MORPHO-PHYSIOLOGICAL SCREENING FOR DROUGHT TOLERANCE AT SEEDLING STAGE IN WHEAT

FINAL YEAR PROJECT UG 09

SUBMITTED BY:

KAINAT RAO |NUST2016 |200571 |BSABSUG09

TUBA MARYAM |NUST2016 |152374 |BSABSUG09

HADIA KHAN |NUST2016 |200977 |BSABSUG09

ROHA ASMAT SAJID |NUST2016 |200700 |BSABSUG09

Atta-Ur-Rahman School of Applied Biosciences (ASAB)

National University of Sciences and Technology (NUST), Islamabad Pakistan

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This is to certify that the Final Year Project Titled

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ROHA ASMAT SAJID |NUST2016 |200700 |BSABSUG09

has been accepted towards the requirements for the undergraduate degree in APPLIED BIOSCIENCES

(Supervisor) MRS. NOSHEEN FATIMA

LECTURER

(ASAB), NUST

DR. HUSSAIN A. JANJUA

PRINCIPAL

(ASAB), NUST

DEDICATION

To our Parents and Teachers who kept motivating us through this whole journey of learning.

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List of Acronyms Used

CAT: Catalase

CMS: Cell Membrane Stability

CRD: Completely Randomized Design

FAO: Food and Agriculture Organization

GDP: Gross Domestic Production

GRI: Germination Rate Index

NARC: National Agricultural Research Centre

PARC: Pakistan Agricultural Research Council

PEG: Polyethylene Glycol

PMD: Pakistan Meteorological Department

ROS: Reactive Oxygen Species

RWC: Relative Water Content

SOD: Superoxide Dismutase

WSD: Water Saturation Deficit

ABSTRACT

Drought is one of the most common prevailing abiotic stress factors which has adverse effects on crop production. Wheat is the staple crop of Pakistan and its production potential is severely hampered by the water scarcity conditions. Water stress limits the growth of wheat by affecting its physiological, morphological, biochemical, and molecular pathways necessary to develop a good crop cover. This study was performed to analyse some of the physio-morphological parameters under the water deficit environments and to evaluate and identify the most tolerant and susceptible wheat genotypes. The traits conducted in the study included germination percentage, root and shoot length, coleoptile length, seedling vigour and water saturation deficit. There were ten genotypes under study and comparisons were made with two control genotypes, known to have better drought tolerance. Water stress was induced by using a 20% polyethylene glycol (PEG 6000) solution. Water stress had a significantly higher effect on shoot, root and coleoptile development affecting the lengths. Based on our study, two potential genotypes were identified for further intensive screening at biochemical and molecular level. Such screening procedures are the basis of breeding programs. Breeders use this information to either improve existing varieties by crossing them with tolerant varieties or by improving yield of tolerant genotypes so that they can be used commercially.

Chapter 1

Introduction

Agriculture serves as the backbone of Pakistan's economy by contributing around 18-25% to the total GDP (Gross Domestic Product). (Pakistan Bureau of statistics, 2018) It is heavily dependent on water availability. About 88% of available water is used in agriculture. (Siddiqi, 2012) That is why it is important to know about the agrarian background of the country.

Pakistan's Agriculture is divided into irrigated and rainfed regions:

- **Irrigated regions** include a system of supplying water to land by means of artificial canals, ditches etc to promote the growth of crops.
- **Rainfed regions** are dependent on rainwater and are further divided into arid and semi-arid regions depending upon the rate of annual rainfall:

a. **Arid regions** get less than 25 cm rainfall annually and include all cold and hot deserts.
   These areas usually lack substantial vegetation due to moisture stress.

b. **Semi-arid regions** receive a yearly rainfall that ranges from 25-75 cm. (Pakistan Journal of Meteorology, 2012)

The crops are grown either with rainwater that has percolated into the ground or using irrigation. Cropping system of Pakistan is divided into the following seasons: (Rehman, 2015)

- **Rabi Crops:** They are sown between October and December and then harvested during the months of April to May. The major cash crops include wheat, lentils, mustard and barley etc.

- **Kharif Crops:** These crops are sown in the months of April to June and harvesting is done during October to December which include cotton, rice, maize and sugarcane etc. (Agrinfobank, 2019)

Among all the major cash crops of rainfed areas, wheat is considered as a staple food crop in Pakistan. Wheat belongs to the genus Triticum, in which the specie *Triticum aestivum* holds a significant economic importance. It is a major source of carbohydrates, thus considered as the most important grain in the world. It is a food source for approximately one-third of the globe's population. (Hussain, 2017). Wheat comprises of 60% diet of an individual in Pakistan. It also occupies a crucial position among the government policies of Pakistan. (PARC, 2015)

Along with Pakistan, wheat is the prime part of agricultural trade globally as well. Its cultivation land covers around 220 million hectares with an estimated production of about 564.6 million tonnes. Pakistan holds the 9th position between other countries on the grounds of per annum wheat production. having a largest cultivation area among all other food crops. (AgroChart, 2019)

As per Pakistan Economic Survey in the year 2017-18, wheat secures 4th position among the list of major crops from the economical viewpoint. The mentioned crop provides around 9.1% to the overall agriculture of Pakistan and about 1.7% to the GDP. (Memon, 2017)

It is evident that major food crops especially wheat have a significant economic and nutritional value, but they also face major productivity losses in Pakistan. These losses are caused by some biotic and abiotic stresses. Abiotic stresses including heat, salinity and drought are a major matter of concern. According to the Pakistan Bureau of Statistics, there have been variations in per annum wheat production in Pakistan in recent past years as well as a significant decrease in the percentage yield of wheat during 2017-18. This decrease in the wheat production is caused by several factors which include:

- Usage of poor-quality seeds with low yield that are prone to diseases and pests
- Inadequate use of the fertilizers.
- **Drought**: shortage of water and weeds infestation that reduce the wheat yield significantly. (PARC, 2015)

Drought is the leading constraint among the others which are causing a decrease in wheat production worldwide. The frequency and severity of drought has intensified because of increased temperature. In reference to the prevalent climate changes, the unpredictable rainfall patterns have severely affected the crop of wheat in Pakistan by decreasing its productivity. In 2018, there was less rainfall than expected during the monsoon season in Pakistan (May-August), causing Sindh to deal with water shortage of 69.5% below average, and Baluchistan 45% below average. Thus, led to reduction of water and food including wheat. (Web, 2019)

According to the report by Pakistan Meteorological Department (PMD), Southern Pakistan has been susceptible to alarming drought conditions which are expected to move towards more deterioration in next four years. (Web, 2019) Moreover, it is assumed that the available amount of water for cultivation would drop from 87-73% in the developing countries. In Pakistan, it is estimated that the water level decrease would be 72-62%. (Khan S. T., Can irrigation be sustainable?, 2013)

Considering the above-mentioned water scarcity, dry spell can influence the harvest yield of wheat significantly. The damage relies upon its severity and the developmental phase of the plant at which dry spell happens. Various stages have various responses to drought; for example, a few phases have the capacity to adapt to stress by holding water potential, turgor pressure or by the proficient usage of water. Drought has various consequences for crop plants including:

- A decrease in the leaf size
- Diminished stem expansion
- Restricted multiplication of roots
- Decreased water retention
- Diminished yield
- Disruption of osmotic balance
- Reduction in photosynthetic capacity due to low photosynthetic pigments. (Nezhadahmadi, 2013).

Shortage of water at the seedling stage, mid-season water stress, terminal stress, or combination of any of them is the most threatening issue in wheat production. Different factors have different effects on the crop yield which include seed germination, seedling vigour, growth rate and mean emergence time. Among all, the germination and seedling growth factors are very crucial in determination of crop yield. (M.H. Chachar, 2014)

According to (Dhanda, 2010), the seed vigour index and shoot length are the most sensitive factors towards drought stress, followed by the root and coleoptiles length, respectively. The seed germination rate and final germination percentage along with the water absorbance capacity of the seeds get lowered significantly with the increase of osmotic stress level. (Heikal, 2011)

There are studies such as selecting drought tolerant plant species or the treatments of seed which are helpful to alleviate the negative effects of water stress on plants. (Iqbal, 2007) Screening of drought tolerant genotypes at early seedling stage to avoid future yield loss is mostly achieved by the induction of simulated drought through chemicals like Polyethylene Glycol (PEG). (M.H. Chachar, 2014)

With a projected ever-rising shortage of water worldwide particularly in Pakistan, the need of the time is to acknowledge drought and its consequences on the crops. Wheat, being the food of more than 35% of the globe's population, is facing drought stress in most of the parts of the world. The requirement of drought tolerant wheat varieties has risen because of prevalent climatic dangers that have reduced crop yield globally. (Nezhadahmadi, 2013)

Hence, in this research, various wheat landraces from Potohar region were collected from National Agricultural Research Council (NARC) Pakistan and screened at germination stage (most sensitive and primitive phase of wheat plant growth) for monitoring their response under the drought conditions and how the drought stress affected the morphological and physiological responses in the wheat.

1.1 Objectives of the Research

- To study drought effects on morphological and physiological characteristics of the wheat landraces.
- Early screening of wheat at germination stage to identify drought tolerant genotypes which can be used further in breeding programs.

Chapter 2

Literature review

2.1 Yield and Consumption of Wheat in Pakistan

Wheat (Triticum aestivum L.) being the major food crop in Pakistan has the largest cultivation rate and lies among the top ten countries which produce wheat on massive scales. Significance of wheat can be analysed by the fact that it has quality nutrition to provide with, while being one of the richest sources of carbohydrates. It is reported to constitute major portion of the diet of the common man in Pakistan.

The following table depicts the taxonomic classification of the genus Triticum:

Kingdom	Plantae
Order	Cyperales
Family	Poaceae (grass family)
Genus	Triticum
Species	Triticum aestivum L.

