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GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES



T.C

NİĞDE ÖMER HALİSDEMİR UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF AGRICULTURAL GENETIC ENGINEERING

KASP MARKER-ASSISTED SELECTION FOR DROUGHT RESISTANT
POTATO BREEDING LINES

GİZEM ÜNAL

FEBRUARY 2021

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Master Thesis

Supervisor

Assoc. Prof. Dr. Ufuk DEMİREL

FEBRUARY 2021

The study titled “**KASP Marker-Assisted Selection for Drought Resistant Potato Breeding Lines**” and presented by Gizem ÜNAL under the supervision of Assoc. Prof. Dr. Ufuk DEMİREL has been accepted as 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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CONFIRMATION:

This thesis has been found appropriate at the date of/....../2021 by the jury mentioned above who have been designated by Board of Directors of Graduate School of Natural and Applied Sciences and has been confirmed with the resolution of Board of Directors dated/....../2021 and numbered

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THESIS CERTIFICATION

I hereby acknowledge that this thesis was written by me. All information mentioned in each part of this thesis is based upon a scientific foundation and in line with the academic rules. Any knowledge I have utilized during the preparation of the thesis and resources benefitted from the literature have been declared in this thesis.



GİZEM ÜNAL

SUMMARY

KASP MARKER-ASSISTED SELECTION FOR DROUGHT RESISTANT POTATO BREEDING LINES

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Department of Agricultural Genetic Engineering

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Potato (*Solanum tuberosum* L.) is one of the major cultivated and the first non-cereal crops in the world. However, it is a susceptible crop against drought conditions. Drought stress causes great yield losses in potato plant. That's why it is very important to develop drought-tolerant potato varieties in plant breeding programs. Thanks to the discovery of molecular marker technology an important step has been taken for plant breeding. However, validated KASP marker related to the yield of potato plants under drought conditions wasn't reported. A validation step has been taken for KASP markers which are associated with yield under drought stress in the scope of this thesis. The 89 potato breeding lines were screened with 7 KASP markers, they were selected based on their high yielding potential for at least 5 KASP markers. Then the results were verified with a field trial. In conclusion, the selection efficiencies of the 7 KASP markers were verified in a %85,71 ratio. The breeding lines, MEÇ1668.05, MEÇ1650.01, and MEÇ1651.03 could be released as new cultivars as having the highest yields under stressed and non-stressed conditions.

Keywords: Potato (*Solanum tuberosum* L.), abiotic stress, drought, Marker Assisted Selection (MAS), KASP markers, yield

ÖZET

KURAĞA DİRENÇLİ PATATES ISLAH HATLARININ KASP MARKÖR YARDIMLI SELEKSİYONU

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Patates (*Solanum tuberosum* L.) tahıllar dışında dünya çapında en çok üretimi yapılan bitkidir. Fakat kuraklık stresine hassastır. Kuraklık stresi patates bitkisinde ciddi verim kaybına neden olmaktadır. Dolayısıyla, kurağa toleranslı patates çeşitlerinin geliştirilmesi bitki ıslahında önem arz etmektedir. Moleküler markörlerin kullanılmaya başlaması ile bitki ıslahında önemli gelişmeler yaşanmıştır. Fakat patates bitkisinde kuraklık stresinde verim ile ilişkilendirilmiş bir markör çalışması bulunmamaktadır. Bu tez çalışması, kuraklık stresi altında yüksek verim ile ilişkilendirilmiş KASP markörlerinin valide edilmesi için önemli bir çalışma olmuştur. İlk olarak, 89 adet patates ıslah hatları 7 adet KASP markörleri ile taranmış, en az 5 KASP markörü ile kurak koşullarda yüksek verim potansiyeline sahip olan ıslah hatları seçilmiş ve sonuçlar tarla denemesi ile doğrulanmıştır. Sonuç olarak 7 adet KASP markörlerinin seleksiyon etkinlikleri %85,71 oranında doğrulanmıştır. MEÇ1668.05, MEÇ1650.01 ve MEÇ1651.03 ıslah hatları hem kontrol hemde su kısıtlaması koşullarında en yüksek verime sahip olarak yeni patates çeşitleri için aday hatlar olarak belirlenmiştir

Keywords: Patates (Solanum tuberosum L.), abiyotik stres, kuraklık, Markör yardımcı seleksiyon (MAS), KASP markörleri, verim

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SYMBOLS AND ABBREVIATION

Symbols	Description
%	Percentage
g	Gram
g/da	Gram per decare
kg/da	Kilogram per decare
L	Liter
L/da	Liter per decare
mg	Milligram
min	Minute
mL	Millilitre
mm	Milli meter
ng	Nanogram
ng/μL	Nanogram per microliter
°C	Degree celsius
rpm	Revolutions per minute
sec	Second
t/ha	Tone per hectare
μL	Microliter
ng/μL	Nanogram per microliter
°C	Degree Celsius
rpm	Revolutions per minute
sec	Second
t/ha	Tone per hectare
μL	Microliter

Abbreviation	Description
A	Adenine
ABA	Abscisic Acid
AFLPs	Amplified Fragment Length Polymorphisms

AM	Association Mapping
C	Cytosine
CAS	Cycloartenol Synthase
COR	Cold Regulated
DArT	Diversity Arrays Technologies
DAE	Days After Emergences
DNA	Deoxyribonucleic Acid
DREB	Dehydration Responsive Element Binding Region
DSI	Drought Susceptibility Index
DT	Discarded Tuber
DTI	Drought Tolerance Index
EF	Elongation Factor
EIN4	Ethylene receptor homolog
FAOSTAT	FAO Corporate Statistical Database
FRET	Fluorescence Resonance Energy Transfer
G	Guanine
HSPs	Heat Shock Proteins
InDels	Insertion-Deletions
KASP	Kompetitive Allele Specific PCR
LEA	Late Embryogenesis Abundant Proteins
LOBD	Lateral Organ Boundaries Domain
MAS	Marker Assisted Selection
MDH	Malate Dehydrogenase
MT	Marketable Tuber
N	Nitrogen
NPK	Nitrogen-Phosphor-Potassium
NT	Number of Tuber
NuUe	Nitrogen Use Efficiency
PAL	Phenylalanine ammonia-lyase
PCR	Polymerase Chain Reaction
PVX	Potato Virus X
q-RT PCR	Quantitative Real-time PCR
QTL	Quantitative Trait Loci

RdDM	RNA directed DNA Methylation
RBB	Russet Burbank
RNA	Ribonucleic Acid
RFLPs	Restriction Fragment Length Polymorphisms
ROS	Reactive Oxygen Species
RT-PCR	Real-time Polymerase Chain Reaction
SCAR	Sequence Characterized Amplified Region
SNPs	Single Nucleotide Polymorphisms
SPAD	Chlorophyll index
SSRs	Simple Sequence Repeats
T	Timine
TPI	Triosephosphate isomerase
TY	Tuber Yield
WUE	Water Use Efficiency
YD	Yield under Drought conditions
YN	Yield under Normal Conditions

CHAPTER I

INTRODUCTION

Potato is one of the four major food crops and ranks at the first in terms of annual production among non-cereal crops in the world (FAO, 2020). It is an all-purpose crop and it is not only consumed as food or feed. It is also used as industrial raw materials and production of fuels (Ortiz and Watanabe, 2004). Potato is a temperate crop that is cultivated worldwide and its optimum temperature requirement is 17-21 °C (Levy and Veilleux, 2007). However, annual potato production has been raised in developing countries, especially in tropic and subtropic regions which have a higher average temperature than the optimum temperature requirement of potato (Demirel et al., 2017). On the other hand, global warming has become a significant problem in the last decade (Kikuchi et al. 2015). It is expected that climate change will cause an increase in the severity of drought in many regions and it will negatively affect drought susceptible crops like potato (Schafleitner et al. 2011; Simelton et al. 2012). Potato is a sensitive plant against especially heat and drought (Monneveux et al. 2013; Levy and Veilleux 2007). These abiotic stress conditions cause high yield losses in plants (Mittler, 2006). In the case of facing abiotic stress factors, plant breeding strategies play a major role to increase crop yield (Simova- Stoilova et al. 2016). The main goal of plant breeding is to obtain new crops which have better yield and quality characters under abiotic stress conditions (Barone, 2004; Simova- Stoilova et al. 2016).

In the past, breeding strategies for abiotic stress tolerance relied on the phenotypic selection in specific environments (Khan et al., 2015). Transfer of desired genes from wild type to cultivated one was based on interspecific hybridization and selection of these hybrids.. In backcrossing, selected hybrids are backcrossed with donor parent in each generation to reduce the unwanted characteristics of the hybrid. (Barone, 2004). However, with the development of molecular marker technology in the 1980s, the fate of plant breeding strategies was changed. (Nadeem et al., 2018). Given current biological issues such as overpopulation and adverse environmental conditions, one of the main concerns should be to seek viable traits for abiotic stress tolerance such as water use efficiency. (Slafer et al., 2005; Pandey et al., 2017). In order to cope with abiotic stress factors, innovative tools including marker-assisted selection (MAS) have

attracted a great deal of attention. These tools tender a good and quicker strategy to withstand adverse environmental conditions via the accurate selection of plant genotypes (Gantait et al., 2019). Marker-assisted selection (MAS) may be shortly defined as “A selection process which the desired trait selected based on a trait-associated molecular (DNA) marker which a specific piece of DNA which shows us the differences between individuals at genome level”. The main idea of molecular markers is the elimination of core limitations of classical breeding programs in which the selection is based on plant morphology and the transferring of either desirable and undesirable traits because of the strong interlinkage (Reddy 2017).

When the plants are exposed to abiotic stress conditions, the expressions of thousands of genes are altered as the response mechanism (Zhang et al 2013; Evers et al., 2010). Conversely, biotic stress resistance is mostly a monogenic trait in plants, abiotic stress is multigenic. It means abiotic stress resistance is controlled by many genes. That’s why engineering and controlling abiotic stress resistance is more difficult than biotic stress resistance (Wang et al., 2003). Thus, to determine desired genotypes, the usage of only one molecular marker is generally not enough for breeding abiotic stress tolerance. On the other hand, potato varieties usually exhibit low fertility, high heterozygosity, multiple ploidy levels, and inbreeding depression. Therefore, breeding programs are time consuming and expensive on potato crops. (Wetterstrand, 2014; Guenther et al., 2001; Slater et al., 2013). In order to reduce cost and save time, researchers started to use molecular genetic markers linked to phenotypic traits (Milbourne et al., 2007). For this purpose, QTL mapping, linkage mapping, and association mapping are widely used. However, linkage mapping-based QTL mapping is more difficult to apply to tetraploid cultivated potato especially for the traits which are non-stressedled by multigene like drought resistance. These mapping techniques require a mapping population that is created by the crossing of two homozygous parental lines. Since the cultivated tetraploid potato, *Solanum tuberosum*, has inbreeding depression, it’s not preferred to breed homozygous lines. Since association mapping studies don’t require a bi-parental mapping population, this method is preferred for developing molecular selection markers in potato.

Accordingly, it was aimed to validate KASP molecular selection markers developed based on association mapping strategy to screen drought tolerant potato breeding lines in this study.

Researchers have developed and used molecular markers to identify the differences between individuals at the DNA level (Botstein et al., 1980). Molecular markers are developed to detect polymorphisms caused by SSRs, InDels, and SNPs (Gupta et al., 1999). When the SNPs were first discovered in the human genome, it proved that SNPs are the most abundant form of the genetic variations between individuals. This is the reason why the use of SNP markers has had a great hegemony lately. SNPs are polymorphic, abundant, and ubiquitous (Ghosh et al., 2002).

Various techniques are used for MAS studies. One of those is the competitive allele-specific PCR (KASP) genotyping assay which is a novel, homogeneous, and fluorescence-based application. It provides identification and computation of SNPs, such as insertions and deletions (He et al., 2014). This method is highly powerful and cost-effective while compared with other techniques, and appropriate to SNP genotyping at 90% ratio (Köseoğlu et al., 2017).

For this study, it has been hypothesized that the KASP markers associated with drought resistance of potato those were developed by our research group in the previous studies can be used for the selection of high-yielding breeding lines for drought conditions.

Therefore, the importance of the thesis was as follows;

- 1) It was aimed to validate the KASP markers in a potato breeding population.
- 2) It was aimed to help potato breeders to develop novel breeding strategies integrated with KASP marker-assisted selection to improve drought-resilient potatoes in the future for saving land, money, and labour.

CHAPTER II

LITERATURE REVIEW

2.1 Potato

Potato is one of the most widely grown and the first non-cereal crops in the world. Potato is not only consumed as food and feed. It is also used as raw material for different industrial products, including bio-fuel (Ortiz and Watanabe, 2004). Worldwide cultivated potato is a temperate crop and its optimum temperature requirement is 17-21 °C (Levy and Veilleux, 2007). The annual potato production was 368.02 million tons on 17.6 million hectare area in 2018 (FAO, 2020). The top potato--producing countries are China, India, Russia, Ukraine, and the U.S.A, respectively.

Due to its high nutritive value and high yield index which is above %75, potato is evaluated as an important crop to prevent hunger, poverty, and malnutrition in the future (Scott et al., 2000; Thiele et al., 2010). Increasing potato production may help to feed the increasing population of world. For this aim, there are two main ways, i) to increase the cultivation area of potato and ii) to increase the yield per unit area. The improved environmental adaptation of potatoes such as tolerance to drought, heat, salt, or combined environmental factors is crucial.

Although potato production has increased in the last 20 years, the average yield is almost stable in developing countries (Ortriz and Watanabe, 2004). Most of the annual potato production is contributed by developing countries that are prone to environmental abnormalities such as heat, drought, and salinity (Scott and Suarez, 2012). However, potato is a cool climate crop requiring 17-21 °C optimum temperature (Struik and Ewing, 1995; Levy and Veilleux, 2007). Due to the increase in potato production in developing countries, especially in tropic and subtropic regions having a higher average temperature than optimum for potato, potato breeders have focused on the development of abiotic stress-tolerant cultivars (Demirel et al., 2017). Also, global warming scenarios give a signal for sustainable potato production in many regions that includes doing traditional potato production in Europe and North America (Hijmans, 2003; Holden et al., 2003; Haverkort and Verhagen, 2008; Van Oort et al., 2012). It is

predicted that global warming will cause drought and an increase in temperature in many regions. Potato is sensitive to abiotic stress factors especially heat and drought (Monneveux et al., 2013; Levy and Veilleux 2007). Since the heat and drought simultaneously occur in Turkey, to develop more proper drought-tolerant cultivars for the environmental conditions of Turkey, the breeding programs should be carried out in Turkey conditions.

2.2 Abiotic Stress

Abiotic stress in agricultural term can be shortly defined as “A negative impact of non-living environmental factors such as drought, heat, cold, mineral deficiency, etc. on plants”. These stress factors cause growth reduction and consequently yield loss on plants (Cramer et al., 2011). It was pointed out that abiotic stress factors might limit crop production up to 70% (Boyer, 1982). In most cases, more than one abiotic stress factors can simultaneously occur. For instance, high temperatures can also cause the reduction of available water in the soil. In this case, the plant roots can face with a high concentration of salt and boron in the soil. Hence, the plants have to cope with heat, drought, and salt stress at the same time (Khan et al., 2015). Global warming and climate change scenarios indicate that today’s agriculture has a big challenge to increase crop productivity over 70% till the 2050s (Friedrich, 2015; Joshi et al. 2016a; Wang et al. 2016b). The fact remains that when the cultivated area is highly affected by abiotic stress factors, crop yield is reduced over the 40% (Pennisi, 2008). Recent analysis has indicated that the average temperature of the world will increase about 2.0-4.9 °C by 2100 (Raza et al. 2019). Because of these climatic changes, scientists and breeders have to focus on breeding abiotic stress tolerance of crops (Nakashima et al. 2014). It’s thought that facing with salt stress has been 97% percent increased even in well-watered areas for the last 20 years (Qadir et al. 2014). Also, global warming will cause changes in evapotranspiration and precipitation patterns because that it is expected to increase the likelihood of drought (Dai, 2011). Burke et al. (2006) have been specified that the areas which are under drought are enlarging and it is predicted to increase in the future. According to some presciences, 90% of the world's area is exposed to abiotic stress factors. As affecting 26% of the arable area, drought may be considered as the main abiotic stress factor (Khan et al. 2015).

As sessile organisms plants can not move from the stress conditions. Therefore, to cope with abiotic stress factors, plants evolved some mechanisms which enable them to tolerate and survive under adverse conditions through either physiological, molecular, and cellular changes (Knight H. and Knight M., 2001). The acclimatization process which has developed along with natural selection helps plants to display either stress tolerance or stress avoidance (Mickelbart et al. 2015). According to available research reports, plants tolerance against abiotic stress conditions could be evaluated as a kind of genetic regulation. In this case, plants can either compete with adverse conditions to maintain their normal growth and development or they can interact with the environment to develop (Gantait et al., 2019).

Potato is considered as a susceptible crop to abiotic stress factors such as drought, heat, and high salt levels in the soil. Climate change and global warming are growing problems in the last decades. These problems will cause irrepressible hungry in the world within the next 50 years. Potato is considered an important crop to prevent hunger, poverty, and malnutrition that are predicted to occur in the future because of its high nutritional value and high yield index. When plants are exposed to abiotic stress conditions, the expressions of thousands of genes are altered as the response mechanism (Zhang et al 2013; Evers et al., 2010). Conversely, biotic stress resistance is mostly a monogenic trait in plants, abiotic stress is multigenic. It means abiotic stress resistance is non-stressedled by many genes. That's why engineering and non-stressedling abiotic stress resistance is more difficult than biotic stress resistance (Wang et al., 2003). In this case, to determine desired genotypes, the usage of only one molecular marker is generally not enough for breeding abiotic stress tolerance.

2.3 Drought Stress

Drought can meteorologically be defined as interactive relation of decrease in the precipitation, reducing of groundwater layer and limited water presentness concomitantly increase in temperature (Singh and Laxmi, 2015; Singh et al., 2015). It is the physiological form of the stressed that can define as the inadequate water availability of the plants as a result of some injuries in plant metabolism (Kumar et al., 2018).

It is expected that worldwide drought impact will increase up to 20% at the end of this century because of climate change especially in Central and South America and Central and Western Europe (Edmeades, 2013; Singh. et al., 2015). Researchers signed that global climate change will lead to an US\$ 84 billion loss of farmers in South Asia (Mendelsohn, 2014). As resulting in at least 50% yield losses, most of the crops are not tolerant to drought stress (Singh. et al., 2015). Mediterranean regions and the Middle East were indicated among the hot spots regions of severe drought (Barlow et al., 2016; Saadi et al., 2015). Similarly, Turkey has been also thought that would severely be affected by drought especially around the Mediterranean and Middle East regions (Aktas, 2012).

2.3.1 Plant response to drought stress

When plants are exposed to drought stress, they show various responses as morphological such as the reduction in leaf area and stem length, leaf senescence, some changes in the root system, etc., physiological such as the decrease in transpiration rate, increase water use efficiency, stomatal closure, and osmotic adjustment, and biochemical such as proline accumulation, increase in activity of nitrate reductase, storage of carbohydrates, etc. These multiple responses make drought stress tolerance more complex to solve (Fig.1) (Haworth et al., 2013; Ammar et al., 2015; Conesa et al., 2016). An increase in the abscisic acid (ABA) accumulation is considered as a key mechanism to adapt drought stress among the various stress responses (Esther et al. 2000; Bano et al. 2012; Brodribb and McAdam 2013). Under drought conditions, cells lose water and this dehydration induces an increase in the accumulation of ABA which triggers to alter the expression of some genes (Abe et al. 1997). When plants are faced with drought, some degradations such as cell injury caused by reactive oxygen species (ROS) accumulation and an increase in cellular temperature can occur. Finally, these degradations are concluded with an increase in the fluidity of cell membranes, changes in protein-protein interactions, aggregation, and denaturation of proteins (Farooq et al., 2008).

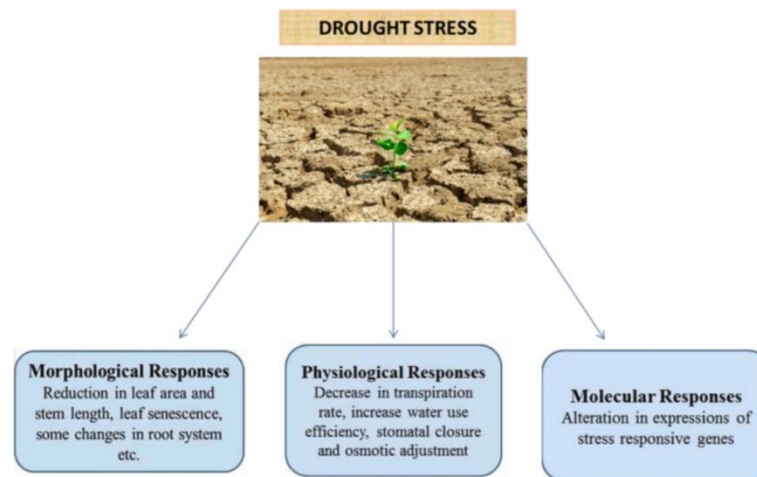


Figure 2.1. General plant response to drought stress

Under drought conditions, some metabolic pathways, photosynthesis, ROS scavenging mechanism, and stomata closure are mainly affected. For acclimatization to drought stress, some biomolecules such as protective proteins like Heat Shock Proteins (HSPs) and Late Embryogenesis Abundant (LEA) proteins (Vierling 1991; Lipiec et al. 2013) and some osmolytes like trehalose and proline (Zhang et al. 2010; Hayat et al. 2012) are accumulated.

Cellular impairments which are followed by an evident decrease in volume of the cell could be considered as the first symptom of dehydration. Additionally, functions of the some enzymes are negatively affected by excess accumulation of solutes. This situation finally, causes a reduction in photosynthesis and water use efficiency (WUE). When the duration of dehydration extends, leaves are rolled and wilted which finally causes to death of the plant (Sahoo et al., 2013). Plants exhibit more susceptibility to drought during reproductive stages such as the flowering period or seed development (Samarah et al., 2009; Alqudah et al., 2011; Samarah and Alqudah, 2011). Breeding crops in the Mediterranean environment which are the likelihood of the seen drought stress is pretty high, leaf area, leaf weight, and leaf growth rate can be considered as proper traits for selection drought-resilient crops (Alqudah and Schnurbusch, 2015; Quan et al., 2016).

