STABILITY AND MOLECULAR DIVERSITY STUDIES FOR YIELD AND IT'S CONTRIBUTING TRAITS IN WHEAT

THESIS

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Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola
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By
LOKESH KUMAR VERMA

DEPARTMENT OF AGRICULTURAL BOTANY
POST GRADUATE INSTITUTE, AKOLA

DR. PANJABRAO DESHMUKH KRISHI VIDYAPEETH,
KRISHINAGAR PO, AKOLA (MS) 444104

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DECLARATION OF STUDENT

I hereby declare that, the experimental work and its interpretation of the thesis entitled **"STABILITY AND MOLECULAR DIVERSITY STUDIES FOR YIELD AND IT'S CONTRIBUTING TRAITS IN WHEAT"** or part thereof has neither been submitted for any other degree or diploma of any University, nor the data has been derived from any thesis / publication of any University or scientific organization. The sources of material used and all assistance received during the course of investigation have been duly acknowledged.

Place: **Akola** **(LOKESH KUMAR VERMA)**

Date: 21/06/2016 Enrolment No. MM-2943

CERTIFICATE

This is to certify that, the thesis entitled **" STABILITY AND MOLECULAR DIVERSITY STUDIES FOR YIELD AND IT'S CONTRIBUTING TRAITS IN WHEAT "** submitted in partial fulfilment of the requirement for the degree of **Master of Science in Agriculture (Agricultural Botany)** of Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola is a record of bonafide research work carried out by **LOKESH KUMAR VERMA** under my guidance and supervision.

The subject of thesis has been approved by the Student's Advisory Committee.

Place : Akola
Date : 21/06/2016

(N. R. Potdukhe)
Chairman
Advisory Committee

Countersigned

21.06.2016

Associate Dean
Post Graduate Institute
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

THESIS APPROVED BY THE STUDENT'S ADVISORY COMMITTEE INCLUDING EXTERNAL EXAMINER (AFTER VIVA–VOCE)

1. Chairman Dr. N. R. Potdukhe
2. Member Dr. Swati G. Bharad
3. Member Dr. S.J.Gahukar
4. Member Shri. R. D. Walke
6. External Member (S. S. Nichal)

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(Lokesh Kumar Verma)
Enrolment No.MM-2943

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(D) Abbreviations

%	:	percent
µl	:	micro liter
µM	:	micro molar
/	:	per
~	:	approximately
+	:	positive
-	:	negetive
AFLP	:	Amplified fragment length polymophism
bp	:	base pairs
cm	:	Centimeter
CTAB	:	Cetyl Trimethyl Ammonium Bromide
^{0}C	:	degree Celsius
C.D.	:	Critical difference
Cm	:	Centimeter (S)
CV	:	Coefficient of variation
d.f.	:	degree of freedom
Diff.	:	Difference
Dr. PDKV	:	Dr. Panjabrao Deshmukh Krishi Vidyapeeth
DAS	:	Days After Sowing
DNA	:	Deoxyribo Nucleic Acid
dNTPs	:	Deoxynucleotide
EI	:	Environmental Index
E1	:	First Environment
E2	:	Second Environment
E3	:	Third Environment
E.M.S.	:	Error Mean Sum Of Square
EDTA	:	Ethylene Diamine Tetra Acetic acid
et al.	:	Et alia (and other)
etc.	:	Etcetra
Fig.	:	Figure
g	:	gravity
gm	:	gram
Ha	:	hectare
HCl	:	hydrochloric acid
hr	:	hour
ISSR	:	Inter-simple sequence repeat
i.e.	:	that is

kD	:	Kilodalton
L	:	liter
M	:	molar
MSS	:	Mean sum of squares
Mbp	:	million base pairs
mg	:	milligram
mg/ml	:	milligram per milliliter
min	:	minute
ml	:	milliliter
mm	:	millimeter
mM	:	millimolar
nm	:	nanometer
No.	:	Number
°C	:	degree Celsius
PCR	:	Polymerase Chain Reaction
pH	:	Negative logarithm of hydrogen ion
Physiol.	:	Physiological
pM	:	picomolar
q	:	Quintal
RAPD	:	Random amplified polymorphic DNA
RFLP	:	Restriction fragment length polymorphism
Rpm	:	rotations per minute
S.E.	:	Standard error
SC	:	SCAR (Sequence Characterized Amplified Region)
SNP	:	Single nucleotide polymorphism
SRAP	:	Sequence-related amplified polymorphism
SSR	:	Simple sequence repeat
TRAP	:	Target region amplification polymorphism
U	:	unit(s)
v/v	:	volume by volume
viz.	:	such as
w/v	:	weight by volume
µM	:	Micro Mole

(F) Thesis Abstract

a)	Title of the thesis	: "STABILITY AND MOLECULAR DIVERSITY STUDIES FOR YIELD AND IT'S CONTRIBUTING TRAITS IN WHEAT"
b)	Full name of student	: LOKESH KUMAR VERMA
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j)	Signature, name and address of forwarding authority	:

Head
Department of Agril Botany
Dr. P.D.K.V.Akola

Head,
Department of Agricultural Botany
Post Graduate Institute,
Dr. Panjabrao Deshmukh Krishi Vidyapeeth,
Akola (M.S.).

ABSTRACT

The present investigation entitled "Stability and Molecular Diversity Studies for Yield and It's Contributing Traits in Wheat" was carried out with the objectives to study the stability of genotypes for yield parameters over diverse environments, to know the effect of yield contributing traits for imparting stability for yield and to characterize the genotypes for genetic diversity and to identify the variation among them using SSR markers.

This experiment was conducted during *rabi* season of 2014-15 on, 16 wheat genotypes comprised of 11 bread wheat (*Triticum aestivum* L.)

and 5 durum wheat (*Triticum durum* Desf.) evaluated in 3 environment viz. Niphad, Parbhani and Akola for 12 traits and molecular study was conducted at Biotechnology Centre Dr. PDKV, Akola.

ANOVA for Niphad, Parbhani and Akola revealed that the mean sum of squares due to genotypes were found highly significant for all the traits except germination per cent.

Mean performance of genotypes in 3 different environments indicated that Niphad environment was the most suitable for the better expression of the traits studied.

ANOVA for stability proffessed that the genotypic differences pooled over 3 environments were significant for all the traits indicating the presence of sufficient amount of buffering capacity of the genotypes for those traits and there is scope for selection of genotypes having desirable traits. Mean sum of squares due to environment alone are highly significant for 5 traits depicting the environmental variability and chances of better selections for yield and its contributing traits under the environments selected for study. While mean sum of squares due to environment for plant height, effective tillers plant^{-1} and test weight were found moderately significant and for trait spikelets spike^{-1} were non-significant there by indicating the presence of moderate and low of variability for these traits under environments selected for study. G×E interaction source is highly significant for days to maturity, grain yield kg plot^{-1} and moderately significant for test weight and straw yield kg plot^{-1}, depicting the differential response of genotypes for these traits in 3 environments. The environment linear component was found highly significant for 10 traits out of 12 and moderately significant for remaining 2 traits, thereby indicating the differences between environments and their considerable influence on all the traits studied. Non-linear component (GXE interactions) is highly significant for days to maturity, test weight, grain yield kg plot^{-1}, straw yield kg plot^{-1} and moderately significant for grains spike^{-1}. These results indicated that the relative ranks of the genotypes differed from one environment to another. Non-significance of non-linear interaction indicated that there was no influence of GXE interaction on the genotypes. Pooled deviation was found highly significant for days to 50 % flowering, plant height,

effective tillers plant^{-1} and grains spike^{-1} while, only significant for test weight indicated that the genotypes differed considerably with respect to their stability for all above mentioned studied traits. Remaining traits exhibited non-significant pooled deviation.

Genotype NIDW-0950 was found to be most stable across the environments and genotypes NIAW-301 and PBN-4881 found stable to unfavorable environment while, NIAW-2595 performed well in desirable environment. Genotypes NIAW-2495, NIAW-2539, AKAW-4800, PBND-5175, PBN-4876, MACS-6478 and PBND-4825 exhibited stable performance merely for one or two traits within the pool of studied genotypes for yield and its contributing traits however their evaluation for quality traits is essential. Besides it can be concluded that the genotypes performing better have potential to be used as parents in breeding programs for production of genotypes having high grain yield for different regions.

In present study six SSR primers were found polymorphic. The PIC value ranged from 0.774 (Xgwm-136) to 0.924 (Xgwm-130) with an average of 0.871 per primer. The highest PIC value was found in primer Xgwm-130 (0.924) followed by XPSP-2999 (0.918). High PIC value indicates high degree of polymorphism among the genotypes which in turns helps to estimate genetic distance and diversity with more precision.

The sixteen genotypes studied were divided into three super clusters on the basis of SSR banding pattern and dendrogram generated by using cluster analysis (NTYSIS) using six SSR primers and found that the maximum diversity was in between PBN-4881 and PBND-4825 genotypes while, maximum similarity was in between PBN-4881 and NIAW-2595 genotypes. Maximum genotypes 9 were grouped in cluster 'I'. In present research following genotypes were found stable across 3 environments (Niphad, Parbhani and Akola) viz. NIDW-0950, NIAW-301, PBN-4881 and NIAW-2595 for more number of characters and exhibited more diversity in similarity matrix while, genotypes NIAW-2495, NIAW-2539, AKAW-4800, PBND-5175, PBN-4876, MACS-6478 and PBND-4825 exhibited stable performance merely for one or two traits.

CHAPTER I

INTRODUCTION

Background Information

Common bread wheat is one of the leading edible cereal grains in all over the world in variety of forms. Global demand for wheat by the year 2020 is forecasted around 950 million tonnes. This target will be achieved only, if Global wheat production is increased by 2.5% per annum. Wheat has always been subjected to extensive and ceaseless research so as to maximize grain production but also to improve grain yield per unit area, however there is considerable room for improvement, especially to amplify efforts for continued genetic improvement of wheat to meet out the growing requirements of an ever increasing population.

Wheat is annual *rabi* season crop commonly known as "Gahu" in Marathi,"Gehon" in Hindi, "Godhi" in Kanada, "Kothumai" in Tamil and "Gam" in Bengali, belongs to the Division: Magnoliophyta, Class: Liliopsida-monocottyledons, Subclass: Commelinidae, Order: Cyperales, Family: Graminae (Sub family-Poaceae), Genus: Triticum, for which there are 50 wild species and three species are being cultivated in India. Cultivation of diploid wheat (genome Am Am, 2n=14) einkorn wheat (*Triticum monococcum spp. monococcum*) and tetraploid wheat (genomes AABB, 2n=28) emmer wheat (*Triticum turgidum spp. dicoccoides*) about 10000 years ago (Tanno and Willcox, 2006) contributed to the 'Neolithic Revolution' (Shewry 2009), a highlight in the evolution of human societies marked by a transition from hunting and gathering of food to agrarian lifestyles (Dubcovsky and Dvorak, 2007). Presently, bread wheat (*Triticum aestivum spp. aestivum* L.) an allohexaploid species (genomes AABBDD, 2n=42) that originated around 8000 years ago following hybridization of emmer wheat with goat grass (*Aegilops tauschii*, genome DD) (McFadden and Sears, 1946), accounts for over 95% of all cultivated wheat (Dubcovsky and Dvorak, 2007).The total wheat genome is in excess of 16,000 megabases in size (Arumuganathan and Earle, 1991), and approximately 80% of it consists of repetitive (non-coding) DNA sequences,

interspersed amongst low-copy-number or singleton genes *Triticum* grown in tropical, subtropical as well as temperate zones. It can tolerate severe cold as well as snowfall and resume growth with grain setting in a warm weathers viz., bread wheat or spring wheat. About 87 per cent of total wheat production is of bread wheat, 12 per cent of durum wheat and very less 1 per cent of *dicoccum* wheat, which is having therapeutic value.

Wheat is naturally self-pollinated crop, which is usually grown to a height of about 3 feet and completing the life cycle within 130-140 days. Inflorescence of wheat is made up of spikelets enclosed by outer lemma and palea, spike takes 2-3 days to complete flowering and opening of the flower starts from the middle spikelets and proceeds both upward and downward. Complete opening of flower requires seldom 20 minutes. Anther dehiscence takes place within 2-3 minutes.

Wheat is a native of South Western Asia. In India, it is second most important food crop after rice in terms of both area and production. Wheat* is a nutritive food crop, having high amount of nutrients. It is rich in carbohydrates 87.1 gm (29%),dietary fiber 14.6 gm (59%),sugars 0.5 gm, total fat 2.2 gm (3%), protein 16.4 gm (33%), vitamins-A 10.8 IU, vitamin-C 0.0 mg, vitamin –D, vitamin-E 1 mg (5%),vitamin-K 2.3 mcg (3%), Thiamin 0.5 mg, Riboflavin 0.3 mg, Niacin 7.6 mg, vitamin B6 0.4 mg, folate 52.8 mcg, vitamin B12 0.0 mg, pantothenic acid 1.2 mg, choline 37.4 mg, beatine 87.4 mg, calcium 40.8 mg, iron 4.7 mg, magnesium 166 mg, phosphorus 415 mg, potassium 486 mg, sodium 6.0 mg, zinc 3.5 mg, magnese 4.6 mg, selenium 84.8 mcg, water 12.3 gm, ash 1.9 gm.(USDA nutrient database SR-21).

*Each 120 gm wheat grain.

The development of improved genotypes, which can be adapted to a wide range of environments, is one of the final goals of researchers in plant breeding program. The interplay between the effects of genotypes and environments is usually known as genotype x environment (GE) interaction (Moll and Stuber, 1974). The G x E interaction is an important restricting factor in the estimation of variance components as well

as in the efficiency of selection programs. The adaptability of a genotype over diverse environmental conditions is tested by the magnitude of its interaction with different locations and over several years (Baker, 1988). A genotype is considered to be more favorable one, if it has a high mean yield with low amount of fluctuations. The G x E interaction reduces the correlation between genotype and phenotype and reduces the effectiveness of selection (Flores *et al.* 1998). The presence of a G x E interaction for seed yield as a quantitative trait can decrease the usefulness of subsequent analysis, restrict the significance of inference and seriously limit the feasibility of selecting favorable genotypes (Sabaghnia *et al.* 2008). Selection of stable genotype, which performs consistently across environments, can reduce the magnitude of G x E interaction.

Many methods have been established to measure stability and the genotype x environment interaction (Comstock and Robinson 1952, Finlay and Wilkinson 1963, Eberhart and Russell 1966, Perkins and Jinks 1968, Hanson 1970, Freeman and Perkins 1971, Tai 1971).Out of these models, the most simple and popular is Eberhart and Russell (1966) according to this model of stability analysis, a variety is a stable, if it has a unit regression over the environments (b=1.00) and minimum deviation from regression ($S^2di=0$). Therefore, a variety with high mean yield over the environments, unit regression coefficient (b=1.00) and deviation from the regression as small as possible ($S^2d1=0$) will be a better choice as stable variety.

The real value of a cultivar depends not only on its productivity or the expression of certain trait, but also on its ability to realize these traits to a high degree and in different growing conditions (Hristov *et al.* 2008) The stability of a given trait is a measure of variability between its genetic potential and realized value in different environments (Heinrich *et al.* 1983).

Estimation of genetic variation level among the accessions is prerequisite for germplasm conservation and breeding program (Fufa *et al.* 2005). Knowledge of genetic diversity in a crop species is fundamental

study for its improvement. Through selection and breeding a large number of alleles have been lost, so that more difficulties have egressed for improvement of wheat in modern Agriculture system. (Allard 1996, Hosington *et al.* 1999). A rich and diverse germplasm collection is the backbone of every successful crop improvement programme (Manjareez-Sandoval *et al.* 1997). The diversity opens the way for plant breeders to produce convoy of genotypes for climate change and population growth. Progress in wheat breeding also requires broad genetic base with an availability of promising germplasm collection, therefore an analysis of genetic diversity among genotypes can be a useful tool to get information about the genetic variability of genotypes and it possibly changes the direction of programe; (Kleshtkina *et al.* 2007). Consequently, such knowledge can also contribute to a purposeful and focused utilization of germplasm. Molecular characterization and genetic manipulation is the best way to raise the wheat production, therefore it is necessary to study and to guesstimate the mode of inheritance and genetic variation in different parameters of plants to start the productive programs of wheat breeding. Molecular markers can provide comprehensive characterization of genetic resources.

Genetic studies using molecular markers in elite material of hexaploid wheat have been restricted both by the limited number of polymorphic markers and by the low level variability within the self – pollinated species

The genetic divergence can be assessed in different ways, such as

(1) Phenotypic parameters, which are characterized by high response to the environment as well as the difficulty of obtaining associated parameters with the economic trials. (2) Isozyme which depends sometimes on particular tissue or defined growth stage. (3) Genetic origin (pedigree), one of most efficient means that used for estimation of the degree of genetic divergence. (4) Molecular techniques. The most important of these techniques that used in the plant and the most prevalent technology is microsatellite. Identification of better wheat cultivar is mainly

based on morphological and physiological characteristics. Even though these descriptors are useful, they are limited in number and may be affected by environment factors. Molecular markers are useful complements for morphological characterization of cultivars because they are plentiful, independent of tissue and allow cultivar identification early in plant development. Molecular markers being independent from environmental conditions have come up as an effective tool for characterization of genetic material (Malik *et al.* 2013, Abouzied *et al.* 2013). Molecular markers provide a direct measure based on agronomic trails or geographic origin .applying molecular markers and recognition of polymorphic nucleotide sequences dispersed throughout the genome have provided new possibility for evaluating genetic relationships (Gostimsky *et al.* 2005, Karakas *et al.* 2010). Molecular markers have been used to characterize genetic diversity in wild relatives (Hammer *et al.* 2000) and in seed bank collection of improved wheat germplasm (Borner *et al.* 2000, Huang *et al.* 2002). The polymerase chain reaction (PCR) technique facilitated the development of a second generation of simple and lower cost molecular markers, including SSR markers. This technology is based on the diagnosis of 2-6 base pair repeated sequence 1-10 base pairs. These parameters spread widely across eukaryotic organism's genome and are frequently high (Zhao and Kochert, 1993). Genetic studies using microsatellite markers have increased rapidly because they are highly polymorphic, heterozygous conserved sequences, which can be used as co-dominant markers (Gupta *et al.* 2004, Zane *et al.* 2002). SSR markers assist in classification of the genotypes through the identification of differences in the numbers and locations of the repeated sequences.

Microsatellite is characterized as having high polymorphism, has a capacity to produce varied bonds significantly compared to other markers and is co-dominant. The most important uses of SSR is to estimate the diversity, genetic mapping and fingerprinting, also it could provide a model for estimation of the variation among isolated generation and assists in improvement of crops efficiently through diagnosis of favourite alleles and collecting them.

In India, wheat crop is grown on an area of 30.37 million hectares with production of 90.71 million tonnes in 2014-2015 and average wheat productivity in India is 29.89 q. ha^{-1}. (Anonymous 2015[a]). However India contributes 12 per cent to the world wheat production.

In Maharashtra State, wheat is grown on an area of 1.067 million hectares with production of 1.308 million tonnes and average productivity is 12.25 q. ha^{-1}. However in Vidarbha region wheat crop is grown on an area of 0.327 million hectares with production of 0.40 million tonnes and average wheat productivity of Vidarbha is 11.68 q ha^{-1}. (Anonymous 2015[b]).

In India, the major wheat producing states are Uttar Pradesh, Punjab, Haryana, Madhya Pradesh, Rajasthan, Bihar, Gujarat, Maharashtra, Uttarakhand and West Bengal.

Importance and the need of study

Wheat varieties are grown in varied agro climatic conditions, which cause fluctuations in yield and quality traits. Therefore it is necessary to develop and identify phenotypically stable genotypes, which could perform better consistently over a wide range of environments. Besides categorization of varieties, it is also essential to identify the suitable genotypes for specific favorable and unfavorable environments for sustainable wheat production.

The joint regression analysis proposed by Perkins and Jinks (1968) bridged the gap between the statistical and general approaches for studying G x E interaction and stability parameters. The present study was undertaken to identify the stable wheat genotypes for morphological, yield and its contributing traits under different environments.

The characterization of genetic diversity in related and closely similar crop germplasm is important tool for the use of genetic resources.The molecular markers have the potential to detect the genetic diversity and characterization of genotypes. Various molecular markers or SSR markers are often chosen as the preferred markers for a variety of

significance and application in plant breeding because the SSR markers have multi-allelic character, relative abundance, co-dominant inheritance and extensive genome coverage.

DNA –based assays have revolutionized and modernized our ability to characterize genetic variation. The first advantage of molecular techniques is their capacity to detect genetic diversity at higher level of resolution than other methods. Furthermore, they are robust and information may be obtained from little amount of plant material at any stage of development. Among the numerous DNA markers (RFLP, RAPD, AFLP, SSR, ISSR, SNP and others). Microsatellites (or SSRs) and SNPs are the markers that are best suited one, providing the robust data necessary in intellectual property and patent rights. An effort will be made in this direction to characterize the genotypes for genetic diversity and to identify the variations among them using molecular markers.

Objectives

1. To study the stability of genotypes for yield parameters over diverse environment.
2. To know the effect of morphological and yeld contributing parameters for imparting stability.
3. To characterize the genotypes for genetic diversity and to identify the variation among them using SSR markers.

Scope and limitation

Wheat is best adopted to cool growing conditions. However, this crop is being increasingly grown in South-East Asia including India, Pakistan, Bangladesh and Nepal, where the ambient temperatures exceed. Optimum temperature for wheat is 15^0C (Chowdhary and Wardlaw, 1978) while moderately high temperature (25-32^0C) for longer duration and very high temperature (33-40^0C) for shorter period are very common in subtropical environments of these South-East Asian countries, particularly during grain filling (Stone and Nicolas, 1994 and Paulsen, 1994). Therefore

heat stress is one of the major constraints in wheat production in many areas of the world.

