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Evaluation of Drought Tolerance in Several Genotypes of Spelt

(*Triticum aestivum* Var. Spelta)

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Evaluation of Drought Tolerance in Several Genotypes of Spelt (*Triticum aestivum Var. Spelta*)

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Estudo da Tolerância à Seca em Vários Genótipos de Espelta (*Triticum aestivum Var. Spelta*)

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Abstract

Domestication of plants and plant breeding have dramatically eroded the allelic variations of crop species which led to an increasing susceptibility of crop plants to environmental stresses, diseases and pests. Drought is a major environmental stress factor that affects the growth and development of plants so the selection of tolerant genotypes becomes increasingly important with respect to the predicted effects of global warming. In this study, several genotypes of Spelt (*Triticum aestivum* var. spelta) were tested under low water supply in soil with the aim of to find Spelt genotypes more resistant than wheat to these conditions, and select them so that in future may be used to improve wheat crops. Morphological analyses were performed and mineral and enzymatic analyses and also dry matter production were calculated. Our results suggests that the genotypes Sp53, Sp96, Sp912, Sp757 and Sp804 are a potential ones to use in breeding programs to improve wheat production. Under drought, these genotypes had growth efficiency of 38%, 45%, 64%, 37%, and 31% respectively and also showed higher biomass than modern wheat and were also mineralogical richer. The genotypes Sp96 and Sp912 showed highest activity of all antioxidants enzymes tested. This work proves that Spelt is a good wheat to continue to study in order to improve wheat crops in dry areas and consequently increase the quality of life and health of the populations living in those areas.

KEYWORDS: Drought, Domestication, Spelt, Wheat, Diet, Nutrition.

Resumo

A domesticação e melhoramento de plantas têm vindo a alterar e reduzir geneticamente as variações alélicas das espécies o que levar a uma suscetibilidade maior dessas plantas a stresses ambientais, doenças e pragas. A seca é um dos mais importantes factores de stress ambiental que afecta o crescimento e desenvolvimento das plantas, deste modo, a selecção de espécies mais tolerantes torna-se cada vez mais importante no que diz respeito aos efeitos previstos de aquecimento global. Neste estudo, vários genótipos de Espelta (*Triticum aestivum* var. Spelta) foram testados sob baixo fornecimento de água no solo com o objetivo de encontrar genótipos mais resistentes do que o trigo a estas condições e selecioná-los para que, no futuro, possam vir a ser usados para melhorar as colheitas de trigo. Para este estudo foram realizadas análises morfológicas mineralógicas e enzimáticas bem como a produção de matéria seca calculadas. Os nossos resultados indicam que os genótipos Sp53, Sp96, Sp912, e Sp757 Sp804 são potenciais escolhas para no futuro serem usados em programas de cruzamento com o intuito de melhorar a produção de trigo. Sob seca, esses genótipos apresentaram uma eficiência de crescimento de 38%, 45%, 64%, 37% e 31%, respectivamente e não só em biomassa, mas também a nível de minerais na sua constituição, obtiveram melhores resultados quando comparando com o trigo moderno. Os genótipos Sp96 e Sp912 revelaram ainda possuir uma maior atividade de todas as enzimas antioxidantes testadas. Este trabalho prova que a Espelta é um bom trigo para continuar a estudar, a fim de melhorar as colheitas de trigo moderno em áreas secas e, consequentemente, aumentar a qualidade de vida e saúde das populações que vivem nessas áreas.

PALAVRAS-CHAVE: Seca, Domesticação, Espelta, Trigo, Dieta, Nutrição.

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List of Abbreviations

AA – Ascorbic Acid

ABA – Abscisic Acid

APX – Ascorbate Peroxidase

ATP – Adenosine Triphosphate

CAT – Catalase

DSP – Desmoplakin

FENS – Faculty of Engieering and Natural Sciences

FW – Fresh Weight

GR – Glutathione Reductase

GSSG – Oxidized Glutathione

ICP – Inductively Coupled Plasma

LEA – Late Embryogenesis Abundant

MDHA – Monodehydroascorbate

MDHAR – Monodehydroascorbate Reductase

$NADP^+$ – Nicotinamide Adenine Dinucleotide Phosphate

NADPH – Nicotinamide Adenine Dinucleotide Phosphate Oxidase

NBT – p-Nitro Blue Tetrazolium Chloride

POD – Peroxidase

ROS – Reactive Oxygen Species

SOD – Superoxide Dismutase

1. Introduction

1.1. Wheat

1.1.1. Origins and Importance

With 620 and 681 million tons produced worldwide in 2006 and 2011, respectively, wheat provides about 20% of the calories consumed by humans, being an important source of protein, vitamins and minerals (Dubcovsky and Dvorak, 2007; Brenchley *et al.*, 2012). Is the universal cereal of the Old World agriculture and the world's foremost crop plant, followed by rice and maize (Peng *et al.*, 2011). Far from being a staple food, in many parts of the world, is a major cereal crop, commonly known as a "King of Cereals" (Datta *et al*, 2009). Thus, wheat is the most important food crop of mankind, whose considerable areas of cultivations are located on low-moistured soils. The consumption of wheat increases each year, especially in developing countries. It is estimated that global wheat production between 2010 and 2020 will rise by 40% (Aliyev, 2012).

About 95% of the wheat crop is common wheat (*Triticum aestivum*), used for making bread, cookies, and pastries and the remaining 5% is durum wheat (*Triticum durum*), used for making pasta and other semolina products (Brenchley *et al.*, 2012). Einkorn wheat (*Triticum monococcum*) and other hulled wheats, namely emmer (*Triticum dicoccum*) and spelt (*Triticum spelta*), are today relic crops of minor economic importance (Fig. 1.1) (Heun *et al.*, 1997; Peng *et al.*, 2011).

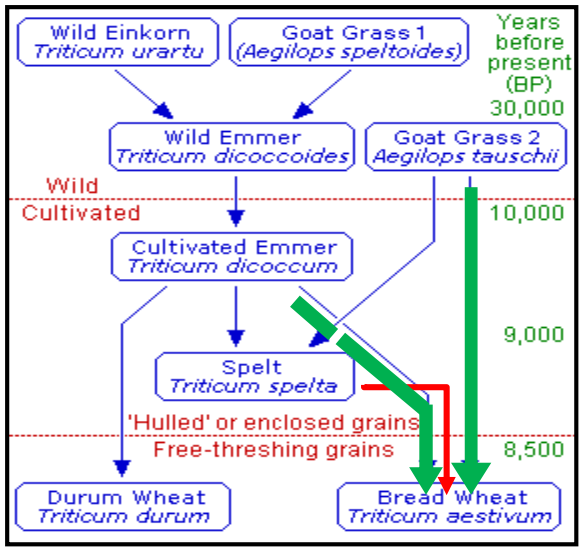

Fig. 1.1 – The evolution of wheat from the prehistoric Stone Age grasses to modern macaroni wheat and bread wheat [1].
Red Arrow – Old theory of wheat evolution.
Green Arrow – New theory of wheat evolution.

For many years, it was believed that bread wheat had evolved from Spelt by mutations that changed the form of the ear however, newer scientific research suggests that it evolved independently about 8,500 years ago but from the same two ancestors, Cultivated Emmer and a Goat Grass (Fig. 1.1) (Marcussen *et al.*, 2014). This created a free-threshing hybrid that differed from Spelt by the ear being roughly square in section, with more grains and a tougher rachis [1].

1.1.2. Domestication of Wheat

The domestication of wheat around 10,000 years ago marked a dramatic turn in the development and evolution of human civilization. The society transited from a huntergatherer and nomadic pastoral, to a more sedentary agrarian one (Eckardt, 2010). Dependent on wild resources for their nutritional requirements, this nomadic lifestyle was dictated by plant availability, and the annual animal cycle. The cultivation of plants and the husbandry of animals enabled humans to obtain a control over their food resources, protecting them from climatic and environmental uncertainty (Brown *et al.*, 2009). This transition was the beginning of agriculture and it caused many changes in human culture — a phenomenon known as the "Neolithic Revolution" (Salamini *et al.*, 2002).

The first humans to pioneer farming practices lived in the Fertile Crescent — a region that spans modern-day Israel, Jordan, Lebanon and western Syria, into southeast Turkey and, along the Tigris and Euphrates rivers, into Iraq and the western flanks of Iran (Fig. 1.2).

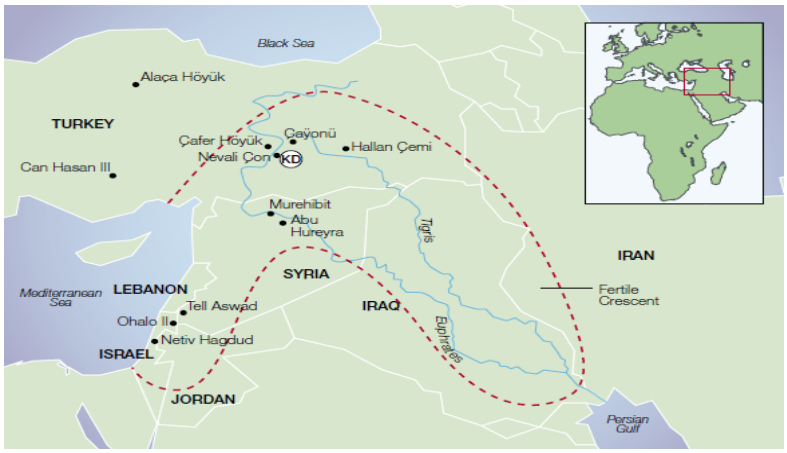

Fig. 1.2 – Fertile Crescent area. From Salamini et al., 2002.

This region is characterized by a variable topography, marked seasonality with cold rainy winters and dry summers, a history of fluctuating precipitation and a rich palaeoflora that is well documented in the fossil pollen record. These features contributed to making this region the cradle of agriculture, there is why in the Fertile Crescent agriculture allowed the development of a dense human population (Salamini *et al.*, 2002).

Domestication of plants (and also animals) is the major factor underlying human civilization and is a gigantic evolutionary experiment of adaptation. Is the outcome of a selection process that results in the increased adaptation to cultivation and use by humans (Brown, 2010) and, as Charles Darwin said, it is a gigantic evolutionary experiment of adaptation and speciation, generating incipient species (Darwin, 1905).

Two of the most important traits in the domesticated bread wheat were an increase in grain size and the development of nonshattering seed (Fig. 1.3). The former has been associated with successful germination and growth of seedlings in cultivated fields, whereas the latter trait (a hallmark of domestication) prevents natural seed dispersal and allows humans to harvest and collect the seed with optimal timing (Eckardt, 2010). These traits contributed to an increase in crop yields.

Fig. 1.3 – Differences between domesticated wheat (top) and ancestral species (bottom). From Eckardt, 2010

1.2. Changes in Society, Lifestyle and Diet

After the Green Revolution, thanks to advances in technology and science during the last decades, people adopted a new lifestyle. Due to this new lifestyle, not only in the developed countries, but also in the less developed countries, people changed their diet. Now people eat more, and more animal products (Chrispels and Sadava, 2003; Capper *et al.*, 2013). For example, in Portugal, between 1961 and 2009 the average of Kcal daily consumed per person increased from 2473 Kcal to 3617 Kcal. From these values, in 1961 the Kcal/day consumed from animal products were 345 Kcal (14% of the total diet), and raised to 1034 Kcal (30% of the total diet) in 2009, which means that in Portugal from 1961 to 2009 the portion of animal products doubled, whereas about 1200 Kcal/day of human total diet was reached (Fig. 1.4) [2].

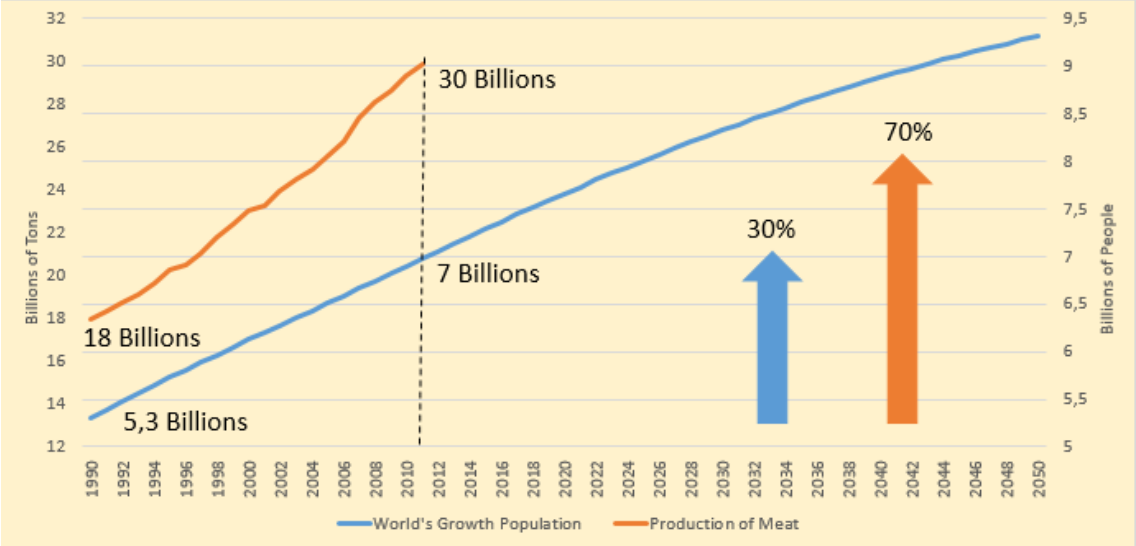

Fig. 1.4 - Comparison between the increases of consumption of meat with the population growth.