As mentioned above, potato is very sensitive to drought stress. Drought susceptibility of potato may be correlated with two main reasons: 1) Due to its shallow root system, the

water uptake is limited. 2) Its photosynthetic machinery is not enough to recover water and heat stresses (Iwama and Yamaguchi 2006).

Under drought conditions, the root system of potato morphologically changes (Wishart et al. 2013, 2014) via increasing of lateral root formation, decreasing of root elongation, increasing of root thickness, and formation of root hairs thus, it can keep root-soil contact (Schmidt et al. 2012; Haling et al. 2013). The shallow root system of potato makes it a susceptible crop to drought conditions, although, potato may grow under varying climatic conditions (Pino et al., 2013). Drought also negatively affects the shoot length, leaf size and number, and photosynthetic rate (Dalla Costa et al. 1997, Deblonde and Lendent 2001, Ekanayake and Midmore 1992, Kiziloglu et al. 2006, Weisz et al. 1994). Also, drought conditions are especially deleterious for potato during the tuberization period because it affects the tuber yield and quality (Deblonde and Lendent 2001, Ekanayake and Midmore 1992, Weisz et al. 1994). Therefore, scientists have focused on breeding potato crops that are tolerant to abiotic stress conditions. It is very important to develop new potato genotypes that are more adapted to adverse environmental conditions.

The effects of drought on tuber yield depend on the time, duration, and severity of the drought (Jeffery, 1995). As the most susceptible periods to drought stress are the emergence and the tuberization period of the potato. During these periods, tuber yield is highly affected (Martinez and Moreno, 1992; Obidiegwu et al., 2015). Drought stress also affects tuber quality via a higher accumulation of toxic glycoalkaloids (Bejarano et al., 2000). On the other hand, some defects such as secondary growth, malformations on the tuber, or brown spots may be seen on the tuber (Harris, 1978).

The Genotype×Environment interaction which has a high effect associated with genetic variance under drought conditions reduces heritability. That's why the selection of drought-tolerant genotypes becomes time-consuming and limited (Blum, 1998).

Water limitation leads to closure of stomatal conductance (Carvalho, 2008). Correspondingly, stomata size, leaf area, and stem length are decreased while cellular death and cell membrane permeability are increased (Monneveux et al., 2013; Zingaretti et al., 2013). Changes in chlorophyll content that is determined via SPAD meter is an

important parameter for drought tolerance. Extension of photosynthetic activity via decreasing or retardation of senescence is defined as a “stay-green effect” that increases yield performance under drought conditions (Berger et al., 2010). Stay green effect has been seen in potato under severe water restriction conditions however yield reduction was also observed (Ramírez et al., 2014).

One of the important effects of drought stress is the higher accumulation of Reactive Oxygen Species (ROS) such as hydrogen peroxide (H_2O_2), superoxide anions ($O_2^{\cdot-}$), hydroxyl radical (OH^{\cdot}), and singlet oxygen (1O_2) (You and Chan, 2015). An increase in the accumulation of ROS can induce some responses like lipid peroxidation of the cell membrane, denaturation of DNA and protein structure, degradations in the pigments of photosynthesis, increase in carbohydrate oxidation, and decrease in some enzyme activities (Noctor and Foyer, 1998). Besides, ROS also has an important role as signaling molecules as regulating the plant metabolism to current environmental conditions (Hancock, 2017). So, it's very important to adjust ROS balance at the cellular level can ensure to survive under adverse conditions (Demirel et al., 2020).

To increase crop productivity, important progress has been made by using conventional breeding methods. Nevertheless, because of the low heritability and multi genetic form of the drought tolerance, narrow genetic variation of the gene pool and high interactions with the environment, breeding of drought-tolerant varieties by using conventional methods are highly restricted (Pardo, 2010; Hill et al., 2013; Jha et al., 2014; Liu et al., 2014).

2.4 Molecular Markers

Molecular selection markers are the DNA fragments that are associated with biological traits. They can be transferred over generations and evaluated as a probe to sign a gene, tissue, chromosome, and even an individual (Xu and Crouch 2008). Thus, they exhibit genetic differences between individuals. As molecular markers take place near to the desired trait or linked them, they don't affect the function of a gene (Sharma S. and Sharma A., 2018).

Currently, there are three common methods to assign genetic markers; 1) QTL mapping, 2) Association mapping, 3) Screening by recurrent selection (Takeda and Matsuoka 2008). Among them, one of the most suitable methods to identify the genes which are involved in drought tolerance is conventional Quantitative Trait Loci (QTLs) analysis to apply molecular markers on bi-parental populations. For this purpose, it is necessary to construct a genetic linkage map and this map is identified based on linkage equilibrium in a biparental population. It means that it requires a designed population. In this case, the resolution of genetic maps and identification QTLs are limited. On the other hand, the detection of QTLs by the conventional QTL analysis is also affected by several factors such as type, size, and structure of the population, the number of sampled environments, calculation method, and the number of molecular markers (Abou-Elwafa, 2016).

The specific linkage phase between desired gene locus and marker locus is considered as the disadvantage of selecting DNA markers. A recombinant event that can occur in a genome can damage this linkage (Lübberstedt et al. 1998; Toojinda et al. 1998). On the other hand, in the case of, a linkage between the target gene and nontargeted genes can be detrimental for breeding programs. For instance, in one study which is carried for wheat elite germplasm lines, the undesired genes which passed to the genome while transferring all QTLs of fusarium resistance affected the agronomic traits (McCartney et al. 2007). Similarly, backcross of study leads to increase plant height in rice (Venuprasad et al. 2012).

However, Association Mapping (AM) studies don't require a designed biparental population. It uses diverse accessions like germplasm collections, breeding lines, or landraces. So, to develop mapping populations, non-stressedled crosses are not required for association mapping.

To establish the mapping populations which are used for traditional QTL mapping needs fewer generations like 6-10 years. That's why they have limited recombination events. Contrastly, the populations which are used for AM passed many generations since domestication. Hereby, the markers that are closely located to QTL can be identified (Flint-Garcia et al., 2003).

Knowledge about genetic variations, levels, and structure of variation within and between populations ensures a great advantage for the utilization of plant individuals in breeding programs (Cole, 2003). To detect levels and structure of these variations, usage of the evolutionary background, the process of gene flow, mating system, and population density are important factors (Hamrick, 1989). To search these factors, different types of morphological and agronomic traits are successfully used. For the last three decades, there is a fast increase regarding the information of the plant genome sequences, and the physiological and molecular role of various plant genes which have integrated with molecular genetics and plant breeding programs (Nadeem, 2018). One of these important integrations is genetic markers and plant breeding (Kebriyae, 2012). The genetic marker that is closely related with the target gene, generally they do not directly symbolize the genes of interest but act like a sign or flag, can define as a gene or DNA sequence which non-stresses a particular gene or trait with a known chromosome location (Collard, 2005). They are extensively classified into two groups as classical markers and DNA/molecular markers (Jiang, 2013). Classical markers can be grouped into three categories as 1) Morphological markers that can visually distinguish such as flower color, seed size, growth habit, and other important agronomic traits (Nadeem et al., 2018). 2) Biochemical markers which are multi-molecular forms of enzymes. They are coded by various genes but have the same functions so they can also be called as isozymes (Bayley, 1983). 3) Molecular markers are the specific DNA sites that show polymorphism between individuals, they can transfer from generation to generation according to inheritance laws (Ruane and Sonnino, 2007). They are examined according to polymorphisms that are found in the nucleotide sequences of different individuals. These polymorphisms are based on insertion, deletion, point mutations, duplication, and translocation (Nadeem et al., 2018). Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSRs), Single-Nucleotide Polymorphism (SNP), and Diversity Arrays Technology (DArT) markers can be given as some examples of molecular markers (Jiang, 2013). However, they are classified into various groups according to their 1) Mode of gene action, they can be co-dominant or dominant markers, 2) Detection methods like hybridization-based molecular markers or polymerase chain reaction (PCR)-based markers, 3) Mode of transmission they can transfer to offsprings via paternal organelle inheritance, maternal organelle inheritance, bi-parental nuclear inheritance or maternal nuclear inheritance (Semagn et al., 2006).

Recently, successful developments and applications of different types of DNA molecular markers have evolved in plant genetics and breeding programs (Nadeem et al., 2019).

Three major groups that can explain the allelic variations of the genome within the same species are 1) differences at tandem repeats at particular locus such as simple sequence repeats (SSRs), 2) Insertions/Deletions (InDels), 3) Single nucleotide polymorphisms (SNPs) (Weber and May, 1989; Ophir and Graur, 1997; Wang et al., 1998).

2.4.1 Marker assisted selection (MAS)

The application of molecular markers to select the plants carrying the traits of interest is called as Marker Assisted Selection (MAS) (Gantait et al., 2019). Difficulties in classical breeding programs can be overcome by this selection approach (Collards et al., 2005). To integrate the desired characters into a specific genotype, the classical breeding strategies require a higher number of breeding series. However, MAS ensures a perspective to pyramid characteristics of desired traits in one genotype. Thus it provides a more authoritative and accurate way with fewer such series (Babu et al., 2004), and it saves time and resources which are used with superior crop productivity (Gantait et al., 2019). Effective usage of MAS ensures doubled genetic gain to plant breeding programs when compared with the phenotypic selection for desired agronomic traits such as yield, resistance to abiotic stress, and other qualitative traits (Oliveira et al., 2008). MAS could be effectively used to identify, monitor, maintain, link, and pyramid multiple genes of interest for stress tolerance (Gantait et al., 2019).

2.4.2 Marker-assisted selection in plant breeding

Thanks to Green Revolution, a great increase occurred in potential yields of rice and wheat by avoiding food crises in the 1960s (Pingali, 2012). Therefore, a strong expectation is that breeding studies should increase gain in yield while decreasing funding. On the other hand, the new crop cultivation system which is based on Green Revolution concluded with an increment of pests and diseases (Tilman et al. 2002). To handle these problems, scientists identified genetic sources from before the 1940s

landraces. In addition to this, they have also brought biotic and abiotic tolerances into high-yielding varieties (Gilliham et al., 2017; Bailey-Serres et al., 2010).

The fundamental aim of a plant breeder is to improve present cultivars that are deficient in one or more traits. To achieve desired traits, the serial crossing is done for generations with this approach, whole genomes are crossed and then the recombinants which have desired traits are selected. As it comprises too many crosses, phenotypic selections, and the possibility of passing undesired traits with desired traits to progeny, this method is time-consuming and laborious. Developing molecular breeding strategies such as using molecular markers, could make it easier to cope with these problems (Sharma S. and Sharma A., 2018).

Owing to growing molecular biology, identifying of heritable variation by describing the genes which include the genetic architecture of desired traits. Today, the selection possibility of directly desired genes by using molecular techniques called as molecular breeding (Moose and Mumm 2008).

Marker-assisted selection technique combines conventional breeding and molecular biology methods. Phenotypic selection is carried out according to the genotype of markers (Sharma S. and Sharma A., 2018).

The objective of MAS is broadly used to clone and identify genes among species (Song et al. 1995; Qu et al. 2006; Ji et al. 2016). To understand the potential value of MAS and its limitations, many genes that have important phenotypic effects were cloned (Cobb et al., 2019).

Recently, the most important aim of plant breeding programs is developing the combination of abiotic stress tolerance, biotic stress tolerance, and agronomic quality characters while improving yield performance and providing farmer's demands. If more than one desired trait is wanted to select for the same breeding program by using the traditional breeding method, it can cause a loss in yield and increase the selection cycle of the breeding program. However, MAS can ensure to combine desired traits with less yield losses by selecting in a fewer cycle (Xu and Crouch, 2008).

Different AFLP, RFLP, and SSR markers are extensively used in breeding programs since the 1980s (Kage et al. 2016). To make faster the transferring of monogenic traits from exotic germplasm to a cultivar, isozyme markers were firstly used at beginning of the 1980s (Tanksley and Rick 1980; Tanksley 1983). Later on, to improve qualitative traits, Beckmann and Soller firstly introduced restriction fragment length polymorphism (RFLP) markers for breeding studies in 1986. Lande and Thompson shed light on theoretical studies of MAS for quantitative traits in 1990.

To increase the impact of traditional breeding programs, the MAS technique has been successfully implemented for various crops such as wheat, sorghum, rice, and maize (Miedaner and Korzun 2012; Mohamed et al., 2014; Jena and Mackill, 2008; Prasanna et al., 2010). For instance, to develop MAS-946-1 which is the first drought-tolerant aerobic rice variety, scientists have been successfully used the MAS technique in India (Gandhi 2007). Also, it was reported that the *sub1* gene which is located on chromosome 9 of rice is associated with submergence tolerance by International Rice Research Institute (IRRI). So, the molecular markers which are tightly linked with this gene were used for submergence tolerance of rice by the International Rice Research Institute (IRRI) (Neeraja et al. 2007). Table 2.1 shows some examples of MAS studies in crop plants.

Table 2.1. Example MAS studies in crop plants

Plant	Traits about MAS studies	References
Maize (<i>Zea mays</i>)	Hybrid vigor	(Stuber, 1995)
Barley	Grain yield	(Schmierer et al., 2004)
Common bean (<i>Phaseolus vulgaris L.</i>)	Anthraco nose resistance	(Miklas et al., 1990).
Soybean (<i>Glycine max</i>)	Cyst nematode resistance	Concibido et al. (1996)
Pearl millet (<i>Pennisetum glaucum (L.)</i>)	Downy mildew resistance	(Navarro et al., 2006).
Rice (<i>Oryza sativa L.</i>)	Good cooking and quality characters	(Hardin, 2000).

Eagles et al. (2001) reported that to improve new wheat cultivars, approximately 20 genes, and chromosome regions were successfully used in Australia. Besides, to improve pest resistance, more than 20 different disease resistance genes and 21 alleles

which are associated with bread and pasta quality characters, MAS has been applied in wheat crop (Dubcovsky, 2004). Hardin (2000) developed two rice (*Oryza sativa* L.) varieties that have specific cooking and other quality characters such as amylose content in the U.S with the help of SSR markers. Another study on rice by MAS was carried in Indonesia. They have developed two rice varieties that are resistant to bacterial blight with high yield performance (Bustamam et al., 2002). In North America, a common bean variety was developed for anthracnose resistance by using SCAR (Sequence Characterized Amplified Region) marker that is tightly linked to the *Co-42* gene which is identified for anthracnose resistance by Balardin and Kelly in 1998. A new pearl millet hybrid that has tolerance to downy mildew was introduced in India by a combined breeding method of MAS and backcross (Navarro et al., 2006). Two main issues make MAS studies less reliable; 1) Possibility of recombination that could occur between marker and trait. 2) Application of MAS requires high cost. Despite these barriers, MAS has been widely used for breeding programs of crops (Taunk et al., 2019).

2.4.3 Marker-assisted selection for abiotic stress tolerance in crop plants

To feed the growing world population, human come across some challenges which arising from abiotic factors such as drought, heat, salt, and cold, and biotic factors such as plant pathogens, insect pests, and weeds. Because of the increasing human population, speedy urbanization, continuous greenhouse-gas emission, and the innovation of so much technology lead to climate change, and the mentioned abiotic stress factors are the outcomes of climate change either directly or indirectly. To maintain crop productivity, plants should tolerate the various abiotic stress conditions. Countless alleles that show resistance or tolerance to abiotic stress conditions have been broadly examined in almost all crop germplasms. However, those alleles were identified in genotypes like landraces, wild relatives, or ancestors. Because of their poor yield, the farmer community doesn't prefer to use these genotypes. However, geneticists and plant breeders attempted to use those genotypes for transferring the tolerance related genes to high yielding crops. According to this attempt, high yielding crops will have a higher tolerance to abiotic stress conditions. In this way, MAS plays an important role in the last few decades (Gantait et al., 2019).

As abiotic stress tolerance is a multigenic trait and genotype×environment interaction also has a big impact on it. Therefore, abiotic stress tolerance is considered as a quantitative and polygenic character that relies on the genetic deviation, heritability, and changing of the environment (Geiger and Heun 1989). Three key perspectives that influence the genetic modification mechanism could be explained as 1) Heritability, the degree of genetic effect on the variation in a resistant trait 2) A variation that arises by chance, like genetic drift, in a resistant trait. 3) Natural selection on the resistant traits (Simms and Rausher 1992). Therefore, to determine these explained issues and designing a breeding program for abiotic stress tolerance, it's needed that mapping of simple inherited trait loci and quantitative trait loci (QTL), comprising candidate gene loci (Gantait et al., 2019). Table 2.2 shows some example studies of MAS in abiotic stress tolerance of plants.

Table 2.2. Some MAS studies in abiotic stress tolerance of crops

Plant	Reported MAS studies	Remarks	References
Wheat (<i>Triticum aestivum</i>)	3 AFLPs	Heat tolerance	Vijayalakshmi et al. (2010)
Grape (<i>Vitis species</i>)	SNPs	Heat tolerance	Lijavetzky et al. (2007)
Chickpea (<i>Cicer arietinum</i>)	4 key candidate genes in the <i>QTL</i> -hotspot	Drought tolerance	Kale et al. (2015)
Rice (<i>Oryza sativa</i>)	2 RFLP	Salt tolerance	Bonilla et al. (2002)
Tomato (<i>Solanum Lycopersicum</i>)	49 QTLs	Salt tolerance	Villalta et al. (2007)
Maize (<i>Zea mays</i>)	32 QTLs	Drought tolerance	Messmer et al. (2011)
Strawberry (<i>Fragaria ananassa</i> Duch.)	Expressed sequence tag (EST) and Amplified fragmentlength polymorphism (AFLP)	Drought tolerance	Razavi et al., (2011)

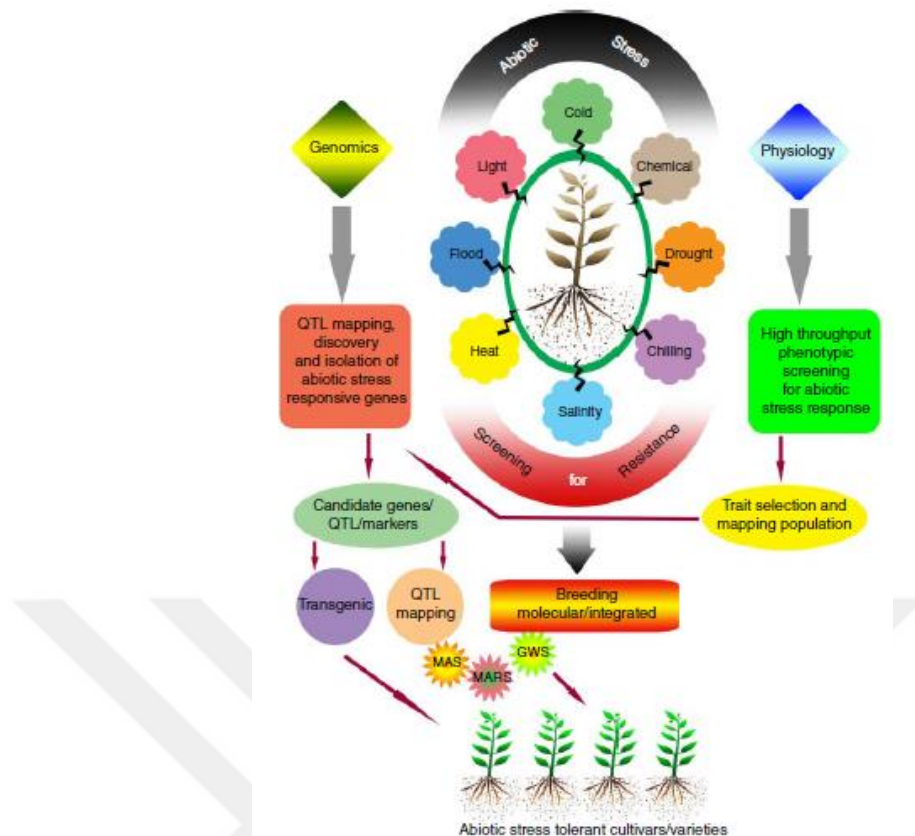


Figure 2.2. Molecular marker-based strategies for improving abiotic stress-tolerant varieties (Gantait et al., 2019)

Sari-Gorla et al. (1999) were carried out a QTL analysis for the flowering period, anthesis-silking interval (ASI), and plant height in maize under well-watered and water restricted conditions. As a result of the study, 153 loci of RFLPs, AFLPs, and SSRs were associated with investigated traits. Also, Ziyomo and Bernardo (2013) suggested some useful selection parameters that are secondary traits, like ASI, leaf senescence, and ears per plant for high grain yield of maize under stressed conditions. Bonilla et al. (2002) reported Saltol which is a major salt tolerance QTL in chromosome 1 of rice to be flanked by two RFLP markers and two microsatellite markers. Giorni et al. (1999) found a significant accumulation of pt59, pao86, and paf93 which are cold regulated (COR) genes and proteins (COR14a and COR14b) in winter cultivars of barley, and they suggested that higher accumulation of COR14 may be associated with the cold resistance of barley. Rapacz et al. (2008) observed a higher expression of COR14b in freezing tolerant plants on the first day of cold acclimation.

As mentioned before, abiotic stress tolerance is a complex character as non-stressed by multiple genes. On the other hand, GenotypeXenvironment interaction has a big impact on it. Also, some epistasis events which can not be predicted can occur. That's why there are still gaps in the breeding of abiotic stress tolerance by MAS (Xu and Crouch, 2008).

2.4.4 Marker-assisted selection for abiotic stress tolerance in potato

For many purposes, the application of molecular markers in potato breeding has been reported. As some example of that kind of studies, cultivar identification (Gebhardt et al. 1989), phylogenetic studies (Kardolns et al.1998), analysis of recombination between genomes (Williams et al. 1993), identification of genes non-stressedling traits (Gebhardt, 1994), and marker-assisted selection (Hamalainen et al. 1997) can be given. As mentioned above, the main objectives of MAS in breeding programs are 1) transferring genes of desired traits between different genotypes and 2) selection of breeding lines with desired traits. For this purpose, the identification of one or more markers that are linked to the gene(s), and the identification of their localization on the molecular map can be evaluated as the first step. One of the most highly saturated maps with different molecular markers is the potato map. This situation ensures great opportunities for MAS in potato breeding (Barone, 2004).

