Salinity tolerance in Bread wheat: Germplasm status, Molecular diversity and in vitro screening for K:Na discrimination levels

by

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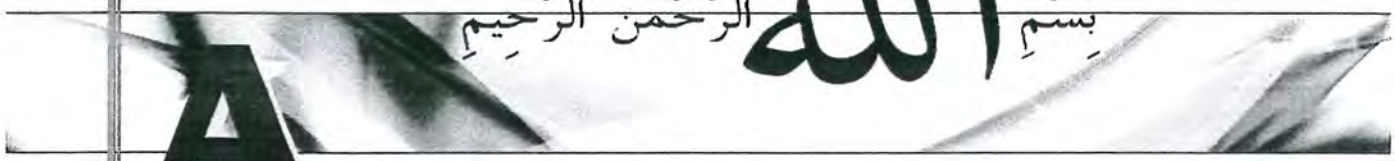

ALLAH THE BENEFICENT THE MERCIFUL I START WITH HIS NAME

"WE WILL SOON SHOW THEM OUR SIGNS IN THE UNIVERSE AND INSIDE THEIR SELVES, UNTIL IT WILL BECOME QUITE CLEAR TO THEM THAT IT IS THE TRUTH. IS IT NOT SUFFICIENT AS REGARDS YOUR LORD THAT HE IS A WITNESS OVER ALL THINGS?"

(QURAN, 41:53)

DEDICATION

I dedicate my humble efforts to...
The most honorable, the most generous, the satisfaction
to my soul and certificate for my salvation
Shaikh-e-Tarriqat Ameer-e-Ahle Sunnat Founder of Dawat-e-Islami
Haztate Allama Moulana

MUHAMMAD ILYAS ATTAR

Qadri Razavi (may he live long)
Whose spiritual guidance has led millions of the gone-astray people
to the right path of salvation in this world
and the world hereafter.
May ALLAH bestow him the age of Khidher (A.S) Ameen.

DECLARATION

This is to certify that this dissertation entitled "**Salinity tolerance in Bread wheat: Germplasm status, Molecular diversity and in vitro screening for K:Na discrimination levels**" submitted by Muhammad Farooq is accepted in its present form by the Department of Plant Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad as fulfilling the thesis requirement for the degree of Master of Philosophy in Plant Physiology.

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LIST OF ABBERVIATIONS

bp	base pair
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide phosphates
EDTA	Ethylenediaminotetraacetic acid, Disodium salt
$MgCl_2$	Magnesium Chloride
µg .	Microgram
µl	Microlitre
µM	Micromolar
PCR	Polymerase chain reaction
RAPD	Random amplified polymorphic DNA
rpm	Revolutions per minute
Taq	Thermus aquaticus
Tris	[Hydroxymethyl] aminomethane
MAS	Marker assisted selection
SSR	Simple sequence repeats
SH	Synthetic hexaploids
K:Na	Pottasium Sodium disrcrimination

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ABSTRACT

Salinity stress is an important abiotic yield limiting factor. Present study covers the characterization of important germplasm comprised of synthetic hexaploids and some historical genotypes important for salinity tolerance studies. The results revealed that synthetic hexaploids are genetically more diverse than tester set germplasm in terms of phonological attributes. *In vitro* screening was also performed at 75mM NaCl salt stress. The genotypes found to be promising under salt stress conditions include SH-13, SH-12 and SH7 among synthetic hexaploids and Kharchia, LU-26S, Calafia, Chinese Spring and Pasban-90. Molecular diversity based on SSR and RAPD markers among these synthetic hexaploids and tester set genotypes also revealed significant results. Results based on SSR better discriminated genotypes as compared to SSRs. Moreover SSRs used in this study were specific to A-genome hence determining the diversity specific to A-genome in the germplasm studied. Higher level of polymorphisms in synthetic hexaploids as compared to tester set genotypes based on A-genome SSRs yielded the information that apart from D-genome diversity these synthetic hexaploids also possess desirable diversity in A-genome as well.

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MUHAMMAD FAROOQ ATTARI

Chapter 1

INTRODUCTION

Wheat (*Triticum aestivum* L.) is an allohexaploid (2n=6x=42) genomically possessing three wild grasses contributing genomes A, B and D all belonging to family Poaceae. About 8000 years ago, the wild wheat plant which human beings selected for domestic purpose was actually the result of a natural cross between two distantly related wild grasses *Aegilops speltoides* (BB or SS) and *Triticum uratu* (AA), occurred probably 1000 years ago somewhere in the Near East. A very likely place of origin is the area known in early historical times as the Fertile Crescent - a region with rich soils in the upper reaches of the Tigris-Euphrates drainage basin (Briggle and Curtis, 1987). The spontaneous F1 hybrid (2n=2x=14, AB) naturally doubled to give rise to the wild emmer or durum wheat with 2n=4x=AABB composition. Around 6000 BC one more hybridization took place in natural conditions between wild emmer and the progenitor or donor of D genome i.e. *A. tauschii* which resulted in modern hexaploid bread wheat i.e. *Triticum aestivum* with 2n=6x=42, AABBDD (Jiang *et al.*, 1994).

Wheat occupies 17% (220 million hectares) of the total cultivated land with 620 million tons annual production worldwide. It provides about one-fifth of the calories consumed by humans so support nearly 35% of world population (Dreisigaker, 2004). To meet the needs of the growing world population, the forecast demand for the year 2020 is projected to be 1 billion tons. Due to land limitations, the enhancement of wheat production must come from higher absolute yields by increasing efforts in plant breeding along with biotechnological tools and expanded genetic diversity (Rajaram, 2001).

In Pakistan wheat is used as staple food and considered as the most important crop of the country. Total area under wheat for year 2006- 2007 was about 8.44 million hectares out of which irrigated area was about 7.33 million hectares and rainfed area was 1.11 million hectares. The national yield of wheat was 23.5 million tons in which the rainfed area's share was 1.39 million tons. The average yield per hectare was about 2.59 tones, being about 1.26 tones in rainfed conditions (Govt. of Pak., 2006-2007).

The stresses which form the backbone of the national wheat breeding programs are separated into two classes; biotic (dynamic) and abiotic (static) (Mujeeb-Kazi and Kimber, 1985). Among these stresses three rusts, karnal bunt, barley yellow dwarf virus, some spot blotch, powdery mildew, aphids, smuts, drought, salinity and heat are of serious concern as cumulatively these are important for food security. Wheat is cultivated in rainfed as well as in irrigated land but later is more important as the world's top wheat grower country like India and China had larger irrigated area (Dixon *et al.,* 2008). It is irrigated land that is believed to be most productive, but unfortunately it faces a great production constraint i.e. salinity. Currently, there is nearly about 20 percent of 275 million ha of irrigated land is salt affected, globally (Flowers and Flowers, 2005). In Pakistan, about 5.30 million ha of land are salt-affected including Indus plain which is considered one of the most important and the largest part of the Pakistan for wheat production (Khan *et al.,* 2009). The scarcity of good quality water, low soil permeability and high cost of amendments, are the other factors which continuously increasing the salinity problems in the country. The reduction in the yield of many crops including wheat by salinity is well documented. The growth of plants may be reduced under salt stressed conditions because of (a) an osmotic stress due to a lowering of the external water potential, or (b) effects of specific ions on metabolic processes ranging from the absorption of nutrients to enzyme activation or inhibition (Kingsbury *et al.*, 1984).

A number of strategies can be applied to salt effected soils for its management and can be brought under cultivation by reclamation. Along reclamation of saline soil, the other method of effective utilization of saline soils is to employ salinity tolerant germplasm. This involves identification of genotypes or cultivars that are tolerant to salinity or use of new genetic resources to introduce new genes for salt tolerance in existing cultivars (Farshadfar *et al.*, 2008). Several approaches to developing salt tolerant lines are available. The simplest which requires little or no physiological or biochemical understanding of the underlying processes is to screen existing cultivars for salt tolerance and use the selected lines as a basis for further breeding (Wyn-Jones *et al.*, 1986).

Salt tolerance in wheat (*Triticum aestivum*) and many other species is associated with the ability to discriminate between Na^+ and K^+ in the soil solution and to preferentially accumulate K^+ and exclude Na^+ (Gorham, 1990a). A high K^+/Na^+ ratio combined with low leaf salt contents is characteristic of salt-tolerant members of the grass tribe *Triticeae*. There is evidence that a high K^+/Na^+ ratio, particularly in the youngest leaf, is associated with salt tolerance in bread wheat cultivars both in the field and in hydroponic studies (Joshi *et al.*, 1979).

The D genome of wheat, derived from the diploid grass *Aegilops squarrosa* carries a gene (or genes) which determines the K^+/Na^+ ratio in the shoots of wheat plants grown in saline hydroponic culture (Wyn-Jones *et al.*, 1984; Shah *et al.*, 1987). The diploid progenitor *Ae. tauschii* (2n=2x=14, DD) accessions has emerged as a priority resource essentially because of its genetic proximity to the D genome of bread wheat. In this regard the D genome synthetic hexaploids are the most important which are genomically AABBDD. Currently 750 accessions of *Ae. tauschii* are available among these 450 accessions form a core collection (Kawahara, 2003). They have been extensively used in synthetic hexaploids at CIMMYT (Mujeeb-Kazi, 2003, Mujeeb-Kazi *et al.*, 2008, Trethowan and Mujeeb-Kazi, 2008). Potentially, new genetic variation among primary synthetics has also been found for tolerance to drought (Villareal *et al.*, 1998) and salinity (Gorham, 1990a). Since the genetic diversity for the traits like drought and salinity is limited in the conventional wheat cultivars so these synthetic hexaploids provide a unique way to incorporate novel resistant alleles for biotic and abiotic stresses into wheat cultivars (Das *et al.*, 2007).

Traditionally, germplasm has been characterized based on agronomic and morphological studies, but recently the use of molecular markers to study diversity within domesticated species has become common (Lage *et al.*, 2003). Molecular markers provide a powerful tool to assess the diversity within and among germplasm and to monitor the flux of diversity over time. Among these DNA based techniques, random amplified polymorphic DNA (RAPD) gained importance due to its simplicity, efficiency and non requirement of sequence information (Gepts, 1993). RAPD provide virtually

limitless set of descriptors with which to compare individual plants and among the population. RAPD markers have also been used for fingerprinting (Nybon *et al.*, 1989) and tagging of genes (Klein-Lankhorst *et al.*, 1991). In particular, SSRs (Simple Sequence Repeats) show potential for large scale DNA finger printing of wheat genotypes due to high level of polymorphism detected (Christiansen *et al.*, 2002).

Microsatellites, also called simple sequence repeats (SSRs) are a PCR-based marker system which exploits the high variability in the repeat number of simple tandemly repeated DNA motifs. In most cases, microsatellites containing dinucleotide or trinucleotide motifs are used for marker development. Microsatellite markers consist of a defined primer pair flanking a specific microsatellite site in the genome, which can be used for PCR amplification. The variation in repeat number of the microsatellites results in PCR products of varying lengths, which are stably inherited and thus can serve as genetic markers (Roder *et al.*1995). A special advantage in wheat is the genome specificity of most microsatellite markers, which allows the analysis of the three homoeologous genomes of this allohexaploid species individually. In wheat breeding, SSRs are increasingly used as the marker backbone for a variety of purposes. These include the localization of individual genes onto the 21 wheat chromosomes such as, for example, biotic and abiotic resistance genes or genes affecting other agriculturally important traits and characterization of wheat lines from germplasm collections for the determination of genetic diversity (Donini *et al.*, 2000).

The objectives of this study were:

i. Study of phenological aspects for identification of entries with good agronomic characters.
ii. In vitro screening for salt tolerance using an evaluation parameter; K:Na discrimination through hydroponic tests.
iii. Assessment of molecular diversity among the entries using A-genome specific SSR markers for the tester set and for synthetic wheat.

Chapter 2

REVIEW OF LITERATURE

2.1 SYNTHETIC HEXAPLOID WHEAT

Wheat (*Triticum aestivum* L.) evolved from three wild grasses, probably somewhere in the Near East about 10,000 years ago and possessing three genomes A, B and D coming from these wild grasses (Briggle and Curtis, 1987). These wheat wild relatives like wild diploid progenitors, *T. urartu; T. baeoticum, Ae. speltoides* and *Ae. tauschii* and tetraploid *T. dicoccoides* might be a valuable and readily accessible source of new genetic diversity for wheat improvement (Valkoun, 2001). Among these wild relatives *Ae. tauschii* the donor of D genome has got attention of the breeder as much potential is available in this wild relative for wheat improvement against different biotic and abiotic stresses (Zohary *et al.*, 1969). The different accessions of *Aegilops* species (*Ae. cylindrica* Host, *Ae. geniculata* Roth and *Ae. neglecta* Req. ex Bertol.) have the potential to be used for wide hybridization programme of wheat for abiotic stresses like drought resistance and high temperature (Zaharieva *et al.*, 2001; Zaharieva *et al.*, 2003; Baalbaki *et al.*, 2006). Among different methods for wheat improvement wide hybridization has received much attention over a last few decades (Mujeeb-Kazi *et al.*, 2008).

In this regard, a core collection of *Ae. tauschii* comprised of 450 accessions (Kawahara *et al.*, 2003) were used in CIMMYT to produce more than 1000 synthetic hexaplod wheats (Mujeeb-Kazi, 2003). These synthetic hexaploid wheats were produced by artificially crossing tetraploid forms such as modern durum wheat (*Triticum turgidum*, 2n=4x=28 AABB) with *Ae. tauschii* (2n=2x=14, DD) (Mujeeb-Kazi *et al.*, 1996). Over the past decade CIMMYT has produced numerous synthetics and their derivatives with exceptional expression for traits relating to agronomic features, resistances/tolerances to abiotic and biotic stresses and quality (Trethowan and Mujeeb-Kazi, 2008). These synthetic hexaploids have been utilized to transfer novel genes conferring resistance to diseases and pests such as leaf rust (Villareal *et al.*, 1992), stripe rust (Ma *et al.*, 1995),

Karnal bunt (Villareal *et al.*, 1994) and greenbug (Lage *et al.*, 2003) from *Ae. tauschii* to bread wheat.

The genetic diversity in synthetic hexaploid wheat derived from *Ae. tauschii* enabled them to show resistance to major wheat diseases and tolerance to abiotic stresses such as drought, heat, water logging, salinity and pre-harvest sprouting. Through experiments it had been identified that synthetic wheat derivatives out yield commercial varieties by 18–30% under rainfed conditions. At the molecular level these new synthetic derivatives had been shown to boost genetic diversity (Van Ginkel and Ogbonnaya, 2007). Synthetic hexaploids are good source for incorporating genes conferring resistance to biotic and abiotic stresses into common bread wheat. Synthetics, having many novel alleles for biotic and abiotic stresses, can be used to introduce new genetic diversity into the wheat gene pool (Lage *et al.*, 2003). Zhang *et al.* 2005). This novel allelic variation can be maintained in SH/BW derivatives through backcross breeding. This indicated that the synthetic hexaploids and its backcross derivatives can also be used for broadening the genetic base of elite wheat breeding program.

