Study the Effect of Salt Stress on Morpho-Molecular Characters of Wheat

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Preface

Presently, there is much conflict between the genetic resources due to the ever growing population coupled with declining in arable land, ecological changes and loss of genetic diversity. These all factor made the biggest challenge to the researchers for improving the cereal crop specifically wheat. Present study was carried out to study the effect of salt stress on Morpho-molecular characters of wheat genotypes. For this the total of fifteen varieties were collected, to evaluate the genetic diversity under the normal field condition and the effect of salt stress on seedling stage the seeds of all varieties were grown in normal field and Hoagland solution under hydroponic system respectively. In the present study, Morpho-molecular physiological data were recorded at pre- harvest stage and at post harvest stage along with physiological characteristics. The study was performed under controlled as well as treatment condition and data recorded viz, germination, shoot length, root length, fresh weight and dry weight in both conditions that shows the resistance genotypes on the basis of morphological data recorded. A collection of fifteen variety of wheat diversified through SSR primer. The SSR primer was given information with higher value of resolving power. These results confirmed the usefulness of SSR –PCR analysis to detect specific molecular markers in wheat genotype. The SSR based dendogram grouped the fifteen genotypes into two main clusters. Cluster showed that the genotypes, the lowest similarity displayed by DBW-16 and KRL-213 analyzed and the genotypes PBW-550 and RAJ 3765 followed by Kharchia-65 displayed the greatest genetic similarity. The range of genetic diversity values broadly indicates the degree of heterogeneity or homogeneity in different genotypes of the plant species**.** Morpho-molecular physiological and genetic information obtained in this might be useful in agriculture.

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"Emotion cannot be expressed in the words because then they are transformed into more formality, our gratitude is many more than what we expressing here"

Pleasure to avail ourself of this opportunity to express our profound sense of veneration and gratitude to our family who have always been our role models, their every help, love affection, moral support at every corner of our life and teachers for their esteemed guidance, untiring supervision, constructive suggestions and encouragement throughout the course of the present investigation and preparation of this manuscript.

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*"At the end we thanks and appreciation to "**GOD"** who helped us at each step to complete this research work smoothly."*

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CHAPTER 1: INTRODUCTION

Wheat **(*Triticumaestivum*L.)** is one of the most important and widely cultivated crops in the world, used mainly for human consumption and support nearly 35% of the world population. Nearly 95% of wheat grown today is hexaploid, used for the preparation of bread and other baked products **(Debasis and Khurana, 2001).** Wheat is a member of the family Poaceae, which includes major cereal crops such as sorghum, maize, wheat, rice, millet and barely **(Briggle and Reitz, 1963).** It has total production in India and world **85** and **654million MT** respectively during 2013 **(FAO**). India share about 13.15% of total production in world **(FAO).** Wheat is becoming increasingly popular and since 1960, its consumption has risen almost 5% per year in developing countries, **(Bushuk,1998)**. By 2020, the world demand for wheat is expected to be 40% higher than that of its level in the latter half of the 1990s **(Rosegrant, 1997).** In view of this the wheat production must increase at a rate of 2% annually to meet 2050 demands **(Gill *et al.*, 2004).**

Wheat grain is a staple food used to make flour for leavened, flat and steamed breads, biscuits, cookies, breakfast cereal, pasta, noodles, bio-fuel, and for fermentation to make alcoholic beverages such as beer and liquors **(Tsegaye& Berg, 2007a)**. Its importance may vary in different countries, partly as a result of the diversity in different species of wheat.

Soil salinity is one of the major abiotic stresses which become a major environmental issue **(Shannon *et al.*, 1994).** Excessive salt accumulation in soils, in fact, has been recognized as a limiting factor for crop production of one-third of the world's limited arable land **(Epstein *et al.*, 1980).** Hence, a detailed Understanding of the basic mechanisms involved in the plant salt tolerance is an important prerequisite to improve the performance of crop plant in saline soils **(Binzel and Reuveni, 1994).** Soil salinity affects germination, crop growth and productivity. Salt stress affects many physiological aspects of plant growth shoot growth was reduced by salinity due to inhibitory effect of salt on cell division and enlargement in growing point **(Mccue and Hanson, 1990).**

Early flowering reduced dry matter, increase root: shoot ratio and leaf size caused by salinity may be considered as possible ways of decreasing yield in wheat under salt stress condition **(Mass *et al.*, 1989).** It is reported that high transpiration rate at leaf surfaces cause high accumulation of salt in leaves which kill them before full maturity **(Dodd, *et al*, 1999).** Net photosynthesis was decreased due to the reduction in photosynthesis and increasing in respiration per unit of leaf area. Crop yields start declining when pH of the soil solution exceeds 8.5 or ECe value goes above 4 dS m_1. Addition of salts to water lowers its osmotic potential, resulting in decreased availability of water to root cells. Salt stress thus exposes the plant to secondary osmotic stress, which implies that all the physiological responses, which are invoked by drought stress, can also be observed in salt stress. It has been estimated that more than 20% of all cultivated lands around the world containing levels of salts high enough to cause salt stress on crop plants **(Boyer, 1982).**

Improving salt tolerant varieties on the other hand, is of major importance and efforts should be focused on finding mechanisms which are involved in salinity tolerance. This may lead us to find gene sources **(Zhu, 2000)** as well as methods for screening large number of genotypes for salt tolerance. Wheat is classified as a semi tolerant crop to salinity. One way to alleviate the problem is the breeding of salt tolerant genotypes that perform better than current sensitive varieties under moderate to high salinity stress. Identifying genotypes that are tolerant to saline conditions is a practical and relatively simple way of improving crop yield and profitability on these difficult soils.

Genetic diversity can be assessed form pedigree analysis, morphological traits or using molecular markers and it is the material basis for crop improvement **(Habash *et, al* 2009)**. It is desirable to have large genetic diversity for the creation of new genotypes. DNA markers are technology that can increase breeding progress, especially for traits that are difficult to select under field conditions and that are controlled by multiple genes. Therefore, the use of biochemical/molecular markers for the evaluation of genetic diversity has received much attention in recent years. Molecular markers provide new dimension, accuracy and perfection in the screening of germplasm **(Taran *et al.*, 2005).** Accordingly, molecular markers emerged as a tool for many applications of value to crop improvement and proved to be an important way to increase selection efficiency **(Bernardo, 2008).** Many kinds of molecular markers based on various DNA analysis methods are being used in present day. Microsatellites are repeating sequences of 2–6 base pairs of DNA and are among the most stable markers of genetic variation and divergence among wheat genotypes because they are multi-allelic, chromosome-specific and evenly distributed along chromosomes **(Roeder *et al.*, 1998).**Microsatellite genotyping is used for genetic biodiversity, population genetics at the level of relatedness, genome mapping, as markers for pathogens, etc. The aim of this research is to determine the genetic diversity of Indian wheat genotypes and their responses under the salinity stress. In this context the following objectives were proposed:

1. **Collection and maintenance of wheat genotypes**
2. **Study the morphological and physiological changes in response to salt stress.**
3. **Study the genetic diversity using microsatellite markers**

CHAPTER-2 REVIEW OF LITERATURE

2.1. Wheat overview

Among cereals, Wheat **(*Triticum aestivum* L.)** is one of the most cultivated crops in the world. It covers the largest surface among cereals (216 million ha) worldwide. The production takes the third place, after maize and rice, with 654 million tons **(FAO 2013(proj.)).** India share about 13.15% of total production of wheat in world **(FAO).** It is the staple food of about two billion people provides nearly 55% of the carbohydrates and 20% of the food calories consumed globally **(Breiman and Graur, 1995).** Wheat breeding programs around the world are working toward improved grain yield with better quality, disease-resistance and agronomic performance. Wheat belongs to the *Triticum* genus of the large Poaceae family, which gathers about 3500 species **(Bonnier *et al.*, 1990).** Although most of wheat cultivars, bread wheat **(*Triticum aestivum*)** are grown in the world that constitutes about 95 percent of total global production of wheat and therest belong to durum wheat **(*Triticum turgidum* L).**Wheat is self-pollinated, hexaploid crop which having 2n= 6x= 42 chromosome. The hexaploid wheat have large genome size, a high proportion of repetitive DNAs, continuous inbreeding caused by self-pollination and a narrow genetic base represent the difficulties for use of molecular markers **(Joshi and Nguyen, 1993).**

2.1.1. The importance of wheat

Bread wheat **(*Triticum aestivum*)** is a globally important grain because it is a key ingredient in foods such as bread, noodles etc. Over 200 million hectares of wheat are cultivated worldwide **(Reynolds *et al.*, 2010)**. Wheat is one of the most important staple food crops of about two billion people (36% of the world population) occupying 17% (one sixth) of crop acreage worldwide, feeding about 40% (nearly half) of the world population and providing 20% (one fifth) of total food calories and protein in human nutrition. Worldwide, wheat provides nearly 55% of the carbohydrates and 20% of the food calories **(Breiman and Graur, 1995).**

2.1.2. Taxonomic status and genome of wheat

Taxonomic status

Wheat (***Triticum aestivum* L.)** is the first important and strategic cereal crop belongs to family ***Poaceae* (*Gramineae*)** in which one to several flowered spikelets are sessile and alternate on opposite side of the rachis forming a true spike. **Linnaeus (1973)** first classified wheat recognized seven species within the genus *Triticum* while the wild relatives were included in the genus *Aegilops*. **In 1918, Sakamura** reported the chromosome number sets (genomes) for each commonly recognized type. This was a turning point in *Triticum* classification. It separated wheat into three groups. Diploids had 14 (n=7), tetraploids had 28 (n=14) and the hexaploids had 42 (n=21) chromosomes. Bread wheat is *Triticum aestivum (2n=42) while, T. durum(2n=28) and T. compactum (2n=28)* are the other major species. All three are products of natural hybridization among ancestral species which are no longer grown commercially **(Briggle, 1967).**

2.1.3. Cultivation and production of wheat

Wheat can be produced in a wide range of climates and soil conditions, grown in areas as far north as the Arctic Circle and as far south as the equator. The production of wheat is so widespread that it is being harvested somewhere in the world in any given month but it grows best in the regions having temperate climates with rainfall between 12 and 36 inches per year.

Wheat made a significant contribution to the increase in global food production during the past four decades as total production arose steadily through the use of higher yielding, water and fertilizer responsive, disease resistant cultivars supported by strengthened input delivery systems, tailored management practices and improved marketing **(Dixon *et al.*, 2006).** Globally, almost 50% of the wheat cultivated in the developing world (50 million ha) is sown under rain fed systems that receive less than 600 mm per annum, the poorest and most disadvantaged farmers of whom live in areas of less than 350 mm per annum **(CIMMYT, 2009).**

India holds the second position (after China) in total world wheat production which is about 27.0 million ha with the productivity of 26.4 q/ha **(CIMMYT, 2009).** Furthermore, even in traditionally irrigated areas, supplementary rather than full irrigation has become common, exposing wheat systems to water stress at various stages during the season. In order to meet growing human needs, wheat grain production must increase at an annual rate of 2%, without any additional land to become available for this crop and to meet this challenge; new level of understanding of the structure and function of the wheat genome is required.