Potato varieties usually exhibit low fertility, high heterozygosity, multiple ploidy levels, and inbreeding depression. That's why breeding programs are time-consuming and expensive in potato plants (Wetterstrand 2014; Guenthner et al. 2001; Slater et al. 2013). To reduce cost and save time, researchers started to use molecular genetic markers linked to phenotypic traits (Milbourne et al. 2007). For this aim, QTL mapping, linkage mapping, and association mapping are widely used. However, QTL mapping and linkage mapping are harder to apply to cultivated potato varieties especially for the traits which are non-stressed by multigene like drought resistance. Because these mapping techniques require mapping population which is created by the crossing of two homozygous parental lines. However, the cultivated potato is tetraploid and has inbreeding depression. That's why it's not allowed to create homozygous lines. Besides, association mapping studies don't require a bi-parental mapping population. Therefore, association mapping is preferred for developing molecular selection markers in potato.

During association mapping studies, the collection of the genotypes which are not highly designed is used as a population (Jannink et al. 2001). Association mapping studies offer higher flexibility while choosing population, for example, gene bank collections of cultivars, breeding lines, germplasm accessions, etc. These populations are good examples for using in association mapping studies (Malosetti et al. 2007).

For resistance to potato cyst nematodes *Globodera pallida* and *G. rostochiensis*, associated 17 genes have been mapped (Bakker et al. 2004). From the Rx gene family, Rx1 from *S. tuberosum* ssp *andigena* and Rx2 from *S. acaule* show resistance to Potato Virus X (PVX) (Ritter et al. 1991; Mori et al. 2011). In addition to these markers which are linked to nematode resistance and potato virus, Zimnoch-Guzowska et al. (2000) reported some AFLP markers for resistance to blackleg (*Erwinia carotovora* ssp. *atroseptica*) in their mapping study. Bormann et al. (2004) and Gebhardt et al. (2006) successfully used the marker NL25 for resistance to potato wart (*Synchytrium endobioticum*) in their studies. However, as resistance to abiotic stress factors non-stressed by multiple interacting genes, MAS for abiotic stress tolerance is not as easier as MAS for disease resistance (Wang et al. 2003; Watanabe et al. 2011). Most gene expression studies have been carried to evaluate genetic characteristics of resistance to cold and drought stress (Bournay et al. 1996; Mane et al. 2008). For instance, Rensink et al. (2005a,b), Schafleitner et al. (2007a,b), Anithakumari (2011), and Demirel et al (2020) have been reported so many drought-induced genes in their comparative gene expression studies under well-watered and drought conditions. They suggested that this information can be used in breeding programs as these QTLs for drought resistance introgressed into susceptible varieties (Ashraf and Foolad, 2013; Hu and Xiong, 2014). Anithakumari et al. (2011) reported a few QTLs that are related to root/shoot ratios for drought resistance and they can be used for marker development. Vasquez-Robinet et al. (2008), Evers et al. (2010), and Demirel et al (2020) have been reported some metabolites which are associated with drought tolerance. Although these studies are highly dependent on the experimental conditions, responses to drought are highly varied (Schafleitner et al. 2007b). Potential development of markers will be facilitated thanks to the availability of the complete sequence of the potato genome.

2.5 Evolution of Molecular Markers

When the RFLPs were discovered in the 1980s, they have been widely used for plant molecular genetics studies. Although they are co-dominant, reproducible, and don't require DNA sequence information, they are expensive and time-consuming (Lander and Botstein, 1989; Madhumati 2014). With the development of PCR technology, disadvantages of RFLP markers were broken and PCR-based markers such as RAPDs, AFLPs, and SSRs have been a new favorite of researchers in the 1990s (Williams et al., 1990). Among them RAPDs are polymorphic but they are dominant and have low reproducibility. As AFLPs are more reliable and have higher reproducibility without DNA sequence knowledge, They are more preferred and still have a popularity for molecular genetic studies. Since they are dominant and time-consuming, they have not been used widely in breeding (Zhang et al., 2011; Nadeem et al., 2018). With the discovery of SSR markers, also known as microsatellites, the disadvantages of RFLPs, RAPDs, and AFLPs have been overcome. SSR markers are co-dominant and highly reproducible. Also, their great abundance in the genome and easy and efficient usability for plant mapping studies, make them “marker of choice” in the plant breeding community (Tautz, 1989; Kalia et al., 2011). The most important disadvantage of SSRs is that they have a high mutation ratio on microsatellite regions which causes alterations in primer binding regions. Finally, the situation leads to unmeaningful allele formations and makes it harder to interpret genotypic and allelic frequencies (Freudenreich et al., 1997). However, when the SNPs were discovered in the human genome at the beginning of the 21st century, the superiority of SSR markers was broken. Because it is proved that they are the most abundant form of genetic variation in plant and animal genome (Ghosh et al., 2002).

2.5.1 Single nucleotide polymorphisms (SNP) markers

The single nucleotide (A, T, G, or C) differences in the specific loci of genome sequence among the individuals of populations called “Single Nucleotide Polymorphisms (SNPs)”. When these differences are detected, it is evaluated as a good marker system for animal and plant breeding (Vignal et al., 2002; Ganai et al., 2012). The presence of sequence knowledge in many species, such as humans, Arabidopsis, and rice, provides a basis to identify and develop SNP markers (Semagn et al., 2013).

Next and third sequencing technologies ease and accelerate the identification of SNPs (Alonso et al., 2016). The selection of true SNPs from the sequence data is a pioneer system for molecular genetics and the breeding field (Bevan et al., 2005). Sequence knowledge provides to identify more than 1 million SNPs which can be used as candidate genetic markers for each species (Morgil et al., 2020). As mentioned above, the nucleotide differences in the genome due to SNPs are the most widely seen form of the genetic variations among individuals. That's why SNPs are the one of latest and the most preferred DNA marker system especially for mapping studies and breeding programs of complex organisms. When SNPs are compared with other DNA markers, their wide abundance in the genome, high sensitive genotyping with low cost, being a co-dominant marker, and less mutation chance make them the most appropriate markers for either ensuring solutions on scientific issues and either for plant breeding studies (He et al., 2014). Table 2.3 shows some example studies about SNP markers in plant breeding.

Table 2.3. Example studies about SNP markers in plant breeding

Plant	Reported SNP Studies	References
Barley	Smut resistance	Lehmensiek, 2008
Maize	Flowering time	Buckler et al., 2009
Wheat	Resistance to leaf rust, stripe rust, and powdery mildew diseases	Lagudah et al., 2009
Soybean	Aphid resistance	Kim et al., 2010
Rice (<i>Oryza sativa</i>)	Salt tolerance	Rana et al., 2019
Wheat	660K SNP array	Sun et al., 2020

Zamora et al. (2013) identified SNP markers that can be used for genotyping, linkage mapping, and phylogenetic studies. An evaluation study was carried for genetic variation and population structure of maize inbred lines which are available for the agro-ecology of Ethiopia by using SNP markers (Ertiro et al., 2017). In another study in maize by Luo et al. (2017) an SNP-based linkage map was constructed and mapped a major QTL on chromosome 1 of the maize plant which is associated with plant height and salt stress. Ren et al. (2016) mapped the *btwd1* gene which is associated with dwarfing in barley by an SNP-based mapping study. Novel SNP markers were identified for *InvGE* and *SssI* genes which are associated with sugar contents and frying color in diploid potato (Delgado et al., 2017). Nineteen QTLs which are associated with nutrient use efficiency (NuUE) were detected by using SNP markers and phenotyping in rice (Mahender et al., 2019). One of the latest studies was carried in common bean by Oladzad et al. (2019). They have identified a new association panel and utilization of SNP collections for mapping desired traits under heat and drought stress conditions. Several SNPs were identified in the maize plant for ZmPP2C-A genes which are associated with drought resistance (He et al., 2019). Deperi et al. (2018) screened 231 potato genotypes by using SNP markers and clustered them according to their ploidy, taxonomy, and origin to make a guide for parental information in breeding programs. Prodhomme et al. (2020) identified a haplotype-specific SNP marker which is linked to the *Sen1* gene that causes wart disease in potato. A significant SNP marker and trait association for tuber size and marketable tuber yield of potato was determined on chromosome 3. It was suggested that the results could be a pioneer for the breeding of drought tolerance in potato (Aliche et al., 2019).

Thanks to developing genomic technologies, many of the SNP genotyping techniques have evolved (Chen and Sullivan, 2003; Gut, 2001; Sobrino et al., 2005; Syvanen, 2001). Among them, genotyping by next-generation sequencing is a featured method. However, this technique is considered a time-consuming and high cost. Also, some missed data could arise (Beissinger et al. 2013). Therefore some other SNP genotyping platforms have evolved. For instance, BeadXpress and GoldenGate (Illumina), SNaPshot, TaqMan SNP Genotyping (Appliedbiosystems), SNPWave (keygene), and Kompetitive Allele-Specific PCR (KASP) (LGC chemistry) are the most widely used techniques for this aim. While selecting the most usable one for the research purpose, some aspects must need to be considered. Such as; sensitivity, accuracy, flexibility,

reproducibility, and cost of technique (Semagn et al., 2014). In such cases, TaqMan and KASP genotyping are considered as the most preferred and suitable methods for genotyping (Neelam et al. 2013). As being low cost, high throughput, highly sensitive, and specific when compared with other techniques (Semagn et al., 2014), the KASP technique was also used in this study.

2.6 KASP (Kompetitive Allele-Specific PCR) Genotyping

KASP (Kompetitive Allele-Specific PCR) is a type of PCR genotyping system which is homogenous and fluorescent-based. KASP technique is simply based on the extension of allele-specific oligonucleotides and signal generation by fluorescence resonance energy transfer (FRET) which is a mechanism to identify energy transduction between two photosensitive molecules (Semagn et al., 2014; Kumpatla et al. 2012). Allele-specific fluorescent-labeled primers ensure endpoint fluorescent labeling. Thus, SNPs at specific loci are scored according to their bi-allelic possibilities (LGC, chemistry). The target SNP region is amplified with allele-specific primers which are labeled with HEX and FAM fluorometric dyes at their 5' end. When allele-specific primer hybridized with DNA, proper fluorescence occurs and genotype (alleles) is identified according to these fluorescent signals (Zhao et al., 2017). The basic workflow of the KASP system is shown in Figure 2.3.

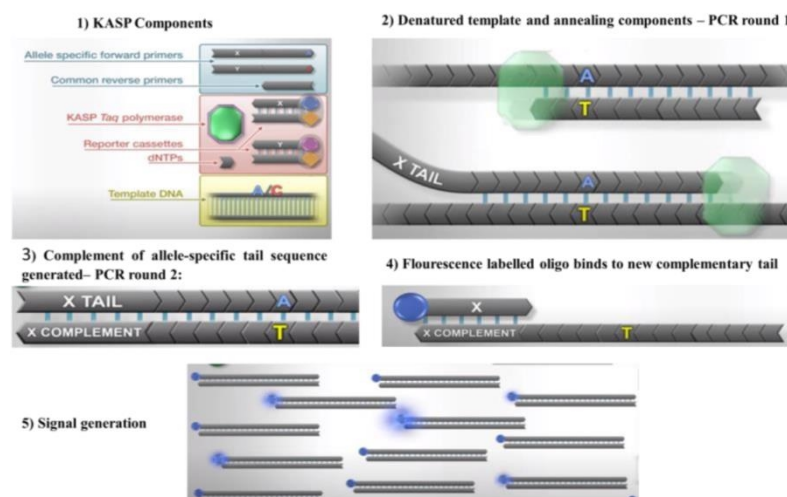


Figure 2.3. The basic workflow of the KASP system (Modified from LGC chemistry KASP genotyping user guide)

This method is highly powerful and cost-effective while compared with other techniques, and appropriate to SNP genotyping at 90% ratio (Köseoğlu et al., 2017). In terms of the number of SNPs and samples, KASP genotyping also has higher accuracy, more reproducibility, and flexibility (LGC, chemistry). That's why KASP has been widely used for SNP genotyping studies in many crop plants. For instance, it was used for genome-wide SNP genotyping in rice (McCouch et al., 2010), MAS breeding for leaf rust resistance in wheat (Neelam et al., 2013), three SNP markers were developed for soybean cyst nematode resistance via KASP assay (Shi et al., 2015). A KASP marker was developed for genotyping of AhFAD2 mutation in peanut (Zhao et al., 2016). Koseoglu et al. (2017) conducted a study in sunflower to downy mildew resistance for MAS studies by using KASP assay. Two KASP markers were developed for resistance to Pea seed-borne mosaic virus (Kylie et al., 2020). The genotyping of *Bemisia tabaci* which is a type of Cassava colonized whitefly was carried by using KASP assay (Wosula et al., 2020). To screen root-knot nematodes for tomato breeding, KASP genotyping was carried to select genotypes that have *Mi-1* gene that ensure resistance to root-knot nematodes (Devran et al. 2016). Also, a KASP marker was validated to screen the *Sw-5* gene which ensures resistance to Tomato spotted wilt virus for tomato breeding (Devran et al., 2019).

Although a limited number of reports are available due to the multigenic nature of abiotic stress tolerance, currently, KASP markers have also started to use for abiotic stress tolerance studies in crop plants. To ease waterlogging tolerance for maize breeding programs in tropical regions, 331 polymorphic SNP markers were genotyped by using KASP assay, and 18 QTLs which are related to waterlogging stress were identified (Zaidi et al., 2015). A KASP marker validation study was carried in the maize plant for heat stress tolerance by using RNA-seq data to identify related SNPs (Jagtap et al., 2020). Two KASP markers were developed with 84% accuracy to select lines that have low cadmium uptake from the soil in durum wheat (Salsman et al., 2018). An association mapping study and subsequent validation of two KASP markers were carried for a wheat diversity panel under well and restricted watered conditions (Khalid et al., 2019). Five KASP markers that are linked to the *qSulf-1* gene enhancing sulfur tolerance in melon were validated (Branham et al. 2020). Three QTLs that are related to Water Use Efficiency (WUE) were detected and validated by using KASP assay in the apple plant (Wang et al. 2018). Three KASP markers were validated for *TaDreb-B1* and

1-feh w3 genes under optimal conditions in the field for grain yield of bread wheat. Although there is no available data under drought conditions in the field, the response of those genes for drought stress tolerance is known (Whei et al., 2009; Zhang et al., 2015b). To improve drought tolerance of cowpea, Marker-assisted backcross breeding was applied via KASP genotyping (Batieno et al., 2016).

2.6.1 KASP studies in potato

The *Sli* gene which ensures self-compatibility was screened by using KASP markers for parental lines and introduced into the production of F1 hybrid seeds in diploid potato breeding programs (Lindhout et al., 2011). To screen four different *Phytophthora infestans* resistance genes, KASP assay was used in diploid potato breeding (Su et al., 2019). The resistance *H2* gene against potato cyst nematode, *Globodera pallida* was mapped on chromosome 5 of tetraploid potato (Strachan et al., 2019). A genome-wide prediction study was carried for allele dosage of specific gravity and fry color traits by KASP assay in tetraploid potato (Endelman, et al., 2018). To validate an SNP marker for the *Sen1* gene which shows resistance to P1 wart disease in potato, a KASP assay was used (Prodhomme et al., 2020).

2.7 Aims and Objectives

Currently, a validated KASP marker related to the yield of potato under drought conditions has not been reported. A genome-wide association study in a potato panel was carried by our research group (Caliskan et al., 2020). They have identified 419 SNP regions related to agronomic and morphological traits and they have indicated that molecular selection markers can be developed by using the KASP technique. Accordingly, 16 KASP markers that are related to yield under drought conditions were designed and tested in a population whose yield potentials are known under drought conditions and seven of them had good potential as a selection marker. This thesis is the next step of validation of those seven KASP markers. Firstly, the 89 potato breeding lines in which their yield potentials have not been known under drought conditions were screened with KASP markers and verified with a field trial.

As consequently, the purposes of this study to validate KASP markers (SNP markers) by the combination of molecular and morphological traits determined under field conditions for their implementation in potato breeding programs for selection drought resistant lines.



CHAPTER III

MATERIALS AND METHODS

3.1 Material

A potato breeding population with 89 breeding lines developed at the Agricultural Genetic Engineering Department was used as plant material in this study. According to the coding system applied in the breeding program carried out by our group, the first two number indicate the first year (e.g. MEÇ17) which is planted to field (e.g. 2017), other numbers until “.” indicate the family number (e.g. MEÇ17023), the lines which belong from the same family is indicated by the numbers after “.” (e.g. MEÇ17023.14). The features of the MEÇ13, MEÇ14, MEÇ15, MEÇ16, and MEÇ17 populations and their pedigree information are presented in Table 3.1 The breeding population was screened by 7 KASP markers for the selection of candidate drought resistant lines based on their genotypic (allelic) variations. Accordingly, 14 breeding lines were selected as high-yielding lines under drought conditions Then, 14 breeding lines and 2 standard cultivars including Agria and Russet Burbank (RBB) which have low yielding under drought conditions were tested in a field trial to confirm KASP assay results under non-stressed and stressed (35% deficit irrigation) conditions.

Table 3.1. The features of the breeding population. The table indicates, code of the breeding population, Pedigree names, and the desired traits of the pedigree which is selected for

No	Breeding Line	Pedigree (♀x♂)	Desired Trait (♀x♂)
1	MEÇ1302.20	C31-5-91 x 07-406	Heat tolerance x Heat tolerance
2	MEÇ1302.18	C31-5-91 x 07-406	Heat tolerance x Heat tolerance
3	MEÇ1301.20	98.120 x (98-428 x Cicero)	Heat tolerance x Heat tolerance
4	MEÇ305.05	99.214 x (Lady Rosetta x S440)	Heat tolerance x Heat tolerance
5	MEÇ1302.05	C31-5-91 x 07-406	Heat tolerance x Heat tolerance
6	MEÇ1406.07	01.509 x Latona	Heat tolerant x High yielding
7	MEÇ1409.09	99.463 x Kolibri	Heat tolerant x High yielding
8	MEÇ1407.08	(GalaxChallenger) x Allegria	High yielding x High yielding
9	MEÇ1407.05	(GalaxChallenger) x Allegria	High yielding x High yielding
10	MEÇ1411.06	Lady Rosetta x Soleia	High yielding x High yielding
11	MEÇ1407.17	(GalaxChallenger) x Allegria	High yielding x High yielding
12	MEÇ1505.07	Amora x CIP43	Industrial x Drought tolerant
13	MEÇ1504.01	Alegria x Amora	Industrial x Drought tolerant

Table 3.1. “(Continue)” The features of the breeding population. The table indicates, code of the breeding population, Pedigree names, and the desired traits of the pedigree which is selected for

14	MEÇ1530.02	Atlantic x CIP43	Industrial x Drought tolerant
15	MEÇ1525.03	Pomqueen x CIP43	Industrial x Drought tolerant
16	MEÇ1525.02	Pomqueen x CIP43	Industrial x Drought tolerant
17	MEÇ1505.06	Amora x CIP43	Industrial x Drought tolerant
18	MEÇ1502.16	01.536 x Hermes	Drought tolerant x Industrial
19	MEÇ1502.15	01.536 x Hermes	Drought tolerant x Industrial
20	MEÇ1501.02	06.62 x Hermes	Drought tolerant x Industrial
21	MEÇ1525.17	Pomqueen x CIP43	Industrial x Drought tolerant
22	MEÇ1505.02	Amora x CIP43	Industrial x Drought tolerant
23	MEÇ1502.25	01.536 x Hermes	Industrial x Drought tolerant
24	MEÇ1502.04	01.536 x Hermes	Industrial x Drought tolerant
25	MEÇ1502.21	01.536 x Hermes	Industrial x Drought tolerant
26	MEÇ1668.05	Lady Olympia x CIP397044.25	High yielding x Drought tolerance
27	MEÇ1642.07	Demon x Bafana	Drought tolerance x High yielding
28	MEÇ1643.01	Demon x CIP397039.51	Drought tolerance x Drought tolerance
29	MEÇ1656.01	IWA-1 x 0704.08	Drought tolerance x High yielding
30	MEÇ1634.01	Chipke x Amora	Drought tolerance x High yielding
31	MEÇ1663.01	Lady Anna x CIP397044.25	High yielding x Drought tolerance
32	MEÇ1684.07	White Lady x CIP397069.11	High yielding x Drought tolerance
33	MEÇ1684.01	White Lady x CIP397069.11	High yielding x Drought tolerance
34	MEÇ1603.08	(Megusta x Lindita) x CIP397039.51	High yielding x Drought tolerance
35	MEÇ1651.02	Fasan x CIP397044.25	High yielding x Drought tolerance
36	MEÇ1651.06	Fasan x CIP397044.25	High yielding x Drought tolerance
37	MEÇ1651.03	Fasan x CIP397044.25	High yielding x Drought tolerance
38	MEÇ1651.08	Fasan x CIP397044.25	High yielding x Drought tolerance
39	MEÇ1651.07	Fasan x CIP397044.25	High yielding x Drought tolerance
40	MEÇ1617.01	06.62 x CIP397069.11	Drought tolerance x Drought tolerance
41	MEÇ1650.01	Fasan x CIP397039.51	High yielding x Drought tolerance
42	MEÇ1654.01	Grandeur x CIP397039.51	High yielding x Drought tolerance
43	MEÇ1654.03	Grandeur x CIP397039.51	High yielding x Drought tolerance
44	MEÇ1683.01	White Lady x CIP397044.25	High yielding x Drought tolerance
45	MEÇ1683.06	White Lady x CIP397044.25	High yielding x Drought tolerance
46	MEÇ1683.10	White Lady x CIP397044.25	High yielding x Drought tolerance
47	MEÇ1625.01	0703.10 x CIP397069.11	High yielding x Drought tolerance
48	MEÇ1615.04	06.62 x CIP397039.51	Drought tolerance x Drought tolerance

Table 3.1. “(Continue)” The features of the breeding population. The table indicates, code of the breeding population, Pedigree names, and the desired traits of the pedigree which is selected for