Table 2.1: Taxonomic classification of bread wheat

Around 9.23mha (million hectares) of the total land is used for wheat production. Punjab holds most of the said land by inhabiting an area of about 6.97mha, while Khyber Pakhtunkhwa, Balochistan and Sindh cover an area of 0.7mha, 0.38 and 1.15 mha respectively. (AgroChart, 2019)

Wheat is among the most grown crops of Pakistan. According to the Pakistan Economic Survey, which was conducted in the year 2017-2018, wheat holds fourth position among the most important crops from economical perspective. (Pakistan Economic Survey, 2017-18). The crop contributes approximately 9.1% to agriculture of Pakistan.

2.2 Life cycle of Wheat plant:

Several decent production decisions are required to grow a good wheat crop. There are certain stages of development that respond best to the inputs. Therefore, in order to manage crops efficiently, it is important to understand the growth and developmental stages of a plant.

The life cycle of wheat is categorized into three stages of development.

1. The vegetative stage (in which leaves, and tillers are developed)
2. The reproductive stage (reproductive organs are formed)
3. The grain fill stage. (Barnard, 2012)

2.2.1 Germination of seed:

To establish good cover, quality seed is essential. The best quality seeds are large and dense kernels. They produce strong seedlings with lush green leaves and strong tillers. This step is said to be the most important for achieving desired yield.

Figure 2.1: Germinating seed showing coleoptile and first roots

2.2.2 Tillering and differentiation of ears:

Temperature, moisture and fertility of soil, cultivar and the rate of seeding germination determine the number of tillers. After four weeks of the emergence of seed from soil, first tiller develops. After development of the first tiller, one or more tillers may form every week. The rate of tillering can be stimulated by early plantation, irrigation and using nitrogen fertilizers. It is disadvantageous to plant late because it reduces the number of tillers. It is an important stage of the wheat crop.

For the development of tillers energy is needed by the plant. Therefore, shortage of nutrients and water leads to reduced tillering, which affects the yield. The process of tillering is water sensitive and it is reduced to half in dry conditions. To change from vegetative phase to reproductive phase, certain stimuli are needed for example the length of day, stress such as heat or drought, vernalisation or combination of these stimuli. The phase changing process occurs after seven weeks of seed emergence.

The time span of vegetative phase may range from 25-50 days which depends on the date of planting and cultivar. (Barnard, 2012)

2.2.3 Flag leaf stage:

Flag leaf is the last leaf which emerges and contributes up to 75% to total leaf area. For achieving desired yield goals, flag leaf protection is the crucial process. During this stage sufficient water and nutrients should be supplied to the plants for increasing energy production, which is in the form of starch and sugar. This stored energy holds special importance to develop kernels with high protein and starch content.

Figure 1.2: Flag leaf stage of wheat crop

It is important for chlorophyll to retain after the formation of the flag leaf stage because this is responsible for energy production through the process of photosynthesis. Reduced water supply during this stage has potentially harmful effect on the yield. (Barnard, 2012)

Development of ear and anthesis:

The development of the ear takes place after two weeks.

First, the number of spikelets is determined through the length of ear and then the number of florets per spikelet is determined by the width of the ear. Pollination and flowering in wheat starts from the middle of the ear and leads to the top and bottom of ears, in a time period of three to five days. After pollination, kernels are formed. More than forty kernels per ear are considered the best. These kernels per ear are determined by the number of pollinated flowers. The process of pollination and pollen formation is very sensitive to environmental conditions, especially increased temperature, drought stress and frosting as well.

Figure 2.2: Wheat height at anthesis stage

2.2.4 Grain filling stage:

Size and weight of the kernel are determined during the grain fill stage. It is a very critical period for producing high yield. The probability for higher yield is greater if this filling period lasts longer. Shortened period will result in low yield.

Many leaves expire after flowering, but glumes, awns and flag leaf persist during this period. 10-20% of the grain weight is provided by the photosynthesis in the awns or beard whereas 70-90% of the grain yield is derived from the products of photosynthesis that are made by the plant.

During this stage, sugar which is deposited in the leaves and stem transfers quickly to the basal grain on the ear of the wheat plant. This sugar will always be present in abundance irrespective of high temperatures and low moisture.

High temperature results in the breakdown of vascular tissues that carry sugar to different parts of the ear. Due to this, kernels present on top of ear do not fill properly which results in low weight of kernels.

2.2.5 Growth and composition of Kernel:

Figure 2.3 : Stages of growth of wheat kernel

There are five stages of kernel growth that are determined by the consistency of the endosperm of the kernel.

2.2.5.1 Watery ripe stage:

The width and length of the kernel are determined during this stage. The size of the kernel increases but it does not gather dry matter. When the kernel is squeezed, a clear fluid can be seen.

2.2.5.2 Milk stage:

In the milk stage, an increase in solids of the liquid endosperm is seen. When the kernel during this stage is squeezed, a white milk like liquid can be seen. After filling of kernels, the endosperm thickens and leads to a soft dough stage.

2.2.5.3 Soft dough stage:

Kernels during this stage are soft and dry. The material squeezed from the kernel no longer remains liquid as water concentration of the kernel decreases. It now has the consistency of dough. The green colour of kernel at the end of this stage begins to fade due to rapid accumulation of starch and nutrients.

2.2.5.4 Hard dough stage:

Kernels in this stage develop hard, firm and are difficult to crush. The level of moisture in the kernel decreases from 40% to 30%, and the kernel attains maximum dry weight. The wheat is now physiologically mature. After this, no extra weight will be added to the grain.

2.2.5.5 Ripening stage:

During this stage, there is high moisture content in the kernel and wheat begins to ripen. The plant now turns to straw color and kernels become hard. Harvest can start when the grain has attained required moisture level. (Barnard, 2012)

The most vital elements of the wheat kernel are starch and proteins. The nitrogen that was previously accumulated in the leaves is the source of most of the proteins, and starch comes from the sugar that was made during the process of photosynthesis. During early grain development, the nitrogen moves into filing kernels to form protein.

Improper filling of kernel leads to the grain having a high protein content, and thus, leading to a low yield. Changes in climate such as high temperature and drought are usually responsible

Climate change has a drastic effect on the sector of Agriculture. There are several factors of climate change which affect agricultural productivity such as patterns of rainfall, increase in temperature, fluctuations in sowing and harvesting time, availability of water, evapotranspiration and suitability of land. (Harry M. et al 1993).

Drought is one of the most common abiotic phenomena which affects the growth and development of crops. It is an important challenge for researchers and plant scientists to cope with. It is expected that around 1.8 billion people will face water shortage by the year 2025. (International decade for action Water for life, n.d.).

In the chart given below, it can be clearly seen that the production of wheat was halted due to the less rainfall in year 2018 during monsoon season. This leads to a decline in water availability followed by food productivity.

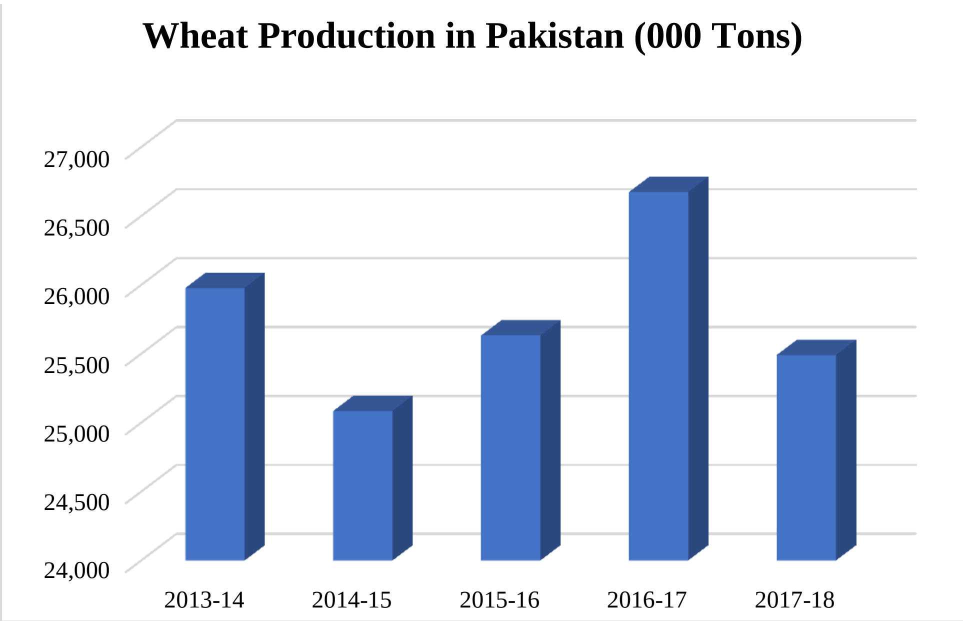

Table 2.2: Wheat Production (tons) in Pakistan in recent years (Statistics, 2018; Statistics, 2018)

2.3 Classification of Drought

Drought is classified into several different. Some of them are:

Meteorological drought: It is defined usually on the basis of to what extent the dryness occurs in comparison to the normal water levels and secondly, for how long it prevails. The meteorological drought is considered to be region specific as the level of precipitation varies from one region to the other.

Agricultural drought: It is when the crops in a certain area are adversely affected due low water supply. However, the amount of water a certain plant requires depends on a few factors such as current weather conditions, plant growth stage, biological traits of the plant and the soil composition.

Socioeconomic drought: It occurs when the economic good demand is more than supply due to low water availability created by low precipitations. Weather strongly determines the supply of several economic goods which may include food supply, power, forage and grains etc. Due to the naturally fluctuating climatic conditions, the supply of water may become sufficient for one year and short for the other. Moreover, the increasing population greatly affects the demand of food and water supply.

Hydrological drought: It is because of low water levels in lakes, rivers and reservoirs. Though the origin of all droughts is mainly due to the decreased precipitation than the normal, the major concern of hydrologists is towards the way this shortage plays out through hydrologic systems. The water coming through these systems is used for several purposes which can complicate the requirements. Hence, the competition among water usage of these storage systems elevates when drought occurs and this increased demand leads to more adverse effects. (National Drought Mitigation Center, n.d.).