The D genome is more drought and salt tolerant as compared to others genomes like A genome and B genome. The D genome contributes to lowers rates of Na^+ accumulation and higher K^+/ Na^+ ratio in leaves, both in diploid *A. tauschii* (DD) and also in hexapliod (AABBDD) wheat. Variation for Na^+ exclusion has also been identified on other genomes (e.g. U and M) with in the genus *Aegilops* and some of these species have also been proposed as sources of salt tolerance for wheat breeding programmes. Wide-hybridization of tall wheatgrass species with wheat appears promising as an avenue to improve salt tolerance. So the hexaploid wheat which is genomically AABBDD exhibits more salt and drought tolerance as compared to other synthesized hexaploids like AAAABB and AABBBB (Xu *et al.*, 2002). Trethowan and Mujeeb-Kazi (2008) also reported importance of exotic germplasm that comprised of the D genome diversity and land races in addressing environmental stresses which limit yield outputs and elucidate their alleviation. Molecular evaluation of these SH help breeders all over the world to target their hybridization programmes around this diversity (Mujeeb-Kazi *et al.*, 2004).

2.2 SALINITY TOLERANCE IN WHEAT

Salinity is the presence of excess salts in the root zone in concentrations that retard plant growth which results in low yield in any crop (Ansari *et al.*, 2007). Rengasamy (2006) defined saline soil as "a soil is considered saline if the electrical conductivity of its saturation extract (EC_e) is above 4 dSm^{-1}". It is estimated that 20 percent of the irrigated land in the world is presently affected by salinity *(Yamaguchi and Blumwald, 2005).* Additionally, there is also a dangerous trend of a 10 percent per year increase in the saline area throughout the world (El-Hendawy *et al.*, 2005). The quick approach to lessen the saline area and to reduce the decline in grain production is, soil management and identification of germplasm that is salt tolerant. Both of the points are important for sustainable agriculture but later is getting more attention as compare to former.

Salt tolerance refers to the ability of plants to maintain the growth under saline conditions. Various tests and evaluation parameters have been used to identify salt tolerant germplasm which include biomass production or yield of plant under saline conditions. However to avoid growing plants for longer periods of time, selection techniques should be based on physiological traits. Salt exclusion and inclusion, Na^+/K^+ discrimination and osmotic adjustment are recognized as different physiological mechanisms for plants to tolerate salinity. Salt exclusion means that the plants have the ability to restrict the uptake of toxic ions into the shoot (Munns, 2002).

The K^+/Na^+ discrimination is a trait associated with salt tolerance and it has been reported in many crops including wheat (Gorham *et al.*, 1991). According to Omielan *et al.* (1991), salt tolerance in *Triticeae* is associated with enhanced ability to discriminate between Na^+ and K^+ in the soil solution and to preferentially accumulate K^+ and exclude Na^+. Salt tolerance in wheat is mostly related to its enhanced ability to discriminate between K^+ and Na^+ during transport of these ions to the shoot (Gorham, 1990a). Shirazi *et al.* (2005) also reported that the genotypes which have the ability of enhanced K^+:Na^+

discrimination perform better under saline conditions when sufficient potassium is applied in the rooting medium.

This trait for salt tolerance is found to be located on D genome of bread wheat. The experiments by Shah *et al.* (1987) on hexaploid bread wheat and its presumed ancestors confirmed that *Ae. Tauschii* and *T. aestivum* had high K^+/Na^+ ratios while *T. dicoccoides* and *Ae. speltoides* had low K^+/Na^+ ratios. These studies demonstrated that the D genome (*Ae. Tauschii*) contains a trait for enhanced K^+: Na^+ discrimination which is located on chromosome 4D. Gorham *et al.* (1987) studied Chinese spring chromosome substitution lines in which each of the D-genome chromosome replaced the homoeologous A- or B-genome chromosome in the tetraploid wheat variety (AABB genome) and found that the trait for K^+:Na^+ discrimination is specifically located on long arm of 4D chromosome. Further ditelosomic analysis confirmed that chromosome 4DL is responsible for Na:K discrimination.. The genetic locus of K^+:Na^+ discrimination in wheat was determined by Dubcovsky *et al.* (1996). They found that the trait is controlled by a single locus, designated *Kna1*, in the long arm of chromosome 4D. The *Kna1* locus was mapped on a short region in the 4DL arm and was completely linked to *Xwg199, Xabc305, Xbcd402, Xpsr567*, and *Xpsr375*; it was also mapped as a quantitative trait. The results of the QTL analysis, based on the K^+/Na^+ ratios in the young leaves of greenhouse-grown plants and flag leaves of field grown plants, agreed with the position of *Kna1* determined as a quantitative trait.

A quantitative trait locus for low Na^+ concentration in leaf blades was mapped to the distal region on the long arm of chromosome 2A and named *Nax1*. A microsatellite marker, *gwm312*, was closely linked to the trait and has been used to accelerate the transfer of this trait into commercial varieties of durum wheat. A second gene, independent of *Nax1*, was suggested to contribute to the full expression of the Na^+ exclusion trait (Lindsay *et al.*, 2004). It was named *Nax2*. James *et al.* (2006) reported the physiological differences of two genes *Nax1* and *Nax2*. *Nax1* differed from *Nax2* by unloading Na^+ from the xylem as it entered the shoot so that Na^+ was retained in the base of the leaf, leading to a high sheath to blade ratio of Na^+ concentration. The *Nax2* line did

not retain Na^+ in the base of the leaf, suggesting that it functioned only in the root. The *Nax2* gene therefore has a similar function to *Kna1* in bread wheat (*Triticum aestivum*).

Synthetic hexaploids with better salt tolerance than their durum parents has been identified by some scientists. This showed that this character has been transferred to synthetics from *Aegilops tauschii*. Schachtman *et al.* (1992) identified synthetic hexaploids with better salt tolerance than their durum parents and this superiority of the synthetics was linked to maintenance of seed weight under saline conditions. Pritchard *et al.* (2002) assessed K^+:Na^+ discrimination in a number of synthetic hexaploid wheat genotypes and durum parents and found that K^+:Na^+ ratios were lower in the durum parents than in the elite synthetics, confirming that the trait was present in the synthetics. Reynolds *et al.* (2005) reported that average value for K:Na discrimination in 95 "Elite I" SH lines was found to be 2.03 while some best lines showed values of 3.4 to 5.2 which can serve as a source for transferring salinity tolerance to salt susceptible germplasm.

Some cultivars are known to perform well in salt stressed conditions these include the Egyptian genotypes Sakha 8 and Sakha 93 and the Indian genotype Kharchia, which were found to be the most tolerant to salinity. Khan *et al.* (2005) studied the effect of salinity on wheat germination under two salinity levels and found that the cultivars Pir Sabak 85, Pak 81 and Sarhad 82 were most tolerant to salinity at germination stage. Hameed *et al.* (2008) reported that cultivar LU-26 exhibited a better protection mechanism against salinity due to lower salt induced proteolysis, higher biomass accumulation and protein content than the relatively sensitive cultivar Pak-81.

Many scientists studied the effect of different levels of salinity on agronomic characters of wheat. Bhatti *et al.* (2004) screened wheat lines for their salinity tolerance under solution culture setup. The salinity was gradually increased from 1.5-30 dS m^{-1}. The results showed that increase in salinity drastically affected the seedling growth i.e. fresh and dry weight of shoot and root 30 days after completion of salinity produced by NaCl. Houshmand *et al.* (2005) evaluated salt tolerant genotypes under both saline and non-saline field conditions as well as under greenhouse condition. They found that

genotypes grown *in vitro* performed comparably with genotypes grown in field for grain yield and dry weight.

Salinity tolerance can also be assessed in terms of grain weight and yield. Rajpar *et al.* (2006) performed pot experiments to determine the effect of salinity on yield and yield components of wheat variety Inqlab-91. They found that increasing soil salinity progressively decreased plant height, spike length, number of spikelets per spike and grain and straw yield. With the increase in electrical conductivity of soil, the concentration of Na^+ increased and that of K^+ decreased. The results suggested that Inqlab-91 can be grown on saline soils with an electrical conductivity upto 6 dS/m. Goudarzi and Pakniyat (2008) evaluated wheat varieties for their salinity tolerance. They recorded several agronomic and physiological characters including 1000-grain weight and reported that tolerant germplasm gave better agronomic performance with high K^+/Na^+ ratio.

2.3 MOLECULAR CHARACTERIZATION THROUGH DNA MARKERS

Molecular biotechnologies helped plant breeders and physiologists to select the genotypes with improved yield under drought conditions (Chang-Xing *et al.*, 2008). Among different molecular technologies, molecular markers provide a powerful tool to assess the diversity within and among germplasm and to monitor the flux of diversity over time (Heckenberg *et al.*, 2002). Although morphological traits can be used to assess genetic diversity but molecular markers are more reliable and receiving much attention (Manfiesto *et al.*, 2001; Huang *et al.*, 2002 and Ahmed, 2002). Detailed characterization of genetic resources can be done by the help of these molecular markers. These markers have a great potential to identify the genetic diversity within and among the accessions, which can help the breeders to optimize the collections, the planning of seed regeneration and the successful implementation of pre-breeding approaches (Borner *et al.*, 2000). Different molecular markers are used to assess the genetic diversity and to characterize the germplasm. It includes randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP) and simple sequence repeats (SSRs).

Among molecular markers RAPD (random amplified polymorphic DNA) technique has been found as an efficient technique for determination of genetic diversity (Joshi and Nguyen, 1993). RAPD anaylsis virtually limitless set of descriptor with which to compare individual plants and among the population. With this innovative tool, genetic diversity can be estimated *(Demeke et al., 1996)* and equally it is possible to carry out large scale screening of genetic resources held in gene banks, natural populations, ecosystems and natural reserves with this quick and rapid technique. RAPD analysis has been extensively used to document genetic variation in Triticum (Coa *et al.*, 1998) suggesting narrow genetic base RAPD markers have also been used for cultivar identification, for finger printing of genomes *(Nybon et al., 1989)* and tagging of genes *(Klein-Lankhorst et al., 1991).* The major disadvantage of this technique is that the results are non reproducible. However these markers are being used for determination of phylogenetic relationship among different groups of wheat.

The molecular markers microsatellite or simple sequence repeats (SSRs) detect high level of polymorphism. In hexaploid wheat there are some difficulties to use molecular markers due to the presence of high proportion of repetitive DNAs, large genome, continuous inbreeding caused by self pollination and narrow genetic base (Joshi and Nguyen, 1993) but high level of polymorphisms detection, ability to be analyzed by automated systems, high accuracy and repeatability make SSRs suitable for large scale DNA finger printing of wheat genotype (Snape, *1998;* Christiansen *et al.*, 2002). The first large set of microsatellite markers for the wheat genome has been published in 1998 (Roder *et al.*, 1998). These microsatellites are PCR-based DNA markers that are highly polymorphic, show codominant inheritance, are evenly distributed throughout the genome and are locus specific (Powell *et al.*, 1996). The majority (80%) of SSR primer sets developed are genome specific and detects only a single locus in one of the three genomes of bread wheat (A, B, or D). Only 20% of the markers detect more than one locus. Ninety-three loci were mapped to the A genome, 115 to the B genome, and 71 to the D genome. The markers are randomly distributed along the linkage map, with clustering in several centromeric regions (Roder *et al.*, 1998).

MATERIALS AND METHODS

The present study was conducted in the field area and the laboratory of Wheat Wide Crosses, National Agricultural Research Center, Islamabad during the year 2009-2010. The experimental material was consisted of 13 synthetic hexaploid wheats and 20 tester set which were studied for its morphological, physiological and molecular characterization.

3.1 PHENOTYPIC EVALUATION

Phenological data were taken from five guarded plants of each accession and then arithmetic means were computed for the following characters.

3.1.1 Plant height (cm)

Plant height was measured by the help of meter rod. It was taken at maturity by measuring the distance from base of the plant to the tip of spike excluding awn. Height of five plants from each genotype was measured and then its mean was computed.

3.1.2 Awn color

Data were recorded for awn color. The parameters used were light brown, white, and dark brown or black.

3.1.3 Pubescence

Pubescence has been considered as a morphological trait that enables moisture retention due to its hairy nature. Pubescence on spike glumes is also known to contribute towards stress resistance. Based upon pubescence, breeders select drought resistant material. Data for pubescence was taken by observing hairs on the base of spikes using a hand lens. The pubescence grading was done as negative (-) or positive (+).

3.1.4 Days to flowering

It was counted as the number of days from sowing to when 50 per cent of the plants in the line flowered.

3.1.5 Physiological maturity

It was counted as the number of days from sowing to when 50 per cent of the plants in the line showed physiological maturity.

3.1.6 Spike length (cm)

Spike length was measured in cm from a point where spike starts originating to the end of last spikelet excluding awns. Spike length of five spike was measured and then its average was computed.

3.1.7 Pigmentation

Anthocyanin pigmentation is genetically controlled and allows one to discern the influence of A and B on the D genome effect. Anthocyanin pigmentation was noted at the base of the tiller for each line. Pigmentation was scored as present (+) or absent (-).

3.2 *IN VITRO* SCREENING FOR SALT TOLERANCE (K:Na TESTS)

In order to determine salinity tolerance of material, a hydroponic salinity test was applied. Thirty three genotypes consisting of both conventional and novel germplasm were screened for their salinity tolerance at 75 mM NaCl. Five plants from each entry were grown in trays having Hoagland solution. After ten days of growth, plants were supplemented with NaCl salt to attain required salinity levels. Salinity was raised gradually in three steps to reach a final level of 75 mol m^{-3} NaCl.

Five days after salinization, recently matured leaf of five plants from each entry excised to determine Na^+ and K^+ uptake. Sap was extracted by boiling the leaf in water bath with 10ml of 100mM acetic acid. Extracts were stored in refrigerator for 24 hours. Dilution of sap was made by taking 1ml of sap and adding 9ml distilled water. Remaining leaf sample was dried and weighted to provide basis for sodium and potassium comparisons.

The Na^+ and K^+ contents were determined, from dilutions, on atomic absorption spectrophotometer. Concentration of sodium was determined at 598nm and of potassium at 766nm absorbance on the atomic absorption spectrophotometer. The concentrations of sodium and potassium extract were calculated according to the method of Yeo and Flower (1983) and Yeo (1992).