2.2. Abiotic stress

Abiotic stresses such as extreme temperatures, low water availability, high salt levels, mineral deficiency and toxicity are frequently encountered by plants in both natural and agricultural systems. Across a range of cropping systems around the world, abiotic stresses are estimated to reduce yields to less than a half of that possible under

ideal growth conditions **(Boyer, 1982).** Abiotic stresses present a major challenge in our quest for sustainable food production as these may reduce the potential yields by 70% in crop plants.

2.2.1. Salinity stress in agriculture

Salinity is a major constraint to food production because it limits crop yield and restricts use of land previously uncultivated. Estimates vary, but approximately 7% of the world's total land area is affected by salinity **(Flowers *et al.*, 1997).** Most importantly, the percentage of cultivated land affected by salt is even greater. Furthermore, there is also a dangerous trend of a 10 % per year increase in the saline area throughout the world **(Pannamieruma, 1984).** In addition, salinity is a problem for agriculture because also only few crop species and genotypes are adapted to saline conditions. In the light of these demographic, agricultural and ecological issues, the threat and effects of salinity become even more alarming. Reducing the spread of salinization and increasing the salt tolerance of crops and improving species or genotypes to salt tolerance, particularly the high yielding ones are, therefore, issues of global importance.

2.2.3. Response of wheat plant towards salinity stress

Salt stress induces many physiological, biochemical and molecular response on plants; so that plants are able to develop tolerance mechanisms which will provide to be adapted to limited environmental conditions **(Arora *et al.*, 2002; Bohnert *et al.*, 2006; Gholamin *et al.*, 2010).** The adverse effects of high concentration of salts for plants are due to the osmotic retention of water and to specific ionic effects on the protoplasm. Water is osmotically held in salt solutions, so as the concentration of salt increased water becomes less and less accessible to the plant.

Soil salinity is known to suppress the growth of most crop species **(Maas & Hoffman, 1977).** It either completely inhibits germination at higher levels or induces a state of dormancy at low levels. Salinity also reduces imbibitions of water because of lowered osmotic potentials of the medium and causes changes in metabolic activity **(Yupsanis *et al.*, 1994).** To get more yields under stressed environment, effect of salinity is being tried to be replenish by different research tools. Seed priming treatments are therefore in practice to reduce the effects of salinity with lesser input of capital and energy **(Taylor *et al.*, 1998).** Many seed invigoration treatments are being used to improve the rate and speed of germination **(Lee & Kim, 2000).**

2.2.2.2. Phenological aspects

One immediate response of plants to elevated salinity is a decrease in the rate of leaf expansion. Consequently, the total leaf area of the plant is reduced. The common decrease in leaf expansion is associated with a loss in cell turgor pressure rather than a salt-specific effect.

Hu and Schmidhalter (1998) showed that wheat growing in 120 mMNaCl reacted with a 25% reduction in growth rate, Na^+ in the growing cells of leaves was at maximum only 20 mM, and Cl^- only 60 mM. In the salt-sensitive genotypes, in which salt is not effectively excluded from the transpiration stream, salt will build up to toxic levels in the leaves, resulting in death of old leaves and new leaves becoming injured and succulent **(Munns and James, 2003).** Consequently, the number of green and healthy leaves will ultimately decline. Although salinity can induce a rapid reduction in root growth **(Neumann, 1995)** shoot growth decreases proportionally more than root growth, causing an increase in the root/shoot ratio. In addition, salinity significantly decreased tiller number and their appearance in wheat **(Mass and Poss, 1989).** Salinity significantly reduces the total dry matter yield, and the degree of reduction in total dry matter depending on genotypes and salt concentrations **(Pessarakli and Huber, 1991).**

In addition, the response of phenological aspects to salinity changes with developmental stages of plant **(Neumann, 1995).** For example, many crops show a reduced tolerance to salinity during seed germination, but greater tolerance during later growth stages and vice versa in other crops. Results of salt tolerance for some crops have shown that wheat, sorghum and cowpea **(Mass and Poss, 1989)** were most sensitive during the vegetative and early reproductive stages, less sensitive during flowering, and least sensitive during the grain-filling stage. Therefore, information on the growth stage response to salinity is important in adopting suitable genetic and management strategies for saline soils.

2.2.4. Mechanisms of salinity tolerance of plants

Salt tolerance refers to the ability of plants to maintain the growth under saline conditions. To achieve this, a plant must have different mechanisms to tolerate salinity. Generally, salt tolerance is not an all-or-nothing phenomenon, and for this reason, some plants or genotypes are more salt-tolerant or sensitive than other. This results in a wide spectrum of plant responses to salinity that are defined by a wide range of adaptations at the whole plant level **(Greenway and Munns, 1980; Munns, 1993).**

2.3. Response of wheat plant to salinity

Wheat is moderately tolerant to salt with threshold without yield loss at 6 dS m -1 and with yield 50% loss at 13 dS m -1 **(Mass and Hoffmann, 1977).** Since the life cycle of wheat is an orderly sequence of development stage, salinity can have a significant effect on the developmental processes that occur at a particular time. The sequence of events has been separated into three distinct but continuous developmental phases **(Francois and Mass, 1994).** In the first phase, which encompasses the early vegetative growth stage, leaf and tiller buds are produced in the axils of the leaves and spikelet primordia is initiated. High salinity at this time reduces the number of leaves per culm, the number of tillers per plant, and the number of spikelet per spike **(Mass and Grieve, 1990).** The differentiation of the terminal spikelet signals completion of this phase. During the second phase, the main stem and tiller culms elongate, and the final number of florets is set **(Kirby, 1988).** Salinity stress during this phase may affect tiller survival and reduce the number of functional florets per spikelet. This phase ends with anthesis. Florets fertilization and grain filling occur during the third phase. Salinity during this phase affects seed number and seed size. The effect of salinity on tiller and spikelet numbers established during the first phase has a greater influence on final seed yield than the effects exerted on yield components in the latter two phases **(Kirby, 1988),** indicating the probability of improving salt tolerance of wheat genotypes during early growth stages.

Ashraf and Foolad, (2007) in their recent review have discussed adverse effect of salt viz. i) ionic effect, ii) osmotic effect, iii) nutrient imbalance, iv) hormonal imbalance, and v) production of reactive oxygen species (ROS). **Saddam, (2013)** studied the adverse effect of salinity on germination and establishment of crop plants. **Aliakbar et al., (2008)** reported that establishment of seedlings at early growth stages of crop plants as one of the most important determinants of high yield is severely affected by soil salinity. Therefore, high germination rate and vigorous early growth under salty soils is preferred. In this study salt tolerance of wheat cultivars were examined at germination and seedling growth stages.

2.3.1. Genomic responses

The analyze of the functions of stress inducible genes is an important tool not only to understand the molecular mechanisms of stress tolerance and the responses of higher plants, but also to improve the stress tolerance of crops by gene manipulation.

2.4. Genetic diversity

Genetic variability is of prime importance for the improvement of many crop species, including wheat, and nearly all crop improvement programs depend on genetic diversity in the available germplasm **(Graner *et al.*, 1994; Sorrells and Wilson, 1997)**.

2.4.1. Molecular markers

Traditional methods of plant breeding have made a significant contribution to crop improvement, but they have been slow in targeting complex traits like grain yield, grain quality and abiotic stress such as drought. In traditional plant breeding, the plant breeder during selection of desirable plant from the segregation population faced the following problems: (i) A large segregating population needs to be screened for a desirable trait e.g., yield and its component, quality, drought tolerance, disease resistance, etc.; (ii) Wait for advanced generations F_6 to start selection for quantitative traits, for which selection in early generation is not effective; (iii) It is very difficult to screen a segregating population for a desired trait when the trait is influenced by environment; (iv) Contrasting forms are often not distinguishable at seedling stage, making it necessary to grow population up to the adult stage; (v) It is difficult to undertake pyramiding of resistance genes, since selection of additional genes in presence of an existing resistance gene would be difficult.

To meet the great increase in food production necessitated by population growth, Biotechnology brings new and powerful molecular tools to plant breeders. Molecular markers allow geneticists and plant breeders to locate and follow the numerous interacting genes that determine a complex trait. Combining marker-assisted selection methods with conventional breeding schemes can increase the overall selection gain and, therefore, the efficiency of breeding program. With the use of molecular techniques it is possible to hasten the transfer of desirable genes between varieties and to introgress novel genes from wild species into crop plants. Availability of tightly linked molecular markers for a trait will facilitate such an indirect selection and help plant breeding by saving time and expense.

The development of molecular marker technologies during the last ten years has revolutionized the genetic analysis of crop plants. A significant progress has been made towards the use of molecular approaches in plant breeding. Molecular marker technology has changed dramatically during the past two decades. The molecular markers, so developed, may be used for improving the efficiency of traditional plant breeding by facilitating indirect selection through molecular markers linked to genes for the traits of interest, because, these markers are not influenced by the environment and can be scored at all stages of plant growth. In addition to these applications,

DNA markers can also be used for germplasm characterization, genetic diagnostics, study of genetic diversity, study of genome organization, etc. **(Rafalaski *et al.*, 1996).**

Molecular markers provide the best estimate of genetic diversity since they are independent of the confounding effects of environmental factors. In recent years, several molecular assays have been applied to assess genetic diversity among wheat cultivars **(Chen *et al.*, 1994).** These molecular methods are different in principle, application, type, amount of polymorphism detected and in task and time requirements. Assays based on the polymerase chain reaction (PCR) are considered to meet both the technical and genetic requirements for the characterization of plant and animal genetic resources **(Powell *et al.*, 1995).**

On the basis of the principles and methods employed, molecular markers can be broadly classified in the following four groups according to **Mohan *et al.*, 1997; Gupta and Varshney, 2000,** (i) hybridization based markers, (ii) PCR-based molecular markers and (iii) sequencing and DNA chip based markers.

2.4.2. PCR-based molecular markers

The development of new methods to perform analysis with molecular markers has been the focus of many recent studies and most of these are based on PCR amplification of genomic DNA **(Kochert, 1994).** Polymerase chain reactions (PCR) have been considered to be the most revolutionary modern technique of molecular biology in 1980s. PCR is a powerful extremely sensitive technique with applications in many fields such as molecular biology diagnostics and population genetics. The process depends on primer sequences of DNA, which match flanking sequences at both ends of targeted sequence. Through repeated denaturing, annealing and synthesized steps, the intervening sequence is synthesized in a 2n amplification.