At present, sustainable development is the most critical goal. To gain benefit from full usage of crop's physiological potential for the purpose of high productivity and safe food with satisfactory quality, the study regarding physiological and biochemical facets of stress tolerance have a comprehensive future for the solution to grain problems on the globe. On the basis of this research, a combination of plant physiology and molecular biology is the keynote of the drought tolerance's mechanism. Hence, more work is required for the identification and manipulation of the genes controlling molecular and physiological traits as well as to gear the research towards the right direction of drought tolerance. (T. Y. Bayoumi, 2008)

The adaptation of plants under stress is of major importance, which can help in planning new methods to devise drought tolerant varieties. The effects of drought on wheat plants has been shown and tested by various different studies. The responses could occur at either physiological, morphological, biochemical or molecular level.

The lack of substantial rainfall over the past two to three seasons has triggered Pakistan drought crisis. According to FAOs drought report, most areas faced severe water shortages. Because of the on-going lack of rainfall in the monsoon season, the situation has worsened since 2018. The national average precipitation rate was below the level of -24.4 percent. As indicated by the Pakistan Meteorological Department (PMD), the majority of the territories did not get any precipitation in the long stretch of August. This circumstance is prompting unfavourable impacts on farming and residential needs of the locals. (ReliefWeb, 2019)

The potency at which dry spell can influence the harvest yield significantly relies upon its seriousness and the development phase of the plant at which the dry spell happens. Various stages have various reactions to drought; for instance, a few phases have the capacity to adapt to stress by holding water potential, turgor pressure or by the proficient usage of water.

Tolerance against water stress is a complex phenomenon. It can be characterised into two classes i.e. drought avoidance and dehydration tolerance. Drought avoidance involves the depth of root, effective and organized water use, and some other adaptations for the plant to survive the stress. Whereas dehydration tolerance is the capability of a plant to dehydrate somewhat under stress conditions and recover back under water availability. (Arash Nezhadahmadi, 2013)

It is the focus of researchers to develop new and improved methods for making stress tolerant plants. There are several factors which affect the responses of plants towards drought stress, for example genotype, stages of growth, duration and severity of drought stress, biological process of growth, patterns of gene expression, respiration activity of plants, photosynthesis machinery and different environmental factors.

As mentioned above, plants have various coping mechanisms to these stresses depending on what growth stage they occur at. These include:

2.4 Biochemical responses towards drought:

Some biochemical changes in plants caused by drought including opening and closing of stomata, changes in proteins, photosynthesis inhibition, lower chlorophyll content and build-up of abscisic acid. Drought also leads to a higher production of reactive oxygen species (ROS), which causes oxidative damage such as lipid peroxidation and membrane destruction in the plants.

Plants have a very intricate defence system consisting of enzymes to avoid such damage by active oxygen, thus making sure that the cell functions remain normal. The balance between reactive oxygen species production and activities of anti-oxidative enzyme control whether oxidative signalling and injury will happen. Two of such ROS-scavenging enzymes include superoxide dismutase (SOD) and catalase (CAT). The enzymatic constituents may directly scavenge ROS or may function by producing a non-enzymatic antioxidant.

Apart from averting oxidative damage, plants have also developed methods to reduce the effects of water loss. Proline is one such osmo-regulatory protein, which enhances the tolerance level of plants under drought conditions by retaining its water potential. It has a major role in stabilizing the membrane and osmotic adjustment in plants. Proline also plays a key role in stabilizing cellular proteins in the presence of high concentrations of osmoticum. (Ghulam Murtaza, 2016)

2.5 Morphological responses towards drought:

Drought conditions can equally affect both the vegetative and reproductive growth of a wheat plant (Arash Nezhadahmadi, 2013). The regulation of these growth patterns is a key factor to determine the sustainability of any crop under varying stress environments. Responses of various morphological traits of wheat under drought conditions are critically analysed as these responses determine the progress of breeding and genetic engineering processes. Major morphological traits of wheat include the structure of leaf i.e. shape, area, size, senescence and waxiness; while of root it includes dry weight, length and the density.

Root and shoot system of any plant is the two most imperative factors. The interlink between the sources and sink limitations of both the organs determine the plant growth by building up an optimum balance. (Ghulam Murtaza, 2016)

As mentioned earlier, roots are one of the vital plant organs that are involved in several functions in plants such as in plant growth and development, anchorage, providing adequate amounts of nutrients and minerals to both stem and leaves. A root structure on whole is referred to as Root System Structure composed of lateral, nodal, seminal and seminal adventitious roots, each of which is responsible for a particular function. These components in spite of being a part of a single root system differ in their origin and age, physiological, morphological and anatomical functions. (Maciej T. Grzesiak, 2019)

In order to enhance the adaptation of plants to water deficit conditions, root system plays a significant role. Some of the major root traits that have the primary role in managing water stress and increasing plant productivity include root depth, root biomass and root length density.

The deeper root system assists the plants to capture the maximum amount of nutrients and moisture present in the deeper soil layer, whereas an increase in root number and volume provides a physical strength to the root system structure allowing greater soil permeability. This increase in surface area via the root hairs not only provides tolerance against drought, but also helps plants in the adaptation of heat stress and salinity conditions. (Vikender Kaur).

Various researches have been conducted to study the response generated by plants under the water deficit conditions and how they adapt morphologically to cope up with drought**.** It has now been a while since researchers started using morphological traits of plants as the screening tools. The root traits can be selected for screening at the seedling stage as they predict the later stage performance of a particular crop. As it is widely known that drought affects the crop productivity in several different ways, the alterations in plants under water deficit conditions improve the resistance at seedling stage against these stresses. One of the benefits include that crops become able to withstand the drought that is most likely to occur right after germination. Moreover, the resistance at the seedling stage reflects how well a certain crop would resist stress conditions if it occurred in later growth stages. Identifying the underground root traits is of prime importance for the effective implementation of root breeding programs.

This will greatly benefit breeders in a way that they will be able to improve the crop yield by selecting supreme root traits as parents. Therefore, they serve as essential prerequisites to maximize the yield in rain fed and water-stressed environments. (Jafar Ahmadi, January 2018)

2.6 Physiological responses towards drought:

Discussing the plant physiology, the water deficit conditions lead to dryness suppressing the normal growth of the plant. Some of the significant variations that occur in wheat plant under stress include stomata closure, reduction in the photosynthetic activity, changes in the cell wall integrity and most importantly the development of oxidative stress, osmotic adjustment, diminished stomatal conductance which results in decrease in internal concentration of CO2. It further leads to the production of toxic metabolites ultimately causing the death of the plant.

One of the physiological traits i.e. Relative Water Content majorly depicts the water supply balance between leaf and the rate at which transpiration occurs. It is related to the volume of cells and has an impact on plant recovery from stress conditions. Another essential indicator of water levels under water deficit conditions is the Leaf relative water content (RWC) which highlights the present level of water content in comparison to the original amount of water a leaf tissue can hold at full turgidity. (A.A.S. Ahmed, 2014).

The maximum value of relative water content is expected to be calculated when the crop is at its vegetative stage. Lower value of relative water content in a crop under water deficit conditions is a sign that there is a decrease in water status and osmotic potential in plants. In order to preserve a proper turgor pressure, osmoregulation happens to play a significant role increasing the soil water absorption and ensuring the survival of plant metabolites. (Almeselmani, 2010).

Various researches have been conducted to study the response generated by plants under the water deficit conditions and how they adapt physiologically to cope up with drought. This involves a high amount of relative and potential water and maintaining the integrity of the cell membrane. Cell-membrane stability (CMS) is also one of the essential components for the selection of drought tolerant genotypes. It is known to depict the plant tissue ability to prevent the leakage of electrolytes on the encounter of drought condition and ensures that cell membrane remains free of damage. Several studies have been conducted to estimate the stability of cell membrane including the assessment of dehydration in leaf tissues and electrolyte leakage in them due to the chemical used for inducing water deficit conditions i.e. Polyethylene Glycol (PEG).

The values of cell membrane stability hold great significance in hybridization programs when we talk about tolerant varieties. The genotypes that exhibit lower values of CMS are more likely to be drought susceptible, whereas, the ones with higher values exhibit drought tolerant behaviour. (Bilal, 2015).

It was also studied that one of the important enzymes involved in regulating the signalling pathways of plants is cysteine proteinase and is directly involved in generating responses under stress. Growth of plants is greatly affected by the variability in the turgor pressure and to overcome that plants opt for osmotic adjustment to mitigate harmful effects. (Arash Nezhadahmadi, 2013)

In the agriculture sector, drought is the most significant stress that affects the yield of crop. Similarly increasing temperature is the most vital constituent of climate change and its harmful effects on yield are expected to be increased in near future. Therefore, there is need of the time to screen genotypes that work best under high stress conditions. Plant breeding programmes primarily emphasized on picking genotypes that have high yield in both normal and stress conditions. There are several shortcomings in this criterion which slows down the process of breeding. (Arnauld A. Thiry, 2016). Consequently, this highlights the importance of screening techniques through physiological, morphological and biochemical processes to determine the genotypes at early seedling stages that work best under high stress conditions.

Chapter 3

Materials and Methods

3.1 Procurement of Seeds

The National Agriculture Research Centre (NARC) in Islamabad was contacted and twelve different wheat genotypes were obtained. These were all given accession numbers and for the ease of documentation, the accessions were numbered from 1-12 and referred as such. The following table lists the accessions according to their reference number. The genotypes 11 and 12 were used as a control, as they were known to have good growth and performance under stress conditions.

Accession Number	Reference Number
11154	1
11156	2
11162	3
11164	4
11176	5
11179	6
11195	7
11214	8
11219	9
11239	10
35989	11
35995	12

Table 3.1: Accession numbers and reference numbers of wheat genotypes

3.2 Sterilization of Seeds

The seeds were sterilized by dipping them in 70% ethanol for 3-5 seconds and then washing them thrice with tap water to completely remove any traces of ethanol. The seeds were then placed in petri plates containing a filter paper and then covered with another filter paper to let them germinate. (Majeed, 2013)

3.3 Experiment Design

In a single petri plate, five seeds of each accession were placed under two conditions (well-watered and stress), with two replications of each. This was a completely randomized design (CRD), with each seed being regarded as a separate unit. The following diagram illustrates the experiment layout for one accession.