Heat stress at late growth stages is a hurdle in 40% of wheat growing areas in the temperate environments (Reynolds *et al.* 2001). Studies conducted under controlled environments revealed that long hours of exposure to moderately high temperature as well as short exposure to very high temperature reduces the productivity of wheat substantially.

It is known that genotypes, environments and their interaction (G x E) have influence on the quality traits of wheat grain. Wheat quality is affected by environmental conditions such as temperature in the growing season, humidity during grain filling, amount and distribution of precipitation, sowing time and date (Blum, 1996, Salinger *et al.* 1995).

Stability of quality characteristics is becoming an important need for the milling industries. Thousand grain weight and test weight traits are used to determine potential flour yield in wheat grain and are accepted as main quality factors by the milling industry (Unver and Kinaci, 1980).

Some cultivars are stable for one trait and are unstable for another suggesting that genetic factors involved in genotype X environment interactions differed between traits (Grasgruber *et al.* 2006). Differences in stability parameters among the different genotypes for the traits *viz.* plant height, grain number per spike, grain weight per spike, 1000 grain weight and grain yield were reported by Mehmet and Yieldirim (2006). While Shirpurkar *et al.* (2006) reported that normal sowing of wheat (8^{th} Nov.) produced significantly higher grain yield (11.39%) than the late sown wheat (26^{th} Nov.). Zaheer Ahmed *et al.* (2009) reported that decreasing trend for heading days, plant height, spikelets per spike, grains per spike, 1000 kernel weight and grain yield under late sown conditions and opined that presence of genotypic interaction showed the possibility of obtaining higher yield under both sowings.

In the recent past, concerted efforts have resulted in the development of new high yielding cultivars in the context of so called green

revolution (Siddiqui and Arain, 1974). The green revolution also brought forward many problems such as the lack of genetic diversity (Tillman 1980, Siddiqui and Arain, 1974). Hence breeding wheat genotypes with diverse genetic base is essential to achieve a level of self-sufficiency and sustainability.

According to Ashraf *et al.* (2001) the adaptability of a variety over diverse environments is usually tested by the degree of its interaction with different environments under which it is grown. A variety or genotype is considered to be more adaptive or stable one, if it has high mean yield but low degree of fluctuation in yielding ability when grown over the diverse environments.

In any breeding program aimed at improvement in genotype in respect to yield or resistance to biotic and abiotic stresses requires genetically diverse germplasm. In the absence of diverse germplasm selection is ineffective that's why there is room for the study like molecular characterization for assessing the diversity of genotypes. Therefore there is much more scope for the study like molecular characterization for assessing diversity, since it asses the diversity within and between the genotypes which considered for the development of promising cultivars compared to existing cultivar, which can give a stable performance in respect of yield and biotic ,abiotic stresses.

Results of molecular markers (SSR) may be used to protect the intellectual property, for crop breeding and selection programs (Gorji and Zolnoori, 2011). However high cost of molecular markers is a serious limitation in marker based molecular diversity studies.

Hypothesis

The objectives of this study are to evaluate stability performance and to determine the effects of yield contributing traits and morphological traits on grain yield in wheat genotypes grown under different environmental conditions and it was assumed that all the

genotypes under study will show a stable performance across the environments.

The conventional methods of wheat genotype identification relied on morphological features; however, it is difficult to distinguish between many wheat cultivars. A variety of techniques based on various molecular analysis like RFLP, RAPD, ISSR-PCR have been used for wheat cultivar identification by the various researchers.

The techniques used to identify genetic diversity in any crop require first of all identification of its extent and distribution. The techniques used to identify genetic diversity can be distinguished into two broad categories, the traditional and the molecular ones. The traditional methods are based on morphological, physiological, phenological and agronomic characters such as leaf, flower and fruit parameters, susceptibility or resistance to biotic and abiotic factors etc. These data are of the great importance as regards the use and management of genetic material and the establishment of a standard type of language.

It assumes that the sequence obtained after using universal primer will show the presence of unique sequence, which helps in identification of specific species of wheat.

CHAPTER II

REVIEW OF LITERATURE

The object of present review is to highlight the salient related research work done on stability and molecular diversity studies in Wheat. Voluminious literature is available on stability parameters and molecular diversity, however attention have been made to review the related literature as below under the following heads and subheads.

2.1 STABILITY ANALYSIS

2.2 MOLECULAR MARKER ANALYSIS

2.1 STABILITY ANALYSIS

Inamullah *et al.* (2007) conducted a study in Pakistan during 2004-05 on wheat cultivars/advance lines to identify the most suitable time of sowing (25 Oct, 5 Nov, 15 Nov, 25 Nov and 5 Dec) and judged their performance under late sowing in the Central Agro Ecological Zone of NWTP, Pakistan. Larger decreases in grain yield despite comparatively smaller decreases in grains per spike and 1000-grain weight indicating the importance of seed germinability and the number of productive tillers per unit area in late sowing. The significant sowing date x genotype interaction for all the parameters particularly grain yield highlighted the importance of further research for evolving new cultivars through crosses, other indigenous sources and/or introduction/selection that give higher yield if sown late in the cropping pattern of central NWFP having maize or sugarcane.

Jawed *et al.* (2007) conducted an experimented on Genotype x environment interaction for grain yield, number of tillers per meter row, number of grains per spike and 1000 gram weight by planting ten wheat varieties in six different sowing dates at Wheat Research Institute, Faisalabad, Pakistan, during 2000-01 and 2001-02 under irrigated conditions. Stability analysis was computed for grain yield, number of grains per spike, number of tillers per meter row and 1000 grain

weight. Stability analysis of variance showed highly significant (P<0.005) differences for environments, genotypes and environment (linear), for all the traits studies except grain yield in which, non-significant result was obtained for genotype.

Khan *et al.* (2007) evaluated 12 bread wheat genotypes including Tararao as a commercial check at eight different locations. Genotype x Environment interaction mean square were highly significant for grain yield. NRL 0405, NRL 0415 and NRL 0418 produced good yield with greater regression coefficient (bi > 1.0) and non-significant mean square deviation suggesting specific adaptation in favorable environments. NRL 0417 with regression coefficient value of 0.874 and grain yield of 29.38 kg/ha performed well in harsh environment. NRL 0409, Tatura, NRL 0412, NRL 0414 and NRL 0416 having bi values close to unity with higher grain yield showed consistent performance over different environments and could be considered as stable and widely adapted.

Rane *et al.* (2007) conducted a study to identify genotype(s) with high yield stability across the environments in general and heat stress environments in particular. Jaipur and Varanasi were hotter than any other locations considered in this study. Considerable intra - location variation in genotypic response pattern was observed over the years and dates of sowing, and this was more conspicuous at Varanasi. Longer crop duration and short grain growth duration at Varanasi were in contrast to shorter crop duration and relatively longer grain growth period that supported better grain growth at Jaipur. The genotype x environment interaction biplots for grain yield revealed that genotypes Raj 3765 and Raj 4027, developed at Jaipur, were more stable across all environments. This was due to their adaptability to high-temperature environments, and hence they are being proposed as promising germplasm sources for late-sown and/or warmer environments.

Shirpurkar *et al.* (2007) studied the effects of sowing date (8 Nov, 30 Nov or 20 Dec) on the performance of 6 wheat genotypes (Raj-4037, GW-322, MACS-2846, HI-977, NIAW-34 and PBW-533) at Niphad,

Maharashtra, India, during the *Rabi* of 2005-06. Sowing on 8 Nov and 30 Nov resulted in the highest yields (41.22 and 40.89 quintal/ha, respectively) and among the genotypes, GW-322 had the highest yield (43.66 q. /ha).

Suraiya Yasmin (2007) conducted wheat performance trial (AYT) with 10 promising lines along with 2 check varieties Kanchan and Shatabdi under optimum seeding time at Dinajpur, Jamalpur, Jessore, Ishurdi and Joydebpur to worked out stability analysis through parametric & non-parametric methods. The experiments were laid out in Randomized Complete Block Design (RCBD) during 2002-2003 with 4 replicates at each location under the supervision of WRC (Wheat Research Center). The yields of wheat varieties are location invariant and hence the high yielding varieties against bi, S^2di values. From the statistical analysis it was observed that no significant differences in rank stability were found among the 10 genotypes grown in 5 environments. Genotypes BAW-1030, BAW-1035, and BAW-1038 are the most stable and well adapted to all environments due to non-significant S^2di value, bi ≤ 1 values than other genotypes with mean yield ≥ grand mean. Only one genotype BAW-28 that response greater resistance to environmental fluctuation and therefore increasing specificity of adaptability to low yielding environments.

Calado *et al.* (2008) studied the performance of 15 wheat genotypes sown at different dates, repeated over 5 years (1994/95 to 1999/00). Despite a great variability between years, the results indicated that satisfactory wheat productivity in the Alentejo can be obtained with sowing dates ranging from the end of October until the end of November. Nonetheless, sowing dates in the first half of November tend to provide higher grain yields. Forwarding the sowing date into December resulted in reduced grain yields, thus compromising the economic viability of wheat growing.

Hristov *et al.* (2008) studied environmental stability of grain weight (GW) per main spike in 20 wheat varieties of different geographic origin and genetic background. Ten cultivars from Serbia and Ten from other countries were tested at the experimental field of the Institute of Field

and Vegetable Crops in Novi Sad over a three-year period. Stability parameters were calculated according to Eberhart and Russell (1966). Regression coefficients showed that most of the cultivars had dissatisfactory stability for the studied trait. Analysis of individual genotype responses to environmental changes revealed significant differences. Comparison of regression coefficients and deviation from regression showed that the varieties Zvezda and Mironovska were the most stable, but they had low GW means. Among the cultivars with high GW means, Lasta and Sana were the most stable.

Singh and Pathania (2008) evaluated 50 genotypes of bread wheat over 8 diverse environments, i.e. 2 years x 2 sowing dates x 2 fertilizer levels, in Ghaziabad, Uttar Pradesh, India, for 6 characters: tiller number, grains per spike, 100-grain weight, grain yield, biological yield and harvest index. Genotype x environment (G x E) interaction was found significant for all the characters. Further, linear component of G x E interaction was significant for biological yield only.

Yadav and Sharma (2008) evaluated 20 bread wheat genotypes for yield and its components for stability over four years under cold arid conditions of Leh, Jammu and Kashmir, India. Seven quantitative traits were studied for the purpose and showed presence of significant G x E interaction for all the traits. The non-linear component of G x E interaction was highly significant for the yield and all contributing traits studied. The results revealed that 12 genotypes, viz., Kailash, Mansarovar, VL770, VL733, HS 365, SWL 3, SWL 5, Sonalika, SEL 494, Singchen, SWL 10 and Leh local exhibited stable performance with respect to grain yield over the years. It was also revealed that stability for grain yield was influenced by stability of yield components, particularly biological yield, and harvest index and 1000-seed weight.

Daniela *et al.* (2009) investigated the stability of 15 traits of quality in 45 winter wheat cultivars of Slovak Republic grown in two seasons. The most stable quality traits in both growing years were identified in the Iiona, Spartakus, Vanda, Cambus, Komfort, KG Margit and

Saturnus, grain weight per spike, grain yield and duration of vegetative period were strongly affected by the environment (Growing years).

Gohil and Jadeja (2009) performed stability analysis for grain yield and some other traits in a diallel crosses of durum wheat (*Triticum durum* Desf.) under conserved soil moisture. The genotype x environment interactions were significant for all the characters. Both linear and non-linear components of G x E interactions were significant for 100-grain weight and grain yield per plant. For remaining five traits, only non-linear component was significant. The stability analysis revealed that none of the genotypes was stable for all the evaluated traits. Based on classification criteria of stability, the parents Bijaga Yellow and A-9-30-1 emerged as stable genotypes across the environments, while GW-1172 was highly responsive to favorable environment, coupled with high stability for grain yield per plant and a few component traits. The five hybrids involving popular variety GW-1 were unstable parent depicted stable performance for yield/plant.

Haman and Khaled (2009) conducted a study for assessing the heat tolerance of 12 wheat genotypes under 6 environmental conditions (two locations and three years). Wheat genotypes were sown in two locations (Sohag and Assiut, Egypt) at two dates: Nov (favorable) and Dec (heat stress) during winter seasons of 2005/2006, 2006/2007 and 2007/2008. The combined analysis of variance showed that the flag leaf area, days to heading, plant height, spike length, 1000-kernel weight, biomass and grain yield were significantly influenced by years, locations, sowing dates, nitrogen fertilizer levels and genotypes. The results showed that sowing at the favorable date using 100 kg/fed Nitrogen fertilizer increased all studied traits. The stability analysis genotype number four and three were high and intermediate yielding and stable for yield, respectively.

Shah *et al.* (2009) evaluated wheat varieties in a different Agro- Ecological zone of Pakistan for stability performance and range of adaptations. The main objective of the study was to determine variety-environment interaction, stability and adaptability of various characters and

effect of different environments relationship of characters with grain yield and grain protein percentage in spring wheat (*Triticum aestivum* L.) Ten varieties were evaluated at nine different locations for three years during 1999-2000, 2000-2001 and 2001-2002 respectively. Variety x Location (SVl), variety x year (sry) and variety x location x year (Svly) interactions were highly significant for all characters. The relative magnitude of interaction variance components indicated that relative performance of varieties for plant height, peduncle length, flag leaf area, productive tillers per meter, 1000 grain weight and grain yield were more inconsistent across the locations than years.

Yadav *et al* (2009) evaluated eighteen genotypes of wheat under normal and saline soil environments over two years in *rabi* seasons in R.B.D. with two replications to study the G X E interaction and to identify stable genotypes. Pooled analysis of variance indicated significant variance due to genotype and G X E interaction for all the characters. Variance due to G X E (lin.) was significant for plant height, spikelets per ear, grain yield per ear, 1000-grain weight and grain yield per plant. The variance due to G X E (lin.) was higher than variance due to pooled deviation for all the characters except days to flowering. Environmental indices were higher under normal as compared to saline environments for all characters except for days to flowering. Out of eighteen genotypes, genotypes KRL 19, Job 673 and Kh 65 showed average response and were highly stable. Thus, these were suitable for high saline conditions. These genotypes should be crossed with high yielding genotypes like Raj 3077 to develop high yielding genotypes suitable for highly saline soils.

Zaheer Ahmad *et al.* (2009) evaluated 18 elite under normal and late planting in Pakistan during 2005-2006. Two local checks Wataq 2001 for normal planting and Faisalabad-85 for late planting along with one long term check (Inqalab 9). The objective of the study was to identify the best promising lines for different cropping pattern. Although there was decreasing trend for heading days, height, spikelet per spike, grains per spike, 1000 kernels weight and grain yield under late sown condition, yet presence of genotypic interaction showed the possibility of obtaining

higher yield under both planting times. Grain yield, heading days and 1000 grain weight had strong and positive interaction with sowing date whereas maturity days showed weak association.

Gowda *et al.* (2010) conducted a study during 2006-08 to assess the stability of genotypes for different physiological and quality parameters using 49 diverse wheat (*Triticum aestivum* L.emend.Fiori & Paol) genotypes. Pooled analysis of variance with respect to all the 12 traits indicated that the variance due to environment was significant for all the twelve traits, which showed distinctly differential effect of the different sowing conditions in the same of environment. The variance for genotypic effect was also highly significant for all the traits under study indicating thereby differential response of all the genotypes selected for the study. The varieties 'HD 2923', 'CBW 14', 'CBW 17', 'CBW 23', 'CBW 12', 'CBW 24', 'RS 951', 'DBW 16', 'DBW 17, 'PBW 559', 'Raj 3765', 'PBW 343 'and 'NIAW 845' have shown higher mean values, desirable regression coefficient and deviation from the regression coefficient for yield, quality parameters and physiological traits. Based on the mean performance, linear regression and S^2d values, the above varieties can be said to be stable.

Killic and Yagbasanlar (2010) conducted a study with an objective to assess genotype x environment (GEI) interaction and to determine stability of 14 durum wheat (*Triticum turgidum* var. durum). Cultivars tested in randomized complete block design with four replications across eight environments of South Eastern Anatolia region of Turkey, Yield performance were analyzed using four parametric stability measures (bi, S^2d_i, R_2). A high GEI was determined for all traits. According to the stability analysis `Balcali-2000', `Firat-93' and `Altintoprak-98 were the most stable for grain yield. The study of genotypic stability showed that `Balcali-2000' cultivar had high stability for quality characteristics and determined to be the best within the pool of the studied cultivars.

Mahboob (2010) evaluated 21 stable wheat mutant lines along with four check varieties under normal and late sowing dates. The

observations were recorded on phonological, morphological and meteorological parameters. Higher yield and improvement in various yield components was recorded at normal sowing dates as compared to late sowing.

Mohmamdi Reza *et al.* (2010) evaluated 20 genotypes in 4 locations for 3 years to identify superior durum wheat genotypes for the rainfed areas of Iran and to determine the existence of different mega environments. Stability of performance was assessed by the Kang's yield stability statistics (Ysi) and 2 new methods of yield regression statistics (Ybi) and yield distance statistics (Ydi). The combined analysis of variance showed that environments were the most important source of yield variability and accounted for 76 per cent of total variation. The first mega environments consisted of environments corresponding to cold locations and a moderately cold location where 'Sardari' was the best adapted cultivars, the second mega environment comprised 'warm' environments, including the Ilam and Kernanshan locations, where the recommended breeding lines Villemun/3/Lahn//gs/stk/4/Dra2/Bcr, Tebro197-4, Stj3//Bcr/ LKS4 produced the highest yield.

Mondal *et al.* (2010) studied Genotype x environment interaction under irrigated conditions in a set of 24 varieties of winter wheat over a period of three years representing three environments. Results indicated significant variation in respect of G x E interaction for yield/plant and nine other characters. Considering the high mean performance, unit linear regression and non-significant mean square deviation as the measure of stability, Diana NS720, WW 21, WW-7, Vir 453-47, Nordesprez and Golden valley were high yielding stable varieties, giving higher yield in favorable years. Genotypes Kanchan, UP-2617, HP-1868, HP-1871, HD-2866, HD-2893 and WH-779 were found to be stable across environments for grain yield. Thus, these genotypes could be included in the hybridization programme to converge the stability characteristics of grain yield for development of a stable cultivar adapted to a wider range of environments.

Mut *et al.* (2010) carried out a study in order to determine some quality traits viz. Thousand grain weight , grain protein contain and stability of quality traits of 25 bread wheat genotypes . The experiment was conducted at 7 environmental conditions during 2 growing periods using randomized complete block design with four replications. The study of genotypic stability showed that Bezostaya advanced lined numbered 11 and 24 had high stability for quality traits and proved to be the best within the pool of the studied genotypes.

Najafian *et al.* (2010) carried out analysis of grain yield stability in hexaploid genotypes grown in temperate region of Iran using additive main effect and multiplicative interaction of 18 lines of wheat along with 2 checks across 9 location for two crop season.

Parveen *et al.* (2010) evaluated 12 spring wheat cultivars for 2 years of 5 diverse locations of Pakistan for stability analysis of tillers/m^2, 1000 grain weight and grain yield. Combined analysis of variance revealed that significant differences among the locations, years and locations x years interaction for these traits. Cultivars x years interaction was highly significant ($p=0.01$) for 1000 grain weight and grain yield. While cultivar x location interaction was highly significant only for productive tillers/m^2. However, cultivars x locations x years interaction existed for all traits ($p=0.01$). Maximum number of productive tillers of 410 /m^2 were produced by wheat cultivar Dirk followed by Fakhre Sarhad (396 tillers/m^2) and Nowshera 96 (395 tillers/m^2). Cultivars Dirk and Nowshera-96 excelled in 1000 grains weight (43 g). Maximum grain yield of 4259 kg/ha was produced by cultivar Nowshera-96 followed by Fakhre Sarhad (4183 kg/ha). Wide range of stability statistics was observed among cultivars for all the three parameters.

Sharma *et al.* (2010) tested one hundred and one advanced winter wheat breeding lines and four check cultivars over a 5-year period (2004-2008) for analysis of stability and genotypic superiority for grain yield using genotype and genotype x environment (GGE) biplot analysis. The experimental genotypes showed high levels of grain yield in each year,

with mean values ranging from 3.9 to 6.7 t ha^{-1}. The more stable high yielding genotypes were ID800994. W/Falke, Agri/Nac//AttilaID800994W/ Vee//F900K/3/Pony/Opata, AU//YT542/N10B/3/II8260/4/JI/Hys/5/ Yunnat Esskiy /6/KS82W409/ Spn and F130-L-1-12/MV12. The findings provide information on wide adaptation of the internationally important winter wheat genotypes, and demonstrate that the IWWIP program is enriching the germplasm base in the region with superior winter wheat genotypes to the benefit of National and International Winter Wheat Improvement Programs.

Soleman Mohamed (2010) evaluated 12 wheat genotypes under four environmental conditions (two sowing dates and two years in Saudi Arabia The combined analysis of variance showed that plant height, spike length, number of kernels per spike harvest index and grain yield was significantly influenced by years, sowing dates and genotypes.

Tivari *et al.* (2010) studied a method to verify the continued of check varieties in multi-location yield trial, a case study in Wheat. Three statistical tools, relative yield, yield responsiveness and Eberhnbart & Russel's parameters were used for assessing 3 Wheat varieties and found PBW-343 more efficient check.

Vali Feiziasl *et al.* (2010) carried out an analysis of yield stability of Wheat genotypes using new crop properties balance index (CPBI) method. The experiment conducted with >7000 data for each traits from national and international trials under rain fed condition for the traits (days to heading , days to physiological maturity , grain filling period, plant height, 1000 grain weight and grain yield). The analysis of adaptation of commonly grown winter wheat varieties showed that 'Sardari' is more suitable for regions with cold winters and cool springs along with spring precipitation; 'Sabalan' variety is adapted to cold winters and relatively temperate spring with abundant spring precipitation; and 'Azar-2' cultivar is suitable for cold falls and temperate springs along with fall-spring precipitation and minimum winter precipitation.