2.4.3. SSR markers: Principles and application in wheat

Molecular markers based on polymerase chain reaction (PCR) methods, such as simple sequence repeats (SSRs) or microsatellites, have become important genetic markers in a wide range of crop species, including wheat **(Ma *et al.*, 1996).** SSR markers are abundant, dispersed throughout the genome, and show higher levels of polymorphism than other genetic markers **(Russell *et al.*, 1997).** These features, coupled with their ease of detection, make them ideal for identifying and distinguishing between accessions that are genetically very similar **(Saker *et al.*, 2005).** DNA sequences with di-, tri-, tetra- or penta-nucleotide tandem repeats are described as microsatellites **(Litt and Luty, 1989),** as simple sequence repeats **(Hearne *et al.*, 1992)** or as short tandem repeats **(Edwards *et al.*, 1991).** These markers appear to be hypervariable, in addition to which their co-dominance and reproducibility make them ideal for genome mapping, as well as for population genetic studies **(Dayanandan *et al.*, 1998).** The number of sites ranged from 103 to 105 depending on the species and repeat motif. Polymorphism produced by a variable number of tandem repeats has been demonstrated in a large number of species. This feature has made microsatellites a very attractive molecular marker for species with a narrow genetic base such as wheat and barley. This methodology is based on the use of primers complementary to SSRs. Multilocus profiles have been generated using different kinds of oligonucleotide containing simple sequence repeats as single primer **(Gupta *et al.*, 1994; Nagaoka and Ogihara, 1997)** or in combination with arbitrary sequence oligonucleotides **(Wu *et al.*, 1994).**

In wheat, **Devos *et al.*, (1995)** searched sequence database and converted two microsatellite sequences into PCR based markers. **Röder*et al.*, (1995); Ma *et al.*, (1996) and Plaschke *et al.*, (1995)** investigated the potential of microsatellite sequences as genetic markers in hexaploid wheat. These markers were genome specific and displayed high levels of variation. More recently, a detailed genetic map of 279 microsatellite loci **(Röder *et al.*, 1998),** another map of 50 loci **(Stephenson *et al.*, 1998),** 65 loci for the D genome **(Pestsova *et al.*, 2000)** and **(Huang *et al.*, 2001)** have been developed for bread wheat. The availability of extensive molecular maps of wheat microsatellites will help in tagging genes of economic importance for marker assisted selection.

Various studies have used SSR markers to investigate genetic diversity in cultivated hexaploid wheat genotypes of T. aestivum L. (**Dreisigacker *et al.*, 2005; Liu *et al.*, 2005; Hao *et al.*, 2006; Salem *et al.*, 2008; Schuster *et al.*, 2009).** SSR markers have been successfully employed to characterize genetic diversity in seed bank collections of improved wheat germplasm **(Bo¨rner *et al.*, 2000; Huang *et al.*, 2002)** and wild relatives **(Li *et al.*, 2000; Hammer, 2000).**

SSRs provide highly informative markers because they are co-dominant (unlike RAPDs) and generally have high polymorphic information content **(Gupta *et al.*, 1996).** Comparative studies of SSR markers in wide range of crop species, like wheat **(Jones *et al.*, 1997)** have generally revealed good congruence between the genetics patterns revealed by the two genetic markers. Our objective was to investigate and compare the genetic relationships among wheat **(*Triticum aestivum* L.)** cultivars, or lines, using molecular data obtained from SSR profiles.

CHAPTER-3 MATERIALS AND METHODS

The present research work was carried out at laboratory of Department of Biotechnology, College of Agriculture, of S.V.P. University of Agriculture and Technology, Meerut during rabi season of 2014. The details of materials used, methods, techniques applied and also climatic and edaphic conditions prevailed during experimentation is described below:

3.1. Collection of Genetic Material

The 15 wheat (*Triticum aestivum* L.) genotypes representing different geographic diversity were obtained from available germplasm in the Crop Research Centre SVPUA&T, Meerut and Central Institute for Soil Salinity Research Kernal. (Table-3.1)

Table 3.1: List of Wheat (*Triticumaestivum* L.) germplasm taken for present study

S.No.	Varieties	S.No.	Varieties	S.No.	Varieties
1.	PBW- 226	**6.**	PBW- 502	**11**	WH- 1021
2.	RAJ- 3765	**7.**	DBW- 16	**12**	Kharchia- 65
3.	PBW- 550	**8.**	DBW- 17	**13**	KRL- 210
4.	PBW-373	**9.**	WH- 711	**14**	KRL- 13
5.	PBW-343	**10.**	HD- 2967	**15**	KRL- 19

3.2. Maintenance of crop plants in field and in pots

The experiment was carried out at the Crop Research Centre and laboratory, department of agriculture biotechnology, SardarVallabhbhai Patel University of Agriculture & Technology, Meerut (U.P.) at latitude of 29^0 40′ North and longitude of 77^0 42′ East and at an altitude of 237 meter above mean sea level. Meerut lies in the heart of Western Uttar Pradesh and has sub-tropical climate. The experimental field had an even topography with good drainage system.

During the season, on dated 24 Dec, 2013 all varieties were sown in field, Sowing was done row. After germination of seeds, fields were irrigated at regular interval of 20-25 days. The crop was maintained in the field using conventional agronomic practices to keep the crop in good condition. Various morphological and physiological parameters were observed during the growth of crop. To study the genetic diversity of wheat genotypes the actively growing leaves were harvested and crushed to fine powder in liquid nitrogen and stored in -80 degree for further experiment.

To study the effect of salt stress on the crop, the seeds of all varieties were also shown in hydroponic solution in two replications, one is on control condition and another was under the treatment of 10 mMNacl for inducing salt stress. (Figure 3.1 A&B)The seeds were maintained in under hydroponic condition under Hoagland nutrient solution.

Hoagland's Complete Nutrient Solution

This is made essentially according to the following reference: D.R. Hoagland and D.I. Arnon. The water-culture

method of growing plants without soil. (**Calif *et al.*, 1950**) with slight modification as described below. Prepare the following stock solutions (1-6) and use the amounts indicated to prepare 1 liter (final volume) of nutrient solution:

Micronutrient stocks: combine the salts (1 M $NH_4H_2PO_4$,1 M KNO_3 ,1 M $Ca(NO_3)_2$,1M $MgSO_4$) in a total volume of one liter of water, and then use 1 mL/L of this entire stock mixture (2.86 gm H_3BO_3, 1.81 gm $MnCl_2$.$4H_2O$, 0.22 gm $ZnSO_4$.$7H_2O$, 0.08 gm $CuSO_4$. $5H_2O$,0.02 gm H_2MoO_4 . H_2O (Assaying 85% MoO_3). Thereafter added iron stock (To make up the iron stock, take 26.1 g EDTA and dissolve in 286 ml water that has ~19 g KOH . Then dissolve 24.9 $FeSO_4.7H_2O$ in ~ 500 ml water. Slowly add the iron sulfate solution to the potassium EDTA solution and aerate this solution overnight with stirring. The pH rises to about 7.1 and the solution is wine red and very little precipitation occurs. Make to 1 liter final volume and store in a bottle covered with foil /dark) to make up a total of 1 L of nutrient solution.

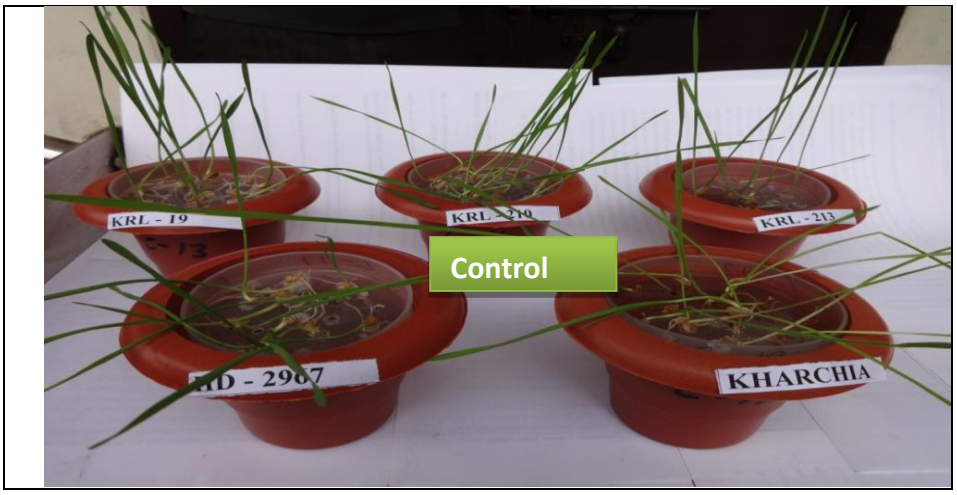
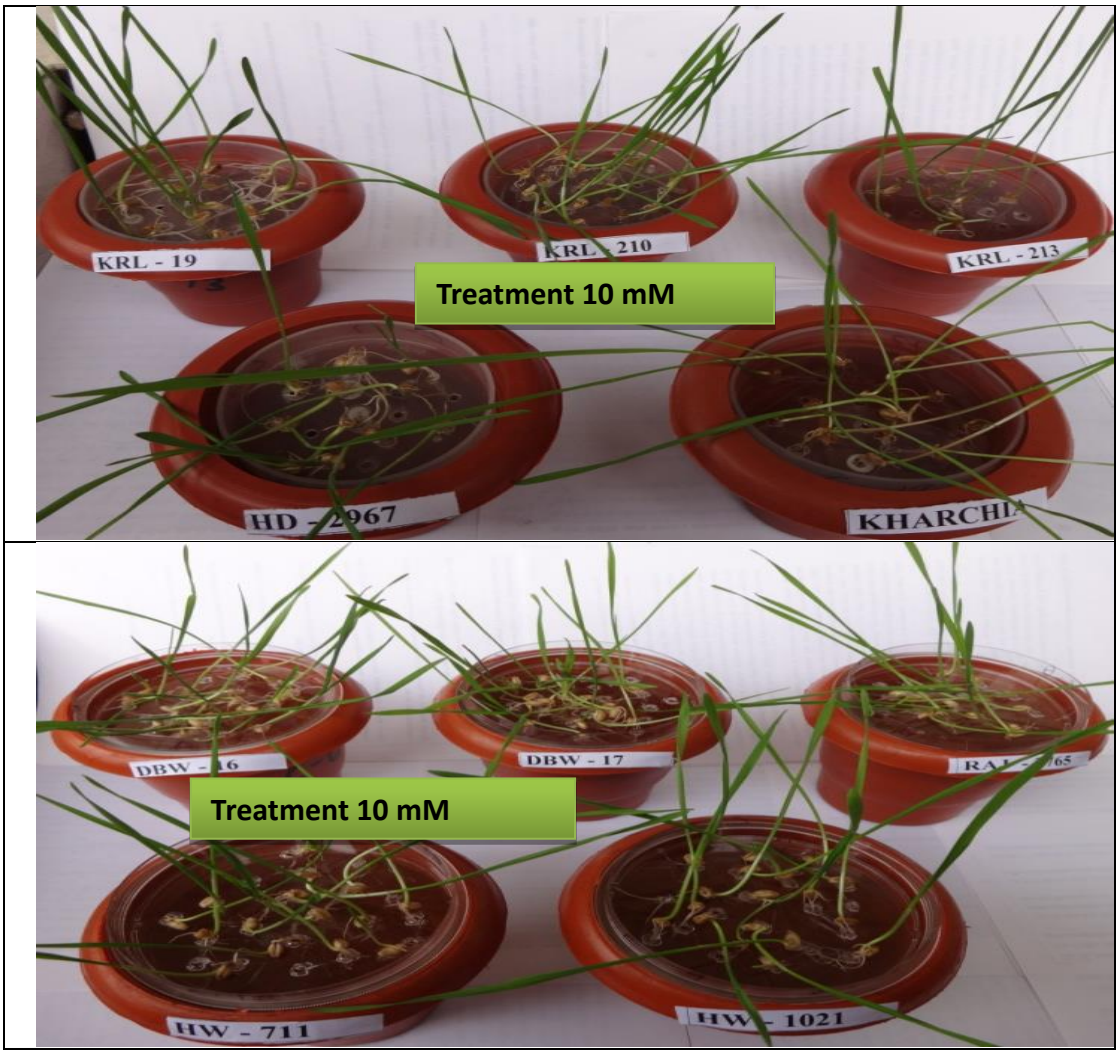