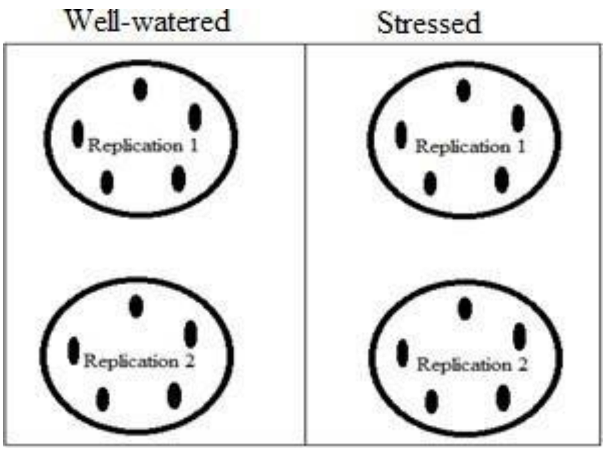

Figure 3.1: Experimental layout for one accession

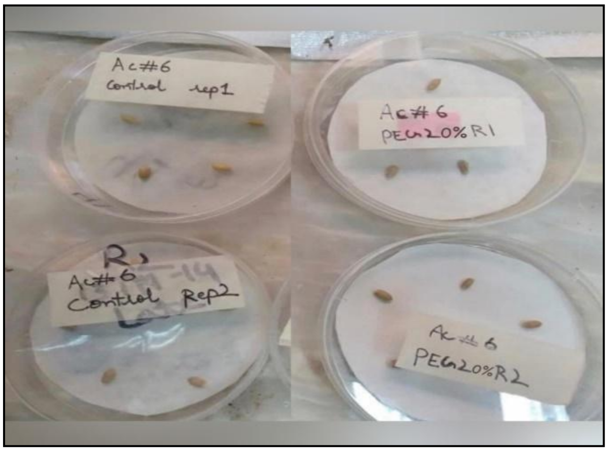

Figure 3.2: Petri plate setup for one accession

3.4 Inducing Stress

For normal well-watered conditions, tap water was used for watering the seeds. 10ml water was used on the first day and then, the plates were sprinkled with water whenever they were noticed to be drying out. For stress conditions, polyethylene glycol (PEG 6000) was used. PEG has a high molecular weight and it cannot cross the plant cell wall. Hence, it induces water stress in plants. (Shivakrishna, 2018) A 20% PEG solution was prepared by mixing 120g of PEG 6000 into

500ml distilled water. 10ml of this solution was used to water the seeds on the first day and afterwards, the seeds were watered with tap water whenever the need was felt.

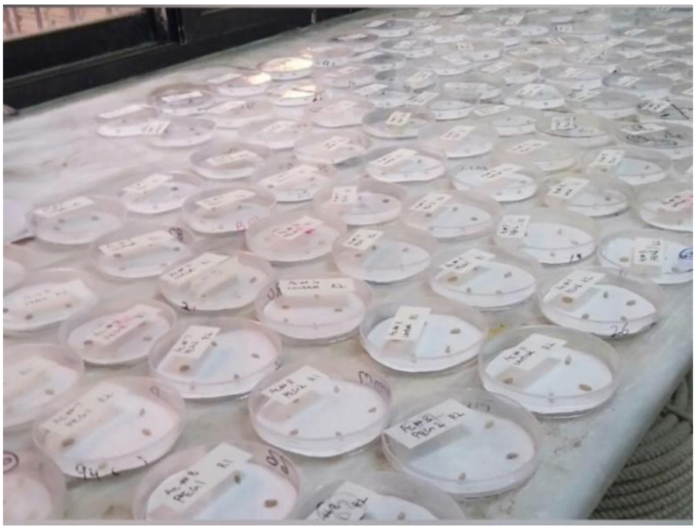

Figure 3.3: Experimental setup for all accessions

All the plates were placed in an incubator set at 22°C.

Figure 3.4: Petri plates in incubator for germination

3.5 Morphological Analysis

3.5.1 Seed germination rate

Seed germination rate is calculated by growing the seeds in a petri plate with moist filtered papers at the bottom. (Al-Ansari and Ksiksi 2016)

The number of germinated seeds was counted on day 5 and the germination rate index was calculated using the following formula:

Percent Germination= No. of seeds germinated / Total no. of seeds × 100

The other physical parameters were measured at day 19 and they included:

3.5.2 Coleoptiles length

Length of protective sheath that covers the shoot during emergence is to be measured. (E.Fosket, 1994)

3.5.3 Shoot length (mm)

3.5.4 Root length (mm)

A 15cm transparent ruler was used to measure the lengths by holding the shoots and roots taut at both ends. A digital scale was used to measure the weights.

Figure 3.5: Coleoptile, shoot and root length measurement

3.5.5 Water saturation deficit

The fresh weights of shoots and roots were measured on day 19 after separating the roots and shoots from the seed using a cutter. They were left in test tubes containing distilled water for 24 hours to obtain saturated weight and it was measured on day 20 after removing the excess water with tissues. The shoots and roots were then placed in dry petri plates for another 48 hours to completely dry them out and the dry weights were measured on day 22.

Water saturation deficit (WSD) was calculated from the measured variables using the following formula (Hellmuth 1970):

WSD= (Saturated weight – fresh weight / saturated weight – dry weight) × 100

3.5.6 Seedling vigour

According to International Seed Testing Association (ISTA), seedling vigour is "the sum of those properties that determine the activity and performance of seedlings of acceptable germination in a wide range of environments". (W.E. Finch-Savage, 2016)

Seedling vigour was calculated using the following formula (Pradeep 2018):

Seedling vigour = [root length + shoot length] × germination %

3.5.7 Percentage reduction over normal treatment

To further stress the effect of increasing drought stress on the morphology of shoots and roots in the wheat, % reduction over normal treatment is calculated by the formula (Manisha Jail, 2014):

[(Mean value in normal plants – mean value in drought treated plants) / Mean value in normal plants] × 100

Figure 3.6: Data collection of calculated readin

Chapter 4

Results and Discussion

4.1 Morphological Analysis

Analyzing the morphology of the plants is studying their physical characteristics, both vegetative and reproductive. However, since this experiment was conducted at the seedling stage, only the vegetative traits have been studied. Morphological analysis has been used in many studies to target the improvement of plant varieties in order to establish early cover. (Borrelli et al. 2009; Sadras and Calderini 2009)

The genotypes 11 and 12 were used as a control to inspect how well the other genotypes performed in comparison to them.

4.2 Effect of Water Stress on Plant Germination

To investigate the effect of water stress on plant germination, the number of germinated seeds at day 5 was counted and the mean germination rate index was calculated for both the treatments respectively. The standard error was also found using Microsoft Excel.

The figure 12 illustrates the findings. As evident from the figure 4, all genotypes performed differently in both treatments. Genotype 8 and 10 had the highest GRI of 1 at normal conditions, while the genotypes 9 and 10 had the lowest GRI of 0.4. All genotypes, except for 6, faced a decrease in the GRI under water stress.