Woldeamlak *et al.* (2010) analyzed yield data of a large set of experiments with barley and wheat in order to assess whether yield stability was greater in mixed cropping than in sole cropping, and to identify which varietal mixture showed most stable grain yields. Stable cropping systems were those having reasonably high mean yield, a regression coefficient (b = 1.0) of the relation between grain yield of the location and the mean yield of each genotype combination I crop ratio or cropping system and a standard deviation (S^2di) of the mean yield as small as possible. Mixed cropping with a mean grain yield of 1744 kg ha-\ regression coefficient (b) of 0.995 and a standard deviation (S^2di) of 0.277 was more stable in grain yield than either barley or wheat sole cropping. This stability test confirmed that mixed cropping was more stable than wheat or barley mono cropping and that some varietal mixtures were more stable than others.

Zeki Mut *et al.* (2010) conducted experiment of stability analysis was conducted at 7 environmental conditions during 2 growing periods (2003-2004 and 2004-2005) using randomized complete block design with four replicates. The ANOVA showed that out of the total sum of squares, 48.4, 28.0 and 23.6% for TGW, 71.4, 14.9 and 13.7% for HW, 54.4, 23.0 and 22.6% for GPC, 44.7, 41.7 and 13.6% for ZSV was attributable to E, G and G x E interaction effects, respectively. Seven stability parameters, covering a wide range of statistical approaches, were used so as to predict the genotypes. The study of genotypic stability showed that Bezostaya and advanced lines numbered 11 and 24 had high stability for quality traits and proved to be the best within the pool of the studied genotypes. Also, 8 and 17 numbered genotypes demonstrated high stability for TGW, HW, GPC and HW, GPC and ZSV, respectively.

Ezatollah Farshadfar *et al.* (2011) studied the non-parametric estimation of phenotypic stability in wheat-barley disomic addition lines. To locate the genes controlling adaptation, disomic addition lines of barley into the genetic background of Chinese Spring wheat were used in a randomized complete block design with 3 replications under 2 different conditions (rainfed and irrigated) for 3 years. Combined analysis of variance showed significant differences for genotypes (G) and GE

interaction indicating variability between genotypes and their effects in the GE interaction and possible chromosomal localization of the genes controlling yield and yield stability in Barley. Nonparametric statistical procedures and rank method indicated that most of the quantitative trait loci (QTLs) involved in controlling phenotypic stability and yield in Barley are located on the chromosomes 3H and 4H. Screening nonparametric estimates using biplot technique classified the stability measures in 3 groups. Group 1 (G1) included NPi (1). The PCs axes separated Si(1) , Si(2), Si (3), Si (6), NPi (2), NPi (4) and YSi (2) in group 2 (G2), YSi (1), NPi (3) , RS and GSI were classified as Group 3 (G3).

Kamal Tripura *et al.* (2011) conducted a study at Indian Agricultural Research Institute, New Delhi using 36 diverse wheat genotypes and three sowing conditions to assess the stability of genotypes for yield and physiological parameters. The pooled analysis of variance with respect to all the traits indicated that the variance due to environment was significant for all the traits. The varieties BACANORAT 88, BHRIKUTI, BL 1804, CHIRIYA-3, CHIRIYA-7, GW-273, GW 326, HD 2189, HD 2819, Kanchan, MP 4010, Nepai 1, C-306 and PBW 175 have shown higher mean values, desirable regression coefficient and deviation from the regression coefficient. Based on the mean performance, linear regression and S^2di values, the above varieties are stable as per the criteria of the stability analysis.

Mehmet Ali Sakin *et al.* (2011) conducted a study to evaluate 25 durum wheat genotype including 12 registered cultivars and 13 advanced breeding lines for their stability grown in three different locations (Tokat-Kazova, Diyarbakir and Sivas-Vias) of Turkey for two growing seasons (2005-2006 and 2006-2007) and to select genotypes having desirable traits to be used in future durum wheat breeding programme. Field trails were conducted in a randomized complete block design with three replications at each location. There was a positive relationship between grain yield and number of spikes per square meter together with test weight, whereas days to heading and spike length were negatively correlated to grain yield. The results of this study also imply that line-5 and

cultivar Gidara among genotypes were the most stable cultivars and can be used as breeding material.

Nouri *et al.* (2011) evaluated 11 durum wheat breeding lines and three checks viz. two durum (Zardak and Saji) and one bread (Sardari) wheat based on grain yield, agronomic traits and frequent tolerance indices under rainfed and irrigated conditions in the West of Iran during the 2008-09 cropping season. The results of analysis of variance for plant height, biomass, number of grains per spike and grain yield in rainfed and irrigated conditions indicated that genotypic differences were highly significant ($P < 0.01$). The check cultivars (Zardak and Sardari) and genotype number 5 were more stable and related to the rainfed environment while genotype number 11 and 4 were highly adapted to the irrigated conditions.

Bigonah (2012) studied fourteen genotypes for grain yield and its stability in different climate of Ardebil, Eqlid, Arak, Zanjan, Tabriz, Mashhad, Jolgerokh, Miandoab, Hamedan and Karadj using randomized complete block design with 3 replications in 2 years in Iran. Results of combined analysis of variance showed that the interaction effects of year × location and genotype × year × location were significant at 1% probability level. For determination of genotypes with high yield and stability, parametric and non-parametric statistics were used among the methods which were used in AMMI model was found more effective than the others. Based on AMMI (AMMI1, AMMI2 and AMMI3) results, genotypes number 2, 5, 6, 7, 8 and 9 were determined as stable in most of the locations, genotypes number 9, 10 and 13 for Karadj, number 2 for Zanjan, number 4 for Jolgerokh and Ardebil, and number 7 for Mashhad showed specific adaptability.

Monika Gupta *et al.* (2012) studied stability analysis of 15 genotypes at three locations of wheat genotypes in agro forestry system of saline alkaline condition for days to heading ,days to maturity , plant height , peduncle length ,No. of spikelet per spike , 1000 grain weight , grain yield per plant and harvest index. The results were very significant statistically. The experiment conducted in saline condition were compared with normal

condition sothat the genotypes of high yield performance can be recommended for commercial cultivation where soil is saline and alkaline.

Kota *et al.* (2013) conducted an experiment with twenty three new plant type (NPT) wheat derivatives with three checks for grain yield and stability under timely (TSI) and late-sown irrigated environments (six environments) at two locations in 2006-07 and 2007-08. Analysis of Variance of stability for grain yield through Eberhart and Russell's model and AMMI analysis revealed highly significant differences among genotypes and environments and significant genotype x environment (G x E) interaction (GEI). Highly significant mean squares due to environment + genotype x environment interactions (E + G x E) in the Eberhart and Russell model revealed that genotype interacted considerably with environmental conditions that existed under TSI and LSI condition. Further partitioning of E + G x E effects indicated that E (linear), G x E (linear) component, and pooled deviation were highly significant for grain yield. Some genotypes showed linear effects over environments, while others showed significant deviation from a linear relationship. Partitioning of G x E interaction into principal components in AMMI analysis revealed that the two interaction principal component axes accounted for 90.4% of the total GEI variation. Genotypes DL 893, DL 901, DL 966 and PBW 343 exhibited high per se performance under TSI, whereas DL 880, DL 882, DL 886, DL 892, DL 893, DL 901 and DL 927 recorded high per se performance under LSI at both locations. Based on per se performance, regression coefficient, and deviations from regression as well as AMMI analysis, genotypes DL 886, DL 901, DL 924, DL 927, DL 966 and DL 960 were found to be stable and are adaptable to both TSI and LSI.

Naser Sabaghnia *et al.* (2013) conducted a field experiments with 20 genotypes for three years (2007-2009). Results showed highly significant GE interaction indicating the possibility of selection for the most stable genotypes. The AMMI analysis indicated that the first five axes were significant based on F-test of Gollob while the other tests (FGH1 and FGH2) identified first three axes as significant AMMI model components. Furthermore, according to F Ratio test and cross validation results, only

Not cited properly

first two axes were significant. According to these distinct numbers of significant axes, sixteen AMMI stability parameters plus ASV (AMMI stability value) were computed. Our results showed that EV- and D-based parameters, displayed G7 and G8, SIPC-based parameters indicated G3 and G4 and AMGE-based parameters identified G15 as the most stable genotypes. Genotypes G15 and G7 were the highest mean yielding genotypes and so they could be regarded as the most favorable durum wheat genotypes.

Singh *et al.* (2013)[a] conducted a field experiment during *rabi* season of 2006-07 and 2007-08 to study the stability analysis of yield and Its component traits in 36 genotypes of durum wheat in eight environments for 12 characters. The analysis of stability parameters revealed significant genotypic differences for all the quantitative traits. A linear component of G x E interaction was highly significant for all the characters. Out of 36 genotypes, 10 genotypes showed better adaptation to rich environment for grain yield and rest of the cultivars showed poor adaptation. Five genotypes (IC 335926, IC 335931, IC 310586, IC 335919 and IC335923) were considered as desirable and stable for dwarf plants while three genotypes (IC 335934, IC 336009 and IC 336010)were Identified as desirable and stable accross 8 the environments for early maturity. Six genotypes(IC 330586, IC 335938, IC 335932, IC 335937, IC 3?5950 and IC 336021) exhibited similarity in response with well adapted varieties and also had high mean yield therefore, these genotypes could be recommended for commercial cultivation.

Singh *et al.* (2013)[b] studied a stability stability of 10 parents and their 45 F_1s and 45 F_2s of wheat were studied at three diverse locations of Uttar Pradesh, India. The stability analysis revealed that the genotypes, environments, genotypes x environments interaction including environments (linear) were highly significant for all the characters, indicating significant variability among the genotypes and significant involvement of environments with different genotypes. The non-linear component (pooled deviation) was also highly significant for all the attributes, exhibiting considerable genetic diversity in yield and its

contributing traits. Among parents, K 9107 was found to be high yielder and stable across environments. The crosses namely, K 68 x K 9107, K 68 x K 7903, K 68 x HP 1633, DL 784-3 x K 9107, DL 784-3 x K 9644, K 9107 x K 9644, K 8027 x C 306, K 8027 x K 9644, C 306 x K 9644 and GW 373 x K 9644 were identified as stable and high yielder across environments in both the generations.

Lule *et al.* (2014) evaluated 15 advanced triticale genotypes and one standard check, Dilfikir, were evaluated at Arjo, Gedo and Shambu localities in 2010 and 2011, and at Getema in 2011, to identify stable high yielding genotypes and the extent of GXE interaction. The tested genotypes respond differently over environments as the test environments are highly variable. Only the first IPCA-I was significant ($p < 0.01$) and contributed to 43.86% of the total genotype by environment interaction. It is found that genotypes TCL-70 and TCL-77 are high yielding next to TCL-76, have IPCA value closer to zero, Genotype Selection Index (GSI) of 4 each and AMMI stability value (ASV) of 0.124 and 0.087, respectively. Analysis using Eberhart and Russell model showed that genotypes TCL-70 and TCL-77 have regression coefficients closer to unity (bi= 1.115 and 1.013) and nearly acceptable deviation from regression (s2di = 0.297 and 0.148), respectively. However, the regression coefficients were significantly different ($P \leq 0.05$) from unity for TCL-76, TCL-67, TCL-64, TCL-60, TCL-63 and Dilfikir. Therefore, both TCL-77 and TCL-70 genotypes are proposed for possible release and are recommended for wider adaptability; the uppermost yielding genotype TCL-76 is recommended for specific environments.

Patel *et al.* (2014) studied stability analysis for grain yield in bread wheat (*Triticum aestivum* L.) for irrigated ecosystems. In bread wheat eight advance lines and four check varieties viz., GW 322, GW 366, GW 496 and LOK-1 were evaluated at three locations to estimate stability parameters for seed yield. Both linear and non-linear (pooled deviation) components of G x E variance were significant; however, linear component was of greater magnitude. The genotype, GW-411 had mean value (2910.22 kg/ha) higher than general mean (2817.35 kg/ha), regression

coefficient around unity and minimum deviation from regression, thereby it was identified as stable genotype across the environments.

Jat *et al.* (2015) conducted a study to ascertain the performance of 8 wheat varieties, their 28 F1s and a check variety LOK 1 bread wheat genotypes under different environmental conditions. The stability parameters were estimated for yield and its contributing trait which exhibited homogeneous error mean square in different environments. The mean squares due to genotypes, E + (G x E), G x E and pool deviation were significant for the studied characters. This suggesting differential response of the genotypes and need to stability analysis. Results revealed that two genotypes PBW 373 and Raj 3765 had non- significant deviation from regression (S^2di) for grain yield per plant. Furthermore, three crosses viz., HD 2687 x Raj 3077, DBW 17 x PBW 373 and PBW 373 x Raj 4037 showed high per se performance with below average response (bi > 1) which indicating suitability for favorable environment. Hence, above mentioned genotypes and crosses could be used to accelerate wheat improvement programmes for different cropping system.

Mahesh Verman *et al.* (2015) carried out a study for analysis 16 bread wheat and 2 durum wheat genotypes under irrigated environment during the Rabi season of 2011-12.The analysis of variance revealed that mean squares sources were significant for genotypes and genotype x environment for all the traits studied except harvest index.

Tyagi *et al.* (2016) conducted a study with 36 genotypes, including checks (HW 2004, HD 2888, PBW 175 and NI 5439) were tested at 12 locations covering four mega wheat growing zones of India, followed by augmented design under drought conditions, during the crop season 2012/13. The main objective of this research was: to estimate variability parameters and correlation; determining the closeness of experimental sites; and identification of site specific adapted wheat genotypes. His research findings revealed higher broad-sense heritability for plant height (89-98%) and thousand grain weight (80-93%) in all four zones of India. Herein positive and significant phenotypic correlation was found between

thousand grain weight and grain yield; while days to heading, days to maturity and plant height were negatively correlated with grain yield under peninsular zone comparable to other zones. Based upon mean yield across locations, the genotypes MACS 6348 (25 q/ha); HD 3043 and AKAW 4635 (24 q/ha each) were found better than others. AMMI analysis revealed that locations; Kota, Sagar, Indore and Niphad were very close to each other and five genotypes were found to be stable in these locations. The locations; Ranchi, Delhi, Gurdaspur and Pune had also been very close to each other and we have identified seven stable genotypes for these locations. Our findings would be very spectacular for wheat breeders conducting multi-location trials. Herein; the promising genotypes identified for different locations could serve as donors to develop the multi-parent advanced generation integrated cross populations to stack genes/alleles conferring drought tolerance.

Verma *et al.* (2016) conducted an experiment during 2012-13 across eleven locations with sixteen barley genotypes. The highly significant effects of environments, genotypes and interactions were observed for forage and grain yield. The environmental effects explained the major portion of the total variance as of 82.3% and 58.8% respectively. Indicated that the environments were diverse and a major part of variation in yield resulted from environmental changes. The highly significant interaction effects partitioned into IPCA1, IPCA2 and IPCA3, IPCA4; which explained 30.4, 19.4, 14.8 & 13.2% for forage and 37.0, 17.2, 16.1 and 12.5% for harvested grain yield. AMMI stability value(ASV) identified promising genotypes UPB 1035, UPB 1034, BH 971 and RD 2857, BH 971 & NDB 1570 for forage and grain respectively. AMMI distance (D) marked RD 2035, BH 970, RD 2857 for former while genotypes RD 2856, NDB 1570 & BH 971 for grain yield. GSI score advocated RD 2857, NDB 1570, RD 2035, RD 2715 and BH 971, RD 2552, AZAD desirable genotypes for selection with forage and grain yield. Genotypes with IPCA-1 scores close to zero identified UPB 1036, BH 971, NDB 1566 and NDB 1570, and RD 2552 for forage and yield respectively would have wider adaptation to the tested environments as per AMMI graphical plots.

2.2 MOLECULAR MARKER ANALYSIS

Roder *et al.* (2002) worked out a study on construction and analysis of a microsatellite-based database of European wheat varieties. A database of 502 recent European wheat varieties, mainly of winter type, was constructed using 19 wheat microsatellites and one secalin-specific marker. All data points were generated in at least two laboratories using different techniques for fragment analysis. An overall level of >99.5% accuracy was achieved. The 199 alleles detected allowed discrimination between all of the varieties except duplicates, and varieties derived from identical parents. Approximately 25% of the varieties showed some heterogeneities, with the highest level of heterogeneity in South-Eastern European material. The highest genetic diversity and the highest number of rare alleles were found in varieties from southern Europe.

Wang *et al.* (2007) studied an assessment of genetic diversity of Yunnan, Tibetan, and Xinjiang wheat using SSR markers. A total of 206 SSR (Simple Sequence Repeats) primer pairs were used to detect genetic diversity in 52 accessions of 3 unique wheat varieties of Western China. A total of 488, 472and 308 allelic variants were detected in 31 Yunnan, 15 Tibetan and 6 Xinjiang wheat accessions with an average of PIC values 0.2764, 0.3082, and 0.1944, respectively. Substantial differences in allelic polymorphisms were detected by SSR markers in all the 21 chromosomes, the 7 homoeologous groups, and the three genomes (A, B, and D) in Yunnan, Tibetan, and Xinjiang wheat. The highest and lowest allelic polymorphisms in all the 21 chromosomes were observed in 3B and 1D chromosomes, respectively. The lowest and highest allelic polymorphisms among the seven homoeologous groups was observed in 6 and 3 homoeologous groups, respectively. Among the three genomes, B genome showed the highest, A the intermediate, and D the lowest allelic polymorphism. The cluster analysis indicated a closer relationship between Yunnan and Tibetan wheat than that between Yunnan and Xinjiang wheat or between Tibetan and Xinjiang wheat.

Strelchenko *et al.* (2008) studied comparative assessment of wheat landraces from AWCC, ICARDA and VIR germplasm collections based on the analysis of SSR markers. Total 976 bread wheat landraces maintained in AWCC (187 accessions), ICARDA (338) and VIR (451) originated from 675 collection sites. These landraces were collected from 48 countries were compared on the structure of 13 SSR loci mapped on different wheat chromosomes. In this study the set of landraces were characterized by a frequency of less than 0.05% and considered as rare alleles and 40 alleles (14.9%) were identified as unique. For each locus, between two to four alleles were detected and had a frequency of more than 0.10%, whilst only for two alleles (Xgwm261176 and Xgwm626104 on chromosomes 2D and 6B respectively) the frequency was more than 0.50%. Based on the analysis of 13 microsatellite loci, 937unique genotypes were identified from 976 landraces.

Uddin and Boerner (2008) carried out a study to evaluate the genetic diversity of a set of hexaploid and tetraploid wheat germplasm at the IPK gene bank, Gatersleben, Germany by applying 13 wheat microsatellites (WMS). The materials consisted of 63 accessions of *Triticum aestivum*, *T. dicoccon* and *T. durum* obtained from different collection missions. In total, 126 alleles were detected with an average of 9.7 alleles per locus. The average PIC values per locus varied from 0.65 for the marker Xgwm192 to 0.88 for the marker Xgwm619. All the primer pairs revealed genetic heterogeneity in more than one genotype. Genetic dissimilarity values between genotypes, calculated by the WMS derived data, were used to produce a dendrogram. In the dendrogram the genotypes were clustered in nine clear groups.

Bahadar Zeb *et al.* (2009) experimented on genetic diversity in Pakistani wheat varieties using simple sequence repeat (SSR) markers. In the present study genetic diversity of 10 varieties of wheat (*T. aestivum*) were analyzed using 14 simple sequence repeat (SSR) primer sets. A high degree of genetic polymorphism was observed among the wheat varieties with average genetic distances ranged from 16 to 67%. SSR primer gdm-3, gdm-19, gdm-61, gdm-62, gdm-64, gdm-86, gdm-88, gdm 93-2a, 93-4b,

gdm-13 and gdm-115 amplified 3, 2.9, 4.1, 4.7, 3, 1.7, 2.7, 3.7, 5.8, 4.1 and 1.4 loci per variety, respectively.

Al-Doss *et al.* (2010) carried out a study to determine the performance of 6 promising durum wheat genotypes for yield stability and molecular characterization under heat stress conditions and to compare the application and utility of SRAP (Sequence-Related Amplified Polymorphism) and TRAP (Target Region Amplified Polymorphism) marker techniques, for analysis of genetic diversity among durum wheat genotypes under heat stress. Field experiments were conducted for 4 sowing dates, over 2 seasons, to expose genotypes to different levels of heat stress during the grain-filling period. Grain yield and yield attributes during the grain filling period were investigated. Results indicated that significant variations were observed among different durum wheat genotypes in respect of all yield attributes. The effect of sowing date on the relative grain yield of durum genotypes was of greater magnitude than the effect of year. On the other hand, under the fourth sowing date (20th January), where heat stress was imposed, line KSUDW104 was the best performing line (3.26 ton/ha) out yielding Benysowef (2.21 ton/ha) by 47.5% and Kronos (2.41 ton/ha) by 35.3%. This line should be recognized as heat tolerant germplasm. The regression coefficients were significant for the six durum genotypes which indicated that they were highly responsive to the change in the average productivity of the growing season. SRAP and TRAP markers, were assayed to determine the genetic diversity of 6 durum wheat genotypes. In SRAP analysis, 45 out of 128 bands (35.16%) were polymorphic while in TRAP analysis, 22 out of 55 bands (40.0%) were polymorphic.

Tahir (2010) carried out a study on germination characteristics and molecular characterization of 11 bread and durum wheat varieties in Sulaimanyah by SSR marker to examine the genomic diversity of wheat (*Triticum aestivum* L. and *Triticum durum* L.). Eleven wheat varieties of diverse origins were analyzed with 12 selected SSRs to provide uniform and maximum genome coverage. A total of 18 bread wheat and 20 durum wheat polymorphic allelic variants were detected, ranging

from 1 to 4 per locus with an average of 1.5 and 1.7, respectively. Genetic similarities calculated from SSR data ranged from 0.286 to 1 for bread wheat and 0.333 to 0.818 for durum wheat.