In this context, if we correlate the population growth with the increase of consumption of meat, we can speculate that we will not be able to produce enough food to feed the world's population. As we can see in the chart above (Fig. 1.4), from 1990 to 2011 the world's population increase 30% whereas, on the other hand, the consumption of meat increase 70% during these 11 years [2]. With the prediction of population growth reach more than 9 billions in 2050, it is expected that the increase in meat consumption increase further. Only in Portugal, from 1990 to 2009 the consumption of meat increase from 62,8Kg per capita per year to 93,4Kg, more than 30Kg.

In addition, to produce 1Kg of meat (pork or beef) we need about 5Kg of cereals plus 15 000 liters of water, so basically, each person in Portugal spend indirectly more than 400Kg of grains and 1 000 000 liters of water per year to eat meat.

For all these reasons, is crucial to improve and develop new techniques that allow to obtain higher yields in crops, since only about 38% of the surface of the Planet Earth is arable (Chrispels and Sadava, 2003).

1.3. Drought Stress

Due to climate change and global warming, crop yields tend to decrease (Nelson *et al.*, 2009; Schlenker and Lobell, 2010). Climate change also impact significantly by increasing water demand, limiting crop productivity and by reducing water availability in areas where irrigation is most needed or has comparative advantage (Turral *et al.*, 2011). Soil productivity is decreasing globally due to enhanced soil degradation in the form of erosion, nutrient depletion, water scarcity, acidity, salinization, depletion of organic mater and poor drainage. Near 40% of the agricultural land has been affected by soil degradation (Cakmak, 2002). Processes such as seed germination, seedling growth and vigour, vegetative growth, flowering and fruit set are adversely affected under drought conditions, reducing yield and quality of the final product (Sairam and Tyagi, 2004). Plants are also more susceptible to drought during flowering and seed development (the reproductive stages), as plant's resources are deviated to support root growth (Oliveira *et al.*, 2013).

On the next figure (Fig. 1.5) we can see how plants responses biochemically, physiologically and molecularly under drought stress:

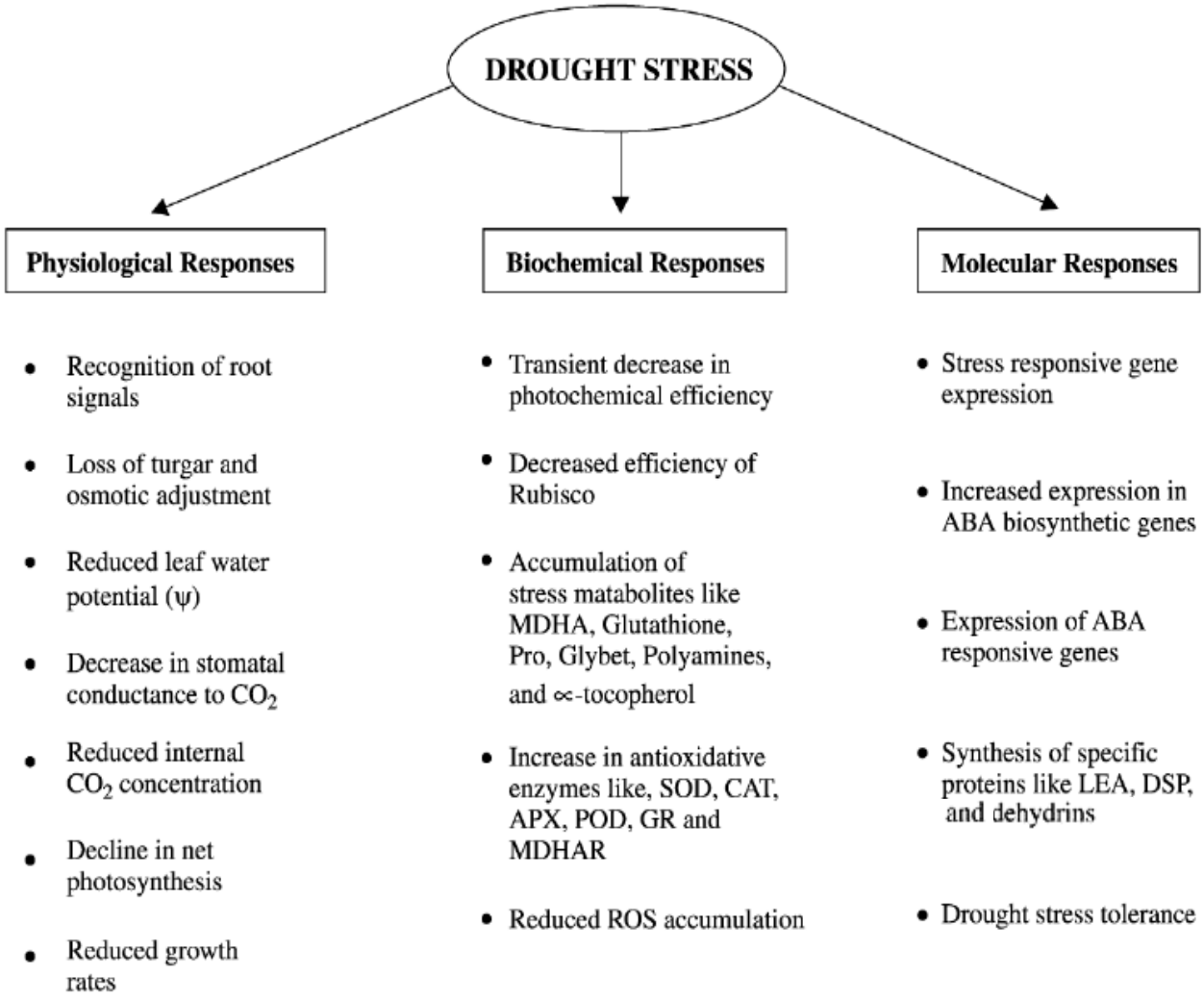

Fig. 1.5 - Physiological and molecular basis of drought stress tolerance. From Oliveira et al., 2013.

One factor contributing to this inadaptation to climate change is the domestication. The genetic changes responsible for the suite of traits that differentiate domesticated plants from their wild ancestors are referred to as the domestication syndrome. In wheat, as in other grains from scattering by wind and facilitating harvesting. Other traits of the wheat domestication syndrome shared by all domesticated wheats are increased seed size, reduced number of tillers, more erect growth, and reduced seed dormancy (Dubcovsky and Dvorak, 2007). Domestication causes also substantial genetic erosion and that erosion was reinforced during modern breeding processes, and thus increased susceptibility and vulnerability to environmental stresses, pests and diseases (Peng *et al.*, 2011).

It has been long known that crop plants are usually exposed to different environmental stresses during their development and these stresses limits their growth and productivity (Moud and Maghsoudi, 2008). The effects of these abiotic stresses on plants in both natural and agricultural settings is a topic that is receiving further attention because of the potential impacts of climate change on rainfall patterns, temperature extremes, salinization of agricultural lands by irrigation and the overall need to maintain or increase agricultural intensity and duration of stress, plant genotype, developmental stage and environmental factors that cause stress (Aliyev, 2012). Among these, drought and salinity are the most severe ones that reduces productivity (Moud and Maghsoudi, 2008).

Drought is a major environmental stress factor that affects the growth and development of plants. In the analysis of a plant's drought response, the mode, timing, and severity of the dehydration stress and its occurrence with other abiotic and biotic stress factors are significant. Furthermore different species, subspecies, and cultivars of crops show variation in their drought tolerance under same conditions, emphasizing the importance of genetic diversity as an underlying factor of drought and its significance in drought-related research. The effects of drought are expected to increase with climate change and growing water scarcity, thus, an understanding of drought stress and water use in relation to plant growth is of great importance for sustainable agriculture (Budak *et al.*, 2013).

The results of drought stress are disorganization of membranes, loss of activity or denaturation of proteins and, as it was said before, oxidative damage by excess production of (ROS). As a consequence, inhibition of photosynthesis, metabolic dysfunction, and damage of cellular structures contribute to growth disorders, reduced fertility, and premature senescence (Krasensky and Jonak, 2012).

Abiotic stress is already a major limiting factor in plant growth and soon, will become even more severe as desertification that covers, day after day, more terrestrial area. Moreover, the faster-than-predicted change in global climate and the different available scenarios for climate change suggest an increase in aridity for the semiarid regions of the globe. Together with overpopulation, which exceeds food supply, this will lead to an overexploitation of water resources for agriculture purposes and increased constraints on plant (Oliveira *et al.*, 2013).

The selection of tolerant genotypes becomes increasingly important with respect to the effects of global warming also in Central Europe, like higher temperatures, lower precipitation and an uneven distribution of precipitation during the growing season. These genotypes may exhibit differences in many physiological reactions such as changes in the osmotic adaptation of plants, changes in the levels of protective proteins and other metabolites and antioxidant capacity of plants. Having been exposed to stress conditions, the plants show induction of a number of biochemical and physiological changes, which lead to the development of protective mechanisms aimed at the efficient utilization of available water or salt excess in soil. Usually, the most resistant genotypes are those that exhibit tolerance at multiple levels at the same time (Truhlářová *et al.*, 2012). Comparing the eight major crops (wheat, barley, corn, sorghum, soybean, oat, potato and sugar beet), wheat is the most sensitive to abiotic stresses involving drought and salinity (Pauk *et al.*, 2012).

For all these reasons, the best strategy for wheat improvement is to utilize the adaptive genetic resources of the wild progenitors, there is why in this study we used Spelt.

1.4. Spelt

Spelt (*Triticum aestivum* var. spelta) is one of the oldest cultivated grains of ancient Europe, and it is now considered a minor crop. The most common uses for spelt is as a substitute for wheat flour in breads, pasta, cookies, breakfast cereal, cakes, mixes for breads, pancakes and so on, and in animal feedstuffs. Spelt has high protein content and makes high-quality bread. It can also be used for making beer and for spelt rice (Neeson, 2011). Spelt shares its genus and species with common wheat and four other hexaploid wheats and has high capacity of adaptation to several adverse soil conditions such as drought stress and salinity but, on the other hand, wheat produces higher yields and is easier to thresh. Spelt it also has inconsistent yields, low test weights, a limited range of adapted cultivars and requires an expensive dehulling process, however, some research suggests that spelt out-performs many traditional grains (such as wheat) under suboptimal growing conditions and is able to better utilize nutrients when grown in a low-input system.

Nutritionally, spelt contain higher levels of protein (12.1–17.1%), soluble dietary fiber, and minerals, such as zinc, selenium, lithium, phosphorus and magnesium than common wheat (Neeson *et al.*, 2011). Results from an EU research project identified spelt varieties with increased protein yield (18%) and higher nitrogen efficiency enabling them to compete with wheat and oats in the livestock feed market. When harvested for forage, yields and protein content of winter spelt were significantly higher than for spring oats (Neeson *et al.*, 2011).

The aim of this study is to find Spelt genotypes with better growth rates and under drought conditions than modern wheat, to be used in the future to improve wheat crops by breeding programs. Accordingly, different genotypes of spelt, randomly selected, and modern wheat (Adana99) were grown at the same time under drought stress. Therefore, morphological differences between spelt and modern wheat as well as enzymatic activity and mineral composition were assessed.

1.5. Vernalization

In this experiment, we will adopt a technique to grow our plants called Vernalization. This technique consists in prolonged exposure to cold temperatures in absence of light in order to promoting flowering, before being sown (Amasino, 2004). A good vernalization should be done with temperatures between 3-8^{o}C (with some exceptions) and the time required for complete vernalization depends of the specie. The length of vernalization treatment required for complete vernalization is related to whether a species has an obligate or facultative vernalization requirement. Many crops of the biennial plants stay vegetative without cold exposure so the cold requirement is obligatory, on the other hand, for some species, vernalization has only a furthering effect on flower induction, therefore the cold requirement for those is called facultative (Kaymak and Güvenç, 2010).