Figure 3.1 A: Wheat genotypes grown in Hoagland solution (Control)



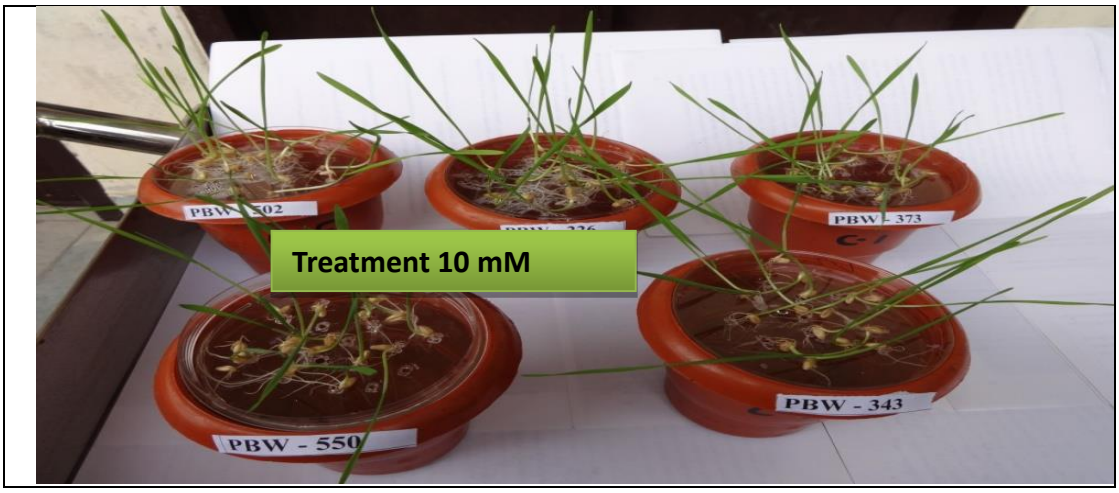


Figure 3.1 B: Wheat genotypes grown in Hoagland solution (Treatment)

3.3. Methods

Observations of Morphological characters were recorded on randomly selected five plants from each introgression lines in each replication at maturity. These plants were harvested and threshed separately. The data were recorded on following characters:

3.3.1. Morphological Data from Hydroponic condition

Seeds of wheat were maintained in Hoagland solution in hydroponic system to study the effect of salt stress. Various morphological attributes were recorded after twenty days from both control and treatment condition on randomly selected five plants from each introgression genotype.

Germination count: After the sowing Germination count were recorded at 24, 48, 72, and 96 hrs. in both control and treatment condition.

- **Shoot Length:** Shoot length is measured in centimeters from the base of root to the tip.
- **Root Length:** Root length is measured in centimeters from the base of shoot to the bottom.
- **Fresh weight:** After the growth of 20 days**,** the Fresh weightwas recorded in gram. For this the plants were randomly selected and blotted on tissue paper and fresh weight was recorded.
- **Dry weight:** After taken the fresh weigh, the plants will dry for overnight in oven and dry weight of whole plants were recoded.

3.3.2. Morphological Data from field condition

3.3.2.1. Pre harvest characters

- **Plant Height:** Plant length is measured in centimeters from bases of the plant to the tip of the spike (excluding awns) at the time of maturity.
- **Tiller:** Wheat seedling of different cultivars in their early stages of growth show marked difference in their growth habit. The character is recorded at 30-45 days after sowing depending upon the growing condition.
- **Length of Spike:** Ear length is measured in centimeter from tips of apical spikelet (excluding awns) to the bases or collar of ear.
- **Spikelets per Spike:** Number of spikelets present on spike is counted and mean of 10 spikes per genotypes is depicted in the text.

3.3.2.2. Post harvest characters

- **Seeds per Spike:** Mean number of seeds counted from 10 randomly sampled spikes at maturity is recorded as seeds per spike.
- **Thousand Grain Weight:** Weight of thousand seeds expressed in grams.

3.4.1 Isolation of genomic DNA

***TrisHcl*[1 M] (pH 8.0) 100 ml:**Dissolved 15.76gm of TrisHcl in 80 ml of distilled water. Adjusted the pH to 8.0 with 0.1 N HCl and made up volume to 100 ml. Autoclaved and stored at room temperature.

***EDTA* [0.5 M] (pH 8.0) 100 ml:** Weighed 18.6 gm EDTA Na_2 and dissolved in 80 ml of distilled water. Adjusted the pH to 8.0 by adding 0.1 N NaOH. Mixed vigorously with a magnetic stirrer for some time to ensure that all the solute have been dissolved. The volume was made up to the volume 100 ml. The solution was then autoclaved and stored at room temperature.

NaCl [5 M] 100 ml: 29.25 gm of NaCl was dissolved in 80 ml of distilled water properly and made up to the volume 100 ml. The solution is then autoclaved and stored at room temperature.

CTAB extraction buffer 10 X (pH 8.0) 500 ml: CTAB extraction buffer was prepared with a final concentration of CTAB 2.0%, NaCl 1.4 M, Tris buffer 100m M, EDTA 20mM, β-mercaptoethanol 0.2% and PVP 1 %. To prepare the 500 ml extraction buffer add 10.0 gm of CTAB, 43.837 gm of NaCl, 50 ml TrisHCl from stock of 100mM, 125 ml of EDTA from stock of 20 mM, and adjusted the pH to 8 and made up the volume to 500 ml. Autoclaved and stored at room temperature. 0.03 ml β-merceptoethanol in 15 ml of buffer and 0.15 gm of PVP in 15 ml of buffer was added just before use.

TE buffer (pH 8.0) 100 ml: Added 1.00 ml of Tris buffer from stock of 1M TrisHCl and 0.2 ml of EDTA from stock of 0.5M to a final concentration of 10mM and 1mM respectively.

Chloroform: Isoamylalcohol: Chloroform and Isoamylalcohol was mixed in the ratio of 24:1 and stored in a brown bottle. A vapour of Isoamyl alcohol is poisonous, so care should be taken.

RNase A: 10 mg/ml: 10 mg RNase A was dissolved in 1 ml of double distilled autoclaved water. Dispensed into aliquots and stored at -20°C.

Ethanol 70 %: 70 ml of ethanol was mixed with 30 ml of distilled water and stored in a tight capped bottle.

For genomic DNA isolation CTAB method (**Doyle & Doyle, 1987**) with some modification was used. CTAB (Cetyl-trimethyl-ammonium bromide) is a cationic detergent, which solubilizes membranes and forms a complex with DNA. For isolation of DNA, 3 gm of fresh leaf sample/ Young frozen leaf were taken & ground to fine powder with mortar and pestle using liquid nitrogen and transferred to the 15ml of pre-warmed (65°C) extraction buffer in a centrifuge tube. The sample was mixed well by occasional shaking. Samples were subjected to I hr. incubation at 65°C. An equal volume of Chloroform: Isoamylalcohol (24:1) was added to the tube and mixed gently for 15-20 minutes. The tubes were then centrifuged (CPR-24, Remi India) at 8,000 rpm for 10 minutes. After centrifugation aqueous phase is transferred to fresh tube and again extracted with chloroform: isoamyl alcohol. Added 0.6 volume of Chilled isopropanol and kept at -20°C for 2 hours. Centrifuge the tubes at 10,000 rpm for 15 minutes at 4°C. After centrifugation, supernatant was discarded and pellet was washed with 70 percent ethanol. Finally the pellet was air dried and was dissolved in 100 µl of TE buffer.

3.4.2 Purification

5.0 µl of RNase (10 mg/ml) was added to 100 µl of DNA solution, and incubated at 37°C for 1 hour. Equal volume of C: I (24:1) was added to the sample and mixed gently by inverting the tube. Tube was spun at 13,000 rpm at room temperature for 1 minute. Aqueous layer was collected and 1 ml of 100% Ethanol was added. Again centrifuge at 13,000 rpm for 10 minutes at 4°C.Threw the supernatant and added 500µl of 70% ethanol. Mixed gently for 2-4 times and spun with 13,000 rpm for 10 minutes at 4°C. Discarded the supernatant and finaly the pellet was air dried at 37°, dissolved in 100 µl of TE buffer and stored at 4°C for further experiments.

3.4.3. Quantification of Genomic DNA

3.4.3.1. Spectrophotometeric determination

The genomic DNA dissolved in TE buffer was quantified by recording A_{260} nm in a UV-VIS Spectrophotometer-119 (Systronic Pvt. Ltd., India). Reference was set against TE buffer as blank and absorbance was recorded at 260 and 280 nm. The ratio of A_{260}/A_{280} provided an estimate of purity of nucleic acid. The ratio of OD_{260}/ OD_{280} was between 1.8-2.0 for pure DNA prepration (**Sambrook *et al.*, 1989**). The concentration of DNA in µgm/ml of the sample was calculated using the extinction coefficient of DNA, i.e. 1 OD unit at 260nm = 50 µg/ml of double stranded DNA (**Sambrook *et al.*, 1989**).

3.4.3.2. Electrophoresis analysis

Electrophoresis buffer [TBE]: Weighed 108.0 gm of Tris base, 27.5gm of Boric acid and 7.44 gm of EDTA and were dissolved in 750 ml double distilled water. Adjust the pH 8.0 with 1N NaOH. Filtered and adjusted the final volume to 1 liter. Autoclaved the buffer and store at room temperature.

DNA loading dye: Weighed 0.0025 gm (0.25% w/v) of bromophenol blue, 0.25 gm (0.25%) xylene cyanol and added 3 ml (30%) glycerol and dissolved in 8 ml double distilled water. Made up the volume to 10 ml with distilled water and set the pH 8.0 with 1 N NaOH. The dye was aliquoted into eppendorf tubes and stored at 4°C.

Ethidium Bromide: Weighed 100mg Ethidium Bromide and dissolved in 10 ml of double distilled water and

stored at 4°C. (Note-EtBr is highly carcinogenic Use gloves while handling).

Agarose gel electrophoresis of the isolated genomic DNA was performed in electrophoresis assembly (Tarson, midi model) to know about the quality of DNA. Larger molecule migrate slower because of greater frictional drag and because they form their way through the pores of gel less efficiently than smaller molecules. As the size of genomic DNA is quite big a 0.8% gel was used to visualize the genomic DNA, as it can resolves DNA molecules in the range of 0.7 to 8.5 kb. 0.8% (w/v) agarose gel was prepared by suspending 0.8g agarose in 100 ml 1x TBE buffer. 8 µl ethidium bromide was added and the gel was casted in a gel tray fixed in a gel caster which was kept on horizontal surface. A comb was placed in such a way that 2 mm gap was maintained between the bottom of the gel and the comb tip. The gel was allowed to solidify and then comb was removed. Gel was placed in the electrophoresis chamber and 1x TBE buffer was added to fill the chamber and flood the surface of the gel

The isolated genomic DNA samples were mixed with a loading dye in and were electrophoreses on 0.8% agarose gel in 1x TBE buffer at 3-5 volt/ cm for 2 hours.