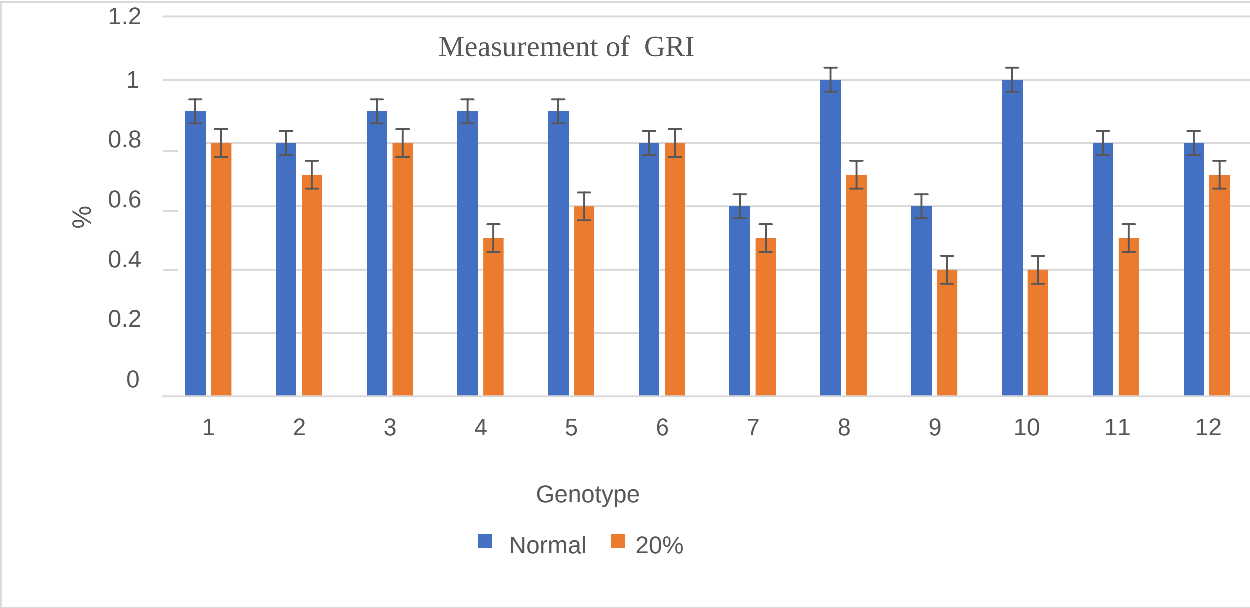

Figure 4.1 : GRI in normal and PEG treated plants

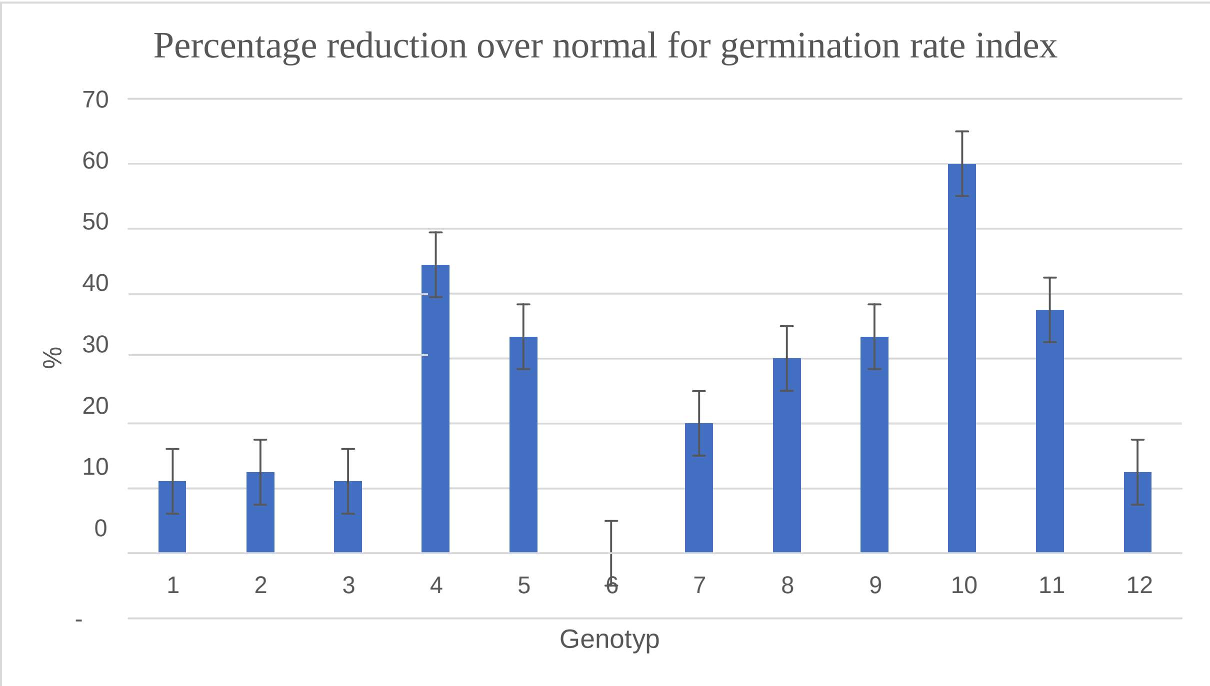

Figure 4.2: Percentage reduction over normal for GRI

Percentage reduction over normal treatment was calculated to quantify the effect of water stress on the GRI and the figure 13 indicates the results. The genotype 6 had no difference in its GRI in both treatments. The highest decrease in the GRI in water stressed plants was seen in genotypes 10 with a percentage reduction of 60%. The control genotype 12 had a 12.5% reduction in its GRI in PEG treated seedlings which corresponds to the genotypes 1, 2 and 3.

Therefore, it can be deduced that water stress decreases the germination rate of wheat seeds as shown in a study conducted in *Hajee Mohammad Danesh Science and Technology University, Dinajpur* in which all wheat varieties faced a decrease in their germination index under water stress. (M.S. Rana, 2017)

4.3 Effect of Water Stress on Shoot Length

The shoot lengths of the wheat seedlings were measured and a mean length was calculated for the normal and PEG treatments, respectively. The figure 14 illustrates the results and it is clearly evident that all genotypes faced a significant decrease in their shoot lengths under water stress.

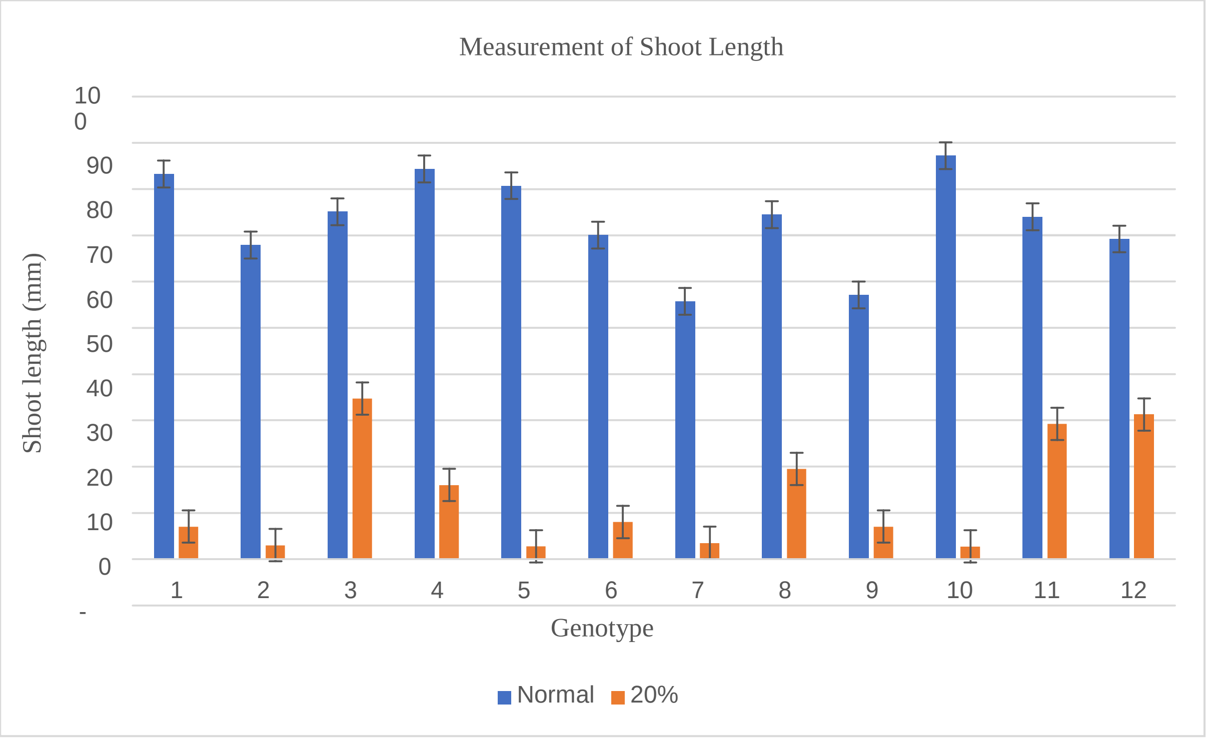

Figure 4.3: Shoot lengths under normal and PEG treatment

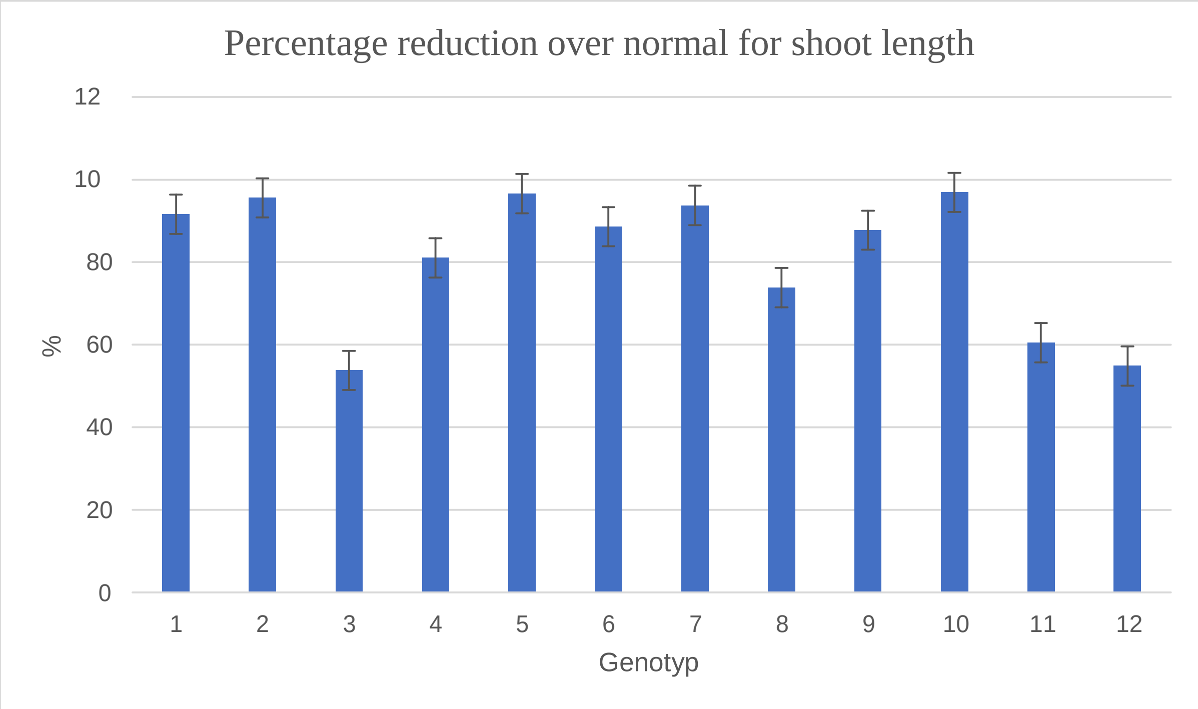

Figure 4.4: Percentage reduction over normal for shoot length

The highest shoot length was recorded in the genotype 10 with a length of 87.2mm and the lowest in genotype 7 with a length of 55.7mm under normal treatment. Genotype 3 had the highest shoot length under water stress (34.7mm), which was close to the length of the control genotype 12 (31.3mm). The genotype 10 had the shortest shoot length of 2.72mm under stress.

As shown in figure 15, genotype 5 faced the highest reduction in its shoot length under water stress by 96.6. The lowest change was seen in the genotype 3 with a reduction of 53.8%, which is close to the reduction faced by the control genotypes 12 (54.8%).