Emon *et al.* (2010) worked out a study on molecular marker-based characterization and genetic diversity of wheat genotypes in relation to Boron use efficiency. In the present study, 21 diverse wheat (*Triticum aestivum* L.) genotypes were subjected to SSR analysis to identify and characterize boron efficient varieties. In the DNA profiling of the 21 genotypes, including two control varieties, Fang 60 (efficient) and SW 41 (inefficient), using 32 SSR loci, a total of 234 alleles were detected. Allele number per locus ranged from 3 to 11, with an average of 7.312, and the PIC value ranged from 0.562 to 0.873 with an average of 0.776. Average genetic diversity over all SSR loci for the 21 genotypes was 0.804, ranging from 0.637 to 0.884. All the loci were polymorphic and clearly distinguished the genotypes. Cluster analyses (NJ tree, UPGMA, PCO) identified a similar pattern of variation.

Gorji and Zolnoori (2011) studied genetic diversity in hexaploid wheat genotypes using microsatellite markers.This study was conducted to study molecular characterization of 38 wheat cultivars through the use of microsatellite markers. Polymorphic information content calculated to assess the information's of each marker. Ranged from 0.19to 0.78 with a mean of 0.49 .23 polymorphic microsatellite markers delineated all of 34 of the samples, revealing a total of 73 SSR allels i.e.an average of 3.2 allels per locus. Genetic distances among 38 genotypes ranged from 0.11 (between genotypes S-83-20, N-83-3).to 0.88 (between genotypes D-83-2 and M-83-5) the mean distance being 0.47.

Mardi *et al.* (2011) studied the comparative assessment of SSAP, AFLP and SSR Markers for evaluation of genetic diversity of durum Wheat (*Triticum turgidum* L. var. durum). Comparative assessment of genetic diversity of 122 durum wheat genotypes (*Triticum turgidum* L. var. durum) was performed using 73 SSAP polymorphic fragments, 123 AFLP polymorphic loci and 104 SSR alleles. SSAP and AFLP data showed a

clear demarcation between the cultivars and landraces and SSR data classified cultivars and landraces according to their origin. Furthermore, the estimated genetic diversity of Iranian landraces was higher compared to the foreign entries and a loss of genetic diversity was observed from landraces to cultivars. This study determined that differences in genetic relationships revealed by SSAP, AFLP and SSR distances could not be attributed solely to differences in the level of polymorphism detected by each marker system.

Zhu Yanfang *et al.* (2011) studied the fingerprinting and identification of closely related wheat (*Triticum aestivum* L.) cultivars using ISSR and fluorescence-labeled TP-M13-SSR markers. In this study, five ISSR and two sets of fluorescence-labeled TP-M13-SSR markers were applied for discrimination of eight closely related wheat cultivars. Five ISSR primers revealed a total of 43 distinct reproducible bands, 29 (67.44%) were polymorphic. The number of polymorphic bands detected by each ISSR primer ranged from 3 to 8 with an average of 4.8 per primer. One ISSR primer UBC 849 was able to identify all the eight wheat cultivars. The two sets of fluorescence-labeled TP-M13-SSR markers produced a total of 29 alleles with an average of 14.5 per locus in the 8 wheat cultivars. All of the 29 alleles were polymorphic, and the numbers and sizes of alleles revealed were used for cultivar discrimination. Combination of the two sets of fluorescence-labeled TP-M13-SSRprim ers could distinguish all the 8 closely related wheat cultivars. Finally, 2 fluorescence-labeled TP-M13-SSR markers successfully fingerprinted the 8 closely related wheat cultivars. The result suggested that fluorescence-labeled TP-M13-SSRmarkers could be used as a potential and valuable method for fingerprinting and identification ion of wheat cultivars, and fluorescence-labeled TP-M13-SSR markers should be given priority in fingerprinting and identification of wheat cultivars compared with ISSR for its high-throughput and high accuracy.

Khavarinejad and Karimov (2012) performed a study with simple sequence repeat (SSR) and random amplified polymorphic deoxyribonucleic acid (RAPD) markers to determine the genetic diversity of

10 bread wheat genotypes cultivated in Mazandaran province of Northern Iran. Numbers of 33 and 17 polymorphic alleles were detected for genotypes by RAPD and SSR markers, respectively. The most polymorphic information content (PIC) value and polymorphism percentage was found in RAPDs by UBC 350 and UBC 109 markers with values of 0.53 and 0.50, respectively. In SSRs, Xgwm 469-6D marker detected 14 bands with five polymorphic alleles but Xgwm 120-2B had the most PIC with 57%. The obtained results show an average of 3.4 polymorphic bands per primer for SSR and 5.5 polymorphic bands per primer for RAPD.

Ratiba Bousba *et al.* (2012) conducted a study to assess in formativeness and efficiency of 26 different molecular markers for genetic diversity among 40 durum wheat comprising landraces, old, moderate and improved durum wheat. High levels of polymorphism were recorded for SSR markers used in this study. A total of 136 fragments were obtained from the 26 SSR primers and all the bands were polymorphic across all the genotypes screened, most of them were polymorphic. The polymorphism information content (PIC) values ranged from 38 % to 94%, with an average of 74%. These findings provide basis for future efficient use of these molecular markers in the genetic analysis of durum wheat.

El-Assal and Ahmed Gaber (2012) studied the discrimination capacity of RAPD, ISSR and SSR Markers and of their effectiveness in establishing genetic relationship and diversity among Egyptian and Saudi eleven wheat cultivars and landraces collected from Egypt and Saudi Arabia, five Egyptian wheat (Sakha 93, Sods 1, Sods 4, Gmiza 9 and Sohag 3) and six Saudi wheat landrace cultivars (Hmees, Al-Kaseem, Hegazi, Abo-Sakr, Dubai 1 and Nagran) were characterized using RAPD, ISSR and SSR molecular markers as efficient tools. Ten and nine oligonucleotide primers of RAPD and ISSR respectively and four primer pairs of SSR were used in wheat samples analysis. The number of alleles ranged from 8-21 per primer, with an average of 14.1 per primer. In ISSR analyses, a total of 78 alleles were detected, along with 36 alleles (46%) were polymorphic. The number of alleles per primer ranged from 5-10 with

an average of 8.6 alleles per ISSR primer. SSR reactions recorded 6 alleles, of which 5 alleles (83%) were polymorphic.

Islam *et al.* (2012) studied molecular characterization of wheat (*Triticum aestivum* L.) genotypes through SSR markers to examine the genetic diversity of 12 wheat (*Triticum aestivum* L.) genotypes, using 4 simple sequence repeats (SSRs). A total of 10 alleles were found and allele number per locus ranged from 2 to 4 with an average of 2.5. The polymorphic information content (PIC) values ranged from 0.2755 to 0.5411 with an average of 0.3839. The average gene diversity over all SSR loci for the 12 wheat genotypes was 0.4688, ranging from 0.3299 to 0.6042. Cluster analysis based on microsatellite allelic diversity discriminated the varieties into different clusters. Genetic diversity was the highest between variety Gourab and Akbar as well as Gourab and BAW-1064, showing a genetic distance value of 0.4697. The genetic distance was lowest between Balaka and Aghrani as well as Triticale and BAW-1036.

Arslan Sheikh Sehgal *et al.* (2012) carried out a study that, the genetic diversity and molecular characterization of 10 wheat genotypes using 27 polymorphic microsatellite screened primers including Simple Sequence Repeats (SSR). About twenty one loci were found. Bioinformatics tools were applied for constructing dendogram. Lr-19 gene was present in all ten wheat genotypes and Sr-15 gene is present in nine genotypes except Uqab- 2000 that cause resistance against wheat rust. Highest genetic diversity was observed between Shalimar-86 and Pak-81 genotypes and showed the similarity of about 95.34%. Distantly related Uqab- 2000 showed minimum genetic diversity and 65.64% dissimilarity with Kohistan-97. Uqab-2000 rust resistant genes may have an insertion or deletion and examined as distantly related to rest of nine genotypes. The current research found that SSR makers identified rust resistant genes in numerous wheat genotypes. Present work also characterized the wheat genotypes at molecular level and found genetic diversity between different wheat genotypes. SSR markers could distinguish and characterize wheat genotypes, more screened primers could be used for saturation of different

regions in further research. Bioinformatics also play a vital role in retrieving, analyzing and interpreting the data for further studies.

Valentina *et al.* (2012) studied genetic diversity of 30 wheat genotypes at the DNA level using 24 simple sequence repeat (SSRs) markers. The number of alleles per locus ranged from 1 to 14 with an average number of 8.44 alleles per locus. The highest number of alleles per locus was detected in the genome A with 7.2, compared to 5.9and 5.0 for genomes B and D, respectively. The highest number of alleles was recorded at chromosome 7 (9.5), while the lowest number of alleles was detected at chromosomes 3 and 4 (5.0 and 5.3). The smallest genetic distance characterized genotypes Super Zitarka and Zitarka, Tena and Osjecanka, Tena and Bezostaja, Lela and Toras, Janica and Alka, Felix and Seka.Genotypes Pipi and Courtot showed the least genetic similarities with rest of the genotypes.

Wang Yajuan *et al.* (2012) conducted a study to assess genetic diversity among 19 *Triticum aestivum* accessions and 73 accessions of closely relate species was analyzed using simple sequence repeat (SSR) markers. Forty-four out of 497 SSR markers were polymorphic. In total 274 alleles were detected (mean 6.32 alleles per locus). The polymorphic information content (PIC) of the loci ranged from 0.3589 to 0.8854 (mean 0.7538). The D genome contained the highest mean number of alleles (6.32) followed by the A and B genomes (6.13 and 5.94, respectively). The correlation between PIC and allele number was significant in all genome groups (0.7540, 0.7361 and 0.7482 for A, B and D genomes, respectively). Among the seven homologous chromosome groups, genetic diversity was lowest in group 7 and highest in group 5. In cluster and principal component analyses, all accessions grouped according to their genomes were consistent with their taxonomic classification. Accessions with the A and D genomes were clustered into two distinct groups and AABB accessions showed abundant genetic diversity and a close relationship. *Triticum durum* and *T. turgidum* were clustered together, consistent with their morphological similarity. Cluster analysis indicated emmer is closely related to hexaploid wheat. Compared

with common wheat, higher genetic variation was detected in spelt, *T. aestivum* subsp. yunnanense and subsp. tibetanum. In addition, a close genetic relationship between *T. polonicum* and *T. macha* was observed.

Hanaa Mahdy Abouzied *et al.* (2013) conducted a study SSR-based genetic diversity assessment in tetraploid and hexaploid wheat populations. Molecular analysis for a set of hexaploid and tetraploid (*Triticum durum*) wheat cultivars was investigated by applying 11 SSR primers *Triticum aestvium* The plant materials consisted of 45 genotypes 15 of which were *Triticum aestivum* and 30 of T. durum obtained from four different regions Egypt, Greece, Cyprus and Italy. The analysis of population structure revealed that genetic diversity within populations (Hs=0.2761) represented 97.7% of the total genetic diversity (HT=0.2827). The proportion of the total genetic diversity that was attributed to the population differentiation was low (Gst=0.0233) within population. ANOSIM (Analysis of Similarities), results showed that R was equal to 0.9048 ($P<0.0001$) indicated that all the most similar samples of genotypes are within the same population. The wheat varieties from the four distinct regions were clustered according to SSR data into two main clusters, durum wheat varieties and bread.

Munir Ahmad *et al.* (2013) worked out a study on morphological and molecular genetic variation in wheat for salinity tolerance at germination and early seedling stage. The present study was conducted to evaluate the level of genetic diversity among 172 (123 Pakistani and 49 exotic) wheat genotypes for salinity tolerance at germination and early seedling stage. Genetic similarity coefficients based on RAPD marker data ranged from 0.38 to 0.95. RAPD primer OPA 2 produced a unique fragment of 1000 bp, whereas OPF 13 generated two fragments of 1200 bp and 1400 bp only in some tolerant genotypes. Genetic similarity coefficients for SSR markers ranged from 0.45 to 0.95. Both RAPD and SSR markers revealed genetic variation in the studied genotypes.

Rekha Malik *et al.* (2013) studied genotypic characterization of elite Indian wheat genotypes using molecular markers and their pedigree analysis. Forty eight elite wheat genotypes including popular cultivars of India were analyzed using 56 SSR and 12 STS markers. Estimated values of coefficient of parentage (COP) for the pair-wise combinations of 48 genotypes ranged from 0.0 to 0.58 with an average of 0.047. The average number of alleles per locus was 2.42 and average polymorphism information content of 0.469.

Apoorva Arora *et al.* (2014) studied the population structure and genetic diversity among Indian wheat varieties using microsatellite (SSR) markers. The current study was performed to assess the status of genetic diversity among 319 Indian wheat varieties. Out of 30 markers applied, 50 polymorphic fragments generated from16 polymorphic primers were selected for diversity studies. The total genetic diversity for all groups (Ht) was 0.38888 and within groups (Hs) was 0.3130 indicating less genetic variability among sub-populations (Gst=0.19). Gene flow (Nm) was also found to be relatively high with a value of 2.0654. Genetic variability parameters computed using POPGENE 1.32 (81%) and AMOVA (78.5%) revealed that the genetic diversity was mainly contained within the populations.

Hakki *et al.* (2014) worked out a study on molecular and elemental characterization of selected Turkish durum wheat varieties. In this study, the extent of diversity among 5 Turkish durum wheat cultivars and their populations has been assessed using 7 microsatellite markers and the elemental analysis together with the differences in their protein content. In molecular analysis, total 23 alleles have been obtained among all the genotypes with middling of 4.6 per primer. As a result of AMOVA performed, the extent of diversity was found to be higher among the genotypes (76%) in comparison to the variability within the genotypes (24%).

Hamdalla (2014) assessed the degree of genetic divergence among 16 wheat bread using SSR Indicators. The present study was

conducted at Nebraska University, Field Crop & Horticulture Department during summer 2013. Ten selected simple sequence repeat (SSR) marker sets were evaluated in 16 accessions of bread wheat (*Triticum aestivum*), 12 of which have been introduced from Mexico, two from USA and two were local genotypes (Latifya and Ibah 95).All the 10 markers were polymorphic and produced 45 alleles (average 4.5). The gmw480 marker had higher percentage of frequency, while xgwm132 and gmw32 markers had lowest percentage of frequency, therefore they showed high efficiency in categorizing the studied genotypes. The PIC values were 0.32 for gmw480 and 0.99 for xgwm132 & gmw32 and gmw 32 markers (average 0.86). The dissimilarity coefficient (diversity) ranged between 0.2 for BW3-9830& BW3-9832 genotypes and 1.0 for many genotypes (average 0.80). The highest dissimilarity coefficient for the introduced genotypes was 0.97 for genotype BW49398 while the lowest value was 0.71 for genotype CW15-6732.

Meriam Nefzaoui *et al.* (2014) studied molecular diversity in Tunisian durum wheat accessions based on microsatellite markers analysis. In this study, they identified microsatellites from A and B genomes of bread wheat (*Triticum aestivum* L.) that are useful for the identification, characterization and genetic relationship estimation of durum wheat varieties and landraces. These markers amplified a total of 24 alleles, with a number of alleles per locus varying from 2 (for Xpsp 2999, Xgwm 193 and Xgwm 130) to 3 (for Xgwm 136, Xgwm 389, Xgwm 610, Xgwm 493, Xgwm 273 and Xgwm 89) and an average of 2.667. The Polymorphism Information Content (PIC) varied between 0.1103 for Xgwm193 to 0.556 for Xgwm493, with an average value of 0.363. Average genetic diversity among the accessions was 0.422.

Wang Li Xin *et al.* (2014) carried out a study to establish method and procedure of wheat variety stability assessment using SSR markers as a replacement for morphological observations. In preliminary study, the methods to identify non-homozygous SSR loci and calculate the homozygous SSR loci ratio (SSR–HLR) of wheat varieties were established. On this basis, the genotypes at 347 molecular markers loci of

20 advanced lines demonstrated that SSR– HLRs were 84.7–94.8 % in the F4 lines, 96.1–99.4 % in the F5 lines, and C98 %in the F6 lines, respectively. Eighty of 347 markers with good polymorphism, stable PCR amplification, and high resolving power of genotyping were recommended to detect SSR–HLR for 633 wheat regional trial varieties. Comparing morphological observation, the varieties with SSR– HLR[95 % were deemed stable; the varieties with SSR–HLR\91 % were deemed unstable; the varieties with SSR–HLR ranging from 91 to 95 % were required field identification for stability assessment. Based on the relationship between the homozygous SSR loci ratio and wheat variety stability, procedures for the assessment of wheat variety stability using SSR markers were established.

Mustafa Erayman *et al.* (2015) worked on diversity analysis of genetic, agronomic, and quality characteristics of bread wheat (*Triticum aestivum* L.) cultivars grown in Turkey. A total of 24 wheat cultivars and 5 wild progenitors of wheat were examined using 24 simple sequence repeat (SSR) primers with a known physical locus on the A, B, and D genomes of hexaploid wheat. A total of 72 bands produced 939 alleles on the wheat cultivars and wild progenitors. Markers were efficient in discriminating the species and the highest genetic diversity information was obtained from the markers Xgwm312 andXgwm372.

Sania Ahmad *et al.* (2014) evaluated forty two elite Indian wheat genotypes representing different agro-climatic zones of India using set of sixteen STS and 36 SSR markers for assessing genetic diversity. In total, 86 alleles were detected and polymorphism information content (PIC) ranged from 0.09 (Xwmc227) to 0.99 (Xgwm165) with a mean of 0.56, indicating sufficient discrimination by markers. Among the seven homologous chromosome groups, GD was highest in group 4 followed by lowest in group 3 and 6. Average number of allele were highest in B-genome (2.6) followed by the A- and D- genomes (2.3 and 2.1, respectively). Cluster analysis based on SSRs and quality protein markers identified three distinct clusters. Principal component analysis (PCA) obtained from molecular data shows that the first two components justified

38.04% of total variance. Further, these elite lines were also characterized for HMW using SDS-PAGE. For the Glu-1 loci, 10 alleles were detected, 3 at the Glu-A1 locus, 5 at the Glu-B1 and 2 at the Glu-D1. The frequency range in 10 HMW-GS combination was 3.2% to 74.2% in the advanced lines. At the Glu-D1 locus, the subunits 1Dx5+1Dy10 was present in 74.2% of the lines followed by the subunit 1Dx2+1Dy12 with a frequency of 25.8%. The subunit 1Dx5+1Dy10 was predominantly observed in these advanced lines having a stronger effect on bread making quality. This study provides evidence about genetic divergence and distinctness including quality traits in elite Indian wheat genotypes evaluated under the umbrella of All India Coordinated Wheat and Barley Improvement Programme and serve as new source of genetic variation for wheat improvement.

CHAPTER III

MATERIAL AND METHODS

The present investigation entitled as "Stability and Molecular Diversity Studies for Yield and It's Contributing Traits in Wheat" was carried out during *rabi*, 2014-2015, in all sixteen genotypes were evaluated at three environments with common planting date 13th November, 2015.

1. Wheat Research Unit of Dr. Panjabrao Deshmukh Krishi Vidhyapeeth, Akola. Geographical situation is 20.42°N latitude and 77.02°E longitude and 307.4 meters above mean sea level.
2. Agricultural Research station, Mahatma Phule Krishi Vidyapeeth, Niphad Dist. Nashik. Geographical situation is latitude 20.07°N and 74.10°E longitude and 569 m. above sea level.
3. Wheat Research Unit, Vasantrao Naik Marathwada Krishi Vidhyapeeth, Parbhani. Geographical situation is at 19.08°N latitude and 76.5°E longitude and 347 m above sea level.

The details of the materials used and methods adopted during the course of investigation are described as under.

Table3.1: The genotypes selected for the study

Sr. no	Genotypes	Source	Important features
1	AKAW -4739	Dr. PDKV, Akola	grain spike^{-1}
2	AKAW- 4798	Dr. PDKV, Akola	grain spike^{-1}
3	AKAW- 4800	Dr. PDKV, Akola	grain spike^{-1}
4	PBN- 4876	M.K.V. Parbhani	Effective tillers plant^{-1}
5	PBN -4881	M.K.V. Parbhani	Spikelets spike^{-1}, grain spike^{-1}
6	NIAW -2495	M.P.K.V., Rahuri	Spike length (cm)
7	NIAW -2539	M.P.K.V., Rahuri	Spikelets spike^{-1}
8	NIAW -2595	M.P.K.V., Rahuri	Test weight (g)
9	AKAW -3722 (C)	Dr. PDKV, Akola	Early maturity
10	MACS- 6478 (C)	A.R.I. Pune	Test weight (g)

11	NIAW -301 (C)	M.P.K.V., Rahuri	Effective tillers plant^{-1}
12	AKDW -4525	Dr. PDKV, Akola	Test weight (g)
13	PBND- 4825	M.K.V. Parbhani	Test weight (g)
14	PBND -5175	M.K.V. Parbhani	Test weight (g)
15	NIDW -0950	M.P.K.V., Rahuri	Grains spike^{-1}
16	NIDW- 295 (C)	M.P.K.V., Rahuri	Effective tillers plant^{-1}, Spikelets spike^{-1}, Grains spike^{-1}, Test weight

3.1.1 Layout of experiments-

Table 3.2: The details of experiment are given in below:

1. Experimental design	: Randomized Block Design		
2. Number of replication	: 3		
3. Number of genotypes	: 16		
4. Plot size	: Gross: 6.0 × 2.4 sq.m.= 14.40 sq.m : Net : 6.0 × 2.0 sq.m =12.00 sq.m		
5. Number of row per plot	: Gross : 12 rows : Net :10 rows		
6. Spacing (Row to Row)cm	: 20 cm		
7. Fertilizer Dose	: 120:60:60 Kg N, P_2O_5 and K_2O ha^{-1}. • Basal dose at the time of sowing 60:60:60 N_2,P_2O_5,K_2O Kg ha^{-1} • Top dressing of 30 Kg N after 21 and of 30 kg N after 42 of DAE		
8. Locations / Date of sowing	Akola	Parbhani	Niphad
	13/11/2014	13/11/2014	13/11/2014

3.1.2 Observations to be recorded

The observations for yield and its contributing traits were recorded on randomly selected five competitive plants in each genotype and in each replication as described below and were averaged to represent the treatment mean per replication.

