC. Since wilting is related to a high osmotic potential caused by drought stress, these results indicate that L1 has a good ability to maintain its water capacity and/or solute in its cells to reduce the osmotic potential under abiotic stress. It is likely that various genetic backgrounds govern the different abiotic stress-response system in each potato line used in this study.

Plants' morpho-physiological responses to abiotic stress are generally controlled by various genes after the plants perceive extracellular stress signals and produce various transcription factors that activate the stress-related genes. The four genes examined in the present study are thought to be involved in abiotic stress tolerance based on their roles in the enhancement of cell membrane integrity (*StnsLTP1* and *StTAS14*), the maintenance of protein folding (*StHSP70*), and the maintenance of the osmotic potential and the scavenging of radical oxygen (*StTAS14*).

Potato plants respond to abiotic stress by increasing some abiotic stress-related genes (*StCEL, StTAS14, StnsLTP1,* and *StHSP70*) in different fold changes. The variation in the genes' expression levels was affected by the abiotic stress treatment, and the different abiotic stresses induced different patterns of the genes' expressions. This was observed in all four of the target genes. The combined drought-heat stress generally induced the largest fold changes of the gene expressions. The morpho-physiological experiment revealed that the combined drought-heat stress had the most negative effects on the abiotic stress-related traits. The more severe damage due to combined drought-heat stress might more strongly affect the abiotic stress-related genes' regulation compared to the single drought stress or heat stress.

In tomato, *TAS14* enhances the osmotic stress tolerance by reducing the osmotic potential and accumulating solute, thus maintaining the cell membrane stability (Munoz-Mayor *et al.* 2012). *TAS14* is a dehydrin that belongs to the family of late embryogenesis abundant (LEA) proteins. The expression of *TAS14* is induced by cell dehydration and ABA (Hanin *et al.* 2011). In the present morpho-physiological assessment, potato lines L1, L3, and L5 exhibited good ability to maintain their RWC and CMS and had the least wilting symptom (L1). However, in this experiment, the *StTAS14* expression was not affected by the potato line, indicating that the ability to maintain those traits does not depend on *StTAS14* gene only, since it is likely that the RWC and CMS are associated not only with the osmotic potential but also with other cellular mechanisms.

In line with the report by Gangadhar *et al.* (2016), it was observed herein that *StnsLTP1* gene expression was highly inducible by drought and heat stress, and the expression was even higher under the combined drought-heat stress. potato plants with the overexpression of *StnsLTP1* showed high survival ability, chlorophyll content, and CMS under drought, heat, and salinity stress (Gangadhar *et al.* 2016). Here, potato line L1 showed the highest survivability under abiotic stress (manifested by the least severe wilting symptoms), and the plants expressed a high level of *StnsLTP1* gene. The high chlorophyll content in the potato plants with the overexpression of *StnsLTP1* was related to its inducible gene, *StHSP70*, the expression of which was also increased under abiotic stress (Gangadhar *et al.* 2016).

As an abiotic stress-inducible gene, *StHSP70* was also increased by heat and combined drought-heat stress. High *HSP70* expression has been suggested to increase the chlorophyll content (Gangadhar *et al.* 2016) since this gene has a function as a chaperone that helps protein folding correction (including chloroplasts as one part of the photosynthetic apparatus). As assessed in the present morpho-physiological experiment, abiotic stress affected the potato plants by increasing their chlorophyll content. The fold change of the *StHSP70* expression was significantly correlated with the chlorophyll content that was measured in the morpho-physiological experiment (R=0.55, p=0.03), and in that experiment, L5 had the highest chlorophyll content.

A thorough understanding of potato plants' responses to abiotic stresses, as investigated herein, is crucial to managing this crop's adaptation to the present and future unfavorable agricultural conditions affected by climate change. Under the challenges of climate change, saving food sustainability requires adaptation practices. Such adaptations could include: (1) adjusting cultivation techniques that are selected or designed based on our knowledge of the changes in the characteristics of potato plants under abiotic stress conditions, and (2) planting stress-tolerant cultivars that can be developed by using the identified genetic materials and abiotic stress-correlated genes. Both the security of staple foods and the sustainability of potato production could also support a wide range of biological-based industries such as snacks, functional food, and bioethanol.