49	MEÇ1641.01	CIP397044.25 x Safari	Drought tolerance x High yielding
50	MEÇ1641.03	CIP397044.25 x Safari	Drought tolerance x High yielding
51	MEÇ1667.04	Lady Olympia x CIP397039.51	High yielding x Drought tolerance
52	MEÇ1616.02	06.62 x CIP397044.25	Drought tolerance x Drought tolerance
53	MEÇ1616.01	06.62 x CIP397044.25	Drought tolerance x Drought tolerance
54	MEÇ1638.01	CIP397044.25 x Bafana	Drought tolerance x High yielding
55	MEÇ1638.02	CIP397044.25 x Bafana	Drought tolerance x High yielding
56	MEÇ1638.03	CIP397044.25 x Bafana	Drought tolerance x High yielding
57	MEÇ1638.05	CIP397044.25 x Bafana	Drought tolerance x High yielding
58	MEÇ17084.01	Nautilus x GH113	High yielding x High yielding
59	MEÇ17080.01	Lady Olympia x Rumba	High yielding x High yielding
60	MEÇ17023.04	Bafana x Pomqueen	High yielding x High yielding
61	MEÇ17011.11	Agria x CIP397055.2	High yielding x Drought tolerance
62	MEÇ17023.01	Bafana x Pomqueen	High yielding x High yielding
63	MEÇ17078.06	Lady Olympia x Navigator	High yielding x High yielding
64	MEÇ17023.03	Bafana x Pomqueen	High yielding x High yielding
65	MEÇ17082.01	Nautilus x Arsenal	High yielding x High yielding
66	MEÇ17053.04	Compass x Alegria	High yielding x High yielding
67	MEÇ17011.03	Agria x CIP397055.2	High yielding x Drought tolerance
68	MEÇ17085.06	Nautilus x Lady Olympia	High yielding x High yielding
69	MEÇ17092.03	Navigator x Galata	High yielding x High yielding
70	MEÇ17011.15	Agria x CIP397055.2	High yielding x Drought tolerance
71	MEÇ17011.18	Agria x CIP397055.2	High yielding x Drought tolerance
72	MEÇ17078.03	Lady Olympia x Navigator	High yielding x Drought tolerance
73	MEÇ17075.08	Lady Olympia x Bafana	High yielding x High yielding
74	MEÇ17011.13	Agria x CIP397055.2	High yielding x Drought tolerance
75	MEÇ17076.?	Lady Olympia x GH113	High yielding x High yielding
76	MEÇ17080.14	Lady Olympia x Rumba	High yielding x High yielding
77	MEÇ17065.02	Hermes x Bafana	High yielding x High yielding
78	MEÇ17076.02	Lady Olympia x GH113	High yielding x High yielding
79	MEÇ17024.02	Bafana x Tessa	High yielding x High yielding
80	MEÇ17080.06	Lady Olympia x Rumba	High yielding x High yielding
81	MEÇ17011.04	Agria x CIP397055.2	High yielding x Drought tolerance
82	MEÇ17011.17	Agria x CIP397055.2	High yielding x Drought tolerance

Table 3.1. “(Continue)” The features of the breeding population. The table indicates, code of the breeding population, Pedigree names, and the desired traits of the pedigree which is selected for

83	MEÇ17023.14	Bafana x Pomqueen	High yielding x High yielding
84	MEÇ17011.01	Agria x CIP397055.2	High yielding x Drought tolerance
85	MEÇ17075.01	Lady Olympia x Bafana	High yielding x High yielding
86	MEÇ17013.03	Agria x Galata	High yielding x High yielding
87	MEÇ17009.01	Agria x Bafana	High yielding x High yielding
88	MEÇ17099.02	??	
89	MEÇ17023.02	Bafana x Pomqueen	High yielding x High yielding

3.2 Method

3.2.1 DNA extraction

DNA extraction was performed separately from each line to screen the breeding population. For this purpose, 2 or 3 tubers of each breeding line were kept in dark conditions at room temperature to break dormancy and initiate sprouting as shown in Figure 3.1. Then, 100-200 mg of the sprouts were used as tissue material for DNA extraction. DNA was extracted from each line by using the GeneJET Plant Genomic DNA Purification mini kit (Thermo Scientific) according to the supplier’s instructions with minor changes. The protocol is presented below.



Figure 3.1. Sprout formation on tubers for DNA extraction

DNA extraction protocol;

1. 100-200 mg of fresh tissue samples were ground in 100 μ l of Lysis Buffer A with the help of mortar and pestle.
2. Ground tissue samples were taken into a 1.5 mL microcentrifuge tube, 250 μ l Lysis Buffer A was added and samples were vortexed for 10-20 seconds.
3. A 50 μ l of Lysis Buffer B and 20 μ l of RNase A were added and mixed via vortex for 5 seconds.
4. Samples were incubated at 65 °C on a shaking dry water bath for 10 minutes.
5. A 130 μ l of Precipitation Solution was added and tubes were inverted 2-3 times to mix well.
6. Samples were incubated on ice for 5 minutes.
7. Samples were centrifuged for 8 minutes at 14,000 rpm at 4 °C
8. The supernatant was taken into a new 1.5 mL microcentrifuge tube and 400 μ L of Plant gDNA Binding Solution and 400 μ L of 96% ethanol were added and mixed well by vortexing.
9. Half of the prepared mixture (600-700 μ L) was transferred to the spin column and centrifuged for 1 minute at 8,000 rpm. The flow-through solution was discarded and the remaining mixture was transferred to the same column and the same step was followed.
10. Wash Buffer, I with ethanol was added to the column. Then, it was centrifuged for 3 minutes at 8,000 rpm, flow-through was discarded and the column was placed back into the same collection tube.
11. Wash Buffer II with ethanol was added to the same column and centrifuged for 8 minutes at 14,000 rpm and flow-through was discarded.
12. The column was placed in an empty collection tube, then it was centrifuged at 14,000 rpm for 3 minutes to clean ethanol residue. The collection tube was discarded and the column was placed into a new 1.5 mL microcentrifuge tube.
13. A 25 μ L of Elution Buffer was added onto the column to elute DNA. It was incubated for 15-20 minutes at room temperature and centrifuged for 5 minutes at 10,000 rpm.

To determine the quantity and quality of DNA samples, 2 μ L of eluted DNA was used for spectrophotometric measurement (BioSpec UV-vis Spectrophotometer,

SHIMADZU) and then 50 ng of each DNA samples were run on %1.8 agarose gel for 1 hour.

3.2.2 KASP genotyping assay

Each DNA sample was screened with 7 different KASP markers for identification of the genotypes of each line as AAAA, AAAB, AABB, ABBB, and BBBB by using a Real-Time PCR instrument (Rotor-Gene Q; QIAGEN). The KASP markers used in the study were developed by the project (115O406) funded by TUBITAK for the selection of high-yielding potato genotypes under drought conditions (Caliskan et al., 2019). List and associated traits of the KASP markers are presented in Table 3.2.

Table 3.2. KASP Markers to be used for screening drought resistant breeding lines. DT: Discarded Tuber; MT: Marketable Tuber; TY: Tuber Yield; NT: Number of Tuber; SPAD: Chlorophyll Index. C or D after the dash is non-stressed or drought conditions, respectively

KASP MARKER	TRAIT	SNP MARKER	FAM Allel	HEX Allel	GENE
KASP2-D	DT-C	solcap_snp_c2_41395	T	A	Triosephosphate isomerase
KASP5-D	DT-D	solcap_snp_c2_20714	A	G	Malate dehydrogenase
KASP7-D	TY-C	solcap_snp_c2_6108	T	C	Suppressor of ty
KASP8-D	NT-C	solcap_snp_c2_598	A	G	LOB domain-containing protein 40
KASP11-D	TY-D	solcap_snp_c2_3452	T	C	Phenylalanine ammonialyase
KASP14-D	NT-D TY-D	solcap_snp_c2_886	A	G	DDRGK domain-containing protein 1
KASP15-D	SPAD-C	solcap_snp_c2_36027	A	C	Cycloartenol synthase

PCR content and thermal cycle which were used for screening are shown in Table 3.3 and Table 3.4, respectively.

Table 3.3. PCR content for screening

Content	Volume (μ l)
DNA (5ng/ μ l)	5,00
2X KASP Master Mix	5,00
KASP Primer Mix (FAM allele, HEX allele)	0,14
Total Volume	10,14

Table 3.4. PCR cycles and reading conditions

	Incubation	Cycle
Enzyme activation	94 °C for 15 min	1
Amplification of target DNA	94 °C for 20 sec	10
	61-55 °C for 60 sec (dropping 0.6°C per cycle)	
Amplification of target DNA	94 °C for 20 sec	26
	55 °C for 60 sec	
Cooling down	35 °C for 2 min	1
Reading	35 °C for 1 min	1

A scatter chart for each KASP marker was done by Microsoft Excel software using the values generated by fluorescent signals from two allele-specific KASP markers. According to KASP assay results, breeding lines were grouped as high, mid, and low yielding under drought conditions.

The selection of candidate high-yielding breeding lines for drought conditions by using the KASP markers was performed according to the key in Table 3.5 Accordingly, a breeding line estimated as high-yielding by at least five KASP markers was selected as a candidate drought resistant. This criterion was pointed out as the most reliable one based on the results of the project (115O406) by Caliskan et al. (2019).

Table 3.5. Genotypes associated with high-yielding under drought for each KASP marker. *A: Adenine, T: Thymine, G: Guanine, C: Cytosine

KASP Marker	Genotype (alleles) Associated with High-Yielding under Drought	Gene
KASP2-D	TTAA	Triosephosphate isomerase
KASP5-D	GGGG AAGG	Malate dehydrogenase
KASP7-D	TCCC	Suppressor of ty
KASP8-D	AAGG	LOB domain-containing protein 40
KASP11-D	TCCC TTCC	Phenylalanine ammonia-lyase
KASP14-D	AGGG	DDRGK domain-containing protein 1
KASP15-D	ACCC AACC	Cycloartenol synthase

3.2.3 Field trial

The selected 14 breeding lines by KASP assay were planted at the Research Station of Faculty of Agricultural Sciences and Technologies, Nigde Omer Halisdemir University on 21 May 2020. Some climatic data and properties of the soil type of experimental area are presented in Table 3.6-8. Climate data according to the 2020 climate values of the trial area was taken from the records of the Faculty of Agricultural Sciences and Technologies Meteorology Station and the average of long years (1935-2019) climate data values were taken from the records of Niğde Meteorology Provincial Directorate. Some physical properties of the trial area soil were analysed at the International Agricultural Research and Training Center Directorate and Ankara University Faculty

of Agriculture Soil Science and Plant Nutrition Department Laboratories. Some physical and chemical properties of the trial area soils were analysed in Bereket Soil and Irrigation Water Analysis Laboratory. As seen on tables, the experimental area has a sandy-clay-silt soil type. Besides, it was not seen any alkalinity and salinity problem as well as limy soil content. The field experiment was carried out according to a split-plot experimental design with four replications under non-stressed and stressed (35% deficit irrigation) conditions. The main plots were consisted of two water levels as 100% and 65% irrigation, whereas breeding lines were sub-plots. After soil preparation, 67.5 kg/ha of Nitrogen (N), Phosphorus (P), and Potassium (K) was applied to the soil by 15-15-15 NPK compound fertilizer. Sub-plots were designed as two rows with 0.7 m distance, and 10 seed tubers were planted on each row with 0.30 m in-row distance for each breeding line. The field trial plan is presented in Figure 3.2. Standard agronomic practices except irrigation were applied throughout the growing period. Plants were exposed to two different irrigation regimes (full and 35% deficit). Stressed (DI) treatment started 35 days after emergence (DAE) and continued until harvest.

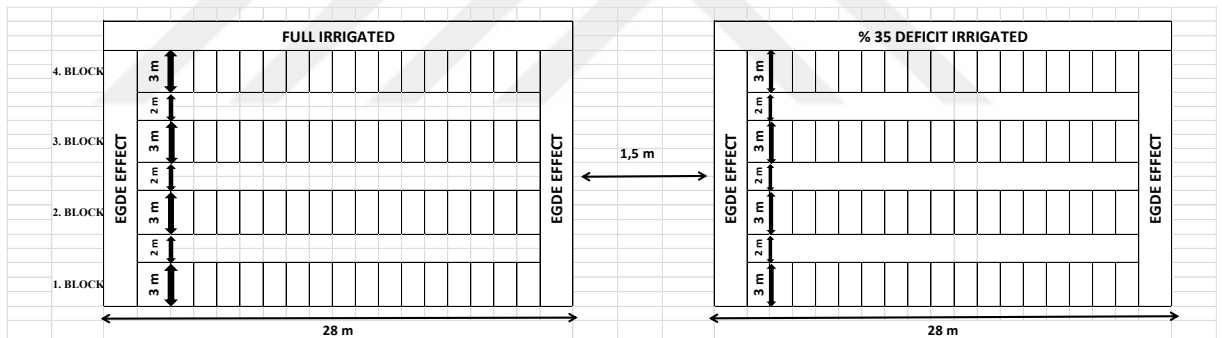


Figure 3.2. Field trial plan

A drip irrigation system was set up in the experimental fields and an irrigation meter was placed to irrigation system (Figure 3.3)



Figure 3.3. Drip irrigation system of the trial

Table 3.6. Climate data according to the 2020 climate values of the trial area and the average of long years (1935-2019). Max: Maximum, Min: Minimum, Temp: Temperature, R.H: Relative Humidity, L.Y.A: Long Years Average

Months	Max. Temp. (°C)		Min. Temp. (°C)		Mean Temp. (°C)		Mean R. H. (%)		Total rainfall (mm)	
	L.Y.A	2020	L.Y.A	2020	L.Y.A	2020	L.Y.A	2020	L.Y.A	2020
May	21.4	22.8	8.4	9.1	15.1	16.0	52.7	52.3	48.7	21.2
June	25.6	26.7	11.8	12.7	19.1	19.8	51.3	47.9	27.9	26.7
July	29.3	31.8	14.7	17.1	22.4	24.6	39.5	40,5	5.2	0.0
August	29.5	30.0	14.5	14.3	22.3	22.5	40.2	35,0	6.6	0.0
September	25.6	29.8	10.3	14.2	18.0	22.0	40.6	43,5	10.5	2.4
Mean	26.3	28.2	9.9	13.5	19.4	21.0	44.9	43.8	19.8	10.1

Table 3.7. Some physical properties of the trial area soil

Depth (cm)	Field Capacity (%)	Fading point (%)	Volume Weight (g/cm ³)	Ratio (%)		
				Clay	Silt	Sand
0-30	31.25	16.36	1.28	45.0	31.3	23.7
30-60	29.13	16.48	1.35	46.2	31.8	22.0

Table 3.8. Some physical and chemical properties of the trial area soils

% Saturation	Ph	Tuz (%)	CaCO ₃ (%)	Organic Matter (%)	K ₂ O (kg/da)	P ₂ O ₅ (kg/da)	N (%)	Micro Elements (ppm)
46.5	7.95	0.01	21.8	3.15	201.19	10.85	0.138	Zn: 2.15 Mn: 10.35 Fe: 3.25 Cu: 1.21 Mg: 582.1 Ca: 5572

Emergences of each genotype were controlled for every two days (Figure 3.5). Half of 14 breeding lines completed their emergences on 17-19 June 2020. That's why the emergence date was considered as 18 June 2020.



Figure 3.4. Field pictures were taken at the emergence of control plots

40 and 60 DAE, urea topdressing was applied twice as 138 kg/ha of pure N for each time. Subsequently, 20 DAE, 40 DAE, and 60 DAE, fungicide with 64% Mancozeb, and 8% Metalaxyl active ingredients were treated as 250 g/da for potato mildew

(*Phytophthora infestans*), Fungicide with 80% Mancozeb active ingredient as 200 g/100L for *Alternaria solani*. 250 g/da Iron chelate which contains 6% iron and 1L/da liquid organic fertilizer which contains 70% of organic matter was applied from the leaves. Moreover, 10 g/da insecticide containing Acetamiprid and Thiamethoxam active ingredients were applied from leaves for Colorado potato beetle periodically.

An equal amount of water was applied at five days intervals for both treatments until the beginning of stress treatment. After emergence of plants, tuberizations of breeding lines were controlled each two days (Figure 3.5). Water restriction treatment was started to apply 35 DAE when more than 50% percent of breeding lines initiated tuber formation. Soil samples were taken from 30 cm and 60 cm depth with the help of a soil drill each five days. The fresh weight of samples was measured and samples were dried in an oven at 105 °C for 24 hours. Then, the dry weight of the samples was measured. Field capacity was calculated according to the gravimetric method and thus irrigation amount necessary for adjusting field capacity was determined as mm³ for non-stressed treatment. Next, 65% water amount of non-stressed treatment was applied to the stressed treatment plants for 15 days before harvesting. All irrigations were applied at five days intervals. 750 mm³ water was given to the non-stressed group whereas 487 mm³ was given to the stressed group from the first day of stress application to 15 days before harvesting.



Figure 3.5. Pictures from the tuberization period

3.2.4 Chlorophyll index (SPAD)

The chlorophyll index value was measured on the youngest full-size leaves of plants (4-5th leaves from the top) with the help of a SPAD meter (Konica Minolta SPAD 502). SPAD measurements were done at 7 days intervals from the first day of drought application to the 35 days before harvesting time in both non-stressed and stressed applications. Three plants of a breeding line in each replication were measured and three measurements were recorded for each plant. Thus, 9 measurements were recorded for a breeding line in each replication.

3.2.5 Canopy temperature

Canopy temperature was measured on the youngest full-size leaves of plants (4-5th leaves from the top) with the help of an infrared thermometer (MASTECH BM380). Plant temperature measurements were done at 7 days intervals from the first day of drought application to the 35 days before harvesting time in both non-stressed and stressed applications. Measurements were done with 3 plants of a breeding line in each replication.

3.2.6 Harvesting

When plants reached harvesting maturity, each breeding line was harvested using a two-row harvesting machine (Figure 3.6). The number of tubers per plant, mean tuber weight (g), percentage (%) of unmarketable (<30 mm) and marketable (>30 mm) tubers, tuber yield per hectare (ton/ha), and marketable tuber yield per hectare (ton/ha) were determined after harvesting.



Figure 3.6. Harvesting of experimental plots

3.2.7 Specific gravity and dry matter content (%)

The specific gravity and dry matter content of tubers were determined using a digital potato hydrometer (PW-2050) weighing 1.5-2 kg of potato tubers in air and water.

3.2.8 Drought tolerance index (DTI)

Drought tolerance indexes of the breeding lines were calculated by Formula 3.1 (Cabello et al. 2013). Accordingly, multiplication of yield under drought conditions (YD) and normal conditions (YN) is divided by the square of mean yields of all genotypes under normal conditions.

$$\frac{YD*YN}{\bar{Y}N^2} \quad (3.1)$$

3.2.9 Drought susceptibility index (DSI)

Drought susceptibility indexes of genotypes were calculated by Formula 3.2 which is given below (Cabello et al 2013).

$$\frac{1-(YD/YN)}{[1-(1-\text{ort}YD/\text{ort}YN)]} \quad (3.2)$$

In all cases; YD,YN, \overline{YD} , \overline{YN} represents the yield of genotype under drought, yield of genotype under well-watered, mean yields of all genotypes under drought and mean yields of all genotypes under normal conditions, respectively.

3.2.10 Statistical analysis

The data were subjected to variance analysis according to the split-plot design by JMP statistical package program, and differences between treatments were compared with Tukey's posthoc tests ($p \leq 0.05$).

CHAPTER IV

RESULTS

4.1 DNA Extraction

The quantity and purity of DNA samples were determined by spectrophotometric measurement. The DNA samples were pure enough based on the A260/A280 ratio and A260/A230 which were in the range of 1.8-2.0 and 2.0-2.2 respectively (Table 4.1). The quality of DNA samples was checked via agarose gel electrophoresis. Next, 1,8% agarose gel was prepared and final concentrations of DNA samples were prepared as 50 ng and run on the gel for 60 min. As shown on the gel images (Figure 4.1-5), the DNA samples of each line were intact.