1. Germination %

Germination % was observed when all wheat seeds sown were emerged out and the number of seedlings were counted and compared to total number of seeds sown and then the germination % was worked out.

2. Days to 50% flowering

Days to 50 per cent flowering (days), when flower opening was observed in the spikes of about 50 per cent plants in a plot, the date was recorded and number of days from the sowing date were counted as days required for 50 per cent flowering.

3. Days to maturity

When the grains in the spikelets becames hard and easily be removed from the glumes, that observation was recorded as maturity. The date was recorded and number of days from the sowing date were counted as days required for maturity.

4. Plant height (cm)

The plant height of randomly selected five plants was measured in centimeters from ground level to the top of the main tiller and sub tillers excluding awns at the time of harvest. Per plant mean height in each treatment per replication was worked out.

5. Effective tillers plant^{-1}

Number of tillers per plant were counted from five plants and their mean was worked out as number of effective tillers per plant and are recorded in each treatment and each replication.

3.3 Statistical analysis

The mean values were worked out from the measurements recorded on randomly selected five plants for different characters used for following statistical analysis-

3.1.3 Analysis of Variance

Environment wise Analysis of Variance was carried out as per the standard method given by Panse and Sukhatme (1967) in order to partition the total variation of different characters under study into its components viz., blocks, treatment and error. The details are given in the following table.

Table 3.3 Analysis of Variance

Sources of variation	d.f.	S.S.	M.S.S. expected
Replications (Blocks)	(r-1)	SSr	$M_1 = MSSr = (\sigma^2 e + g \sigma^2 r)$
Treatments(Genotypes)	(g-1)	SSg	$M_1 = MSSg = (\sigma^2 e + r \sigma^2 g)$
Error	(r-1)(g-1)	SSe	$M_3 = MSSe = (\sigma^2 e)$

3.1.4 Stability Analysis

Model of Eberhart and Russell (1966)

Eberhart and Russell model is an improvement upon the model of Finley and Wilkinson (1963). According to this model, the regression of the variety mean on environmental index and a function of the squared deviations from this regression would provide estimates of the desired stability parameter. The term stable variety has been used for a variety that performs above average in all environments. Hence the stable variety has high mean (Xi), unit regression ($b_i \approx 1.0$) and the deviation from the regression line should be as small as possible ($S^2d_i \approx 0$).

The analysis is based on the mathematical model as

$$Yij = \mu i + bilj + \delta ij$$

Where

Yij is the mean of the ith variety (i = 1 to g) at the j^{th} environment (j = 1 to n).

μi is the overall mean of the i^{th} variety over all environments.

bi is the regression of the i^{th} variety to varying environments.

Ij is the environmental index obtained as the mean of all varieties at the j^{th} environment minus the grand mean.

δij is the deviation from the regression of the i^{th} variety in the environment.

$$Ij = \frac{Y.j}{g} - \frac{Y..}{gn} \qquad \Sigma jIj = 0$$

a) Environment (linear) SS for 1 degree of freedom.

$$\text{Environment (linear) SS} = \frac{1}{g} (\Sigma j\, Y.j\ Ij)^2 / \Sigma jI^2j$$

b) G x Env. (linear) item equals to regression SS and is calculated in the same way

$$G \times E \text{ (linear)} = \Sigma i\, [\, (\Sigma j\, Yij\, Ij)^2 / \Sigma jI^2j$$

C) Pooled deviations sum of square is obtained either by subtraction of a and b items from the item c or by summing all the remainder sum of squares of individual lines.

Table3.4: Analysis of variance of multi-environmental data when stability parameters are estimated following Eberhart and Russell (1966) model.

Sr.No.	Sources	d.f.	SS	MS	F
1	Total	(gn -1)	$\Sigma i \Sigma j Y^2 ij - \frac{Y^{2..}}{Gn}$	MS_2	MS_2/MS_4
2	Genotypes	g -1	$\Sigma i Y^2 i./n - \frac{Y^{2..}}{gn.}$		
3	Environments + G x Env.	(n -1)+ (g-1)(n-1) =g(n-1)	$\Sigma i \Sigma j Y^2 ij - 1/n \Sigma i Y^2 i.$		
A	Env. (linear)	1	$\frac{1}{g} (\Sigma j Y.j \; lj)^2 / \Sigma j \; l^2 j$ or $\frac{1}{g}(SP^2)/SSEnv$ Indexes.		
B	G x E (linear)	g-1	$\Sigma i[(\Sigma j \; Yij \; lj)^2 / \Sigma j \; l^2 j] -$ Env. (Linear)SS	MS_3	MS_3/MS_4
C	Pooled deviations	g (n-2)	$\Sigma i \; \Sigma j \; \delta ij = \Sigma i[\Sigma j \; Y^2 ij - Y^2 i.] - (\Sigma j \; Yij \; lj)^2 / \Sigma j \; l^2 j \quad n$	MS_4	MS_4/MS_5
	Genotype 1	n-2	$[\Sigma j \; Y_1{}^2 j - \frac{Y^2_{1.}}{n}] - \Sigma j \; Y_1 j \; lj)^2 / \Sigma j \; l^2 j = \Sigma j \; \delta_1 j$		
	Genotype g	n-2	$[\Sigma j \; Y^2 gj - \frac{Y^2_{g}}{n}] - (\Sigma j \; Ygj \; lj)^2 / \Sigma j \; l^2 j = \Sigma j \; \delta gj$		
4	Pooled error	n(r-1)(g-1)	Pooled replications X genotypes SS over environments= M`e	MS_5= M`e	

g =number of genotypes

n = number of environments

r = number of replications

l =environmental index

Yij =basic observation, i.e. mean of the ith variety over replications

In the jth environment.

M`e = pooled $\sigma^2 e/r$

The regression for individual genotypes proceeds exactly in the same manner as for Finley and Wilkinson (1963) analysis.

To illustrate, the regression coefficient of genotype 1 is obtained as:

$b_1 = \Sigma j\ Yij\ Ij / \Sigma j\ I^2 j$

The appropriate F- tests have to be performed, since there is no item exclusively attributable to G x E, the genotype MS has been tested against the pooled deviation MS. This test approximates to the test of genotype MS against G x E MS. The G x E (linear) item is equivalent to regression MS in Finley and Wilkinson model and represents the differences among linear regressions. This item is significant at $P \leq 0.001$ against pooled deviation and both these are significant against pooled error.

3.2 Molecular diversity studies

The present study entitles "Stability and Molecular Diversity Studies for Yield and It's Contributing Traits in Wheat" has some aspects on molecular breeding as – (To characterize the genotypes for genetic diversity and to identify the variation among them using SSR markers.) Which were undertaken in the Molecular Breeding Laboratory of Biotechnology centre, Department of Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

3.2.1 List of instruments used

The various instruments used in the present study are listed below

i. Autoclave
ii. Deep freezer
iii. Digital Water Bath
iv. Gel documentation system
v. Horizontal Electrophoresis Unit
vi. Ice flaking machine
vii. Magnetic stirrer

viii. Microwave oven

ix. Programmable Thermal Cycler

x. Pipettes

xi. pH meter

xii. Refrigerated centrifuge

xiii. Vertical Electrophoresis Unit

xiv. Vortex Stirrer

xv. Water purification system

3.2.2 Chemicals and reagents

Chemicals used for genomic DNA extraction, polymerase chain reaction and electrophoresis are listed in Table 3.5.

Reagents:

1. 10 µM SSR primers
2. 5units/µl $^{-1}$ *Taq* DNA polymerase
3. 10X *Taq* DNA polymerase buffer with KCl
4. 25 mM $MgCl_2$
5. 10 mM dNTPs

Table 3.5. List of chemicals used for the molecular analysis

Chemical	Molecular Formula	Molecular Weight (g/mol)	Company
B-mercaptoethanol	C_2H_6OS	78.13	HiMedia
6x loading Dye			Fermentas
Acetic acid	CH_3COOH	60.05	Qualigens
Acrylamide	C_3H_5NO	71.08	HiMedia
Agarose-moderate EEO			Amresco
Ammonium persulphate	$(NH_4)_2\ S_2O_8$	228.2	Qualigens
Bis-Acrylamide	$C_7H_{10}N_2O_2$	154.17	SDFCL

Boric acid	H_3BO_3	61.84	HiMedia
Bromophenol blue	$C_{19}H_9BR_4O_5Sna$	0.25%	Fermentas
Chloroform	$CHCL_3$	119.38	Qualigens
CTAB (Cetyl trimethyl ammonium bromide)	$C_{19}H_{42}NBr$	364.45	Amresco
EDTA disodium salt	$C_{10}H_{14}K_2N_2O_8.2H_2O$	372.2	HiMedia
Ethanol	C_2H_5OH		Omnis
Ethidium bromide	$C_{21}H_{20}N_3Br$	394.32	HiMedia
Formaldehyde	CH_2O	30.02%	SDFCL
Isoamyl alcohol	$(CH_3)_2$ $CHCH_2CH_2OH$	88.15	Qualigens
Isopropyl alcohol	C_3H_8O	60.10	Rankem
Silver nitrate	$AgNO_3$	168.87	HiMedia
Sodium chloride	NaCl	58.44	HiMedia
Sodium hydroxide flakes	NaOH	40.00	HiMedia
Sterile distilled water	H_2O	18	
TEMED	$C_6H_{16}N_2$	116.21	Amresco
Tris buffer (hydroxymethyl methylamine)	$NH_2.C(CH_2OH)_3$	121.14	Qualigens
Tris hydrochloride	$(C_4H_{11}NO_3HCl)$	157.6	HiMedia
Tris, Free base	$C_4H_{11}NO_3$	121.14	HiMedia
Urea	NH_4CONH_2	60.06	HiMedia
Xylene cyanol	$C_{25}H_{27}N_2O_6S_2Na$	538.6	HiMedia

3.2.3 Buffers and solutions

Solutions: Different solutions used along with their composition are given in Table 3.6.

Table 3.6. Composition of different solutions

Sr. No.	Solutions	Composition
1.	10% Polyacrylamide	Urea 100 g, Acrylamide 95 g, Bis acrylamide 5 g, 10 x TBE 100 ml, distilled water 700 ml (Total Vol. 1 lit.)
2.	10% APS	0.1 g Ammonium per sulphate was dissolved in 1 ml distilled water
3.	0.5 M EDTA Na_2	18.61 g Disodium Ethylene Diamine Tetra Aceatate was dissolved in 100 ml distilled water by stirring vigorously and pH was adjusted to 8.0 with NaOH. Solution was sterilized by autoclaving.
4.	Ethidium Bromide	1 g Ethidium bromide was added to 100 ml of distilled water and stirred for few hours to ensure that the dye has dissolved. The container was wrapped in aluminum foil and stored at 4°C.
5.	5 M NaCl	29.2 g of NaCl was dissolved in 100 ml of distilled water and volume adjusted to 100 ml and solution was sterilized by autoclaving.
6.	1 M Tris HCl	15.76 g of Tris base was dissolved in 100 ml of distilled water by stirring vigorously and pH was adjusted to 8.0 by adding concentrated HCl. The volume was adjusted to 100 ml and solution was sterilized by autoclaving.
7.	Loading dye	6X loading dye(0.04 % bromophenol blue, 0.04% xylene cyanol FF, 5 % glycerol in water)
8	Chloroform Isoamyl Mixture (24 : 1)	96 ml chloroform was mixed with 4 ml of isoamyl alcohol
9	70 % Ethenol	70 ml of absolute ethanol was mixed with 30 ml sterile water

Buffers:

Table 3.7. Various buffers used along with their composition

Sr. No.	Buffers	Composition
1.	Extraction buffer	2 % w/v. CTAB,1.4 M NaCl,20 mM EDTA,100 mM Tris-Cl, 0.2 % β- mercaptoethanol (added at the time of use). The extraction buffer was autoclaved before addition of β - mercaptoethanol.
2.	10XTBE	0.9 M Tris base, 0.9 N Boric acid, 0.02 M EDTA.
3.	TE Buffer	10 mM Tris HCl (pH 8.0), 1 mM Na2 EDTA (pH 8.0)

3.2.3 Raising of seedlings

1) Seeds of each genotype grown in pots surface sterilized with 1 per cent (W/V) mercuric chloride and 70 per cent ethanol and then rinsed with deionized water and grown for two weeks.

2) Two gram fresh leaves of two week old germinated seedlings grown in field were used for DNA extraction.

3.2.4 Extraction of DNA

DNA was extracted using Cetyltrimethyl ammonium bromide (CTAB) protocol given by Sharma *et al.* (2002) with some modifications.

3.2.5 Protocol for DNA isolation

i. Two gram fresh leaf sample from five seedlings was grinded in liquid nitrogen to obtain fine powder.

ii. The powder was immediately transferred to 50ml polypropylene centrifuge tubes containing 15 ml pre warmed (60°C) extraction buffer and was mixed by inversion.

iii. The mixture was incubated for 60 min at 65^0C in hot water bath followed with intermediate shaking after every 10 min.

iv. 15 ml of Chloroform ($CHCl_3$): Isoamyl alcohol (24:1) was added and mixed gently but thoroughly to emulsify both the components.

v. Centrifugation was carried out at 8000 rpm for 10 min at room temperature.

The upper aqueous phase was transferred into a new 50 ml polypropylene centrifuge tubes with a wide bore pipette.

vi. 0.6 volume of ice-cold isopropanol was added and mixed by inversions. CTAB-DNA complexes formed a fibrous network.

vii. Alternately after mixing with isopropanol, the samples were centrifuged at 8000 rpm at room temperature for 15 min.

viii. After centrifugation a pellet was formed at the bottom of the polypropylene centrifuge tubes.

ix. The supernatant was removed and the pellet was washed with 70 per cent ethanol.

x. The pellet was air-dried for 30 minutes and then dissolved in 0.5 ml of TE buffer.

xi. The pellets were allowed to dissolve completely overnight at 4^0C without agitation.

3.2.5 Ribonuclease A treatment

RNA was removed by giving Ribonuclease A treatment. RNAse A (10 mg/ml) was added to the DNA sample @ 100 µg/ml and incubated at 37^0C for 1 hour.

3.2.6 DNA quantification

i. The DNA obtained after extraction was confirmed by running it on 0.8 per cent agarose gel containing ethidium bromide 10 µl (10 mg/ml for 100 ml) in a horizontal gel electrophoresis system.

ii. 2µl of genomic DNA of each genotype + 3µl loading dye + 5µl sterile water was loaded in the each well.

Iii. After completion of 5 cm run, the gel was observed under UV light and the DNA yield and quality was confirmed. After confirmation of the DNA yield extracted, it was quantified on spectrophotometer.

Procedure

i. One ml of TE buffer was taken in a cuvette and the spectrophotometer was calibrated at 260 nm as well as 280 nm wavelengths as control.

ii. For DNA quantification 2 to 5 µl of DNA sample was taken in 998 to 995 µl DEPC treated distilled water. The sample was mixed properly and the optical density was recorded (D) at both 260 and 280 nm.

iii. The DNA concentration was estimated employing the following formula.

$$\text{Amount of DNA } (\mu g/\mu l) = \frac{\text{(OD) 260 X 50 X dilution factor}}{1000}$$

iv. The quality of DNA was judged from the ratio of the OD values recorded at 260 and 280 nm.

3.2.7 PCR Amplification

Out of Various primers tried by various workers, different markers were selected for present investigation on the basis of its polymorphic nature in wheat species and molecular characterization work was carried out. The list of primers are given below-

Table 3.8. The list of identified SSR primer used for Amplification

Sr. No.	Primer Name	Sequence (5'-3')	Direction
1	Xgwm-130	AGCTCTGCTTCACGAGGAAG	F
		CTCCTCTTTATATCGCGTCCC	R
2	Xgwm-136	GACAGCACCTTGCCCTTTG	F
		CATCGGCAACATGCTCATC	R
3	Xgwm-193	CTTTGTGCACCTCTCTCTCC	F
		AATTGTGTTGATGATTTGGGG	R
4	Xgwm-493	TTCCCATAACTAAAACCGCG	F
		GGAACATCATTTCTGGACTTTG	R
5	Xgwm-610	CTGCCTTCTCCATGGTTTGT	F
		AATGGCCAAAGGTTATGAAGG	R

6	Xgwm18	GGA GTC ACA CTT GTT TGT GCA	F
		CAC TGC ACA CCT AAC TAC GTG C	R
7	Xgwm-133	ATCTAAACAAGACGGCGGTG	F
		ATCTGTGACAACCGGTGTGA	R
8	Xgwm-120	GATCCACCTTCCTCTCTCTC	F
		GATTATACTGGTGCCGAAAC	R
9	Xgwm-469	CAACTCAGTGCTCACACAACG	F
		CGATAACCACTCATCCACACC	R
10	Xgwm-325	TTTCTTCTGTCGTTCTCTTCCC	F
		TTTTTACGCGTCAACGACG	R
11	Xgmw-106	CTG TTC TTG CGT GGC ATTA AA	F
		AAT AAG GAC ACA ATT GGG ATG G	R
12	Xgwm-533	AAG GCG AAT CAA ACG GAA TA	F
		GTT GCT TTA GGG GAA AAG CC	R
13	Xgwm-389	ATC ATG ATC TCC TTG ACG	F
		TGC CAT GCA CAT TAG CAG AT	R
14	Xgwm-219	GAT GAG CGA CAC CTA GCC TC	F
		GGG GTC CGA GTC CAC AAC	R
15	Xgwm-459	ATG GAG TGG TCA CAC TTT GAA	F
		AGC TTC TCT GAC CAA CTT CTC G	R
16	Xgwm-508	GTT ATA GTA GCA TAT AAT GGC C	F
		GTG CTG CCA TGA TAT TT	R
17	Xgwm-518	AAT CAC AAC AAG GCG TGA CA	F
		CAG GGT GGT GCA TGC AT	R
18	XPSP-2999	TCCCGCCATGAGTCAATC	F
		TTGGGAGACACATTGGCC	R
19	XPSP-3000	GCA GAC CTG TGT CAT TGG TC	F
		GAT ATA GTG GCA GCA GGA TAC	R
20	gwm18	GTGAGGCAGCAAGAGAGAAA	F
		CAAAGCTTGACTCAGACCAAA	R
21	gwm 133	CAAATGGATCGAGAAAGGGA	F
		CTGCCATTTTTCTGGATCTACC	R

3.2.8 Preparation of reaction mixture for PCR

The PCR was carried out in PCR tubes, containing a reaction volume of 20 µl. The mixed tubes were kept in Thermal cycler (Applied

Biosystems Gene Amp PCR System 2700) with protocol. For PCR reaction, master mix was prepared as given in Table 3.9.

Procedure of preparation of reaction mixture for PCR:

1. Prepare and label a 300 µl micro centrifuge tube for the PCR cocktail of 18 reactions. This number of reactions is recommended when using 16 samples to accommodate pipetting error.

2. Defrost all components of the cocktail at room temperature, except the polymerase which has to be kept at −20°C at all times prior to use.

3. Prepare the PCR cocktail for 20 reaction volume by adding the components in order as listed below.

4. Vortex the mix and centrifuge at 1,000 × *g* force briefly.

5. Dispense 19 µl of the PCR cocktail in each PCR tubes using the same tip [replace tip occasionally (every 16 wells) to reduce pipetting error].

6. Add 1 µ l of the sample DNA (50 ng/ µl) to each tube. Use a fresh tip for each DNA sample.

7. Centrifuge the tubes at 1,000 × *g* force for 1 min.

9. Place the tubes into the thermo-cycling block, close it, and apply the appropriate PCR program.

Table 3.9. Master Mix for 1x of 20µl reaction

PCR Mix	1x	18x
10x *Taq* polymerase assay buffer with $MgCl_2$	2.5 µl	45 µl
$MgCl_2$	1 µl	18 µl
dNTPs (10 mM)	0.5 µl	9 µl
Taq polymerase (5 U/µl)	0.2 µl	3.6 µl
Sterile distilled water	13.8 µl	248.4 µl
Primer (Forward)	0.5µl	9 µl
Primer (Reverse)	0.5 µl	9 µl
Template DNA (50 ng)/ µl	1µl	
Total Reaction volume	20 µl	342 µl

Table 3.10 PCR reactions

PCR Stages		Parameters	Time
Initial Denaturation		94°C	5 min
35 cycles	Denaturation	94°C	30 sec
	Annealing	60- 62°C	45 sec
	Extention	72°C	1 min
Final Extension		72°C	10 min

After completion of the cycles samples were kept at 4°C till electrophoresis.

3.2.9 Separation of amplified product by Polyacrylamide gel electrophoresis

i. DNA amplification was carried out using one SSR primer pair at a time, as per PCR cycling regime.

ii. The composition of PCR reaction mixture is given in the Table 3.9.

iii. The amplified products were resolved on 10 per cent PAGE on a Vertical Gel Electrophoresis System.