4.2. Conclusion and future perspective

In a growth room environment, drought, heat, and combined drought-heat stress affected the growth and physiological traits of plants, and the combined drought-heat stress had a greater effect on some traits. Some morphological and physiological characteristics are genotype-dependent. The potato lines studied herein showed different responses to each type of abiotic stress, indicating that they have different levels of sensitivity or tolerance to each abiotic stress. The information about the potato lines' genetic profiles can be used in further studies and abiotic stress breeding programs, e.g., as parental materials. The simple abiotic stress-related traits investigated in this study could be used to differentiate stress-tolerant and stress-susceptible potato lines, and the use of these traits will be helpful in screening that involves a large number of accessions.

As climate change increases temperatures and decreases precipitation in some regions, potato line L1 (84.194.30), which was observed herein to be relatively tolerant to abiotic stress, could be used in potato breeding programs in heat- and drought-affected regions, such as in the potato production areas in tropical (i.e., South India and the highlands of Southeast Asia) and sub-tropical (North India and South China) regions that are most affected by high temperatures (Figure 4.1) and in the mid-latitude and subtropical dry regions which will suffer from less moisture due to low precipitation such as India, Northeast China, East China, Southeast China, and some parts of Europe and the U.S. (Figure 4.2).

Abiotic stress induced the expressions of *StCEL*, *StTAS14*, *StnsLTP1* and *StHSP70*. Generally, combined drought-heat stress induced the highest level of abiotic stress-related gene expression. It is likely that compared to drought stress and heat stress, the combination of drought and heat stress has a stronger effect on the signaling cascade in the cells and then results in a higher level of gene expression. Under the three types of abiotic stress examined, the different potato lines expressed *StCEL* and *StnsLTP1* in different patterns. I suspect that these genes each have a role in the different potato lines' responses to each abiotic stressor since, based on the results of the first experiment, the potato lines exhibited differing levels of tolerance or sensitivity to each abiotic stressor.

The present findings clarified that the potato plants responded to drought stress by closing their stomata and increasing their expression of *StTAS14,* which is an ABA-induced gene. These findings will lead to further investigations, particularly regarding the relationship between ABA (as a key abiotic stress-responded phytohormone) and phenotypic responses (e.g., stomatal aperture and ABA biosynthesis) and the expression of ABA-related genes in potato plants. The possibility of interactions between ABA-dependent and ABA-independent pathways under abiotic stress should also be considered. Endo (2016) isolated and characterized *StCEL* as a *DREB1A*-like, and this gene is speculated to regulate the downstream genes (e.g., *TAS14*), since *StCEL* binds to the DRE in the promoter region of *TAS14* in potato. Genes or transcription factors that have both DRE and ABRE can integrate different abiotic stress signaling pathways, thus allowing cross-tolerance to combined stress (Roychoudhury *et al.* 2013).

Studies of the effects of combined drought-heat stress on potato plants have not been as numerous as those of other plant species and have provided limited information. Since the combined drought-heat stress affected mostly the potato plants' morpho-physiological parameters, and since this type of abiotic stress will also become more widespread due to climate change, further studies are warranted to determine the plants' adaptation and tolerance mechanisms in response to drought-heat. Research that is designed to reveal how the abiotic stress-related genes are involved in combined drought-heat stress adaptation and tolerance mechanisms is needed. The future studies could be conducted by using overexpression transgenic potato plants or gene-silencing mutant potato plants in a controlled growth room, coupled with morpho-physiological and biochemical assessments. The correlation between gene expression level and abiotic stress correlated trait could be a consideration in the utilization of the gene or the traits over the diploid potato line in future studies in 4x × 2x crosses to transfer the genetic attributes.