The plants in the trays were allowed to grow for 21 days after attaining the final salinity level. After a total of 35 days growth, plants were harvested and biomass was recorded which included shoot length, root length, shoot dry weight and root dry weight. Data for five replication of each entry was recorded on each of these parameters and then arithmetic mean was taken.

3.4 MOLECULAR EVALUATION

The germplasm was subjected to molecular evaluation for determining their DNA based diversity. This was done using RAPD and SSR markers following protocol described below:

3.4.1. DNA Extraction

Seeds of selected genotypes were sown in growth chamber under controlled conditions of temperature, humidity and light. Young leaf tissue was taken from all the genotypes for DNA extraction.

In the growth room 5 to 7 cm long piece of fresh leaf material was cut from the plants (3 week-old seedlings) and placed in 1.5 ml eppendorf tubes. The tubes were subsequently dropped in the liquid Nitrogen to rapidly freeze the leaf material. The plant material was then crushed to a fine powder with a micro pestle. Five hundred micro-liter DNA extraction buffer (1% SDS, 100mM NaCl, 100mM Tris base, 100mM Na_2EDTA, pH: 8.5 by HCl) was added to each eppendorf tube containing the crushed leaf material and mixed well with the help of a micro pestle. 500µl phenol:chloroform:isoamylalcohol (in the ratio of 25:24:1) was added and tubes well shaken until a homogenous mixture

was made. Samples were then centrifuged at 10,000 rpm for 5 minutes. The aqueous phase (supernatant) was transferred to a fresh tube. To precipitate the DNA 50 µl 3M sodium acetate (pH= 4.8) and 500µl isopropanol was added to the tube and mixed gently. To make the DNA pellet, samples were centrifuged at 10,000 rpm for 5 minutes. After discarding the supernatant, pellet was washed with 70% ethyl alcohol. Pellet dried at room temperature for an hour and re-suspended in 40 µl TE buffer (10mM Tris, 1mM EDTA and pH: 8.0) (Weining and Langridge, 1991). To remove RNA, DNA was treated with 40µg RNAse-A at 37°C for 1 hour. After RNAse treatment, DNA samples were run on 1.0% gel to check the quality of DNA and then stored at 4°C. To use in Polymerase Chain Reaction (PCR) a 1:5 dilution of DNA was made in doubled distilled, deionized and autoclaved water.

3.4.2. Polymerase Chain Reaction

For RAPDs, PCR reactions were carried out in 25 µl reaction tube, containing 50-100 ng total genomic DNA template, 0.25 µM of each primer, 200 µM of each dATP, dGTP, dCTP, dTTP, 50 mM KCl, 10 mM Tris, 1.5 mM $MgCl_2$ and 2.5 units of *Taq* DNA polymerase (Dweikat *et al.*, 1993). For SSR analysis 92 SSR primers specific for A genome were used. For SSRs, PCR reactions were carried out in 20 µl reaction containing 4 µl total genomic DNA template, 2 µl of each primer, 4 µl of each dNTP (dATP, dGTP, dCTP, dTTP), 2 µl Taq buffer + KCl, 1.2 µl $MgCl_2$, DEPC water 6.6µl and 0.2 µl of Taq DNA polymerase (Dweikat *et al.*, 1993)

3.4.3. Amplification Conditions

For RAPD analysis the amplification conditions were as; an initial step of denaturation for 4 minute at 94°C followed by 40 cycles each consisting of a denaturation step of 1 minute at 94°C, an annealing step of 1 minute at 36°C and an extension step of 2 minute at 72°C. Seven minutes were given after the last cycle to the extension step at 72°C to ensure the completion of the primer extension reaction. Amplitronyx 6 was used for all amplification reactions while for SSR analysis the amplification conditions were as; an initial step of denaturation for 1 minute at 93°C followed by 30 cycles each consisting of a denaturation step of 30 seconds at 93°C, an annealing step of 1 minute at 60°C and an extension step of 1 minute at 72°C. Five minutes were given after the last

cycle to the extension step at 72°C to ensure the completion of the primer extension reaction.

3.4.4 Gel Electrophoresis

For electrophoresis of the amplification products using RAPDs, 1.5 % agarose/TBE gel was used. While electrophoresis of the amplification products using SSR using 2.0 % agrose/TBE gel.Gels were visualized by Ethidium Bromide under the UV light chamber and observed using the computer program UVIPhotoMW.

3.5 STATISTICAL ANALYSIS

For statistical analysis, all the scorable bands were considered as a single locus / allele. The loci were scored as present or absent. Bivariate 1-0 data matrix was generated. Genetic distances (Nei and Li, 1979) were calculated using Unweighted Pair Group of Arithmetic Means (UPGMA) procedure as follows:

$$GD_{xy} = 1 - d_{xy} / d_x + d_y - d_{xy}$$

Where,

GD_{xy} = Genetic distance between two genotypes.

d_{xy} = Total number of common loci (bands) in two genotypes.

d_x = Total number of loci (bands) in genotype 1.

d_y = Total number of loci (bands) in genotype 2.

The 1-0 bivariate data matrix for each set of wheat lines based on the data of SSR primer sets was used to construct dendrogram using computer program "NTsys-PC" version 2.1.

Table 3.1 List of entries in the Tester set

S. No.	Name of Entries
1	SHORAWAKI
2	SAKHA 8
3	WH 157
4	KHARCHIA 65
5	LU26 S

6	PDW 34
7	SNH.9
8	KRL 1-4
9	GALVEZ S 87
10	OASIS F 86
11	CHINESE SPRING
12	CIANO T 79
13	YECORA F 70
14	MEPUCHI
15	PBW 343
16	COCHIMI
17	CALAFIA
18	KRL-19
19	PERICU

Table 3.2 Pedigree of Synthetic Hexaploid entries

Synthetic Hexaploid Number	Pedigree
SH-1	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.TAUSCHII (781)
SH-2	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE. TAUSCHII (882)
SH-3	68112/WARD//AE. TAUSCHII (369)
SH-4	ALTAR 84/ AE. TAUSCHII (224)
SH-5	ALTAR 84/ AE. TAUSCHII (502)
SH-6	ALTAR 84/ AE. TAUSCHII (220)
SH-7	ALTAR 84/ AE. TAUSCHII (211)
SH-8	ALTAR 84/ AE. TAUSCHII (JBANGOR)
SH-9	CETA/ AE. TAUSCHII (1027)
SH-10	CETA/ AE. TAUSCHII (895)
SH-11	CROC 1/ AE. TAUSCHII (224)
SH-12	D67.2/P66.270// AE. TAUSCHII (220)
SH-13	D67.2/P66.270// AE. TAUSCHII (213)

RESULTS

4.1 PHYSIOLOGICAL SCREENING FOR SALT TOLERANCE

Values obtained from different parameters like K^+:NA^+ discrimination, shoot length, root length, shoot dry weight and root dry weight helped to screen out the salt tolerance germplasm at 75 mM NaCl stress. Results for screening germplasm in comparison with tester set were given in Table 4.1 and 4.2 respectively.

4.1.1 K^+: NA^+ Discrimination

Wheat plants having the ability to exclude Na and accumulate K at high NaCl concentration show good tolerance against salt in field condition. So accumulation of more K ions as compared to Na ions under saline condition is a salt tolerant character and most of the breeders and physiologist used this criterion to improve wheat plant.

Average K^+:NA^+ value for tester set was 3.37 which ranged from 1.1 to 6.74. Maximum value for K^+:NA^+ was recorded for Calfia while PDW 34 had minimum value. This showed that Calfia had greater ability among the tester set to tolerate salinity while PDW 34 was not so good as compared to others. Other genotypes which showed good tolerance were LU26 S, Chinese Spring, Shorawaki, Galvez S 87 and Kharchia having 5.75, 5.24, 5.20, 4.74 and 3.54 K^+:NA^+ value respectively. These genotypes can be considered as highly salt tolerant genotypes.

There are some genotypes which showed medium tolerance at 75 mM NaCl. These include Cochimi, Mepuchi, Sakha 8, KRL 1-4, Pasban, Oasis F 86, Pericu and Ciano T 89. Their K^+:Na^+ value ranged from 2.33 to 3.59. K^+:Na^+ for PDW-34 and PBW-343 were recoreded to less than 2 which indicates that these genotypes could not perform well under salt stress condition.

In case of synthetic hexaploid wheat the average value of 3.02 was recorded for K^+:Na^+ discrimination. It ranged from 2.02 to 4.58. All the synthetic hexaploids showed

medium tolerance towards salinity except SH-13 which showed high tolerance at 75 mM NaCl concentration. The value of K^+:Na^+ for this genotypes was 4.58 which was recorded to be the maximum. There were five synthetic hexaploids (SH-3, SH-4, SH5, SH-11 and SH-13) which showed K^+:Na^+ value greater than 3 and rest all having values below 3. The lowest value of K^+:Na^+ was observed in SH-1 however its value was higher from those of PBW 343 and PDW 34 which have lowest K^+:Na^+ value in tester set. These results showed that although these synthetic hexapliods wheats have not tolerant to salinity as compared to tester set yet it can provide us the diversity upon which we can work and pyramid the genes of tolerance into spring wheat varieties and could get good results.

4.1.2 Agronomic Parameters under Salt Stress Conditions

To secreen the germplam not only K^+:NA^+ discrimination was done but some other parameters like shoot length, root length, shoot and root dry weight were recorded on 35 days old seedlings under salt stress of 75 mM NaCl. These parameters along with K^+:NA^+ gave a clear picture about the germplasm status towards salinity tolerance.

Data for tester set showed that average shoot and root dry weight were 0.07 and 0.02 respectively however the values for these parameters ranged from 0.153 to 0.045 and from 0.054 to 0.015 respectively. Highest shoot dry weight was observed in Shorawaki while Lu 26S had the maximum root dry weight. Overall Shorawaki had the highest biomass production under stress condition as compared to other members of tester set. The lowest values of shoot and root dry weights were observed for Pericu and WH 157 respectively whereas Pericu had the minimum biomass.

Data for shoot and root length under stress condition showed that Shorawaki had the maximum shoot and root length among all the genotypes of tester set. The average value of shoot and root lengths were recorded to be 30.02 cm and 20.33 cm respectively. This showed that Shorawaki performed well under stress conditions. Genotypes with shortest shoot and root length were PDW 34 and Chinese Spring whose values for these parameters were 25.8 cm and 13.6 cm respectively. Among tester set Shorawaki, LU26 S

and Calafia performed well but the genotypes Pericu and PDW 34 were found to be agronomical poor with low biomass production.

Regarding agronomic data of synthetic hexaploids, genotype SH-4 had highest shoot dry weight and biomass production and this genotypes performed well under stress conditions. Genotype SH-5 had highest dry root weight. SH-1 and SH-9 had the lowest shoot and root dry weight respectively. SH-9 had the minimum biomass production and agronomically performed poor in salt stress conditions. SH-7 had the maximum shoot and root length as compared to other genotypes while the average shoot length and root length for these synthetic hexaploid were 28.65 cm and 12.69 cm respectively. Based on agronomically performance SH-4, SH-5, SH-7 and SH-13 were found to be the good genotypes having some potential to perform well under saline conditions.

On comparing the two sets of genotypes the tester set and the synthetic hexaploids the former performed well and have good values for all the parameters that are used by most of the breeders and physiologist to select ideotype for salinity affected land. The genotypes Calafia, Shorawaki, Kharachia, LU 26S and SH-13, SH-4 can be used in breeding programmes to develop varieties suitable for salt affected soils.

4.2 PHENOLOGICAL EVALUATION

Both the sets i.e. tester set and the synthetic hexaploid wheats were characterized phenologically. Data were recorded from five plants of each entry and then arithmetic mean was computed. Results of phonological evaluation is given in Tables 4.3 & 4.4.

4.2.1 Growth Habit

Growth habit was recorded as prostrate, moderately prostrate or erect. Prostrate growth habit is a wild character found in *Ae. Tauschii* and from it, transferred to synthetic hexaploids. All genotypes in the Tester set were erect showing the absence of prostrate habit in conventional/domesticated germplasm.

Table 4.1: Mean values of agronomic parameters and K^+/Na^+ values of Tester set at 75 $molm^{-3}$ NaCl

Name of Entry	*K/Na*	*SDW(g)*	*RDW(g)*	*SL(cm)*	*RL(cm)*
Shorawaki	5.20±1.2	0.153	0.037	39.3	25
Sakha 8	3.19±0.98	0.089	0.017	31.3	21.5
WH 157	2.17±0.87	0.079	0.015	31.9	15.7
Kharchia 65	3.54±1.1	0.088	0.026	31.3	24.6
LU26 S	5.76±1.5	0.081	0.054	33.8	21.4
PDW 34	1.10±0.63	0.059	0.018	25.8	17.9
SNH.9	2.0±0.54	0.085	0.029	31.9	21.3
KRL 1-4	3.03±0.25	0.079	0.040	27.5	19.9
Galvez S 87	4.74±0.41	0.090	0.025	31.9	23.4
Oasis F 86	3.0±0.65	0.069	0.020	26.2	22.9
Chinese Spring	5.24±0.74	0.070	0.018	33.6	13.6
Ciano T 79	2.33±0.25	0.068	0.019	27.9	23.4
Mepuchi	3.24±0.65	0.070	0.020	31.8	19.8
PBW 343	1.94±0.41	0.058	0.018	25.9	17.9
Cochimi	3.59±0.84	0.062	0.022	26.6	18.4
Calafia	6.74±1.6	0.089	0.024	30.8	20.8
KRL-19	1.62±0.32	0.081	0.040	27.8	18.8
Pericu	2.59±0.52	0.045	0.016	27.1	19.8
Pasban	3.02±0.85	0.081	0.019	28.0	20.2