1.6. Mineral Content

Mineral composition will be obtained by ICP analysis. Among other minerals, the contents of Na^{+}, K^{+} and Ca^{2+} were assessed. K/Na and (K+Ca)/Na ratios has been used by scientists because are important to help us to understand if the genotypes have good growth rates under drought stress (Chhipa and Lal, 1995). When under drought stress, the uptake of water is low and a high concentration of salt is absorbed by plants. A lower ratio of K/Na in plants has been reported as a more sensitivity to stress (Lopez and Satti, 1997). Even in other plants as rice, there is a strong evidence of correlation of this ratio and yields losses in crops (Asch *et al.*, 2000).

Intracellular K^+ and Na^+ homeostasis is important for the activities of many cytosolic enzymes, and for maintaining membrane potential and an appropriate osmoticum for cell volume regulation. Na^+ stress due to drought, disrupts K^+ uptake by root cells, accumulates to high levels inside plant cells and becomes toxic to enzymes (Zhu, 2003). Even in halophytic plants, which accumulate large quantities of Na^+ inside the cell, their cytosolic enzymes are just as sensitive to sodium as enzymes of glycophytic ones, which implies that halophytes have to compartmentalize the Na^+ into the vacuole. K^+ deficiency inevitably leads to growth inhibition because K^+, as the most abundant cellular cation, plays a critical role in maintaining cell turgor, membrane potential and enzyme activities (Zhu, 2007).

When intracellular Na^+ are in toxic levels, there is a cytoplasmatic trigger in Ca^{2+} signal wich is needed to remove Na^+ from cells (Zhu, 2002). Increased Ca^{2+} supply has a protective effect on plants under Na^+ stress, because Ca^{2+} sustains K^+ transport and K^+/Na^+ selectivity in sodium-changelled plants. This beneficial effect of Ca^{2+} is mediated through an intracellular signaling pathway that regulates the expression and activity of K^+ and Na^+ transporters. Ca^{2+} may also directly suppress Na^+ import mediated by nonselective cation channels. Usually, the symptoms of damage by this stress are growth inhibition, accelerated development and senescence, and of course, death during prolonged exposure (Zhu, 2007).

1.7. Enzymatic Activity

Enzymatic activity as glutathione reductase (GR), ascorbate peroxidase (APX), catalase (CAT) and superoxide dismutase (SOD) are also extremely important to support our search of genotypes resistant to drought stress. It will help to elucidate if the plants are under stress.

When under drought stress, to optimize the water use efficiency of plants, there is a reduction in CO_2 assimilation due to stomatal closure. Under these conditions, limited quantities of Oxidized Nicotinamide Adenine Dinucleotide Phosphate ($NADP^+$) are available to accept electrons, therefore oxygen can function as an alternative electron acceptor, although this pseudocyclic pathway for electron transport provides additional ATP and it can result in the production of superoxide. Superoxide is one toxic oxygen molecule from the group known as Reactive Oxygen Species (ROS) and upon reaction with chloroplast components can produce more reactive oxygen products such as the hydroxyl radical, which can results in lipid peroxidation, inhibition of fixation of CO_2 and the photooxidation of chloroplast pigments.

Glutathione reductase is important to scavenge and remove these toxic products before cellular damage occurs because it plays an essential role in the protection of chloroplasts against oxidative damage by oxidation of essential thiol groups, inactivating these enzymes (Gamble and Burke, 1984). This enzyme along with the other ones provide highly efficient machinery for detoxifying O^{2-} and H_2O_2. For example, the balance between SODs and the different H_2O_2 scavenging enzymes in cells is considered to be crucial in determining the steady-state level of O^{2-} and H_2O_2 (Hakeem and Ahmad, 2012). SOD is the primary scavenger, which converts O^{2-} to H_2O_2 and then is eliminated by APX in association with GR, which helps in regeneration of ascorbic acid (AA). H_2O_2 is also scavenged by CAT, though the enzyme is less efficient than APX/GR system (Sairam and Srivastava, 2002). High concentrations of these enzymes will mean that plants are under stress.

Putting together all these results it will be possible conclude the efficiency under drought stress of plants tested.

2. Materials and Methods

All experiments were conducted on Greenhouse of Faculty of Engineering and Natural Sciences of Sabancı University, Tuzla/Istanbul, Turkey (40°53'26¨N/29°22'42¨E). The soil used was collected from Central Anatolia region of Turkey. All experiments were performed between January and July of 2014.

All plants were grown in pots with soil from Central Anatolia of Turkey (39°90'N 35°00'E). This soil is characterized as highly calcareous and semi-arid, because this area is the driest region in Turkey, with an annual precipitation of 325 mm (Cakmak *et al.*, 1996). Additionally, these soils also face nutrients deficiency (Bagci *et al.,* 2007; Cakmak, 2008).

Adana99 was used as reference genotype was because is one of the most used seeds in Anatolian soil, due to have better growth rates and high yields in this type field (Mazid *et al.*, 2009).

2.1. Plant culture and treatments

2.1.1. 1st Experiment

Eight spelt wheat genotypes (Sp2, Sp41, Sp492, Sp563, Sp732, Sp757, Sp804 and Sp912) and one cultivar of modern wheat (Adana99), used as reference genotype, were selected to screen them for drought tolerance. Two different treatments (drought tolerance and control) were formed and each genotype were cultivated in 3 pots (triplicate) by using about 10 seeds per pot. Previously, each pot was filled with about 2.2Kg of soil enriched with 200 ppm of N, 100 ppm of P, 30 ppm of K, 5 ppm of Zn and 5 ppm of Fe. The water supply was reduced from 70% to 30% of field capacity after 3 weeks of germination. The whole duration of this experiment, since sown till harvest was 50 days.

2.1.2. 2nd Experiment

Eight spelt wheat genotypes (Sp53, Sp67, Sp69, Sp92, Sp96, Sp225, Sp382 and Sp801) and one cultivar of modern wheat (Adana99), used as reference genotype, were selected to screen them for drought tolerance. The whole procedure was identical to the first experiment, with the follow differences: Before sowing, all seeds were vernalized for 3 weeks at 3-4°C. The whole duration of this experiment, since sown till harvest was 30 days.

2.1.3. 3rd Experiment

Eight spelt wheat genotypes (Sp41, Sp67, Sp69, Sp92, Sp96, Sp563, Sp732 and Sp912) and one cultivar of modern wheat (Adana99), used as reference genotype, were selected to screen them for drought tolerance. The whole procedure was identical to the second experiment. The whole duration of this experiment, since sown till harvest was 30 days.

2.2. Determination of Dry Matter Production

After harvesting, all plants from each pot (sample) were washed with water, placed in paper bags and storage at 55°C, during 1 week. Then, each sample was weighed (Sartorius CP3202S, d=0.01g) to determination of dry matter.

2.3. Determination of Mineral Nutrients

Whole shoot and root samples were dried at 70 °C. All samples were milled to a fine powders in an agate vibrating cup mill (Pulverisette 9; Fritsch GmbH; Germany) during around 1 minute at 750rpm, digested and sent to ICP analysis for determination of macro (K, Ca, Na, P, S and Mg) and micronutrients (Zn, Fe, Mn, Cu, B and Al).

To digest, each sample were weighed (0.30g ± 0.10g) and transferred to a digestive tube, which was filled with 2mL of 30% H_2O_2 and 5mL of 65% HNO_3 and then all samples were acid digested in closed-vessel microwave system (MarsExpress; CEM Corp., Matthews, NC, USA). After this process, 13mL of double-deionized water were added in each tube to brought up the volume of 20 mL and then all samples were filtered and storage. A blank was added to our set of samples, and also a reference of Tomato Leaf (NIST 1573a) (0.20g ± 0.00g).

Inductively coupled plasma optical emission spectrometry (ICP-OES; Vista-Pro Axial; Varian Pty Ltd, Mulgrave, Australia) was used to determine the mineral concentrations of the samples. Measurements were checked by using certified standard reference materials obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA).

2.4. Determination of Enzymatic Activity

A sample of approximately 0.5g (Sartorius CP3202S, d=0.01g) of fresh leaves were collected from each pot and kept at -80°C. All samples were milled with help of liquid nitrogen and quartz powder in a porcelain mortar. Then, 5 mL of 50 mM potassium phosphate (K-P) buffer solution was added to the samples. The K-P buffer was prepared by mixing 50 mM KH_2PO_4 and 50 mM K_2HPO_4 and the pH was adjusted to 7.6. After, 0.1 mM EDTATitriplex-III was added to this mixture for the homogenization step. The homogenates were then centrifuged at 15000g for 30 min, and the supernatants were used for protein and enzyme analysis. Protein concentrations in the crude extracts were measured by using the Bradford assay as described by Bradford (1976). SOD activity was measured by a slightly modified version of the photochemical method described by Giannopolitis and Ries (1977). This assay is based on the inhibition of the photochemical reduction of p-nitro blue tetrazolium chloride (NBT) by SOD and its spectroscopic measurement at 560 nm. One tube of reaction mixture contains 500 µl 50 mM Na_2CO_3, 500 µL 12 mM L-methionine, 500 µL 75 µM NBT and 500 µL 2 µM riboflavin as well as enzyme extracts (50-150 µL). The total volume was brought up to 5 ml with K-P (pH 7.6) containing 0.1 mM Na-EDTA. The reaction was started by adding the riboflavin to the mixture and placing the vials under the lights in growth chamber for about 8 min. One unit of SOD activity is defined as the SOD activity that results in a 50 % decrease in the NBT reduction. Glutathione reductase activity was determined by recording the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm according to Foyer and Halliwell (1976) with a few modifications. The 1 mL reaction mixture consisted of 100 µL of 0.5 mM oxidized glutathione (GSSG), 100 µL of 0.12 mM NADPH, 50-150 µL of the enzyme extract and 650-750 µl of 50 mM K-P buffer (pH 7.6) with 0.1 mM Na-EDTA. Results were adjusted for the non-enzymatic oxidation of NADPH by observing the decrease of absorbance at 340 nm in the absence of GSSG. APX activity was measured according to Nakano and Asada (1981) by monitoring the decrease in absorbance of ascorbic acid at 290 nm. The 1 mL reaction mixture contained, 100 µL of 12 mM H_2O_2, 100 µL of 0.25mM ascorbic acid, 50-150 µL of the enzyme extract in addition to 650-750 µL of 50 mM K-P buffer (pH 7.6) containing 0.1 mM Na-EDTA. Finally, CAT activity was determined by monitoring the decrease in the absorbance of H_2O_2 at 240 nm. The reaction mixture contained 100 µL of 100 mM H_2O_2 dissolved in K-P buffer, 50-150 µL of the enzyme extract and sufficient 50 mM K-P buffer (pH 7.6) containing 0.1 mM Na-EDTA to bring up the total volume to 1 mL.

3. Results and Discussions

3.1. 1st Experiment

After submit the genotypes to drought stress, morphologically it was quite easy to choose which ones were the best candidates as stress tolerant plant. For the first experiment, the genotype Sp41 and Sp757 were the better ones under stress, on the other hand, the two worst were Sp563 and Sp732.

In the following table (Table 3.1) we can see the results of mineral content from ICP analysis for K, Ca and Na and the respective ratios as well as the drought efficiency:

Table 3.1 – Mineral composition of K, Ca and Na from all genotypes studied of the first experiment, as well as, drought efficiency and K/Na and (K+Ca)/Na ratios with respective standard deviation (STD).

	Genotype	Dry matter (g.plant^{-1})	Drought Efficiency (%)	K (%)	Ca (%)	Na (%)	K/Na	(K+Ca)/Na
Control	Sp757	1.25 ± 0.26	-	5.144 ± 0.100	0.615 ± 0.038	0.021 ± 0.003	243	272
	Sp41	1.17 ± 0.11	-	5.247 ± 0.200	0.669 ± 0.077	0.027 ± 0.002	196	221
	Sp804	1.46 ± 0.31	-	4.471 ± 0.333	0.748 ± 0.143	0.053 ± 0.002	85	99
	Sp563	1.08 ± 0.29	-	5.308 ± 0.198	0.721 ± 0.064	0.018 ± 0.002	297	337
	Sp2	1.18 ± 0.16	-	5.222 ± 0.160	0.614 ± 0.039	0.014 ± 0.002	363	406
	Sp492	1.07 ± 0.16	-	5.559 ± 0.174	0.730 ± 0.046	0.031 ± 0.007	179	203
	Sp912	1.01 ± 0.02	-	5.016 ± 0.235	0.648 ± 0.005	0.011 ± 0.001	444	501
	Sp732	1.13 ± 0.17	-	5.126 ± 0.475	0.738 ± 0.094	0.013 ± 0.003	388	443
	Adana99	1.17 ± 0.07	-	5.083 ± 0.351	0.684 ± 0.048	0.012 ± 0.002	419	475
Drought Stress	Sp757	0.44 ± 0.09	35	5.619 ± 0.144	0.802 ± 0.016	0.028 ± 0.002	316	361
	Sp41	0.37 ± 0.01	31	5.855 ± 0.221	0.956 ± 0.157	0.026 ± 0.006	228	265
	Sp804	0.45 ± 0.23	31	6.226 ± 0.007	0.825 ± 0.079	0.019 ± 0.000	329	373
	Sp563	0.33 ± 0.04	30	6.132 ± 0.073	1.099 ± 0.035	0.023 ± 0.005	267	314
	Sp2	0.34 ± 0.04	28	5.825 ± 0.068	0.845 ± 0.021	0.022 ± 0.001	267	305
	Sp492	0.30 ± 0.05	28	6.033 ± 0.059	1.041 ± 0.051	0.025 ± 0.003	242	284
	Sp912	0.28 ± 0.05	28	5.750 ± 0.131	0.863 ± 0.056	0.023 ± 0.003	248	285
	Sp732	0.29 ± 0.07	25	5.839 ± 0.025	1.069 ± 0.036	0.023 ± 0.003	249	295
	Adana99	0.34 ± 0.03	29	5.064 ± 0.137	0.742 ± 0.021	0.018 ± 0.001	286	328

The data shown are approximations. The calculations were done with the real values.