3.4.4. PCR amplification.

3.4.4.1. The following solutions were used for PCR amplification:

Template DNA: DNA was extracted from leaves samples. It was than diluted in sterile distilled water and prepared the working solution of 25ng/µl.

Taq DNA polymerase

heTaq enzyme used for polymerization was obtained from Bangalore Genei (5 units/µl). It was stored at -20°C.

Assay Buffer: A readymade assay buffer TAPS 100mM, KCL 500mM, MgCl2 15mM and Gelatin 0.1% supplied with Taq Polymerase enzyme by Bangalore Genie was used.

dNTPs: dNTPs mix from Bangalore Genei was used for PCR amplification. The concentration for each dNTP (dATP, dTTP, dCTP, dGTP) was 2.5 mM. It was also stored at –20°C.

Primers: A set of SSR primers were custom synthesized by IDT (USA). The primers were dissolved in appropriate amount of TE as per concentration given with the primers. The primers were diluted with the TE to make the working solution of 10µM concentrations. Sequences of primers are given in table 3.2.

Table- 3.2: Primer sequence forward and reverse

S.N.	Primer Code	Primer Sequence 5'-3'
1.	CWM122	F-ATCGCTGGCAGCAGGCTAGCTA
		R-TCTTCCTCGAACAACCCTAG
2.	CWM119	F-GTCAACAACAACGCCTGG
		R-TAAGCGGAAGAAAGATG
3.	CWM107	F-GCCGGCTCGCCATGTTCTCCA
		R-CTCATCATCTCGACTCGCCCT
4.	Xgwm276-7A	F-ATTTGCCTGAAGAAAATATTT
		R-AATTTCACTGCATACACAAG
5.	CWM115	F-CCTTTCTCATCCTTGCCCTCC
		R-GTTGTTGTTGTGGTCGAAATGGTT
6.	Xgwm11-1D	F-GGATAGTCAGACAATTCTTGT
		R-GTGAATTGTGTCTTGTATGCTT
7.	Xgwm332-7A	F-AGCGAGCAAGTCACCAAAAC
		R-ATTCGCTGGAAAAGAGTGCCAA
8.	CWM118	F-TTTCGCAGCCGCAACTACC
		R-TGATCTTCCACGCCGCTATG
9.	Xgwm293-5A	F-TACTGGTTCACATTGGTGGTGCG
		R-TCGCCATCACTCGTTCAAG
10.	CWM110	F-TCAGGGAAGCACCGTGTAGAG
		R-CGGCAGTGAGCGCGGGTAAT

P**CR-Reaction**

DNA amplification reaction for SSR was performed in a total volume of 20 µl. The components used are described in table 3.3 and were mixed gently in 0.2 ml thin walled PCR-tubes. A master mix was prepared except template DNA to avoid pippeting error. Master mix was then distributed equally into tubes and finally template DNA was added. The mixture was mixed by spinning for a short time and placed in (Long Gene) for amplification. The amplification was performed by using the thermal profile as describe in table 3.4.

Table 3.3 Components of PCR reaction mixture

Components	**Stock Concentration**	**Final Concentration**
Taq Buffer	10x	1X
$MgCl_2$	25 mM	2.5Mm
dNTPs mix	10mM	1mM
Primer 5’	100µM	10µM
Primer 3’	100µM	10µM
Taq Polymerase	5U/µl	0.5U/ µl
DNA	25ng	5ng
Water (Milli Pore)	To make up final volume	20µl
Total		**20 µl**

Table 3.4. Thermal profile for PCR reaction

Step	**Temperature (°C)**	**Time**
Initial Denaturation	95	2 min.
Denaturation	95	1 min.
Annealing*	Variable with primer	50 min.
Extension	72	50 min.
Cycles	35	
Final Extension	72	7 min.
Final Hold	4	------

*Annealing differed primer to primer.

3.4.3.2. Gel electrophoresis for amplified DNA

Amplified fragments were of small size hence, 2 % of agarose gel was used to resolve them. 2 % (w/v) agarose gel was prepared by suspending 8gm agarose in 400 ml, 1x TBE buffer. 40 µl ethidium bromide was added and the gel was casted in a trough (Bangalore Genei), kept on horizontal surface. The gel was allowed to solidify and then comb was removed carefully. Gel was placed in the electrophoresis assembly (Bangalore Genei) and filled with 1x TBE buffer to flood the surface of the gel.

The amplified DNA samples were mixed with a loading dye in and were loaded into wells. The gel was then allowed to run at 250V for 3-4 hours. After electrophoresis the gel was visualized and photographed with Alpha Innotech (Alphaimager) System.

3.4.4. Diversity Analysis using SSR primers

In order to assess the ability of primers to resolve the different varieties the resolving power (Rp) for each primer was calculated following **Prevost and Wilkinson's (1999)** method as Rp = Ib (band information). Resolving Power is calculated as 1-[2 x (0.5-p)], p being the proportion of the 40 varieties containing the bands and Gene Diversity is calculated as $1-\sum pi^2$ **(Anderson *et al.*, 1993).**

The bands were scored as present (1) or absent (0) for each DNA sample with the 10 SSR primers. Amplification was performed twice and only reproducible amplifications products were included in the data analysis. Similarity matrix using the similarity coefficient of **Jacquard (1910)** was constructed from the whole data. Pair wise distances between DNA accessions were calculated and analysed using the Unweighted Pair Group Method Arithmetic average (UPGMA) **(Sneath and Sokal, 1973)**. Clusters were analysed using the computer program NTSYS-PC, version 2.11s **(Rohlf, 2000)**.

CHAPTER-4RESULT AND DISCUSSION

4.1. Collection of wheat germplasm

For study of Morphological, genetic diversity analysis and to study the effect of salt stress on Indian wheat cultivars a total of fifteen varieties were collected viz.PBW-373, PBW-343, PBW-550, PBW-226, PBW-502, DBW-16, DBW-17, RAJ-3765, WH-1021, WH-711, HD-2967, KHARCHIA-65, KRL-19, KRL-210 and KRL-213.

To study the general morphological and physiological attributes along with genetic diversity analysis the seeds of all varieties were sown on experimental station of Department of Biotechnology, SVPUA&T, Meerut. The crop was maintained in good condition using recommended agronomic conditions. Various morphological characters were observed at different stages of plant growth to understand the responses of all genotypes under normal conditions.

Simultaneously to study the effect of salt stress on seedling stage the seeds of all varieties were grown in Hoagland solution under hydroponic system as already shown in material and methods. Seeds of all genotypes were grown in two sets of pots; one for control with normal Hoagland solution and another with 10% saline Hoagland solution. The seeds were regularly monitored and various observations were recorded at different stages of growth.

4.2. Morphological Characterization

4.2.1. Pre-harvest characters

Plant height: It is a crucial factor for understanding the development of plant. Plant height was recorded in cm. in all the genotypes at the time of maturity (Table 4.1). Variation in plant height was observed in different wheat genotypes and varied from 66cm in PBW502 to 108cm in KRL210.

Number of Productive Tillers: The numbers of productive tillers per plants were noted at the time of maturity. The data was recorded from five plants of each variety and their mean is shown in table (4.1). As per the result presented in table, the no. of productive tillers per plant was varied from 6.2 in DBW 17 to 2.4 in Raj 3756.

4.2.2. Post-Harvest characters

Length of spike: The length of spikelets is directly contributed to yield component. Therefore the spikelet of five different plant of same variety was measured for their length and there mean is shown in table (4.1). The result showed that the length of spike varied from 11.8 cm in PBW 502 to 15.94 cm in PBW226 genotype.

Spikelets per spike: This parameter is also directly related to plant yield. The no. of spikelets per spike varies from 10.8 in PBW502 to 18.80 in KRL 213.

Seeds per spike: Seed per spike is direct measure of yield/plant and also economically important. It varies from variety to variety as shown in Table 4.1. The maximum seeds/spike observed in HD 2967 i.e. 51.33. On the other hand the minimum no of seeds were observed in PBW 502 i.e. 25.20.

Table- 4.1 Morphological observations of wheat genotypes

S.N.	Variety	Plant ht. (cm)	No. of Tillers	Spike Length (cm)	No. of Spikelet/ spike	No. of Grains / Spike	Test Wt. (gm)
1.	**PBW 373**	76.4	4.4	14.12	14.2	33.75	37.0
2.	**PBW 343**	84.4	4.6	12.88	16.6	36.00	46.3
3.	**PBW 550**	87.0	4.4	15.78	17.6	48.00	33.2
4.	**PBW 226**	93.6	4.2	15.94	15.0	38.66	42.8
5.	**PBW 502**	66.0	2.6	11.80	10.8	25.20	29.9
6.	**DBW 16**	92.8	5.4	13.24	12.8	28.20	33.6
7.	**DBW 17**	88.2	6.2	14.02	15.6	41.33	38.2
8.	**Raj 3765**	74.6	2.6	13.66	11.6	31.40	38.6
9.	**WH 1021**	95.2	4.4	15.44	17.6	45.20	31.8
10.	**WH 711**	79.6	4.2	12.86	17.8	39.33	35.0
11.	**HD 2967**	90.6	4.2	13.26	17.2	51.33	33.5
12.	**Kharchia 65**	100	4.6	14.40	15.0	30.00	28.6
13.	**KRL 19**	86.4	3.8	14.98	15.20	37.25	36.3
14.	**KRL 210**	108.6	3.8	15.32	17.60	29.20	29.4
15.	**KRL213**	90.0	4.0	13.36	18.80	49.66	49.66

Thousand Grain weight: The actual yield of the genotype is measured by thousand grain weight as the yield of plant depends upon size as well as weight of seeds of genotype. In table 4.1 test Weight of the grain varies from 29.4 gm in KRL-210 to 49.7 in KRL-213.

4.3. Effect of salt stress on seedling stage

To study the effect of salt stress the seeds of all variety were grown under Hogland solution in a hydroponic system. The seeds were treated with 10mM saline Hogland solution for inducing salt stress. The seeds were allowed to grow along with control plants in petri plates. Various observations were recorded at different stage of germination and seedling growth.

Germination count: Germination count was recorded at 24 hrs, 48 hrs, 72 hrs and 96 hrs with a regular interval of 24 hrs. (Table 4.2). The seeds of all variety took different time to germinate as well as show different percent of seed germination in control and treatment conditions. Initially the 15 seeds all variety was planted in solution under control and treatment conditions. As the result indicated that under control condition, most of the seeds were germinated within 24 hrs. The variety PBW 502 and HD 2967 have given a quick response in germination and out of 15, 13 seeds were germinated within 24 hrs. Whereas the variety KRL-210 and KRL 213 were seems to be slow germinator as only 2 seeds out of 15 were germinate within first 24 hrs. Under the treatment condition the germination was significantly affected by salt stress as the germination was found to be delayed as compared to control plants. The seeds of any variety did not germinate within first 24 hrs. Under the effect of salt stress, the seeds starts germinated after 48 hrs. Among them the variety HD 2967 shows a good response under salts stress. As 10 seeds out of 15 were germinated in 48 hrs. Overall the all the seeds were germinated in control and treatment conditions. However the time taken for germination is different for each genotype.