Hence, it can be noted that water shortage acts as a major stress which leads to a decreased shoot length as shown by studies conducted by Ibrahim Bolat and Tahar Boutraa, respectively. Both studies indicate a significant decrease in the lengths of the plants under moderate and severe drought. (Boutraa et al. 2010; Bolat et al. 2014)

4.4 Effect of Water Stress on Root Length

The root lengths of the wheat seedlings were measured and a mean length was calculated for the normal and PEG treatments, respectively. The figure 16 illustrates the findings of the study for the root lengths. As shown in the figure 16, genotype 6 had the greatest root length of 153.9mm under normal treatment. The genotypes 7 had the shortest root length of 75.6mm in the same conditions. Under PEG treatment, the control genotype 12 had the highest root length of 73.3mm. Genotype 3 also showed good growth under water stress with a root length of 61.3mm. Genotype 10 had the shortest root length under stress (7mm).

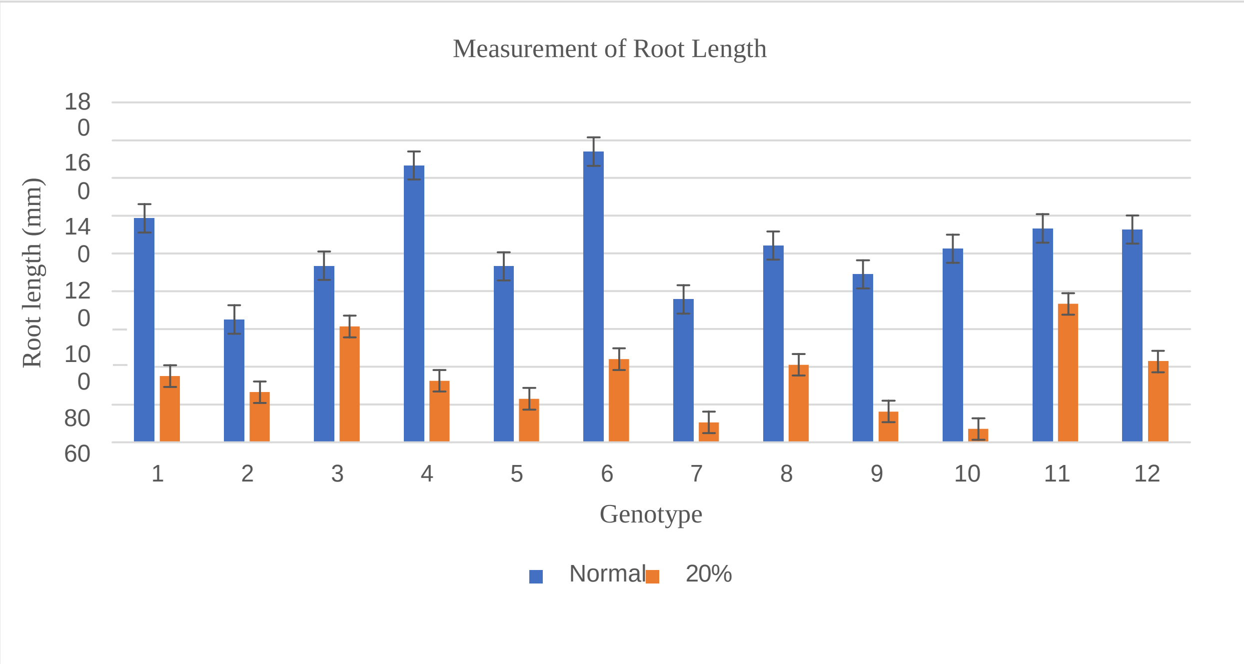

Figure 4.5: Root lengths under normal and PEG treatment

Figure 16 illustrates the percentage reduction over normal for the root lengths. The highest reduction was seen in the genotype 10 by 93%. The lowest change in the root length under PEG treatment was seen in genotype 3 in which the reduction was by a 34.4% only. This corresponds to the reduction faced by the control genotype 12 (35.3%).

The same pattern of decrease in the root lengths were also seen in the study conducted by Tahar Boutraa, in which the total length of the plants had decreased under water stress. (Boutraa et al. 2010)

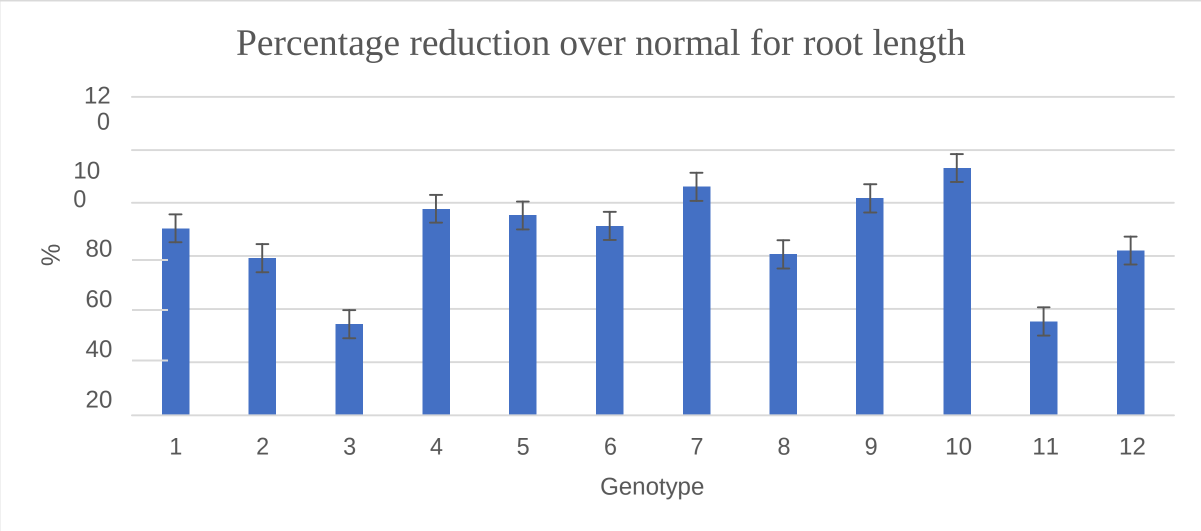

Figure 4.6: Percentage reduction over normal for root length

4.5 Effect of Water Stress on Coleoptile Length

The coleoptile lengths of the wheat seedlings were measured and a mean length was calculated for the normal and PEG treatments, respectively. Figure 18 illustrates the results and it can be seen that the highest coleoptile length was seen in genotype 5 (43.9mm) under normal treatment. The control genotypes 11 and 12 also showed moderate lengths of 25 and 22.9mm each under normal conditions. The lowest coleoptile length was seen in genotype 7 (19.2mm) under the same conditions.

Under PEG treatment, genotype 3 had the highest coleoptile length of 25.6mm, which was close to that of the control genotypes 12 (25mm). The genotype 8 also showed some moderate coleoptile growth of 17mm under water stress. Genotype 2 had a very little coleoptile of only 0.63mm.

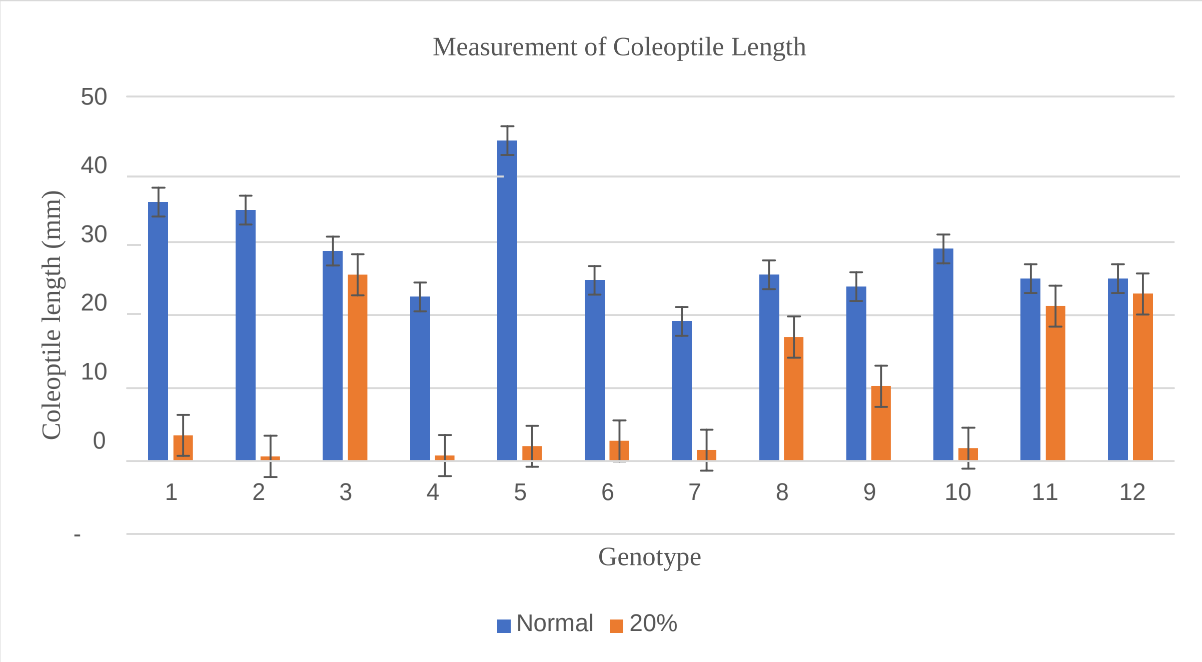

Figure 4.7: Coleoptile lengths under normal and PEG treatment

The percentage reduction over normal treatment was also calculated for the coleoptile lengths, as shown in figure 18.

