



T.R.

NİĞDE ÖMER HALİSDEMİR UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

DEPARTMENT OF AGRICULTURAL GENETIC ENGINEERING

IDENTIFICATION OF PHYSIOLOGICAL AND BIOCHEMICAL
TRAITS RELATED TO HIGH TUBER YIELDING IN POTATO
UNDER DROUGHT CONDITIONS.

Saad AHSAN

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CONDITIONS

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Master Thesis

Supervisor

Assoc. Prof. Dr. Ufuk DEMİREL

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The study titled “**Identification of physiological and biochemical traits related to high tuber yielding in potato under drought conditions**” by **Saad AHSAN** under the supervision of **Assoc. Prof. Dr. Ufuk DEMİREL** has been recognized as a master thesis by the jury at the Department of Agricultural Genetic Engineering of Niğde Ömer Halisdemir University, Graduate School of Natural and Applied Sciences.

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DECLARATION OF THESIS

I declare that the thesis has been written by myself and I confirm that all information included in this thesis is scientific and is in accordance with the University's rules and regulations. Any materials that I have used from external sources as well as help received and all sources used in finalizing this research work and preparing this thesis, all have been acknowledged in the thesis.



Saad AHSAN

SUMMARY

IDENTIFICATION OF PHYSIOLOGICAL AND BIOCHEMICAL TRAITS RELATED TO HIGH TUBER YIELDING IN POTATO UNDER DROUGHT CONDITIONS

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This study was conducted for the identification of physiological and biochemical traits related to high tuber yielding in potatoes under drought conditions. A total of 25 potato genotypes, 9 high-yielding, 9 moderate-yielding, and 7 low-yielding under drought and control conditions, were used. Tubers were planted in pots, and during the tuber initiation phase, they were separated into two sets: one as the control group and the other subjected to drought conditions by withholding irrigation for a duration of 12 days. The experiment was conducted two times and data for biochemical traits, physiological parameters and enzymatic activity of different potato genotypes control and drought conditions were determined. The findings revealed that when subjected to drought conditions, all genotypes exhibited an increase in MDA, proline, SOD, POD, and APX levels. The CAT, RWC, Photosynthesis rate, transpiration rate and stomatal conductance and tuber yield were significantly higher under control conditions as compared with drought conditions. The chlorophyll content and leaf temperature were not affected significantly under both normal and drought conditions.

Keywords; Potato, genotypes, drought, quality, physiology, biochemical traits

ÖZET

KURAKLIK KOŞULLARINDA PATATESTE YÜKSEK YUMRU VERİMİ İLE İLGİLİ FİZYOLOJİK VE BİYOKİMYASAL ÖZELLİKLERİN BELİRLENMESİ

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Bu çalışma kurak koşullarda patateslerde yüksek yumru verimi ile ilgili fizyolojik ve biyokimyasal özelliklerin belirlenmesine yönelik yapılmıştır. Kuraklık ve kontrol koşullarında 9 adet yüksek verimli, 9 adet orta verimli ve 7 adet düşük verimli olmak üzere toplam 25 adet patates genotipi kullanılmıştır. Yumrular saksılara ekilmiş ve yumru oluşum aşamasında kontrol ve kuraklık olmak üzere iki gruba ayrılmış ve bitkilere 12 gün ara sulama ile kuraklık uygulanmıştır. Deneme iki kez gerçekleştirilmiş ve normal ve kurak koşullarda farklı patates genotiplerinin (yüksek verimli, orta verimli ve düşük verimli genotipler) biyokimyasal özellikleri, fizyolojik parametreleri ve enzimatik aktivitelerine ilişkin veriler belirlenmiştir. Sonuçlar, kuraklık koşullarında tüm genotiplerde MDA, prolin, SOD, POD ve APX içeriklerinin arttığını göstermiştir. CAT, RWC, Fotosentez hızı, terleme hızı ve stoma iletkenliği ve yumru verimi, kuraklık koşullarına kıyasla kontrol koşullarında önemli ölçüde daha yüksekti. Klorofil içeriği ve yaprak sıcaklığı hem normal hem de kurak koşullarda önemli ölçüde etkilenmemiştir.

Anahtar kelimeler: Patates, genotipler, kuraklık, kalite, fizyoloji, biyokimyasal özellikler

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SYMBOLS AND ABBREVIATIONS

Symbols

%

<

=

>

°C

cm

g

h

ha⁻¹

kg

L

m

m²

mM

Abbreviations

Percentage

Less than

Equal

Greater than

Centigrade

Centimeter

Gram

Hour

Hectare

Kilogram

Liter

Meter

Square meter

Millimolar

Abbreviations

CAT

NPK

POD

SOD

Descriptions

Catalytic Activity

Nitrogen, Phosphorus, Potassium

Peroxidase Dismutase

Superoxide Dismutase

CHAPTER I

INTRODUCTION

Potato (*Solanum tuberosum* L.) holds significant global importance as a staple food crop, ranking fourth in both cultivated acreage and worldwide production. (Reddy et al., 2018; Demirel et al., 2020). It is a valuable food crop and is cultivated on a large scale due to its wide range of utilization in developed and developing countries throughout the World (Lutaladio and Castaldi, 2009). Potatoes offer substantial yield potential from relatively small cultivation areas, thanks to their high productivity, short growth cycle, and adaptability to diverse environmental conditions. They serve as a staple food in various regions, including urban areas of South Africa (Reddy et al., 2018) and other tropical countries (Millam, 2006). It can be used directly by adding into different food items such as vegetables, and meat or in processed forms such as chips or canned potatoes (Bagri et al., 2018). Additionally, the use of potato tubers for animal feed, industrial starch and alcohol production are important (Kirkman, 2007; McGregor, 2007).

Potato is a crop that prefers temperate climates and performs poorly in hot climates (Haverkort and Verhagen, 2008; Pulatov et al., 2015). The better climate for crop growth is provided by sunny days and chilly nights. Potatoes also fit in multiple intercropping systems worldwide due to their short-growing nature (Nasir and Toth, 2022). Varying soils with a pH range of 5-7.5 can be used to grow potatoes, but high organic matter containing loamy soils are best (Reddy et al., 2018). For potato propagation, seed tubers with two to three healthy eyes are typically used. Larger seed tubers are divided into 3.5–5 cm-diameter sections containing a few eyes each for use in propagation (Reddy et al., 2018). The process of preparing a seedbed comprises plowing to a depth of 20–35 cm and then tilling (Salavati et al., 2018). Soil turning and maintaining it loose are other practices used in hot weather to lessen weed and pathogen problems (Reddy et al., 2018). The most widely used irrigation technique is furrow irrigation, and seed tubers are placed into ridges (Singh et al., 2021).

Potatoes can reach heights of up to 3.5 feet depending on the variety, thus earthing up is also advised after 30 days of planting.

The initial domestication of potatoes took place around 10,000 years ago in the Andean mountains of South America (Stadel, 2005). Potatoes arrived in Europe and Asia in the late 1500s from America (Camire et al., 2009), where they quickly gained importance for human sustenance. Almost 80% of potatoes are produced in Asia and the European continents. China stands as the leading potato producer worldwide, followed by India, the Russian Federation, the United States, and Ukraine , globally (FAO, 2022). Türkiye is 16th number among potato production countries and produces almost 5 million tons of potatoes each year to fulfill their demand but the yield per unit area is low compared to the United States of America (FAO, 2022). The central Anatolian region of Türkiye is the most relevant area for potato production.

Several factors affect the growth, yield, and quality of potatoes including weeds (Jabran et al., 2023), insect pests (Naqqash, 2023), diseases (Adolf et al., 2020; Tsrör, 2023), and some other abiotic stresses (Demirel et al., 2020; Demirel, 2023). Also, some areas where there is less water or rainfall are facing problems of low yield due to drought stress (Vadez et al., 2012). Drought stress occurs when there is inadequate rainfall and a shortage of soil water throughout the entire crop growing season (Wahid et al., 2007; Sekhon et al., 2010; Vadez et al., 2012). The effects of drought stresses are mostly in higher proportions in shallow-rooted crops such as potatoes. Also, potato plant has a wider and dense canopy due to which they require a high rate of water, especially at the development stages.

Drought stress negatively affects potato growth and development resulting in low yield and quality (Rykaczewska, 2017; Riao et al., 2022; Demirel, 2023). The impact of drought on potato tuber yield is contingent upon the timing, duration, and intensity of the stress. The key stages at which water stress exerts the most significant influence on tuber yield are during plant germination and emergence, the initiation of tuber formation, and the bulking stages (Martinez and Moreno, 1992; Obidiegwu et al., 2015). The severity, timing, and duration of the drought all negatively affect potato tuber yield (Jeffery, 1995). The yield of tuber under drought is affected by a combination of physiological and morphological processes that include photosynthesis, leaf expansion, tuber initiation, and tuber bulking (Monneveux et al., 2013). Also, the enzymatic activity is disturbed under drought conditions (Schafleitner et al., 2007).

There are some studies about physiological traits related to abiotic stress which are explained below. Numerous studies have unveiled that one significant factor contributing to yield reduction in crops under abiotic stress is the excessive production of reactive oxygen species (ROS) (Gill et al., 2010; You and Chan, 2015; Hasanuzzaman et al., 2020). Elevated concentrations of reactive oxygen species (ROS), which encompass hydrogen peroxide (H_2O_2), hydroxyl radicals (OH), superoxide anions (O_2^-), and singlet oxygen ($^1O_2$), have the potential to induce peroxidation of cellular membrane lipids, degradation of photosynthetic pigments, oxidation of carbohydrates, denaturation of proteins, DNA damage, and a decrease in enzyme activities (Noctor and Foyer, 1998). One advantage of reactive oxygen species (ROS) is that they serve as signaling molecules, prompting plants to adapt their metabolic processes in response to challenging environmental conditions (Hancock, 2017). That's why the capacity of plants to control homeostasis of ROS is very important for plant survival under critical environmental conditions.

There is some previous literature available about the yielding potential of used varieties under drought conditions (Spiers and Schapendonk, 1990; Vayda, 1994; Rykaczewska, 2017; Riao et al., 2022) but the novelty of this study is that to find out the secondary traits that are related to yield under drought stress conditions and later on use these traits for breeding programs. The main hypothesis of the study is that we can use some physiological and biochemical traits to identify drought-tolerant potato genotypes with high yields and more tolerability.

Currently, there is a huge need to breed varieties that have stress-tolerant properties, enabling them to thrive across a wider spectrum of environmental conditions. Also, breeding potatoes for stress tolerant especially drought tolerance is resource-intensive and time-consuming process (Monneveux et al., 2013; Tiwari et al., 2022). Developing screening techniques in potatoes is crucial for identifying high-yielding genotypes under drought. Furthermore, the screening tools chosen for assessing drought tolerance should have the capacity to reflect the yield potential of genotypes when they are exposed to drought conditions. Screening genotypes at an early developmental stage under controlled environmental conditions is favored due to its advantages, such as applicability in off-season periods and quicker results (Sprenger et al., 2015; Tiwari et

al., 2022). The early identification of physiological traits related to yield has been a subject of significant interest for both physiologists and breeders.

Hence, our objective was to identify physiological traits related to yield and to acquire insights into certain traits that could be used for evaluating drought resistance in potatoes. This was achieved through the application of Principal Component Analysis (PCA) to assess physiological traits associated with yield. Principal Component Analysis has been frequently employed to investigate the interrelationships among physiological, biochemical, and enzymatic traits in the context of crop yield and plant performance (Bao et al., 2019; Liu et al., 2020). In the studies, PCA serves as a valuable statistical tool to identify the most influential factors and their contributions to overall yield variability. By reducing the dimensionality of the dataset, PCA enables a clearer understanding of how these traits collectively impact yield, aiding in the identification of key factors for crop improvement (Liu et al., 2020). In this study, our goal was to discern the critical mechanisms and processes triggered by drought stress through a comparative analysis of cultivars that exhibit resistance and susceptibility to drought.

In several agricultural and environmental research, PCA has been applied to study the interplay between photosynthesis, respiration, transpiration, and antioxidant enzyme activities (SOD, POD, CAT) and their influence on crop yield (Singh et al., 2018; Zhang et al., 2020). PCA allows to identify key variables and their contributions to variations in crop yield.

Until now, a large number of research on potatoes and their drought-coping ability have been found (Zhang et al. 2014; Banik et al. 2016; Cioloca et al. 2016; Kabira and Muthoni 2016). Mostly these studies have concentrated on examining the physiological and biochemical reactions of tubers to drought-induced stress, as well as elucidating the signaling pathways that play a role in this process. The study gives us knowledge about physiological and biological processes associated with drought response in potatoes, to breed drought-resistant potato varieties. Therefore, we investigated some physiological and biochemical characteristics of 25 potato genotypes with low-yielding, moderate-yielding, and high-yielding under drought and control conditions. Our objective is to assist plant breeders in discovering straightforward and expeditious screening techniques for the identification of drought-resistant potato plants from extensive

segregating populations and germplasm collections, facilitating their incorporation into breeding programs.

The primary objectives of this study encompass:

1. To determine high-yield-related secondary traits in potatoes under normal and drought conditions.
2. To find physiological traits associated to yield and gain knowledge about some traits for screening drought resistance in potato.
3. To determine some screening tools for high-yielding potato genotypes under drought with superior-quality breeding programs.

CHAPTER II

LITERATURE REVIEW

2.1 Importance of Potato

Potato is a valuable crop and serves as a staple food in different countries worldwide. Potatocultivation plays a crucial role in providing sustenance, generating employment opportunities, and serving as a source of income, especially in rural areas (Scott et al., 2000; Nanbol and Mamo, 2019). In actuality, potatoes are a significant staple food all over the world and generally offer a more favorable nutrient-to-price ratio compared to many other vegetables (Drewnowski 2013, IPC 2018). It is a major food item that has a greater potential for current and future food security (Nanbol and Mamo, 2019). Potato plants have an edible biomass content of about 85%, which is substantially higher than the edible biomass content of cereals, which is just 50% (Lutaladio and Castaldi, 2009). The potato fits in well with many intercropping systems because of relatively brief growth cycle. Potato grows well in well-drained soil due to its expanding nature in loose soil and mostly dislikes soggy soils.

Potato is the major source of starch and several other nutrients and vitamins. The presence of vitamin C, potassium, and dietary fiber are just a few of the essential elements that potatoes bring to the table (McGill et al. 2013). Recent studies show that potatoes contribute 5% of overall magnesium intake, with a medium (5.3 oz) potato with the skin providing 48 mg of magnesium (Freedman and Keast 2011). While the iron content in potatoes may not be notably high (1.3 mg), their bioavailability of iron is relatively superior to many other iron-rich vegetables. This enhanced bioavailability is attributed to the exceptionally low or negligible presence of antinutrients, chelators, and ligands that typically hinder iron absorption (e.g., tannins, oxalates, phytates). Additionally, the elevated levels of vitamin C in potatoes contribute to this improved iron absorption (Woolfe, 1987).

2.2 Historical Perspectives of Potatoes

The potato was first domesticated in the New World, where their wild ancestors still exists (Spooner et al., 2004). Around 8000 years ago, potato farming started in South America (Lutaladio and Castaldi, 2009), and during the Columbian exchange, Spanish conquistadors introduced potatoes to Europe (Lutaladio and Castaldi, 2009). Potatoe crop was introduced into countries such as the United Kingdom and Ireland by the conclusion of the 16th century (Bradshaw and Ramsay, 2009). Nearly a century after its cultivation began in Europe (Bradshaw and Ramsay, 2009), late blight decimated Irish potatoes on a huge scale due to monocultural farming practices (Lutaladio and Castaldi, 2009). Breeders focused their efforts on creating cultivars that were resistant and had great yields as a result (Lutaladio and Castaldi, 2009).

Potato is an annual herbaceous plant and a member of the Solanaceae family. Around 4500 potato varieties have been developed since they were first introduced from the Andean highlands and have been adapted to various conditions (Pieters and Judd, 2014). These cultivars are divided into early varieties (harvesting in 75–90 days), mid varieties (harvesting in 90–100 days), and late varieties (harvesting in 100–150 days).

Potato rank as the fourth most significant crop globally in terms of production. It is a widely adopted crop due to its huge yield potential from a small unit area, and short life cycle (Raja et al., 2020). Potatoes are also capable of adopting a wide range of environmental conditions that increase their adaptability to small land-holding farmers and kitchen gardeners. The potato was domesticated first in the Andean mountains of South America about 10,000 years ago and then reached Asia and the European continent in late 1500 BC (Stadel, 2005).

2.3 Climatic Conditions for Potato

Potato is a crop that thrives in chilly climates and does poorly in hot climates (Haverkort and Verhagen, 2008; Pulatov et al., 2015). A better growing environment for crops is created by sunny days and cool nights; yet different temperatures are needed for potato vegetative growth and tuber formation. While 4 to 18 °C is thought to be ideal for the tuber initiation and bulking stages, 16 to 25 °C is thought to be ideal for vegetative

development (Reddy et al., 2018). After 20–25 days of seeding, tuber production begins, and plants produce blooms that range in color from white to purple and may or may not fall off depending on the weather. Plants require between 350 and 550 mm of water during their life cycle, depending on the soil type, climate, and variety (Reddy et al., 2018). Before harvesting, irrigation is stopped 10 to 15 days in advance, and harvesting is done before temperatures rise beyond 30 °C.

2.4 Yield of Potatoes Worldwide and in Türkiye

During the year 2020, potatoes were cultivated across an area of approximately 18.13 million hectares (m ha) and production reached 376.11 million metric tons (MT) (FAO, 2022). Asia and Europe are the largest potato-producing continents accounting for 43.4 and 37.3% of potato production while the Americas, Africa, and Oceania produced 12.8, 5.9, and 0.5% respectively. China stands as the largest potato-producing country globally, followed by India, the Russian Federation, the United States, and Ukraine in descending order of production (Table 2.1). Türkiye produced 138153 tons in a 5100000 ha area accounting 16th topmost potato-producing country and 2nd among Middle East countries after Iran (FAO, 2023). Potatoes are produced in nearly all provinces (71 out of 81) of Türkiye. The central Anatolian region is famous for its cultivation due to its cool temperate climate.

Table 2.1. Area and yield of the top 10 potato-producing countries and Turkey during the year 2021 (FAOSTAT, 2023)

Country	Area (ha)	Production (tons)
China, mainland	5780000	72683682.57
India	2248000	34030646.43
Russian Federation	1142111	28406484.84
United States of America	378670	20311535.71
Ukraine	1283200	19508660.71
Poland	235830	13391512.68
Germany	258300	11131666.71
Belarus	249500	7453765.46
Netherlands (Kingdom of the)	159040	7021220.68
France	211590	6980724.03
Turkey	5100000	138153

2.5 Stresses in Potato

Normally potato has a high yield, but it suffers yield losses due to various factors such as weeds (Jabran et al., 2023) insect pests (Alyokhin et al., 2022) diseases (Charkowski et al., 2020), and other stresses like drought stress (Obidiegwu et al., 2015). Given its global significance and the escalating challenges posed by drought resulting from climate change, conducting research to enhance drought resistance in potatoes is of paramount importance. Potato is efficient in water usage and requires a huge quantity of water during the whole growing season (Shahnazari et al., 2007; Vreugdenhil et al., 2007). Generally, potato is drought-sensitive (Vasquez-Robinet et al., 2008), and a decline in production under drought while the yield loss can reach 79% if less water is available (Binod et al., 2015).

Plants have developed an antioxidant defense system that includes non-enzymic substances like ascorbate, glutathione, tocopherol, carotenoids, and flavonoids as well as enzymes like superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), glutathione reductase (GR), and polyphenol oxidase (PPO) (Agarwal and Pandey 2004, Núñez et al. 2003/4, Sergi and Alegre 2003). These enzymatic and non-enzymic substances effectively guard against ROS by cooperating with each other (Scebba et al. 1998).

2.6 Drought Stress in Potato

Drought-induced reductions in crop productivity occur due to their impact on photosynthetic processes, which can be observed at various levels such as the canopy, leaf, or chloroplast. This effect can be direct or result from feedback inhibition when the transport of photosynthate to sink organs is restricted. (Jones and Corlett, 1992). The way plants respond to drought stress is influenced by factors such as the severity, duration, and speed at which the drought condition develops (Pinheiro and Chaves, 2011). In mild to moderate drought conditions, there is an impact on stomatal characteristics, leading to a reduction in biomass. However, under severe drought conditions, non-stomatal factors can become more influential in causing detrimental effects (Liu et al., 2010) constraining photochemical efficiency and Rubisco activity and

This influence, in turn, has repercussions on both biochemical and physiological metabolisms (Xu et al., 2010).