Drought efficiency was calculated by dividing the dry matter of drought stress plants, by the dry matter of control plants. Genotypes were ordered by drought efficiency and Adanna99 was placed last.

For a better interpretation of the results, the chart with efficiency was drawn as it can be seen in Fig.3.1:

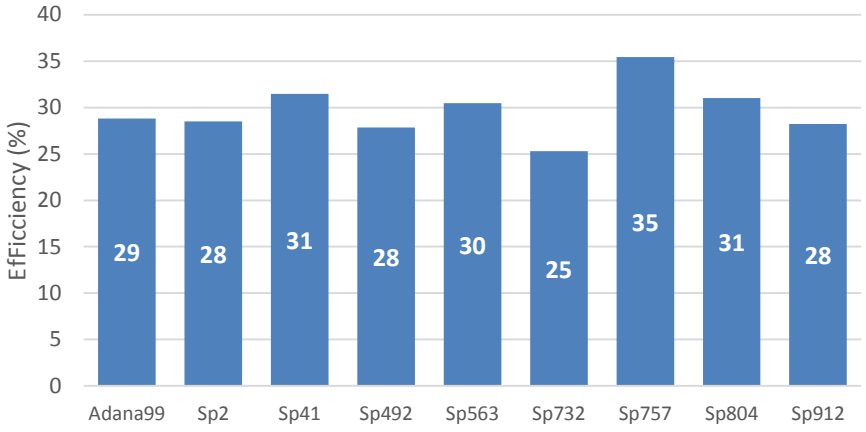

Fig. 3.1 – Drought efficiency results from the first experiment.

These results shows that the genotypes Sp41 (31%), Sp563 (30%), Sp757 (35%) and SP804 (31%) have better efficiency when compared with the reference genotype Adana99. However only these results doesn't prove that the previously spelt genotypes hold some traits that confer resistance to drought stress.

After determination of mineral content, the ratio K/Na was calculated (Fig.3.2):

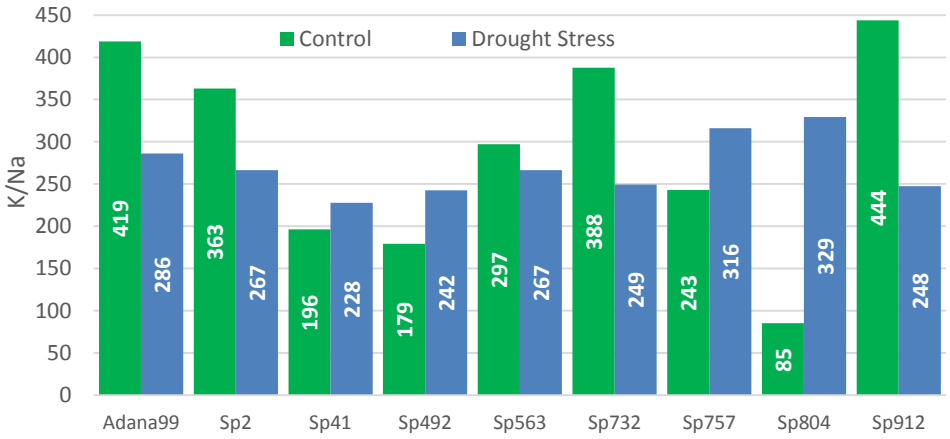

Fig. 3.2 – K/Na ratio from all genotypes tested from the first experiment under drought stress and control conditions.

The previously image clearly shows the different response of the same genotype when submitted to stress. Under control conditions (Green Bars) only the genotype Sp912 have better ratio than reference genotype, on the other hand, when the same genotypes were submitted to drought stress (Blue Bars) a totally different scenario happened. This time the genotypes Sp757 and Sp804 are the ones that have better ratio than reference genotype. This result enforces what was said earlier about the genotype Sp757 and Sp804 being better than modern wheat when submitted to drought stress. Although, despite the genotypes Sp41 and Sp563 have better drought efficiency than modern wheat, the K/Na ratio was lower.

A high cytosolic K/Na ratio is very important is because intracellular K^+ and Na^+ homeostasis is important for the activities of many cytosolic enzymes, and for maintaining membrane potential and an appropriate osmoticum for cell volume regulation (Zhu, 2003). The results shows that Na^+ contents increased under drought conditions, and this increase results in a decrease of K/Na ratio in all genotypes studied (Fig. 3.2).

In 2003, Zhu described that high concentration of minerals as Na^+ due to low concentration of water disrupts K^+ uptake by root cells, and when Na^+ enter in the cells and accumulates to high levels, it becomes toxic to enzymes. To prevent growth cessation or cell death, excessive Na^+ has to be expelled or compartmentalized in the vacuole. This compartmentation system not only lowers Na^+ concentration in the cytoplasm but also contributes to osmotic adjustment to maintain water uptake from saline solutions. Other organelles, such as plastids and mitochondria, may also accumulate some Na^+ (Zhu, 2003).

Plants limiting the uptake of toxic ions or maintaining normal nutrient ion contents could show greater tolerance (Khan *et al*., 2009) which is the case of some genotypes already mentioned in the present study. Also, some plants have better capacity to close stomata under drought stress, which permits to reduce the losses of water by transpiration. Abscicic acid (ABA) is the hormone response, besides other functions, to open and close stomata (Trejo *et al*., 1993). ABA have a crucial regulatory function under drought environments, so plants that are have higher capacity to sense drought and produce ABA are more adapted to dry soils, because plants response to water deficit with the accumulation of ABA in leaves, which induces stomatal closure and inhibits opening (Wilkinson and Davies 2002). This is a fundamental process in the plant's capacity to maintain plant water status under conditions of low soil moisture content and high evaporative demand.

The remaining macronutrients (P, S and Mg) and micronutrients (Zn, Fe, Mn, Cu, B and Al) were also analyzed. A brief analysis in the Table 3.2 show us that in general, under control conditions, Adana99 has more minerals in its composition, on the other hand, under drought stress Adana99 loses more nutrients than spelt genotypes. This is a very good result at a nutritional level because in regions as Sub-Saharan Africa, where drought is a big problem and there are lot of health problems due to nutritional deficiencies on people's diet, there is a need to implement new strategies to solve those problems (Chrispels and Sadava, 2003).

Table 3.2 – Mineral composition from all Spelt genotypes studied in the first experiment and Adana99, with respective STD.

	Genotype	P	S	Mg	Zn	Fe	Mn	Cu	B	Al
		(%)			(mg.kg^{-1})					
Control	Sp757	0.544 ± 0.074	0.390 ± 0.034	0.180 ± 0.010	40 ± 3	51 ± 5	96 ± 14	9 ± 0	21 ± 3	9 ± 2
	Sp41	0.429 ± 0.038	0.380 ± 0.012	0.187 ± 0.015	47 ± 0	53 ± 6	126 ± 14	9 ± 1	20 ± 2	9 ± 1
	Sp804	0.422 ± 0.029	0.376 ± 0.026	0.164 ± 0.003	42 ± 1	43 ± 4	113 ± 19	7 ± 0	19 ± 0	8 ± 1
	Sp563	0.559 ± 0.152	0.422 ± 0.064	0.198 ± 0.022	53 ± 11	50 ± 4	118 ± 12	9 ± 1	19 ± 3	10 ± 0
	Sp2	0.425 ± 0.061	0.386 ± 0.014	0.176 ± 0.003	44 ± 2	49 ± 2	111 ± 12	8 ± 1	17 ± 3	8 ± 1
	Sp492	0.473 ± 0.030	0.398 ± 0.039	0.201 ± 0.005	52 ±3	49 ± 2	123 ± 6	9 ± 0	19 ± 0	8 ± 2
	Sp912	0.412 ± 0.042	0.395 ± 0.026	0.175 ± 0.008	41 ± 2	51 ± 2	103 ± 6	8 ± 1	19 ± 1	10 ± 1
	Sp732	0.507 ± 0.060	0.449 ± 0.026	0.201 ± 0.025	51 ± 7	49 ± 4	117 ± 15	9 ± 1	19 ± 2	6 ± 1
	Adana99	0.481 ± 0.055	0.378 ± 0.012	0.183 ± 0.012	45 ± 6	53 ± 2	116 ± 9	9 ± 0	19 ± 1	10 ± 1
Drought Stress	Sp757	0.394 ± 0.015	0.371 ± 0.025	0.185 ± 0.015	37 ± 1	55 ± 2	130 ± 23	9 ± 0	15 ± 1	8 ± 2
	Sp41	0.364 ± 0.020	0.356 ± 0.023	0.198 ± 0.019	45 ± 3	55 ± 3	157 ± 9	11 ± 1	17 ± 3	10 ± 2
	Sp804	0.381 ± 0.008	0.359 ± 0.005	0.194 ± 0.002	51 ± 4	57 ± 3	131 ± 13	10 ± 0	15 ± 2	10 ± 2
	Sp563	0.398 ± 0.024	0.435 ± 0.015	0.223 ± 0.005	44 ± 3	56 ± 3	151 ± 7	10 ± 0	19 ± 3	9 ± 2
	Sp2	0.322 ± 0.006	0.359 ± 0.017	0.187 ± 0.003	33 ± 0	52 ± 1	130 ± 5	8 ± 0	16 ± 1	10 ± 2
	Sp492	0.371 ± 0.015	0.362 ± 0.021	0.220 ± 0.015	40 ± 1	55 ± 2	156 ± 21	9 ± 0	16 ± 1	10 ± 0
	Sp912	0.348 ± 0.006	0.386 ± 0.007	0.188 ± 0.011	42 ± 1	61 ± 2	136 ± 20	10 ± 1	13 ± 3	13 ± 2
	Sp732	0.378 ± 0.023	0.393 ± 0.030	0.225 ± 0.013	41 ± 1	53 ± 1	149 ± 14	11 ± 0	17 ± 2	10 ± 1
	Adana99	0.380 ± 0.018	0.326 ± 0.013	0.179 ± 0.006	43 ± 2	52 ± 6	149 ± 20	9 ± 0	14 ± 0	7 ± 0

However, due to problems of seed germination in this experiment, it is not possible to state with certainty that the results reflect reality. For this reason in the second experiment all seeds were vernalized before sowing. Other genotypes were selected for this experiment to extend our range of genotypes.

3.2. 2nd Experiment

In the second experiment, morphologically, the best two genotypes chosen were Sp92 and Sp96 and the worst were Sp67 and Sp801.

In the following table (Table 3.3) we can see the results of mineral content from ICP analysis for K, Ca and Na and the respective ratios as well as the drought efficiency for the second experiment:

Table 3.3 – Mineral composition of K, Ca and Na from all genotypes studied of the second experiment, as well as, drought efficiency and K/Na and (K+Ca)/Na ratios with respective STD.