Table- 4.2: Germination count

S.N.	Variety	24 hours		48 hours		72 hours		96 hours	
		C	T	C	T	C	T	C	T
1	PBW-373	8	_	5	6	2	7	_	2
2	PBW-343	6	_	6	5	3	8	_	2
3	PBW-550	10	_	5	6	_	6	_	3
4	PBW-226	11	_	4	7	_	7	_	1
5	PBW-502	13	_	-	7	2	6	_	2
6	DBW-16	10	_	5	5	_	8	_	2
7	DBW-17	4	_	9	7	2	5	_	3
8	RAJ-3765	9	_	6	3	_	8	_	4
9	WH-1021	9	_	4	8	2	6	_	1
10	WH-711	8	_	5	6	2	5	_	4
11	HD-2967	13	_	2	10	_	4	_	1
12	KHARCHIA-65	5	_	9	4	1	9	_	2
13	KRL-19	4	_	5	3	6	9	_	3
14	KRL-210	2	_	10	2	3	10	_	3
15	KRL-213	2	_	13	2	_	9	_	4

Root length: Similar to shoot length, the root lengths of all genotypes were measured after the 17 days of growth of plantlets. The root length varies from 4.24 in PBW550 to 11.64 in PBW343 (Table 4.3, figure 4.4 A). The effect of salt treatment was observed significant in all genotypes. Whereas, the genotypes KRL-19 and KRL 213 showed very little effect of salt stress.

Shoot length: The shoot length of the plantlets was measured after 17 days of growth. It was found to be varied from a lower value of 5.52 in DBW-17 to a higher value of 21.96 in Kharchia 65 genotypes (Table 4.3, Figure 4.4 B). The shoot length of all genotypes were reduces significantly after the treatment of salt except the genotypes PBW-502, DBW-16, DBW-17 and Raj 3756 where it found to increase a little bit of remain almost equal to the control. Hence these genotypes can be considered to salt tolerant genotypes.

Fresh weight: Fresh weight was measured of all the genotypes and found to be varied from 0.689 in Kharchia 65 to 1.186 in HD-2967. The salt stress greatly reduced the fresh weight in all the genotypes (Table 4.3, Figure 4.4 C).

Dry weight: After drying the whole plant, the dry weight was recorded in all the genotypes. The dry weight was varied from 0.075 to 0.776 (Table 4.3, Figure 4.4 D). The two genotypes shows exceptionally very high dry weight i.e. PBW-373 and PBW-502. It may be due to some experimental error or the genotypes were so good as they give this much of dry weight biomass. This can be confirming after repeating the experiment. But it is not possible in due course of time.

Table- 4.3- Comparative morphological attributes of control and treatment

S.N	Variety	Root Length (cm)		Shoot Length (cm)		Fresh weight (gm)		Dry weight (gm)	
		C	T	C	T	C	T	C	T
1	PBW-373	7.58	2.80	12.88	6.96	0.906	0.619	0.776	0.137
2	PBW-343	11.64	3.74	15.58	6.50	0.937	0.591	0.183	0.147
3	PBW-550	4.24	5.28	13.54	8.24	0.999	0.712	0.168	0.129
4	PBW-226	5.50	4.48	16.18	10.22	0.867	0.576	0.179	0.135
5	PBW-502	6.42	3.52	6.42	6.70	0.889	0.776	0.520	0.113
6	DBW-16	7.48	3.44	7.48	9.14	0.881	0.594	0.154	0.115
7	DBW-17	5.52	4.40	5.52	8.78	1.151	0.854	0.197	0.159
8	RAJ-3765	9.00	5.24	9.00	9.62	1.135	0.847	0.156	0.114
9	WH-1021	11.44	3.18	11.44	8.74	0.871	0.523	0.166	0.127

10	**WH-711**	6.80	3.88	16.46	10.18	1.176	0.889	0.183	0.143
11	**HD-2967**	7.06	3.34	14.24	8.72	1.186	0.900	0.179	0.140
12	**KHARCHIA-65**	7.48	4.92	21.96	18.02	0.689	0.402	0.075	0.036
13	**KRL-19**	8.60	8.62	14.12	9.40	0.939	0.642	0.129	0.090
14	**KRL-210**	6.48	5.22	12.08	9.89	0.721	0.425	0.078	0.040
15	**KRL-213**	5.14	5.22	14.10	10.04	0.764	0.472	0.094	0.055

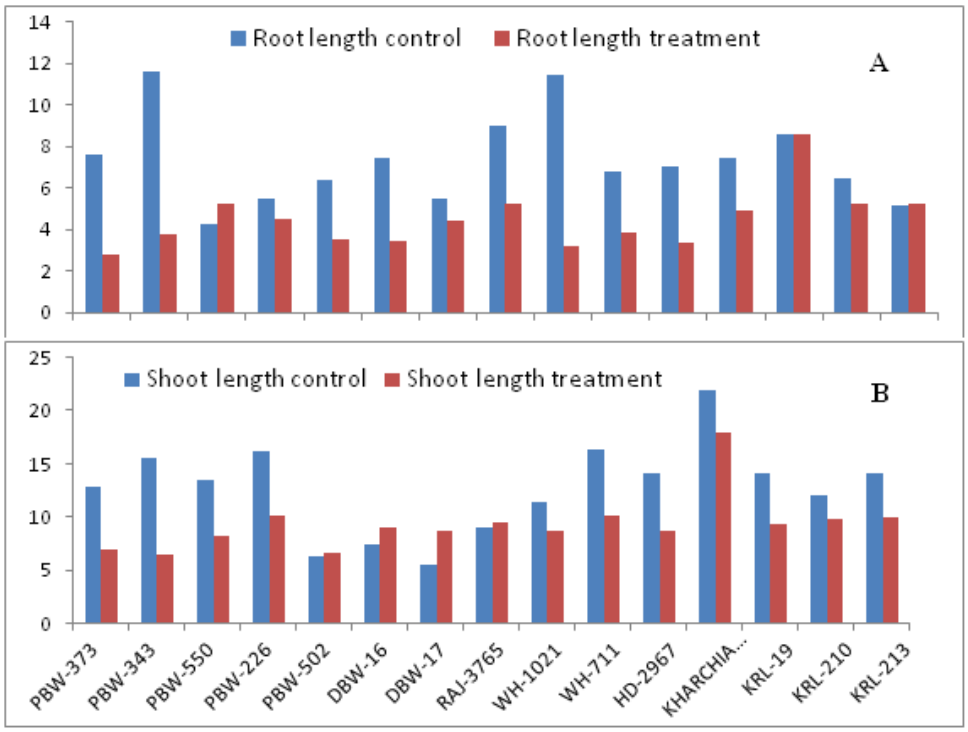


Figure 4.4 A, B:Bar graph of root and shoot length of control and treatment

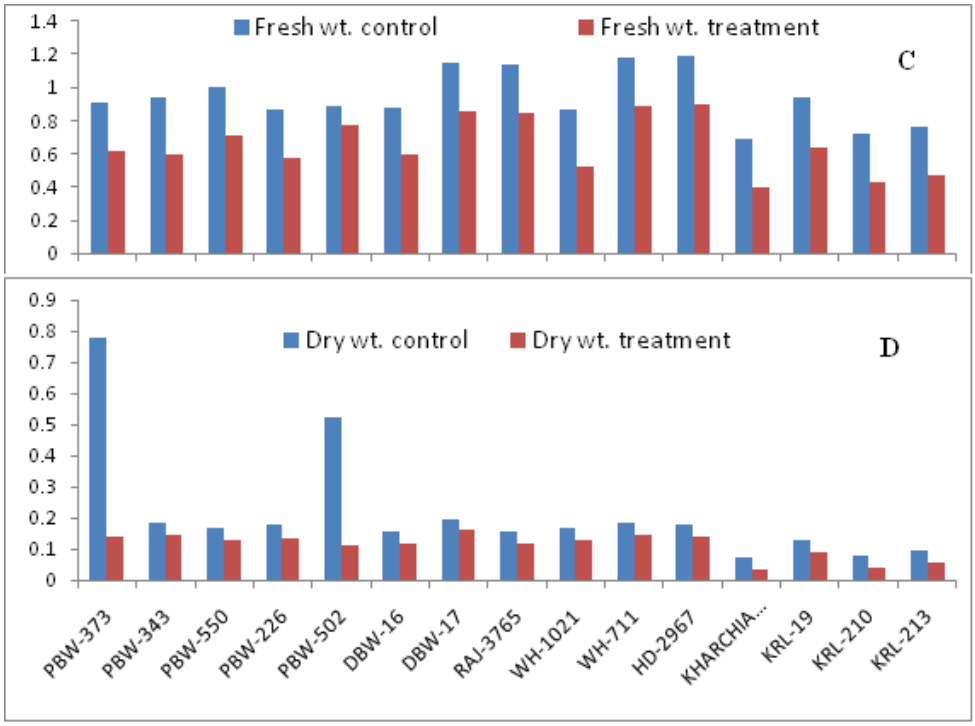


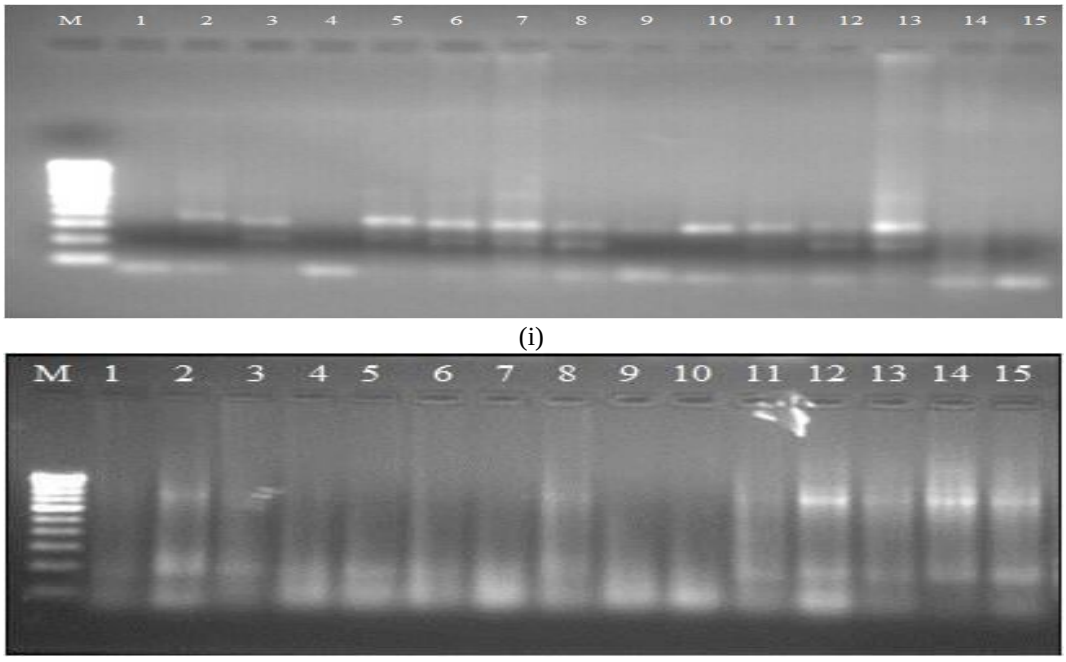
Figure 4.4 C, D: Bar graph of fresh and dry weight of control and treatment

4.4. Characterization of wheat genotypes using molecular markers

Fifteen varieties of wheat which were normally grown under field condition were characterized at molecular level using SSR markers.