Drought has a variety of effects on plant growth depending on the length, severity, and stage of the drought as well as the plant's development (Pinheiro and Chaves, 2011). Stomatal closure, an increase in leaf sugar content, and reactive oxygen species are all effects of drought. Under drought stress, stomatal closure aids in water conservation and maintains leaf water potential, also decreases CO₂ uptake of the plant, which has an impact on photosynthesis (Chaves et al., 2002; Serrai et al., 2004; Mafakheri et al., 2010; Muthoni). The feedback reduction of photosynthesis that results from a rise in leaf tissue sugar content also has an impact on plant yield and growth yield (Basu et al., 1999). Additionally, under drought stress, reactive oxygen species including hydrogen peroxide buildup and superoxide radicals also rise. Reactive oxygen species bond to oxygen molecules, depriving cells of oxygen, which can result in severe oxidative damage and cell death (Finkel and Holbrook, 2000).

Plants cultivated in drought conditions often exhibit reduced stomatal conductance. This adaptation helps in water conservation and sustains an acceptable leaf water status. However, it also leads to a decrease in the internal CO₂ concentration within leaves, ultimately affecting the process of photosynthesis (Chaves et al., 2002). In potato cultivation, the yield of tubers is associated with both the harvest index (HI) and dry matter content. Consequently, in breeding programs, it is crucial to comprehend the extent of genotypic variability concerning the distribution of assimilates and the production of dry matter (Jefferies and Mackerron, 1987, 1993; Mackerron and Jefferies, 1988; Gregory and Simmonds, 1992; Jefferies, 1993a; Tourneux et al., 2003).

The quantity and rate of photosynthesis in plants are impacted by drought stress. The amount of photosynthesis is impacted by changes in leaf area and leaf number (Gaur et al., 2017). On the other side, the rate of photosynthesis is slowed down by a lack of CO₂ and water. Potato leaves under drought stress have a lower relative water content, which raises the intercellular ionic concentration (Lawlor, 2002). Elevated intercellular ionic concentration prevents ATP synthesis, which has an impact on the synthesis of ribulose biphosphate. During photosynthesis, ribulose biphosphate (RuBP) serves as the main

carbon dioxide acceptor. Thus, a decrease in RuBP production has a direct impact on photosynthesis (Tezara et al., 1999; Flexas et al., 2006).

Physiological responses to drought are frequently more strongly linked to "predawn" water potential rather than daytime water potential (Jones, 2007). One of the physiological response strategies to drought involves the presence of drought-sensitive stomata, which lead to reduced transpiration rates. This adaptation results in a relatively higher intrinsic water use efficiency (WUE), regardless of radiation levels, ultimately facilitating plant growth in regions with limited water availability (Coleman, 2008). Transpiration rates are influenced by various factors, including leaf area, the ratio of root-to-leaf, leaf orientation, leaf shape, surface characteristics of leaves (such as pubescence), leaf thickness, and the distribution pattern of stomata.

One of the primary physiological consequences of water scarcity in crops is a decrease in the photosynthetic rate of per unit leaf area (Moorby et al., 1975; Vos and Oyarzun, 1987; Schapendonk et al., 1989; Dallacosta et al., 1997; Deblonde and Ledent, 2001; Germ et al., 2007). The reduced net photosynthesis occurs as a result of either partial or complete closure of stomata (Vos and Oyarzun, 1987; Trebejo and Midmore, 1990; Haverkort et al., 1991; Jefferies, 1994; Liu et al., 2005). Both the decrease in net photosynthesis due to stomatal closure and the reduction in leaf area under water stress or drought conditions significantly contribute to yield losses in drought-affected crops (Legay et al., 2011).

Plants capable of sustaining an adequate relative water content (RWC) over an extended period of drought exposure are more likely to maintain their metabolic processes and survival. Research has shown that cultivars exhibiting greater drought resistance are capable of retaining higher cellular hydration levels when faced with drought conditions (McCann and Huang, 2008). The measurement of relative water content (RWC) has proven effective in distinguishing between drought-resistant and drought-susceptible potato cultivars (Coleman, 1986).

The metabolite accumulation during drought stress does not necessarily correlate with an increase in resistance levels or distinguish resistance differences between genotypes. Elevated metabolite levels can occur due to an increased degradation rate or reduced

biosynthesis of another metabolite, without necessarily conferring a protective effect against drought stress.(Degenkolbe et al., 2013).The metabolic response to drought stress varies among different genotypes, and research has indicated varying levels of accumulation of osmotically active solutes in response to drought. The ability to respond to stress in this manner has also been employed as an indicator of drought resistance in numerous model plants and crop species (Schafleitner et al., 2007; Vasquez-Robinet et al., 2008; Evers et al., 2010). Research conducted on potatoes has shown that drought stress triggers the accumulation of osmotically active solutes, which may include proline, inositol, raffinose, galactinol, and trehalose (Vasquez-Robinet et al., 2008; Evers et al., 2010; Legay et al., 2011; Kondrak et al., 2012) These findings indicate that these accumulated solutes, such as proline, inositol, raffinose, galactinol, and trehalose, may serve as osmo-protectors or play an osmoregulatory role in response to drought stress (Schafleitner et al., 2007; Teixeira and Pereira, 2007). Proline functions as a scavenger of reactive oxygen species (ROS) and serves as a non-enzymatic antioxidant, effectively mitigating the harmful effects of various ROS components. This role of proline helps in the survival of plants under water stress conditions by acting as an osmo-protectant (Vanková et al., 2012).

In addition to the timing of water availability, different mature cultivars respond differently to drought (Chang et al., 2018). Late maturing cultivars may be less impacted by late drought than early maturing cultivars, according to a thorough analysis of 103 potato cultivars (Aliche et al., 2018). Varieties that mature later have longer vegetative growth periods. They can further delay the onset of full canopy cover when faced with late drought stress, hence reducing its consequences (Romero et al., 2017). However, because plants already develop their maximum number of stems prior to the onset of the late drought, potato stem numbers may be less affected (Lahlou et al., 2003). Deblonde and Ledent (2001) similarly reported a less significant impact of the late drought on the plant height of early cultivars.

Proline can also serve as a signaling molecule, influencing mitochondrial functions, regulating cell proliferation or cell death, and initiating specific gene expression. These functions of proline are crucial for facilitating plant recovery from stress conditions (Szabados and Savouré, 2010). Just as metabolic responses do, biochemical and enzymatic responses also undergo alterations in reaction to both biotic and abiotic

stresses. Win et al. (1991) reported that the use of antitranspirants that increased leaf water potential and modified leaf-to-tuber potential gradients in water-stressed potato plants resulted in higher calcium (Ca^{2+}) accumulation in the tubers. This application also reversed tuber necrosis caused by calcium deficiency. Studies by Lefevre et al. (2012) demonstrated an elevation in the concentration of most analyzed cations in numerous cultivars when subjected to water deficits. Interestingly, this study identified two cultivars that not only maintained stable yields but also exhibited high mineral content under water deficit conditions (Lefevre et al., 2012).

Malondialdehyde (MDA) is an important colorless liquid organic compound that is highly reactive in nature and considered as a measure of lipid peroxidation in plant tissues (Nair et al., 2008; Tulkova and Kabashnikova, 2022). It is found naturally in different fruits and vegetables and used as an index of oxidative stress (Oral et al., 2006; Gobel et al., 2009). Exposure of potatoes to adverse conditions like drought, high temperatures, or disease can trigger the production of reactive oxygen species (ROS) within plant cells. These ROS can instigate lipid peroxidation, leading to the generation of malondialdehyde (MDA) as a byproduct.

Superoxide dismutase (SOD) are vital metalloenzymes which have a vital role in defense mechanisms under several stress conditions (Rosa et al., 2021). Peroxidase (POD) is an isoenzyme that has a greater role in effective defense systems due to its huge enzymatic activity (Saffar et al., 2009). This enzymatic system plays a pivotal role in initiating the scavenging of reactive oxygen species molecules, thereby offering protection against various stresses and preventing oxidative damage (Jaleel et al., 2008; Rosa et al., 2021). All stress treatments increased peroxidase activity in all genotypes, with the exception of Agria, where no changes were seen. APX is another important parameter that is influenced under drought conditions and results of a study showed that for the majority of the cultivars, stress treatment increased APX activity; however, in the case of Agria, no discernible changes were seen (Demirel et al., 2020).

The biochemical responses of potatoes to drought stress are intricate, and the levels of antioxidants can exhibit a range of outcomes, including increases, decreases, or no significant effect. These responses vary depending on the specific genotype and the type of antioxidant being considered (Andre et al., 2009; Wegener and Jansen, 2013).

Elevation in the activity of enzymes such as peroxidase, superoxide dismutase, and catalase in response to oxidative stress in potatoes (Boguszewska et al., 2010) Yellow tuber-bearing cultivars are less significantly impacted by drought treatment when compared to non-yellow tuber-bearing varieties. The latter group tends to exhibit substantial variations in antioxidant content that are dependent on the specific cultivar (Andre et al., 2009).

A low photosynthetic rate may accompany the accumulation of ROS within the chloroplast stroma which can be detoxified through the action of superoxide dismutase (SOD) and ascorbate peroxidase (APX) located within the plastid. Ascorbate peroxidase activity or SOD will increase or not in our cultivars under drought stress conditions. Peroxidase (POD) activity increases or not in varying genotypes. Also, the proline in all genotypes under drought stress give us the idea that the proline level is related to drought stress in our cultivars or not.

2.7 Aims and Objectives

The main purpose of this study is to collect information about several traits in potato genotypes related to drought stress. Traits associated with high yields under drought conditions can serve as valuable secondary criteria for selection in potato breeding programs. Besides, several molecular markers that are linked with these traits could be developed and they would be used in the breeding programs. Targeted breeding approaches need a basic information about plant species response to different abiotic stresses faced during the completion of their growth cycle. That's why there is a huge need to study those responses that are linked with abiotic stresses under field conditions and to clarify molecular mechanisms involved in maintaining crop yield and quality during such stress conditions. This would further assist to define molecular markers that described the efficient potato cultivars under control and stress conditions.

Physiological data allowed us to know about the photosynthesis rate that may change due to leaf transpiration rate under drought conditions. Malondialdehyde (MDA) is a colorless liquid organic compound that is highly reactive (Nair et al., 2008) and found naturally in different fruits and vegetables and used as a major element for oxidative

stress (Oral et al., 2006; Gobel et al., 2009). Their accumulation occurs mostly under drought treatment in any kind of genotypes.

Also, this study is helpful to identify some physiological, biochemical traits, and enzymatic as well as non-enzymic activity in potatoes under drought conditions and the relation of these traits with tuber yielding in potatoes under drought conditions and note down the differences between different tolerant and susceptible species. The relative water content, chlorophyll content (SPAD values), photosynthesis rate, transpiration rate, leaf temperature, proline content, malondialdehyde (MDA) content, enzymatic activities of superoxide dismutase (SOD), peroxidase (POD), and ascorbate peroxidase (APX) have been determined in this study. The tuber yield has been also determined under drought and normal conditions. This study allows us to know the traits that are superior for the stress conditions in potato cultivars, also the difference in the traits between susceptible and tolerant species under drought and normal conditions. Besides, the traits related to high yielding under drought and control conditions have been determined and then these traits can be used later in potato breeding programs.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

This study was conducted for the identification of physiological and biochemical traits related to high tuber yielding in potato under drought conditions. The experiments were conducted under greenhouse conditions at Nigde Omer Halisdemir University, Nigde, Türkiye (37.9415° N, 34.6243° E). A Total of 25 potato genotypes containing nine high-yielding, nine moderate-yielding, and seven low-yielding under drought were used in the study. These genotypes were chosen from the results of a previous study (Çalışkan et al., 2019). The yield for all these selected genotypes is shown in Table 3.1.

Table 3.1. The yield for all the high-yielding, moderate-yielding, and low-yielding genotypes in the year 2017-18 (Çalışkan et al., 2019)

High-yielding genotypes		Moderate-yielding genotypes		Low-yielding genotypes	
Genotypes	Yield (t/ha)	Genotypes	Yield (t/ha)	Genotypes	Yield (t/ha)
DT13085.01	43.60	LAURA	26.84	DT14042.24	14.77
DT14046.19	39.09	PELICAN	26.05	DT11088.01	13.18
DT14016.01	41.20	WENDY	27.54	DT14083.28	11.67
DT13052.23	43.13	JELLY	24.43	DORUK	14.38
DT13124.21	46.79	43-111-11	29.01	DT11108.02	9.90
DT13045.74	48.37	99-115-11	27.61	BETTINA	10.27
DT13143.54	44.92	40-103-11	26.00	FASAN	15.42
DT13100.02	48.98	DT13037.31	25.47		
DT13136.22	42.38	Solist	26.64		

3.2 Methods

3.2.1 Experimental design

Pot experiments were conducted using a split-plot design. The experiment was conducted two times that is, the study was repeated according to the time scale, and data for physiological and biochemical traits and enzymatic activity was recorded accordingly. Tubers from each genotype were sown in pots (small plastic pots, 5 L) to

get plants of each genotype. Potato plants were grown in the greenhouse under optimum conditions for 45 days (till the tuber initiation stage). The number of replications was three. Plants grown from each genotype were divided into two sets (control and drought stress). Six plants from each genotype were planted with a division of three plants as control and three for drought application. A total of 150 plants for 25 genotypes were planted.

3.2.2 Treatments

Control Treatment

Plants selected as control were grown under normal/optimum growth conditions as mentioned above until sample harvesting time. Plants were grown in the greenhouse under optimum conditions for 45 days (till the tuber initiation stage) (Gervais et al., 2021). Until the start of the drought, all plants received regular irrigation, and following emergence. A recommended fertilizer (N-P-K: 18%-18%-18%) dose was administered twice to all plants.

Drought Treatment

Plants were subjected to drought treatment by withholding irrigation for a total of 12 days (till the relative water content of potato under drought treatment drops to 70-75%) (Papadopoulos and Hao, 2000).



Figure 3.1. Set of potato pots before the application of drought treatment

3.3 Physiological Measurements

The differences in some physiological traits of all genotypes during drought treatment were evaluated by comparing control plants at both the initial and final stages. Gaseous exchange capacity (Stomatal Conductance, Photosynthetic Rate, Transpiration Rate) and other physiological parameters (relative water content, chlorophyll content, and plant leaf temperature) were investigated to test changes during drought treatment within all replicates.

3.3.1 Gaseous exchange capacity

Stomatal Conductance

Stomatal conductance measurements in the control and drought treatment were performed at both the initial tuber initiation and the last day of stress application stages. Stomatal conductance measurements were carried out in all replications under a constant light intensity ($1000 \mu\text{mol}/\text{m}^2/\text{sec}$), CO_2 amount ($400 \mu\text{mol}$), and airflow ($500 \mu\text{mol}/\text{s}$) using a portable photosynthesis system (LI-6400 XT Licor) in mid-day hours

(Figure 3.2). The apical leaflet of the second or third leaves of the potato plants was used for determining stomatal conductance measurement within all replicates.

Photosynthesis Rate

Photosynthetic rate measurements in the control and drought stress applications were performed at both the initial tuber initiation and the last day of stress application under constant light intensity ($1000 \mu\text{mol}/\text{m}^2/\text{sec}$), CO_2 amount ($400 \mu\text{mol}$), and airflow ($500 \mu\text{mol}/\text{s}$) using a portable photosynthesis system apparatus (LI-6400 XT Licor) in mid-day hours (Figure 3.2). Photosynthesis rate measurements were carried out in all replications. The apical leaflet of the second or third leaves of the potato plants was used for photosynthesis measurement.

Transpiration Rate

Transpiration rate measurements in control and drought applications were performed at the initial tuber initiation and the last day of stress application stages under a constant light intensity ($1000 \mu\text{mol}/\text{m}^2/\text{sec}$), CO_2 amount ($400 \mu\text{mol}$), and airflow ($500 \mu\text{mol}/\text{s}$) using a portable photosynthesis system (LI-6400XT Licor) in mid-day hours (Figure 3.2). The measurements were carried out in all replications. The apical leaflet of the second or third leaves of the potato plants was used for the transpiration rate.



Figure 3.2. Determination of physiological traits of potatoes by using a portable photosynthesis system

3.3.2 Relative water content

Measurement of relative water content in control and drought applications was performed during drought treatment at the tuber initiation and the last day of stress application stages from the apical leaflet of the second or third leaves of the potato plants. Relative water content (RWC) measurements were carried out in replications. The harvested leaf samples were quickly transferred to the laboratory and fresh weight was immediately measured by using a sensitive scale. The fresh leaves were kept in pure water for at least 18 hours to obtain turgor, and then the turgor weights of the leaves were measured. After turgor weighing, the leaves were dried in a microwave at 500W for 10 minutes and then kept at 95 °C for 2-3 hours in order to ensure complete drying of the leaves and then weighed dry weights. Following these operations, the RWC of plants was determined according to the equation below:

$$\text{RWC: } [(\text{Fresh weight} - \text{Dry weight}) / (\text{Turgor weight} - \text{Dry weight})] \times 100 \quad (3.1)$$

3.3.3 Chlorophyll index

Leaf chlorophyll index measurements in control and drought stress applications were determined at the initial and final stages. A Chlorophyll-Meter (Konica Minolta SPAD-502 Plus Chlorophyll meter) in mid-day hours was used for determining spad values. The measurements were carried out in all the replications. The apical leaflet of the second or third leaves of the potato plants was used for leaf chlorophyll index measurements.



Figure 3.3. Measurement of chlorophyll content by using a Spadmeter

3.3.4 Leaf temperature (°C)

Plant leaf temperature measurements in control and drought applications were measured during drought treatment at the tuber initiation and the last day of stress application stages with an IRT instrument (MASTECH BM380 Laser Temperature Measuring

Instrument) in mid-day hours. The apical leaflet of the second or third leaves of the potato plants was used for plant temperature measurements.

3.4 Biochemical Assays on Frozen Leaves Samples

On the twelfth day of drought application, upper 3rd and 4th compound leaves were harvested from three replicates, separately. Samples of the harvested leaves were promptly frozen in liquid nitrogen and kept in a freezer at -80 °C. Proline and MDA contents, and antioxidant enzyme activities were determined by using specific protocols (Figure 3.5).

3.4.1 Proline content

On the 12th day of the first drought application, leaf samples of drought-applied and control plants were cut and collected for analysis. To determine the proline content, the 2nd, 3rd, and 4th leaves were collected from the completed top. The samples were replicated in 3 replications for each treatment.

In each replication, leaf samples were taken from 3 plants, and 3 leaf samples were taken from each plant. Leaf samples were instantly frozen in liquid nitrogen, then ground into powder with the help of a mortar and pestle and stored at -80 °C.

Proline content was observed by applying a modified method identified by Bates et al. (1973). Proline content was measured as the following steps:

1. A 100 mg potato leaf sample was grounded in 2 mL of 3% sulfosalicylic acid, and then the sample was mixed by vortex.
2. The mixed samples were precipitated by centrifugation in 10,000xg for 10 min at 4 °C.
3. 0.2 mL of the supernatant was transferred to a new 1.5 mL centrifuge tube.
4. Freshly prepared 0.2 mL ninhydrin solution (1.25 ml ninhydrin, 30 mL glacial acetic acid, 20 mL 6 M orthophosphoric acid) was added.
5. The mixture was inverted 3-4 times for 15 seconds.