SDW: Shoot dry weight **RDW:** Root dry weight

SL: Shoot length **RL:** Root length

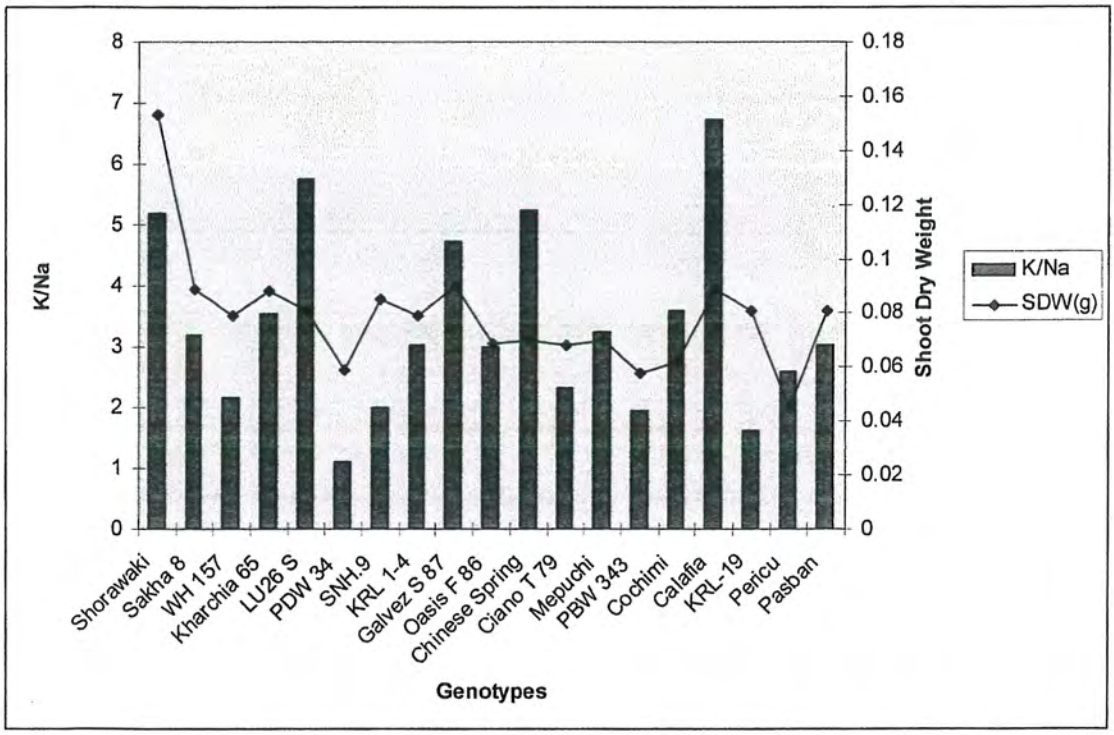

Fig. 4.1 Comparison of K/Na value and shoot dry weight for Tester set

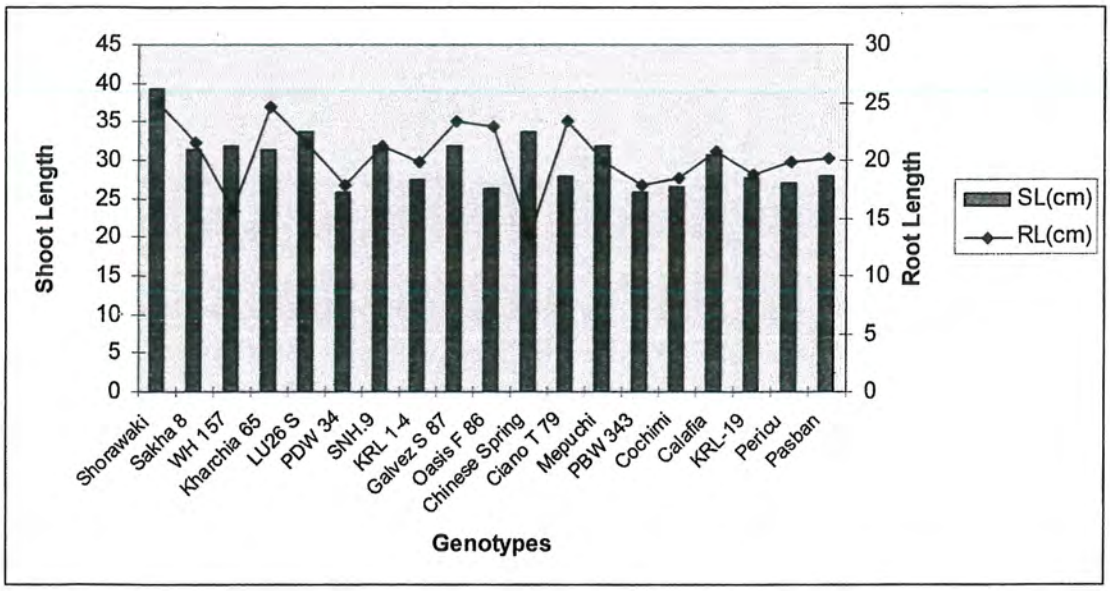

Fig. 4.2 Shoot length and Root length of Tester set

Table 4.2: Mean values of agronomic parameters and K^+/Na^+ values of Synthetic hexaploids at 75 mM NaCl

Name of Entry	*K/Na*	*SDW(g)*	*RDW(g)*	*SL(cm)*	*RL(cm)*
SH-1	2.02±0.5	0.043	0.013	21.5	11.6
SH-2	2.85±0.13	0.061	0.018	25.8	12.1
SH-3	2.93±0.98	0.058	0.023	27.9	17
SH-4	3.27±1.1	0.073	0.029	30.5	11.9
SH-5	3.25±0.64	0.059	0.031	29.3	14.2
SH-6	3.00±1.1	0.067	0.023	30.1	8.8
SH-7	2.80±0.2	0.069	0.026	35.9	17.9
SH-9	2.45±0.33	0.045	0.005	23.9	8.2
SH-10	2.67±0.42	0.064	0.024	28.2	11.2
SH-11	3.59±0.12	0.058	0.021	26.9	15.6
SH-12	2.89±0.45	0.061	0.030	32.3	9.6
SH-13	4.58±0.65	0.065	0.019	31.6	14.2

SDW: Shoot dry weight **RDW:** Root dry weight

SL: Shoot length **RL:** Root length

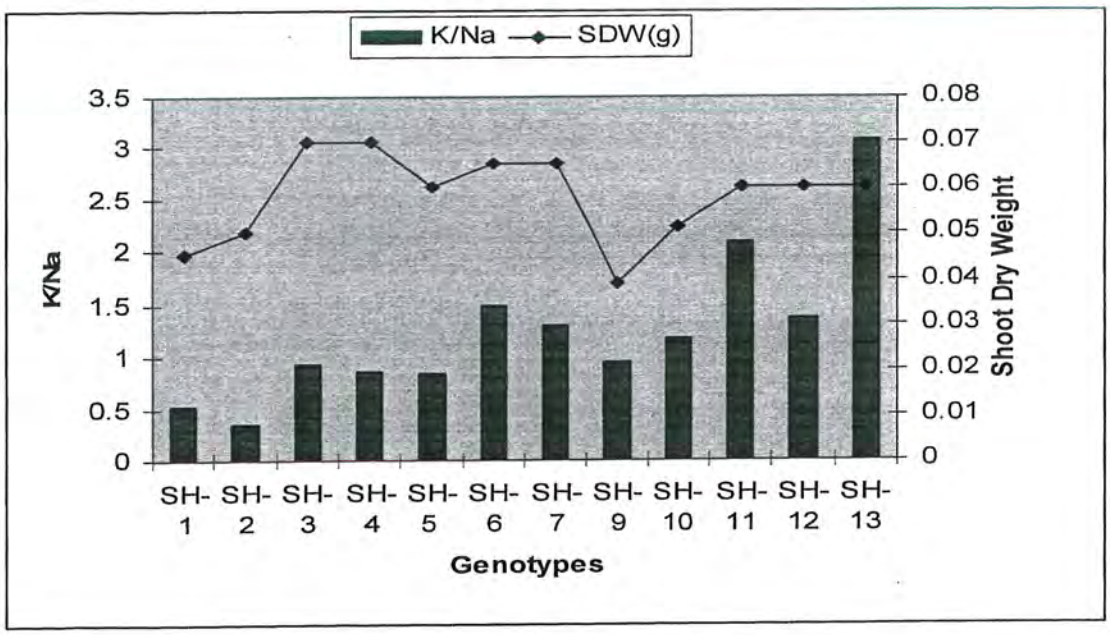

Fig. 4.3 Comparison of K/Na value and shoot dry weight for Synthetic Hexaploids

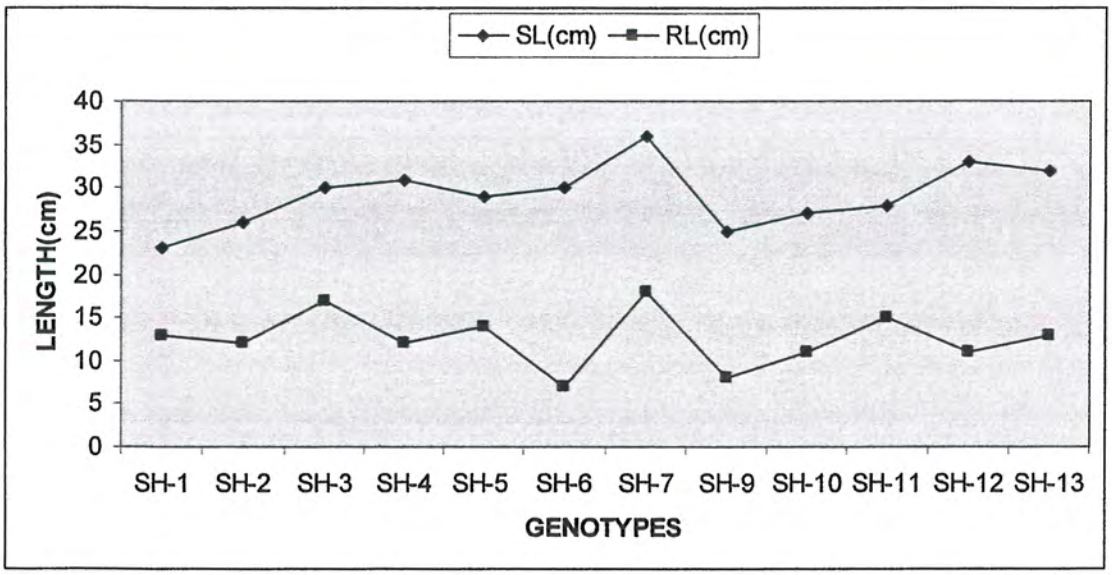

Fig. 4.4 Shoot length and Root length of Synthetic hexaploids

Among synthetics only three genotypes showed prostrate habit, four were moderately prostrate and rest of other were erect. Growth habit in synthetics is an important character to study gene action regarding this particular trait.

4.2.2 Pubescence

Pubescence has been considered as a morphological trait that enables moisture retention due to its hairy nature. Pubescence on spike glumes is also known to contribute towards stress resistance (Erdei *et al.*, 1990). Data on pubescence was taken by observing hairs at the base of spikes *i.e.* the peduncle. Most of the genotypes from tester as well as from synthetic hexapliod set lack pubescence. Among tester set, genotypes Sakha 8, Ciano T 79, Mepuchi, PBW 34 and Chochimi while among synthetics SH-1, SH-6 and SH-8 showed presence of pubescence.

4.2.3 Plant Height (cm)

Plant height indirectly contributes towards yield as lodging depends upon the height of wheat plant. Average plant height in case of tester set was 97 cm which ranged from 86cm to 111cm (Table). Tallest genotype was WH 157 and smallest genotypes were LU26 S and PBW 343. Synthetics were found to be taller as compared to conventional material showing a range of 83cm to 156cm. All the synthetics showed height greater than 100cm except SH-9 (83cm).

4.2.4 Awn Color

Awn is a qualitative character which contribure towared drought resistance and increase in yield. Awn color in both type of material was found to be brown, yellow, amber white (Table 4.3 & 4.4). Majority of entries in tester set showed amber white awn color while in synthetics it was brown in most of the entries. Only one genotype, Chinese Spring was awnless.

4.2.5 Days to Heading

Results shown in Table 4.3 & 4.4 indicate that synthetics took more days to head as compared to tester set. Tester set entries showed early heading with day to heading

ranging from 110(PDW34) to 126 (LU26 S). In case of synthetics, it ranged from 119(SH-2 and SH-6) to 144(SH-4).

4.2.6 Days to Physiological Maturity

Average days which tester set took to physiological mature were 177 days. Its value ranged from 173-186 days with majority of genotypes maturing in duration of 174-180 days. However synthetics were late to mature as compared to tester set. In synthetics its value ranged from 173-189 days. Only two synthetics were early maturing which are SH-6 and SH-13.

4.2.7 Spike Length (cm)

Spike length is an important character as it determines yield in terms of spikelets and grains produced. There was no significanct difference observed between the average spike lengths of two sets. In tester set it ranged from 10-15cm while synthetics showed longest spikes with 10-17cm length (Table 4.3 & 4.4).

4.2.8 Number of Spikelets per Spike

Number of spikelets per spike in case of tester set ranged from 19 (Kharchia 65) to 26 (Ciano T 79) and in synthetics it ranged from 16 (SH-9) to 26 (SH-10 and 13). There were more spikelets in genotypes having long spikes.

4.2.9 Number of Grains per Spike

Significant difference was observed between the two sets for number of grains per spike. Tester set has more number of spikes as compared to synthetics. This shows that seed set is less in synthetics as compared to conventional material. Number of grains per spike ranged from 34-79 in tester set while 18-66 in synthetics (Table 4.3 & 4.4).