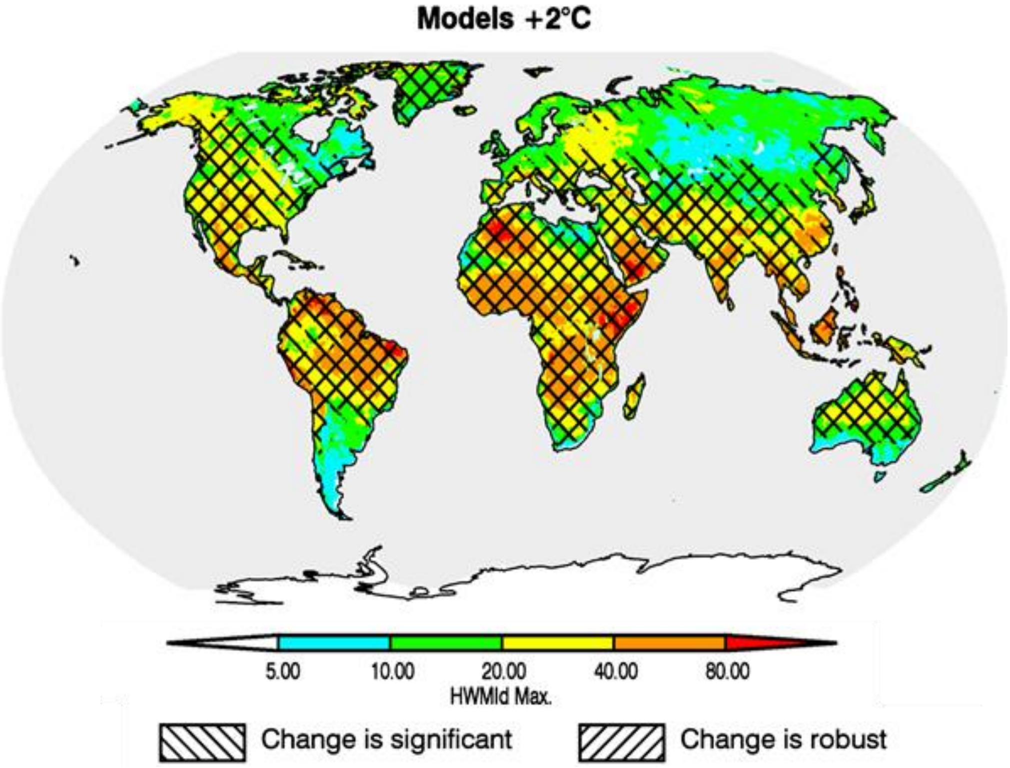

Figure 4.1. Projected future distribution of heat waves due to climate change. Southeast Asia, South Asia, East Asia, South America, and Africa are the most affected by the temperature increase (Dosio *et al.* 2018). China and India, as the larger potato producer countries, are in that affected area, as are tropical highlands, which are potato production areas in the tropical regions.

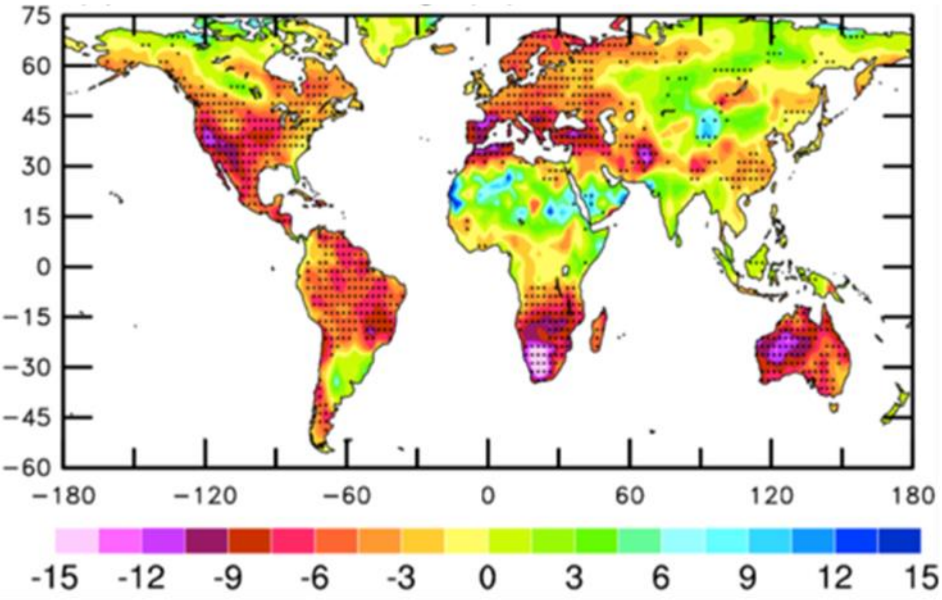

Figure 4.2. Projected future changes in soil moisture content.

This projection was made for 2080–2099 minus 1980–1999 as the percentage in the top-10-cm layer in association with changes of precipitation due to climate change. The Americas, Europe, Australia, Southern Africa, and East Asia will show the most changes in soil moisture content due to shifts in precipitation and an increased risk of drought (Dai 2013).