	Genotype	Dry matter (g.plant^{-1})	Drought Efficiency (%)	K (%)	Ca (%)	Na (%)	K/Na	(K+Ca)/Na
Control	Sp53	0.43 ± 0.03	-	5.233 ± 0.064	0.686 ± 0.016	0.013 ± 0.001	416	471
	Sp92	0.47 ± 0.02	-	5.191 ± 0.119	0.700 ± 0.018	0.015 ± 0.000	353	400
	Sp96	0.40 ± 0.01	-	5.346 ± 0.117	0.656 ± 0.038	0.009 ± 0.001	578	649
	Sp225	0.40 ± 0.01	-	5.169 ± 0.067	0.659 ± 0.032	0.010 ± 0.002	530	597
	Sp382	0.35 ± 0.03	-	5.161 ± 0.231	0.746 ± 0.033	0.011 ± 0.002	472	541
	Sp67	0.41 ± 0.02	-	5.130 ± 0.128	0.589 ± 0.029	0.014 ± 0.001	362	403
	Sp801	0.35 ± 0.02	-	4.963 ± 0.216	0.791 ± 0.038	0.010 ± 0.001	505	585
	Sp69	0.43 ± 0.03	-	4.992 ± 0.083	0.696 ± 0.031	0.012 ± 0.000	402	458
	Adana99	0.46 ± 0.02	-	4.552 ± 0.082	0.621 ± 0.039	0.010 ± 0.000	462	525
Drought Stress	Sp53	0.16 ± 0.02	38	5.728 ± 0.045	0.781 ± 0.041	0.008 ± 0.001	761	865
	Sp92	0.17 ± 0.01	36	5.575 ± 0.093	0.732 ± 0.115	0.009 ± 0.001	590	667
	Sp96	0.14 ± 0.00	36	5.619 ± 0.157	0.762 ± 0.028	0.008 ± 0.000	722	820
	Sp225	0.14 ± 0.01	36	5.406 ± 0.199	0.799 ± 0.040	0.008 ± 0.001	642	737
	Sp382	0.12 ± 0.02	36	5.611 ± 0.201	0.861 ± 0.090	0.008 ± 0.001	691	797
	Sp67	0.14 ± 0.01	34	5.474 ± 0.129	0.764 ± 0.025	0.009 ± 0.001	630	718
	Sp801	0.12 ± 0.02	34	5.359 ± 0.171	1.073 ± 0.077	0.008 ± 0.000	712	854
	Sp69	0.13 ± 0.03	30	4.986 ± 0.703	0.778 ± 0.229	0.008 ± 0.001	618	714
	Adana99	0.17 ± 0.01	37	4.912 ± 0.041	0.753 ± 0.018	0.008 ± 0.001	604	697

The data shown are approximations. The calculations were done with the real values.

Once again, for a better interpretation of the results, the chart with efficiency was drawn and it's shown below in Fig. 3.3:

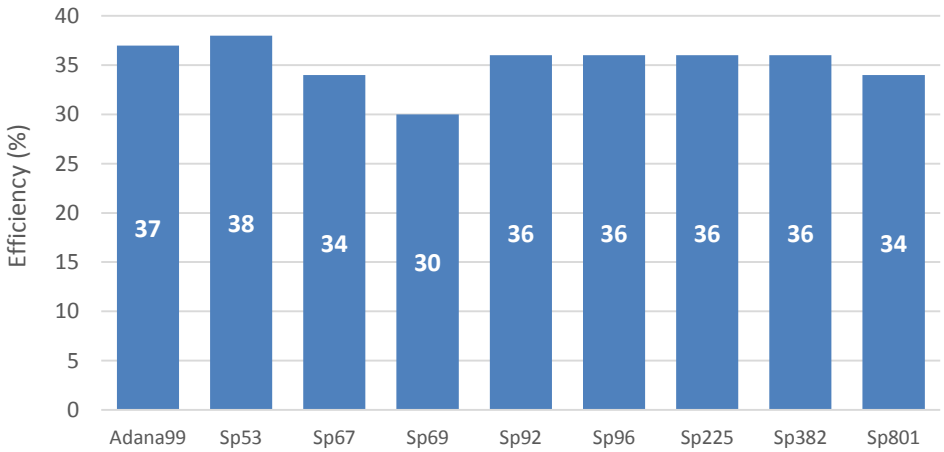

Fig. 3.3 – Drought efficiency from second experiment.

This time, the results shows that only the genotype Sp53 (38%) have a better efficiency when compared with the reference genotype.

Again, after determination of mineral content, the ratio K/Na was calculated and it is shown on the next figure (Fig. 3.4):

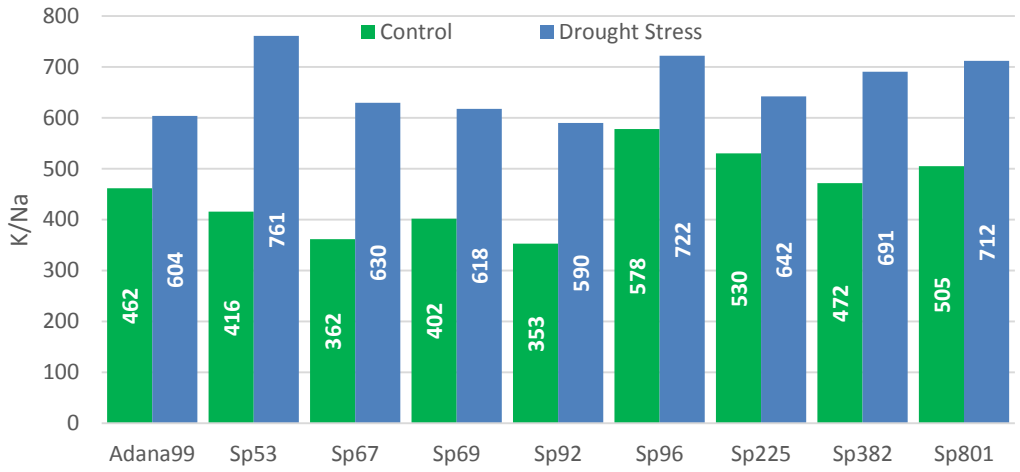

Fig. 3.4 – K/Na ratio from all genotypes tested from the second experiment under drought stress and control conditions.

This time if we take a look in Fig. 3.4, under control conditions only the genotype Sp92 have lower K/Na ration than reference genotype. Under drought stress conditions the genotypes Sp53, Sp96 and Sp801 got a higher score however the genotype 801 have a low drought efficiency as it was shown in Fig. 3.3. Comparing this results with the previous, the genotypes Sp53 and Sp96 could be a better genotypes under drought stress when compared with modern wheat.

It is known that drought stress lead to cellular dehydration, which causes osmotic stress and removal of water from the cytoplasm, resulting in a reduction of the cytosolic and vacuolar volumes (Bartels and Sunkar, 2005). The major difference between the low-water-potential environments caused by salinity versus drought is the total amount of water available. During drought, a finite amount of water can be obtained from the soil profile by the plant, decreasing soil water potential. In most saline environments, a large amount of water is at a constant, but under low water potential (Leksungnoen, 2012).

As in the previous experiment, the remaining Macro and Micronutrients were also analyzed. This time, such as occurred on drought efficiency, the results were not as good as expected as it can be seen in the Table 3.4:

Table 3.4 – Mineral composition from all Spelt genotypes studied in the second experiment and Adana99, with respective STD

	Genotype	P	S	Mg	Zn	Fe	Mn	Cu	B	Al
		(%)			(mg.kg^{-1})					
Control	Sp53	0.384 ± 0.035	0.176 ± 0.003	0.362 ± 0.017	42 ± 1	60 ± 1	77 ± 3	9 ± 0	17 ± 3	11 ± 3
	Sp92	0.376 ± 0.009	0.182 ± 0.005	0.399 ± 0.015	51 ± 2	53 ± 2	93 ± 2	8 ± 0	17 ± 3	5 ± 1
	Sp96	0.450 ± 0.026	0.176 ± 0.004	0.367 ± 0.006	53 ± 1	59 ± 1	105 ± 3	10 ± 0	15 ± 1	6 ± 1
	Sp225	0.460 ± 0.009	0.201 ± 0.006	0.379 ± 0.019	58 ± 8	61 ± 2	104 ± 6	10 ± 0	20 ± 1	6 ± 1
	Sp382	0.410 ± 0.009	0.213 ± 0.006	0.435 ± 0.008	59 ± 2	61 ± 1	89 ± 3	10 ± 1	19 ± 3	7 ± 1
	Sp67	0.398 ± 0.030	0.175 ± 0.008	0.369 ± 0.011	59 ± 5	59 ± 1	103 ± 6	10 ± 0	16 ± 2	6 ± 0
	Sp801	0.501 ± 0.043	0.193 ± 0.004	0.377 ± 0.008	68 ± 4	59 ± 2	109 ± 3	10 ± 1	18 ± 2	7 ± 2
	Sp69	0.474 ± 0.032	0.191 ± 0.006	0.348 ± 0.006	49 ± 1	56 ± 1	92 ± 3	9 ± 0	16 ± 2	5 ± 1
	Adana99	0.450 ± 0.021	0.175 ± 0.008	0.374 ± 0.021	57 ± 3	47 ± 2	111 ± 8	8 ± 0	15 ± 1	5 ± 0
Drought Stress	Sp53	0.309 ± 0.017	0.176 ± 0.005	0.338 ± 0.013	28 ± 3	62 ± 2	76 ± 2	9 ± 0	16 ± 1	18 ± 2
	Sp92	0.283 ± 0.011	0.172 ± 0.002	0.370 ± 0.011	33 ± 2	56 ± 4	87 ± 4	8 ± 0	18 ± 4	13 ± 4
	Sp96	0.326 ± 0.005	0.161 ± 0.012	0.346 ± 0.020	35 ± 6	61 ± 2	97 ± 22	9 ± 0	17 ± 2	13 ± 4
	Sp225	0.312 ± 0.009	0.184 ± 0.007	0.353 ± 0.014	39 ± 2	58 ± 1	103 ± 5	9 ± 0	19 ± 1	10 ± 1
	Sp382	0.314 ± 0.003	0.196 ± 0.011	0.402 ± 0.014	39 ± 1	64 ± 5	90 ± 7	10 ± 0	17 ± 1	13 ± 9
	Sp67	0.316 ± 0.013	0.176 ± 0.007	0.340 ± 0.013	42 ± 1	61 ± 1	111 ± 3	9 ± 0	17 ± 1	10 ± 1
	Sp801	0.315 ± 0.010	0.175 ± 0.012	0.313 ± 0.009	38 ± 3	56 ± 4	101 ± 4	9 ± 1	19 ± 0	12 ± 6
	Sp69	0.444 ± 0.198	0.168 ± 0.023	0.312 ± 0.008	27 ± 6	49 ± 9	83 ± 19	8 ± 2	19 ± 6	8 ± 0
	Adana99	0.337 ± 0.012	0.170 ± 0.004	0.315 ± 0.014	36 ± 0	49 ± 2	92 ± 3	8 ± 0	16 ± 1	10 ± 1

For better evaluation of our results, the Efficiency vs K/Na chart was calculated from both experiments and it's shown in Fig. 3.5 and Fig. 3.6. This can elucidate us to conclude which genotypes might have better yields under drought conditions and also if they're more adaptive than modern wheat (Adana99) to survive in these conditions.

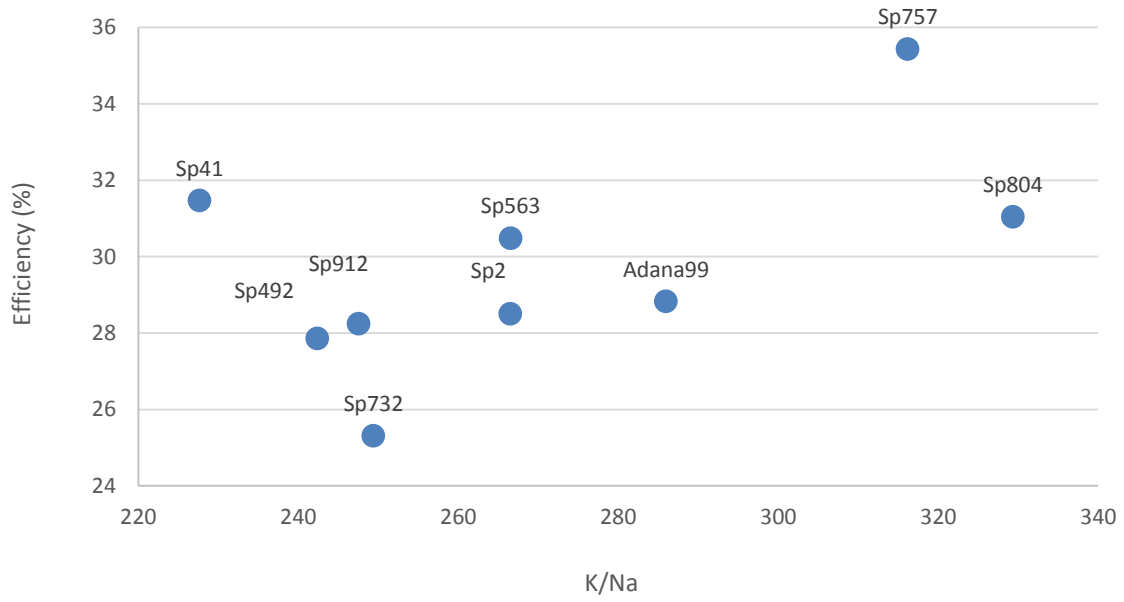

Fig. 3.5 – Efficiency vs K/Na from the results from the first experiment.