Table 4.1. Spectrophotometric measurement results of breeding lines

NO	BREEDING LINES	260/280	260/230	Conc. (ng/ul)
1	MEÇ1302.20	1.835	2.136	316.4
2	MEÇ1302.18	1.87	2.225	101.0
3	MEÇ1301.20	1.878	2.126	223
4	MEÇ1305.05	1.888	2.158	105.1
5	MEÇ1302.05	1.901	2.161	150.6
6	MEÇ1406.07	1.786	1.67	92.05
7	MEÇ1409.09	1.87	2.114	212.9
8	MEÇ1407.08	1.888	2.219	105.1
9	MEÇ1407.05	1.904	2.222	113.65
10	MEÇ1411.06	1.732	1.367	53.15
11	MEÇ1407.17	1.829	1.804	132.45
12	MEÇ1505.07	1.878	2.057	98.9
13	MEÇ1504.01	1.904	2.127	131.55
14	MEÇ1530.02	1.887	2.152	130.7
15	MEÇ1525.03	1.928	1.839	156.3
16	MEÇ1525.02	1.91	2.045	50.45
17	MEÇ1505.06	1.899	2.127	133.35
18	MEÇ1502.16	1.86	1.991	61.75
19	MEÇ1502.15	1.895	1.64	86.9
20	MEÇ1501.02	1.899	2.162	99.9
21	MEÇ1525.17	1.786	1.823	103.85
22	MEÇ1505.02	1.769	1.901	62.2
23	MEÇ1502.25	1.897	2.048	127.35

Table 4.1. “(Continue)” Spectrophotometric measurement results of breeding lines

24	MEÇ1502.04	1.86	1.955	87.7
25	MEÇ1502.21	1.906	2.181	176.15
26	MEÇ1668.05	1.799	1.753	64.85
27	MEÇ1642.07	1.845	2.061	437.25
28	MEÇ1643.01	1.952	1.857	191.45
29	MEÇ1656.01	1.922	2.021	258.7
30	MEÇ1634.01	1.864	1.766	121.35
31	MEÇ1663.01	1.89	2.115	110.4
32	MEÇ1684.07	1.862	1.893	187.6
33	MEÇ1684.01	1.854	2.08	56.95
34	MEÇ1603.08	1.858	1.969	207.75
35	MEÇ1651.02	1.874	2.068	308.9
36	MEÇ1651.06	1.936	1.609	225
37	MEÇ1651.03	1.88	1.999	181.85
38	MEÇ1651.08	1.92	2.298	170.05
39	MEÇ1651.07	1.912	2.403	66.65
40	MEÇ1617.01	1.904	2.242	238.35
41	MEÇ1650.01	1.908	2.134	232.25
42	MEÇ1654.01	1.856	2.032	364.9
43	MEÇ1654.03	1.842	1.418	96.05
44	MEÇ1683.01	1.863	1.823	73.4
45	MEÇ1683.06	1.877	2.105	208.8
46	MEÇ1683.10	1.894	2.193	179.35
47	MEÇ1625.01	1.892	2.146	207.3
48	MEÇ1615.04	1.879	1.919	214.35
49	MEÇ1641.01	1.894	2.112	115.15
50	MEÇ1641.03	1.887	2.117	203.6
51	MEÇ1667.04	1.865	1.965	170.5
52	MEÇ1616.02	1.879	2.16	86.35
53	MEÇ1616.01	1.89	2.162	323.75
54	MEÇ1638.01	1.923	1.94	128.9
55	MEÇ1638.02	1.876	1.981	217
56	MEÇ1638.03	1.891	2.16	269.45
57	MEÇ1638.05	1.914	2.177	77.8
58	MEÇ17084.01	1.892	2.161	130.65
59	MEÇ17080.01	1.904	2.098	166.25
60	MEÇ17023.04	1.898	2.048	132.3
61	MEÇ17011.11	1.881	1.968	227.3
62	MEÇ17023.01	1.889	2.002	127.2

Table 4.1. “(Continue)” Spectrophotometric measurement results of breeding lines

63	MEÇ17078.06	1.902	2.014	247.65
64	MEÇ17023.03	1.839	1.801	235.6
65	MEÇ17082.01	1.891	2.053	170.1
66	MEÇ17053.04	1.894	1.977	169.4
67	MEÇ17011.03	1.895	2.052	106.7
68	MEÇ17085.06	1.798	1.686	126.05
69	MEÇ17092.03	1.861	2.059	397.5
70	MEÇ17011.15	1.911	2.309	97.45
71	MEÇ17011.18	1.71	1.656	30.2
72	MEÇ17078.03	1.891	2.182	95.55
73	MEÇ17075.08	1.878	1.863	185.85
74	MEÇ17011.13	1.886	2.105	234.7
75	MEÇ17076.?	1.882	2.256	209.2
76	MEÇ17080.14	1.897	2.191	151.8
77	MEÇ17065.02	1.724	1.567	37.2
78	MEÇ17076.02	1.904	2.153	247.75
79	MEÇ17024.02	1.897	2.155	119.85
80	MEÇ17080.06	1.856	2.048	276.7
81	MEÇ17011.04	1.863	2.113	409.35
82	MEÇ17011.17	1.919	2.205	141.1
83	MEÇ17023.14	1.852	1.994	377.5
84	MEÇ17011.01	1.837	1.855	177
85	MEÇ17075.01	1.876	1.961	183.5
86	MEÇ17013.03	1.886	2.135	335.2
87	MEÇ17009.01	1.764	1.41	51.35
88	MEÇ17099.02	1.889	2.142	340.55
89	MEÇ17023.02	1.528	1.792	44.25

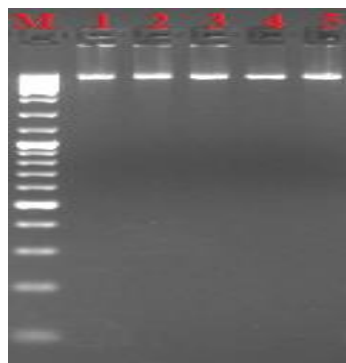


Figure 4.1. Gel image of MEÇ13 breeding lines

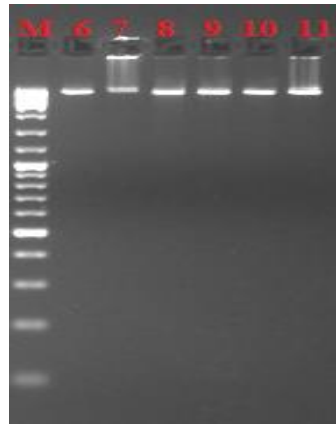


Figure 4.2. Gel image of and MEÇ14 breeding lines

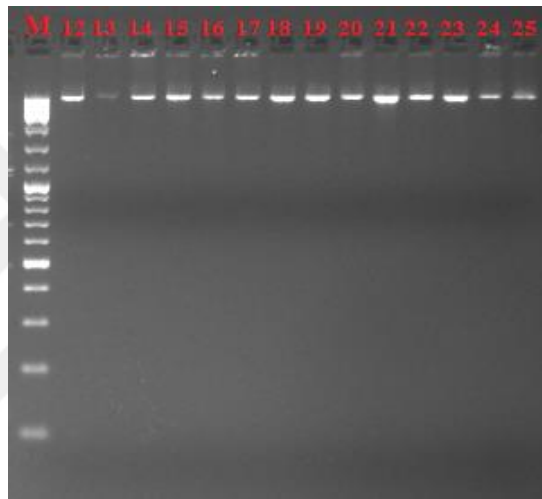


Figure 4.3. Gel image of MEÇ15 breeding lines

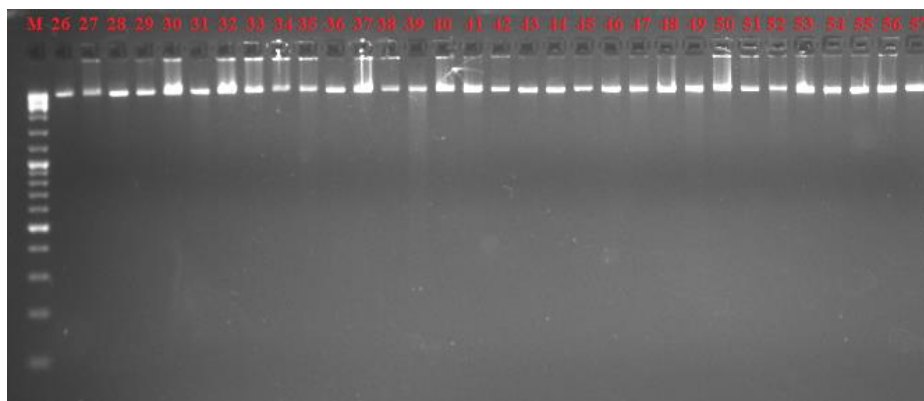


Figure 4.4. Gel image of MEÇ 16 breeding lines

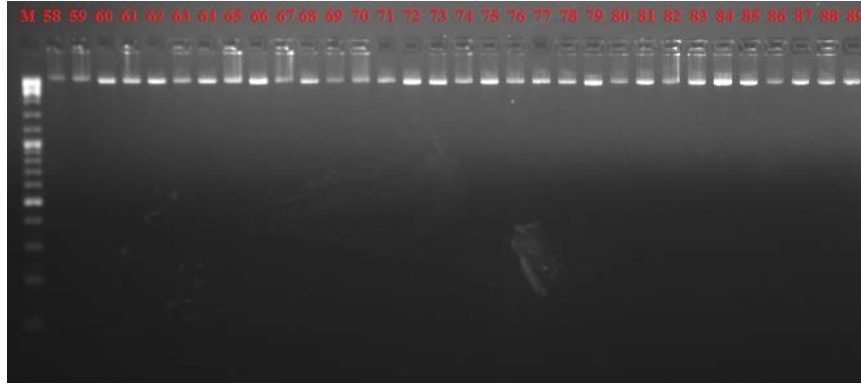


Figure 4.5. Gel image of MEÇ17 breeding lines

4.2 KASP Genotyping Assay

Previously developed 7 KASP markers for identification of high-yielding potatoes under drought were tested in a population which consists of 89 individuals. A two-step evaluation method was used for the selection of candidate high-yielding breeding lines for drought conditions. At the first step, a breeding line having at least one of the following alleles, TTAA for KASP2-D, AAGG for KASP4-D, GGGG and AAGG for KASP5-D, TCCC for KASP7-D, AAGG for KASP8-D, TCCC and TTCC for KASP11-D, AGGG for KASP14-D, ACCC and AACC for KASP15-D is considered as potentially high-yielding under drought (Table 3.5). For that, a scatter chart for each KASP marker was done by Microsoft Excel software using the values generated by fluorescent signals from two allele-specific KASP markers. The genotypes (alleles) which identify high-yielding breeding lines were written with red color (Figure 4.6-12).

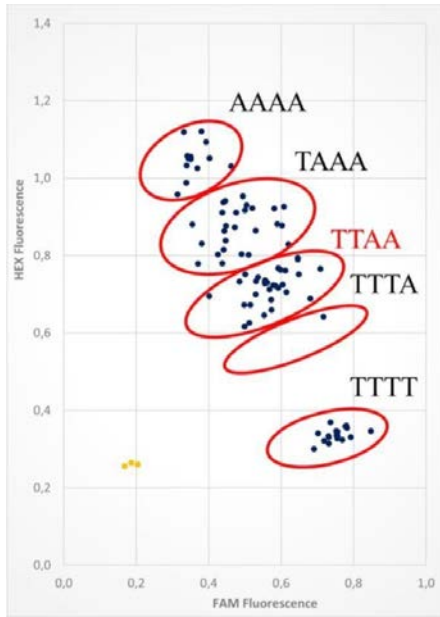


Figure 4.6. KASP2-D screening results

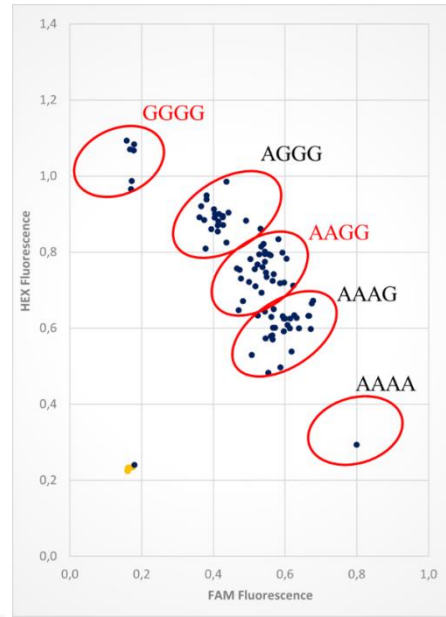


Figure 4.7. KASP5-D screening results

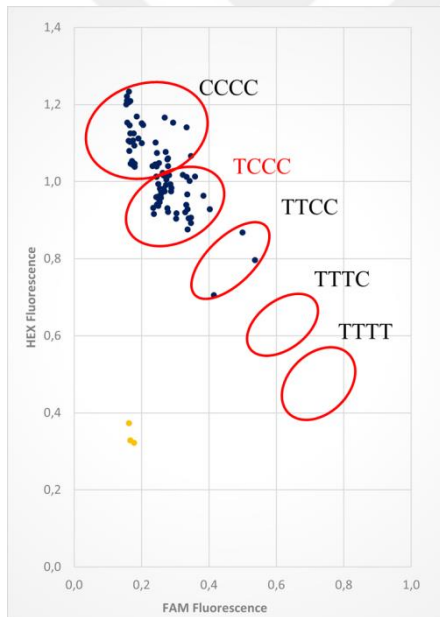


Figure 4.8. KASP7-D screening results

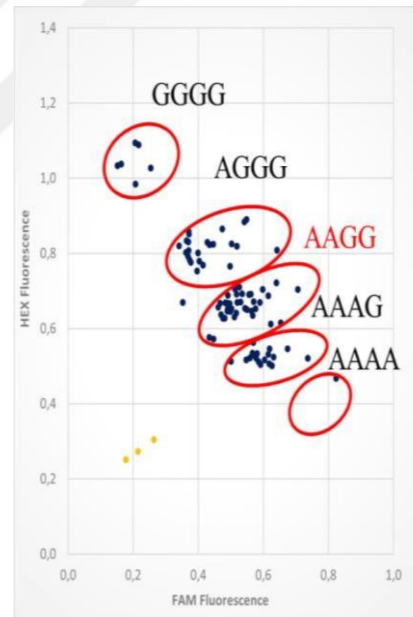


Figure 4.9. KASP8-D screening results

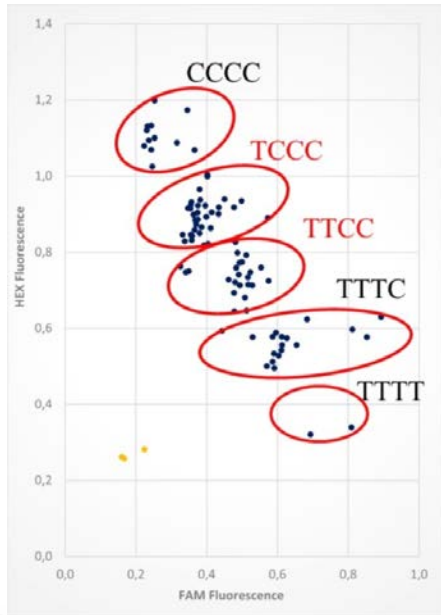


Figure 4.10. KASP11-D screening results

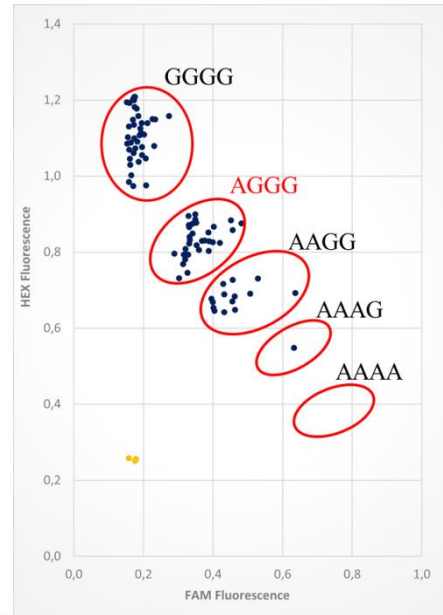


Figure 4.11. KASP14-D screening results

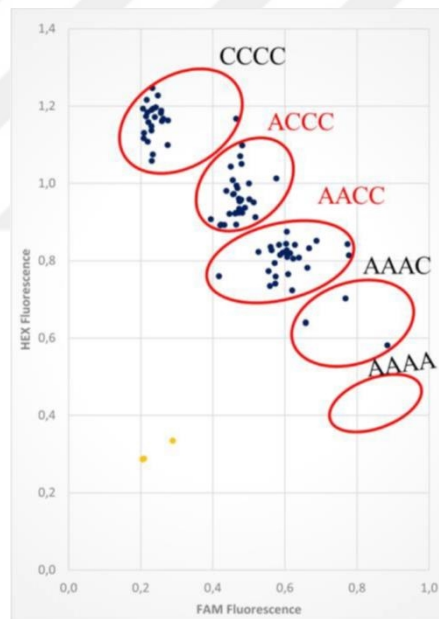


Figure 4.12. KASP15-D screening results

As a result of the first evaluation step, the number of breeding lines that were considered as potentially high-yielding under drought was as follows, 32 genotypes according to screening by KASP2-D, 37 genotypes according to screening by KASP5-D, 43 genotypes according to screening by KASP7-D, 39 genotypes according to screening by KASP8-D, 56 genotypes according to screening by KASP11-D, 34

genotypes according to screening by KASP14-D and 59 genotypes according to screening by KASP15-D.

Caliskan et al. (2020) pointed out using the combined results of five or more markers together is more reliable. Accordingly, a breeding line estimated as potentially high-yielding by at least five KASP markers was selected as a candidate drought resistant line at the second step of evaluation. Therefore, 14 potato breeding lines were selected as high-yielding by KASP marker-assisted selection approach. The list of selected breeding lines is presented in Table 4.2. The breeding lines selected by which KASP markers are presented in Table 4.3. The accuracy of the markers was tested by the evaluation of yield performances of the selected breeding lines in both stressed and non-stressed conditions.

Table 4.2. The selected breeding lines after KASP assay screening

No	GENOTYPE	No	GENOTYPE
1	MEÇ1651.03	9	MEÇ1525.03
2	MEÇ17023.14	10	MEÇ1684.01
3	MEÇ17082.01	11	MEÇ17011.04
4	MEÇ1650.01	12	MEÇ1638.05
5	MEÇ1651.07	13	MEÇ1654.03
6	MEÇ1668.05	14	MEÇ1656.01
7	MEÇ1638.03	15	AGRIA
8	MEÇ17085.06	16	RBB

Table 4.3. The selected breeding lines by which KASP markers

KASP2-D	KASP5-D	KASP7-D	KASP8-D	KASP11-D	KASP14-D	KASP-15D
MEÇ1668.05			MEÇ1668.05	MEÇ1668.05	MEÇ1668.05	MEÇ1668.05
MEÇ1650.01	MEÇ1650.01	MEÇ1650.01	MEÇ1650.01	MEÇ1650.01	MEÇ1650.01	MEÇ1650.01
MEÇ1651.03			MEÇ1651.03	MEÇ1651.03	MEÇ1651.03	MEÇ1651.03
MEÇ1651.07	MEÇ1651.07	MEÇ1651.07	MEÇ1651.07	MEÇ1651.07	MEÇ1651.07	MEÇ1651.07
MEÇ1684.01		MEÇ1684.01	MEÇ1684.01	MEÇ1684.01	MEÇ1684.01	MEÇ1684.01
	MEÇ1656.01	MEÇ1656.01	MEÇ1656.01	MEÇ1656.01	MEÇ1656.01	MEÇ1656.01
MEÇ17011.04	MEÇ17011.04	MEÇ17011.04		MEÇ17011.04		MEÇ17011.04
	MEÇ1638.03	MEÇ1638.03	MEÇ1638.03	MEÇ1638.03		MEÇ1638.03
MEÇ17082.01	MEÇ17082.01	MEÇ17082.01		MEÇ17082.01	MEÇ17082.01	
MEÇ17023.14	MEÇ17023.14	MEÇ17023.14		MEÇ17023.14		MEÇ17023.14
MEÇ1654.03			MEÇ1654.03	MEÇ1654.03	MEÇ1654.03	MEÇ1654.03
MEÇ1638.05	MEÇ1638.05			MEÇ1638.05	MEÇ1638.05	MEÇ1638.05
MEÇ1525.03	MEÇ1525.03	MEÇ1525.03	MEÇ1525.03	MEÇ1525.03		MEÇ1525.03
MEÇ17085.06		MEÇ17085.06	MEÇ17085.06		MEÇ17085.06	MEÇ17085.06

4.3 Chlorophyll Index (SPAD)

The alteration in chlorophyll index of non-stressed and stressed groups on the first day of stress application (Day-0) and the 30th day of deficit water application (Day-30) is presented in Figure 4.14. During the water stress application, it was observed that the stress application group had darker green leaf color when compared with the non-stressed group. On Day-0, the mean SPAD value of the non-stressed and stressed group was almost the same for all genotypes. On Day-30, the mean SPAD value of the non-stressed group was around 17% less comparing to Day-0, while the stress application group was almost the same. The SPAD value of all breeding lines and cultivars was higher on the Day-30 under stress compared with non-stressed groups, and the increases in SPAD value were as followed MEÇ17023.14 23.2% MEÇ1656.01 22.5%, AGRIA 28.9%, MEÇ1651.03 15.44%, MEÇ1525.03 4.3%, RBB 23.4%, MEÇ1638.03 16.5%, MEÇ1651.07 11. %2, MEÇ17011.04 22.5%, MEÇ17085.06 34.1%, MEÇ1650.01 13.1%, MEÇ1668.05 37.80%, MEÇ17082.01 12.0%, MEÇ1638.05 25.7%, MEÇ1684.01 27%, MEÇ1654.03 14. 6%.

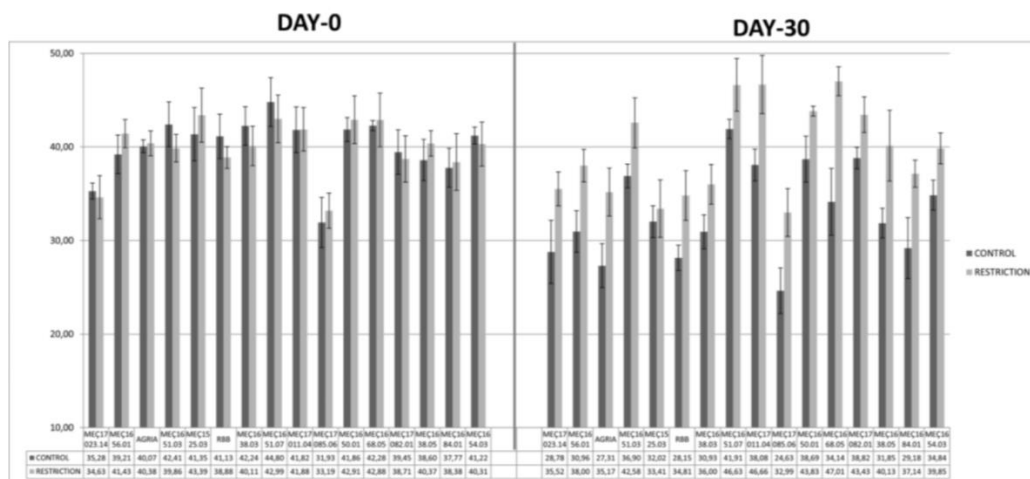


Figure 4.14. Alteration of SPAD values on Day-0 and Day-30

4.4 Canopy Temperature

The alteration in canopy temperature of non-stressed and stressed groups at Day-0 and Day-30 is presented in Figure 4.15. On Day-0, the mean temperature value of the non-stressed and stressed group was almost the same for all genotypes. On Day-30, the mean temperature value of the stressed group was around 28% higher compared with non-stressed groups. Besides, the average temperature value of the non-stressed group was almost the same on Day-0 and Day-30. The average temperature of the stress group increased by around 22% from Day-0 to Day-30. The temperature values of the breeding lines and cultivars grown under stressed conditions were higher on Day-30 compared to non-stressed conditions, and the increase was as followed MEÇ17023.14 20.8%, MEÇ1656.01 26.8%, AGRIA 24.7%, MEÇ1651.03 34.7%, MEÇ1525.03 29%, RBB 21%, MEÇ1638.03 29%, MEÇ1651.07 24%, MEÇ17011.04 26%, MEÇ17085.06 30.8%, MEÇ1650.01 29.5%, MEÇ1668.05 25.1%, MEÇ17082.01 34.4%, MEÇ1638.05 32.8%, MEÇ1684.01 30.2%, MEÇ1654.03 33.7%.