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Summary (概要)

バレイショにおける乾燥、高温及び乾燥と高温複合ストレス： 形態生理と分子反応の研究

非生物的ストレスは、作物の成長と発達を妨げることによる作物収量の最も制限的な要因です。干ばつストレスと熱ストレスは、世界の食料生産を妨げる主要な非生物的ストレスです。干ばつと熱ストレスの強度と分布は、現在の気候変動により厳しくなっています。平均的な全球気温の上昇は、熱ストレスイベントの上昇を引き起こしますが、中緯度および亜熱帯地域の年間平均降水量の減少は、水不足にもつながります。さらに重要なのは、干ばつと熱ストレスが自然に一緒に発生する場合です。ジャガイモ(*Solanum tuberosum* L.）は、世界で3番目に重要な食用作物です。この作物は、さまざまな環境と季節の広い農業気候帯で世界中で栽培されています。しかし、ジャガイモ植物は成長と塊茎の生産に特定の生理学的条件を必要とします。したがって、高温と水不足がジャガイモ生産の最も深刻な制約になります。ジャガイモに対する干ばつストレスまたは熱損傷の影響に関する実質的な研究が行われているが、干ばつと熱の組み合わせによるストレスの影響に関する報告はめったにない。非生物的ストレスに敏感な栽培ジャガイモに加えて、様々な非生物的ストレスに対する耐性を提供する可能性のある在来種と野生の親類 からなるジャガイモの遺伝資源についても報告されていませんでした。

二倍体育種系統と四倍体の市販ジャガイモを使用して、干ばつストレスと熱ストレスに個々におよび組み合わせてどのように反応するかを研究しました。二倍体育種系統には、さまざまな在来種や野生の近縁種の遺伝的背景があります。第2章では、PEGによる干ばつストレス、熱ストレス、および干ばつと熱の組み合わせストレスに対するジャガイモの形態学的および生理学的応答について説明しました。非ストレスおよび非生物的ストレス処理下の成長室環境で、非生物的ストレス関連特性を評価しました。結果は、すべてのジャガイモ系統が干ばつに反応し、植物の高さを減らすことで干ばつと熱のストレスを組み合わせることを示しました。ただし、草丈に対する熱ストレスへの反応は系統間で異なっていました。一部は増加し、他は減少しました。一方、すべての系統の葉サイズは、干ばつ、熱、および干ばつと熱の組み合わせストレスの各ストレス条件下で、非ストレス条件よりも小さくなりました。ジャガイモ植物は、クロロフィル含有量を増加させ、相対水分含有量（RWC）を減少させることにより、干ばつストレスと干

ばつ熱ストレスに反応しました。しおれ症状に関連する水分量の減少は、それぞれ干ばつおよび干ばつと熱の組み合わせストレス下で示されています。ライン L1（84.194.30）は、非生物的ストレスに対して比較的耐性があると思われる、制御に対する小さな RWC の変化に裏付けられた、すべての非生物的ストレスで最低レベルの萎を示しました。

一部の植物転写因子（TF）および遺伝子は、非生物的ストレスによって誘導され、植物の非生物的ストレス耐性の改善に大きな役割を果たします。干ばつ、熱、および干ばつと熱の組み合わせストレス下での非生物的ストレス関連遺伝子（*StCEL*、*StTAS14*、*StnsLTP1*、および *StHSP70*）の発現レベルの評価については、第3章で説明します。発現レベルの研究は RT-qPCR を使用して行いました。サンプルは、成長室で PEG 誘導の干ばつストレス、熱ストレス、および組み合わせた干ばつ熱ストレスの下で植物から採取されました。結果は、非生物的ストレスが、様々なレベルのジャガイモ植物において *StCEL*、*StTAS14*、*StnsLTP1*、および *StHSP70* 遺伝子発現を誘導したことを示した。一般的に、干ばつと熱の組み合わせストレスは、テストされた非生物的ストレス関連遺伝子の最高レベルを誘発します。異なる非生物的ストレスの下で、異なるジャガイモ系統は、*StCEL* および *StnsLTP1* 発現レベルの異なるパターンを示した。

第4章は一般的な議論です。一般に、ジャガイモ系統は、各非生物的ストレスに対して異なる反応を示し、各非生物的ストレスに対する感受性または耐性のレベルが異なることを示しています。したがって、ジャガイモ系統は、他の研究および非生物的ストレス育種プログラムで利用できます。この研究で使用される単純な非生物的ストレス関連の特性は、耐性と感受性のジャガイモ系統を適切に区別することができます。したがって、スクリーニングで特性が役立つアプリケーションには、多数の登録が必要です。*StCEL*、*StTAS14*、*StnsLTP1*、および *StHSP70* の発現レベルは、非感受性ストレスに対するジャガイモ系統の異なる感度または耐性と関連している可能性があります。したがって、特に干ばつと熱の複合ストレス下で、これらの遺伝子のより多くの役割を探るには、深い研究が必要です。遺伝子発現レベルと非生物的ストレス関連形質との相関関係は、遺伝的特性を伝達するための 4x×2x 交配での将来の研究における遺伝子または形質の利用または二倍体ジャガイモ系統の利用における考慮事項となり得る。

List of publication

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