4.2.10 1000 Grain Weight (gram)

In present study synthetics had more 1000 grains weight when compared with tester set. That's the reason synthetics were considered to attribute towards increase in

Table 4.3: Mean values for phenological parameters of tester set genotypes

Name of Entry	PUB	DH	DPM	PH	SL	SL/S	G/S	1000 GW	GC	AC
Shorawaki	-	120	179	94	13	24	34	29.4	B	B
Sakha 8	+	112	177	103	14	20	65	39	DB	AW
WH 157	-	120	179	111	12	20	46	33.4	B	AW
Kharchia 65	-	114	174	93	13.5	19	52	40	LB	AW
LU26 S	-	126	186	86	9.6	18	46	46	B	Y
PDW 34	-	110	179	102.6	15.5	22	53	34.4	LB	B
SNH.9	-	114	177	101.3	13.25	22	66	39.2	LB	AW
KRL 1-4	-	116	183	91.3	14.5	26	58	33.2	W	AW
Galvez S 87	-	116	173	95.6	13.25	25	70	29.4	LB	AW
Oasis F 86	-	125	178	89.3	15	24	79	32	DB	AW
Chinese Spring	-	123	174	120	10.25	23	67	24.4	B	Awnless
Ciano T 79	+	122	183	96	12.75	26	72	25.2	DB	AW
Yecora F 70	-	116	182	100.6	10.5	22	45	38.6	DB	AW
Mepuchi	+	118	179	105	12.25	20	62	42	LB	AW
PBW 343	+	116	174	86	15	22	58	38.6	B	AW
Cochimi	+	118	177	90	11.25	20	58	21	B	AW
Calafia	-	123	176	99	13.5	23	75	41.8	LB	AW
KRL-19	-	122	174	99.6	13.25	21	47	31.6	DB	AW
Pericu	-	120	174	90.3	13.5	24	66	39.6	B	AW
Pasban 90	-	117	180	95	11.5	19	52	38	LB	AW

GW: grain weight. (g)

PH: plant height. (cm)

PUB: pubescence, negative (-) or positive (+)

DH: Days toheading

DPM: Days to physiological maturity

G/S: No. of grains/spike

SL: Spike length (cm)

SL/S: spikelets per spike

GC: grain color (DB = dark brown, LB = light brown, B = brown,, W = white)

AC: Awn color (DB = dark brown, LB = light brown, B = brown, AW = amber white, W = white, Y= yellow)

Table 4.4: Mean values of phenological parameters of Synthetic Hexaploids

Name of Entry	GH	PUB	DH	DPM	PH	SL	SL/S	G/S	1000 GW	GC	AC
SH-1	-	+	122	181	156	12.16	22	37	38.4	LB	B
SH-2	-	-	119	189	115	12.4	24	42	45.6	B	AW
SH-3	M	-	142	189	132.3	14	22	38	67	DB	B
SH-4	M	-	144	189	116.6	10.66	21	23	40.4	LB	AW
SH-5	M	-	142	186	118	9.16	18	19	62	DB	B
SH-6	-	+	119	173	103	13.67	24	42	38	B	B
SH-7	+	-	142	186	121	15.67	24	30	57.6	DB	Y
SH-8	+	+	142	186	109.6	11.67	20	39	71.8	DB	Y
SH-9	+	-	141	186	83	10.67	16	18	37.8	DB	B
SH-10	-	-	138	184	137.6	17.16	26	60	52.6	DB	DB
SH-11	-	-	138	186	144.6	17.16	24	66	48.2	DB	B
SH-12	M	-	139	186	115.3	12.33	18	22	55.2	B	B
SH-13	-	-	126	173	117.6	11.33	26	46	43	LB	AW

GW: grain weight. (g)

PH: plant height. (cm)

PUB: pubescence, negative (-) or positive (+)

DH: Days toheading

DPM: Days to physiological maturity

G/S: No. of grains/spike

GH: Growth habit, prostrate (+), erect (-)

SL: Spike length (cm)

SL/S: spikelets per spike

GC: grain color (DB = dark brown, LB = light brown, B = brown,, W = white)

AC: Awn color (DB = dark brown, LB = light brown, B = brown, AW = amber white, W = white, Y= yellow)

yield of wheat varieties as among yield components this parameter has highest LU26 S. Entries of synthetic hexaploids showed a relatively high grain weight ranging from 37.8g - 71.8g. Highest grain weight was given by genotype SH-8. Based on grain weight a genotype can be scored as high or low yielding.

4.2.11 Grain Color

Grain color is very important character from economical point of view as in Pakistan white grain wheat is given preference over red grain wheat. In addition to this grain color is also used for characterization and identification of genotypes. Its study is also important to determine gene action. In both type of germplasm grain color was found to be brown, dark brown or light brown.

Based on morphological parameters and yield attributes Kharchia 65, LU26 S, Mepuchi, Oasis F 86, PBW 343, Calafia and Pericu showed good performance for days to heading, days to maturity, plant height, grains per spike and grain weight. On the other hand genotypes SH-3, SH-5, SH-7, SH-8 and SH-12 showed highest grain weight and more grains per spike with relatively tall plants.

The above mentioned genotypes are suggested as agronomically good with high yield potential. This morphological analysis showed that there is little diversity among the entries of tester set while entries of synthetic set were found to be morphologically more diverse.

4.3 MOLECULAR CHARACTERIZATION USING SSRs

Two different molecular marker system i.e. RAPDs (Randomly amplified polymorphic DNAs) and SSRs (Simple sequence repeats) were used to elucidate genetic diversity among the genotypes of tester set and synthetic hexaploids. The results are given below:

4.3.1 Genetic Diversity Evaluation in Tester Set Using SSR markers

A total of 92 SSR primers consisting of di-, tri- nucleotides with multiple repeats motifs were used for screening 20 genotypes of tester set. A total of 195 loci were

amplified with these 92 SSR primers with an average of 2.11 alleles per locus. The number of alleles per locus ranged from 1 to 14. Maximum alleles were amplified with primer Xgwm674-2A i.e. 14 while the minimum alleles were amplified by the primers Xgwm473-3A, Xgwm-683-4A, Xgwm-285-4A, Xgwm33-1A, Xgwm32-1A, Xgwm635-5A, Xgwm417-5A, Xgwm494-3A, Xgwm415-3A, Xgwm293-2A, Xgwm282-2A, Xgwm276-2A, Xgwm179-1A, Xgwm160-1A, Xgwm60-1A, Xgwm156-2A, Xgwm601-6A and Xgwm164-2A. The highest number of scorable bands was obtained with the primer Xgwm154-2A i.e. 49 and the minimum number of scorable bands were obtained by the primers Xgwm32-1A, Xgwm482-4A i.e. 1. The size of amplification product ranged from 50 bp to 1000bp. Maximum alleles were amplified in the variety Shorawaki i.e. 82 and the minimum alleles were amplified in the variety Pericu i.e. 5.

4.3.2 Similarity Matrix Interpretation

For the estimation of genetic relatedness among 20 genotypes of tester set, SSR data were subjected to generate similarity matrix as shown in table 4.5. The value of similarity co-efficient of these genotypes ranged from 0.569 (56.9%) to 0.908 (90.8%). Minimum similarity of 56.9% were shown by

- Shorawaki & Genotype No. 3 with Pasban-90

While the genotypes showed a value of maximum similarity of 90.8% are

- Calafia with Krl-19
- Calafia with Pericu

Table 4.5: Similarity matrix showing co-efficient of similarity among 20 tester set genotypes based on SSRs

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*
1	1.000																			
2	0.738	1.000																		
3	0.703	0.728	1.000																	
4	0.738	0.764	0.738	1.000																
5	0.708	0.733	0.79	0.754	1.000															
6	0.713	0.738	0.628	0.841	0.749	1.000														
7	0.662	0.646	0.744	0.749	0.821	0.764	1.000													
8	0.636	0.631	0.636	0.764	0.672	0.749	0.749	1.000												
9	0.687	0.692	0.708	0.795	0.754	0.759	0.81	0.785	1.000											
10	0.662	0.667	0.733	0.708	0.728	0.692	0.733	0.687	0.841	1.000										
11	0.641	0.656	0.692	0.708	0.708	0.723	0.723	0.677	0.831	0.856	1.000									
12	0.662	0.679	0.703	0.718	0.796	0.744	0.723	0.708	0.779	0.805	0.856	1.000								
13	0.672	0.667	0.672	0.687	0.718	0.692	0.713	0.646	0.759	0.774	0.795	0.795	1.000							
14	0.621	0.656	0.651	0.679	0.667	0.733	0.713	0.667	0.769	0.723	0.774	0.795	0.795	1.000						
15	0.631	0.646	0.651	0.667	0.677	0.703	0.682	0.626	0.628	0.733	0.785	0.795	0.836	0.826	1.000					
16	0.667	0.682	0.677	0.692	0.733	0.728	0.749	0.713	0.774	0.749	0.759	0.831	0.810	0.800	0.800	1.000				
17	0.626	0.651	0.626	0.713	0.723	0.749	0.79	0.754	0.764	0.677	0.677	0.749	0.738	0.708	0.738	0.708	1.000			
18	0.656	0.651	0.636	0.754	0.703	0.8	0.769	0.733	0.754	0.697	0.718	0.749	0.718	0.718	0.759	0.785	0.908	1.000		
19	0.574	0.631	0.615	0.692	0.754	0.769	0.779	0.723	0.723	0.636	0.656	0.708	0.687	0.687	0.718	0.795	0.908	0.877	1.000	
20	0.569	0.574	0.569	0.656	0.687	0.713	0.754	0.677	0.697	0.621	0.631	0.682	0.651	0.651	0.692	0.759	0.821	0.81	0.841	1.000

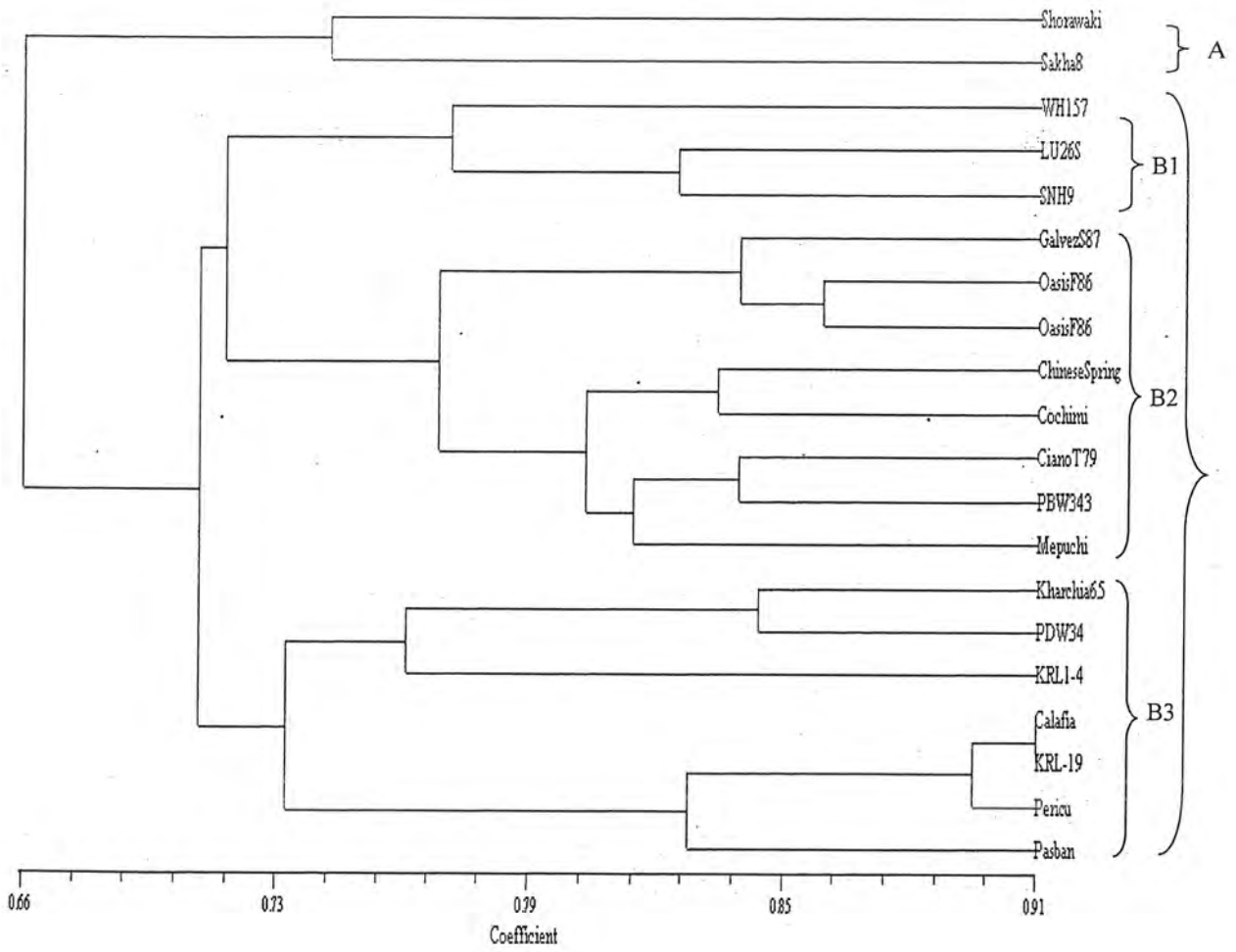

Fig 4.5: Dendrogram representing clustering of 20 genotypes of Tester set using SSR markers

4.3.3 Dendrogram interpretation

In dendrogram genotypes are grouped on the basis of their genetic similarities and differences using UPGMA. Dendrogram showing clustering pattern of 20 conventional wheat genotypes is given in Fig 4.5 Dendrogram contains two main cluster which are named as cluster A and cluster B.

Cluster A: This cluster includes two genotypes Shorawaki and Sakohe-8. The genetic similarity between these two genotypes is **73.8%** (Table 4.5).

Cluster B: This cluster include remaining 18 genotypes. The most diverse genotypes of the cluster are WH-157 and Pasban-90. This cluster is further divided into three sub-clusters sub-cluster B1, sub-cluster B2 and sub-Cluster B3.

Sub-cluster B1: This sub-cluster included three genotypes WH-157, LU-26 'S' and SNH-9. Genetic similarity revealed by Nei and Lei's co-efficient between WH-157 and LU-26 'S' **is 79%,** between WH-157 and SNH-9 is 74.40% and between LU-26 'S' and SNH-9 is 82.10%.

Sub-cluster B2: This cluster include seven genotypes Kharchia, PDW-34, KRL 1_4, Calafia, KRL-19, Pericu and Pasban-90. This sub-cluster had a further very clear subdivision partitioning the genotypes Kharchia, PDW-34 and KRL 1_4 from Calafia, KRL-19, Pericu and Pasban-90.

Sub-cluster B3: This cluster had 8 genotypes including Galvez 'S' 87, OASIS F 86, Chinese Spring, CIANO T-79, Yecora F-70, Mepuchi, Codimi, and PBW-343. Further partitioning separated Galvez 'S' 87, OASIS F-86 and Chinese Spring from other genotypes in this sub-cluster.

4.3.4 Genetic diversity evaluation in synthetic hexaploids using SSR markers

A total of same 92 set of SSR primers as applied on tester set were also applied used to elucidate genetic diversity among the 13 synthetic hexaploid genotypes. Out of these 92 SSR primers only 32 gave amplified product. These 32 primers amplified 66 loci with an average of 2.06 loci per primer. The number of alleles per locus ranged from 1 to 7. Maximum alleles were amplified with Xgwm71.2-3A while 20 different primers amplified only one allele. Maximum number of alleles were amplified in the genotype

SH-1 i.e. 44 while the minimum number of alleles were amplified in the genotype SH-7 i.e. 8.

4.3.5 Similarity matrix interpretation

Similarity matrix (Table 4.6) revealed by Nei and Lei's simple matching co-efficient depicted that genetic similarity among these synthetic hexaploids ranged from 0.348 (34.8%) to 0.848 (84.8%). Genetic diversity among synthetic hexaploids is more than diversity among genotypes in tester set. Minimum genetic similarity was observed 0.348 (34.8%) between:

- SH-1 and SH-7

While the maximum genetic similarity was observed 0.848 (84.8%) between

- SH-12 and SH-13

4.3.6 Dendrogram Interpretation

Dendrogram was constructed using UPGMA method to group the genotypes with same genetic structure on the basis of SSRs (Fig. 4.6). **At 50%** similarity level dendrogram is divided into two main clusters, Cluster A and Cluster B.

Cluster A: Cluster-A include only one genotype SH-1, indicating that this genotypes is more diverse to other genotypes in this group. Maximum genetic diversity was found between SH-1 and SH-7 i.e. 65.2%.