4.5.1 Number of tubers per plant

After harvesting, the number of tubers per plant (total tuber number/total plant number) was determined for each genotype (Figure 4.16). Variance analysis results of the number of tubers per plant are presented in Table 4.5. Differences in the number of tubers per plant values were significant ($p < 0.05$) in terms of Genotype, Water Treatment, and Genotype \times Water Treatment. Besides, the values of genotypes were grouped with Tukey post-hoc test. (Table 4.6). The results of the number of tubers per plant under non-stressed and stressed conditions are presented on a graph (Figure 4.16).

Table 4.5. Variance analysis of the number of tubers per plant

Source	SS	MS	DF	F Ratio
REPLICATE	1.96	0.65	3.00	1.06
GENOTYPE	154.58	10.31	15.00	6.7751**
W.TREATMENT	86.77	86.77	1.00	56.3456**
REPLICATE*GENOTYPE	27.68	0.62	45.00	0.97
W.TREATMENT*GENOTYPE	23.10	1.54	15.00	2.4286*

** $p \leq 0,01$, * $p \leq 0,05$

Table 4.6. Tukey test analysis results for number of tubers per plant under w.treatment \times genotype interaction

Treatment	Breeding Line	Group	Mean
Non-stressed	MEÇ1651.03	A	9.00
Non-stressed	MEÇ1650.01	A	8.23
Non-stressed	MEÇ1684.01	A	7.66
Non-stressed	MEÇ1656.01	A	7.41
Non-stressed	MEÇ1668.05	A	7.35
Non-stressed	MEÇ1638.05	A	7.16
Water Restriction	MEÇ1651.03	A	6.89
Non-stressed	MEÇ1525.03	B	6.70
Water Restriction	MEÇ1668.05	B	6.62
Water Restriction	MEÇ1656.01	B	6.57
Non-stressed	MEÇ1651.07	B	6.44
Non-stressed	MEÇ17011.04	B	5.99

Non-stressed	MEÇ17082.01	C	5.77
Non-stressed	MEÇ17085.06	C	5.76
Water Restriction	MEÇ1650.01	C	5.67
Non-stressed	AGRIA	C	5.44
Water Restriction	MEÇ1684.01	C	5.43
Non-stressed	MEÇ1654.03	D	5.31
Non-stressed	RBB	D	5.22
Non-stressed	MEÇ17023.14	E	5.07
Water Restriction	MEÇ17011.04	F	4.72
Water Restriction	MEÇ1525.03	F	4.72
Water Restriction	RBB	G	4.41
Water Restriction	MEÇ1654.03	H	4.28
Water Restriction	MEÇ17082.01	H	4.26
Non-stressed	MEÇ1638.03	H	4.20
Water Restriction	MEÇ1651.07	I	4.07
Water Restriction	MEÇ17023.14	I	4.05
Water Restriction	AGRIA	I	3.90
Water Restriction	MEÇ1638.03	I	3.81
Water Restriction	MEÇ17085.06	J	3.60
Water Restriction	MEÇ1638.05	K	3.37

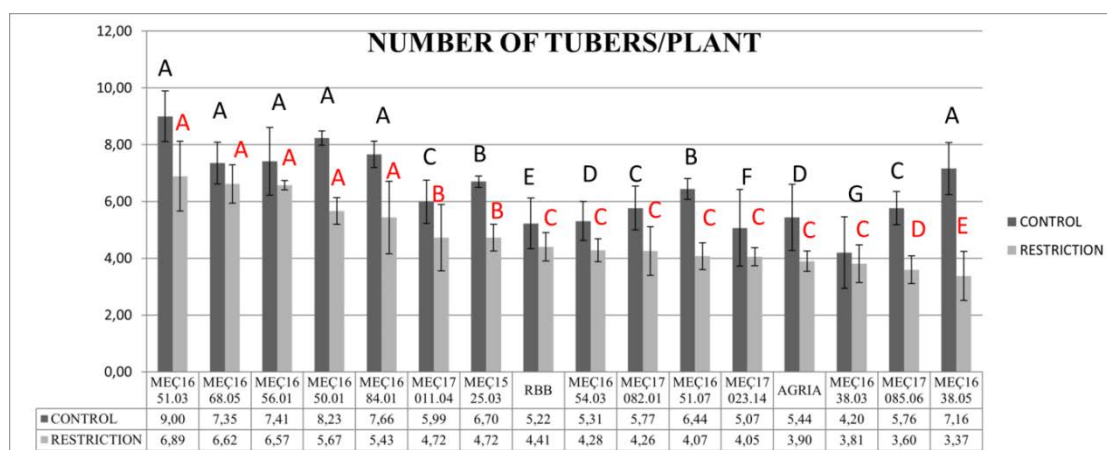


Figure 4.16. Alteration in the number of tubers per plant between non-stressed and stressed groups. The black letters indicate the groups of the breeding line under non-stressed and the red letters indicates the groups of the breeding line stressed conditions

The number of tubers per plant reduced for each breeding line and cultivar in stressed groups. The number of tubers per plant were in the range of between 9 and 4 under non-stressed conditions whereas, 6.8 and 3.7 under deficit irrigated conditions. The 11 out of 14 breeding lines that were selected by KASP markers had higher number of tubers than standard cultivars under non-stressed conditions while 7 of them had a higher value than RBB and 11 of them had a higher value than Agria. The breeding lines showed the highest value under non-stressed conditions as follow; MEÇ1651.03 (9.0), MEÇ1650.01 (8.0), MEÇ1684.01 (7.2), MEÇ1668.05 (7.0), MEÇ1656.01 (6.7), MEÇ1638.05 (6.5) whereas, MEÇ1651.03 (6.2), MEÇ1656.01 (6.0), MEÇ1668.05 (6.0), MEÇ1650.01 (5.5), MEÇ1684.01 (5.2) had the highest values under stressed conditions.

The average of number of tubers per plant in non-stressed group was found 6.4 unlike the stress group with 4.8. MEÇ1651.03, MEÇ1668.05, and MEÇ1656.01 breeding lines showed a decrease of 24%, 10%, and 13%, respectively, under stressed conditions. Besides, the MEÇ1638.05 genotype exhibited the highest loss (53%) in terms of the number of tubers per plant under stressed conditions.

4.5.2 Mean tuber weight (g/tuber)

Variance analysis results of mean tuber weight are presented in Table 4.7. As seen on the table, mean tuber weight values were statistically significant ($p < 0.05$) in terms of Genotype, Water Treatment, and Genotype×Water Treatment. Besides, the values of genotypes were grouped with Tukey post-hoc test (Table 4.8). The results of mean tuber weight under non-stressed and stressed conditions are presented on a graph (Figure 4.17).

Table 4.7. Variance analysis of mean tuber weight

Source	SS	MS	DF	F Ratio
REPLICATE	9.21	3.07	3.00	0.04
GENOTYPE	32936.50	2195.77	15.00	5.1619**
W.TREATMENT	13694.20	13694.20	1.00	36.3748**
REPLICATE*GENOTYPE	3564.97	79.22	45.00	2.61
W.TREATMENT*GENOTYPE	5647.14	376.48	15.00	12.4185**

** $p \leq 0.01$, * $p \leq 0.05$

Table 4.8. Tukey test analysis results for mean tuber weight under w.treatment×genotype interaction

Treatment	Breeding Lines	Group	Mean
Non-stressed	MEÇ1668.05	A	141.75
Non-stressed	MEÇ1651.07	A	127.47
Non-stressed	MEÇ17023.14	B	122.96
Non-stressed	MEÇ1638.03	B	117.93
Non-stressed	MEÇ1654.03	B	114.94
Non-stressed	MEÇ1656.01	B	114.06
Water Restriction	MEÇ1638.03	B	112.27
Non-stressed	MEÇ1650.01	C	108.12
Non-stressed	MEÇ17082.01	D	104.11
Water Restriction	MEÇ1656.01	D	103.25
Water Restriction	MEÇ1651.07	E	101.58
Non-stressed	MEÇ1651.03	E	101.22
Non-stressed	MEÇ1525.03	E	99.70
Non-stressed	MEÇ17011.04	F	95.24
Water Restriction	MEÇ17023.14	F	94.66
Water Restriction	MEÇ1650.01	F	94.64
Water Restriction	MEÇ1651.03	F	93.52
Non-stressed	MEÇ1684.01	G	89.57
Water Restriction	MEÇ17011.04	H	87.94
Non-stressed	MEÇ1638.05	H	87.66
Water Restriction	MEÇ1668.05	I	87.18
Water Restriction	MEÇ17082.01	I	86.11
Non-stressed	RBB	J	83.94
Water Restriction	MEÇ1638.05	J	82.65
Water Restriction	MEÇ1654.03	K	79.24
Non-stressed	AGRIA	L	78.80
Non-stressed	MEÇ17085.06	L	78.26
Water Restriction	MEÇ1525.03	M	76.51
Water Restriction	RBB	N	65.84
Water Restriction	MEÇ17085.06	N	64.06
Water Restriction	AGRIA	O	54.27
Water Restriction	MEÇ1684.01	O	51.03

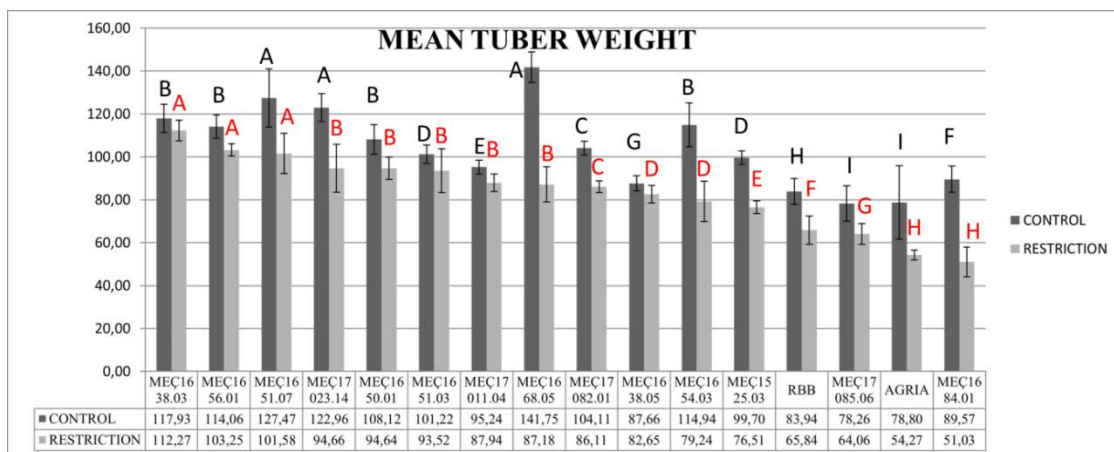


Figure 4.17. Alteration of mean tuber weight (g) between non-stressed and stress groups. The black letters indicate the groups of the breeding line under non-stressed and the red letters indicates the groups of the breeding line under stressed conditions

Although MEÇ1668.05 had the highest value, under non-stressed conditions in terms of mean tuber weight, it has shown a severe reduction under stressed conditions as 38.5%. MEÇ1638.03 genotype showed the highest value under stress conditions. Besides, it had a near value in the non-stressed group and didn't show a considerable reduction in stress groups.

Mean tuber weight values were in the range of between 141.75 g and 78.26 g under non-stressed conditions whereas 112.27 g and 51.03 g under stressed conditions. All breeding lines which were selected by KASP markers, except MEÇ17085.06, had higher mean tuber weight than standard cultivars under non-stressed conditions while 12 of them had a higher value than RBB and Agria, MEÇ17085.06 had a higher value than only Agria and MEÇ1684.01 had the lowest value under deficit irrigated conditions. The breeding lines which had the highest value under non-stressed conditions as follow; MEÇ1668.05 (141.25 g), MEÇ1651.07 (126.75 g), and MEÇ17023.14 (122.5 g) whereas, MEÇ1638.03 (111.75g), MEÇ1656.01 (102.75g), MEÇ1651.07 (101.0 g) had the highest values under stressed conditions.

4.5.3 Marketable tuber ratio (%)

After harvesting, tubers were grouped according to their size, shape, and forms. The tubers which were bigger than 30 mm and oval were evaluated as a marketable tuber. The tubers which have smaller than 30 mm size, rotted, and malformed ones were

evaluated as unmarketable. Accordingly, the marketable tuber ratio (%) of genotypes under non-stressed and stress conditions were presented on a graphic (Figure 4.18). Besides, variance analysis of this trait was presented in Table 4.9. As seen on the table, marketable tuber ratios were found statistically significant ($p < 0.05$) in terms of Water Treatment and Genotype*Water Treatment but not significant for Genotype. Also, the values of genotypes were grouped with Tukey post-hoc (Table 4.10).

Table 4.9. Variance analysis table of marketable tuber ratio (%)

Source	SS	MS	DF	F Ratio
REPLICATE	8.843	2.948	3.000	0.160
GENOTYPE	2070.860	138.057	15.000	2.505
W.TREATMENT	3852.660	3852.660	1.000	66.4148**
REPLICATE*GENOTYPE	827.761	18.395	45.000	0.864
W.TREATMENT*GENOTYPE	870.136	58.009	15.000	2.7245**

** $p \leq 0.01$, * $p \leq 0.05$

Table 4.10. Tukey test analysis results of marketable tuber ratio (%) for w.treatment \times genotype interaction

Treatment	Level	Group	Mean
Non-stressed	MEÇ1651.07	A	98.29
Non-stressed	MEÇ1654.03	A	98.17
Non-stressed	RBB	A	97.41
Non-stressed	MEÇ1638.03	A	96.80
Non-stressed	MEÇ1651.03	A	96.01
Non-stressed	AGRIA	A	95.23
Non-stressed	MEÇ1684.01	A	94.47
Non-stressed	MEÇ1668.05	A	93.94
Non-stressed	MEÇ1656.01	A	92.92
Water Restriction	MEÇ1638.03	A	92.68
Non-stressed	MEÇ1650.01	A	91.15
Non-stressed	MEÇ17082.01	A	90.92
Non-stressed	MEÇ17011.04	A	90.65
Non-stressed	MEÇ1638.05	A	90.15
Water Restriction	MEÇ1651.03	A	89.79
Non-stressed	MEÇ17023.14	A	89.65
Water Restriction	MEÇ1656.01	A	87.31
Water Restriction	MEÇ1668.05	A	86.65
Water Restriction	MEÇ1651.07	A	86.43
Non-stressed	MEÇ1525.03	A	86.22

Non-stressed	MEÇ17085.06	A	86.16
Water Restriction	MEÇ1650.01	B	84.90
Water Restriction	MEÇ1684.01	C	84.27
Water Restriction	MEÇ1654.03	D	82.62
Water Restriction	MEÇ17011.04	E	81.27
Water Restriction	MEÇ17023.14	F	79.82
Water Restriction	MEÇ1638.05	G	79.51
Water Restriction	MEÇ17085.06	H	77.97
Water Restriction	RBB	I	76.73
Water Restriction	MEÇ17082.01	J	75.67
Water Restriction	MEÇ1525.03	J	75.13
Water Restriction	AGRIA	K	71.83

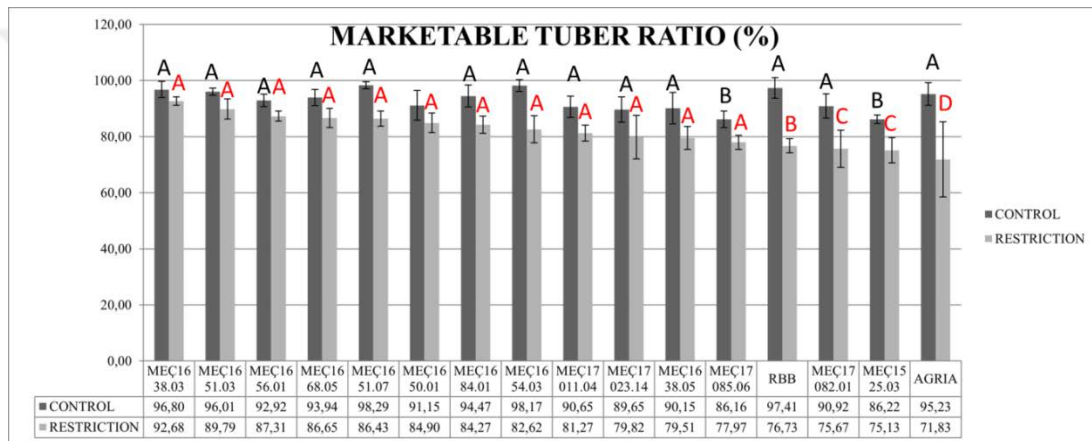


Figure 4.18. Marketable tuber ratio under non-stressed and stressed conditions. The black letters indicate the groups of the breeding line under non-stressed and the red letters indicates the groups of the breeding line under water restriction conditions

Marketable tuber ratio values were in the range of between 98.28% and 86.16% under non-stressed conditions whereas 92.68% and 71.83% under stressed conditions. Only 2 out of 14 breeding lines which were selected by KASP markers, MEÇ1651.07 and MEÇ1654.03 had a higher ratio than both standard cultivars, and MEÇ1638.03 and MEÇ1651.03 had a higher ratio than Agria under non-stressed conditions while 12 of them higher ratio than RBB and Agria under stressed conditions. Besides, MEÇ17082.01 and MEÇ1525.03 had a higher ratio than Agria and a lower ratio than RBB under stressed conditions. All breeding lines and cultivars were in group A under non-stressed conditions, except MEÇ17085.06 and MEÇ1525.03. Apart from MEÇ17082.01 and MEÇ1525.03, all breeding lines were in group A under stressed conditions.

4.5.4 Discarded tuber ratio (%)

After harvesting, tubers were grouped according to their size, shape, and forms. The tubers which were bigger than 30 mm and oval in shape were evaluated as a marketable tuber. The tubers which have smaller than 30 mm size, rotted, and malformed ones are evaluated as discarded tubers. Accordingly, the discarded tuber ratio (%) of genotypes under non-stressed and stressed conditions were presented on a graphic (Figure 4.18). Also, variance analysis of this trait was presented in Table 4.11. As seen on the table, discarded tuber ratios were statistically significant ($p < 0.05$) in terms of Water Treatment and Genotype×Water Treatment but not significant for Genotype. Also, the values of genotypes were grouped with Tukey post-hoc test (Table 4.12).

Table 4.11. Variance analysis table of discarded tuber ratio (%)

Source	SS	MS	DF	F Ratio
REPLICATE	8.84	2.95	3.00	0.16
GENOTYPE	2070.86	138.06	15.00	2.51
W.TREATMENT	3852.66	3852.66	1.00	66.4148**
REPLICATE*GENOTYPE	827.76	18.39	45.00	0.86
W.TREATMENT*GENOTYPE	870.14	58.01	15.00	2.7245**

** $p \leq 0,01$, * $p \leq 0,05$

Table 4.12. Tukey test analysis results of discarded tuber ratio (%) for w.treatment×genotype interaction

Treatment	Breeding Line	Group	Mean
Water Restriction	AGRIA	A	28.17
Water Restriction	MEÇ1525.03	A	24.87
Water Restriction	MEÇ17082.01	A	24.33
Water Restriction	RBB	A	23.27
Water Restriction	MEÇ17085.06	A	22.03
Water Restriction	MEÇ1638.05	A	20.49
Water Restriction	MEÇ17023.14	A	20.18
Water Restriction	MEÇ17011.04	A	18.73
Water Restriction	MEÇ1654.03	A	17.38
Water Restriction	MEÇ1684.01	A	15.73
Water Restriction	MEÇ1650.01	A	15.10
Non-stressed	MEÇ17085.06	B	13.84
Non-stressed	MEÇ1525.03	B	13.78
Water Restriction	MEÇ1651.07	B	13.57

Water Restriction	MEÇ1668.05	B	13.35
Water Restriction	MEÇ1656.01	B	12.69
Non-stressed	MEÇ17023.14	C	10.35
Water Restriction	MEÇ1651.03	C	10.21
Non-stressed	MEÇ1638.05	D	9.85
Non-stressed	MEÇ17011.04	D	9.35
Non-stressed	MEÇ17082.01	D	9.08
Non-stressed	MEÇ1650.01	E	8.85
Water Restriction	MEÇ1638.03	F	7.32
Non-stressed	MEÇ1656.01	F	7.08
Non-stressed	MEÇ1668.05	G	6.06
Non-stressed	MEÇ1684.01	H	5.53
Non-stressed	AGRIA	H	4.77
Non-stressed	MEÇ1651.03	I	3.99
Non-stressed	MEÇ1638.03	I	3.20
Non-stressed	RBB	J	2.59
Non-stressed	MEÇ1654.03	K	1.83
Non-stressed	MEÇ1651.07	K	1.71

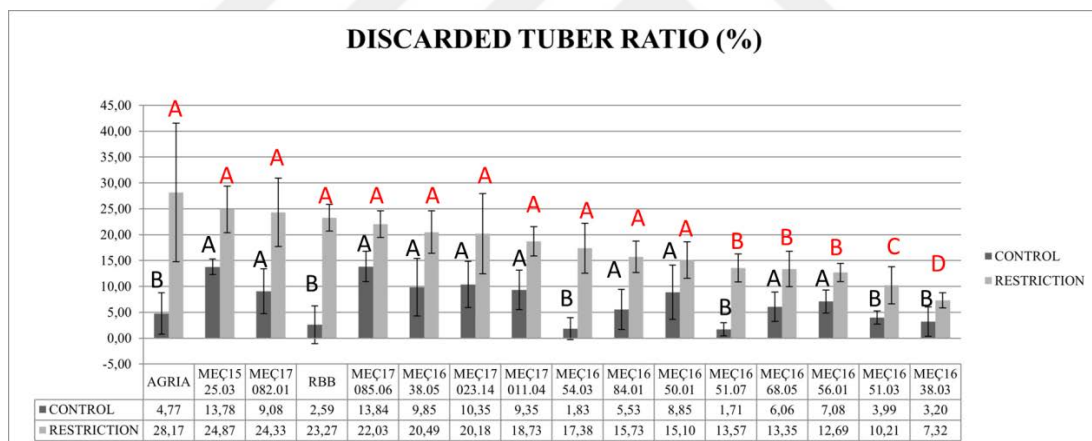


Figure 4.19. Discarded tuber ratio under control and stress conditions. The black letters indicates the groups of breeding line under non-stressed and the red letters indicates the groups of breeding line under water restriction conditions

Discarded tuber ratio values were in the range of between 13.83% and 1.71% under non-stressed conditions whereas 22.03% and 13.56% under stressed conditions. 10 out of 14 breeding lines which are selected by KASP marker have higher ratio than both standard cultivars. MEÇ1638.03 and MEÇ1651.03 has higher ratio than RBB but lower than Agria under non-stressed conditions while 10 of them have lower ratio than RBB and Agria under stressed conditions. Besides, MEÇ17082.01 and MEÇ1525.03 have

higher ratio than RBB and higher ratio than Agria under stressed conditions.