3.2.9.1 Gel preparation

i. The glass plates were first washed with tap water and then with distilled water and air-dried. The dried plates were rinsed with methanol and again air-dried.

ii. The plates with spacers were assembled together with the help of clamps.

iii. 40 ml of 8 per cent PAGE was mixed with 300ml of 10 per cent freshly prepared APS and 35 µl volume of TEMED was added at the end and the solution was quickly poured between the glass plates (taking precaution not to introduce any air bubble) and the comb was inserted and the gel was allowed to polymerize for about 1 hour.

iv. After polymerization the comb was removed carefully and the wells of gel were carefully cleaned with the distilled water.

v. The gel plates were then mounted on the vertical gel electrophoresis system.

vi. Plates were allowed to pre run for about one hour.

vii. 20 µl of the amplified product loaded in each well and the electrophoresis was carried out on voltage of 70 V for 4.15 min.

viii. After electrophoresis, the gels were stained using silver staining (0.3gm Silver Nitrate)

3.2.9.2 Silver staining

i. To resolve the SSR products, the silver staining was carried out following with some modifications. Following steps were involved.

ii. After electrophoresis, the gel plates were carefully removed from the glass plates and transferred to a tray containing double distilled water and kept for 5 min with gentle shaking.

iii. The distilled water in the above tray was replaced with fixing solution containing 15 ml Methanol, 750 µl Glacial acetic acid and 135 ml double distilled water, kept for another five min with gentle shaking.

iv. Silver solution i.e staining solution was now poured in the tray. The silver solution was prepared by dissolving 0.3 g AgNo3 powder in 150 ml of 10 per cent Methanol solution with 750 µl Glacial acetic acid.

v. The solution was kept for 5 min with gentle shaking.

vi. The silver solution was removed from the tray and retained for further use; gel was rinsed for a while in distilled water.

vii. The gel was transferred to a developing solution (prepared by dissolving 4.5 g NaOH pellets in 150 ml distilled water with 450 µl of Formaldehyde.

viii. The solution in the tray was shaken gently for five to ten min allowing appear the DNA alleles.

ix. The staining was stopped by rinsing the gel for five min in the fixing solution which was retained after step IV.

x. The gel was placed on the platform of Gel documentation system and photographed under EPI white light.

3.6.6 Data scoring and analysis

1. DNA bands were scored which were formed in the range of expected amplified product size given for each particular primer.
2. Amplified bands were numbered according to their migration within the gel.
3. For each genotype, the presence or absence of each bands was determined and scored '1' if present and '0' if absent.
4. Similarities between any 2 genotypes were estimated according to Jaccard (1908) as.

The similarity of sample was calculated as-

$$S_{AB} = \frac{2\,N_{AB}}{N_A + N_B}$$

Where,

S_{AB} = The similarity index

N_{AB} = The number of bands shared by individuals A and B

N_A and N_B = The total number of bands in individual A and B respectively

5. Dendrogram was constructed based on the S_{AB} values by adopting the Sequential Agglomerative Hierarchical Non-overlapping (SAHN) clustering technique of unweighted pair groups method of arithmetic mean (UPGMA). These computations were performed using the statistical analysis package NTSYS-pc vr 2.02 i.
6. Polymorphic information content (PIC) refers to the value of a SSR marker for detecting polymorphism within a population. PIC depends on the number of detectable alleles and the distribution of their frequency. The value was calculated as,

$$PICi = 1 - \sum_{j=1}^{n} P_{ij}^2$$

Where,

PICi is the polymorphic information content of a marker i, P_{ij} is the frequency of the j^{th} pattern for marker i and the summation extent over n pattern.

7. The polymorphic percentage of the obtained bands was calculated by using following formula,

   Polymorphic % = (No. of polymorphic bands/ Total bands) X 100.

CHAPTER IV

RESULTS AND DISCUSSION

The present investigation entitled "Stability and Molecular Diversity Studies for Yield and It's Contributing Traits in Wheat" was carried out during *rabi*, 2014-2015 and results obtained are presented in the following sections.

4.1 Analysis of Variance

4.2 Mean performance of genotypes over environments

4.3 ANOVA for stability Analysis

4.4 Stability parameters

4.5 Molecular diversity

4.6 Correlation between genetic diversity and stability performance of genotypes

4.1 Analysis of Variance

The Analysis of Variance worked out for three environments Niphad, Parbhani and Akola is presented in table 4.1, 4.2 and 4.3.

The ANOVA for first environment i.e. Niphad, revealed that differences due to genotypes were highly significant for 10 characters out of 12 characters and two are moderately significant for germination per cent and straw yield kg plot^{-1}.

The ANOVA for second environment i.e. Parbhani, manifested that the genotypes differences for almost all the characters were found highly significant except germination per cent wherein, the differences were moderately significant.

The ANOVA for third environment, Akola proffessed that the genotype differences expressed in terms of genotypes mean sum of squares were found highly significant for the characters viz. days to 50 % flowering, days to maturity, plant height (cm), effective tillers plant^{-1}, tillers sq. meter^{-1}, spikletes spike^{-1}, spike length (cm), grains spike^{-1}, test weight (g), grain yield kg plot^{-1}, straw yield kg plot^{-1} however, the differences were moderately significant for germination percentage

Table 4.1 Analysis of variance for grain yield and it's contributing traits.

Sources	Df	Mean sum of squares (MSS)											
		Germination %			Days to 50 % flowering			Days to maturity			Plant height (cm)		
		Niphad	Parbhani	Akola	Niphad	Parbhani	Akola	Niphad	Parbhani	Akola	Niphad	Parbhani	Akola
Replications	2	6.250	8.333	10.938	0.146	1.750	0.083	1.750	0.396	0.083	0.298	0.573	1.161
Genotypes	15	10.799*	9.410*	8.750*	39.165**	65.194**	66.311**	47.954**	19.643**	23.194**	145.951**	52.781**	157.489**
Error	30	4.028	3.889	4.279	0.724	0.594	0.728	1.283	0.885	0.794	2.052	2.395	5.470

* Significant at 5% probability level, ** Significant at 1% probability level.

Table 4.2 Analysis of variance for grain yield and it's contributing traits.

Sources	Df	Mean sum of squares (MSS)											
		Effective tillers plant^{-1}			Tillers sq.m^{-1}.			Spikelets spike^{-1}			Spike length (cm)		
		Niphad	Parbhani	Akola	Niphad	Parbhani	Akola	Niphad	Parbhani	Akola	Niphad	Parbhani	Akola
Replications	2	0.190	0.079	0.047	45.646	110.333	218.896	0.188	0.063	0.333	0.070	0.097	0.031
Genotypes	15	0.728**	0.549**	1.058**	2933.110**	1973.017**	2719.165**	8.288**	5.954**	6.800**	6.796**	7.195**	6.983**
Error	30	0.114	0.135	0.127	242.601	311.600	324.140	0.521	0.463	0.778	0.039	0.127	0.038

* Significant at 5% probability level, ** Significant at 1% probability level.

Table 4.3 Analysis of variance for grain yield and it's contributing traits.

Sources	Df	Mean sum of squares (MSS)											
		Grains spike^{-1}			Test weight (g)			Grain yield kg plot^{-1}			Straw yield kg plot^{-1}		
		Niphad	Parbhani	Akola	Niphad	Parbhani	Akola	Niphad	Parbhani	Akola	Niphad	Parbhani	Akola
Replications	2	1.320	14.313	8.313	1.415	3.647	0.694	0.001	0.023	0.014	1.366	0.400	0.657
Genotypes	15	217.911**	80.554**	70.354**	33.867**	46.245**	36.847**	1.479**	0.709**	0.939**	2.292*	8.796**	6.529**
Error	30	5.100	17.046	13.046	2.337	2.166	0.731	0.069	0.022	0.053	0.874	0.488	1.165

* Significant at 5% probability level, ** Significant at 1% probability level.

4.2 Mean performance of genotypes in different environments

Data recorded on each of the morphological, yield and its contributing characters for sixteen genotypes of wheat over three environments were analyzed to find out the mean, range and environmental indices for different traits and are presented in Table 4.4

4.2.1 Germination percentage

Among three environments mean for germination percentage was maximum in Niphad (94.19) followed by Akola (91.86) and Parbhani (89.77). The range was observed same in all environments and environmental indices were ranged from -2.174 in Parbhani to 2.243 in Niphad.

4.2.2 Days to 50% flowering

Highest mean for days to 50% flowering was recorded in Niphad (67.27) followed by Parbhani (58.38) and Akola (54.33), maximum range was observed in Akola (43.33 to 66.67) and minimum in Niphad (60.00-70.67). Environmental indices were ranged from -5.660 in Akola to 7.278 in Niphad.

4.2.3 Days to maturity

Mean for days to maturity were recorded 119.81 days, 115.21 days, 114.52 days in Niphad, Parbhani and Akola respectively. Niphad recorded highest mean followed by Akola and Parbhani, while maximum range was observed in Niphad (112 -127) and minimum in Akola (111.33-119.33). Environmental indices were ranged from -1.993 in Parbhani to 3.299 in Niphad.

Table 4.4 Mean, range, environment index estimated for yield and it's contributed traits in wheat

Sr. No.	Character	NIPHAD			PARBHANI			AKOLA		
		Mean	Range	EI	Mean	Range	EI	Mean	Range	EI
1	Germination %	94.19	92-97	2.243	89.77	88-93	-2.174	91.88	90-95	-0.069
2	Days to 50% flowering	67.27	60-70.67	7.278	58.38	46-67	-1.618	54.33	43.33-67.67	-5.660
3	Days to maturity	119.81	112-127	3.299	114.52	110-119	-1.993	115.21	111.33-119.33	-1.306
4	Plant height (cm)	90.98	76.67-107.00	2.956	84.73	79.13-93.33	-3.290	88.36	79.90-105.80	0.335
5	Effective tillers plant^{-1}	3.70	2.30-5.30	0.278	3.26	2.66-4.27	-0.163	3.31	2.70-4.70	-0.115
6	Tillers sq. m.$^{-1}$	366.48	303.33-407.33	15.340	348.54	288-381	-2.597	338.40	307.33-377.33	-12.743
7	Spikelets spike^{-1}	15.31	12.33-18.33	0.319	14.81	12.33-16.67	-0.181	14.85	13-19	-0.139
8	Spike length (cm)	8.63	6.29-10.73	0.905	7.38	5.11-9.34	-0.342	7.16	5.02-9.14	-0.564
9	Grains spike^{-1}	54.06	36.45-74.22	8.750	43.69	27-60	-1.625	38.19	31.33-46	-7.125
10	Test weigh (g)	42.87	36.89-48.28	0.881	41.75	35.05-49.33	-0.239	41.35	30-51	-0.642
11	Grain yield kg plot^{-1}	3.97	3.10-5.16	0.758	2.84	2.07-3.72	-0.368	2.82	1.96-4.05	-0.390
12	Straw yield kg plot^{-1}	8.12	6.88-10.05	-1.103	9.78	7.37-13.68	-0.553	9.77	6.06-12.56	0.550

EI- environmental index

4.2.4. Plant height (cm)

Average plant height was maximum (90.98 cm) in Niphad followed by Akola (88.36 cm) and Parbhani (84.73 cm). The wide range for this character was observed in Akola (79.90-105.80) and narrow range was observed in Parbhani (79.13-93.33). Environmental indices were ranged from -3.290 in Parbhani to 2.956 in Niphad.

4.2.5 Effective tillers plant^{-1}

Out of three environments the average number of tillers plant^{-1} were maximum in Niphad (3.70) followed by Akola (3.31) and Parbhani (3.26) while maximum range was recorded in Akola (2.70 to 4.70) and minimum in Parbhani (2.66-4.27). Environmental indices were ranged from -0.163 in Parbhani to 0.278 in Niphad.

4.2.6 Tillers sq. meter^{-1}

Amongst three environments average number of tillers sq. meter^{-1} were maximum in Niphad (366.48) followed by Parbhani (348.54) and Akola (338.40). Similarly, maximum range was recorded in NIphad (303.33-407.33) and minimum in Parbhani (307.33-377.33). Environmental indices were ranged from -12.743 in Akola to 15.340 in Niphad.

4.2.7 Spikelets spike^{-1}

Out of three environments the maximum mean (15.31) was observed for number of spikelets spike^{-1} in Niphad and minimum in Parbhani (14.81), while maximum range was recorded in Niphad (12.33-18.33) and minimum in Parbhani (12.33-16.67). Environmental indices were ranged from -0.181 in Parbhani to 0.319 in Niphad.

4.2.8 Spike length (cm)

Highest mean for spike length was observed in Niphad (8.63 cm) and minimum in Akola (7.16 cm). Maximum (5.11-9.34 cm) and minimum range (5.02-9.14) were observed in Parbhani and Akola respectively. Environmental indices were varied from -0.564 in Akola to 0.905 in Niphad.

4.2.9 Grains spike^{-1}

Out of three environments maximum mean (54.06) for this character was observed in Niphad and minimum (38.19) in Akola. Similarly maximum (36.45-74.22) range was recorded in Niphad and minimum (31.33-46) in Akola. Environmental indices were ranged from -7.125 in Akola to 8.750 in Niphad.

4.2.10 Test weight (g)

This character exhibited maximum mean in Niphad (42.87) followed by Parbhani (41.75) and Akola (41.35). Maximum range was observed in Parbhani (35.05-49.33) and minimum in Niphad (36.89 - 48.28). Environmental indices were ranged from -0.642 Akola to 0.881 in NIphad.

4.2.11 Grain yield kg plot^{-1}

Highest grain yield kg plot^{-1} was observed in Niphad (3.97) followed by Parbhani (2.84) and Akola (2.82). Range for this trait was maximum in Niphad (3.10-5.16) and minimum in Parbhani (2.07-3.72). The environmental indices were ranged from -0.390 in Akola to 0.758 in Niphad.

4.2.12 Straw yield kg plot^{-1}

The average straw yield kg plot^{-1} was more (9.78) in Parbhani and less (8.12) in NIphad. While, maximum range was recorded (7.37 to 13.68) in Parbhani and minimum (6.88 to 10.05) in Niphad. Environmental indices were ranged from -1.103 in Niphad to 0.550 in Akola.

4.3 Stability Analysis

4.3.1 Analysis of Variance for stability

Analysis of Variance was carried out as per Eberhart and Russell Model for twelve characters to partition the total variation in genotype, environment, interaction of G X E and other sources.

Analysis of Variance for Stability

The ability of a genotype to produce a narrow range of phenotype in different environments can be called as stable (Lewis 1954).

The genotype will be stable in the absence of the environmental influences as well as genotype X environment interaction.

Eberhart and Russell Model of stability analysis was used for the assessment of environmental influence and genotype X environment interaction influence on genotypes for each character. When genotype X environment interactions were significant for the characters, then partitioning of total sum of squares due to genotype X environment interaction into predictable and unpredictable sources of variation was done using the procedure provided by Eberhart and Russell (1966).

Analysis of Variance (Table 4.5) for stability proffessed that the genotypic differences pooled over three environments were significant for germination per cent, days to 50% flowering, days to maturity, plant height (cm), effective tillers plant^{-1}, tillers sq.meter^{-1}, spikelets spike^{-1}, spike length (cm), grains spike^{-1}, test weight (g), grain yield kg plot^{-1} and straw yield kg plot^{-1}.

Environment and (G × E) differences are highly significant for germination per cent, days to 50 per cent flowering, days to maturity, spike length (cm), grains spike^{-1}, test weight (g), grain yield kg plot^{-1} and straw yield kg plot^{-1}. While for the character tillers sq.meter^{-1}, environment and (G × E) differences are moderately significant.

Mean sum of squares due to environment alone are highly significant for germination percentage, days to 50 per cent flowering, days to maturity, tillers sq.meter^{-1}, spike length (cm), grains spike^{-1}, grain yield kg plot^{-1} and straw yield kg plot^{-1}. While mean sum of squares due to environment for plant height (cm), effective tillers plant^{-1}, and test weight (g) were found moderately significant and for spikelets spike^{-1} were non-significant.

While variation due to G × E interaction source are highly significant for days to maturity and grain yield kg plot^{-1} and are moderately significant for test weight (g) and straw yield kg plot^{-1} only and for rest of the characters difference due to G × E interactions are non-significant.

The environment linear component was found highly significant for 10 characters out of 12 which includes germination percentage, days to 50 per cent flowering, days maturity, plant height (cm), tillers sq. meter^{-1}, spike length (cm), grains spike^{-1}, test weight (g), grain yield kg plot^{-1}, straw yield kg plot^{-1} and for two characters viz. effective tillers plant^{-1} and spikelets spike^{-1} the environment linear component was found moderately significant.

While the G × E linear component highly significant for days to maturity, test weight (g), grain yield kg plot^{-1}, straw yield kg plot^{-1} and moderately significant for grains spike^{-1}, the G X E linear component was found to be non-significant for rest of the characters.

Pooled deviation was found highly significant for days to 50 % flowering, plant height (cm), effective tillers plant^{-1} and grains spike^{-1} while moderately significant for test weight (g). Remaining traits viz. germination per cent, days to maturity, tillers sq.meter^{-1}, spikelets spike^{-1}, spike length (cm), grain yield kg plot^{-1} and straw yield kg plot^{-1} (kg) the pooled deviation was found non-significant.

Table 4.5 Analysis of Variance for stability analysis

Sources	DF	Germination %	Days to 50% flowering	Days to maturity	Plant height (cm)	Effective tillers plant^{-1}	Tillers sq. m^{-1}	Spikelets spike^{-1}	Spike length (cm)	Grains spike^{-1}	Test weight (g)	Grain yield kg Plot^{-1}	Straw yield kg plot^{-1}
		1	2	3	4	5	6	7	8	9	10	11	12
Rep within Env.	6.00	2.84	0.22	0.25	0.23	0.04	41.65	0.06	0.02	2.66	0.64	0.00	0.27
Genotypes	15.00	7.65 **	51.09**	21.67 **	83.09 **	0.62**	2440.73 **	6.55**	6.92 **	49.94 *	27.48 **	0.35 **	4.56 **
Env.+ (G*X E)	32.00	5.82**	46.53 **	12.31 **	26.55	0.14	249.59 **	0.29	0.66 **	99.21 **	6.01 **	0.75 **	1.53 **
Environments	2.00	78.09**	700.93 **	132.46 **	157.41 **	0.94 **	3235.64 **	1.23 *	10.03 **	1039.84 **	9.95 **	6.90 **	14.60**
G*X E	30.00	1.00	2.90	4.30 **	17.82	0.08	50.52	0.23	0.04	36.50	5.75 **	0.35 **	0.66 **
Environments (Lin.)	1.00	156.17 **	1401.87**	264.92 **	314.81**	1.88**	6471.28 **	2.46 **	20.07**	2079.68**	19.91 **	13.80**	29.20**
G*X E.(Lin.)	15.00	0.66	3.39	8.13 **	16.22	0.03	36.20	0.18	0.04	51.31 *	10.21 **	0.68 **	1.15 **
Pooled Error	90.00	1.35	0.23	0.33	1.10	0.04	97.59	0.20	0.02	3.91	0.58	0.02	0.28
Pooled Deviation	16.00	1.26	2.25 **	0.43	18.21 **	0.13 **	60.78	0.27	0.03	20.33 **	1.21 *	0.01	0.16
Total	47.00	6.40	47.98	15.30	44.59	0.29	948.89	2.29	2.66	83.48	12.87	0.63	2.50

*Significant at 5% probability level, **Significant at 1% probability level

4.4 Stability parameters

The three stability parameters viz., mean, regression coefficient (bi) and deviation from linear regression line (s^2di) were estimated for twelve characters and the results obtained are presented in Table 4.6, 4.7 and 4.8.

4.4.1 Germination percentage

Mean value and stability parameters for this trait are presented in Table 4.6.

Out of sixteen genotypes PBN-4881 and AKDW-4525 had highest germination percentage (94.44%) while three genotypes AKAW-4798, AKAW-4800 and NIAW-2495 had lowest germination percentage (90.00% each) and mean over three environments was (91.94%). The genotypes AKAW-4739, AKAW-4798, AKAW-4800, PBN-4881, NIAW-2495, NIAW-2539, NIAW-2595, MACS-6478, NIAW-301 and PBND-5175 exhibited regression values lesser than unity. The deviation from regression line was significant for the genotype NIAW-301. Considering three parameters together; mean, regression value and deviation from regression value, genotype NIAW-2595 (Mean 92.78, bi 0.917, s^2di 0.200), PBND-5175 (Mean 92.22, bi 0.893, s^2di 0.619) and PBN-4881 (Mean 94.44, bi 0.917, s^2di 0.200) exhibited better stability in all the environments for germination percentage.

4.4.2 Days to 50% flowering

Genotype NIDW-295 required maximum number of days to 50% flowering (67.00), while AKAW-3722 required minimum number of days to 50% flowering (50.33) followed by AKAW 4798 (53.11) as compared to the mean over three environments. (59.99).