Table 4.6: Similarity matrix showing co-efficient of similarity among 13 synthetics based on SSRs

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
1	1												
2	0.621	1											
3	0.47	0.545	1										
4	0.5	0.727	0.576	1									
5	0.439	0.636	0.788	0.697	1								
6	0.455	0.712	0.682	0.682	0.833	1							
7	0.348	0.515	0.697	0.667	0.818	0.803	1						
8	0.515	0.652	0.621	0.682	0.682	0.758	0.712	1					
9	0.485	0.682	0.651	0.682	0.682	0.667	0.591	0.758	1				
10	0.515	0.803	0.47	0.652	0.621	0.636	0.53	0.636	0.758	1			
11	0.53	0.758	0.576	0.788	0.606	0.652	0.636	0.742	0.773	0.803	1		
12	0.545	0.682	0.621	0.652	0.652	0.697	0.621	0.697	0.667	0.727	0.742	1	
13	0.515	0.595	0.595	0.652	0.652	0.727	0.773	0.788	0.697	0.667	0.742	0.848	1

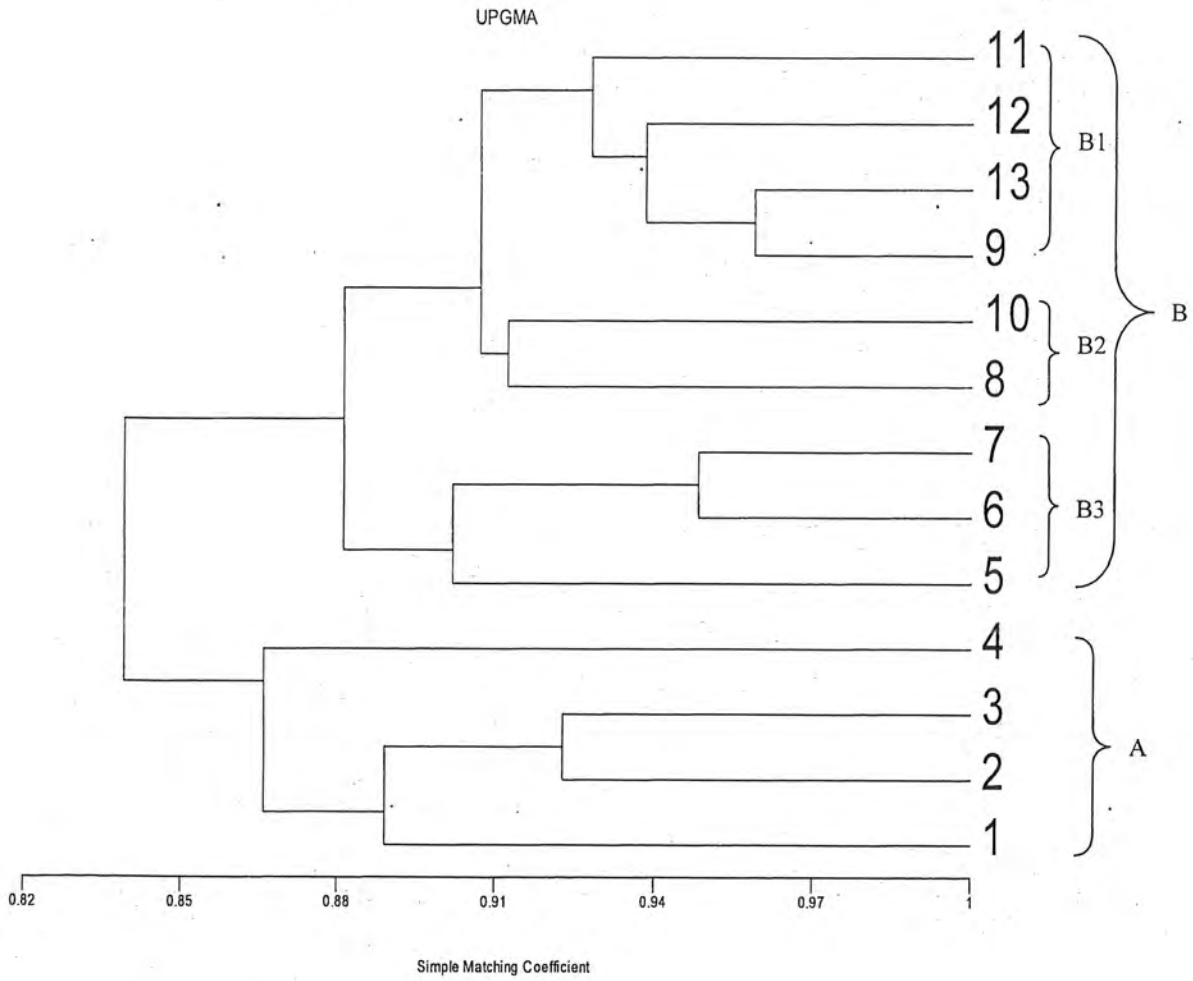

Fig 4.6: Dendrogram representing clustering of 13 genotypes of Synthetics using SSR markers

Cluster-B: All the remaining 12 genotypes were included in Cluster-B. For the ease of understanding cluster-B is further divided into three sub-clusters at about 70% similarity level (Fig. 4.6).

Sub-cluster-B1: This sub-cluster had four genotypes SH-2, SH-4, SH-10 and SH-11. SH-2 and SH-10 are more similar as compared to SH-4 and SH-11 (Table 4.6).

Sub-Cluster-B2: This sub-cluster includes four genotypes SH-8, SH-9 SH-12 and SH-13. SH-8 and SH-9 are more diverse as compared to SH-12 and SH-13 (Table 4.6).

Sub-cluster-B3: This sub-cluster had four genotypes SH-3, SH-5, SH-6 and SH-7. SH-3 is comparatively diverse from the other genotypes in this sub-cluster (Fig. 4.6).

4.4 MOLECULAR CHARACTERIZATION USING RAPDs

4.4.1 Genetic diversity evaluation in tester set using RAPD primers

To estimate the genetic diversity among tester set genotypes another molecular markers system RAPD was sued. 50 RAPD decamer primers out of which 20 were from OPQ series, 20 were of OPR series while 10 were from OPS series. Out of these 50 primers 22 primers gave amplification while the rest were non-informative. All the amplified primers were found polymorphic. These RAPD primers amplified 70 loci with an average of 3.18 loci per primer. Maximum loci were amplified in the genotype SAKHA-8 i.e. 22 while the minimum were amplified in Pericu i.e. 8. Among the primer series used RAPD primers from the OPQ series were amplified comparatively more than the other two series. Number of the bands generated by these primers was ranged from 1 to 51. Maximum numbers of bands were generated by the OPR-11 while the minimum numbers of bands were generated by the primer OPQ-3.

4.4.2 Similarity matrix interpretation

Similarity matrix (Table 4.7) was prepared using simple matching co-efficient by Nei and Lei's method based on data generated by RAPD primers. Similarity level among

these genotypes ranged from 0.5 (50%) to 0.929 (92.9%). Maximum similarity 92.9% was shown by

- Chinese Spring and Mepichu

While the minimum similarity 50% was found in

- Sakha and KRL-19
- SNH-9 and Calafia

4.4.3 Dendogram interpretation

At about 65% similarity level the genotypes were classified into 3 clusters. Cluster-A had nine genotypes, Cluster-B had only one genotype while the Cluster-C had 10 genotypes (Fig 4.7).

Cluster-A: Cluster-A had nine genotypes it is further sub-divided into two sub-clusters

Sub-clusterA1: It had four genotypes Shorawaki, Kharchia, Galvez and OASIS.

Sub-clusterA2: it had also four genotypes Sakha, KRL, PDW34 and WH-157.

Cluster B: Cluster B had only one genotype SNH-9 indicating that this genotype is diverse from all the other genotypes.

Cluster C: Cluster C had ten genotypes and this cluster is sub-divided into two sub-clusters partitioning the genotype KRL-19 from the other nine genotypes in this cluster.

4.4.4 Genetic diversity evaluation in synthetic hexaploids using RAPD primers

Same set of 50 RAPD primers were also used to elucidate genetic diversity among the synthetic hexaploid accessions. Out of these 50 RAPD primers only 14 gave clear scorable products. These 14 RAPD primers amplified 50 loci with an average 3.57 loci per primer. Maximum loci were amplified in SH-2 i.e. 33 while the minimum loci were amplified in the genotype SH-9, SH-10 and SH-13 i.e. 7. Maximum number of

Table 4.7: Similarity matrix showing co-efficient of similarity among 20 tester set genotypes based on RAPDs

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*
1	1.000																			
2	0.743	1.000																		
3	0.757	0.757	1.000																	
4	0.829	0.771	0.786	1.000																
5	0.771	0.800	0.843	0.800	1.000															
6	0.786	0.786	0.857	0.757	0.843	1.000														
7	0.700	0.700	0.714	0.586	0.700	0.714	1.000													
8	0.786	0.871	0.771	0.814	0.814	0.857	0.714	1.000												
9	0.800	0.714	0.729	0.857	0.686	0.729	0.614	0.814	1.000											
10	0.743	0.629	0.671	0.743	0.571	0.671	0.557	0.729	0.886	1.000										
11	0.729	0.557	0.657	0.643	0.671	0.657	0.600	0.657	0.757	0.729	1.000									
12	0.729	0.586	0.657	0.671	0.643	0.686	0.543	0.629	0.729	0.729	0.857	1.000								
13	0.600	0.486	0.586	0.543	0.571	0.586	0.500	0.529	0.571	0.600	0.757	0.814	1.000							
14	0.671	0.529	0.629	0.586	0.643	0.629	0.571	0.629	0.729	0.700	0.914	0.771	0.729	1.000						
15	0.743	0.600	0.671	0.657	0.657	0.671	0.614	0.671	0.771	0.743	0.929	0.843	0.714	0.900	1.000					
16	0.686	0.514	0.586	0.600	0.571	0.614	0.529	0.614	0.714	0.714	0.843	0.871	0.771	0.843	0.829	1.000				
17	0.657	0.543	0.614	0.571	0.571	0.643	0.500	0.586	0.657	0.657	0.786	0.757	0.771	0.786	0.771	0.771	1.000			
18	0.614	0.500	0.600	0.557	0.586	0.600	0.514	0.514	0.643	0.614	0.743	0.743	0.729	0.743	0.700	0.729	0.757	1.000		
19	0.743	0.571	0.700	0.629	0.629	0.700	0.671	0.671	0.771	0.771	0.871	0.814	0.743	0.843	0.886	0.771	0.771	0.757	1.000	
20	0.714	0.600	0.729	0.657	0.657	0.729	0.614	0.671	0.771	0.743	0.814	0.814	0.743	0.786	0.829	0.743	0.771	0.757	0.886	1.000

bands were generated by the primer OPR-12 i.e. 34 while the minimum number of bands were generated by the primer OPQ-15 i.e. 2.

4.4.5 Similarity matrix interpretation

Similarity matrix revealed that similarity level ranged from 0.32 (32%) to 0.92 (92%) indicating diversity among these genotypes is ranged from 8% to 68%. The level of diversity in these synthetic hexaploids is more than the diversity among the tester set genotypes. Maximum similarity was observed between the genotypes (Table 4.8)

- SH-9 and SH-13

While the minimum similarity was recorded between the genotypes.

- SH-2 and SH-13

4.4.6 Dendrogram interpretation

At about 55% similarity level genotypes are classified into two clusters (Fig 4.8).

Cluster-A: Cluster-A had six genotypes. At about 62% similarity level this cluster is sub-divided into two sub-clusters.

Sub-cluster A1: This sub-cluster had five genotypes which is further bifurcated into two clusters, one had SH-1, Sh-6 and SH-7 while the other had only one genotype SH-3 (Fig 4.8).

Sub-cluster A2: This sub-cluster had only one genotype SH-2.

Cluster B: This cluster had seven genotypes and this cluster is further divided into two sub-clusters.

Sub-cluster B1: This sub-cluster only had one genotype SH-5 at about 68% similarity level.

Sub-cluster B2: This sub-cluster had six genotypes and its further sub-division partitioned SH-12, SH-11 from Sh-10, SH-13 and SH-9.

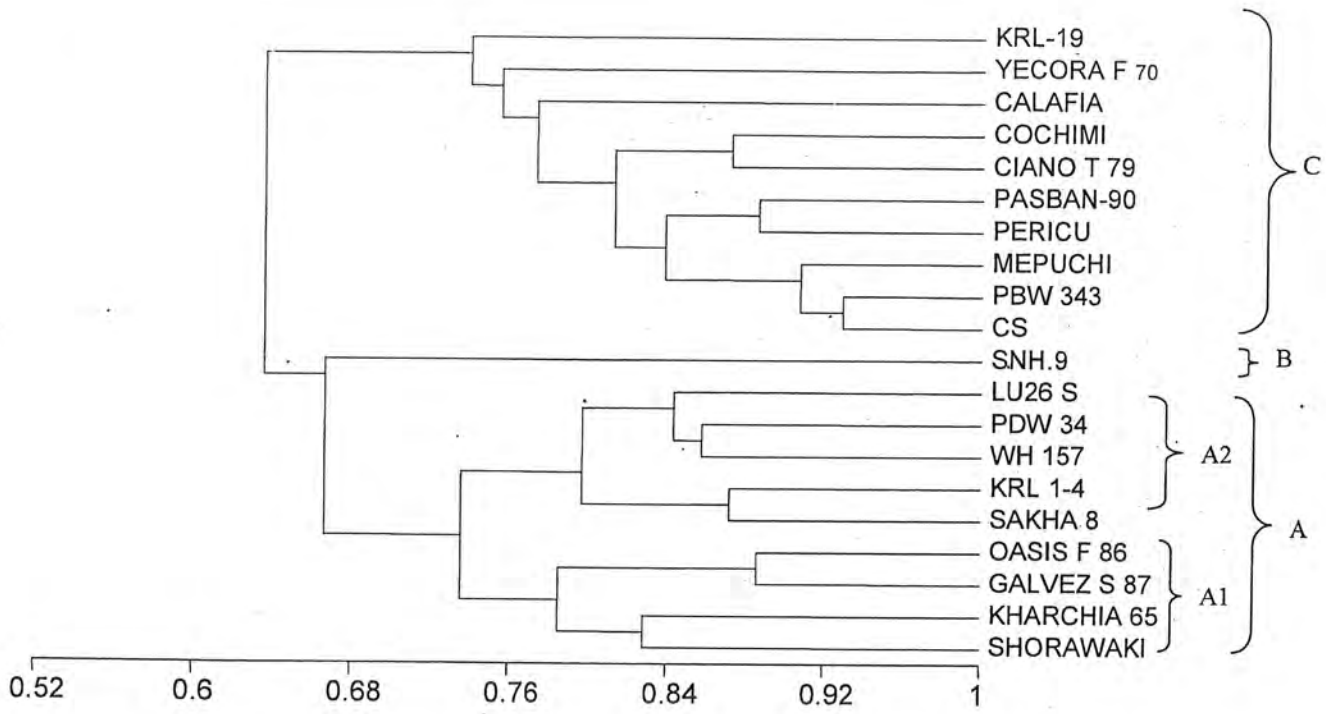

Fig 4.7: Dendrogram representing clustering of 20 genotypes of Tester set using RAPD markers