4.5.5 Total yield (t/ha)

Variance analysis of the total yield (t/ha) of genotypes under non-stressed and stressed conditions were presented on Table 4.13. As seen on table, total yield (h/da) values were statistically significant ($p < 0.05$) in terms of Genotype, Water Treatment and Genotype×Water Treatment. Also, the values of genotypes were grouped with Tukey post-hoc test (Table 4.14). Alteration of total yield values between non-stressed and stress groups were presented on a graph (Figure 4.20).

Table 4.13. Variance analysis table of total yield (t/ha)

Source	SS	MS	DF	F Ratio
REPLICATE	28.79	9.60	3.00	0.63
GENOTYPE	9088.58	605.91	15.00	18.069**
W.TREATMENT	3258.43	3258.43	1.00	86.8009**
REPLICATE*GENOTYPE	682.88	15.18	45.00	0.79
W.TREATMENT*GENOTYPE	563.09	37.54	15.00	1.9571*

** $p \leq 0.01$, * $p \leq 0.05$

Table 4.14. Tukey test analysis results of total yield (t/ha) for w.treatment*genotype interaction

Treatment	Level	Group	Mean
Non-stressed	MEC1668.05	A	51.80
Non-stressed	MEC1651.03	A	44.20
Non-stressed	MEC1650.01	A	41.27
Non-stressed	MEC1651.07	B	38.88
Non-stressed	MEC1656.01	B	36.36
Non-stressed	MEC1638.05	B	36.33
Water Restriction	MEC1668.05	B	36.31
Water Restriction	MEC1650.01	B	32.40
Non-stressed	MEC1684.01	B	32.13
Water Restriction	MEC1651.03	C	30.26
Non-stressed	MEC17082.01	D	28.55
Non-stressed	MEC17011.04	D	27.19
Non-stressed	MEC17023.14	D	26.93
Non-stressed	MEC1638.03	E	25.48
Water Restriction	MEC1651.07	E	24.82

Water Restriction	MEC1684.01	F	23.23
Water Restriction	MEC1656.01	F	22.95
Non-stressed	MEC1654.03	F	22.32
Non-stressed	MEC1525.03	F	21.53
Water Restriction	MEC17011.04	F	20.57
Non-stressed	RBB	F	20.20
Non-stressed	MEC17085.06	F	19.95
Water Restriction	MEC1638.03	G	19.50
Water Restriction	MEC17082.01	G	19.32
Water Restriction	MEC17023.14	G	18.14
Water Restriction	MEC1654.03	H	15.94
Non-stressed	AGRIA	H	15.60
Water Restriction	MEC1638.05	H	15.29
Water Restriction	RBB	H	15.07
Water Restriction	MEC1525.03	I	13.34
Water Restriction	MEC17085.06	J	11.01
Water Restriction	AGRIA	K	9.15

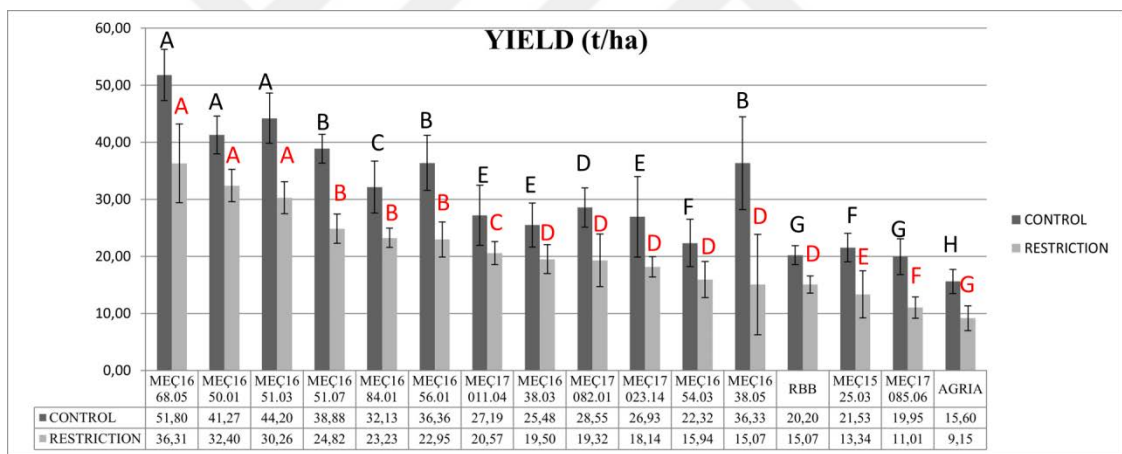


Figure 4.20. Alteration of total yield values between non-stressed and stressed groups. The black letters indicate the groups of the breeding line under non-stressed and the red letters indicate the groups of the breeding line under water restriction conditions

Total yield values were in the range of between 51.80 (t/ha) and 15.60 (t/ha) under non-stressed conditions whereas 36.31 (t/ha) and 9.15 (t/ha) under stressed conditions. 13 out of 14 breeding lines that were selected by the KASP marker had a higher yield than both standard cultivars. MEC17085.06 had a higher yield than Agria but lower than RBB under non-stressed conditions while 12 of them had a higher yield than RBB and Agria under stressed conditions. Besides, MEC1525.03 and MEC17085.06 had a lower yield than RBB and a higher yield than Agria under stressed conditions. The breeding

lines which had highest yield under non-stressed conditions as follows: MEÇ1668.05 (51.00 t/ha), MEÇ1651.03 (44.00 t/ha), MEÇ1650.01 (40.75 t/ha) whereas MEÇ1668.05 (35.75 t/ha) MEÇ1650.01 (32.00 t/ha), MEÇ1651.03 (30.00 t/ha) under stressed conditions.

As seen on the graph, water deficiency caused significant yield losses for all genotypes. MEÇ1668.05 genotype had the highest yield either under non-stressed and stressed conditions. MEÇ1650.01 genotype had the second-highest value under stressed conditions. Also when these two genotypes were compared, MEÇ1668.05 had around 30%, MEÇ1650.01 had around 20%, MEÇ1651.03 had 32% yield loss under stressed conditions. Although the MEÇ1638.05 genotype is also higher yield than standard cultivars (RBB and Agria), It showed the highest yield loss around 60% under the stressed condition when compared with the non-stressed condition.

4.5.6 Marketable yield (t/ha)

Variance analysis of the marketable yield (t/ha) of genotypes under non-stressed and stressed conditions was presented in Table 4.15. As seen on the table, marketable yield (h/da) values were statistically significant ($p < 0.05$) in terms of Genotype, Water Treatment, and Genotype×Water Treatment. Also, the values of genotypes were grouped with Tukey post-hoc (Table 4.16). Alteration of total yield values between non-stressed and stressed groups were presented on a graph (Figure 4.21).

Table 4.15. Variance analysis table of marketable yield (t/ha)

Source	SS	MS	DF	F Ratio
REPLICATE	26.77	8.92	3.00	0.91
GENOTYPE	8160.75	544.05	15.00	6.681**
W.TREATMENT	3214.36	3214.36	1.00	38.0815**
REPLICATE*GENOTYPE	440.48	9.79	45.00	0.77
W.TREATMENT*GENOTYPE	1266.11	84.41	15.00	6.6135**

** $p \leq 0.01$, * $p \leq 0.05$

Table 4.16. Tukey test analysis results of marketable yield (t/ha) for w.treatment*genotype interaction.

Treatment	Level	Group	Mean
Non-stressed	MEÇ1668.05	A	49.81
Non-stressed	MEÇ1651.03	A	43.46
Non-stressed	MEÇ1650.01	B	38.90
Non-stressed	MEÇ1651.07	B	37.69
Non-stressed	MEÇ1638.05	B	35.58
Non-stressed	MEÇ1656.01	B	34.26
Non-stressed	MEÇ1684.01	C	31.91
Water Restriction	MEÇ1651.03	C	30.02
Water Restriction	MEÇ1650.01	C	29.55
Non-stressed	MEÇ17011.04	D	27.02
Non-stressed	MEÇ17082.01	D	26.89
Water Restriction	MEÇ1668.05	D	25.56
Non-stressed	MEÇ1638.03	D	25.47
Water Restriction	MEÇ1651.07	E	23.65
Water Restriction	MEÇ1684.01	E	22.85
Non-stressed	MEÇ1654.03	E	22.31
Water Restriction	MEÇ1656.01	E	21.87
Water Restriction	MEÇ17011.04	F	20.31
Non-stressed	RBB	G	19.52
Non-stressed	MEÇ1525.03	G	19.49
Non-stressed	MEÇ17023.14	G	19.49
Water Restriction	MEÇ1638.03	H	18.85
Water Restriction	MEÇ17082.01	H	18.70
Water Restriction	MEÇ17023.14	H	17.07
Water Restriction	MEÇ1654.03	I	14.76
Non-stressed	AGRIA	I	14.69
Water Restriction	RBB	I	14.36
Non-stressed	MEÇ17085.06	J	13.07
Water Restriction	MEÇ1525.03	K	12.41
Water Restriction	MEÇ1638.05	L	10.97
Water Restriction	MEÇ17085.06	M	9.94
Water Restriction	AGRIA	N	8.32

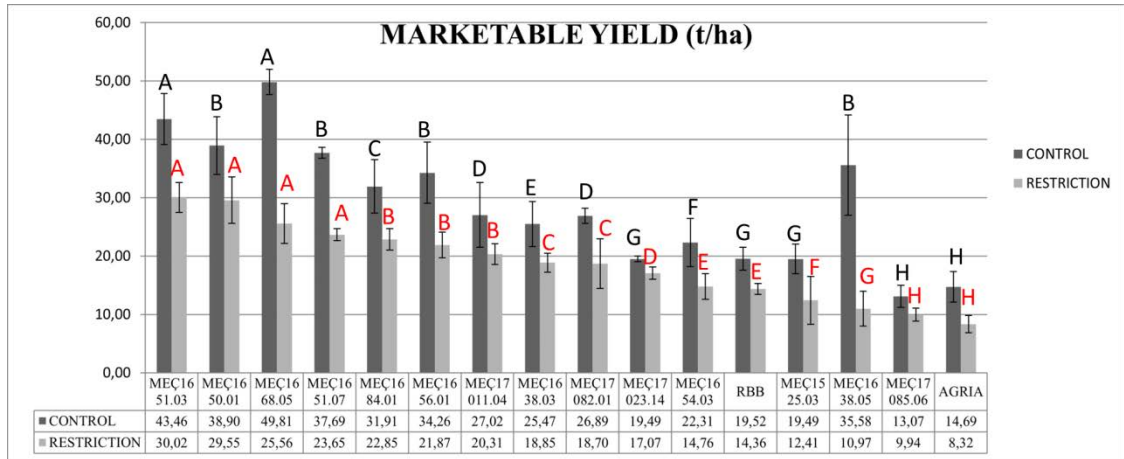


Figure 4.21. Alteration of marketable yield values between non-stressed and stressed groups. The black letters indicate the groups of the breeding line under non-stressed and the red letters indicate the groups of the breeding line under water restriction conditions

Marketable yield values were in the range of between 49.81 (t/ha) and 13.07 (t/ha) under non-stressed conditions whereas 30.02 (t/ha) and 8.32 (t/ha) under stressed conditions. 11 out of 14 breeding lines that were selected by the KASP marker had higher marketable yield than both standard cultivars. MEÇ1525.03 and MEÇ17023.14 had a higher yield than Agria but lower than RBB and MEÇ17085.06 had the lowest marketable yield under non-stressed conditions while 11 of them had a higher yield than RBB and Agria under stressed conditions. Besides, MEÇ1525.03, MEÇ1638.05, and MEÇ17085.06 had lower marketable yield than RBB and higher marketable yield than Agria under stressed conditions. The breeding lines which had the highest marketable yield under non-stressed conditions are; MEÇ1668.05 (49.00 t/ha) and MEÇ1651.03 (42.75 t/ha) whereas MEÇ1651.03 (29.75 t/ha), MEÇ1650.01 (29.00 t/ha) and MEÇ1668.05 (25.00 t/ha) under stressed conditions.

4.5.7 Dry matter content and specific gravity

The variance analysis of dry matter content and specific gravity of genotypes under non-stressed and stressed conditions was presented in Table 4.17-18, respectively. As seen in tables, dry matter content and specific gravity values were statistically significant ($p < 0.05$) in terms of Genotype. However, it wasn't statistically significant ($p > 0.05$) in terms of Water Treatment and Genotype×Water Treatment interaction. The values of genotypes were grouped with Tukey post-hoc test. (Table 4.19-20). Alteration

of dry matter content and specific gravity values between non-stressed and stressed groups were presented on a graph (Figure 4. 22-23).

Table 4.17. Variance analysis table of dry matter content

Source	SS	MS	DF	F Ratio
REPLICATE	15.84	5.28	3.00	6.11
GENOTYPE	443.88	29.59	15.00	43.9076**
W.TREATMENT	0.10	0.10	1.00	0.09
REPLICATE*GENOTYPE	38.90	0.86	45.00	0.67
W.TREATMENT*GENOTYPE	16.49	1.10	15.00	0.85

**p≤0.01, *p≤ 0.05

Table 4.18. Variance analysis table of specific gravity

Source	SS	MS	DF	F Ratio
REPLICATE	0.000	0.000	3.000	2.490
GENOTYPE	0.007	0.000	15.000	13.464**
W.TREATMENT	0.001	0.001	1.000	12.591
REPLICATE*GENOTYPE	0.001	0.000	45.000	0.755
W.TREATMENT*GENOTYPE	0.001	0.000	15.000	1.971

**p≤0,01, *p≤ 0,05

Table 4.19. Tukey test analysis results of dry matter content for w.treatment*genotype interaction

Treatment	Level	Group	Mean
Water Restriction	MEÇ17011.04	A	23.66
Water Restriction	MEÇ1651.07	A	23.44
Non-stressed	MEÇ1651.07	A	23.06
Water Restriction	MEÇ1668.05	A	22.96
Non-stressed	MEÇ17011.04	A	22.75
Non-stressed	MEÇ1668.05	A	22.28
Non-stressed	MEÇ1651.03	A	21.87
Water Restriction	MEÇ1651.03	A	21.16
Water Restriction	MEÇ1684.01	A	20.73
Water Restriction	MEÇ1654.03	A	20.71
Non-stressed	MEÇ1684.01	B	20.31
Water Restriction	MEÇ17085.06	C	19.96
Non-stressed	MEÇ1638.05	C	19.90
Non-stressed	MEÇ1654.03	D	19.76
Non-stressed	MEÇ17085.06	E	19.67

Non-stressed	RBB	F	19.48
Water Restriction	MEÇ1638.05	F	19.47
Water Restriction	MEÇ17082.01	F	19.46
Non-stressed	MEÇ1656.01	F	19.24
Water Restriction	MEÇ1650.01	F	19.23
Non-stressed	MEÇ17023.14	F	19.08
Water Restriction	MEÇ1656.01	F	19.08
Non-stressed	AGRIA	G	18.97
Water Restriction	AGRIA	G	18.88
Non-stressed	MEÇ1650.01	H	18.56
Water Restriction	RBB	H	18.50
Non-stressed	MEÇ17082.01	H	18.38
Water Restriction	MEÇ17023.14	I	17.91
Non-stressed	MEÇ1525.03	I	17.61
Water Restriction	MEÇ1638.03	J	17.00
Non-stressed	MEÇ1638.03	J	16.92
Water Restriction	MEÇ1525.03	K	16.60

Table 4.20. Tukey test analysis results of specific gravity for w.treatment*genotype interaction

Treatment	Breeding Line	Group	Mean
Water Restriction	MEÇ17011_04	A	1.096
Water Restriction	MEÇ1651.07	A	1.095
Water Restriction	MEÇ1668.05	A	1.095
Control	MEÇ17011_04	A	1.093
Control	MEÇ1668.05	A	1.088
Water Restriction	MEÇ1684_01	A	1.084
Water Restriction	MEÇ1654_03	B	1.082
Water Restriction	MEÇ1638_05	C	1.081
Water Restriction	MEÇ1651.03	C	1.081
Water Restriction	MEÇ17085_06	D	1.079
Control	MEÇ1684_01	D	1.078
Control	MEÇ1654_03	D	1.078
Control	MEÇ17085_06	D	1.078
Control	MEÇ1651.07	D	1.077
Water Restriction	MEÇ1650.01	D	1.077
Control	MEÇ1638_05	D	1.077
Water Restriction	MEÇ1656_01	D	1.076
Control	MEÇ1651.03	D	1.076
Water Restriction	MEÇ17082.01	E	1.075
Control	MEÇ17023.14	E	1.074
Water Restriction	AGRIA	E	1.074

Control	MEÇ17082.01	E	1.074
Water Restriction	RBB	E	1.074
Control	RBB	E	1.074
Control	MEÇ1656_01	E	1.073
Water Restriction	MEÇ17023.14	E	1.072
Control	AGRIA	E	1.072
Control	MEÇ1650.01	F	1.071
Water Restriction	MEÇ1638.03	F	1.070
Control	MEÇ1525_03	G	1.066
Water Restriction	MEÇ1525_03	H	1.065
Control	MEÇ1638.03	I	1.064

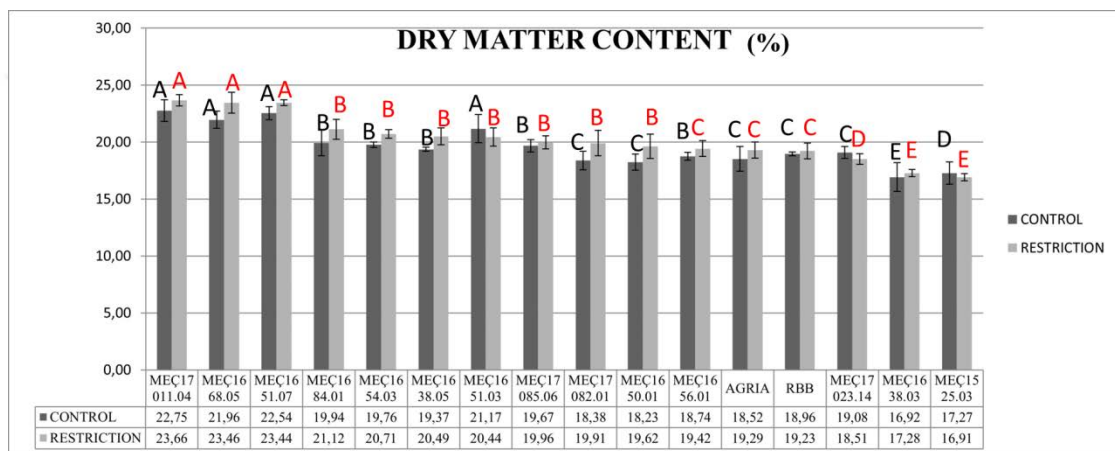


Figure 4.22: Alteration of dry matter content values between non-stressed and stressed groups. The black letters indicate the groups of the breeding line under non-stressed and the red letters indicate the groups of the breeding line under water restriction conditions

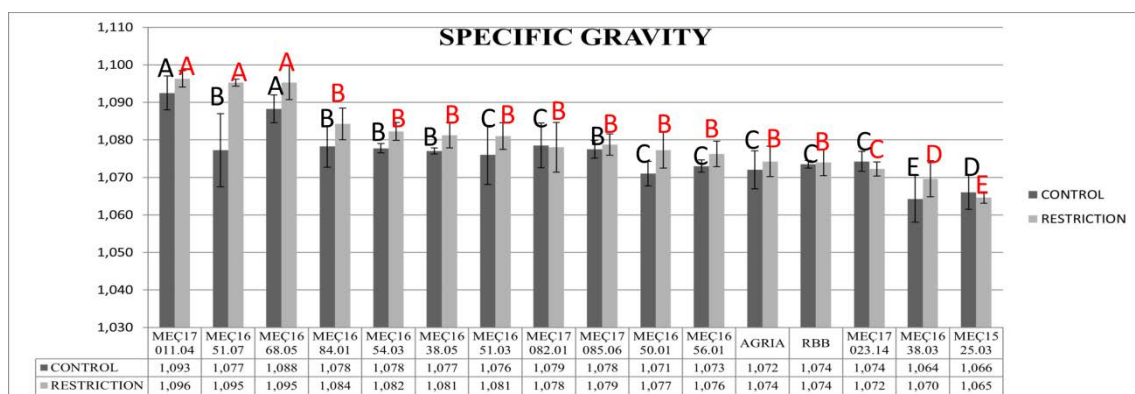


Figure 4.23: Alteration of specific gravity values between non-stressed and stressed groups. The black letters indicate the groups of the breeding line under non-stressed and the red letters indicate the groups of the breeding line under water restriction conditions

Although it wasn't statically significant, the dry matter content of all genotypes, except MEÇ1525.01 and MEÇ1651.03, are increased under stressed conditions. The average value of trial was 19.57 under non-stressed conditions while 20.21 under stressed conditions. Dry matter content values were in the range of between 22.75 and 16.92 under non-stressed conditions whereas 23.66 and 16.91 under stressed conditions. 11 out of 14 breeding lines that were selected by the KASP marker had higher dry matter and specific gravity values than both standard cultivars. MEÇ1656.01 had higher dry matter content than Agria but lower than RBB and MEÇ17082.01, MEÇ1650.01 and MEÇ1525.03 had lower dry matter content than both standard cultivars under non-stressed conditions while 11 of them had higher dry matter content than RBB and Agria under stressed conditions. Besides, MEÇ17023.14, MEÇ1638.03, and MEÇ1525.03 had lower dry matter content and specific gravity values than both standard cultivars under stressed conditions. The breeding lines which had the highest dry matter content and specific gravity values under stressed conditions are MEÇ17011.04 (23.25/1.096), MEÇ1651.07 (23.00/1.095), and MEÇ1668.05 (23.00/1.095).