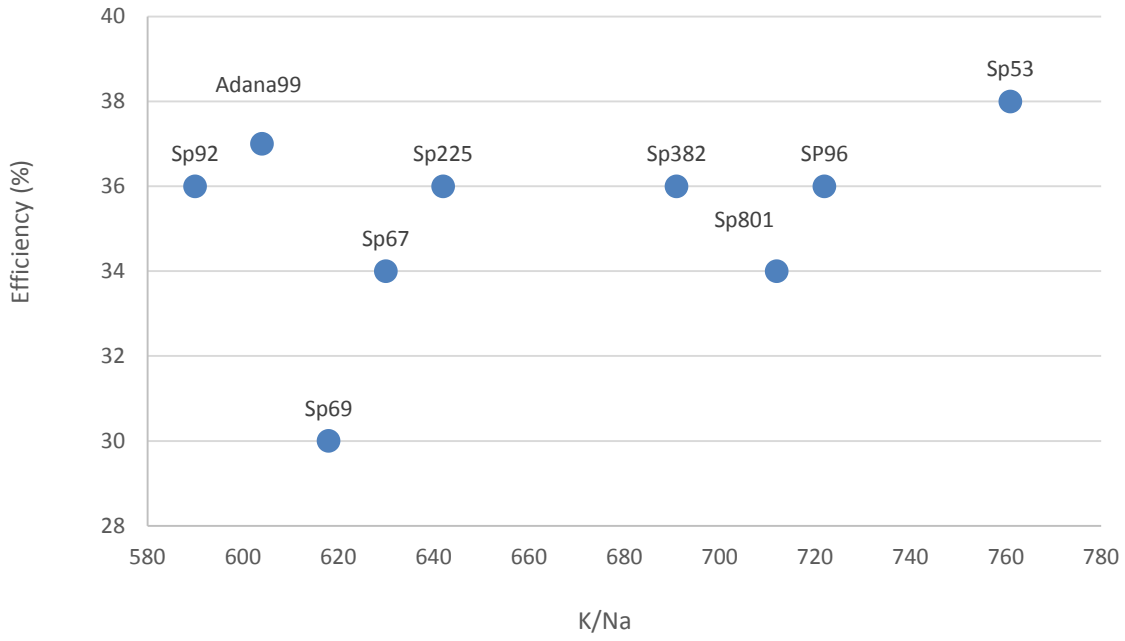

Fig. 3.6 – Efficiency vs K/Na from the results from the second experiment.

The previous charts help us to conclude which genotypes could come to be more resistant under drought stress. Using this information we can say that from the first experiment genotypes Sp804 and Sp757 are good candidates, and from the second experiment the genotypes Sp53 and Sp96 are the ones that might be more resistant under drought stress.

3.3. 3rd Experiment

For the third experiment, we selected the best two and worst genotypes from the previous experiments. The genotypes selected as good were the Sp96 and Sp563 and as bad were Sp41, Sp67, Sp69, Sp92, Sp732 and Sp912.

Unfortunately the genotypes Sp53, Sp757 and Sp804 which were the better candidates as drought tolerant plants, as it can be seen in our results, couldn't be selected for this experiment due to the lack of stock of seeds in our bank of seeds. We hope that in future experiments we can repeat these tests using those genotypes.

In this experiment, after morphological analysis we concluded that the worst genotypes under drought stress were the Sp41 and Sp96 and the best genotypes were the Sp67 and Sp732. In the next figures it is possible to analyze the genotypes under drought stress with the control conditions and compare the good ones with the bad ones (Fig. 3.7; Fig. 3.8; Fig. 3.9):

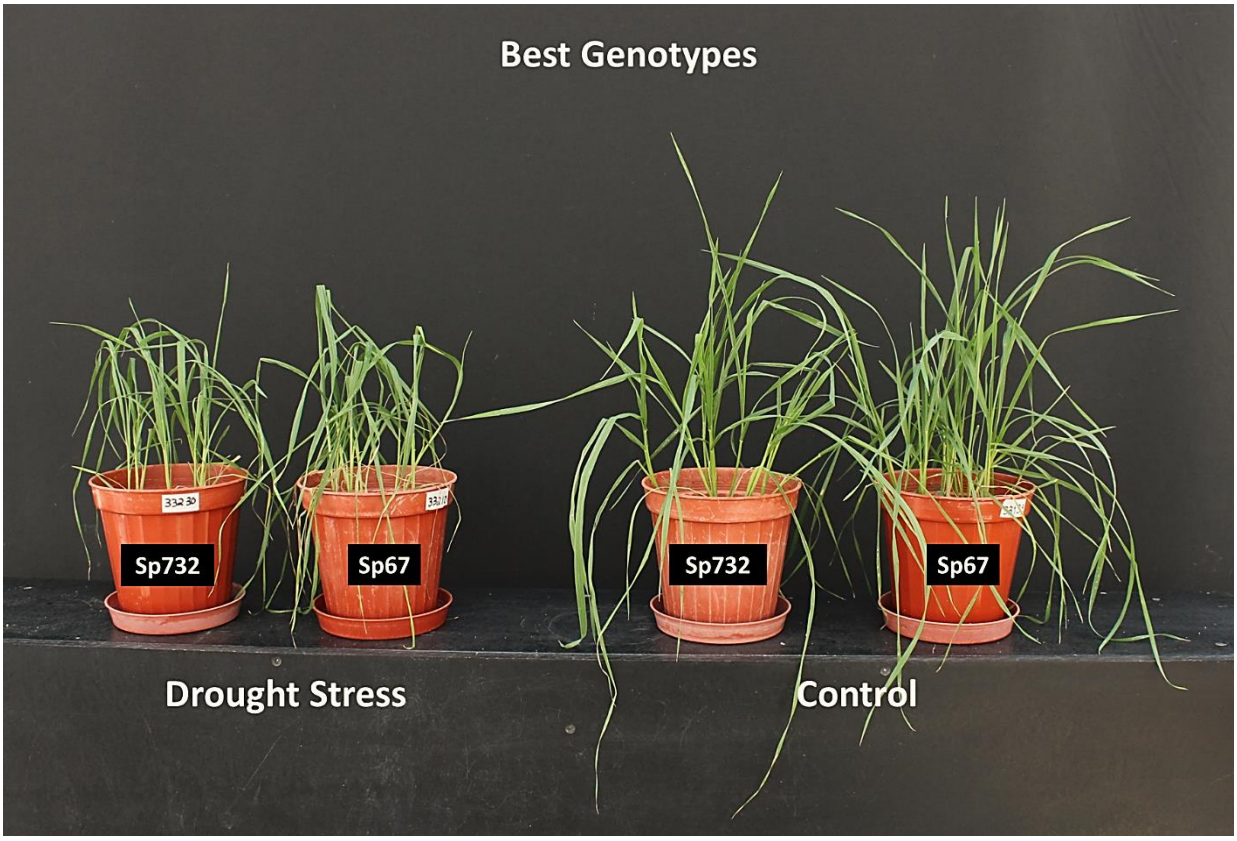

Fig. 3.8 – Comparison of the best genotypes under drought stress with the respective controls.

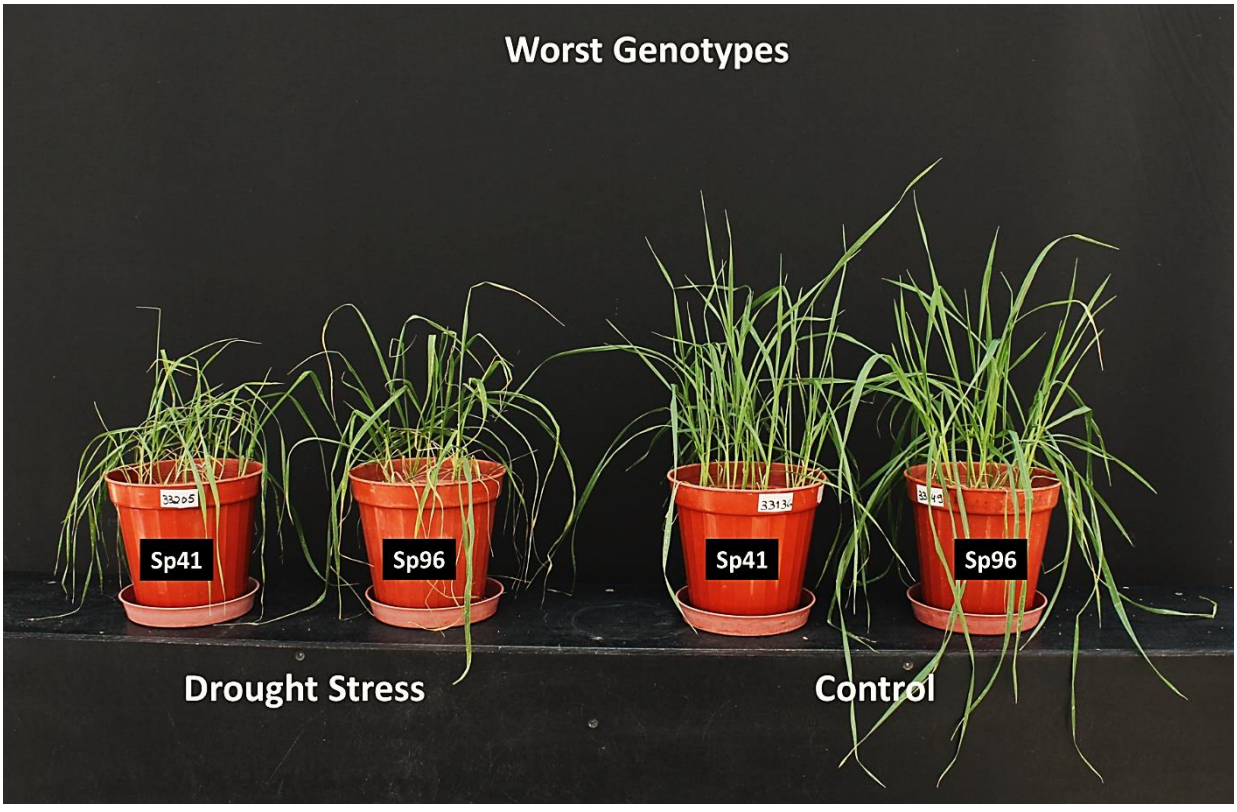

Fig. 3.7 – Comparison of the worst genotypes under drought stress with the respective controls.

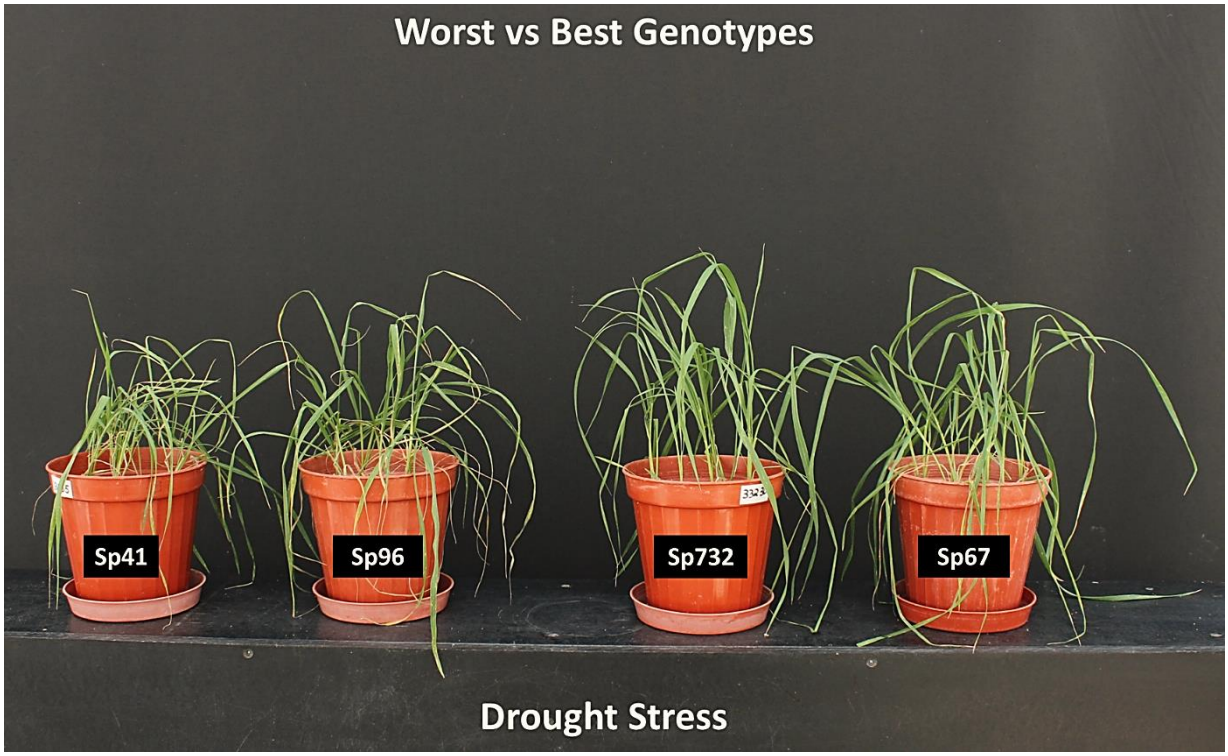

Fig. 3.9 – Comparison between the worst (on the left side) and best (on the right side) genotypes under drought stress.