6. The mixture was incubated at 90 °C for 1 hour, and then the reaction was stopped on ice.
7. A 1 mL of toluene was added to each reaction mixture and then mixed for 15 seconds by vortex.
8. The mixture was incubated at room temperature keeping in a dark place for 20 min.
9. The toluene phase was then carefully collected and added into test tubes and absorbance was measured at 520 nm in a spectrophotometer.
10. The spectrophotometer was blanked by adding 1 mL of toluene.
11. The proline contents were determined by using a standard curve (Figure 3.4) with the help of the following equation:

$$\text{Proline } \mu\text{mol/g FW} = (A_{520} + 0.0039) / 0.0178 \times \text{Dilution Factor} \times (1/\text{tissue weight g}) / 115.13 \quad (3.2)$$

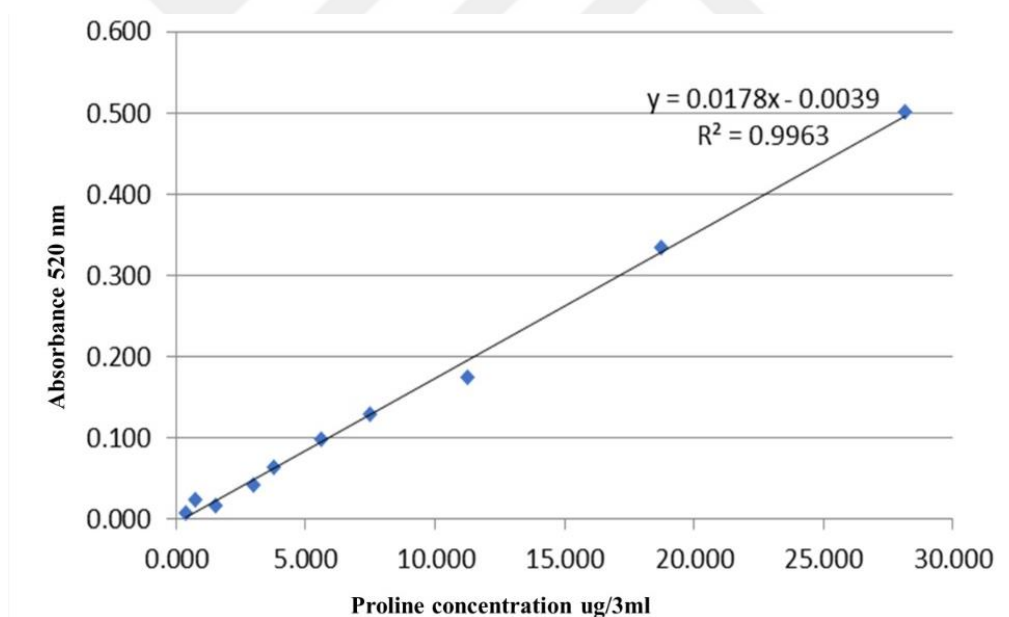


Figure 3.4. Standard curve for determining proline content

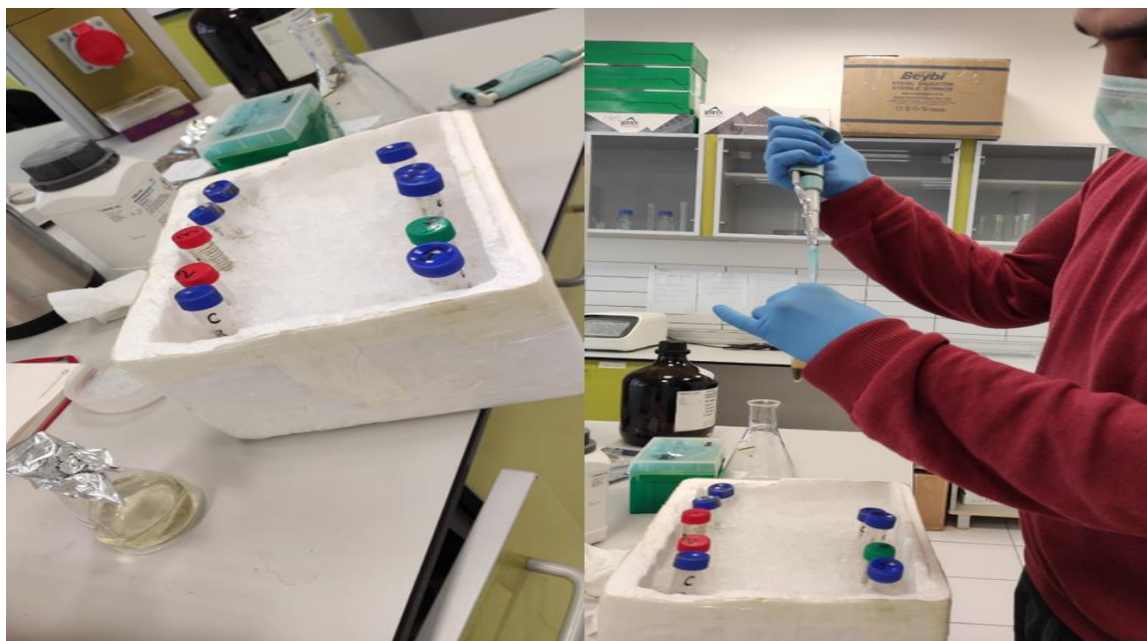


Figure 3.5. Biochemical assay of potato genotypes under normal and drought conditions

3.4.2 Malondialdehyde (MDA) content

MDA content was determined by using the method of Heath and Packer (1968) with minor amendments. A solution was prepared containing 200 ml of 0.1% TCA (Trichloroacetic Acid), 0.5% TBA (Thiobarbituric Acid), and 20% TCA. To create the solution with 0.5% TBA and 20% TCA, 20 g of TCA and 500 mg of TBA were weighed and dissolved in 70 ml of autoclaved distilled water, resulting in a total volume of 100 ml.

For the analysis, the following steps were performed:

1. A plant sample weighing 300 mg was dissected in 5 ml of 0.1% TCA solution (w/v), and the resulting homogenate was transferred to a 15 ml centrifuge tube.
2. The sample was then centrifuged at $10,000 \times g$ for 20 minutes at 4°C .
3. From the obtained supernatant, 4 ml was transferred to a new 15 ml centrifuge tube.
4. To this tube, 4 ml of a 20% (w/v) TCA solution containing 0.5% (w/v) TBA was added, and the mixture was incubated in water at 100°C for 30 minutes to stop the reaction. The tubes were subsequently cooled on ice.

5. Next, the samples were centrifuged at 10,000 ×g for 5 minutes at 4 °C, and the resulting mixture was thoroughly mixed. Absorbance was measured at 532 nm and 600 nm.
6. In the absorbance measurement, a blind sample consisting of 20% TCA solution containing 0.5% TBA was used as a reference.
7. The amount of MDA was calculated using the formula with a coefficient value of 155 mM⁻¹ cm⁻¹."

Results were calculated by using the following equation,

$$\text{MDA } (\mu\text{mol/g fresh tissue}) = ((A_{532} - A_{600}) / 155) \times (V_1 / V_2) \times (V_3 / V_4) \times (1 / W) \times \text{DF} \times 1000 \quad (3.3)$$

V1: Total volume of the reaction (8ml in our experiment)

V2: The volume of the measured sample in the cuvette (3 ml)

V3: The volume of 0.1% TCA used for extraction of tissue sample (5ml in our experiment)

V4: The volume of homogenate used for the reaction (4ml in our experiment)

W: The weight of tissue sample used for enzyme extraction (about 0.2-0.3 g)

DF: dilution factor of enzyme in reaction in the cuvette (If you did) (it is 1 in our case)

3.5 Enzyme Assays

The typical byproduct of plant cellular metabolism is the production of reactive oxygen species (ROS). According to Ergin et al. (2012) and Krishnamurthy and Rathinasabapathi (2013), oxidative stress in plants is brought on by a variety of environmental conditions, such as drought, which results in excessive production of ROS. According to Ershova et al. (2011) and Sharma et al. (2012), the ROS block enzymes and negatively impact crucial cellular components. Enzymatic systems made composed of superoxide dismutase (SOD), peroxidases (POD), and ascorbate peroxidase (APX) are among the most important ROS-scavenging processes. According to earlier research (Yin et al., 2008; Huang et al., 2010), alterations in antioxidant enzymes and antioxidants helped plants survive abiotic stress. To measure the activity of these enzymes following protocols were used for each enzyme.

3.5.1 Superoxide dismutase (SOD)

SOD activity was demonstrated by nitro blue tetrazolium (NBT) under light and in the presence of riboflavin. It was determined by measuring its ability to inhibit photochemical reduction. the activity of SOD that causes 50% of the inhibition of NBT reduction was determined under a 560 nm light wave (Giannopolitis and Ries, 1977).

For the measurement of the superoxidase enzyme, the following protocol was used.

A reaction mixture (3 ml) was prepared by adding 1.5 ml 100 mM K-Phosphate Buffer (pH 7.8), 300 1 mM EDTA-Na₂ (pH8.0), 0.629 ml 71 mM Methionine, 0.461 ml 410 µM NBT, 90 µl enzyme extract and 0.02 ml 1.2 mM Riboflavin, therefore the final concentration of each component in the final volume of the mix was adjusted to 50 mM K-Phosphate Buffer, 0.1 mM EDTA-Na₂ 14.9 mM Methionine, 63 µM NBT, and 8 µM Riboflavin. Then, the mix was incubated for five minutes at 4000 lux light intensity. Additionally, two blank samples devoid of enzyme extract were generated. Blank 1 without the enzyme extract was incubated for 5 min in the dark and Blank 2 without the enzyme extract in the light. NBT's photochemical reduction was halted by putting the tubes in the dark. Then, at 560 nm, the absorbance of the samples and the controls was determined. The quantity of the enzyme that inhibits NBT decrease by 50% is known as one unit (U) of SOD activity using the following formula.

$$\text{(Unit/g fresh tissue)} = \frac{((B2-B1)-(S-B1))}{(B2-B1)} \times 100 \times (1/50) \times ((V1 \times V2)/V3) \times (1/W) \times DF \quad (3.4)$$

B1 (Blank 1): Incubated in the dark for 5 minutes, Mixture does not include enzyme extract.

B2 (Blank 2): Incubated in light for 5 minutes, Mixture does not include enzyme extract.

S (Sample): Incubated in light for 5 minutes, Mixture includes enzyme extract

V1: Total volume of reaction mixture in the cuvette

V2: The volume of the extracted enzyme from the tissue sample

V3: The volume of enzyme extract in the reaction in the cuvette

W: The weight of tissue sample used for enzyme extraction

DF: dilution factor of enzyme in reaction in the cuvette

3.5.2 Peroxidase dismutase (POD)

POD effectiveness was measured according to the method developed by Chance and Maehly (1955). The oxidation of guaiacol in the reaction mixture was assessed for peroxidase (POD) activity by quantifying the rate of change of absorbance measured at 470 nm for two minutes. 2.84 ml of K-phosphate buffer (10 mM, pH 7.0), 0.05 ml of guaiacol (20 mM), and 0.09 ml of enzyme extract made up the reaction mixture. The addition of 0.02 ml H₂O₂ (40 mM) started the reaction. The blank included 2.93 ml of K-phosphate buffer (10 mM, pH 7.0), 0.05 ml of guaiacol (20 mM), and 0.02 ml H₂O₂ (40 mM) by employing an extinction coefficient of 26.6 mM per cm, POD activity was estimated. For the estimation of POD activity, the following formula was used.

$$(\text{Unit/g fresh tissue}) = ((\Delta A/E) \div t) \times ((V1 \times V2)/V3) \times (1/W) \times DF \quad (3.5)$$

$$\Delta A = \text{Abs}_2 - \text{Abs}_1 \quad (3.6)$$

E= molar extinction coefficient of Ascorbic acid at 470 nm with 1 cm diameter cuvette;
26.6 mM

t= reaction time (2 min)

V1: Total volume of reaction mixture in the cuvette

V2: The volume of the extracted enzyme from the tissue sample

V3: The volume of the enzyme extract in the reaction in the cuvette

W: The weight of tissue sample used for enzyme extraction

DF: dilution factor of enzyme in reaction in the cuvette

3.5.3 Ascorbic peroxidase (APX)

For measurement of the ascorbic peroxidase enzyme, the following protocol was used. APX activity was assessed by using the rate of change in absorbance at 290 nm to determine the amount of ascorbate that decreased throughout two minutes (Nakano and Asada, 1981). 50 mM K-phosphate buffer (pH 7.0), 1 mM EDTA-Na₂, 0.5 mM ascorbic acid, 0.1 mM H₂O₂, and 25 µl enzyme extract were all included in one microliter of the

reaction mixture. An attenuation coefficient of 2.8 mM per cm was used to determine the ascorbate peroxidase activity.

3.5.4 Catalytic activity (CAT)

CAT antioxidant enzyme was determined as μmol of H_2O_2 per min per milligram H_2O_2 breakdown and observed as a change in absorbance up to 0.01, practicing the method given by Chance and Maehly et al. (1955). A 30 μL of enzyme extract was used to prepare reaction mixture with the addition of 2670 μL of 50 mM potassium phosphate buffer (pH 7.0) and 100 mM H_2O_2 (300 μL) was added at the end to initiate the reaction. The absorbance was measured at 240 nm via spectrophotometer (UV-1800 Shimadzu). Reaction mixture was used as blank with no enzyme extract.

3.6 Yield/pot

For determining the final yield under both drought and normal conditions, potatoes were uprooted separately from all the pots, and tubers were separated from the soil. The yield of potato from each pot was determined by separating each potato from each pot and taking their weight by using a weighing balance.

3.7 Statistical Analysis

This experiment was conducted two times and the results for both repeats were statistically similar, so their means were used for further analysis. Data were analyzed using Statistix 8.1 software. Analysis of variance was performed by using a factorial design where genotypes were one factor while drought and control conditions were the second factors. Correlation analysis was done to compare the interaction of physiological, biochemical, and enzymatic activities among varieties and applied treatments. Principle Component Analysis was determined by using Past software (2001 version 4.13). Three PCA was done showing PCA under normal conditions, PCA under drought conditions and PCA under both normal and drought conditions. Cultivars that had +PC1 and +PC2 scores were classified as resistant, those with +PC1 and +PC2 scores as moderately resistant, those with -PC1 and +PC2 scores as moderately

susceptible, and those with -PC1 and -PC2 scores as susceptible according to Kakani et al.(2005).



CHAPTER IV

RESULTS

Physiological and biochemical parameters and enzymatic activity of different potato genotypes (high-yielding, moderate-yielding, and low-yielding genotypes) under normal and drought conditions were determined in this study.

Data for physiological parameters (stomatal conductance, photosynthesis rate, transpiration rate, relative water contents (RWC), chlorophyll contents, and leaf temperature), biochemical parameters (proline and malondialdehyde), enzymatic activity (superoxide dismutase, peroxidase, catalase, and ascorbate peroxidase) and yield are shown in Table 4.1 and 4.2.

Table 4.1. Data for biochemical and physiological parameters of different potato genotypes under normal and drought conditions

Genotypes	MDA		Proline		SOD		POD		APX		CAT		RWC	
	Normal	Drought	Normal	Drought	Normal	Drought	Normal	Drought	Normal	Drought	Normal	Drought	Normal	Drought
DT13085.01	18.93 c-e	23.80 a	3.76 l	14.21 a-c	684.2 f-l	775.4 ab	0.71 hi	1.02 d-g	1.12 m-p	2.21 a-f	1.99 h-n	2.49 a-c	88.99 a-e	64.07 n-p
DT14046.19	19.54 c-e	23.12 a	3.12 l	14.68 ab	683.9 f-m	769.1 a-e	0.72 h	1.01 e-g	1.01 n-p	2.10 a-h	2.03 f-m	2.51 ab	87.44 b-f	63.60 op
DT14016.01	18.26 c-e	23.67 a	3.25 l	14.43 ab	685.7 f-k	778.2 a	0.70 h-j	0.99 fg	0.98 op	2.03 b-h	2.04 e-m	2.44 a-f	90.82 ab	55.87 r
DT13052.23	18.83 c-e	24.10 a	3.70 l	13.93	678.1 f-m	769.5 a-d	0.70 hi	0.99 e-g	0.91 p	1.98 d-i	2.06 d-m	2.44 a-f	89.26 a-d	61.58 pq
DT13124.21	19.49 c-e	25.15 a	3.13 l	15.72 a	679.5 f-m	777.3 a	0.68 h-k	1.06 a-g	1.27 l-p	2.38 a-d	2.01 g-n	2.58 a	89.35 a-d	58.13 qr
DT13045.74	19.65 c-e	25.11 a	4.02 l	15.67 a	674.8 f-m	779.3 a	0.71 hi	1.01 e-g	1.13 m-p	2.29 a-e	2.01 g-n	2.48 a-d	80.04 g	55.52 r
DT13143.54	18.96 c-e	24.11 a	4.12 l	14.24 a-c	678.9 f-m	770.9 a-c	0.70 h-j	1.03 b-g	1.15 m-p	2.25 a-e	2.11 b-k	2.43 a-f	87.70 b-f	57.80 qr
DT13100.02	18.94 c-e	24.47 a	3.37 l	14.00 a-c	672.0 f-m	774.9 ab	0.73 h	1.02 c-g	1.13 m-p	2.13 a-g	2.08 c-l	2.44 a-f	87.73 b-f	61.86 pq
DT13136.22	18.75 c-e	24.88 a	3.66 l	15.57 a	678.8 f-m	780.5 a	0.63 h-l	0.97 g	1.27 l-p	2.47 a-c	1.94 i-o	1.95 i-p	86.06 c-f	63.73 op
LAURA	18.29 c-e	23.98 a	4.70 l	10.78 d-f	680.3 f-m	715.1 a-h	0.56 i-l	1.06 a-g	1.30 l-p	2.55 a	2.08 c-k	1.60 n-s	85.43 d-f	67.43 l-o
PELIKAN	17.50 de	23.53 a	3.63 l	11.92 c-e	622.1 k-m	713.9 a-i	0.51 l	1.11 a-g	1.23 l-p	2.5 ab	1.88 j-q	1.38 s	92.05 a	67.34 l-o
WENDY	17.71 c-e	23.20 a	4.07 l	9.71 e-h	650.9 h-m	712.9 a-i	0.54 j-l	1.12 a-g	1.16 m-p	2.56 a	2.05 e-m	1.56 o-s	88.67 a-e	67.66 l-o
JELLY	18.64 c-e	23.42 a	5.12 j-l	10.73 d-f	605.1 m	705.3 a-j	0.60 h-l	1.12 a-g	1.67 g-l	2.54 a	1.77 j-s	1.45 rs	87.43 b-f	68.40 j-m
43-111-11	18.92 c-e	23.31 a	4.05 l	12.67 b-d	675.3 f-m	710.7 a-j	0.58 h-l	1.12 a-g	1.26 l-p	2.46 a-c	2.14 b-j	1.52 o-s	88.06 a-f	68.05 k-n
99-115-11	19.56 c-e	23.38 a	4.56 l	10.43 d-g	657.3 g-m	738.9 a-g	0.54 kl	1.14 a-f	1.38 k-o	2.45 a-c	1.86 j-r	1.49 q-s	88.37 a-f	66.37 m-o
40-103-11	19.01 c-e	23.26 a	4.99 kl	11.12 d-f	647.7 h-m	738.9 a-f	0.54 kl	1.12 a-g	1.46 j-n	2.49 a-c	2.05 e-m	1.51 p-s	87.70 b-f	66.94 m-o
DT13037.31	18.94 c-e	24.05 a	4.02 l	11.18 d-f	605.4 lm	711.3 a-j	0.53 kl	1.06 a-g	1.37 k-p	2.52 a	1.87 j-q	1.55 o-s	87.79 b-f	69.93 h-m
Solist	17.31 e	22.64 ab	4.44 l	10.49 d-g	605.6 lm	711.4 a-j	0.53 kl	1.13 a-f	1.25 l-p	2.53 a	2.08 c-k	1.59 n-s	89.00 a-e	66.76 m-o
DT14042.24	18.16 c-e	20.15 bc	3.60 l	8.37 g-i	662.8 f-m	691.9 d-k	0.50 l	1.18 a-d	1.27 l-p	1.89 e-j	2.34 a-i	1.69 k-s	86.73 b-f	71.33 h-l
DT11088.01	19.74 c-e	20.27 bc	3.75 l	9.10 f-i	635.6 i-m	690.4 e-k	0.50 l	1.19 ab	1.56 i-m	1.78 f-k	2.38 a-h	1.64 m-s	85.10 ef	69.50 h-m
DT14083.28	19.90 c-e	24.05 a	3.63 l	7.99 hi	633.1 j-m	698.1 b-k	0.54 j-l	1.15 a-e	1.53 i-m	2.11 a-g	2.33 a-i	1.58 n-s	89.70 a-c	73.51 h
DORUK	18.20 c-e	19.31 c-e	4.11 l	8.07 hi	633.2 j-m	698.1 b-k	0.53 kl	1.21 a	1.35 k-p	1.63 h-l	2.19 a-j	1.64 m-s	89.30 a-d	72.23 h-j
DT11108.02	19.44 c-e	19.73 c-e	3.76 l	7.43 h-j	646.9 h-m	692.8 c-k	0.53 kl	1.21 a	1.06 n-p	2.03 c-h	2.49 a-c	1.65 l-s	88.67 a-e	72.93 hi
BETTINA	18.72 c-e	19.94 cd	3.74 l	7.51 hi	655.5 h-m	679.3 f-m	0.53 kl	1.18 a-c	0.99 n-p	1.86 e-j	2.46 a-e	1.64 m-s	87.71 b-f	69.19 i-m
FASAN	19.25 c-e	20.15 bc	3.34 l	7.28 i-k	647.1 h-m	689.5 f-k	0.53 kl	1.21 a	0.92 op	1.91 e-j	2.35 a-i	1.53 o-s	84.43 f	72.04 h-k
Tukey _(0.05)	2.64		2.33		84.28		0.16		0.46		0.40		3.86	