4.6 Drought Tolerance Index

The drought tolerance indexes of genotypes were presented in Table 4.19.

Table 4.21. Drought tolerance index values

GENOTYPE	D.T.I
MEÇ1651.03	1.46
MEÇ1668.05	1.44
MEÇ1650.01	1.11
MEÇ1651.07	1.03
MEÇ1656.01	0.96
MEÇ1684.01	0.79
MEÇ17082.01	0.70
MEÇ17011.04	0.59
MEÇ1638.03	0.54
MEÇ1654.03	0.52
MEÇ17023.14	0.46
MEÇ1638.05	0.39
MEÇ1525.03	0.36
RBB	0.33
MEÇ17085.06	0.22
AGRIA	0.18

The breeding lines which have a higher drought tolerance index are evaluated as having better stress tolerance. Drought tolerance index values were in the range of between 1.46 and 0.18. 13 out of 14 breeding lines that were selected by the KASP marker had a higher drought tolerance index than both standard cultivars. MEÇ17085.06 had a higher drought tolerance index than Agria but lower than RBB. The breeding lines which had the highest drought tolerance index were MEÇ1651.03 (1.46) MEÇ1668.05 (1.44), and MEÇ1650.01 (1.11), these genotypes also had the top three highest yield values under stressed conditions.

4.7 Drought Susceptibility Index

The drought susceptibility indexes of genotypes were presented in Table 4.20.

Table 4.22. Drought susceptibility index values

GENOTYPE	D.S.I
MEÇ1638.05	1.03
MEÇ1525.03	0.80
MEÇ1668.05	0.74
MEÇ17085.06	0.60
MEÇ1651.07	0.56
MEÇ1656.01	0.53
MEÇ1650.01	0.48
MEÇ1654.03	0.47
MEÇ1651.03	0.46
AGRIA	0.44
MEÇ1684.01	0.41
MEÇ1638.03	0.39
MEÇ17023.14	0.36
RBB	0.35
MEÇ17082.01	0.35
MEÇ17011.04	0.27

The breeding lines which had a higher drought tolerance index had a lower drought susceptibility index. Drought susceptibility index values were in the range of between 1.03 and 0.27. 11 out of 14 breeding lines that were selected by the KASP marker had a higher drought susceptibility index than both standard cultivars. MEÇ1684.01, MEÇ1638.03, and MEÇ17023.14 had higher D.S.I than RBB but lower than Agria.

Besides MEÇ17082.01 and MEÇ17011.04 had lower drought susceptibility than both standard cultivars.

4.8 Correlation Analysis

Correlation analysis of all variables with the total yield variable was carried. The results were presented in Table 4.21.

Table 4.23. Correlation analysis results of variables by yield variable

VARIABLE	by	Correlation	Signif Prob
MARKETABLE YIELD	YIELD	0.945	**p≤0.01
MARKETABLE TBER RATIO	YIELD	0.510	**p≤0.01
MEAN TUBER WEIGHT	YIELD	0.603	**p≤0.01
NUMBER OF TUBERS PER PLAT	YIELD	0.756	**p≤0.01
DRY MATTER	YIELD	0.376	**p≤0.01
DISCARDED TUBER RATIO	YIELD	-0.510	**p≤0.01
SPECIFIC GRAVITY	YIELD	0.212	*p≤ 0.05
SPAD	YIELD	0.139	p>0.05
CANOPY TEMPERATURE	YIELD	-0.399	**p≤0.01

Except for the Chlorophyll index (Spad) value, all variables have significantly correlated with the yield variable. Discarded tuber ratio (%) and canopy temperature values were negatively correlated with yield.

CHAPTER V

DISCUSSION

Growing molecular biology ensures identifying heritable variation by describing the genes which include the genetic architecture of desired traits. So, using molecular techniques for the selection possibility of desired genes are widely used in plant breeding and it is called as molecular breeding (Moose and Mumm 2008).

Marker-assisted selection technique combines conventional breeding and molecular biology methods. Phenotypic selection is carried out according to the genotype (alleles) of markers (Sharma S. and Sharma A., 2018). Recently, the most important aim of plant breeding programs is developing the combination of abiotic stress tolerance, biotic stress tolerance, and agronomic quality characters while improving yield performance and providing farmer's demands.

In the present study, it was aimed to select high-yielding potato breeding lines under drought conditions by marker-assisted selection. Eighty-nine potato breeding lines whose yield potentials have not been known under drought conditions were screened with 7 KASP markers and 14 breeding lines were selected. Finally, performance of the selected breeding lines were tested with a field trial.

One of the KASP markers used in the present study, KASP2-D, was developed based on the single nucleotide polymorphism (T/A) on the Triosephosphate isomerase (TPI) gene in the chloroplast. Chloroplastic TPI is an enzyme that has important roles for many metabolisms such as the Calvin cycle, glycolysis, sucrose synthesis, and secondary metabolisms (Rohmer 1999; Tambasco-Studart et al. 2005). Rathinasabapathi et al. (2006) showed that TPI links to arsenic resistance in the fern and Thimm et al. (2001) proved Fe deficiency induces the TPI gene activity. So TPI gene could be important under stress. The overexpression of TPI in spinach increased the photosynthetic yield by speed up the Rubisco rate (Ma et al., 2007). Drought stress highly affects the plant carbohydrate metabolism. Ge et al. (2012) proved that TPI expression is increased during grain development of wheat plants under stressed conditions. They have suggested that the genotypes which have higher TPI expression

during grain development can produce more energy due to sucrose biosynthesis, and therefore can have higher drought stress tolerance. The chloroplast and seedling development decreased in *Arabidopsis* plants which are mutant in the case of the chloroplastic TPI gene. Also, a significant reduction in starch accumulation was observed in these plants (Chen and Thelen, 2010).

Based on these findings, the TPI gene affects photosynthesis activity, plant growth, and starch accumulation. In the present study, 12 out of 14 breeding lines had TTAA genotype (Table 5.1), therefore, they were considered as potentially high-yielding according to the KASP2-D marker screening. As a result of the field trial, 10 of the 12 had a higher yield and marketable tuber ratio than standard cultivars, Agria and RBB. For instance, MEÇ1651.03 and MEÇ1668.05 being in the second and fourth rank, respectively, in terms of marketable tuber ratio under stressed conditions. They had very less, 5%, and 8%, marketable tuber loss under stressed conditions. So it could be considered that TPI induces starch accumulation in tubers and affects the tuber size and shape.

KASP5-D marker was designed based on the A/G polymorphism in the cytoplasmic malate dehydrogenase (MDH) gene. MDH is an enzyme that has an important role in the oxidative pathway. It uses NAD^+ or NADP^+ as a cofactor and catalyzes the interconversion of malate and oxaloacetate to each other (Íñiguez et al. 2016). Therefore it produces NADPH which is needed for energy-consuming reactions in the cytoplasm (Selinski ve Scheibe, 2019). The major role of cytoplasmic MDH (cyMDH) is the ensuring of NAD^+ and malate accumulation in the cytosol (Tomaz et al., 2010). On the other hand, Chen et al. (2015) demonstrated that overexpression of the cyMDH gene ensures a high manganese tolerance in a tropical legume. Similarly, the reductive activity of cyMDH increased in wild type and transgenic apple lines under salt and cold stress treatments. However, this increase was higher in transgenic plants when compared with wild type. So, they have suggested that, cyMDH could firstly catalyze malate synthesis under stress conditions and this situation can enhance the tolerance to abiotic stresses (Wang et al., 2016b). Besides, it was observed that the polymorphism in the MDH gene affected the tuber size and yield, and the individuals that have GGGG or AAGG alleles in the case of the MDH gene had high yielding under drought conditions in our previous study. In the present study, 8 out of 14 breeding lines had GGGG or

AAGG genotype in case of KASP5-D (Table 4.3) marker screening. When these genotypes were examined in case of mean tuber weight, marketable tuber, and yield potentials, except MEÇ1525.01, results have overlapped. Although the MEÇ1638.03 genotype had a low total tuber number in both normal and stress conditions, it had the highest mean tuber weight and marketable tuber ratio among individuals. It is thought that this genotype produced bigger and oval-shaped tubers due to the low number of tuber per plant.

KASP7-D marker was designed based on the solcap_snp_c2_6108 SNP marker. The sequence of this marker has similarity either with putative transcription elongation factor SPT5 homolog 1 (putative ty gene) and RNA-directed DNA methylation 3 gene (RdDM3). SPT5-1 regulates the transcription elongation with RNA polymerase II. The knockdown of SPT5 caused serious damages to plant growth and reduced the plant weight in Arabidopsis (Dürr, 2013). RdDM3 gene is responsible for DNA methylation. As plants have been developed some mechanisms to cope with abiotic stresses by alteration in gene expressions, RdDM is one of these mechanisms (Yaish et al. 2013). It has been demonstrated that the AtMYB74 gene is firstly regulated by RdDM under salt stress conditions (Xu et al. 2015). Although the specific gene for the KASP7-D marker is not exactly clear, 88.9% of the individuals having TTTC genotype (alleles) in solcap_snp_c2_6108 SNP marker were high or medium-yielding under drought conditions in our previous study. In the present study 10 out of the 14 selected breeding lines for the field, the trial had TTTC genotype according to screening results of the KASP7-D marker (Table 4.3). The 5 genotypes which had the highest mean tuber weight under stress condition have TTTC genotype. Except for MEÇ1525.03 and MEÇ17085.06 genotypes, results are overlapped. Among the 5 genotypes which had the highest yield under stressed condition, MEÇ1650.01 (at 2nd rank) and MEÇ1651.07 (at 4th rank) have TTTC genotype. Although MEÇ1525.03 and MEÇ17085.06 also have TTTC genotype, they are the two breeding lines that had the lowest yield even lower than standard cultivar RBB.

KASP8-D marker was designed based on the A/G polymorphism on the Lateral Organ Boundaries Domain 40 (LOBD40) gene. It is one of the members of plant-specific transcription factors and has an important role in plant growth and development. (Zhang et al. 2020). Although the main function of LOBD proteins in lateral organ development

(Majer et al. 2011), they also have roles in plant defense mechanism (Thatcher et al., 2012), pathogen resistance (Thatcher et al. 2012; Hu et al., 2014 and Cabrera et al. 2014), hormone response (Zentella et al. 2007) and metabolism regulations (Rubin et al. 2009). It was indicated that LOBD genes' expressions increased under salt and drought stress conditions in sorghum (Wang et al. 2010). The expression profiles of LOBD genes were decreased in potato stolons in response to nitrogen stress (Tiwari et al. 2020). Harsseelaar et al. (2017) indicated that the relative expression of LOB domain-containing protein slightly increased in growing potato tubers. They suggested that LOBD proteins have an important role in starch biosynthesis in potato tubers. Based on these findings, the LOBD40 gene may have a role in abiotic stress tolerance, development of tuber, and tuber yield in potato. In our previous study, the individuals having AAGG genotype for this gene were high-yielding under drought conditions. In the present study, 10 out of the 14 breeding lines which were tested in the field had AAGG genotype for KASP8-D (Table 4.3). 9 out of the 10, except MEÇ1525.03, breeding lines had a higher marketable tuber ratio than standard cultivars, and the three of them, MEÇ1638.03, MEÇ1651.03, and MEÇ1651.03 produced the highest marketable tuber ratio under drought conditions. Besides, the three breeding lines having the highest mean tuber weight, MEÇ1638.03, MEÇ1656.01, MEÇ1651.07 had AAGG genotype. Although, two breeding lines, MEÇ17085.06 and MEÇ1684.01 had AAGG genotype they produced lower mean tuber weight than standard cultivars. Eight of the 10 breeding lines had a higher yield than standard cultivars. Six breeding lines having the highest total and marketable yield, MEÇ1668.05, MEÇ1650.01, MEÇ1651.03, MEÇ1651.07, MEÇ1684.01, MEÇ1656.01, had AAGG genotype. Although two breeding lines, MEÇ1525.03 and MEÇ17085.06 had AAGG genotypes they produced lower total and marketable yield than standard cultivar RBB.

KASP11-D marker was designed based on the T/C single nucleotide polymorphism on the Phenylalanine ammonia-lyase (PAL) gene. It plays a major role in the synthesis of polyphenolic compounds such as lignin and flavonoids (Camm et al. 1973). Besides, it has also important roles in response to biotic and abiotic stresses and growth regulators (Hyodo et al. 1971; Zucker et al. 1972). Transcriptional analysis in a medicinal orchid showed that PAL activity slightly increased in response to salt and wound stresses (Nag et al. 2018). Besides, an association between PAL activity and phosphate deficiency was found in rice (Gho et al. 2020). Similarly, a relationship between this gene and

drought tolerance has been found in our previous study. The individuals having TCCC and TTCC genotypes had high or medium-yielding under drought conditions. In the present study, all of 14 breeding lines had TCCC or TTCC genotype. Eleven or twelve of them had higher values than standard cultivars in terms of yield, marketable yield, the number of tubers per plant, marketable tuber ratio, and mean tuber weight. Only two breeding lines, MEÇ1525.03 and MEÇ17085.06 produced lower tuber yield than standard cultivars. In addition to MEÇ1525.03 and MEÇ17085.06 breeding lines, MEÇ1638.05 also produced a lower marketable yield than standard cultivars.

KASP14-D marker was designed based on A/G polymorphism in the DDRGK domain-containing protein 1 gene. Although the function of this gene isn't known exactly, is widely seen in plants and some studies have demonstrated that it has roles in various signalling pathways (Wu et al. 2010). Besides, it was specified that the individuals which have AGGG genotype in this gene region were associated with the number of tuber and tuber yield. Also, %75 of the individuals having the AGGG genotype were high or medium-yielding under drought conditions in our previous study. In the present study, 10 out of 14 selected breeding lines for field trials had AGGG genotype in this gene region (Table 4.3) and 9 of them produced higher yield value than standard cultivars. Among them, 3 breeding lines, MEÇ1668.05, MEÇ1650.01, and MEÇ1651.03 had the highest yield under drought conditions. 8 of them produced higher marketable yield than standard cultivars. Although MEÇ17085.06 and MEÇ1638.05 had AGGG genotype, they have produced lower marketable yield than RBB. All of 10 genotypes had a higher value than standard cultivars in terms of mean tuber weight. However, 5 of them, MEÇ1651.03, MEÇ1668.05, MEÇ1656.01, MEÇ1650.01, and MEÇ1684.01 had a higher value than both standard cultivars in terms of the number of tubers per plant, whereas 3 of them, MEÇ1654.03, MEÇ17082.01 and MEÇ1651.07 produced higher number of tuber only than Agria cultivar.

KASP15-D marker was designed based on the A/C single nucleotide polymorphism on the Cycloartenol synthase (CAS) gene region. CAS plays a role in male gametophyte and plastid formation thus ensures the carotenoid and chlorophyll formation in plants (Babychuk et al., 2008). Essentially, its major role in the biosynthesis of sterol in plants (Babychuk et al., 2008). Sterols are involved in plant growth and development as the pioneer of some plant hormones. Moreover, sterols form lipid microdomains and thus

take a part in signal transduction (Valitova et al. 2016). In our previous study, 73.3% of the individuals which had AAAC and AACC genotype in this gene region were high or medium-yielding under drought conditions. In the present study, except for MEÇ17082.01, all selected breeding lines had AAAC and AACC genotype in this gene region (Table 4.3). 11 of 13 breeding lines produced a higher yield than standard cultivars. Although MEÇ1525.03 and MEÇ17085.06 had also been selected by the KASP8-D marker, they produced a lower yield than standard cultivar RBB. In the case of marketable yield, 10 of 13 breeding lines produced higher marketable yield than standard cultivars. Although MEÇ1525.03, MEÇ1638.05, and MEÇ17085.06 had also been selected by the KASP8-D marker, they produced a lower marketable yield than RBB.

Overall, 14 breeding lines among 89 were selected as potentially high-yielding under drought conditions by KASP marker-assisted selection approach. For that, a breeding line was considered as potentially high-yielding based on the result of at least five KASP markers. Twelve out of 14 selected breeding lines produced higher total tuber yield than standard cultivars under deficit irrigated field conditions. This result indicated that 7 KASP markers tested in the study could be used with an 85.7% accuracy in potato breeding for selection high-yielding potatoes for deficit water conditions.

On the other hand, Deblonde et al. (2000) reported that the later potato cultivars have better drought tolerance than earlier cultivars. In the present study, MEÇ1668.05 is one of the medium-early breeding lines with 91 days vegetation period (Table 4.4) produced the highest yield under water restricted conditions. However, it had around 30% of yield loss when compared with non-stressed conditions. MEÇ1650.01 had the second-highest yield under water restricted conditions and it was an earlier genotype than MEÇ1668.05. it had around 20% of yield loss when compared with the non-stressed condition. As having the third-highest yield under stress condition MEÇ1651.03 was one of the medium early genotypes and also had more than 30% yield loss when compared with the non-stressed condition. MEÇ1638.05 had the highest marketable yield loss with 69.16% and it was one of the early breeding lines in the case of marketable yield. MEÇ1668.05, MEÇ17011.04, and MEÇ1651.03 had medium earliness, they had 48.67%, 24.83%, and 25.98% marketable yield loss under stressed

conditions. MEÇ1651.07, MEÇ1684.01 and MEÇ17085.06 were very early breeding lines and they had loss of 37%, 28%, and 23.92% marketable yield under stressed conditions. Although MEÇ1684.01 had a higher yield loss, it was one of the high yielding breeding lines under stressed conditions with having 22.85 t/ha marketable yield and MEÇ1651.07 had 23.65 t/ha marketable yield under stressed conditions. It is thought that the breeding lines which were very early or medium early had less affected by drought when compared with early breeding lines in this study.

Staying green might be a positive character due to protecting or increasing the chlorophyll content of potato plants under drought conditions (Rolando et al. 2015). Thus, it extends photosynthetic activity, and it can give rise to higher yields under drought conditions (Berger et al., 2010). However, Ramirez et al. (2014) reported that the SPAD values of potato plants increased under severe drought conditions but a negative correlation was found with tuber yield. Similarly, Rudack et al. (2017) also reported that chlorophyll content increased in their study but wasn't correlated with drought. In the present study, the breeding lines having higher tuber yield under stressed field conditions had higher SPAD values compared to breeding lines with lower yield under stressed conditions. However SPAD values were not significantly correlated with yield values under stressed conditions.

Stomata closure of leaves under drought stress is well known. It leads to reduce transpiration rate thus plant temperature increases (Kramer, 1983; Nanda et al., 2018). However, different factors may influence the measurement of plant temperature by infrared thermometers such as air temperature and humidity. So, plant temperature results can lead to misleading results. In the present study, while the plant temperatures of non-stressed and stressed groups were almost the same on the first day of stressed application, plant temperatures under stressed dramatically increased on the 30th day of application. However, plant temperatures of non-stressed groups were almost the same as the first day of the application. Besides canopy temperature values were negatively correlated with yield values.

Some studies revealed that potato plants produce a higher amount of total sugar and dry matter content under water and moisture stress conditions compared with normal conditions (Levy, 1983; Steckel and Gray 1979). However, Levy (1983) encountered a

situation in which some cultivars had higher dry matter content under stress conditions while some of them were higher in non-stressed conditions. On the other hand, Heuer and Nadler (1998), indicated that the dry matter content of potato plants increased approximately 2 times under stress conditions. However, in the present study, although the mean value of dry matter contents increased in stress groups, all breeding lines didn't show increasment under water-stressed conditions. While differences between dry matter content values of the breeding lines were significant, no significant difference was observed in treatments and G×T interactions.

The genotypes which have lower drought susceptibility index (DSI) and higher drought tolerance index (DTI) are evaluated as more tolerant to drought conditions. As DSI highly depends on the genome of the material, DTI has a better ability to select the high-yielding potential of genotypes under drought conditions (Cabello et al., 2013). Similarly, Saba et al. (2001) proposed that DSI had lower narrow-sense heritability than DTI. Besides, the severity and duration of the stress also are considered when the efficiency of these parameters is evaluated (Cabello et al., 2013). The drought susceptibility index is lower in the case of low differences between yield values under stress and normal conditions. However, drought susceptibility index increases even in the case of yield values are higher under both non-stressed and stressed conditions. Therefore, DSI is not proper to select high-yielding genotypes (Ramirez and Kelly, 1998). In the present study, MEÇ17011.04 had the lowest drought susceptibility index but it was at the 8th rank of the drought tolerance index. Similarly, MEÇ1651.03 had the highest drought tolerance index but it was at the 9th rank of DSI. When DTI was evaluated, all breeding lines having a higher yield than standard cultivars under drought conditions exhibited higher DTI than standards.

CHAPTER VI

CONCLUSION

In the present thesis study, the selection efficiency of 7 KASP markers was detected. It was hypothesized that the KASP markers associated with drought resistance of potato those were developed by our research group in the previous studies can be used for the selection of high-yielding breeding lines for drought conditions.

Firstly 89 potato breeding lines in which their yield potentials were not known under drought conditions were screened by 7 KASP markers. Next, 14 breeding lines were selected as potentially high-yielding under drought conditions according to results of at least 5 KASP markers. Then, 14 selected breeding lines were tested under both full and deficit irrigated field conditions.

In the result of the study, MEÇ1668.05 with 36.31 t/ha, MEÇ1650.01 with 32.40 t/ha, and MEÇ1651.03 with 30.26 t/ha exhibited the highest tuber yield under stressed conditions. Also, their yields were the highest under non-stressed conditions, too. Therefore, these MEÇ1668.05, MEÇ1650.01, and MEÇ1651.03 breeding lines could be released as new cultivars with the characteristics of high-yielding under both full and deficit water conditions.

In the study, the combined results of KASP marker-assisted selection and the field experiments showed that while 12 out of 14 breeding lines had higher yield than standard cultivars under deficit water conditions, two breeding lines, MEÇ1525.03 and MEÇ17085.06 produced tuber yield in between two standard cultivars. The results showed that high and mild-yielding potato breeding lines for drought conditions could be determined with 85.7% accuracy by using the 7 KASP markers tested in the study. However, to get more reliable results for validation of the effectiveness of the KASP markers, the field experiment should be repeated for at least one year more and in two locations. Furthermore, it is expected to develop novel breeding strategies integrated with KASP marker-assisted selection for improving drought resilience of potato cultivars for saving land, money, and labor in the future.

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