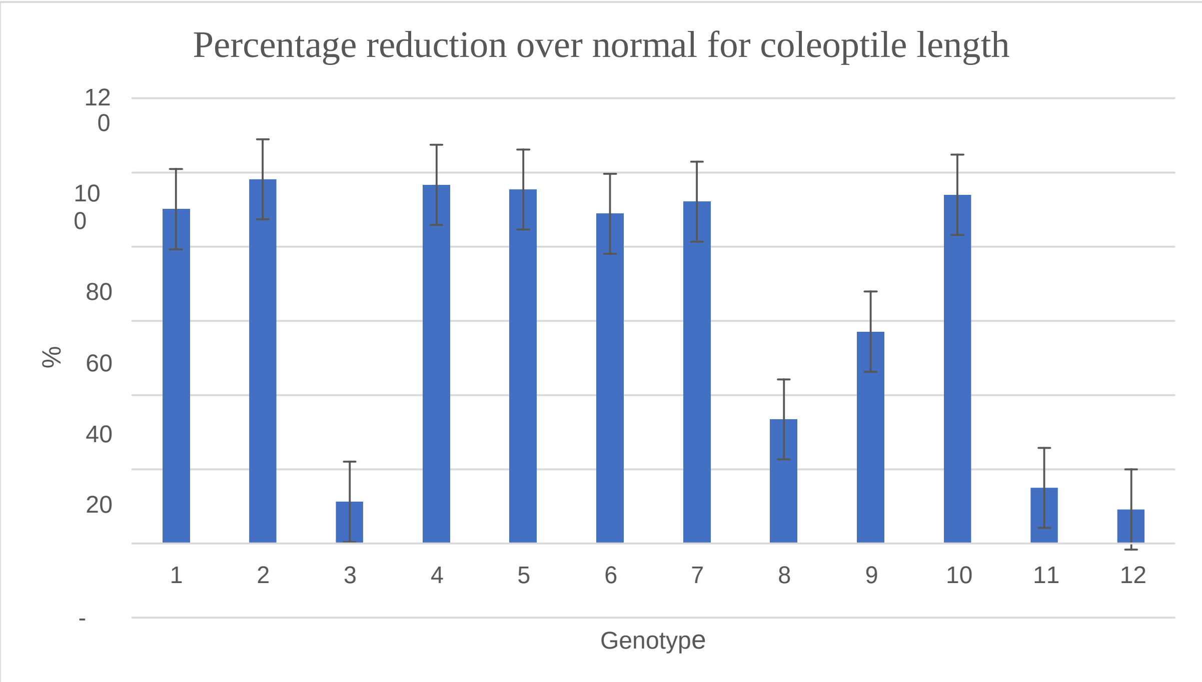

Figure 4.8: Percentage reduction over normal for coleoptile length

As evident from the figure, very high reductions were seen in all genotypes except for 3 (11.3%) and the control 12 (9.2%). Genotype 8 had a moderate reduction of 33.5% and the highest reduction was seen in genotype 2 by 98%.

Hence, it can be summed up that water stress decreases the coleoptile length significantly as it was also seen in a study conducted by Aradhna Kumar in which all wheat genotypes faced a decrease in their coleoptile length under 20% PEG treatment. (Kumari et al. 2014)

4.6 Effect of Water Stress on Seedling Vigour

Seedling vigour is influenced by multiple genetic and environmental factors. In this study, it was calculated using the length of the seedling and the germination percentage that it exhibited. (Seedling Vigor: Why Is It Important? (Collins, Edmisten, Stewart, York, & Reisig))

Figure 20 depicts the results of the calculation of seedling vigour for all genotypes in both normal and PEG treatments. Under normal conditions, the genotype 4 had the highest seedling vigour of 2080. The lowest seedling vigour was seen in genotype 7 (662.5) under normal conditions.

Under PEG treatment, genotype 3 showed the highest seedling vigour of 626. This were close to the seedling vigour exhibited by the control genotype 12 (557). The lowest seedling vigour under water stress was seen in genotype 10 (39).

Figure 21 illustrates the percentage reduction over normal treatment for seedling vigour. The most decrease in seedling vigour was seen in genotype 10 by 98%. Genotype 3 and 17 had the least percentage reduction over normal of 58.7%. This were close to the reductions faced by the control genotype 12 (59.5%).

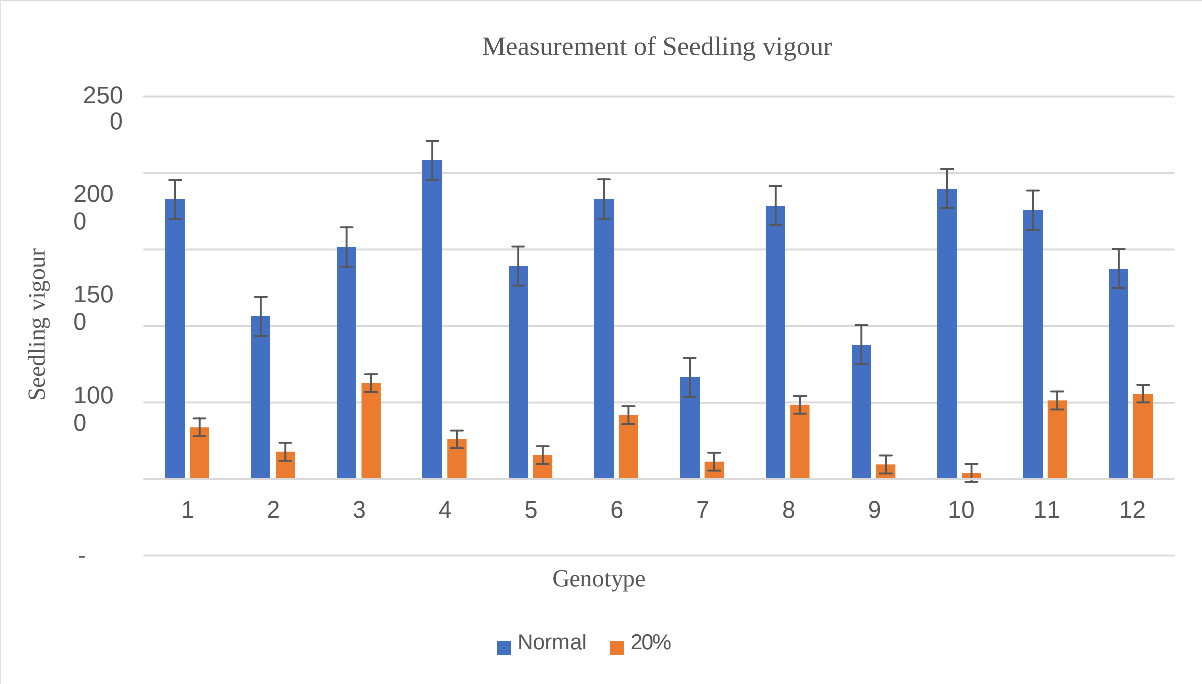

Figure 4.9: Seedling vigour under normal and PEG treatment

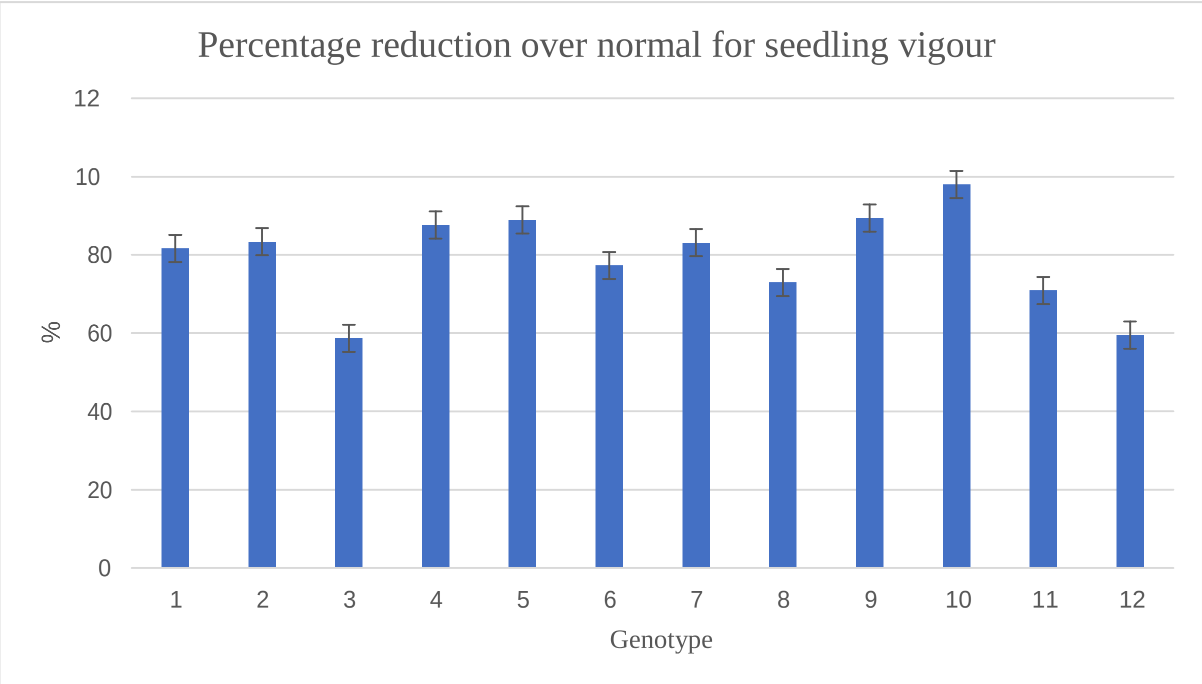

Figure 4.10: Percentage reduction over normal for seedling vigour

Hence, it can be said that water stress decreases the seedling vigour of wheat plants to a considerable degree.

4.7 Effect of Water Stress on Water Saturation Deficit

Water saturation deficit (WSD) is the alteration of water content from the plant compared to the saturation level of that plant at a given instance. A higher WSD indicates that the plants are subjected to a greater degree of water deficit. (Tasmina, 2016)

The WSD was calculated by measuring the fresh, saturated and dry weights of the seedlings and the results are illustrated in the figure 22. Under normal conditions, the highest WSD was recorded in genotype 9 of 53.6. This were close to the WSD of the control genotype 12 (57.8). The lowest WSD of 31.4 under control was seen in the genotype 10.