Table 4.2. Data for physiological and yield parameters of different potato genotypes under normal and drought conditions

Genotypes	Photosynthesis		LT		SC		Transpiration		Spad		Yield	
	Normal	Drought	Normal	Drought	Normal	Drought	Normal	Drought	Normal	Drought	Normal	Drought
DT13085.01	9.47 e-o	6.7 g-o	21.63	21.00	0.07 c	0.04 c	1.68 c-e	1.53 de	32.93	32.19	291.5 bc	170.42 o
DT14046.19	9.50 e-o	6.00 h-o	21.67	21.90	0.10 c	0.08 c	1.32 e	1.80 c-e	28.56	26.40	230.2 ij	170.2 o
DT14016.01	12.53 c-i	9.30 e-o	21.76	21.00	0.13 bc	0.11 c	2.51 b-e	2.18 b-e	27.50	27.67	256.3 fg	153.3 p
DT13052.23	26.73 a	3.26 m-o	24.67	21.78	0.16 a-c	0.09 c	2.30 b-e	2.36 b-e	34.70	36.36	296.4 b	138.7 q
DT13124.21	15.76 b-f	2.70 o	22.32	23.40	0.12 bc	0.08 c	2.90 b-e	4.15 ab	31.67	33.23	276.1 de	168.5 o
DT13045.74	18.43 bc	3.63 l-o	22.00	23.76	0.12 bc	0.10 c	2.93 b-e	2.85 b-e	29.86	29.40	227.1 if	192.3 m
DT13143.54	11.63 c-k	4.83 i-o	22.39	23.40	0.10 c	0.11 c	3.81 abc	2.53 b-e	34.86	32.13	266.0 ef	168.2 o
DT13100.02	11.50 c-k	4.90 i-o	24.45	21.46	0.08 c	0.06 c	2.84 b-e	1.96 b-e	35.23	32.10	268.9 d-f	143.6 pq
DT13136.22	18.16 b-d	3.46 m-o	24.56	23.76	0.10 c	0.07 c	3.34 a-e	2.63 b-e	33.06	32.73	334.0 a	186.3 mn
LAURA	10.90 c-m	6.67 g-o	21.56	22.43	0.06	0.08 c	2.57 b-e	2.66 b-e	33.00	28.16	280.8 cd	66.82 uv
PELIKAN	10.03 e-o	5.43 i-o	20.34	21.56	0.10 c	0.10 c	3.54 a-d	1.70 c-e	31.06	29.86	291.0 bc	38.17
WENDY	13.33 b-h	3.13 m-o	24.98	23.59	0.12 bc	0.11 c	3.15 b-e	3.13 b-e	33.33	34.16	270.4 de	72.24 u
JELLY	9.16 f-o	8.50 f-o	23.17	22.49	0.27 a	0.14 bc	3.18 b-e	4.14 ab	33.96	27.63	262.5 e-g	91.48 st
43-111-11	13.49 b-h	2.95 no	22.34	24.09	0.24 ab	0.04 c	3.33 a-e	2.18 b-e	33.76	27.20	237.7 hi	72.17 u
99-115-11	8.20 f-o	3.53 m-o	23.71	22.43	0.05 c	0.06 c	3.34 a-e	2.62 b-e	35.23	31.16	266.6 ef	99.35 rs
40-103-11	10.53 d-n	3.53 m-o	22.82	22.00	0.07 c	0.15 bc	2.14 b-e	2.31 b-e	31.53	29.00	250.52 gh	105.52 r
DT13037.31	20.90 ab	3.16 m-o	23.61	22.73	0.09 c	0.07 c	2.42 b-e	2.269 b-e	29.53	29.83	189.54 mn	50.88 wx
Solist	12.13 c-j	2.70 o	22.47	23.84	0.06 c	0.08 c	2.07 b-e	2.09 b-e	27.16	31.40	177.2 no	67.47 uv
DT14042.24	11.90 c-k	3.56 m-o	22.67	23.98	0.09 c	0.05 c	2.07 b-e	2.09 b-e	33.50	28.03	152.1 pq	78.67 tu
DT11088.01	17.00 b-e	5.13 i-o	21.09	22.87	0.10 c	0.16 abc	2.66 b-e	2.51 b-e	38.06	42.36	210.6 kl	48.63 w
DT14083.28	18.33 bc	10.70 c-n	21.45	24.81	0.07 c	0.11 c	3.10 b-e	2.19 b-e	29.53	36.30	199.4 lm	48.83 wx
DORUK	11.36 c-l	4.30 k-o	24.98	21.38	0.07 c	0.07 c	3.05 b-e	1.65 c-e	42.86	32.56	192.8 m	104.3 rs
DT11108.02	15.03 b-f	4.43 j-o	24.76	21.63	0.07 c	0.07 c	3.07 b-e	1.73 c-e	28.96	38.50	223.31 jk	55.95 vw
BETTINA	14.23 b-g	3.03 no	21.382	21.78	0.06 c	0.08 c	3.31 a-e	2.04 b-e	30.76	30.06	150.75 pq	98.94 rs
FASAN	15.26 b-f	3.03 no	24.38	24.01	0.05 c	0.07 c	5.47 a	2.80 b-e	37.60	41.90	21.61 jk	91.63 st
Tukey (0.05)	8.18		Ns		0.12		2.08		ns		14.9	

LT= Leaf temperature; SC= Stomatal conductance; ns = non significant

4.1 Physiological Parameters

4.1.1 Stomatal conductance

Stomatal conductance was determined for all the genotypes at both initial and final stages under both control and drought conditions. Data showed a statistically significant difference among the high-yielding, moderate-yielding, and low-yielding genotypes and applied treatments at both initial and final stages (Table 4.3, Figure 4.1 A, B). It has been observed that under drought conditions, the stomatal conductance decreased in all the genotypes. In general, all the genotypes responded to a greater extent under drought conditions as compared with normal conditions (Figure 4.1 B).

The highest stomatal conductance was observed in the DT13052.23 genotype under drought conditions while the DT14016.01 genotype under normal growth conditions and the lowest stomatal conductance was observed in the DT14046.19 under drought and DT13124.21 under drought conditions, among the high-yielding genotypes (Figure 4.1 B). Among the moderate-yielding genotypes, the 43-111-11 genotype produced a higher stomatal conductance under drought and normal conditions while the WENDY genotype produced a lower stomatal conductance under normal and drought conditions (Figure 4.1). Genotype DT14042.24 had the highest stomatal conductance under control conditions while the DT11088.01 genotype had the highest stomatal conductance under drought conditions and the DT11108.02 genotype produced the lowest stomatal conductance under both control and drought conditions among low-yielding genotypes (Figure 4.1 B).

Table 4.3. Analysis of variance for stomatal conductance

Biochemical traits	Source of variation	Mean square	<i>F</i>	<i>P</i>
Stomatal conductance	Genotypes	0.0066	6.70	0.00
	Treatment (Control/Drought)	0.0095	6.94	0.011
	Interaction (G×T)	0.0046	3.37	0.0001

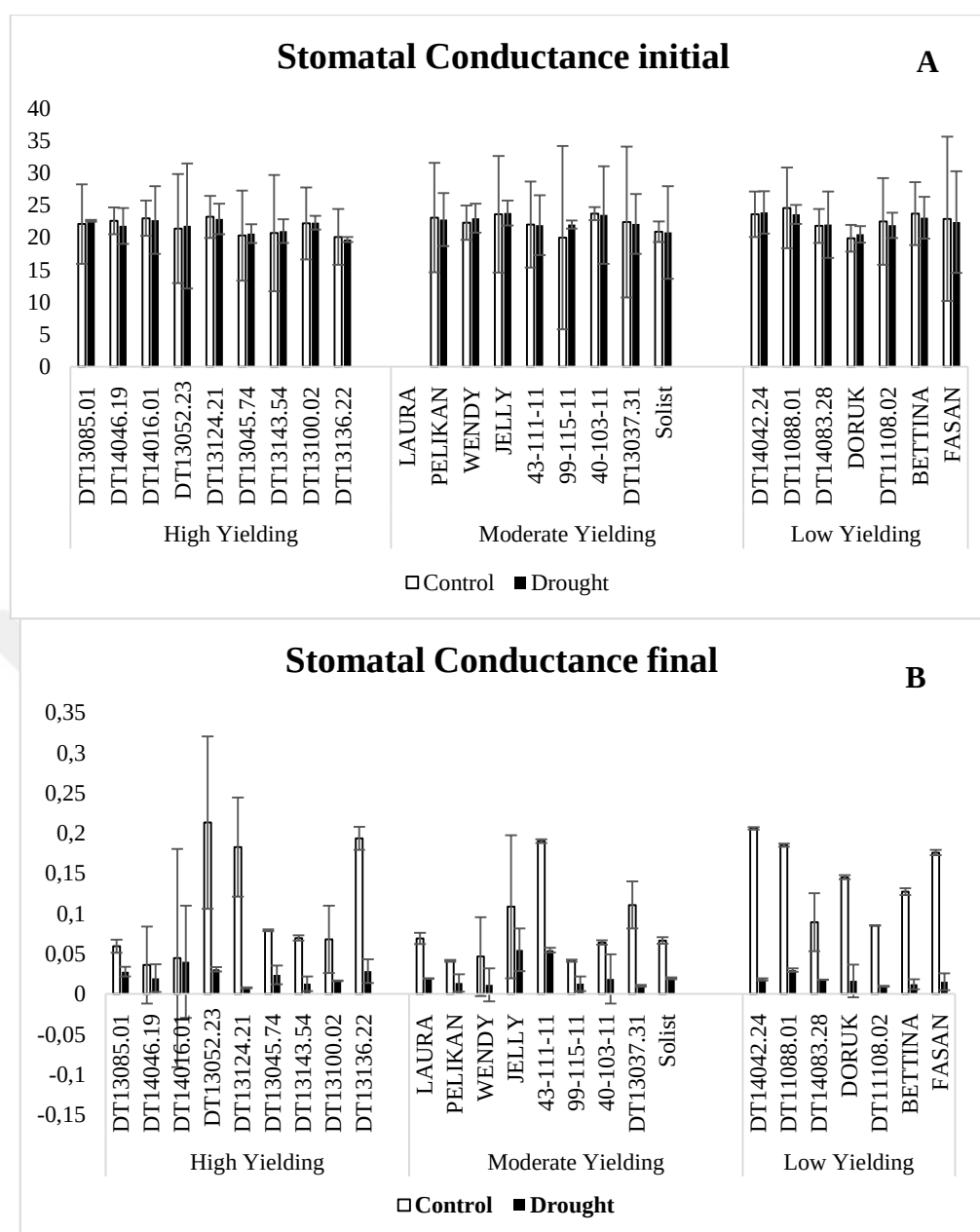


Figure 4.1. A, B. Stomatal conductance rate of different potato genotypes under control and drought conditions on day 0 and day 12

4.1.2 Photosynthetic rate

Photosynthetic rate was determined for all the genotypes at both initial and final stages under both control and drought conditions. Data showed a statistically significant difference among the high-yielding, moderate-yielding, and low-yielding genotypes and applied treatments at both initial and final stages (Table 4.4, Figure 4.2 A, B). It has been observed that under drought conditions, the photosynthetic rate decreased in all the

genotypes. In general, all the genotypes responded to a greater extent under drought conditions as compared with normal conditions (Figure 4.2 B).

Among genotypes, the highest photosynthetic rate was observed in high-yielding genotypes, especially in DT13052.23 while the lowest was presented in genotypes DT13085.01 and DT14046.19 under drought conditions while DT14016.01 genotype produced the highest and genotype DT13124.21 had the highest photosynthetic rate under normal conditions (Figure 4.2 B). Among the moderate-yielding genotypes, DT13037.31 had the higher photosynthetic rate under drought conditions and the lowest at normal conditions respectively (Figure 4.2 B). Genotype DT11088.01 had the highest photosynthetic rate under control and drought conditions while the FASAN genotype produced a minimum photosynthetic rate under normal and DT11108.02 genotype produced the lowest photosynthetic rate under drought conditions among low-yielding genotypes respectively.

Table 4.4. Analysis of variance for photosynthetic rate

Biochemical traits	Source of variation	Mean square	<i>F</i>	<i>P</i>
Photosynthetic rate	Genotypes	32.10	6.37	0.00
	Treatment (Control/Drought)	2951.80	313.27	0.00
	Interaction (G×T)	40.69	4.32	0.00

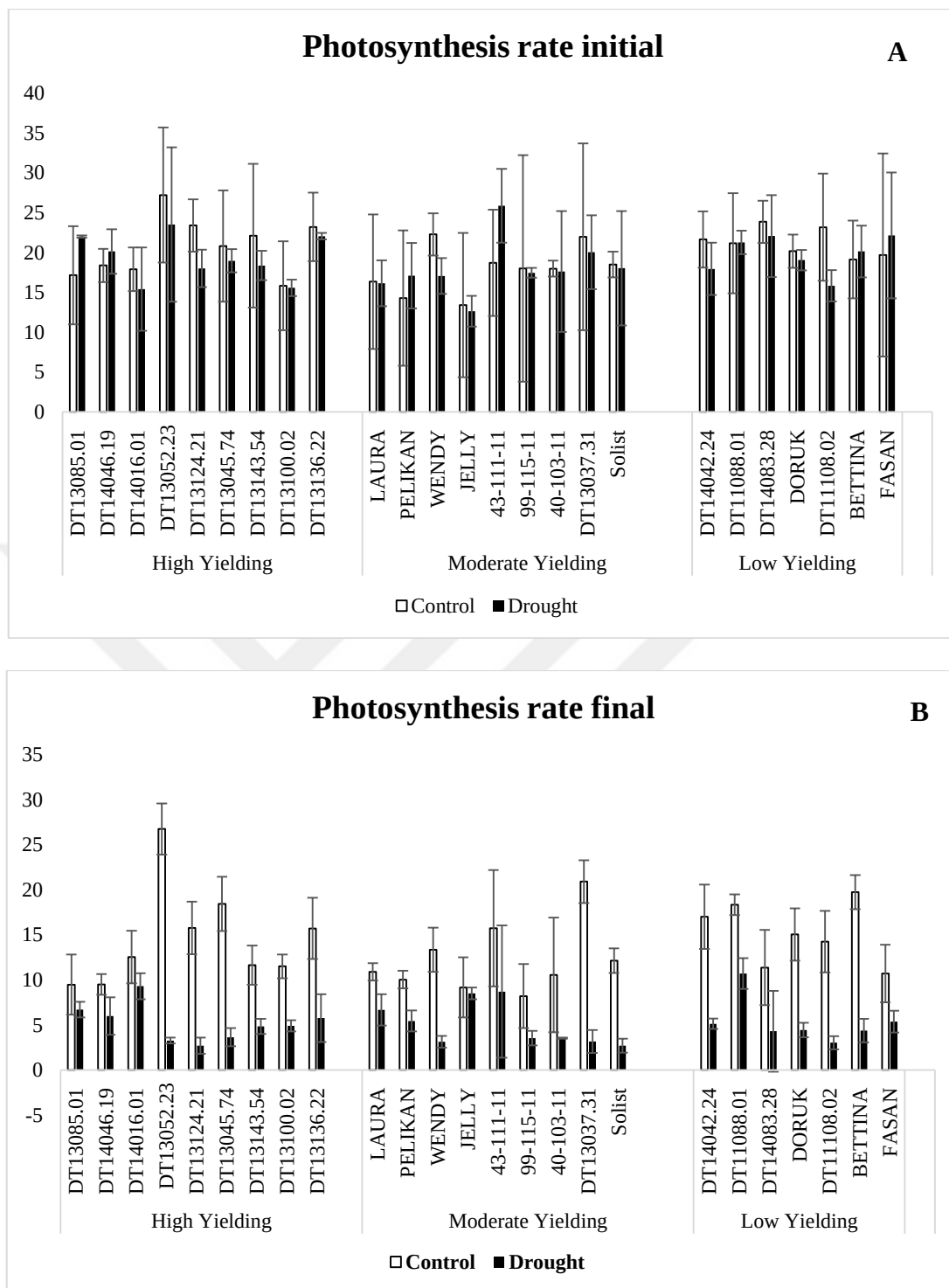


Figure 4.2. A, B. Photosynthetic rate of different potato genotypes under control and drought conditions on day 0 and day 12

4.1.3 Transpiration rate

Transpiration rate varied significantly among all the high-yielding, moderate-yielding, and low-yielding genotypes treatments as well as under both normal and drought

treatments at both initial and final stages (Table 4.5, Figure 4.3 A, B). Data showed that under drought conditions, the transpiration rate decreased among all high-yielding, moderate-yielding, and low-yielding genotypes (Figure 4.3).

Among the high-yielding genotypes, the highest transpiration rate was observed in the DT13136.22 genotype under both drought and normal growth conditions while the lowest was observed in the DT14046.19 under normal and DT13052.23 under drought conditions (Figure 4.3 B). Among the moderate-yielding genotypes, 40-103-11 M and PELIKAN genotypes produced higher transpiration rates under drought conditions and normal conditions respectively while PELIKAN and Solist genotypes produced the lowest transpiration rate under normal and drought conditions (Figure 4.10). Genotype BETTINA had the highest photosynthetic rate under control conditions while the DORUK genotype had the highest photosynthetic rate under drought conditions and DT14042.24 and DT11088.01 genotypes produced the lowest photosynthetic rate under normal and drought conditions among low-yielding genotypes respectively (Figure 4.3 B).

Table 4.5. Analysis of variance for transpiration rate

Biochemical traits	Source of variation	Mean square	<i>F</i>	<i>P</i>
Transpiration rate	Genotypes	1.9957	4.42	0.00
	Treatment (Control/Drought)	7.1607	19.75	0.00
	Interaction (G×T)	1.2721	3.51	0.0001

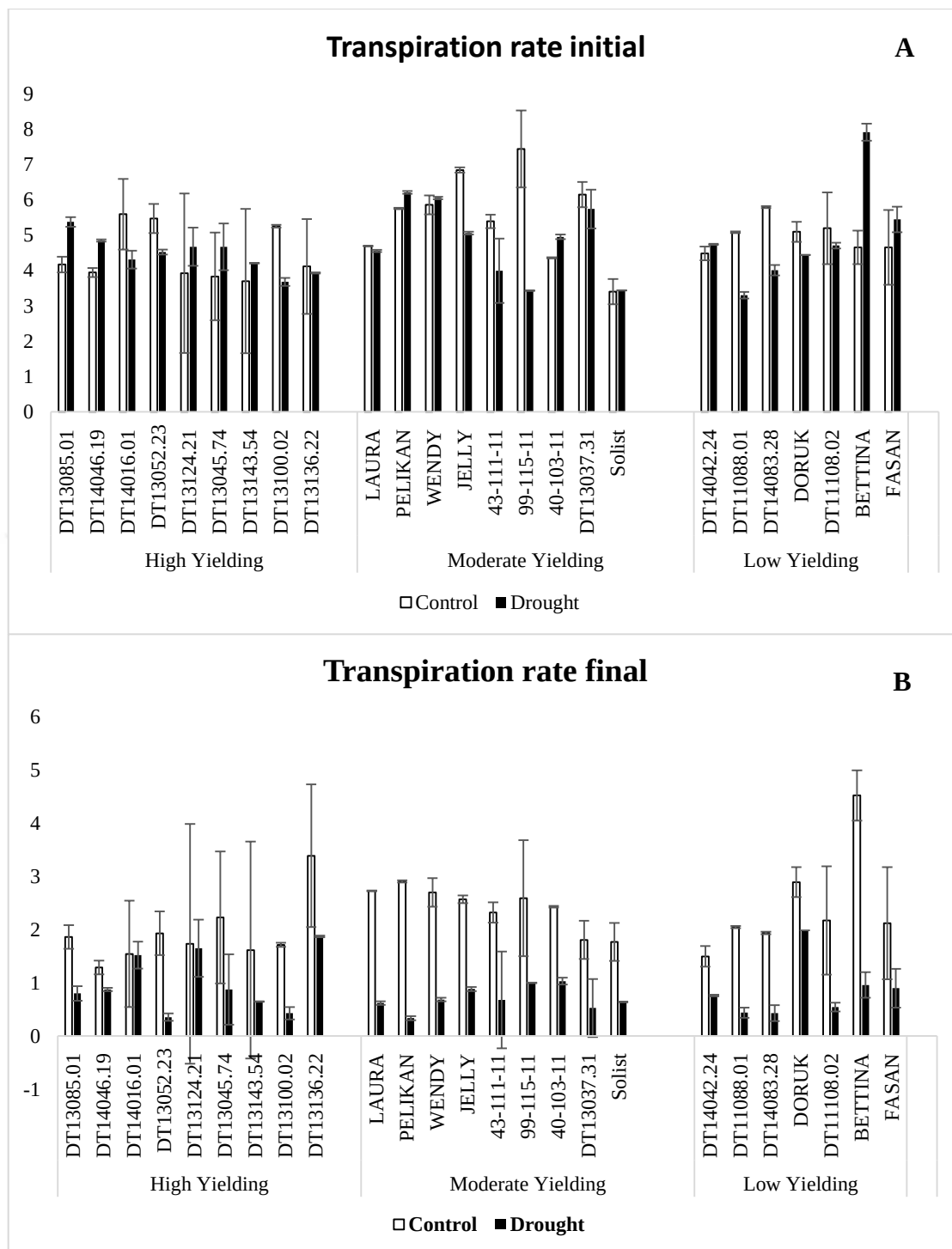


Figure 4.3. A, B. Transpiration rate of different potato genotypes under control and drought conditions on day 0 and day 12