4.4.1. Molecular profiling using SSR

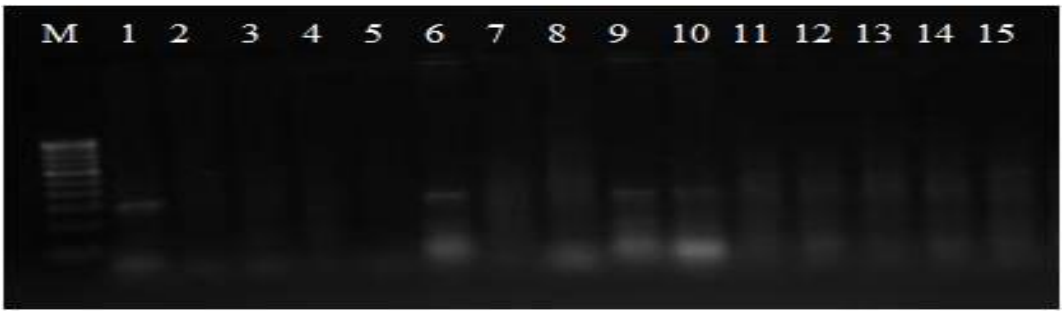
12 SSR primers were used to amplify the genomic DNA of 15 wheat genotypes. Out of 12 SSR primers used, 10 (83.3%) primers resulted in polymorphic, scorable and reproducible results (Figure 4.5 A, B, C, D and E). Hence, they were used for the analysis of genetic diversity of wheat genotypes.



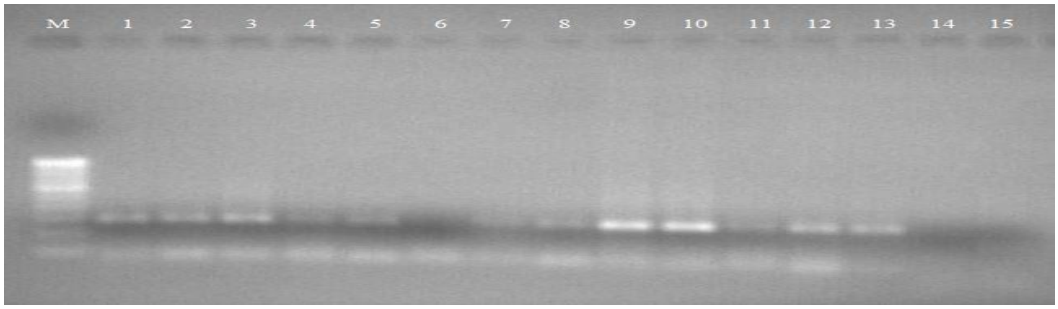
(i)

(ii)

Figure 4.5 A: 1.5% Agarose gel showing PCR amplification of 15 different genotypes of wheat (1-15) using SSR marker (i)CWM122 and (ii)CWM119

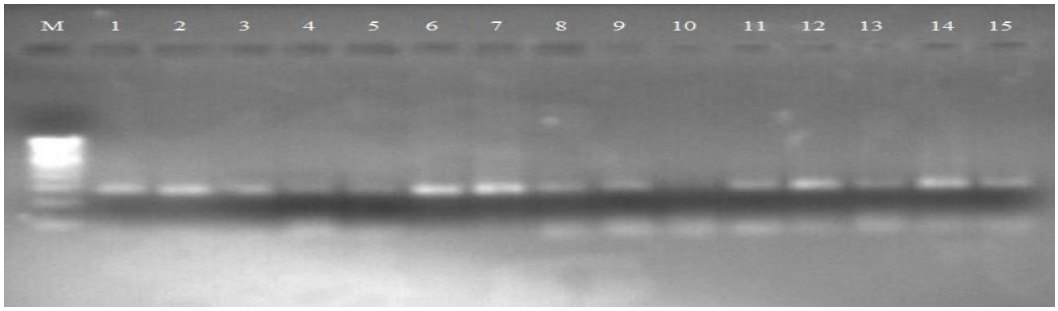


(i)

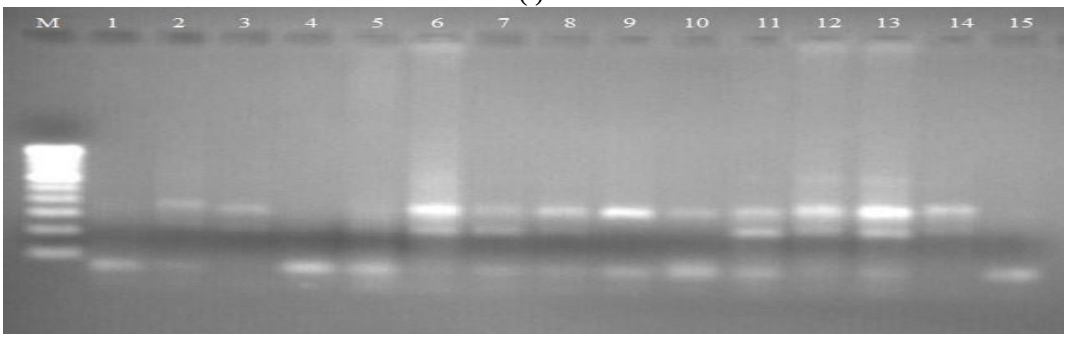


(ii)

Figure 4.5 B: 1.5% Agarose gel showing PCR amplification of 15 different genotypes of wheat (1-15) using SSR marker (i) CWM107and (ii) Xgwm276-7A

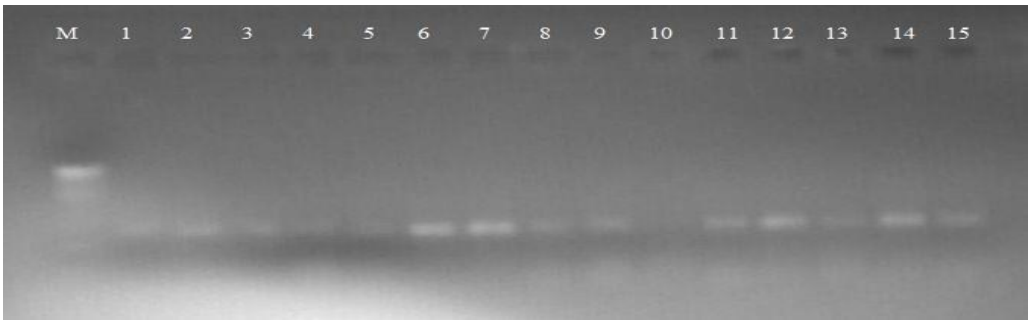


(i)

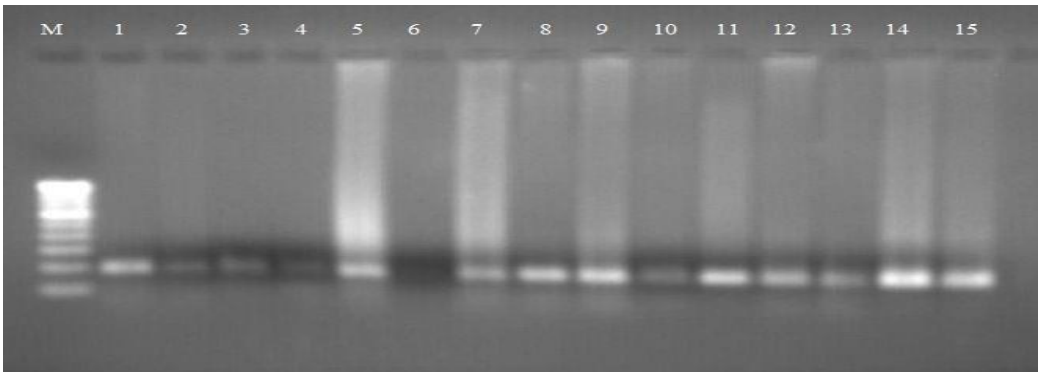


(ii)

Figure 4.5 C: 1.5% Agarose gel showing PCR amplification of 15 different genotypes of wheat (1-15) using SSR marker (i) CWM115and (ii) Xgwm11-1D

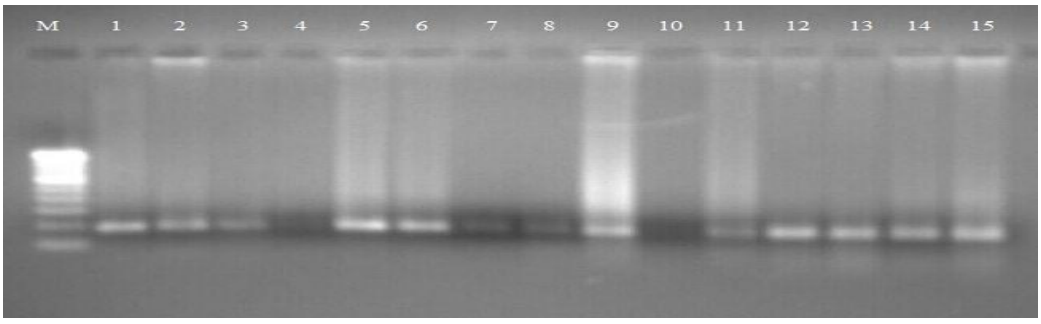


(i)

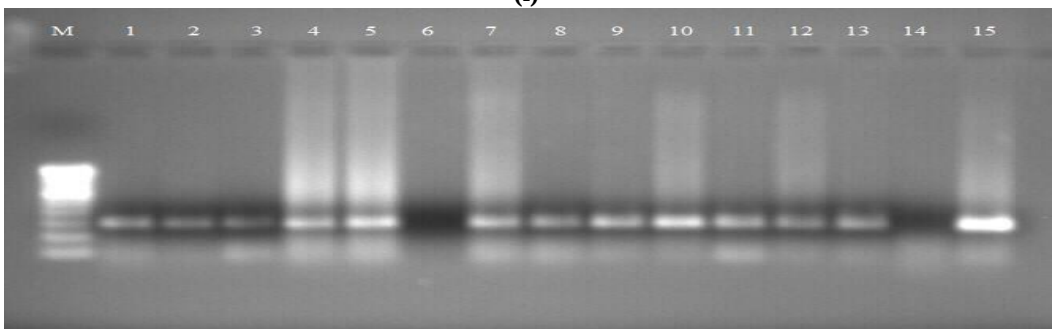


(ii)

Figure 4.5 D: 1.5% Agarose gel showing PCR amplification of 15 different genotypes of wheat (1-15) using SSR marker (i) Xgwm332-7A and (ii) CWM118



(i)



(ii)

Figure 4.5 E: 1.5% Agarose gel showing PCR amplification of 15 different genotypes of wheat (1-15) using SSR marker (i) Xgwm293-5A and (ii) CWM110

4.4.2. Expected gene diversity

Gene diversity was calculated for all the ten polymorphic primers, which varied from 0.12 to 0.95 values with a mean diversity of 0.46 (Table 4.4). The gene diversity of wheat shows to be in the wide range with higher mean gene diversity in comparison to the other crop species. The higher mean gene diversity can be explained as the genotypes of wheat are collected from various regions and diverse enough. This reflects the major reason of higher gene diversity in wheat genotypes taken.