Table 4.8: Similarity matrix showing co-efficient of similarity among 13 synthetics based on RAPDs

	SH-1	*SH-2*	*SH-3*	*SH-4*	*SH-5*	*SH-6*	*SH-7*	*SH-8*	*SH-9*	*SH-10*	*SH-11*	*SH-12*	*SH-13*
SH-1	1.000												
SH-2	0.560	1.000											
SH-3	0.720	0.680	1.000										
SH-4	0.580	0.620	0.660	1.000									
SH-5	0.620	0.460	0.540	0.600	1.000								
SH-6	0.740	0.660	0.740	0.560	0.720	1.000							
SH-7	0.720	0.640	0.720	0.660	0.740	0.860	1.000						
SH-8	0.500	0.420	0.500	0.520	0.680	0.600	0.660	1.000					
SH-9	0.600	0.400	0.560	0.420	0.740	0.740	0.640	0.820	1.000				
SH-10	0.640	0.480	0.640	0.460	0.660	0.740	0.680	0.820	0.880	1.000			
SH-11	0.600	0.360	0.560	0.460	0.660	0.660	0.640	0.660	0.760	0.760	1.000		
SH-12	0.620	0.420	0.620	0.480	0.720	0.720	0.700	0.680	0.780	0.780	0.860	1.000	
SH-13	0.560	0.320	0.520	0.380	0.700	0.660	0.600	0.780	0.920	0.840	0.840	0.780	1.000

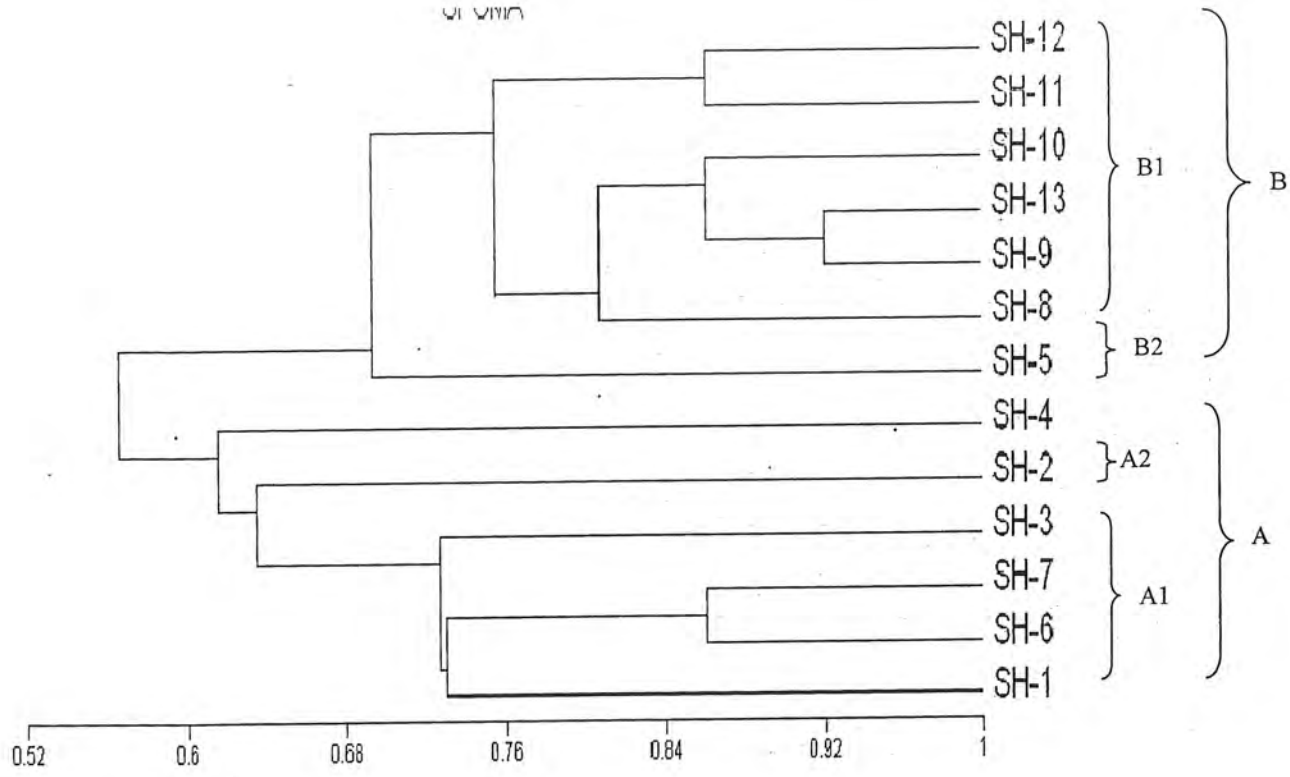

Fig 4.8: Dendrogram representing clustering of 13 genotypes of synthetics RAPD markers

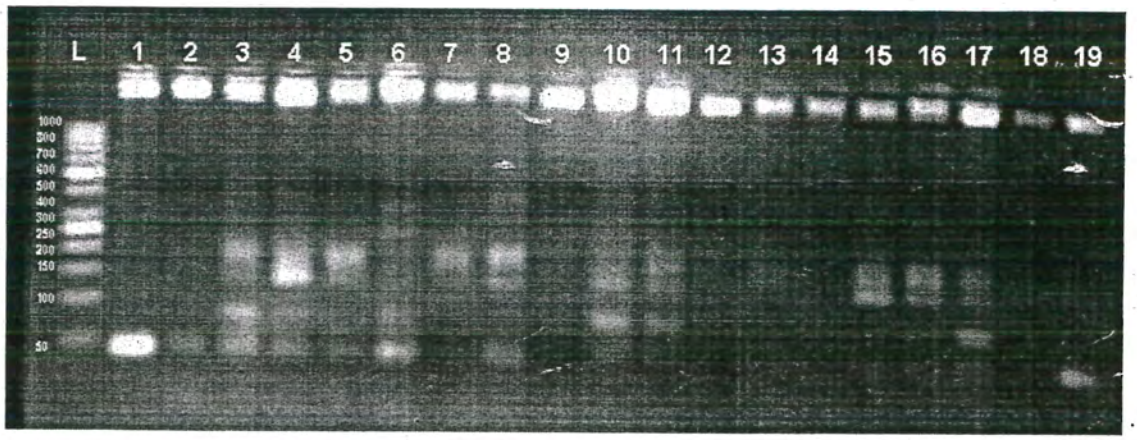

Fig. 4.9 Amplification pattern of 19 Tester set genotypes produced by primer *Xgwm* 595-5A

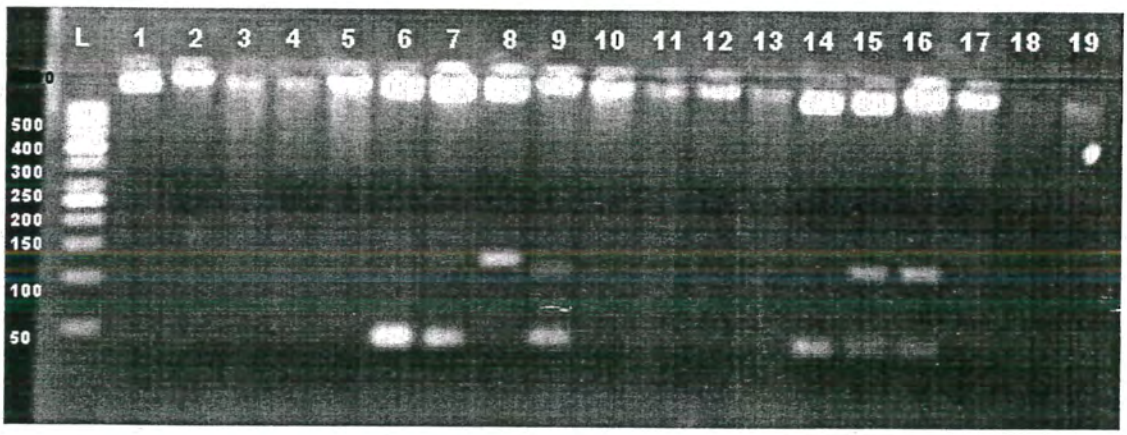

Fig. 4.10 Amplification pattern of 19 Tester set genotypes produced by primer *Xgwm* 95-2A

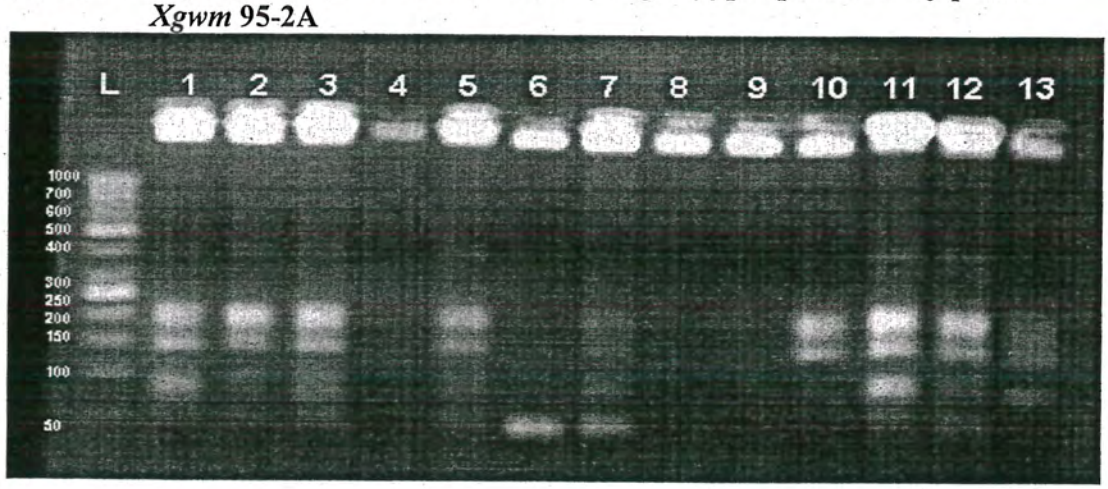

Fig. 4.11 Amplification pattern of 13 synthetic hexaploids by primer *Xgwm* 666-1A

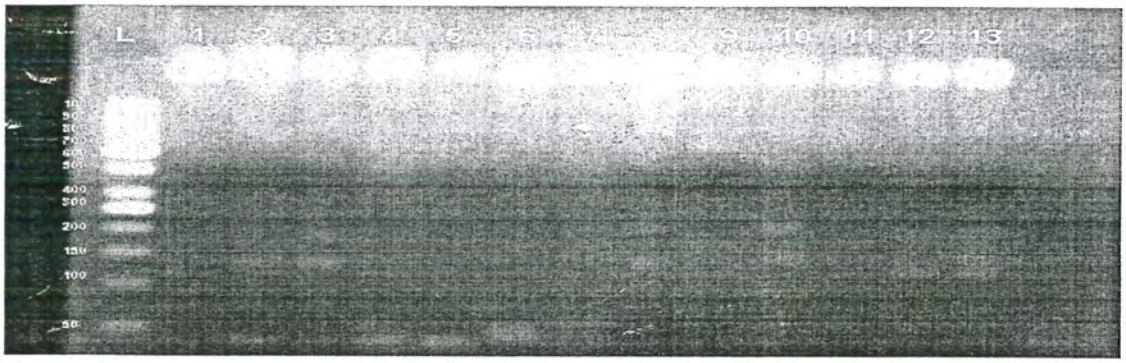

Fig. 4.12 Amplification pattern of 13 synthetic hexaploids by primer *Xgwm* 311-2A