4.1.4 Relative water contents (RWC)

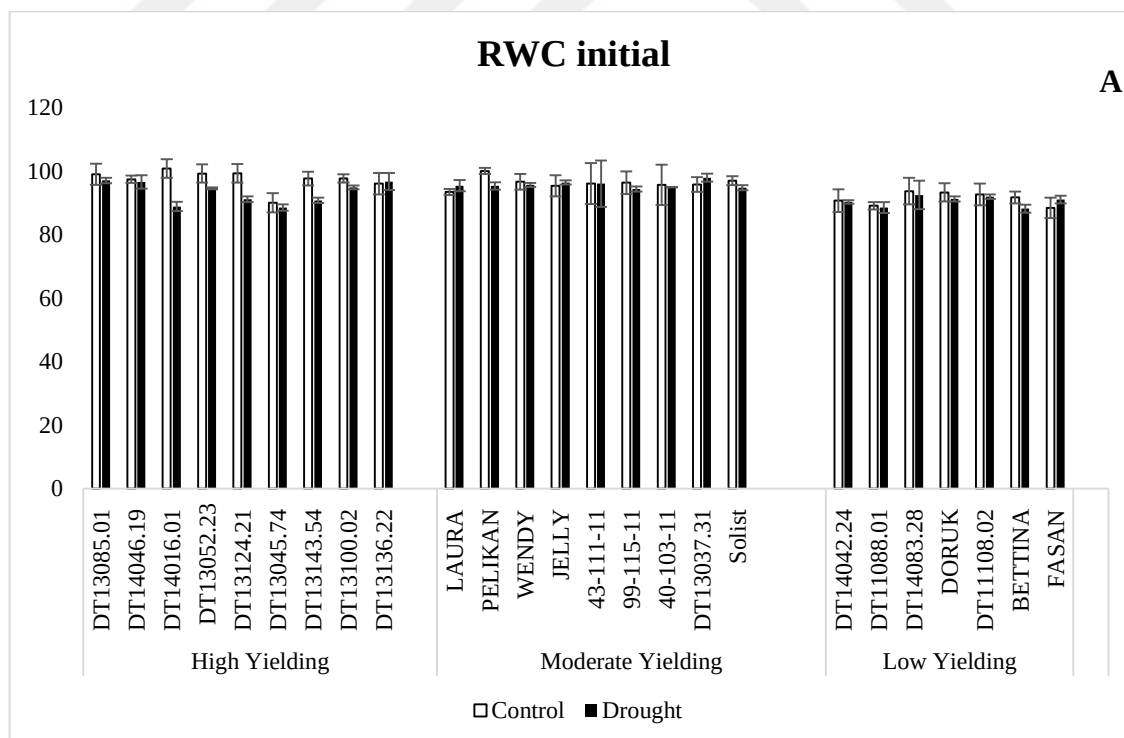
Relative water contents (RWC) varied between the high-yielding, moderate-yielding, and low-yielding genotypes at both initial and final stages (Table 4.6, Figure 4.4 A, B).

Among all genotypes, low-yielding genotypes had the highest RWC compared with high-yielding and moderate-yielding genotypes (Figure 4.4 B). High-yielding genotypes reduced the RWC to a greater extent.

Among the high-yielding genotypes, DT14016.01 and DT13085.01 genotypes produced higher RWC under normal and drought conditions while the genotype DT13045.74 produced lower contents under both conditions (Figure 4.4, B). Genotypes PELIKAN and DT13037.31 had the highest RWC under control and drought conditions while LAURA and 40-103-11 genotypes produced minimum RWC among moderate-yielding genotypes respectively.

Table 4.6. Analysis of variance for RWC

Biochemical traits	Source of variation	Mean square	<i>F</i>	<i>P</i>
RWC	Genotypes	107.0	30.99	0.00
	Treatment (Control/Drought)	35220.3	12474.9	0.00
	Interaction (G×T)	90.0	31.88	0.00



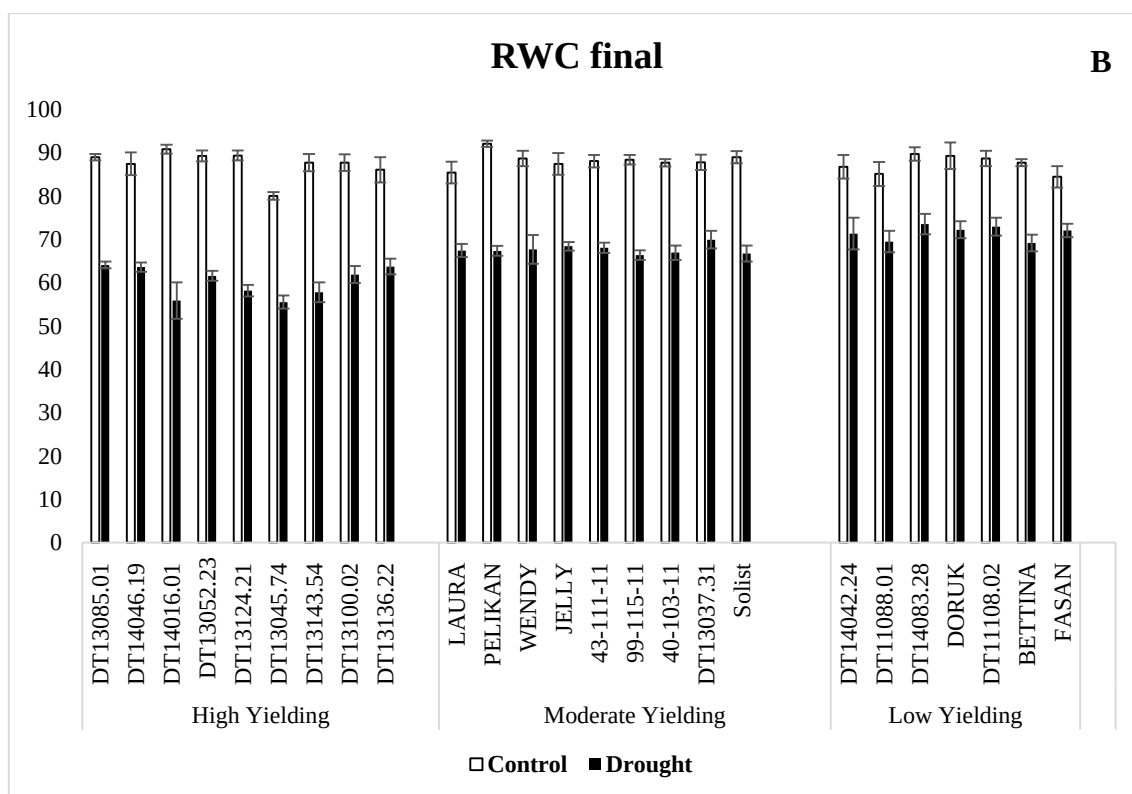


Figure 4.4. A, B. RWC contents of different potato genotypes under control and drought conditions on day 0 and day 12

4.1.5 Chlorophyll contents

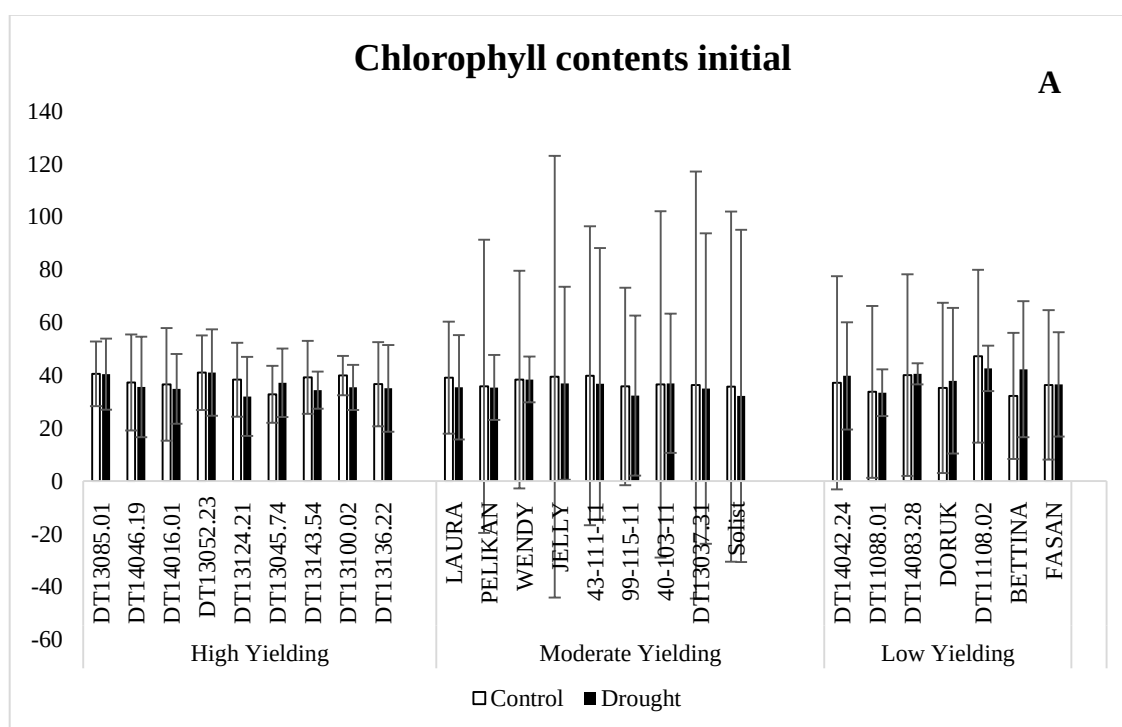
Chlorophyll contents (SPAD value) under normal and drought conditions were examined in high-yielding, moderate-yielding, and low-yielding genotypes of potatoes at both initial and final stages. Results for chlorophyll contents are shown in Table 4.4 and Figure 4.5 A, B. Analysis of variance showed a significant difference between observed genotypes and applied treatments at both initial and final stages (Table 4.7). Among all genotypes, low-yielding genotypes had the highest chlorophyll contents under control and drought conditions as compared with high-yielding and moderate-yielding genotypes (Figure 4.5).

Among the high-yielding genotypes, the DT13052.23 genotype produced higher chlorophyll content under normal and drought conditions while the genotype DT14016.01 produced lower contents under both conditions (Figure 4.5). Genotype JELLY had the highest chlorophyll content under control and drought conditions while Solist genotype produced minimum chlorophyll content among moderate yielding

genotypes respectively. Among the low-yielding genotypes, the DORUK genotype produced higher chlorophyll content under normal and drought conditions while the genotype DT14042.24 produced lower contents under both conditions (Figure 4.5).

Table 4.7. Analysis of variance for chlorophyll contents

Biochemical traits	Source of variation	Mean square	<i>F</i>	<i>P</i>
Chlorophyll contents	Genotypes	74.5075	3.19	0.0003
	Treatment (Control/Drought)	8.6400	0.32	0.0014
	Interaction (G×T)	24.6699	0.93	0.00



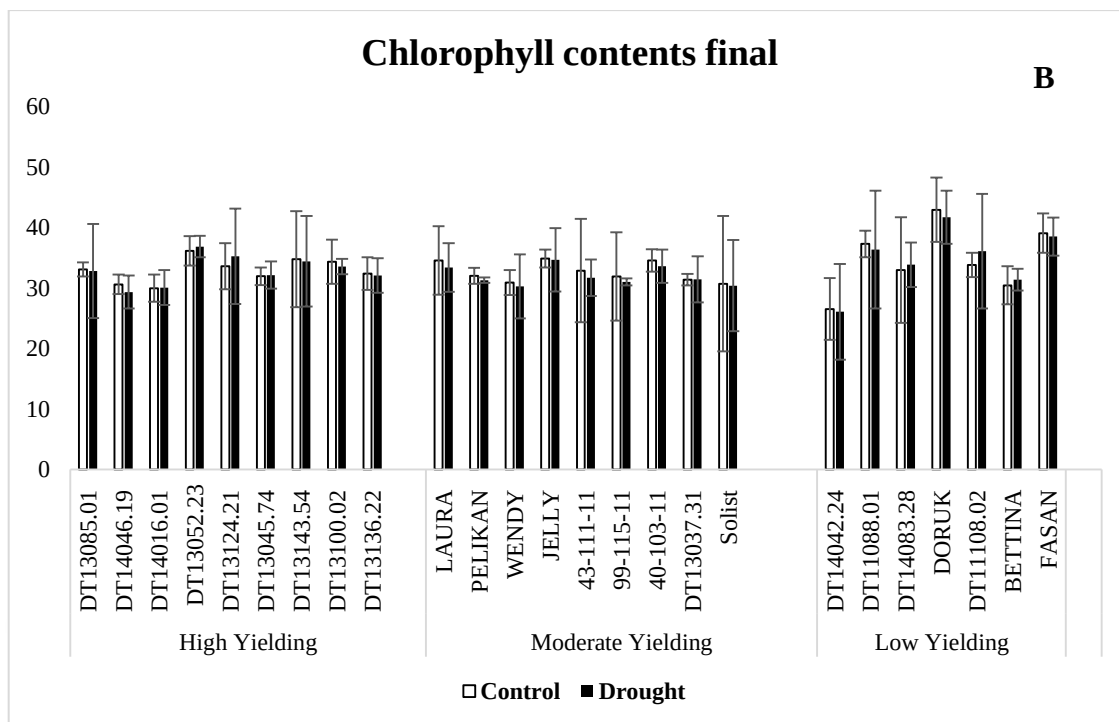


Figure 4.5. A, B. Chlorophyll contents of different potato genotypes under control and drought conditions on day 0 and day 12

4.1.6 Leaf temperature

Leaf temperature under normal and drought conditions was examined in high-yielding, moderate-yielding, and low-yielding genotypes of potatoes. Results for leaf temperature values at both initial and final stages are shown in Table 4.8 and Figure 4.6 A, B. Analysis of variance showed a non-significant difference between observed genotypes and applied treatments (Table 4.8). It has been observed that under drought conditions, the leaf temperature increased in all the genotypes as compared with normal conditions (Figure 4.6 B).

Among the genotypes, moderate and low-yielding genotypes had higher leaf temperatures as compared with the high-yielding genotypes. Genotype WENDY had the highest leaf temperature under both drought and normal conditions while genotype 43-111-11 had the lowest values of temperature for both treatments (Figure 4.6 B).

Table 4.8. Analysis of variance for leaf temperature

Biochemical traits	Source of variation	Mean square	<i>F</i>	<i>P</i>
Leaf Temperature	Genotypes	2.904	1.74	0.051
	Treatment (Control/Drought)	0.028	0.04	0.8485
	Interaction (G×T)	1.606	2.05	0.016

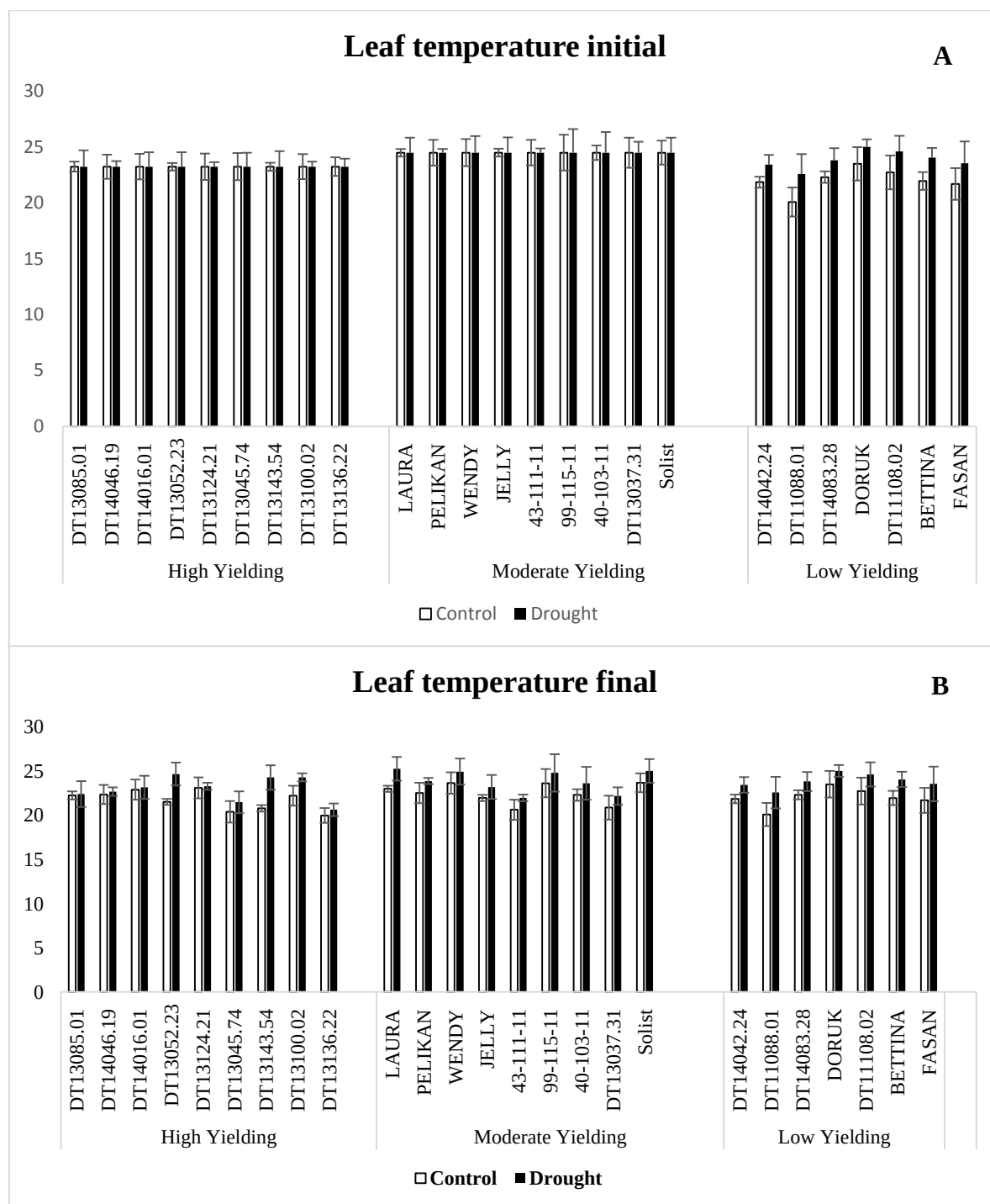


Figure 4.6. A, B. Leaf temperature of different potato genotypes under control and drought conditions on day 0 and day 12

4.2 Biochemical Parameters

4.2.1 Proline content

Data for proline contents also showed a significant difference between low-yielding, moderate-yielding, and high-yielding genotypes as well as applied treatments (Table 4.9, Figure 4.7). Results showed that under drought conditions, proline contents increased at a huge rate among all high-yielding, moderate-yielding, and low-yielding genotypes. The high-yielding genotypes responded to a greater extent and higher proline contents were produced under drought conditions as compared with moderate and low-yielding genotypes (Figure 4.7).

The highest proline content was observed in DT13052.23 while the lowest was in DT14016.01 among high-yielding genotypes. In moderate-yielding genotypes, the highest proline content was observed in 43-111-11 while the lowest was observed in the WENDY genotype, while in low-yielding genotypes, the highest proline content was observed in DT11088.01 while the lowest was observed in the BETTINA genotype (Figure 4.7).