In the Fig. 3.7 we can clearly see that under drought stress the leaves become more yellow, thinner and wrinkled when compared with the control, on the other hand, the difference between the two best genotypes under drought stress with the control (Fig. 3.8) are not so extreme. The leaves are thicker and more vigorous of the genotypes Sp67 and Sp732 and this difference between good and bad genotypes under stress is more notable in the Fig. 3.9. However, as we said in the previous experiments, only with morphological analyzes it is not possible to conclude if those genotypes really have better yields under drought stress conditions.

Again, as in the first and second experiment, the efficiency was calculated (Fig. 3.10):

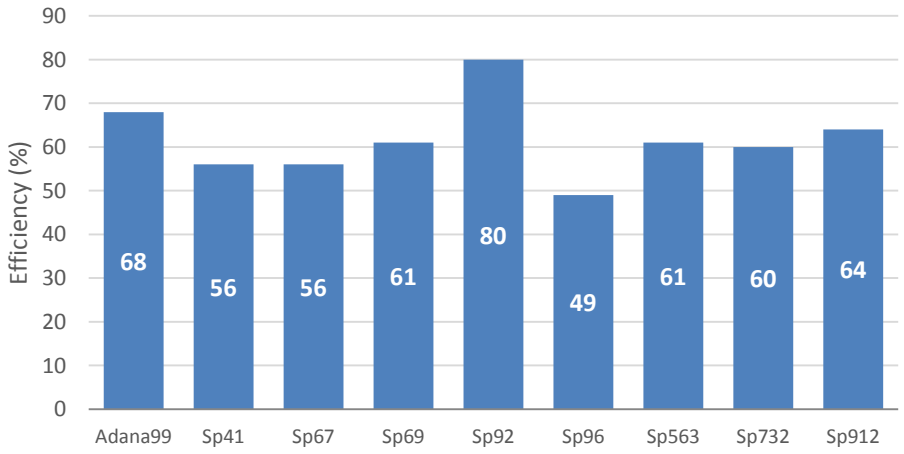

Fig. 3.10 – Drought efficiency results from the third experiment.

Using the previous results, we can affirm that the genotypes Sp92 (80%) and Sp912 (64%) have the better efficiency under drought stress, on the other hand, the genotypes Sp41 (56%), Sp67 (56%) and Sp96 (49%) have the lower efficiency. For the worst genotypes, the results coincide with the morphological analyzes, but for the best the results are not the same. An interesting thing that was noted was that the two genotypes with the better efficiency in this experiment had the lowest efficiency in the previous experiments. This fact can be explain with the duration of the experiments. In the first experiment the number of days under drought stress was longer than in the third experiment, and also in the first experiment we didn't proceed to vernalization. The reason why the efficiencies are so high in this experiment is because of the short period of drought stress applied. It is important to study drought stress during long periods because some plants can tolerate better drought stress than others but only in short periods of time, and that may have happened in our experience.

As in the previous experiments, the following table (Table 3.5) shows the results of mineral content from ICP analysis for K, Ca and Na and the respective ratios as well as the drought efficiency:

Table 3.5 – Mineral composition of K, Ca and Na from all genotypes studied of the third experiment, as well as, drought efficiency and K/Na and (K+Ca)/Na ratios with respective STD

	Genotype	Dry matter (g.plant^{-1})	Drought Efficiency (%)	K (%)	Ca (%)	Na (%)	K/Na	(K+Ca)/Na
Control	Sp92	0.28 ± 0.03	-	5.053 ± 0.718	0.530 ± 0.104	0.008 ± 0.002	603	667
	Sp912	0.29 ± 0.03	-	5.524 ± 0.094	0.573 ± 0.025	0.009 ± 0.002	606	669
	Sp563	0.27 ± 0.03	-	5.282 ± 0.185	0.581 ± 0.042	0.011 ± 0.001	475	528
	Sp69	0.30 ± 0.05	-	5.241 ± 0.135	0.559 ± 0.039	0.007 ± 0.001	733	811
	Sp732	0.36 ± 0.11	-	4.790 ± 0.613	0.647 ± 0.121	0.011 ± 0.002	451	712
	S67	0.36 ± 0.09	-	5.290 ± 0.098	0.521 ± 0.035	0.007 ± 0.000	747	820
	S41	0.28 ± 0.03	-	5.103 ± 0.118	0.631 ± 0.031	0.008 ± 0.001	640	720
	Sp96	0.32 ± 0.02	-	5.460 ± 0.029	0.507 ± 0.025	0.007 ± 0.001	730	798
	Adana99	0.27 ± 0.06	-	4.653 ± 0.042	0.507 ± 0.038	0.008 ± 0.001	556	617
Drought Stress	Sp92	0.22 ± 0.02	80	5.727 ± 0.085	0.549 ± 0.021	0.008 ± 0.001	678	743
	Sp912	0.19 ± 0.02	64	6.078 ± 0.080	0.617 ± 0.093	0.009 ± 0.001	708	779
	Sp563	0.17 ± 0.02	61	5.824 ± 0.058	0.645 ± 0.055	0.009 ± 0.001	618	687
	Sp69	0.18 ± 0.02	61	5.585 ± 0.184	0.570 ± 0.054	0.009 ± 0.001	608	670
	Sp732	0.22 ± 0.02	60	5.603 ± 0.112	0.637 ± 0.048	0.009 ± 0.001	610	679
	Sp67	0.20 ± 0.04	56	5.731 ± 0.138	0.511 ± 0.060	0.010 ± 0.001	592	645
	Sp41	0.16 ± 0.03	56	5.535 ± 0.106	0.691 ± 0.057	0.009 ± 0.001	646	727
	Sp96	0.16 ± 0.01	48	6.007 ± 0.246	0.560 ± 0.054	0.008 ± 0.001	735	803
	Adana99	0.18 ± 0.01	68	5.200 ± 0.108	0.615 ± 0.023	0.008 ± 0.001	688	770

The data shown are approximations. The calculations were done with the real values.

With mineral contents obtained from ICP analyses showed in the previous table, K/Na ratio was calculated (Fig 3.11):

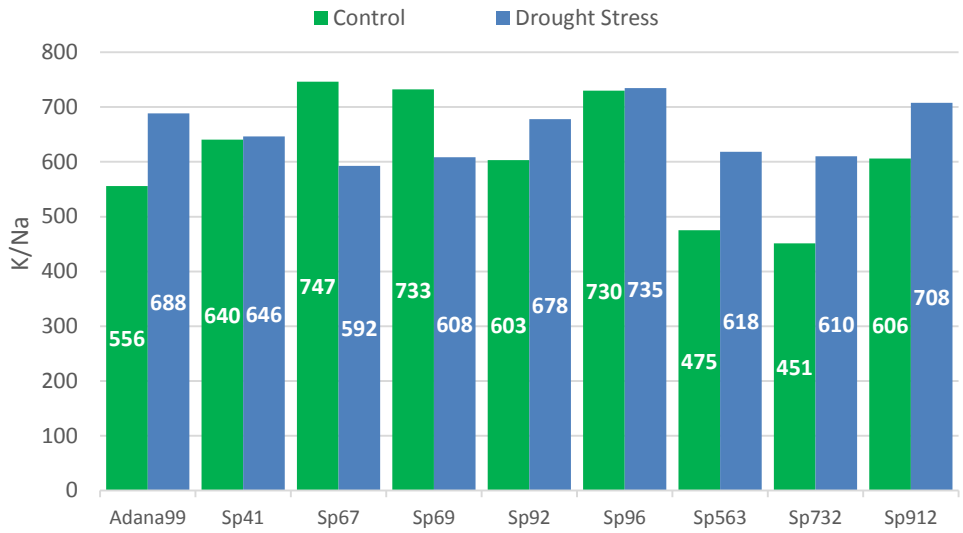

Fig. 3.11 – K/Na ratio from all genotypes tested from the third experiment under drought stress and control conditions.

This time, the highest K/Na ratio under stress belongs to Sp96 and Sp912, and the lowest to Sp67 and Sp69. These results together with the previous proves that the genotypes Sp67 and Sp69 are not good under drought conditions, on the other hand, the genotypes Sp92 and Sp912, and the genotype Sp96, which were the worse and better respectively in the previous experiments, this time shows the best results for efficiency and K/Na ratio, as we can see in the Fig. 3.12:

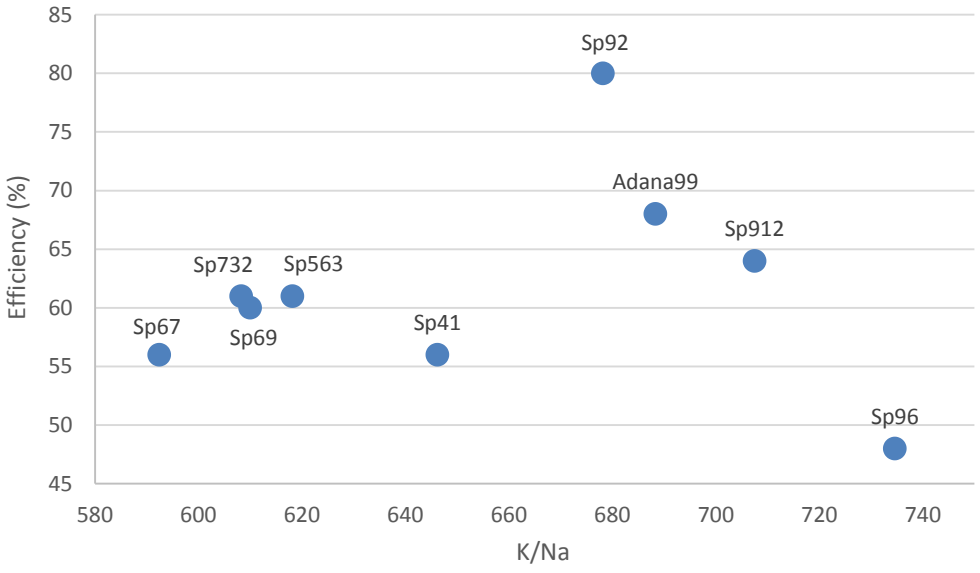

Fig. 3.12 – Efficiency vs K/Na from the results from the third experiment.

As we referred before, these results are slightly controversial and it is not possible for us conclude certainty about which genotypes are more adapted to drought stress conditions. The reason why could be because of vernalization and/or the duration of stress on these experiments.

However, there is a strong evidence in these results that spelt is a more adapted type of wheat under drought conditions. One more time, the genotypes Sp92, Sp96 and Sp912 are candidates to the more tolerant genotypes under these conditions.

The following table (Table 3.6) presents the remaining macronutrients and micronutrients studied in this experiments as we did before:

Table 3.6 – Mineral composition from all Spelt genotypes studied in the third experiment and Adana99, with respective STD.

	Genotype	P	S (%)	Mg	Zn	Fe	Mn	Cu (mg.kg^{-1})	B	Al
Control	Sp92	0,422 ± 0.065	0,335 ± 0.042	0,155 ± 0.017	74 ± 9	59 ± 8	106 ± 23	10 ± 1	12 ± 1	9 ± 1
	Sp912	0,417 ± 0.037	0,347 ± 0.014	0,162 ± 0.011	81 ± 7	55 ± 1	102 ± 10	10 ± 0	15 ± 2	5 ± 1
	Sp563	0,449 ± 0.013	0,366 ± 0.016	0,180 ± 0.012	94 ± 7	55 ± 4	110 ± 4	10 ± 1	13 ± 1	7 ± 1
	Sp69	0,449 ± 0.013	0,305 ± 0.008	0,170 ± 0.007	58 ± 3	59 ± 1	98 ± 10	10 ± 1	14 ± 2	12 ± 2
	Sp732	0,414 ± 0.034	0,355 ± 0.042	0,174 ± 0.014	91 ± 10	48 ± 6	116 ± 7	12 ± 1	14 ± 3	6 ± 1
	Sp67	0,409 ± 0.017	0,307 ± 0.011	0,143 ± 0.006	67 ± 2	64 ± 12	104 ± 13	10 ± 1	14 ± 1	12 ± 1
	Sp41	0,375 ± 0.016	0,311 ± 0.006	0,151 ± 0.013	67 ± 5	63 ± 6	104 ± 12	10 ± 1	16 ± 1	14 ± 3
	Sp96	0,473 ± 0.036	0,322 ± 0.011	0,153 ± 0.006	68 ± 5	59 ± 3	104 ± 15	10 ± 0	13 ± 1	7 ± 2
	Adana99	0,462 ± 0.031	0,359 ± 0.021	0,154 ± 0.006	80 ± 5	50 ± 2	120 ± 5	10 ± 1	15 ± 3	6 ± 1
Drought Stress	Sp92	0,393 ± 0.012	0,355 ± 0.016	0,152 ± 0.004	68 ± 4	59 ± 1	103 ± 11	9 ± 1	16 ± 3	6 ± 1
	Sp912	0,418 ± 0.039	0,369 ± 0.005	0,153 ± 0.004	70 ± 1	59 ± 3	88 ± 8	10 ± 0	17 ± 3	9 ± 1
	Sp563	0,397 ± 0.022	0,381 ± 0.012	0,168 ± 0.004	77 ± 3	54 ± 1	106 ± 19	10 ± 1	17 ± 3	4 ± 1
	Sp69	0,423 ± 0.059	0,300 ± 0.010	0,149 ± 0.011	50 ± 3	55 ± 3	85 ± 18	9 ± 0	15 ± 2	5 ± 0
	Sp9732	0,402 ± 0.029	0,375 ± 0.023	0,174 ± 0.012	82 ± 3	53 ± 2	114 ± 19	12 ± 1	15 ± 2	5 ± 1
	Sp67	0,404 ± 0.017	0,321 ± 0.009	0,144 ± 0.005	55 ± 4	58 ± 1	107 ± 7	9 ± 1	15 ± 1	6 ± 1
	Sp41	0,357 ± 0.011	0,310 ± 0.006	0,157 ± 0.004	67 ± 6	54 ± 1	119 ± 4	9 ± 0	16 ± 2	7 ± 1
	Sp96	0,439 ± 0.013	0,342 ± 0.009	0,141 ± 0.008	60 ± 4	57 ± 2	95 ± 6	10 ± 0	10 ± 1	6 ± 1
	Adana99	0,377 ± 0.007	0,312 ± 0.009	0,150 ± 0.003	69 ± 3	51 ± 1	118 ± 7	9 ± 0	18 ± 2	5 ± 0

With the previous table, we can see that in general spelt is quantitatively richer than Adana99 as it was already mentioned and concluded in this work.