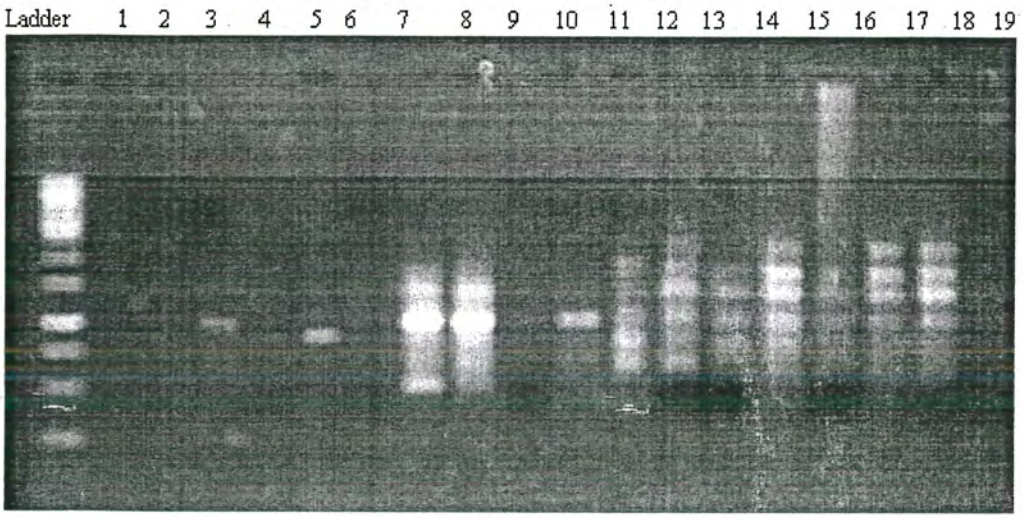

Fig 4.13 Amplification pattern of 20 tester set genotypes by RAPD primer OPR-13

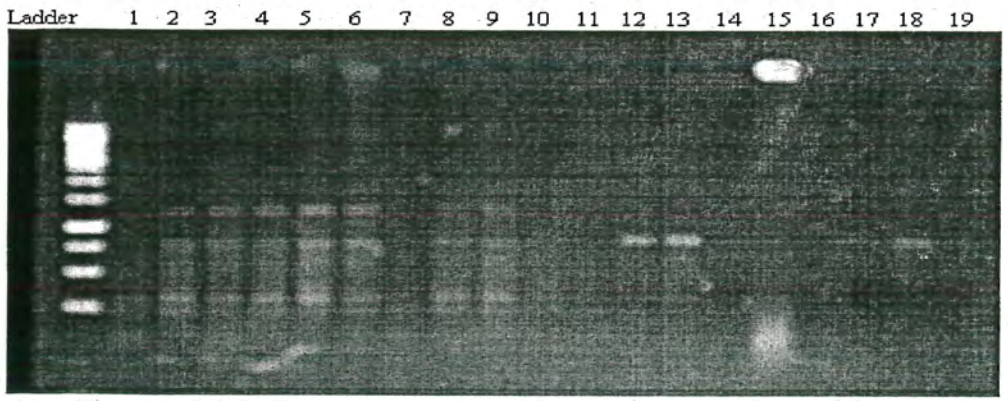

Fig 4.14 Amplification pattern of 20 tester set genotypes by RAPD primer OPR-12

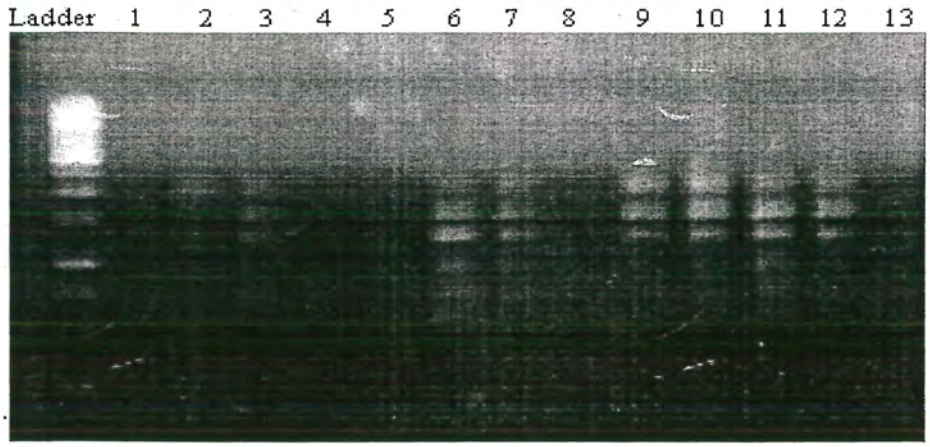

Fig 4.15 Amplification pattern of 13 synthetic hexaploids by RAPD primer OPR-13

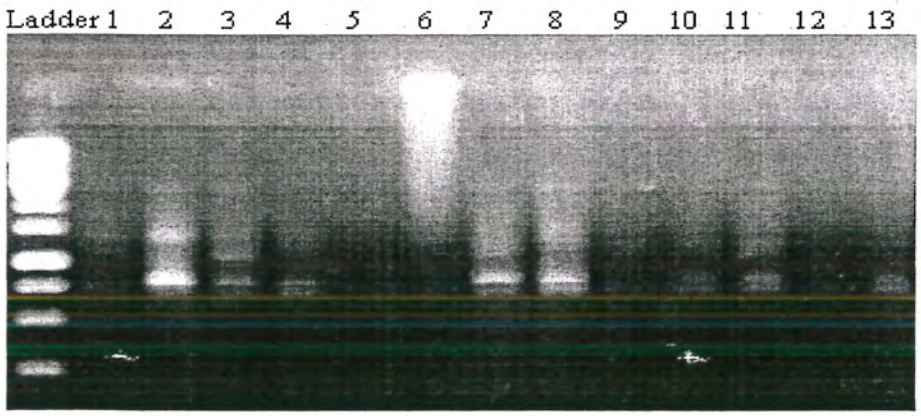

Fig 4.16 Amplification pattern of 13 synthetic hexaploids by RAPD primer OPR-6

Chapter 5

DISCUSSIONS

To meet the increasing world wide demand for food and combating severe food security issues, there is need to increase yield and production of food crop. Wheat being the staple food of about 35% of world population is very important in this respect. Sustaining the world productivity has been associated with genetic diversity of stress resistances (Mujeeb-Kazi *et al.*,1998)

Major constrains to wheat production are biotic and abiotic stresses which causes significance losses to grain yield and quality. In order to achieve high yield targets and to obtain varieties with multiple stress resistances, efforts to expand the allelic diversity are essential (Mujeeb Kazi & Raja Ram, 2002)

Among abiotic stresses to wheat the main focus is on salinity and drought but in addition heat, water logging and minor abiotic factors are also important (Mujeeb-Kazi et al 2009). Soil salinity being one of the serious problems of irrigated land, is major limitation to crop production in these areas. About 7% of the world's soils are saline which covers 950 million hectares of land (Shannon *et al.*, 1982) and this problem continues to increase at a very fast rate (Froster *et al.*, 1990)

Many wheat production areas of world are under salinity which causes huge yield losses towards major food crop. Salt effected soils can be brought under cultivation by producing germplasm tolerant to salinity. This involves identification of genotype or cultivars that are tolerant to salinity or use of new genetic resources to introduce new genes for salt tolerance in existing cultivars (Farshad *et al.*, 2008). Also significant genetic diversity is related among the existing material to incorporate into new finished varieties.

The genetic enhancement of germplasm pools in breeding programs has largely resulted from the wider utilization of plant genetic resources (Rajaram & Van-Ginkel, 1996). Bread wheat offers an enormous potential for the incorporation of genetic resources, because its allopolyploid nature enables the introgression of genes from wild species by recombination of the homoeologous chromosomes (Valkoun, 2001).Excellent sources represent particularly *T. durum* and *T. tauschii,* which was the most closely

related wild ancestors. Due to the small number of independent cross between *T. durum* and *T. tauschii* during evolution of *T. aestivum*, the contribution of total diversity from these species were small (Davorak *et al.*, 1998). Present investigation focused on determination of genetic diversity among available salt tolerant germplasm and among some synthetics wheat lines know to be tolerant to salinity.

A good genetics of germplasm helps breeding programs by facilitating the introgression of gene bank accession in the development of new crosses, thus taking advantage of alleles not found in the conventional material. Quantification and classification of diversity in the germplasm collection is very important therefore, and DNA markers represent a powerful tool to expand on available information, which is based principally on morphological data. Moreover, once a DNA marker is identified for a specific gene of interest, it can be used as a tool for marker assisted selection in the gene bank accessions and segregating populations, enhancing efficiency of the breeding programs.

5.1 Salt tolerance

K^+:Na^+ discrimination is an indicator test for the assessment of salt tolerance potential and has been used for determination of tolerance in wheat at seedling stage (Shah *et al.*, 1987; Omielan *et al.*, 1991; Bhatti *et al.*, 2004). This test relies on the ability of germplasm to discriminate between Na^+ and K^+ uptake. Salinity tolerance in wheat is associated with the accumulation of K^+ and exclusion of Na^+ under saline conditions (Gorham *et al.*, 1985). This trait is located on chromosome 4D of bread wheat (Gorham *et al.*, 1987), had been proposed as a criterion for selecting salt-tolerant genotypes in bread wheat (Gorham and Wyn Jones, 1993). This trait is controlled by a single locus (*Kna1*) and linked with RFLP markers on chromosome 4DL (Dubcovsky *et al.*, 1996). This ability of wheat and other members of *Triticeae* to tolerate salinity is due to HKT transporters (High-affinity K^+ Transporter) that play a key role in regulation of Na^+ and K^+ homeostasis in plants (Munns and Tester, 2008). These are essential factors for controlling the movement of Na^+ from roots to shoots and are important in determining the salt tolerance of plants (Munns and Tester, 2008).

K^+:Na^+ discrimination of synthetic hexaploids was determined by different scientist at different salinity levels (Gorham, 1990a; Schachtman *et al.*, 1992; Pritchard *et*

al., 2002). In present study genotypes were screened at 75mM NaCl. Calafia, LU26 S, Chinese spring, Shorawaki, Galvez S 87 and Kharchia 65 were found to be most tolerant to salinity with K^+/Na^+ values 6.5, 6, 5, 4.96, 4.5 and 3.75 respectively and among synthetics genotype SH-13 was found to be most tolerant having K^+/Na^+ value 3.08 followed by SH-11(2.98). Average K^+/Na^+ value for tester set was 3.20 and for synthetics was 1.24. Synthetic germplasm showed deviation from previous results where Pritchard *et al.* (2002) found comparable values of K^+/Na^+ among tester set and elite synthetic hexaploids at 100mol/m^3. Average value of K^+/Na^+ among synthetics was 2.04 while that of conventional germplasm was 2.26. Schachtman *et al.*, (1992) also reported high ability of synthetic hexaploids to tolerate salinity at 150mol/m^3. El-Hendawy *et al.* (2005) reported that Sakha 8 and Kharchia 65 were tolerant for high level of salinity.

5.2 Phenological studies

Based on phenological performance and yield attributes, entries of tester set showed good performance for days to heading, days to maturity, plant height, grains per spike and grain weight. On the other hand synthetic hexaploids were tall with late maturity however these showed highest grain weight and more grains per spike with longest spikes. Lage and Trethowan (2008) reported that synthetic hexaploid wheats are superior to the local cultivars, producing heavier, longer spikes with better spikelet fertility and more yields.

Average thousand grain weight for tester set was 34.6 g and for synthetic hexaploids was 50.5 g. Villareal *et al.* (1994a) found a slight greater thousand grain weight for synthetic hexaploids which was 56.9 g. Blanco *et al.* (2001) also reported that synthetic hexaploids possess several important agronomic characters and can be used as a valuable source of alleles to improve kernel weight.

5.3 Genetic diversity based on SSR markers

In terms of genetic diversity studies, SSRs currently represent the most powerful marker technique in wheat (Plaschke *et al.*, 1995; Ni *et al.*, 2002). High levels of SSRs polymorphism have been reported in wheat (Huang *et al.*, 2002; Roder *et al.*, 2002). SSR markers are now being widely used for genomic characterization, assessment of genetic

diversity and study of relationship among wild relatives (Sharma *et al.*, 2002; Kudryavtsev, 2006; Naghavi *et al.*, 2009).

In present study genetic diversity of the germplasm was assessed using highly specific SSR primers. The 92 primers yielded a total of 261 polymorphic bands with an average of 3.47 bands per primer. Ni *et al.*(2002) reported similar results with synthetic hexaploids using 23 D-genome specific SSR primers, with an average of 4 bands per primer. Saleem *et al.* (2001) reported the same level of polymorphism in wheat varieties using SSR primers which was 3.1 bands per primer. Chen and Li (2007) used 45 microsatellite markers to investigate the potential genetic diversity in evaluated ninety-five synthetic hexaploid wheats. A total of 261 alleles were detected by these microsatellite primer pairs, with an average of 2.83 alleles per locus. The values of similarity co-efficient for tester set ranged from 34.8% to 84.8%. Average value of similarity co-efficient was 0.529 (52.9%) showing that average diversity in this group is 47.1%. Average value for similarity coefficient for synthetics was 0.42 which showed that genotypes in this group were 42% similar or 52% diverse. Analysis of similarity matrices of both groups of genotypes revealed that genetic diversity among synthetics is more as compared to tester set genotypes.

Use of these molecular tools in the exploitation of genetic diversity holds great promise for further genetic improvement of crops. Together with innovative breeding strategies like the development of synthetic hexaploids in wheat, it might then be possible to achieve a steady increase in yield potential. Present study demonstrated that SSR based diversity studies provide reliable estimates of genetic diversity among conventional as well as novel germplasm for its further exploitation in wheat breeding programs. Both synthetic and conventional germplasm showed different levels of salinity tolerance and genetic diversity. Some genotypes were found to be tolerant with good phonological performance and high genetic diversity. These genotypes are suggested as valuable resources for use in cultivar breeding programs and for further studies.

5.4 Genetic diversity based on RAPD markers

RAPDs being the dominant markers had been used as an important source to estimate genetic diversity in plants. RAPDs have been used for a variety of purposes,

including the construction of genetic linkage maps (Reiter *et al.*, 1992) gene tagging, identification of cultivars (Nybom, 1994) assessment of genetic variation in populations and species (Nesbitt *et al.*, 1995), study of phylogenetic relationships among species, subspecies and cultivars and for many other purposes in a large number of plant species including wheat. RAPD analysis has been extensively used to document genetic variation in Triticum (Coa *et al.*, 1998) suggesting narrow genetic base RAPD markers have also been used for cultivar identification, for finger printing of genomes (Nybon *et al.*, 1989) and tagging of genes (Klein-Lankhorst *et al.*, 1991). The major disadvantage of this technique is that the results are non reproducible. However these markers are being used for determination of phylogenetic relationship among different groups of wheat. In this study RAPD 22 out of 50 RAPD primers gave clear amplification and amplified 70 loci in the tester set genotypes. While in the case of synthetic hexaploids only 14 primers gave clear amplification and amplified 50 loci. The similarity among genotypes in tester set genotypes ranged from 50-92% while in synthetic hexaploids 32-92%. Forapani et al. (2001) compared hemp varieties using RAPDs. They found a mean of 97.1% polymorphism over all varieties and loci. Mukhtar *et al.* (2002) observed 445 DNA fragments amplified with 50 random primers in 20 wheat varieties; they found 64.38% polymorphism.

Teshale *et al.* (2003) used RAPD markers to study the genetic relationship and genetic diversity among 27 Indian wheat accessions (17 hexaploids and 10 tetraploids). The size of PCR amplified products ranged between 0.03 and 3.0 kb. Out of the 103 amplified total RAPD bands, 82(79.6%) were polymorphic. Within hexaploids, of the total 98 amplified bands, 64(65.3%) were polymorphic whereas within tetraploids, of the total 103 bands, 78(75.7%) were polymorphic. The similarity coefficient between hexaploids and tetraploids ranged from 0.630 to 0.952 and 0.400 to 0.966, respectively.

Study on genetic divergence among wheat germplasm including synthetic hexaploids was carried out by Ali et al., (2008) using cluster analysis. They grouped synthetics hexaploids in a separate cluster from rest of bread wheat hexaploid genotypes. Similarly RAPD technique, regardless of its sensitivity to reaction conditions and problems with repeatability and amplifying of non-homologous sequences (Devos and

Gale 1992), has been successfully used for the assessment of genetic diversity in diploid, tetraploid and hexaploid wheat (He *et al.*, 1992; Myburg *et al.*, 1998; Sivolap *et al.*, 1999).

From present study it was found that conventional material provide better tolerance to salinity as compared to synthetics, however, synthetics were agronomically good with a high level of genetic diversity which is a pre-requisite of any crop improvement program. It is therefore concluded that these synthetic hexaploids as well as conventional germplasm are valuable source for genetic improvement of wheat for salinity tolerance.

Conclusion:

The results of the study are concluded as:

- The tester set genotypes had better salinity tolerance as compared to synthetic hexaploids in terms of K:Na discrimination.
- All the synthetic hexaploids had salinity tolerance as well as better performance in terms of yield components.
- The genetic diversity revealed better discrimination in synthetic hexaploid genotypes based on DNA based polymorphism.
- The SSR marker system gave better results than RAPDs and showed polymorphism in genotypes in which RAPDs were fail to identify any polymorphism.

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