Under PEG treatment, most genotypes faced an increase in their water saturation deficits. Figure 23 depicts the percentage change in the WSD for all genotypes under water stress as compared to normal conditions. Genotype 4 had the highest increase in WSD by 96% while the genotype 2 had the smallest change in its WSD by only 2.7%.

According to previous researches, WSD should increase under water stress treatments. (Tasmina, 2016)

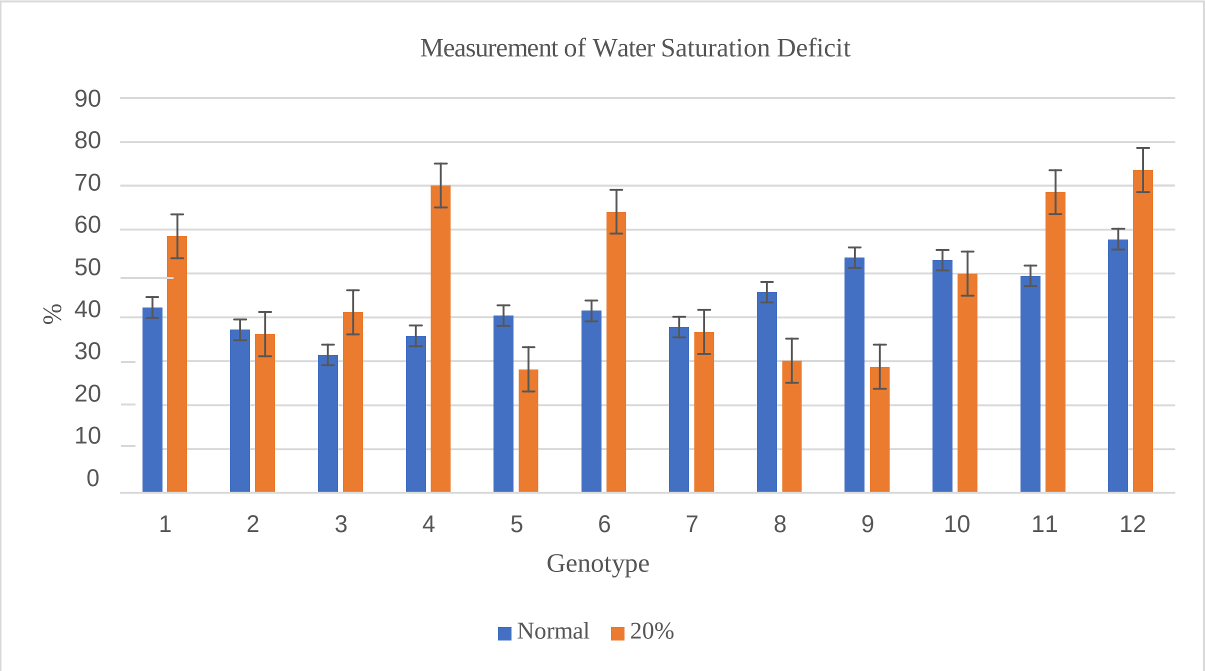

Figure 4.11: WSD for normal and PEG treatment

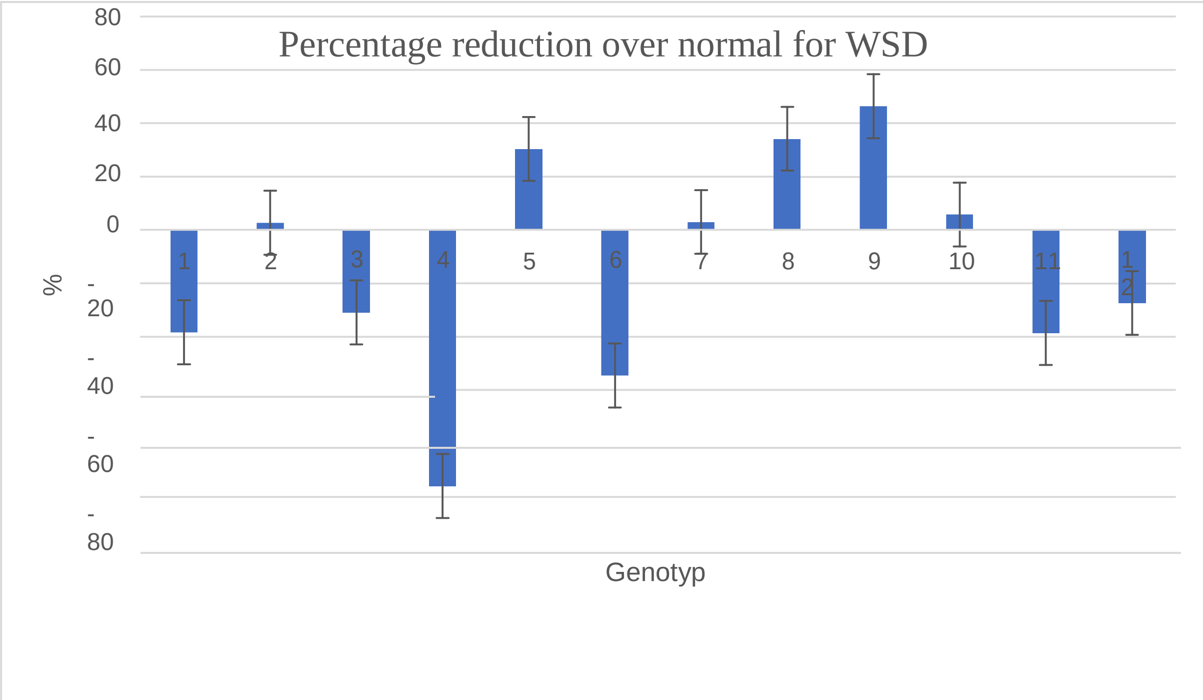

Figure 4.12: Percentage reduction over normal for WSD

4.8 Correlation Studies

Correlation studies were also carried out by generating a correlation matrix using Statistix 8.1, as shown in figure 24.

	Gerimination Rate Index	Shoot Length	Root Length	Coleoptile Length	Seedling vigour	Water Saturation Deficit
Gerimination Rate Index	1					
Shoot Length	0.688064474	1				
Root Length	0.648382023	0.907929481	1			
Coleoptile Length	0.588834022	0.879906951	0.72744147	1		
Seedling vigour	0.782274281	0.950151717	0.943812036	0.766439964	1	
Water Saturation Deficit	-0.06801164	-0.091353076	-0.023694468	-0.081304788	-0.0925442	1

Figure 4.13: Correlation Matrix

The coefficient of correlation is a mathematical calculation of the degree of association between the relative movements of two variables. Its values vary from -1.0 to 1.0. A value of precisely 1.0 means the two parameters have a completely positive relation. There is also a positive increase in the second parameter, for a positive increase in one parameter. A value of -1.0 means the two parameters have a completely negative relation. This shows that the parameters move in opposite directions - in the second parameter, a decrease occurs for a positive increase in one parameter. If the correlation between two variables is 0, they are not linearly related.

Analysts don't find correlations significant until the value reaches at least 0.8. A coefficient of correlation with an absolute value of 0.9 or higher would represent a very strong relationship. (Ganti 2003)

In this study, water saturation deficit had a negative correlation with every parameter. However, the values are not significant. The most significant positive correlation was seen between seedling vigour and shoot length (0.95). Seedling vigour and root length also had a highly significant positive correlation of 0.94. This explains that when the root and shoot lengths increased, so did the seedling vigour.

Another significant positive correlation was seen between root and shoot length of 0.91. An increase in the root length also caused an increase in the shoot length.

Chapter 5

Conclusion and Future Prospects

Drought is one of the most alarming abiotic stresses and a global problem that happens to limit crop productivity at a larger scale. Among several other crops, wheat crop is majorly affected by water deficit conditions leading to a decline in its productivity and quality. These drought conditions can equally affect both the vegetative and reproductive growth of a wheat plant. The potency at which dry spell can influence the harvest yield significantly relies upon its seriousness and the development phase of the plant at which the dry spell happens.

Various stages have various responses to drought; for instance, a few phases have the capacity to adapt stress by holding water potential, turgor pressure or by the proficient usage of water. But due to the prevailing climate changes, the effects are found to be more adverse and disastrous. The regulation of these growth patterns is a key factor to determine the sustainability of any crop under varying stress environments. Several researches have been conducted to study the tolerance mechanism of wheat crops and efforts have been made to maximize its productivity by developing genotypes that show maximum tolerance against drought conditions. An early screening based on morphological traits helps understand the process of early cover formation of the wheat plants. Plants that were tolerant to water stress during these early processes would be able to withstand drought in the early stages of growth and lead to an increase in the total yield of the crop.

In the present study, responses of various morphological traits of wheat under drought conditions were critically analyzed as these responses determine the progress of breeding and genetic engineering processes. Results of the study demonstrated that all the genotypes under study faced a decrease in their morphological characteristics by a considerate amount during water stress, induced by PEG treatment.

The genotypes 1, 2, 3 and 6 had the least reduction in their germination rates under stress. Water stress had a significantly higher effect on shoot length as compared to germination rates and only the genotypes 3 and 8 faced a somewhat lesser decrease in their shoot lengths. The same applied for root lengths as well since the decrease was considerably greater in most genotypes, except for 2, 3 and 8. All the genotypes under study apart from 3, 8 and 9 faced a major decrease in their coleoptile lengths, under stress, as well. Calculations for seedling vigour were also carried out and the results demonstrated that only the genotypes 3, 6 and 8 maintained a portion of their seedling vigour under water stress. Calculations for the water saturation deficit showed that only the genotype 3 had a trend similar to that of the control genotypes.

Based on the performance of the genotypes in all their morphological characteristics, it can be concluded that the genotypes 3 and 8 were more tolerant to water stress as compared to the rest of the genotypes under study.

The genotypes exhibiting maximum tolerance under water-deficit conditions can be further screened for biochemical and molecular parameters for the identification of them being used as potential candidates for breeding purposes. This will greatly benefit breeders in a way that they will be able to improve the crop yield by selecting supreme traits as parents. Therefore, they serve as essential prerequisites to maximize the yield in rain fed and water-stressed environments.

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