Table 4.9. Analysis of variance for proline contents

Biochemical traits	Source of variation	Mean square	<i>F</i>	<i>P</i>
Proline	Genotypes	23.30	23.03	0.00
	Treatment (Control/Drought)	4314.11	4192.07	0.00
	Interaction (G×T)	27.37	26.60	0.00

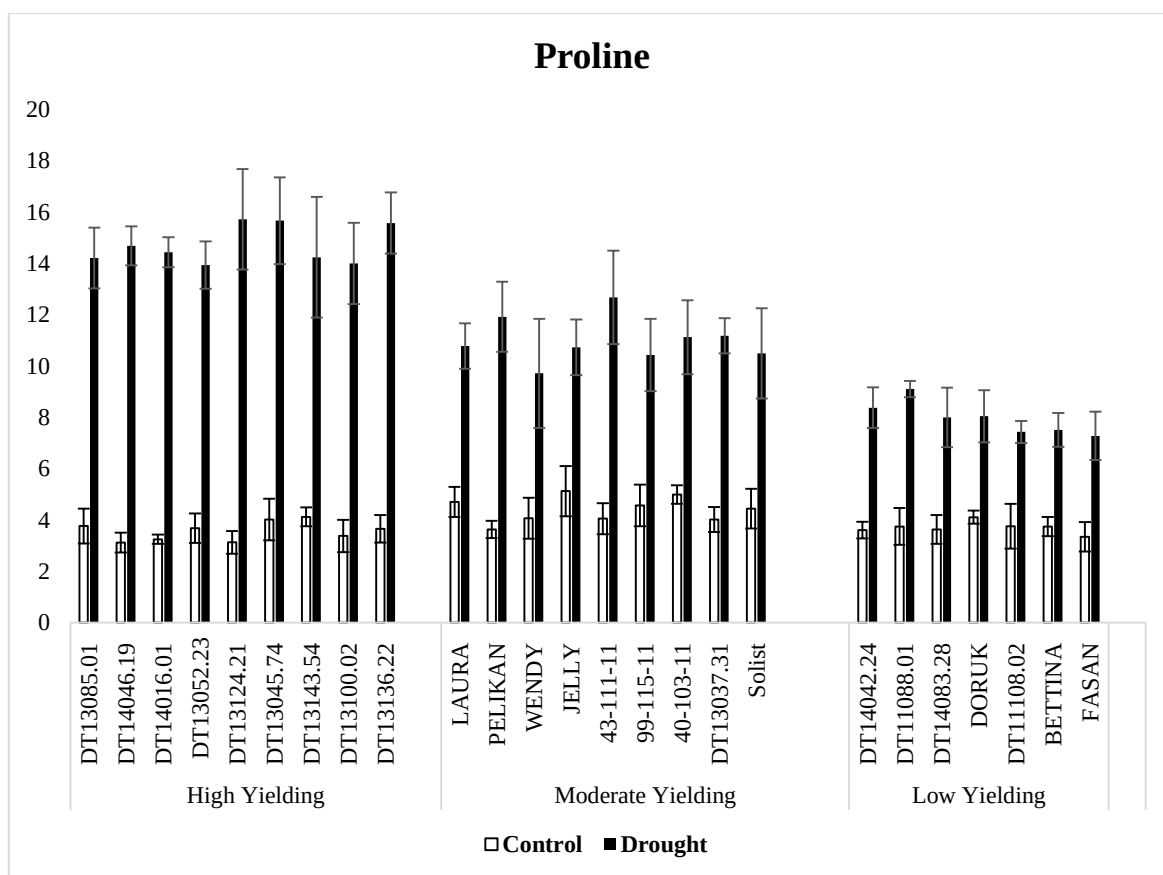


Figure 4.7. Proline contents of different potato genotypes under control and drought conditions on day 12

4.2.2 Malondialdehyde (MDA)

Malondialdehyde (MDA) levels under normal and drought conditions were examined in high-yielding, moderate-yielding, and low-yielding genotypes of potatoes. Results for MDA levels are shown in Table 4.10 and Figure 4.8. Data showed a significant difference between observed genotypes and applied treatments. It has been observed that under drought conditions, MDA contents increased in all high-yielding, moderate-yielding, and low-yielding genotypes. The high-yielding genotype responds to a greater extent under drought conditions as compared with moderate-yielding and low-yielding genotypes (Figure 4.8).

The highest MDA was observed in DT13045.74 H while the lowest was in DT14046.19 among high-yielding genotypes. In moderate-yielding genotypes, the highest MDA content was observed in LAURA while the lowest was observed in the Solist genotype,

while in low-yielding genotypes, the highest MDA content was observed in DT14042.24 while the lowest was observed in the DORUK genotype (Figure 4.8).

Table 4.10. Analysis of variance for MDA

Biochemical traits	Source of variation	Mean square	<i>F</i>	<i>P</i>
MDA	Genotypes	12.48	10.91	0.00
	Treatment (Control/Drought)	1162.58	878.52	0.00
	Interaction (G×T)	11.89	8.98	0.00

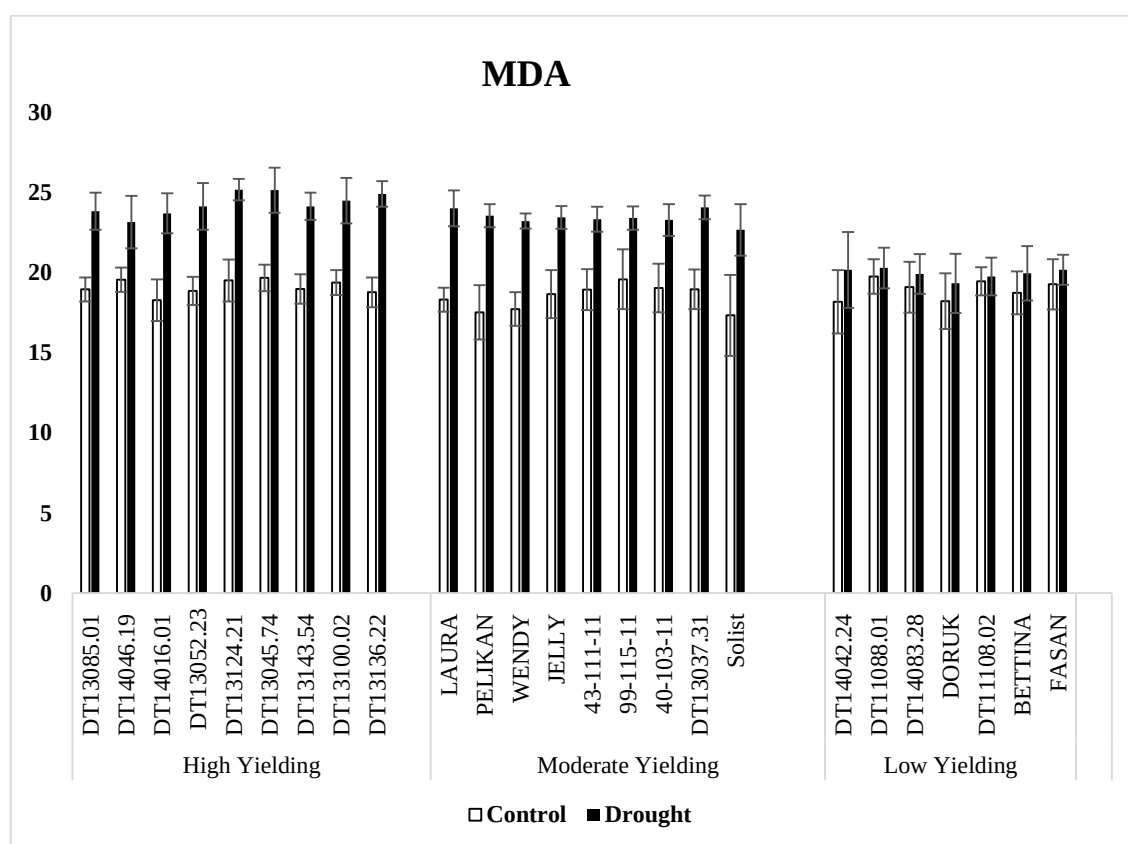


Figure 4.8. MDA contents of different potato genotypes under control and drought conditions on day 12

4.2.3 Superoxide dismutase (SOD)

Superoxide dismutase (SOD) activity varied among low-yielding, moderate-yielding, and high-yielding genotypes as well as under both normal and drought treatments (Table 4.11, Figure 4.9). Data showed that under drought conditions, SOD contents increased among all high-yielding, moderate-yielding, and low-yielding genotypes.

Among genotypes, the highest SOD activity was observed in high-yielding genotypes, especially in DT13136.22 while the lowest was presented in low-yielding genotypes DT14083.28 and DT11088.01 under both drought and normal growth conditions. Among the moderate-yielding genotypes, 99-115-11 and 40-103-11 M genotypes produced higher SOD activity under drought conditions while LAURA and 43-111-11 genotypes produced the highest SOD activity under normal conditions and DT13037.31 genotype produced the lowest SOD activity under both conditions (Figure 4.9).

Table 4.11. Analysis of variance for SOD

Biochemical traits	Source of variation	Mean square	<i>F</i>	<i>P</i>
SOD	Genotypes	9020	11.28	0.00
	Treatment (Control/Drought)	410433	276.78	0.00
	Interaction (G×T)	2293	1.55	0.00

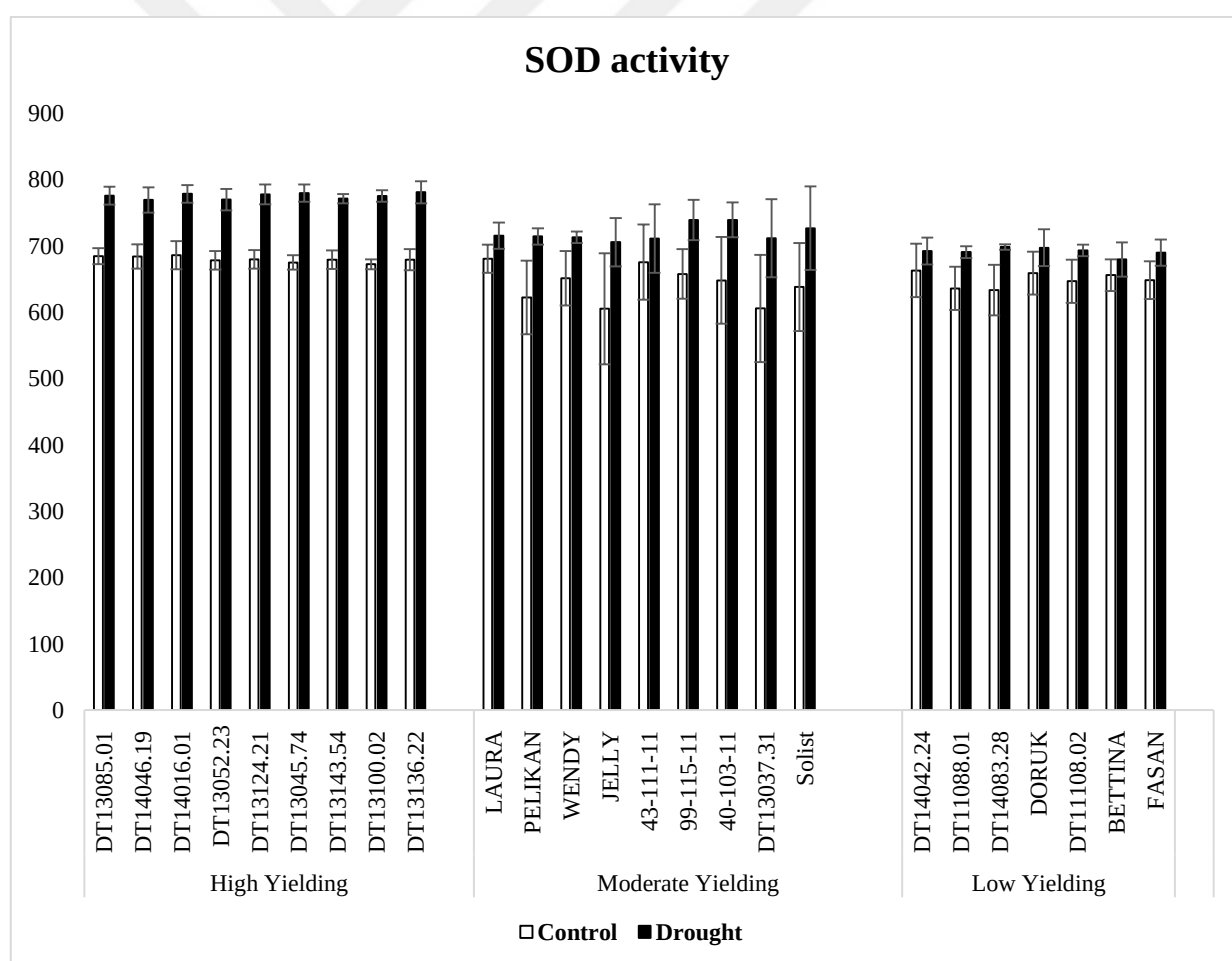


Figure 4.9. SOD contents of different potato genotypes under control and drought conditions on day 12

4.2.4 Peroxidase (POD)

Peroxidase (POD) activity showed a significant difference between low-yielding, moderate-yielding, and high-yielding genotypes as well as applied treatments (Table 4.12, Figure 4.10). Data for POD activity showed that under drought conditions, its activity increased at a huge rate among all high-yielding, moderate-yielding, and low-yielding genotypes. The low-yielding genotypes responded to a greater extent under drought conditions as compared with moderate-yielding and high-yielding genotypes (Figure 4.10).

The highest POD activity was observed in FASAN while the lowest was in DT14083.28 among low-yielding genotypes under drought conditions. Under normal conditions, the highest POD activity was observed in DT13100.02 while the lowest was observed in the DT13136.22 genotype among high-yielding genotypes. (Figure 4.10).

Table 4.12. Analysis of variance for POD

Biochemical traits	Source of variation	Mean square	<i>F</i>	<i>P</i>
POD	Genotypes	0.0067	1.74	0.00
	Treatment (Control/Drought)	18.8321	3504.95	0.00
	Interaction (G×T)	0.0698	13.00	0.00

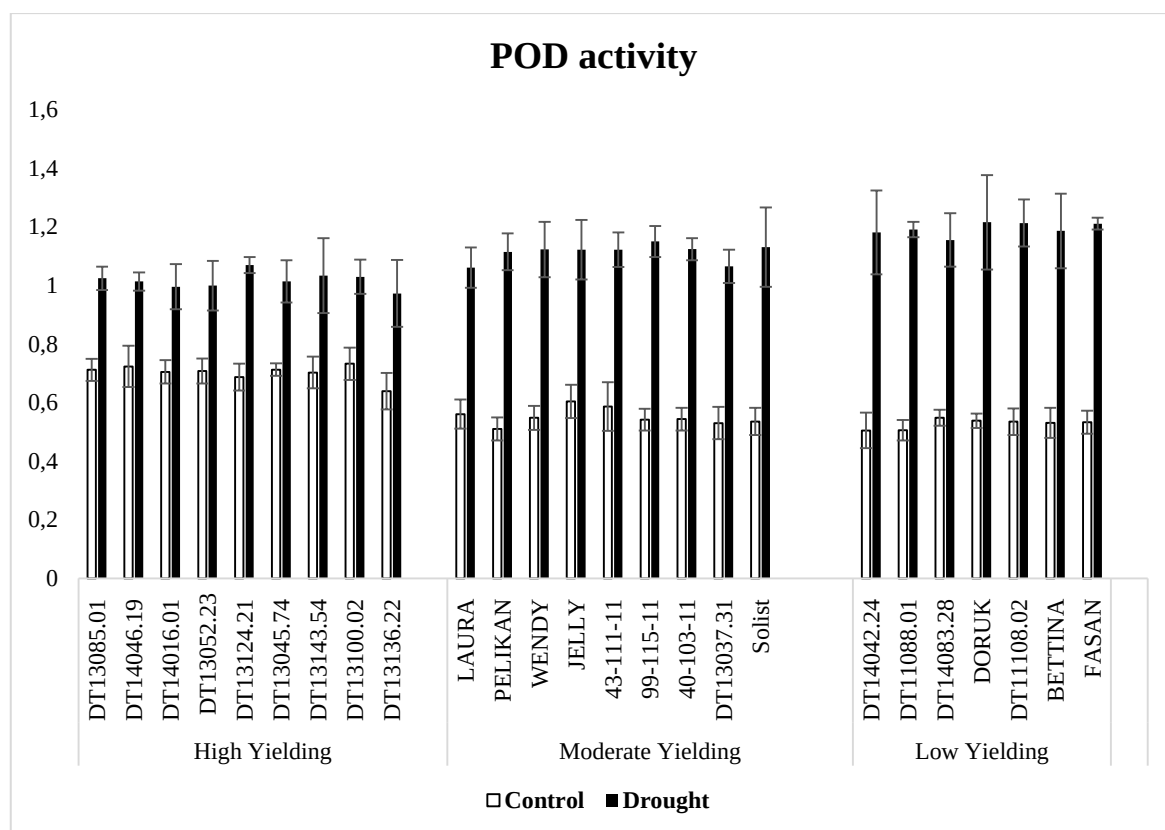


Figure 4.10. POD contents of different potato genotypes under control and drought conditions on day 12

4.2.5 Ascorbate peroxidase (APX)

Ascorbate peroxidase (APX) activity under normal and drought conditions was examined in high-yielding, moderate-yielding, and low-yielding genotypes of potatoes. Results for APX contents are shown in Table 4.13 and Figure 4.11. Analysis of variance showed a significant difference between observed genotypes and applied treatments. It was observed that under drought conditions, APX activity increased in moderate-yielding genotypes as compared with all high-yielding, and low-yielding genotypes. In general, all the genotypes responded to a greater extent under drought conditions as compared with normal conditions (Figure 4.11).

Among genotypes, the highest APX activity was observed in moderate-yielding genotypes, especially in LAURA while the lowest was presented in genotypes 99-115-11 and 43-111-11 under both drought and normal growth conditions. Among the low-

yielding genotypes, DT14083.28 and DT13136.22 genotypes produced higher APX contents under both normal and drought conditions respectively (Figure 4.11).

Table 4.13. Analysis of variance for APX

Biochemical traits	Source of variation	Mean square	<i>F</i>	<i>P</i>
APX	Genotypes	0.4615	11.74	0.00
	Treatment (Control/Drought)	74.4789	1848.92	0.00
	Interaction (G×T)	0.2552	6.34	0.00

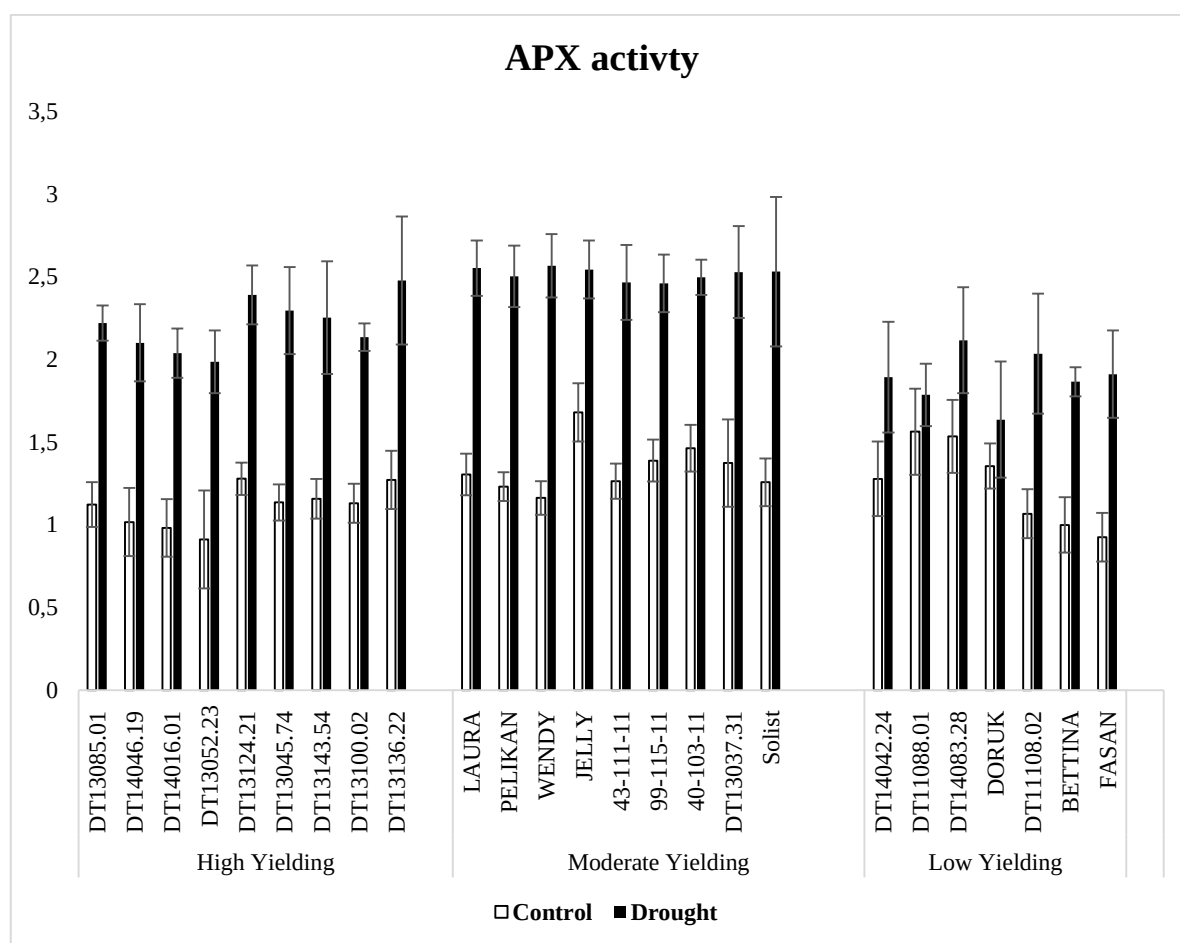


Figure 4.11. APX contents of different potato genotypes under control and drought conditions on day 12

4.2.5 Catalase (CAT)

The catalase activity of potatoes is shown in Table 4.14 and Figure 4.12. Data for analysis of variance showed a highly significant difference between the CAT activity of potato genotypes under control and drought conditions. Data showed a huge variation

among high-yielding, low-yielding, and moderate-yielding genotypes under drought conditions (Figure 4.12). It was observed that under drought conditions, CAT activity decreased in moderate-yielding and low-yielding genotypes while in the high-yielding genotypes, it increased at a huge rate as compared with normal conditions.