The genotypes AKAW-4800, PBN-4876, PBN-4881, NIAW-2539, AKDW-4525, PBND-4825, PBND-5175 and NIDW-295 recorded regression value lesser than unity. The regression values are significant for genotypes AKAW-4739 (bi =1.187) and AKAW-4798 (bi =1.132)

Table 4.6 stability parameters estimated for wheat genotypes

Sr. No.	Genotypes	Germination %			Days to 50% flowering			Days to maturity			Plant height (cm)			Effective tillers plant^{-1}		
		Mean	Bi	s^2di	Mean	Bi	s^2di	Mean	Bi	s^2di	Mean	bi	s^2di	Mean	bi	s^2di
1	AKAW-4739	93.889	0.534	-1.043	60.000	1.187*	-0.217	115.000	2.404	0.169	87.643	1.221	28.667**	3.200	1.839	-0.019
2	AKAW-4798	90.000	0.905	-1.441	53.111	1.132*	-0.222	112.333	0.213	-0.187	88.996	1.714	59.047**	3.516	0.939	-0.041
3	AKAW-4800	90.000	0.905	-1.441	62.667	0.969	2.167**	118.444	-0.212	0.342	89.089	0.517	-0.385	3.400	0.755	0.041
4	PBN-4876	93.889	1.288	-1.121	62.222	0.978	-0.226	118.556	0.068	1.007*	85.460	1.575	3.430*	4.578	1.207	-0.012
5	PBN-4881	94.444	0.917	0.200	55.444	0.637	-0.127	116.000	1.302	-0.164	86.601	-0.100	11.394**	3.467	1.338	-0.036
6	NIAW-2495	90.000	0.905	-1.441	60.222	1.095	0.091	115.333	1.103	-0.233	85.256	1.149	46.230**	2.844	0.428	-0.040
7	NIAW-2539	91.111	0.522	-0.921	58.000	0.987	0.334	115.222	1.421	0.547	85.030	0.745	-0.525	3.267	0.226	0.168*
8	NIAW-2595	92.778	0.917	0.200	60.444	1.129	1.501**	114.444	0.757	0.046	87.736	1.220	11.039**	2.889	0.466	-0.008
9	AKAW-3722	90.556	1.288	-1.121	50.333	1.304	0.259	111.333	0.227	0.379	81.079	-0.664	0.337	3.758	1.473	-0.038
10	MACS-6478	91.667	0.905	-1.441	61.556	1.126	-0.115	115.889	1.328	0.099	82.149	-0.896	3.950*	3.579	0.718	0.077
11	NIAW-301	91.111	0.881	6.380*	59.889	1.129	0.607	117.222	0.837	-0.294	89.317	0.900	14.855**	3.689	0.913	0.044
12	AKDW-4525	94.444	1.276	-0.824	60.889	0.997	0.356	116.000	0.533	0.534	89.016	1.915	32.086**	2.979	0.715	-0.038
13	PBND-4825	91.667	1.300	2.284	61.556	0.908	21.901**	119.778	1.567	-0.043	100.573	2.165	4.238*	2.979	1.901*	-0.041
14	PBND-5175	92.222	0.893	0.619	64.111	0.895	6.080**	118.556	1.648*	-0.315	99.379	1.064	54.608**	3.379	0.546	0.049
15	NIDW-0950	90.556	1.288	-1.121	62.444	1.000	-0.180	118.444	1.197	0.037	84.178	1.395	3.735*	3.200	1.160	0.071
16	NIDW-295	92.778	1.276	-0.824	67.000	0.526	0.194	121.667	1.608	-0.227	86.877	2.082	1.919	4.042	1.377	1.173**
	Population Mean	**91.944**			**59.993**			**116.514**			**88.024**			**3.423**		

* Significant at 5% probability level, ** Significant at 1% probability level
Mean, bi = Regression coefficient, s^2di = Deviation from regression line

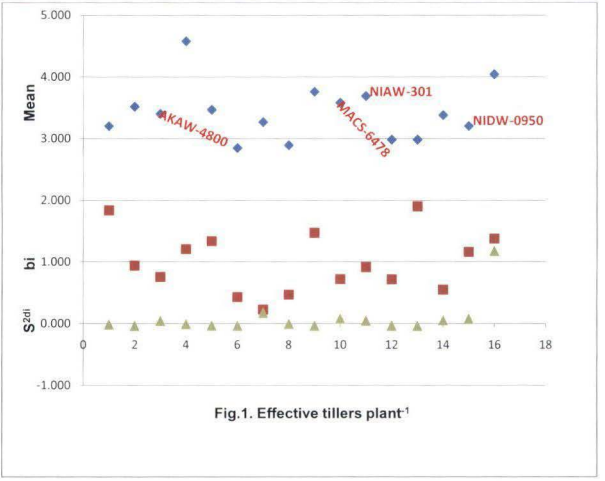


Fig.1. Effective tillers plant^{-1}

Table 4.7 stability parameters estimated for wheat genotypes

Sr. No.	Genotypes	Tillers sq.m.$^{-1}$			Spikelets spike^{-1}			Spike length (cm)		
		Mean	**bi**	**s^2di**	**Mean**	**bi**	**s^2di**	**Mean**	**bi**	**s^2di**
1	AKAW-4739	323.889	0.996	-85.945	16.556	0.692*	-0.187	8.262	1.257	0.061
2	AKAW-4798	369.111	0.754	-89.002	13.000	1.083	-0.146	7.172	1.315	-0.014
3	AKAW-4800	331.889	0.859	-89.870	16.556	2.556	0.214	9.508	1.017	0.038
4	PBN-4876	382.889	1.141	-83.767	15.889	0.301	-0.127	9.611	0.821*	-0.023
5	PBN-4881	373.333	1.059	-70.516	16.667	0.992	-0.117	8.551	0.655	0.107*
6	NIAW-2495	299.000	0.778	585.627**	14.111	0.602	0.053	8.900	0.992	0.133*
7	NIAW-2539	308.778	0.859	-93.147	16.111	1.865	0.240	8.972	1.012	-0.018
8	NIAW-2595	358.222	1.687	44.509	14.778	1.684	-0.106	9.012	1.184	-0.014
9	AKAW-3722	374.556	1.055	-94.045	13.222	-0.782	0.015	7.060	1.034	-0.021
10	MACS-6478	367.667	0.493	-79.760	15.000	1.895	0.815*	9.220	0.768	-0.012
11	NIAW-301	380.778	1.599	-45.404	14.889	1.474	-0.003	8.177	1.057	-0.019
12	AKDW-4525	333.000	1.169	-80.577	12.667	-0.902	0.354	5.651	1.069	-0.023
13	PBND-4825	368.889	0.903	-84.153	13.667	1.083	-0.146	5.541	1.042	-0.012
14	PBND-5175	309.333	0.865	-92.283	15.333	0.902	0.354	5.607	1.035**	-0.023
15	NIDW-0950	365.889	0.891	-91.722	13.889	1.383	-0.186	5.594	0.784*	-0.023
16	NIDW-295	371.000	0.893	-83.041	17.556	2.556	0.214	6.716	0.957	-0.022
	Population Mean	**351.139**			**14.993**			**7.722**		

* Significant at 5% probability level, ** Significant at 1% probability level
Mean, bi = Regression coefficient, s^2di = Deviation from regression line

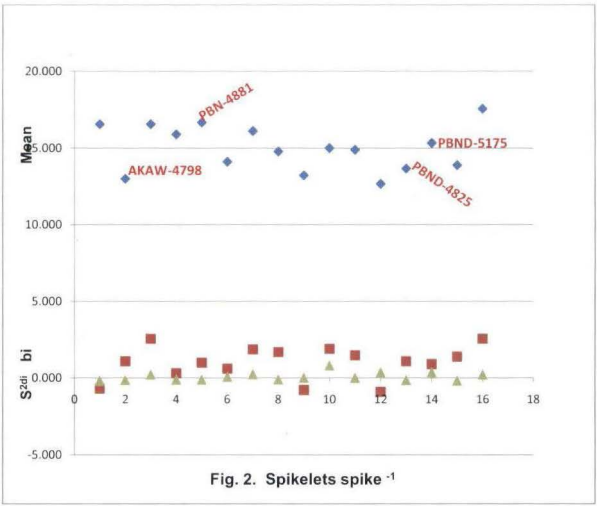


Fig. 2. Spikelets spike $^{-1}$

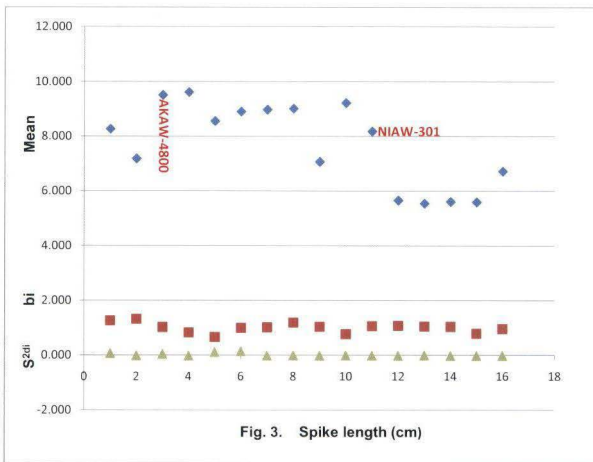


Fig. 3. Spike length (cm)

Table 4.8 stability parameters estimated for wheat genotypes

Sr. No.	Genotypes	Grains spike^{-1}			Test weight (g)			Grain yield kg plot^{-1}			Straw yield kg plot^{-1}		
		Mean	bi	s^2di	Mean	Bi	s^2di	Mean	bi	s^2di	Mean	bi	s^2di
1	AKAW-4739	54.297	2.210	0.668	38.984	5.664	1.978*	3.906	1.656	-0.002	9.200	0.148	-0.265
2	AKAW-4798	36.482	0.215	43.553**	42.306	3.682	-0.377	2.922	0.233**	-0.015	9.059	0.869	-0.274
3	AKAW-4800	50.444	-0.034	75.650**	39.726	-3.093	-0.298	3.264	0.040**	-0.015	10.894	1.984	-0.266
4	PBN-4876	45.444	1.353	50.243**	38.404	6.076	5.245**	2.968	2.380*	-0.014	8.096	0.788	-0.166
5	PBN-4881	48.260	0.781	2.304	37.226	-0.050	-0.520	3.201	1.699*	-0.013	7.904	0.658	-0.262
6	NIAW-2495	42.074	0.146	5.861	41.231	0.258	-0.086	3.292	-0.166**	-0.015	10.084	1.374	-0.272
7	NIAW-2539	43.704	0.800	21.813*	42.161	1.390	-0.498	3.647	0.389**	-0.015	7.242	0.289*	-0.277
8	NIAW-2595	44.592	1.058	3.165	48.406	0.148	0.919	3.444	1.506*	-0.015	8.546	0.421	-0.195
9	AKAW-3722	44.074	1.474	0.526	39.887	-0.612*	-0.573	3.197	1.928	0.001	7.294	0.379	-0.265
10	MACS-6478	44.037	1.152	32.349**	43.762	-4.276	4.346**	3.747	-0.294	0.019	9.923	1.133	-0.274
11	NIAW-301	44.556	0.720	-0.860	42.501	1.913	-0.253	3.222	-0.073*	-0.012	11.131	2.750	1.835**
12	AKDW-4525	43.002	1.050	25.719**	42.667	2.673	0.494	2.681	0.571**	-0.015	9.466	1.010	-0.206
13	PBND-4825	44.926	1.529	-3.349	45.143	1.519	-0.479	3.107	1.799*	-0.014	8.851	0.799	-0.260
14	PBND-5175	41.962	1.246	9.509	46.411	-2.666	0.707	3.047	1.582*	-0.014	8.898	0.071*	-0.277
15	NIDW-0950	49.148	1.896	-2.074	39.580	1.250	-0.371	2.739	0.926	0.034	9.962	2.478*	-0.256
16	NIDW-295	48.000	0.404	-1.039	43.493	2.124	-0.226	2.982	1.824	-0.011	10.991	0.849	-0.273
	Population Mean	**45.313**			**41.993**			**3.210**			**9.221**		

* Significant at 5% probability level, ** Significant at 1% probability level
Mean, bi = Regression coefficient, s^2di = Deviation from regression line

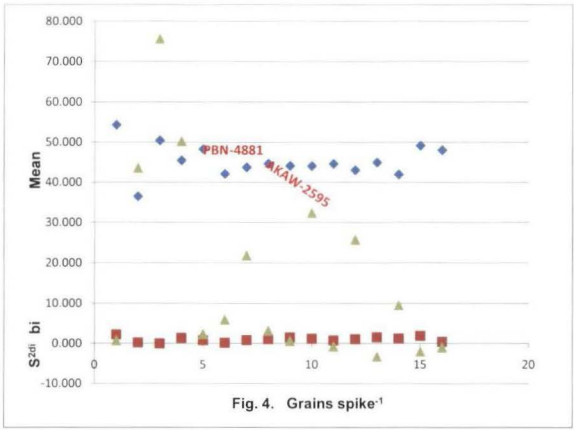


Fig. 4. Grains spike^{-1}

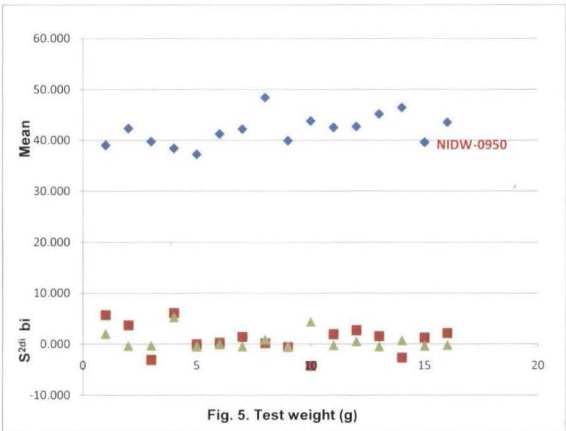


Fig. 5. Test weight (g)

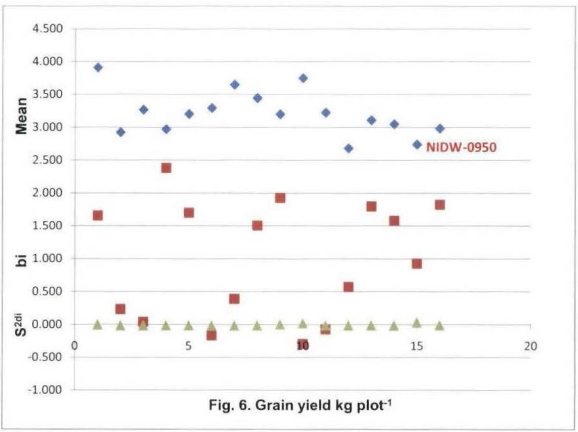


Fig. 6. Grain yield kg plot^{-1}

indicating their unpredictability for days to 50% flowering. The deviation from regression line are significant for the genotypes AKAW-4800, NIAW-2595, PBND-4825 and PBND-5175. According to the Eberhart and Russell model the genotypes NIDW-0950 (Mean 62.44, bi 1, s^2di -0.180) showed absolute stability because of required value is perfect unity. Genotypes, NIAW-2495 (Mean 60.22, bi 1.095, s^2di 0.091), NIAW-301(Mean 59.89, bi 1.129, s^2di 0.607) exhibited stability to favorable (rich) environment and PBN-4876 (Mean 62.22, bi 0.978, s^2di -0.226) exhibited most stable performance over the environments for the character days to 50 % flowering.

4.4.3 Days to maturity

Amongst sixteen genotypes NIDW-295 required maximum number of days to maturity (121.67) while genotypes AKAW-3722 required minimum number of days to maturity (111.33) as compared to the mean over three environments (116.51).

For the character days to maturity, genotypes AKAW-4798, AKAW-4800, PBN-4876, NIAW-2595, AKAW-3722, NIAW-301, AKDW-4525, recorded regression value lesser than unity. The regression value is significant in respect of PBND-5175 (bi=1.648) and deviation from regression line was significant for the genotypes PBN-4876 indicating their unpredictability for days to maturity. Considering the stability parameters together genotypes NIDW-0950 (Mean 118.44, bi 1.197, s^2di 0.037), NIAW-2495 (Mean 115.33, bi 1.103, s^2di -0.233) were found stable genotypes to favorable (rich) environment, while NIAW-301 (Mean 117.22, bi 0.837, s^2di -0.294) exhibited stability to unfavorable (poor) environment for days to maturity.

4.4.4 Plant height (cm)

The genotype PBND-4825 with a plant height of (100.57 cm) was the tallest one and genotype AKAW-3722 with plant height (81.08 cm) was the dwarfest one amongst sixteen genotypes and average plant height over the environments was 88.02 cm. For the character plant height AKAW-4800, PBN-4881, NIAW-2539, AKAW-3722, MACS-6478, NIAW-

301 had regression values lesser than unity. The deviation from regression line was significant for the genotypes AKAW-4739, AKAW-4798, PBN-4876, PBN-4881, NIAW-2495, NIAW-2595, MACS-6478, NIAW-301, AKDW-4525, PBND-4825, PBND-5175 and NIDW-0950 indicating that the response of these genotypes may change in different environments. According to the Eberhart and Russell (1966) all sixteen genotypes found unstable for plant height (cm).

4.4.5 Effective tillers plant^{-1}

Amongst sixteen genotypes maximum number of effective tillers plant^{-1} (4.58) were recorded by the genotype PBN-4876 and minimum (2.84) by NIAW-2495 compared to the general mean (3.42). For the character effective tillers plant^{-1} AKAW-4798, AKAW-4800, NIAW-2495, NIAW-2539, NIAW-2595, MACS-6478, NIAW-301, AKDW-4525, PBND-5175 exhibited regression values lesser than unity. The regression values was significant for PBND-4825 (bi =1.901), and genotypes NIAW-2539 and NIDW-295 exhibited significance for deviation from regression line. Considering the stability parameters together genotypes to NIDW-0950 (Mean 3.20, bi 1.160, s^2di 0.071) was found stable to favorable (rich) environment and genotypes AKAW 4800 (Mean 3.40, bi 0.755, s^2di 0.041) and MACS-6478 (Mean 3.58, bi 0.718, s^2di 0.077) were found stable genotypes to unfavorable (poor) environment for effective tillers plant^{-1} (fig. 1).

4.4.6 Tillers sq.meter^{-1}

Maximum effective tillers sq.m^{-1} (382.89) were recorded by the genotype PBN-4876 and minimum (299.00) by genotype NIAW-2495 as compared to the general mean (351.14). For this character effective tillers sq.m^{-1} genotypes AKAW-4739, AKAW-4798, AKAW-4800, NIAW-2495, NIAW-2539, MACS-6478, PBND-4825, PBND-5175, NIDW-0950, NIDW-295 exhibited regression values lesser than unity. Amongst sixteen genotypes NIAW-2495 exhibited significant regression coefficient value, and significant deviation from regression line indicating it's unpredictability for effective tillers sq.m^{-1}. According to the Eberhart and Russell model the genotypes NIAW-2595 (Mean 358.22, bi 1.687, s^2di 44.509) was the most

stable in favorable environment and NIAW-301, PBN-4881, AKDW-4525, PBN-4876, MACS-6478, AKDW-4525, NIDW-295, PBND-4825, PBND-4876, AKA-4800, NIDW-0950, PBND-5175, NIAW-2595 and AKAW 3722 were exhibited moderate stability performance for number of tillers $sq.m^{-1}$. Only one genotype AKAW-4739 (Mean 323.89, bi 0.996, s^2di -85.95) exhibited stable performance over the environments for character nu tillers $sq.m^{-1}$.

4.4.7 Spikelets $spike^{-1}$

Out of sixteen genotypes maximum spikelets $spike^{-1}$ (17.56) were exhibited by genotype NIDW-295 and minimum by the genotype AKDW-4525 (12.67) and over the environments mean value for spikelets $spike^{-1}$ was recorded to be (14.99). For the character spikelets $spike^{-1}$ genotypes AKAW-4739, PBN-4876, PBN-4881 NIAW-2495, AKAW-3722, AKDW-4525, PBND-5175 exhibited regression values lesser than unity. The regression value was significant for genotype AKAW-4739 (bi =0.692), indicating it's unpredictability for spikelets $spike^{-1}$. Out of sixteen genotypes MACS-6478 exhibited significant deviation from regression line. According to the Eberhart and Russell (1966) model the genotypes PBND-4825 (Mean 13.67, bi 1.083 s^2di -0.146),, AKAW-4798 (Mean 13.00, bi 1.083, s^2di -0.146) were exhibited stability in favorable (rich) environment, while PBN-4881 (Mean 16.67, bi 0.992, s^2di -0.117) and PBND-5175 (Mean 15.33, bi 0.902, S^2di 0.354) found most stable performance over the environments. Genotypes NIAW-301, NIAW-2595, PBN-4876 NIDW-0950 and AKAW-4739 genotypes exhibited medium of stability for spikelets $spike^{-1}$ (fig. 2).

4.4.8 Spike length (cm)

Genotype PBN-4876 exhibited maximum (9.61 cm) and genotype PBND-4825 exhibited minimum (5.54 cm) spike length, while the average over three environments was observed to be (7.72 cm).

For the character spike length (cm) genotypes PBN-4876, PBN-4881, NIAW-2495, MACS-6478, NIDW-0950, NIDW-295 exhibited regression values lesser than unity. The regression values are significant for PBN-4876 (bi =0.821), PBND-5175(bi=1.035), NIDW-0950 (bi=0.784)

indicating their unpredictability for spike length (cm). Amongst sixteen, two genotypes PBN-4881 and NIAW-2495 exhibited significant deviation from regression line, indicating their unpredictability for spike length (cm). Considering the stability parameters together genotypes NIAW-301 (Mean 8.18, bi 1.057, s^2di -0.019) AKAW-3722 (Mean 7.06, bi 1.034, s^2di -0.021) AKAW-4800 (Mean 9.51, bi 1.017, s^2di 0.038), NIAW-2539 (Mean 8.97, bi 1.012, s^2di -0.018) were exhibited stability to favorable (rich) environments, while MACS-6478 (Mean 9.22, bi 0.768, s^2di -0.012) was exhibited stable performance in unfavorable (poor) environment, whereas PBND-4825, NIAW-2595, AKAW-4798, AKDW-4525, NIDW-295, PBN-4876, NIDW-0950 and PBND-5175 exhibited medium level of stability for spike length (cm) over the environments (fig. 3).

4.4.9 Grains spike^{-1}

Amongst sixteen genotypes tested over three environments AKAW-4739 and AKAW-4798 exhibited maximum (54.30) and minimum (36.48), grains spike^{-1} respectively and pooled mean over three environments was observed to be 45.31 grains spike^{-1}.

For the character grains spike^{-1} genotypes AKAW-4798, AKAW-4800, PBN-4881, NIAW-2495, NIAW-2539, NIAW-301, NIDW-295 recorded regression values lesser than unity. Genotypes AKAW-4798, AKAW-4800, PBN-4876, NIAW-2539, MACS-6478 and AKDW-4525 recorded significant deviation from regression line indicating the differential response of each genotype to the environments. As regards three parameters together; mean, regression value and deviation from regression value, genotype NIAW-2595 (Mean 44.59, bi 1.058, s^2di 3.165) was exhibited stability in favorable (rich) environment while PBN-4881 (Mean 48.26, bi 0.781, s^2di 2.304), was exhibited stability in unfavorable (poor) environment. Genotypes NIAW-301 and NIDW-295 were exhibited medium stability over the environments for grains spike^{-1} (fig. 4).