As expected, under drought stress, protein concentration decreases because the decrease in osmotic potential under drought stress reflects the increased hydrolysis of macromolecules such as proteins (Chutia and Borah, 2012). As suggested by earlier workers, protein degradation might be the result of increased activity of protease or other catabolic enzymes, which were activated under drought stress, or due to fragmentation of proteins due to toxic effects of reactive oxygen species resulting in reduced protein content. A decrease in the protein concentration would be a typical symptom of oxidative stress and has frequently been observed in drought stressed plants (Mafakheri *et al*., 2011). On the other hand, under drought stress, other proteins called dehydrins are also synthesized in response to drought stress. The dehydrin family of proteins accumulates in a wide range of plant species under dehydration stress (Mohammadkhani and Heidari, 2008). Dehydrins, CAT, GR, SOD, APX and other proteins are produced in high quantities under drought stress

Based on this, it is expected that the most tolerant genotypes to drought stress are the ones which had a higher protein concentration under stress compared with control conditions (Table 3.7). For enzymatic activity tests we selected the genotypes Sp41, Sp67, Sp92, Sp96 and Sp912 for being the best/worst genotypes under drought stress:

Table 3.7 – Protein concentration from the 5 selected genotypes studied on the third experiment.

Protein Concentration (mg g^{-1} FW)					
Condition	**Genotype**	**Protein Concentration ± STD**			**SE%**
Control	Sp41	35.0	±	4.5	13.0
	Sp67	33.2	±	6.7	20.3
	Sp92	29.7	±	2.1	7.2
	Sp96	25.5	±	1.8	6.9
	Sp912	27.5	±	4.4	16.1
Drought Stress	Sp41	32.3	±	1.1	3.3
	Sp67	34.5	±	4.3	12.3
	Sp92	32.8	±	2.5	7.6
	Sp96	28.0	±	2.1	7.3
	Sp912	35.5	±	4.2	11.8

From the previous table we can see that under drought stress the genotype Sp92 and Sp912 has an increase of about 10% and 23% respectively of protein concentration, which could mean this genotype is more adapted to drought stress, producing high quantities of proteins responsible for drought tolerance and subsequently maintaining normal cell activities. The remaining had a lower increase or even a decrease of protein concentration.

Now, if we analyse individuality the activity of the main enzymes involved in drought response, there are an evidence again that the genotypes Sp92 and Sp912 are better adapted to drought conditions (Table 3.8):

Table 3.8 – SOD Activity from the 5 selected genotypes studied on the third experiment.

SOD Activity (U g^{-1} FW)						
Condition	**Genotype**	**SOD Activity ± STD**			**SE%**	**Increased Activity%**
Control	Sp41	44.0	±	1.4	3.2	-
	Sp67	46.2	±	1.2	2.6	-
	Sp92	45.0	±	5.0	11.0	-
	Sp96	40.8	±	7.3	17.8	-
	Sp912	43.3	±	3.3	7.7	-
Drought Stress	Sp41	51.0	±	1.7	3.2	15.9
	Sp67	48.8	±	1.4	2.8	5.6
	Sp92	51.6	±	1.3	2.6	14.7
	Sp96	50.9	±	1.3	2.6	24.8
	Sp912	51.8	±	1.6	3.0	19.6

On the previous table (Table 3.8) we see that under stress SOD activity from all genotypes increases because the plant is under stress and as we said before, SOD is the primary scavenger, which means is the first enzyme to be activated under stress conditions. SOD converts O^{2-} to H_2O_2 which are eliminated by APX in association with GR, so if there are a high production of this enzyme could mean that plant will remove easily those toxic molecules under stress. The genotype Sp96 and Sp912 are the ones which had a higher increase, on the other hand the genotypes Sp67 and Sp92 had the lower.

The following tables shows the activities of GR, CAT and APX enzymes (Fig. 3.9, Fig. 3.10 and Fig. 3.11):

Table 3.9 – GR Activity from the 5 selected genotypes studied on the third experiment.

GR Activity

(µmol [NADPH] g^{-1} FW min^{-1})

Condition	Genotype	GR Activity ± STD			SE%	Increased Activity%
Control	Sp41	22.3	±	9.2	41.2	-
	Sp67	16.3	±	2.0	12.1	-
	Sp92	15.2	±	2.1	13.6	-
	Sp96	11.7	±	1.6	13.5	-
	Sp912	12.9	±	1.0	7.9	-
Drought Stress	Sp41	11.7	±	4.1	34.8	- 47.5
	Sp67	14.5	±	3.8	26.2	- 11.0
	Sp92	15.9	±	2.2	14.1	4.6
	Sp96	13.5	±	2.6	19.6	15.4
	Sp912	19.2	±	2.2	11.6	48.8

Table 3.10 – CAT Activity from the 5 selected genotypes studied on the third experiment.

CAT Activity

(µmol H_2O_2 g^{-1} FW min^{-1})

Condition	Genotype	CAT Activity ± STD			SE%	Increased Activity%
Control	Sp41	2538	±	749	30	-
	Sp67	2758	±	506	18	-
	Sp92	2474	±	492	20	-
	Sp96	1969	±	134	7	-
	Sp912	1813	±	142	8	-
Drought Stress	Sp41	2136	±	337	16	- 15.8
	Sp67	2717	±	499	18	- 1.5
	Sp92	2397	±	435	18	- 3.1
	Sp96	2065	±	106	5	4.9
	Sp912	2254	±	429	19	24.3

Table 3.11 – APC Activity from the 5 selected genotypes studied on the third experiment.

APX Activity ($\mu mol\ H_2O_2\ g^{-1}\ FW\ min^{-1}$)						
Condition	**Genotype**	**APX Activity ± STD**			**SE%**	**Increased Activity%**
Control	Sp41	37.0	±	4.3	11.5	-
	Sp67	37.6	±	5.4	14.4	-
	Sp92	33.4	±	2.4	7.0	-
	Sp96	31.3	±	1.8	5.9	-
	Sp912	32.0	±	1.6	4.9	-
Drought Stress	Sp41	40.2	±	3.5	8.6	8.6
	Sp67	41.6	±	0.7	1.7	10.6
	Sp92	42.8	±	2.0	4.6	28.1
	Sp96	39.5	±	3.5	9.0	26.2
	Sp912	50.6	±	5.7	11.3	58.1

The previous results reinforces what was already mentioned about the genotypes Sp92 and Sp912 being better crops to use in dry areas, once these genotypes presents a higher enzymatic activity under drought stress. Higher protein concentration means more tools to fight and survive under these conditions, resulting in better grow rates.

Conclusions and Future Aspects

During evolution, plants have adapted to certain rainfall regimes and soil conditions, and not all crops are adapted to drought and adverse soil conditions to produce acceptable yield. The old solution to the problem of "not enough water and nutrients" was to irrigate and fertilize, but a new solution is to try to understand which genes offer adaptation to drought and to try to breed plants to overcome these major climatic and soil constraints.

Due to climate change and global warming, difficulties in agriculture are increasingly affecting the yields and nutritional quality. Drought, one of the most important factor of crop loses was tested in this work in several genotypes of Spelt Wheat. The results showed that Spelt is naturally more enriched than Wheat in macronutrients and micronutrients and also has a better growth efficiency under drought conditions. Some genotypes such as Sp41, Sp67 and Sp69 are very sensitive under these conditions, which means that are not good genotypes to use in future breeding programs to cross with modern wheats (for example Adana99), in order to confer more resistance to these wheats. High periods of drought reduce significantly biomass production, which is easily observed by morphological analyses, where the root and shoot are shorter and leaves are thinner, weak, dried, wrinkled and yellowish. It is noteworthy the genotypes Sp53, Sp96, Sp912, Sp757 and Sp804, had interesting results and thus is crucial to study further, due to the results obtained in growth efficiency and mineral content. Only a few parameters were studied, which results in some contradictory results and for these reason it is important to continue this work to get a more consistence results. Performing these tests in reproductive stages and seed formation is also very important because those are the most sensitive stages of plant's life cycle and a crucial factor for surviving.

It is important continue to study Spelt genotypes in order to find better ones which could be used in breeding programs. The consequences of succeed in this research can be not only improve the yields of crops in dry areas, where is extremely hard to practice agriculture, but also enhance the diet and health of people who live in these areas.

Conventional and biotechnological breeding are complementary approaches and can be expected to enhance the efficiency of breeding for stress resistance and yield. The use of physiological knowledge and powerful tools of molecular genetic analysis requires a systems approach, with agronomists, physiologists, breeders, and biotechnologists working together with farmers to raise crop yields and farmer income in stressful environments, particularly in marginal soils of the tropics.

Conclusões e Propostas Futuras

Ao longo da evolução, as plantas têm vindo a adaptarem-se a certos regimes de chuva e condições do solo, contudo nem todas as culturas estão adaptadas à seca e a condições adversas do solo de modo a produzirem rendimentos aceitáveis. A antiga solução para o problema de "não há suficiente água nem nutrientes" era irrigar e fertilizar, contudo a nova solução é tentar entender quais os genes que oferecem melhor adaptação à seca e cultiva-los de modo a superar estas grandes limitações climáticas e do solo.

Devido às alterações climatéricas e aquecimento global, problemas na agricultura são mais frequentes e têm vindo a afectar os rendimentos e a qualidade nutricional. A seca, um dos mais severos factores de perdas na agricultura, foi testada em vários genótipos de Espelta. Os resultados mostraram que Espelta é naturalmente mais enriquecida que o trigo em macro e micronutrientes, bem como uma melhor eficiência de crescimento sob condições de seca. Alguns genótipos como Sp41, Sp67 e Sp69 são muito sensíveis nestas condições, o que significa que não são bons genótipos a usar em programas de cruzamento com trigos modernos (por exemplo Adana99), a fim de conferir maior resistência a estes trigos sob estas condições. Longos períodos de seca reduzem significativamente a produção de biomassa, sendo facilmente observado morfologicamente, onde a raiz e o caule são bastante curtos e as folhas mais finas, fracas, secas, enrugadas e amareladas. Há que salientar que os genótipos Sp53, Sp96, SP912, Sp757 e Sp804, tiveram resultados interessantes e, deste modo, é crucial um estudo mais aprofundado. Neste trabalho apenas alguns parâmetros foram testados, o que levou a algumas contradições e por essa razão é importante continuar para obter resultados consistentes. A realização destes testes em fases reprodutivas e formação de sementes também é muito importante, pois essas são as fases mais sensíveis do ciclo de vida da planta e um fator crucial para a sobrevivência.

É importante continuar a estudar genótipos de Espelta, a fim de seleciona-los para programas de melhoramento genético. As consequências do sucesso nesta pesquisa podem não só melhorar o rendimento de culturas em áreas secas, onde é extremamente difícil a prática da agricultura, mas também melhorar a dieta e a saúde das pessoas que vivem nessas áreas.

O melhoramento genético convencional e biotecnológico são abordagens complementares e o conhecimento fisiológico e as técnicas de análise genética molecular juntamente com agrónomos, fisiologistas, e biotecnólogos em conjunto com os agricultores, aumentarão a produtividade em ambientes sob grande seca.

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