Among the high-yielding genotypes, the DT13124.21 genotype had the maximum CAT activity under normal conditions while genotype DT13143.54 showed the highest CAT activity under drought conditions (Figure 4.12). Among moderate-yielding genotypes, the Solist genotype had the highest CAT activity under both normal and drought conditions. DT11108.02 genotype showed the maximum CAT activity among low-yielding genotypes under both conditions while FASAN and DORUK genotypes had the lowest activity under drought and normal conditions respectively (Figure 4.12).

Table 4.14. Analysis of variance for CAT

Biochemical traits	Source of variation	Mean square	<i>F</i>	<i>P</i>
CAT	Genotypes	0.60324	17.10	0.00
	Treatment (Control/Drought)	4.10647	131.00	0.00
	Interaction (G×T)	0.76333	24.35	0.00

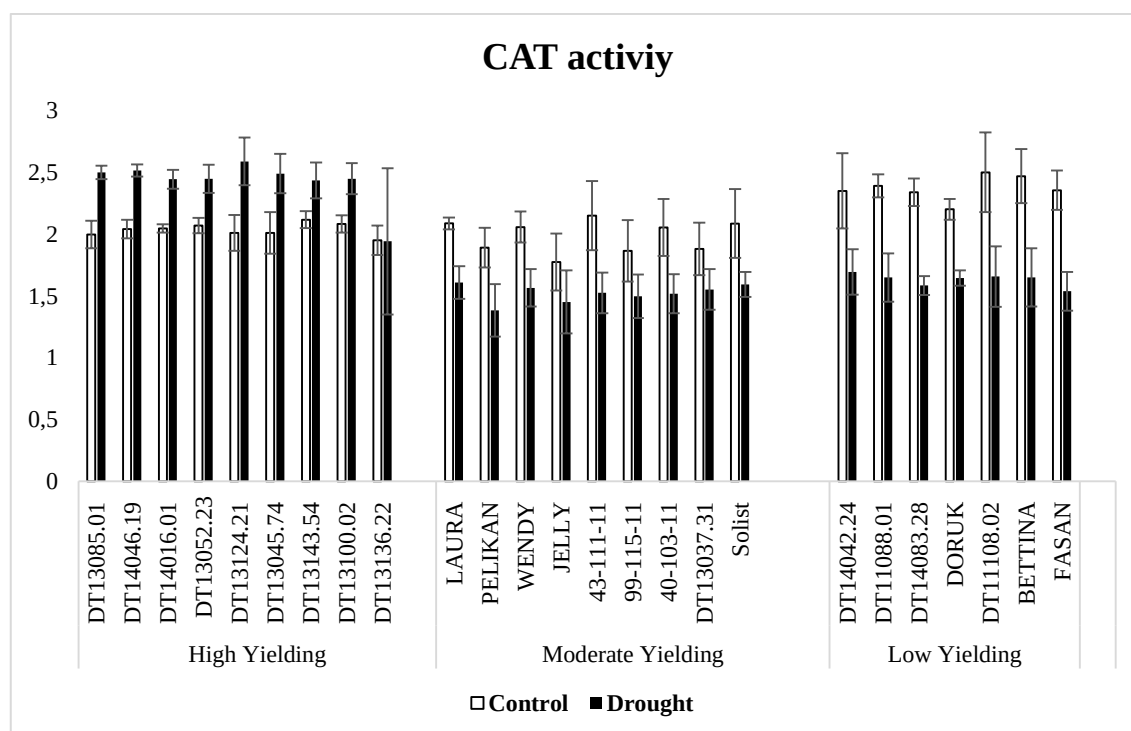


Figure 4.12. CAT contents of different potato genotypes under control and drought conditions on day 12

4.3 Yield/pot

The yield/pot under normal and drought conditions was examined in high-yielding, moderate-yielding, and low-yielding genotypes of potatoes (Figure 4.13). Analysis of variance showed a significant difference between observed genotypes and applied treatments (Table 4.15). It was observed that under drought conditions, the final yield decreased in all the genotypes as compared with normal conditions (Figure 4.13).

From all the observed genotypes, high- and moderate-yielding genotypes had higher yields as compared with the low-yielding genotypes. Genotype DT13136.22 had the highest yield under both drought and normal conditions while genotype DT13052.23 had the lowest yield for both treatments (Figure 4.13). Among the moderate and low-yielding genotypes, the PELIKAN and DT11088.01 genotypes produced a lower yield under drought conditions while the 40-103-11 and DORUK genotypes produced a higher yield under normal and drought conditions.

Table 4.15. Analysis of variance for yield

Biochemical traits	Source of variation	Mean square	F	P
Yield/pot	Genotypes	9732	473.74	0.00
	Treatment (Control/Drought)	671834	28596.8	0.00
	Interaction (G×T)	3954	168.30	0.00

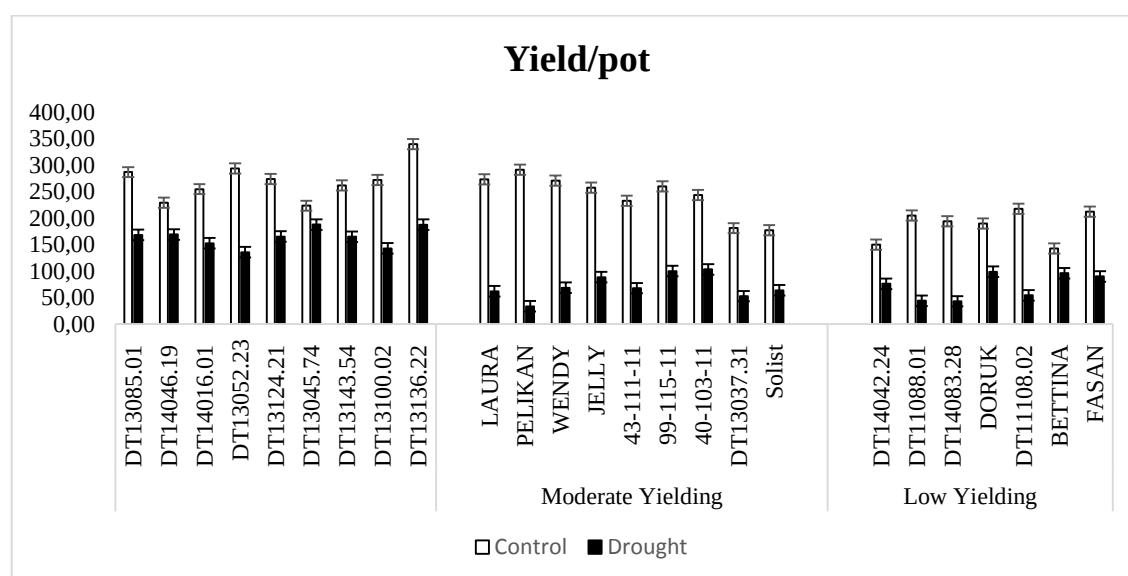


Figure 4.13. Yield/pot of different potato genotypes under control and drought conditions

4.14 Correlation analysis

Correlation analysis was done for determining the relationship between the physiological parameters (transpiration rate, photosynthetic rate, leaf temperature, stomatal conductance, chlorophyll contents (spad value), and relative water content) and biochemical traits (MDA, proline) and enzymatic activities (SOD, POD, APX, and CAT) and final yield (Figure 4.14).

Data for correlation analysis showed positive, negative and some neutral values of correlations among all the biochemical traits and physiological parameters (Figure 4.14). A strong positive correlation was observed in APX values while a strong negative correlation was observed in RWC. Biochemical traits such as proline, MDA, POD, SOD and APX contents showed a highly positive correlation. Other growth and biochemical parameters such as chlorophyll contents (spad values), transpiration rate, photosynthetic rate, stomatal conductance, leaf temperature and CAT activity showed a mixed positive and negative response.

Table 4.16. Correlation analysis results of measured variables

		Prolinec	Prolined	SODc	SODd	PODc	PODd	APXc	APXd	CATc	CATd	RWCc	RWcd	SPADc	SPADd	Phc	Phd	LTc	LTd	STc	STd	Trc	Trd	YieldPc	YieldPd	YieldFd
Prolinec	C	1																								
	Sig.																									
Prolined	C	-.222	1																							
	Sig.	.286																								
SODc	C	-.376	.562**	1																						
	Sig.	.064	.003																							
SODd	C	-.240	.934**	.644**	1																					
	Sig.	.248	.000	.001																						
PODc	C	-.344	.850**	.668**	.891**	1																				
	Sig.	.092	.000	.000	.000																					
PODd	C	.187	-.917**	-.526**	-.889**	-.827**	1																			
	Sig.	.370	.000	.007	.000	.000																				
APXc	C	.582**	-.226	-.564**	-.320	-.423*	.291	1																		
	Sig.	.002	.277	.003	.119	.035	.158																			
APXd	C	.496	-.358	-.184	.239	.014	-.353	.306	1																	
	Sig.	.012	.079	.378	.250	.947	.083	.137																		
CATc	C	-.324	-.624**	.055	-.541*	-.358	.600**	-.230	-.710**	1																
	Sig.	.114	.001	.792	.005	.079	.002	.269	.000																	
CATd	C	-.519**	.772**	.718**	.845**	.933**	-.730**	-.504*	-.185	-.158	1															
	Sig.	.008	.000	.000	.000	.000	.000	.010	.376	.449																
RWCc	C	-.131	-.057	-.133	-.028	-.079	.057	-.007	.057	-.128	-.086	1														
	Sig.	.534	.788	.526	.896	.706	.787	.972	.788	.543	.682															
RWcd	C	.211	-.884**	-.607**	-.894**	-.838**	.808**	.357	-.196	.461	-.822**	.116	1													
	Sig.	.310	.000	.001	.000	.000	.000	.080	.348	.020	.000	.581														
SPADc	C	.134	-.196	-.064	-.148	-.046	.262	.129	-.353	.136	-.079	-.091	.197	1												
	Sig.	.523	.347	.760	.480	.828	.206	.539	.083	.518	.709	.666	.346													
SPADd	C	.051	-.210	-.080	-.144	-.019	.276	.069	-.392	.222	-.018	-.050	.179	.961**	1											
	Sig.	.807	.313	.704	.491	.929	.182	.742	.052	.287	.930	.813	.393	.000												
Phc	C	-.227	.036	.047	-.044	.014	-.079	-.216	-.318	.248	.151	-.179	-.067	.010	.113	1										
	Sig.	.275	.864	.823	.835	.945	.709	.299	.122	.232	.471	.391	.752	.962	.591											
Phd	C	-.035	.077	.062	-.074	.068	-.064	.206	-.165	.016	-.022	-.081	-.022	.098	-.014	-.164	1									
	Sig.	.870	.715	.768	.723	.746	.760	.324	.430	.941	.917	.701	.917	.642	.947	.432										
LTc	C	.126	-.272	-.022	-.108	-.169	.264	-.049	.087	-.071	-.143	.539**	.145	-.036	-.026	-.448	-.360	1								
	Sig.	.550	.188	.918	.606	.418	.203	.818	.678	.735	.496	.005	.489	.865	.902	.025	.077									
LTd	C	.249	-.475	-.094	-.308	-.283	.399	-.083	-.110	.212	-.238	.399	.253	.213	.223	-.180	-.375	.744*	1							
	Sig.	.231	.016	.654	.134	.170	.048	.693	.601	.310	.252	.048	.223	.306	.283	.390	.065	.000								
STc	C	-.231	-.107	.059	-.210	-.134	.187	.036	-.371	.334	-.048	-.214	.194	.261	.306	.644*	.132	-.487*	-.293	1						
	Sig.	.266	.611	.779	.314	.523	.371	.863	.068	.103	.819	.304	.352	.208	.137	.001	.529	.014	.156							
STd	C	.221	.249	.047	.092	.232	-.220	.206	.099	-.240	.014	-.054	-.137	.033	-.032	.013	.723*	-.344	-.408*	.227	1					
	Sig.	.289	.229	.825	.662	.265	.291	.323	.637	.248	.948	.798	.513	.877	.878	.951	.000	.092	.043	.274						
Trc	C	.288	-.312	-.159	-.364	-.396	.274	.068	.054	.089	-.463	-.045	.284	.106	.108	.108	-.050	-.005	.058	.083	-.055	1				
	Sig.	.163	.129	.448	.073	.050	.185	.747	.799	.672	.020	.829	.169	.614	.608	.607	.812	.980	.784	.693	.792					
Trd	C	-.086	.202	.339	.257	.156	-.094	.012	-.123	-.148	.175	.008	-.216	.191	.189	-.056	.011	.136	-.227	.196	.035	.245	1			
	Sig.	.683	.333	.098	.215	.456	.656	.956	.558	.479	.402	.968	.299	.359	.365	.791	.958	.518	.275	.348	.868	.238				
YieldPc	C	-.001	.655**	.359	.647**	.514	-.642**	-.089	.457	-.596**	.352	.143	-.504*	.113	.084	-.224	.112	-.044	-.141	-.135	.199	.004	.134	1		
	Sig.	.995	.000	.078	.000	.009	.001	.673	.022	.002	.085	.496	.010	.589	.689	.281	.595	.836	.502	.520	.341	.985	.523			
YieldPd	C	-.297	.781**	.718**	.867**	.869**	-.721**	-.409	-.047	-.314	.854**	-.241	-.798**	-.038	-.021	.023	-.043	-.209	-.395	-.016	.084	-.170	.491	.447*	1	
	Sig.	.149	.000	.000	.000	.000	.000	.043	.824	.126	.000	.245	.000	.858	.919	.913	.837	.316	.051	.939	.689	.416	.013	.025		
YieldFd	C	-.201	.960**	.600**	.959**	.893**	-.904**	-.318	.335	-.610**	.814**	-.073	-.906**	-.145	-.160	-.003	-.038	-.158	-.308	-.169	.152	-.347	.155	.666**	.811**	1
	Sig.	.336	.000	.002	.000	.000	.000	.122	.102	.001	.000	.728	.000	.489	.443	.987	.856	.450	.134	.418	.467	.089	.461	.000	.000	

4.15 Principal component analysis (PCA)

Principal component analysis of both positively and negatively correlated parameters collectively and separately was used to differentiate genotypes for their drought resistance ability (Figure 4.15). Three PCA was done for determining the relationship between genotypes (high, medium, and low yielding) and among both positively and negatively correlated physiological and biochemical traits. PCA 1 was about normal while PCA 2 was about drought conditions and PCA 3 showed both normal and drought conditions. PC1 and PC2 accounted for 47% under Normal, 61.15% for drought, and 46.53% for both normal and drought (Figure 4.15). Cultivars were classified into four groups based on PC1 and PC2 values. Cultivars with +PC1 and +PC2 were graded as resistant, with +PC1 and -PC2 as moderately resistant, with -PC1 and +PC2 as moderately susceptible, and with -PC1 and -PC2 as susceptible.

PCA1 using the correlated characters of control pot treatment (SODc, PODc, and CATc) with the tuber yield under drought field conditions classified the genotypes DT13052.23, DT13045.74, DT13136.22, Fasan, DT14042.24, 43-111-11, Bettina, DT11088.01, and DT11108.02 as resistant (Figure 4.15). Among these 9 genotypes, 3 (DT13052.23, DT13045.74, DT13136.22) have high tuber yield, 1 (43-111-11) has moderate tuber yield, and 5 (Fasan, DT14042.24, Bettina, DT11088.01, and DT11108.02) have low tuber yield under drought field conditions. As a result, PCA1 could not effectively diagnose the genotypes having high tuber yield under drought field conditions.

PCA2 using the correlated characters of drought pot treatment (Prolined, SODd, PODd, CATd, and RWCd) with the tuber yield under drought field conditions classified the genotypes DT14016.01, DT13136.22, DT13085.01, DT14046.19, DT13085.01, and 43-111-11 as resistant (Figure 4.16). Among these 6 genotypes, 5 (DT14016.01, DT13136.22, DT13085.01, DT14046.19, DT13085.01) have high tuber yield, and 1 (43-111-11) has moderate tuber yield. As a result, PCA2 could effectively diagnose the genotypes having high tuber yield under drought field conditions.

PCA3 using the correlated characters of both control and drought pot treatments (Prolined, SODc, SODd, PODc, PODd, CATc, CATd, and RWCd) with the tuber yield

under drought field conditions classified the genotypes DT13124.21, DT13136.22, DT13143.54, DT13100.02, DT13052.23, and 43-111-11 as resistant. Among these 6 genotypes, 5 (DT13124.21, DT13136.22, DT13143.54, DT13100.02, and DT13052.23) have high tuber yield, and 1 (43-111-11) has moderate tuber yield. As a result, PCA3 could effectively diagnose the genotypes having high tuber yield under drought field conditions.

Among 3 different PCA, PCA2 (with the characteristic at drought) and PCA3 (with the characteristic at both drought and control) can be used for estimation of potential high-yielding genotypes under drought field conditions without a field trial.

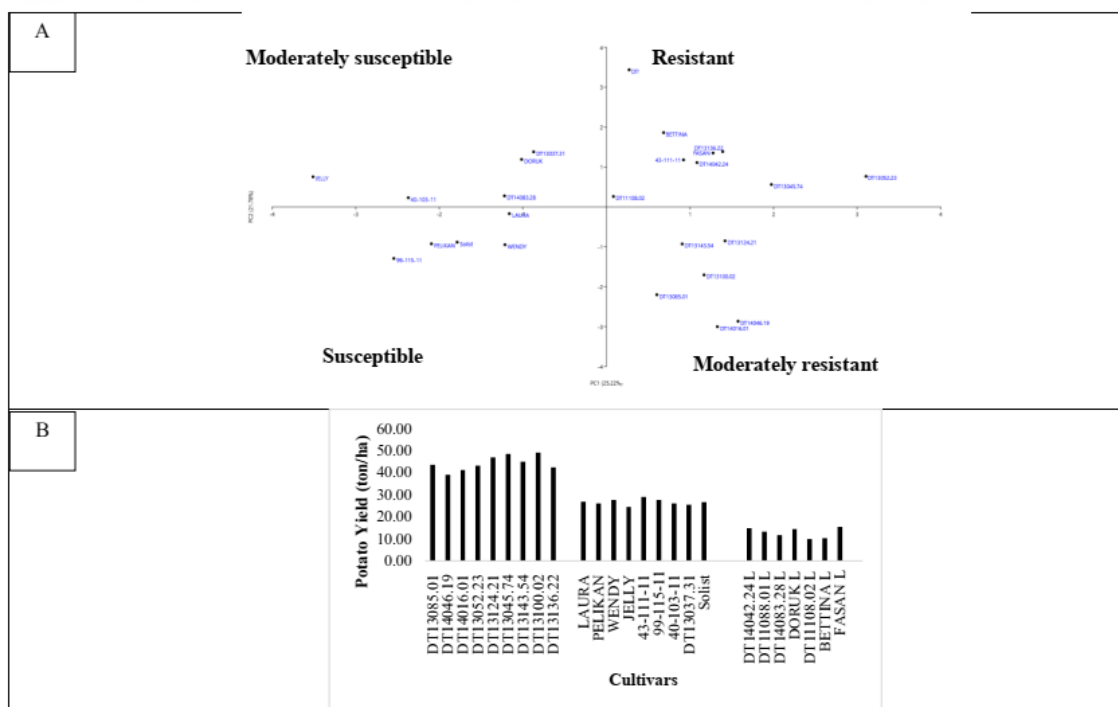


Figure 4.15. A: PCA1 by using the correlated characters under the control pot w with the yield under drought field conditions. B: Tuber yield under drought field conditions.