Table- 4.4: SSR code, molecular wt. and no. of polymorphic alleles, no of monomorphic allele, resolving power and PIC value

S.N.	Primer name	Molecular weight range (bp)	No. of poly-morphic bands	No. of mono-morphic bands	Poly-morphism %	PIC	RP
1	**CWM122**	100, 300, 400	3	0	100	0.66	3.46
2	**CWM119**	200, 800	2	0	100	0.89	0.46
3	**CWM107**	300	0	1	0	0.96	0.70
4	**Xgwm276-7A**	300	0	1	0	0.55	1.33
5	**CWM115**	300	0	1	0	0.12	1.86
6	**Xgwm11-1D**	100, 250, 400	3	0	100	0.66	1.99
7	**Xgwm332-7A**	200	0	1	0	0.12	1.86
8	**CWM118**	200	0	1	0	0.12	1.86
9	**Xgwm293-5A**	200	0	1	0	0.25	1.73
10	**CWM110**	300	0	1	0	0.25	1.73

4.4.3. Resolving Power

Resolving power of the 10 SSR primers ranged from 0.4 to 3.46 with an average 1.7 (Table 4.4). Based on resolving power and the ability of primers to differentiate all accessions, the primers CWM122 and XGWM-11 were found to be most informative with resolving power 3.46 and 1.99 respectively. Thus, the significant value of resolving power indicated the ability of primers to resolve the different closely related genotypes of wheat.

4.4.4. Genetic similarity matrix and cluster analysis

Genetic variability in the germplasms is the important raw material in generating new plant ideotypes having desired traits that help to increase in crop production level which help in the improvement of human nutrition percentage **(Singh, 1991).** About 15 allels were amplified by the ten primers in 10 genotypes taken. The result shows that the SSR markers is a viable approach for the examination of genetic diversity of wheat genotypes. The number of amplified allels observed ranged from minimum 1 to maximum 3.SSR gene specific primers were scored for presence (1) and absence (0) in all wheatgenotypes for every primer. The pair wise genetic similarities among all pairs of samples were estimated with Jaccard's coefficient **(Jaccard, 1910).** The statistical analysis was carried out by using NTSYS-PC software (version 2.11s) **(Rohlf, 2000).** In order to group genotypes into discrete clusters a dendrogram was constructed by employing UPGMA method **(Sneath and Sokal, 1973)**.

SSR data were used to make pair wise comparison of the accessions based on shared and unique amplification products to generate a similarity matrix with NTSYS-PC (version 2.11s). Based on the distance matrix expressed as similarity coefficient a dendrogram was generated by the UPGMA method. Similarity value for all the 10 accessions ranged from 0.11 to 0.88. The lowest similarity displayed by DBW-16 and KRL-213. Of 15 samples analyzed, the genotypes PBW-550 and RAJ 3765 ie 100 % followed by Kharchia-65 displayed the greatest genetic similarity ie 0.88 (Table-4.5).

The UPGMA based cluster shows that all the genotypes are interlinked with each other and exhibited high genetic similarity. The cluster grouped all the samples into two main clusters with different subclusters (Figure 4.6). The cluster one grouped seven genotypes. Within cluster one the two genotypes viz. PBW373 and PBW- 226 shows 100% similarity which suggest that the primers used in the present study were not able to differentiate these two genotypes. Similarly the main cluster two grouped eight genotypes in it. Within cluster the two genotypes viz. PBW-

550 and RAJ3765 were similar with respect to primer used in the present study.

Table 4.5: Jaccards coefficient of similarity derived from SSR data obtained by 15 wheat genotypes and 10 primers.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1.0														
2	0.428	1.0													
3	0.285	0.571	1.0												
4	0.750	0.500	0.333	1.0											
5	0.333	0.428	0.800	0.400	1.0										
6	0.250	0.333	0.571	0.125	0.428	1.0									
7	0.125	0.375	0.667	0.142	0.500	0.833	1.0								
8	0.285	0.571	1.00	0.333	0.800	0.571	0.666	1.0							
9	0.666	0.500	0.571	0.500	0.428	0.500	0.375	0.571	1.0						
10	0.500	0.571	0.428	0.333	0.285	0.375	0.250	0.428	0.571	1.0					
11	0.125	0.571	0.428	0.142	0.285	0.571	0.666	0.428	0.222	0.250	1.0				
12	0.300	0.666	0.555	0.333	0.444	0.500	0.555	0.555	0.500	0.400	0.555	1.0			
13	0.200	0.555	0.625	0.222	0.500	0.555	0.625	0.625	0.400	0.300	0.625	0.888	1.0		
14	0.285	0.571	0.250	0.333	0.125	0.222	0.250	0.250	0.375	0.250	0.428	0.555	0.444	1.0	
15	0.333	0.428	0.125	0.400	0.142	0.111	0.125	0.125	0.250	0.125	0.285	0.444	0.333	0.800	1.0

Lane details: 1.PBW-373, **2.**PBW-343, **3.**PBW-550, **4.**PBW-226, **5.**PBW-502, **6.**DBW-16, **7.**DBW-17, **8.**RAJ-3765, **9.**WH-1021, **10.**WH-711, **11.**HD-3967, **12.**Kharchia, **13.**KRL-19, **14.**KRL-210, **15.**KRL-213

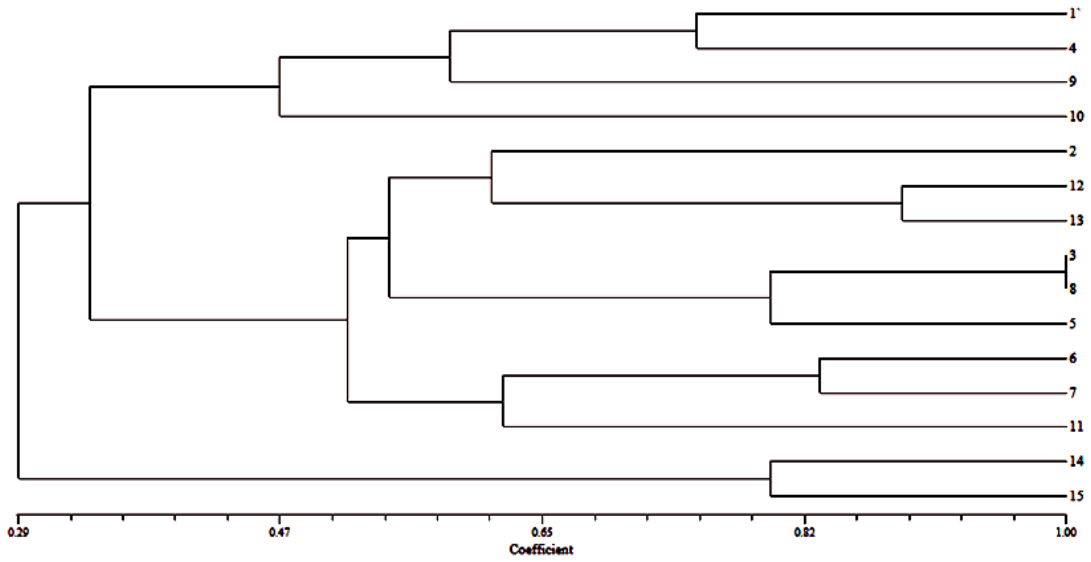


Figure 4.6: Cluster based grouping of 15 wheat varieties on the basis of SSR primers

Lane details: 1.PBW-373, **2.**PBW-343, **3.**PBW-550, **4.**PBW-226, **5.**PBW-502, **6.**DBW-16, **7.**DBW-17, **8.**RAJ-3765, **9.**WH-1021, **10.**WH-711, **11.**HD-3967, **12.**Kharchia, **13.**KRL-19, **14.**KRL-210, **15.**KRL-213

This showed that climatic conditions and physical parameters may affect the plant genome as the plant is adapted and these changes are inherited through genome to the next generation. The range of genetic diversity values broadly indicates the degree of heterogeneity or homogeneity in different genotypes of the plant species **(Goswami and Ranade, 1999*)***.

Cluster analysis clearly indicates that Human intervention, which makes partitioning and distribution of variability complex is cited as reason for the grouping of samples to one cluster suitable for different regions. The present study suggests that SSR is appropriate for analysis of genetic variability in closely related genotypes. Moreover, SSR could able to amplify the different loci of all the 15 genotypes.

CHAPTER-5 Summary and Conclusion

To study of Morphological, genetic diversity analysis and to study the effect of salt stress on Indian wheat cultivars a total of fifteen varieties were collected viz.PBW-373, PBW-343, PBW-550, PBW-226, PBW-502, DBW-16, DBW-17, RAJ-3765, WH-1021, WH-711, HD-2967, KHARCHIA-65, KRL-19, KRL-210 and KRL-213. And the seeds of all varieties were sown on experimental station and molecular biology laboratory of Department of Biotechnology, SVPUA&T, and Meerut.

To evaluate the genetic diversity under the normal field condition and effect of salt stress on seedling stage the seeds of all varieties were grown in Hoagland solution under hydroponic system. One for control with normal Hoagland solution and another with 10 mM saline Hoagland solution.

Among the morphological characteristics of wheat genotypes under normal field condition the characters as Plant height; numbers of Productive tillers/ plants, Leaf area, Days of Maturity, Length of Spikelets and Spikelets per Spike were recorded at pre harvest stage and Seeds per Spike, Thousand Grain Weight and Grain yield per plant were recorded at post harvest stage. In the present study, significant reduction in yield components like spiklets per spike, number of filled and unfilled seeds per spike and final grain yield was observed in all the test genotypes. when salt stress was imposed at seed stage by treating with salt the wheat variety, after salt treatment condition the germination was significantly affected by salt stress as the germination was found to be delayed as compared to control plants. Under the effect of salt stress, the seeds starts germinated after 48 hrs. Among them the variety HD 2967 shows a good response under salts stress. The genotypes KRL-19 and KRL 213 showed a very little effect of salt stress. The genotypes PBW-502, DBW-16, DBW-17 KRL-19 and KRL 213 Raj 3756 can be considered to salt tolerant genotypes. Thus these genotypes could be considered better in resisting themselves under stress condition.

The yield of genomic DNA obtained from all the genotypes (which were grown on normal field condition) of sufficient amount for PCR amplification indicating the suitability of protocol for extracting high quality genomic DNA from the leaves of wheat. The SSR primers used in the present study was enough informative with higher value of resolving power. These results confirmed the usefulness of SSR-PCR analysis to detect specific molecular markers for salt tolerant trait in wheat.

Out of fifteen samples, the lowest similarity displayed by DBW-16 and KRL-213 analyzed and the genotypes PBW-550 and RAJ 3765 ie 100 % followed by Kharchia-65 displayed the greatest genetic similarity ie 0.88.

This showed that climatic conditions and physical parameters may affect the plant genome as the plant is adapted and these changes are inherited through genome to the next generation. The range of genetic diversity values broadly indicates the degree of heterogeneity or homogeneity in different genotypes of the plant species**.**

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