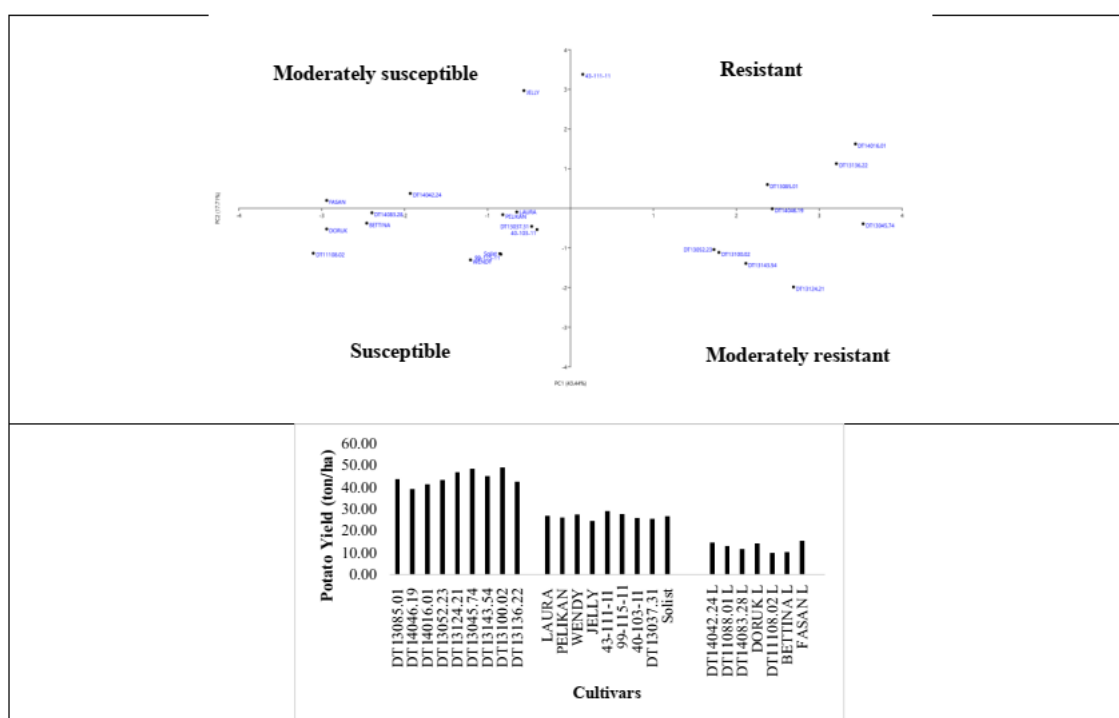


Figure 4.16. A: PCA2 by using the correlated characters under the drought pot with the yield under drought field conditions. B: Tuber yield under drought field conditions.

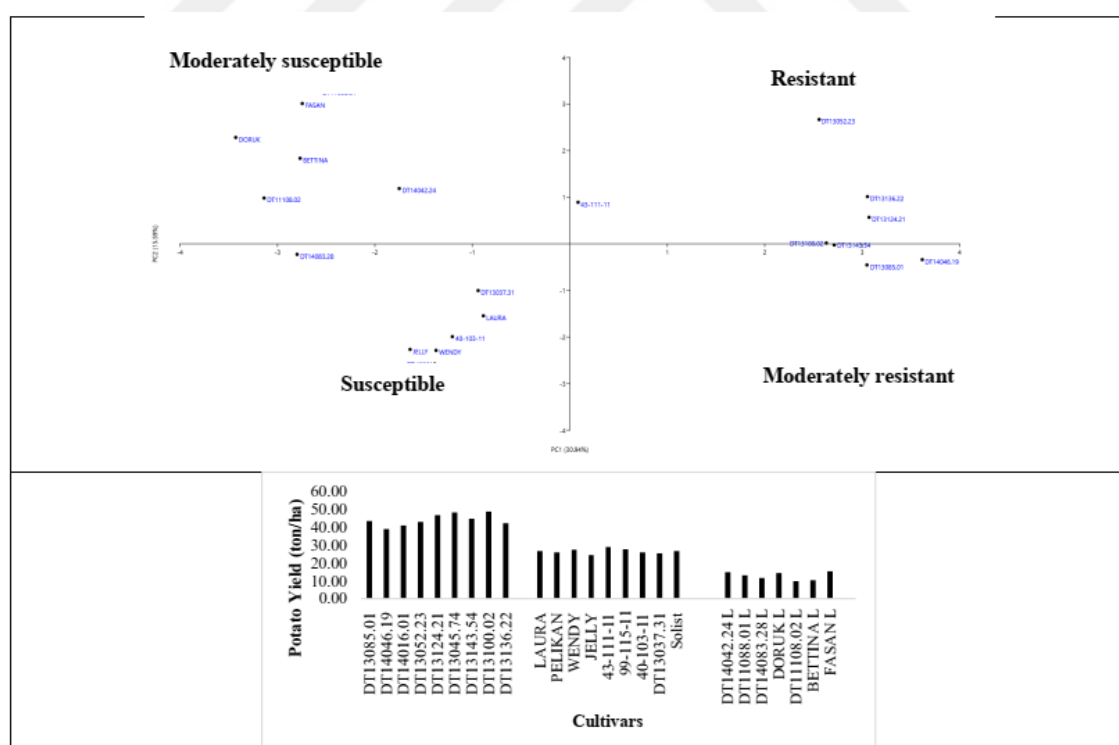


Figure 4.17. PCA3 by using the correlated characters under both control and drought pots with the yield under drought field conditions. B: Tuber yield under drought field conditions

CHAPTER V

DISCUSSION

Physiological and biochemical traits and enzymatic activities of different potato genotypes (high-yielding, moderate-yielding, and low-yielding genotypes) under normal and drought conditions were determined in this study. Traits such as a higher photosynthetic rate, increased chlorophyll content, and improved CO₂ assimilation contribute to higher yields of potato tuber.

Crop plant productivity is reduced by drought either directly or indirectly through photosynthetic processes at the canopy, leaf, or chloroplast level. Feedback inhibition occurs when there is insufficient transport of photosynthate to sink organs (Jones and Corlett, 1992; Jones, 2014). According to Evers et al. (2010) and Obidiegwu et al. (2015), early growth stage drought is regarded as the most detrimental since it significantly reduces total leaf area, slows down photosynthetic rates, and assimilates partitioning to tubers, all of which result in poor tuber formation, bulking, and tuber production. The severity, length, and rate of advancement of drought all influence how plants respond to it (Pinheiro and Chaves, 2011). Drought not only declines the yield but also decreases the quality of tubers by initiating different tuber diseases such as scab etc (Mane et al., 2008).

Stomatal conductance is an essential process that has a critical role in regulating CO₂ uptake for photosynthesis and plant growth as well as water losses through transpiration (Damour et al., 2020). It was observed that under drought conditions, the stomatal conductance decreased in all the genotypes. All the genotypes varied to a greater in their stomatal conductance activity at both initial and final stage under both normal and drought conditions (Figure 4.1 A, B). In general, all the genotypes responded to a greater extent under drought conditions as compared with normal conditions (Figure 4.1 B). The reason is that under water stress, stomatal conductance decreases while holding water potential close to values obtained in non-stressed circumstances (Liu et al., 2005) demonstrating that stress starts out slowly.

Photosynthesis is an essential process that makes the transition of light energy into chemical energy and a fundamental practice in plant productivity carried out in most plants (Siddique et al., 2016). Photosynthetic rate is greatly influenced by the environmental conditions especially shortage of water. It has been observed that under drought conditions, the photosynthetic rate decreased in all the genotypes. In general, all the genotypes responded to a greater extent under drought conditions as compared with normal conditions (Figure 4.2 B). The main reason is the effects of drought stress on growing plants include dehydration, decreased stomatal conductance, altered chlorophyll fluorescence, photoinhibition of photosystem II (Athar, 2005). According to Plich et al. (2020), the main cause of the decrease in tuber production during a drought is thought to be the obstruction of photosynthesis.

Transpiration rate is the amount of loss from a plant surface in the form of vapour and is a critical element for the transportation of salutes and resource management (Wang et al., 2013). Data for transpiration rate showed that under normal conditions, the transpiration rate was higher but varied among all high-yielding, moderate-yielding and low-yielding genotypes (Figure 4.3 A) but under drought conditions, the transpiration rate decreased among all high-yielding, moderate-yielding and low-yielding genotypes (Figure 4.3 B). Previous studies also showed that the highest values of transpiration rate belonged to the normal condition (Pourasadollahi et al., 2019)

Relative water content (RWC) is referred to as the amount of water in a leaf at the moment of sampling in relation to the maximum amount of water a leaf can hold (Arndt et al., 2015). In investigations of water relationships, it is a crucial metric because, for instance, it enables the computation of the osmotic potential at full turgor. RWC decreased under drought conditions in all genotypes. Among all genotypes, low-yielding genotypes had the highest RWC and compared with high-yielding and moderate-yielding genotypes (Figure 4.4). These results are in line with the study of Soltys-Kalina et al. (2016) who observed a huge decline in RWC under drought conditions and this decline varied among genotypes.

Leaf temperature plays an important role in determining transpiration and photosynthetic rate and also helps in leaves and stem turgidity (Monteiro et al., 2016). It is also hugely dependent on the moisture contents of a substrate (Grant et al. 2007). It

has been observed that under drought conditions, the leaf temperature increased in all the genotypes as compared with normal conditions (Figure 4.5). The reason for the increase in leaf temperature is due to the closing or decreasing of water content which may cause the decline of photosynthesis (Lawlor and Tezara, 2009). The increase in leaf temperature under drought conditions is supported by Banik et al. (2016) who observed that leaf temperatures of several potato genotypes decline under low water conditions.

Chlorophyll is a green color antioxidant component of green leaf plants inside the chloroplast, which is primarily found in the green portions of leaves, stems, flowers, and roots (Mirza et al., 2013; Srichaikul et al., 2011). However, the creation of chlorophyll, which is the primary source of energy for plants, is mostly dependent on the penetration of sunlight (Srichaikul et al., 2011). Among all genotypes, some low-yielding genotypes had the highest chlorophyll contents under control and drought conditions as compared with high-yielding and moderate-yielding genotypes (Figure 4.6). These results are consistent with Monneveux et al. (2013) who observed a decline in chlorophyll contents under drought conditions.

Proline is an essential and compatible osmolyte and the osmoprotective molecule that has been postulated to function in the osmotic adjustment as a molecular chaperone and safeguard cellular membranes, structures and proteins from several osmotic stresses and other stress-causing agents (Terao et al., 2003). Abraham et al. (2010) also define proline as an important osmolyte that acts as a cellular structure safeguard agent and stress conditions trigger the accumulation of proline that especially increases upon drought arrival. Our study revealed an increase in proline content under drought as compared with control conditions (Figure 4.7) and is consistent with Sirait et al. (2013) who observed that higher levels of polyethylene glycol (PEG), a drought-causing agent, caused an increase in the proline content in potato plants (Sirait et al., 2013).

Malondialdehyde (MDA) is a colourless liquid organic compound that is highly reactive in nature (Nair et al., 2008). It is found naturally in different fruits and vegetables and used as an index of oxidative stress (Oral et al., 2006; Gobel et al., 2009). Data for MDA showed a significant difference between observed genotypes and applied treatments. Under drought conditions, MDA contents increased in all high-yielding, moderate-yielding and low-yielding genotypes as compared with normal conditions

(Figure 4.8). These results are consistent with Zhang et al. (2018) who observed an increase in MDA contents in the potato seedlings under drought conditions as compared with normal conditions.

Superoxide dismutases (SOD) are vital metalloenzymes that have a significant role in defense mechanisms under several stress conditions (Rosa et al., 2021). In our study, SOD activity increased in all genotypes under drought conditions (Figure 4.9) and these results are supported by Lu et al., 2010 who observed an increase in SOD activity in transgenic sweet potatoes under drought conditions. The reason for the increase in SOD activity is due to the converting the highly accumulated superoxide (O_2^-) to H_2O_2 which is an important catalyzation for reducing the damaging effect of superoxide in the cell (Sies et al., 2017; Rosa et al., 2021).

Peroxidase (POD) has a greater role in effective defense systems due to its huge enzymatic activity (Saffar et al., 2009). This enzymatic system triggers the scavenging of reactive oxygen species molecules thus protecting from several stresses and oxidative damage (Jaleel et al., 2008; Rosa et al., 2021). Data for POD activity showed that under drought conditions, its activity increased at a high rate among all high-yielding, moderate-yielding, and low-yielding genotypes (Figure 4.10) and these results are consistent with the outcomes of Rosa et al. (2023) who observed an increase in POD activity under drought conditions.

Ascorbate peroxidase (APX) is another enzyme having a major role in the catalyzation process of water from hydrogen peroxide by using an electron donor in the form of ascorbate (Chen et al., 2012; Correa-Aragunde et al., 2013). It has been observed that under drought conditions, APX activity increased in moderate-yielding genotypes as compared with all high-yielding, and low-yielding genotypes. In general, all the genotypes responded to a greater extent under drought conditions as compared with normal conditions (Figure 4.11).

CAT directly convert the hydrogen peroxide to water without using other substrate, thus the oxidative molecule, H_2O_2 , is extinguished. Data showed a high variation in CAT activity among high-yielding, low-yielding and moderate-yielding genotypes under drought conditions (Figure 4.12). It was observed that under drought conditions CAT

activity increased in high-yielding genotypes while it was decreased in moderate- and low-yielding genotypes. These results are in line with Mohamed et al. (2015) who studied potato genotype screening under drought conditions and observed a decline in CAT activity under drought as compared with normal conditions. due to decline in catalytic activity, plant's ability to cope various stresses also decreases and plant become prone to diseases that ultimately decline their yield.

Potato yield can be significantly affected by drought conditions. Drought stress can negatively impact the growth, development, and overall productivity of potato plants (Nasir and Toth, 2022). Drought causes stunted growth, shallow root development, altered physiological processes, increased susceptibility to diseases and pests, and reduced tuber formation. Under drought conditions, the yield per pot decreased in all the genotypes as compared with normal conditions under greenhouse conditions (Figure 4.13). These results are consistent with Rykaczewska (2017) and Lahlou et al. (2003) who observed a decline in tuber yield under drought conditions. Lowered tuber quantity and yield are a result of fewer stolons per stem caused by drought during tuberization (Eiasu et al., 2007). Potato plants will produce fewer and smaller-sized tubers if they are subjected to drought during the tuber bulking period. However, it has been hypothesized that the beginning of the stolon and the development of the tuber are the most crucial phases of drought stress (Aliche et al., 2020). Besides, high-yielding potato genotype under drought field conditions performed higher tuber yield per pot under greenhouse conditions compared to moderate- and low-yielding genotypes.

Correlation analysis is a vital tool for hypothesis testing, predictive modeling, and decision-making in data-driven research (Gujarati & Porter, 2009). From correlation analysis, it has been determined that MDA, proline, SOD, POD, stomatal conductance and yield was positively correlated. Genotype DT13136.22 had the highest yield under both drought and normal conditions and considered resistant while genotype DT13052.23 had the lowest yield for both treatments (Figure 4.15). Among the moderate and low yielding genotypes, the PELIKAN and DT11088.01 genotypes produced a higher yield under drought and normal conditions and have drought-tolerant traits while the Solist and DT14042.24 genotype produced a lower yield under normal and drought conditions. The classification outcomes for 25 genotypes regarding drought resistance, utilizing traits demonstrated a correlation with the yield performance of these

genotypes when grown under drought conditions. Previously Liu et al. (2006) and Demirel et al. (2016) have employed correlation for genotype classification based on drought resistance and separate the genotypes on the base of observed traits and their ultimate impact on the final tuber yield.

Principle component analysis (PCA) is recognized as a valuable statistical tool for analyzing multivariate data sets characterized by high correlations among variables (Johnson, 1998). PCA was used to differentiate genotypes for their drought resistance ability (Figure 4.15). A total of three PCA done for determining the relationship between genotypes (high, medium and low yielding) and among the positively and negatively correlated parameters (photosynthetic rate, stomatal conductance, MDA, proline, SOD, POD, APX contents with final field yield (Figure 4.15). In the analysis, PCA1 was used to assess the relationship between certain attributes (SODc, PODc, and CATc) related to control pot treatment and tuber yield under drought field conditions. It classified nine genotypes, including DT13052.23, DT13045.74, and DT13136.22, as resistant. Among these genotypes, three had high tuber yields, one had a moderate yield, and the remaining five had low yields under drought conditions. Notably, PCA1 was less effective in identifying genotypes with high tuber yields in these challenging drought conditions.

PCA2, utilizing the correlated characteristics associated with drought pot treatment (Prolined, SODd, PODd, CATd, and RWCd), in combination with tuber yield under drought field conditions, categorized the genotypes DT14016.01, DT13136.22, DT13085.01, DT14046.19, DT13085.01, and 43-111-11 as being resistant (as depicted in Figure 4.16). Among these six genotypes, five (DT14016.01, DT13136.22, DT13085.01, DT14046.19, DT13085.01) displayed high tuber yields, while one (43-111-11) showed a moderate tuber yield. Consequently, PCA2 effectively identified the genotypes characterized by high tuber yields under drought field conditions.

PCA3, using the correlated characteristics from both control and drought pot treatments (Prolined, SODc, SODd, PODc, PODd, CATc, CATd, and RWCd), in conjunction with tuber yield under drought field conditions, classified the genotypes DT13124.21, DT13136.22, DT13143.54, DT13100.02, DT13052.23, and 43-111-11 as resistant. Among these six genotypes, five (DT13124.21, DT13136.22, DT13143.54,

DT13100.02, and DT13052.23) exhibited high tuber yields, while one (43-111-11) displayed a moderate tuber yield. Consequently, PCA3 proved to be effective in identifying the genotypes characterized by high tuber yields under drought field conditions.

Out of the three PCA approaches considered, PCA2, which focuses on drought-related characteristics, and PCA3, which incorporates both drought and control characteristics, offer effective means to predict high-yielding genotypes under drought field conditions without the necessity of conducting field trials. Previous studies (Kakani et al., 2005; Liu et al., 2006; Demirel et al., 2016) have employed PCA for genotype classification based on drought resistance. the. genotypes into four groups including susceptible, moderate susceptible, resistant and moderate resistant.

CHAPTER VI

CONCLUSION

Abiotic stress factors such as high temperature, water deficit/drought, and salinity have become a significant danger in potato production areas. Water deficit is one of the abiotic stress factors that have a negative impact on the growth, physiology, and enzymatic activity of potatoes. Different genotypes have different abilities to cope with stresses by maintaining normal growth, yield, and quality traits. The current study was conducted to evaluate some physiological, biochemical, and enzymatic traits' correlation with tuber yield under drought conditions. Traits such as a higher photosynthetic rate, increased chlorophyll content, and higher SOD and POD contents contribute to higher yields. Other traits such as transpiration rate and relative water content decline the tuber yield.

Our study showed a significant difference among all genotypes (high-yielding, moderate-yielding, and low-yielding genotypes) and the treatments (normal and drought conditions). Data for correlation analysis showed a positive correlation in proline content, MDA accumulation, SOD, POD, and APX activities for all genotypes under drought. However, while CAT activity increased in high-yielding genotypes under drought, it was decreased in moderate- and low-yielding genotypes under drought. Besides, stomatal conductance, photosynthetic rate, and RWC were decreased in all genotypes under drought conditions. It was concluded in this study that measurement of antioxidant enzymes and other biochemical traits is a feasible screening approach to characterize tolerant and susceptible potato cultivars

Among 3 different PCA, PCA2 (with the characteristic at drought) and PCA3 (with the characteristic at both drought and control) can be used for estimation of potential high-yielding genotypes under drought field conditions without a field trial.

Overall, this study provided an insight into potato drought resistance at physiological and biochemical level. However, further studies are needed to further validate presented results.

The negatively correlated values such as RWC, photosynthetic rate, transpiration rate and stomatal conductance were the important traits that were least influenced in high yielding varieties and play role in increasing yield of tuber. Leaf temperature does not effect under normal or drought conditions so there is no impact of leaf temperature on yielding potential of genotypes.



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