4.4.10 Test weight (g)

Stability parameters presented in Table 4.6 revealed that maximum (48.41g) and minimum (37.23 g) values for 1000 grain weight were recorded by the genotypes NIAW-2595 and PBN-4881 respectively

as compared to the general mean (41.99 g). For the character test weight (g) genotypes PBN-4881, NIAW-2495, NIAW-2595, AKAW-3722, AKAW-4800, MACS-6478 and PBND-5175 exhibited regression values lesser than unity. Amongst sixteen genotypes AKAW-4739, PBN-4876 and MACS-6478 exhibited significant deviation from regression line, while AKAW-3722 recorded significant regression value indicating their unpredictability for test weight (g). Considering the three parameters together, mean, regression value and deviation from regression value, genotypes NIAW-2539 (Mean 42.16, bi 1.390, s^2di -0.498) and NIDW-0950 (Mean 39.58, bi 1.250, s^2di -0.371) were found most stable in favorable (rich) environment for test weight (fig. 5).

4.4.11 Grain yield kg plot^{-1}

Stability parameters for grain yield kg plot^{-1} are presented in Table 4.6. Genotype AKAW-4739 exhibited highest grain yield (3.91 kg) while AKDW-4525 exhibited lowest grain yield (2.68 kg) as compared to the pooled mean (3.21 kg).

Genotypes AKAW-4798, AKAW-4800, NIAW-2495, NIAW-2539, MACS-6478, NIAW-301, AKDW-4525 and NIDW-0950 exhibited regression values lesser than unity. All sixteen genotypes exhibited non-significance for deviation from regression line indicating their predictability for grain yield kg plot^{-1}. Considering the three parameters together; mean, regression value and deviation from regression value, genotype AKAW-3722 (Mean 3.20, bi 1.928, s^2di 0.001) was performed more stable in favorable (rich) environment. Genotype NIDW-0950 (Mean 2.74, bi 0.926, s^2di 0.034) exhibited most stable performance over the grain yield kg plot^{-1} though the mean yield was little beat less than average yield (fig. 6).

4.4.12 Straw yield kg plot^{-1}

The genotype NIAW-301 showed highest straw yield kg plot^{-1} (11.13) while NIAW-2539 showed lowest straw yield kg plot^{-1} (7.24) as compared to the pooled mean (9.22 kg plot^{-1}). For the character straw yield kg plot^{-1} genotypes AKAW-4739, AKAW-4798, PBN-4876, PBN-4881, NIAW-2539 NIAW-2595, AKAW-3722, PBND-4825, PBND-5175 and NIDW-295 exhibited regression values lesser than unity. The regression

values were significant for NIAW-2539, PBND-5175 and NIDW-0950 and only one genotype NIAW-301 exhibited significant value of deviation from regression line indicating their unpredictability for straw yield kg plot^{-1}. According to the Eberhart and Russell model the all genotypes showed medium stability for straw yield kg plot^{-1} and a single genotype viz.NIDW-295 exhibited highly stable performance over environments for straw yield kg plot^{-1}.

4.5 Molecular marker analysis

Twenty one SSR primers of Xgwm, gwm and XPSP series were used to evaluate sixteen wheat genotypes. Out of them six primers viz., Xgwm-130, Xgwm-136, Xgwm-193, Xgwm-493, Xgwm-610 and XPSP-2999 were found polymorphic. Four primers viz., Xgwm-469, Xgwm-389, gwm-18 and gwm-133 were found monomorphic and remaining eleven primers had produced no amplification or fuzzy bands and did not show clear amplification.

The PCR amplified products of each primer was resolved on 10% polyacrylamide gel by electrophoresis and the number of the amplified alleles were scored for genetic variability analysis. The details of these six polymorphic primers viz. Xgwm-130, Xgwm-136, Xgwm-193, Xgwm-493, Xgwm-610 and XPSP-2999 are presented in Table 4.9.

Table 4.9 Characteristics of the amplification products with polymorphic SSR primers among 16 wheat genotypes.

Sr. No.	Primers	Total number of amplicons	Monomorphic amplicons	Polymorphic amplicons	Per cent polymorphism	PIC Value
1	Xgwm-130	20	0	20	100.00	0.924
2	Xgwm-136	13	1	12	92.31	0.774
3	Xgwm-193	14	0	14	100.00	0.860
4	Xgwm-493	14	0	14	100.00	0.893
5	Xgwm-610	15	1	14	93.33	0.862
6	XPSP-2999	17	0	17	100.00	0.918
	Total (polymorphic amplicons)	93	2	91	585.64	5.231
	Average (polymorphic amplicons)	15.5	0.33	15.1	97.61	0.871

The polymorphic information content (PIC) value of SSR loci was calculated across sixteen wheat genotypes and is presented in Table 4.7 for the six polymorphic primers viz., Xgwm-130, Xgwm-136, Xgwm-193, Xgwm-493, Xgwm-610 and XPSP-2999.

The six polymorphic primers amplified a total of 93 alleles with an average of 15.5 alleles per marker. Amplifield allels ranged from 13 (Xgwm-136) to 20 (Xgwm-130). The Xgwm-130 primer amplified heigher number of alleles i.e. 20 followed by primer XPSP-2999 with 17 number of allels. Primer Xgwm-610 amplified 15 number of allels while, primers Xgwm-193 and Xgwm-493 amplified 14 number of allels. Primer Xgwm-136 amplified lowest number of allels i.e 13. The PIC values calculated for these six polymorphic primers were in the range of 2.211 (Xgwm-136) to 5.694 (XPSP-2999) with an average of 3.7315 per primer (Table 4.9).

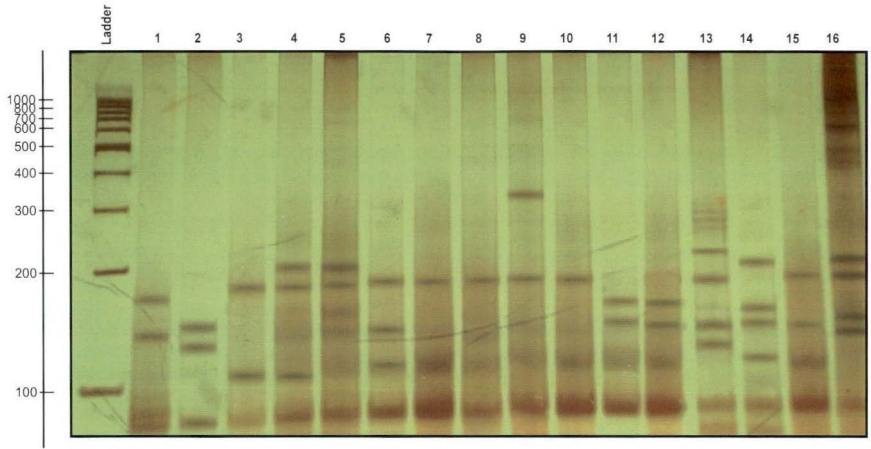
Primer Xgwm-130 amplified 20 allelic bands among 16 genotypes as presented in plate 4.1. The PIC value was 0.924 with 100 per cent polymorphism for all sixteen genotypes included in this study.

Primer Xgwm-136 amplified 13 allelic bands as presented in plate 4.2. PIC value for this primer was 0.774 with 92.31 per cent polymorphism. Xgwm-193 primer amplified 14 allelic band among 16 genotypes as presented in plate 4.3. The PIC value calculated was 0.860 with 100 per cent polymorphism. Primer Xgwm-493 also amplified 14 band among 16 genotypes as presented in plate 4.4 with PIC value 0.893. This primer exhibited 100 per cent polymorphism.

Primer Xgwm-610 amplified 15 allelic bands as presented in plate 4.5. PIC value for this primer was 0.862 with 93.33 per cent polymorphism. Similarly primer XPSP-2999 amplified 17 allelic band among 16 genotypes as presented in plate 4.6 with calculated PIC was 0.918 with 100 per cent polymorphism.

4.6. Dendrogram based on polymorphic SSR primers

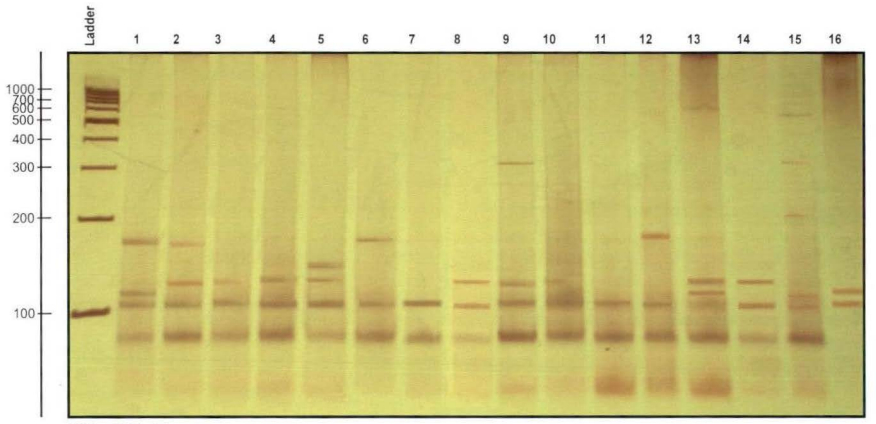
The grouping of different wheat genotypes were done on the basis of SSR banding pattern and dendrogram was prepared by using



100 bp DNA Ladder

1.PBND-4825	5 MACS-6478	9 NIAW-301	13 NIAW-2539
2.AKAW-4798	6 AKAW-4739	10 AKAW-3722	14 PBN-4881
3.AKDW-4525	7 AKAW-4800	11 NIAW-2595	15 NIAW-2495
4.NIDW-295	8 PBND-4876	12 PBND-5175	16 NIDW- 0950

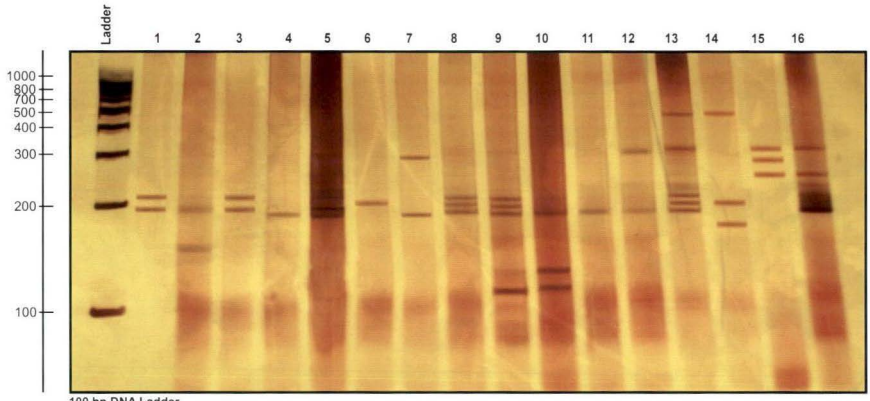
Plat 1. Amplification of 16 wheat genotypes with SSR primer Xgwm-130



100 bp DNA Ladder

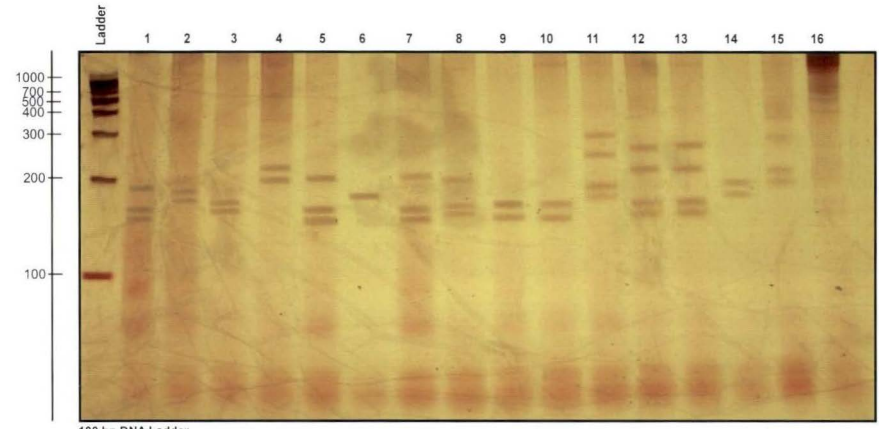
1.PBND-4825	5 MACS-6478	9 NIAW-301	13 NIAW-2539
2.AKAW-4798	6 AKAW-4739	10 AKAW-3722	14 PBN-4881
3.AKDW-4525	7 AKAW-4800	11 NIAW-2595	15 NIAW-2495
4.NIDW-295	8 PBND-4876	12 PBND-5175	16 NIDW- 0950

Plat 2. Amplification of 16 wheat genotypes with SSR primer Xgwm-136



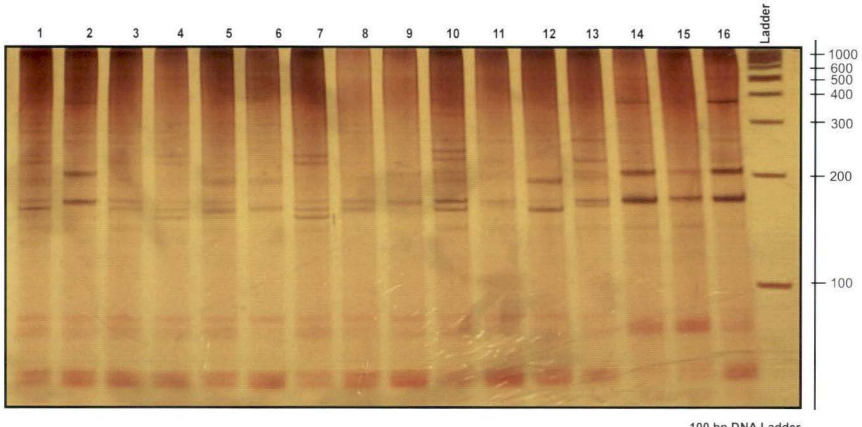
1.PBND-4825	5 MACS-6478	9 NIAW-301	13 NIAW-2539
2.AKAW-4798	6 AKAW-4739	10 AKAW-3722	14 PBN-4881
3.AKDW-4525	7 AKAW-4800	11 NIAW-2595	15 NIAW-2495
4.NIDW-295	8 PBND-4876	12 PBND-5175	16 NIDW- 0950

Plat 3. Amplification of 16 wheat genotypes with SSR primer Xgwm-193



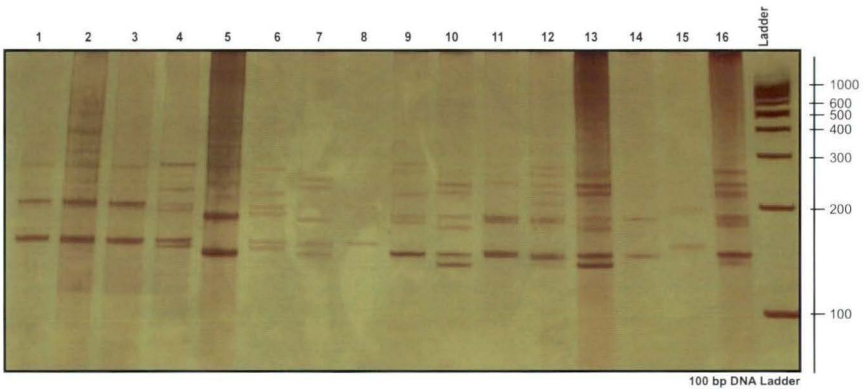
1.PBND-4825	5 MACS-6478	9 NIAW-301	13 NIAW-2539
2.AKAW-4798	6 AKAW-4739	10 AKAW-3722	14 PBN-4881
3.AKDW-4525	7 AKAW-4800	11 NIAW-2595	15 NIAW-2495
4.NIDW-295	8 PBND-4876	12 PBND-5175	16 NIDW- 0950

Plat 4. Amplification of 16 wheat genotypes with SSR primer Xgwm-493



1.PBND-4825	5 MACS-6478	9 NIAW-301	13 NIAW-2539
2.AKAW-4798	6 AKAW-4739	10 AKAW-3722	14 PBN-4881
3.AKDW-4525	7 AKAW-4800	11 NIAW-2595	15 NIAW-2495
4.NIDW-295	8 PBND-4876	12 PBND-5175	16 NIDW- 0950

Plat 5. Amplification of 16 wheat genotypes with SSR primer Xgwm-610



1.PBND-4825	5 MACS-6478	9 NIAW-301	13 NIAW-2539
2.AKAW-4798	6 AKAW-4739	10 AKAW-3722	14 PBN-4881
3.AKDW-4525	7 AKAW-4800	11 NIAW-2595	15 NIAW-2495
4.NIDW-295	8 PBND-4876	12 PBND-5175	16 NIDW- 0950

Plat 6. Amplification of 16 wheat genotypes with SSR primer XPSP-2999

cluster analysis (NTYSIS) based on similarity coefficient generated using six SSR markers.

Table 4.10 Grouping of genotypes at 80 per cent and 75 per cent cut off level of similarity

Sr. No.	Cluster at cut of 75%	Sub cluster at cut of 80%	Genotype
1	I	A	PBND-4825, AKDW-4525, PBN-4876, AKAW-4798
		B	NIAW-2595, PBN-4881
		C	NIAW-301
		D	MACS-6478, AKAW-4800
2	II	A	NIDW-295, AKAW-4739
3	III	A	AKAW-3722, PBND-5175
		B	NIAW-2539
4	Single		NIAW-2495
5	Single		NIDW-0950

On the basis of similarity index and the dendrogram (Fig. 4.1) generated, it is revealed that the sixteen wheat genotypes were grouped into three main clusters at 75% cut off level. Main cluster 'I' subdivided into A, B, C and D sub-clusters at 80% cut level. Subcluster 'A' comprised of PBND-4825, AKDW-4525, PBN-4876 and AKAW-4798 genotypes while, sub-cluster 'B' comprised of NIAW-2595 and PBN-4881 genotypes similarly, sub-cluster 'C' comprised of NIAW-301 while, sub-cluster 'D' comprised of MACS-6478 and AKAW-4800. Main cluster 'II' comprised of NIDW-295, AKAW-4739 genotypes. Similarly main cluster 'III' sub-clustered into A anb B. Subcluster 'A' comprised of AKAW-3722 and PBND-5175 while, sub-cluster 'B' comprised of NIAW-2539 genotype. Genotypes NIDW-0950 and NIAW-2495 were remained single in dendrogram. (Table 4.10)

Table 4.11 Similarity matrix of SSR primer analysis

	PBND-4825	AKAW-4798	AKDW-4525	NIDW-295	MACS-6478	AKAW-4739	AKAW-4800	PBN-4876	NIAW-301	AKAW-3722	NIAW-2595	PBND-5175	NIAW-2539	PBN-4881	NIAW-2495	NIDW-0950
PBND-4825	1.00															
AKAW-4798	0.817	1.000														
AKDW-4525	0.882	0.849	1.000													
NIDW-295	0.731	0.742	0.828	1.000												
MACS-6478	0.710	0.720	0.785	0.742	1.000											
AKAW-4739	0.731	0.785	0.785	0.806	0.677	1.000										
AKAW-4800	0.731	0.720	0.785	0.784	0.806	0.742	1.000									
PBN-4876	0.828	0.796	0.882	0.753	0.774	0.774	0.774	1.000								
NIAW-301	0.731	0.763	0.785	0.699	0.742	0.699	0.742	0.839	1.000							
AKAW-3722	0.699	0.731	0.753	0.688	0.731	0.710	0.753	0.785	0.798	1.000						
NIAW-2595	0.796	0.785	0.806	0.742	0.763	0.763	0.785	0.839	0.806	0.796	1.000					
PBND-5175	0.720	0.731	0.753	0.731	0.731	0.753	0.774	0.742	0.731	0.806	0.796	1.000				
NIAW-2539	0.710	0.656	0.699	0.656	0.699	0.634	0.699	0.731	0.699	0.742	0.720	0.774	1.000			
PBN-4881	0.731	0.763	0.763	0.699	0.742	0.742	0.720	0.817	0.763	0.731	0.828	0.688	0.699	1.000		
NIAW-2495	0.710	0.720	0.742	0.742	0.720	0.742	0.763	0.731	0.677	0.688	0.763	0.688	0.656	0.742	1.000	
NIDW-0950	0.688	0.699	0.699	0.656	0.677	0.677	0.677	0.688	0.677	0.688	0.720	0.731	0.699	0.699	0.720	1.000

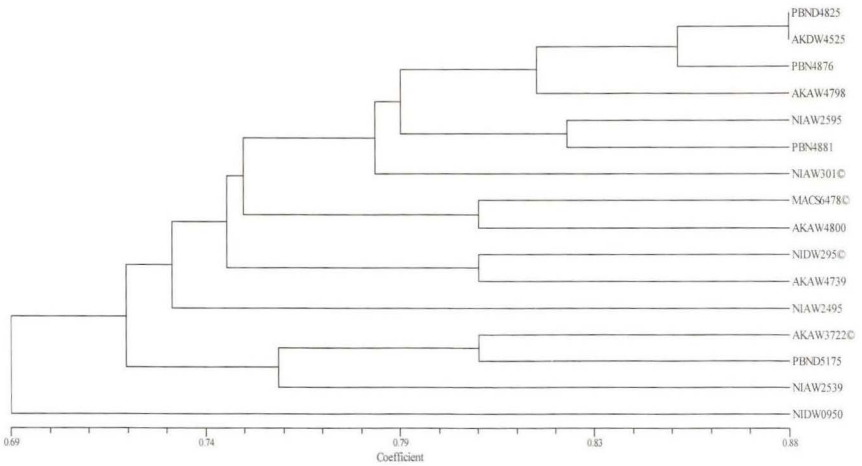


Fig. 7. Dendrogram of wheat genotypes using SSR markers based on Jaccard's similarity coefficient

DISCUSSION

Analysis of variance for Niphad, revealed that the mean sum of squares due to genotype were found highly significant for 10 characters viz., days to 50 per cent flowering, days to maturity, plant height (cm), effective tillers plant^{-1}, tillers sq.meter^{-1}, spikelets spike^{-1}, spike length (cm), grains spike^{-1}, test weight (g) and grain yield kg plot^{-1} which indicates of the presence of sufficient variability among the genotypes for these character. Similarly for characters germination per cent and straw yield kg plot^{-1} the genotype source differences were moderately significant indicated the presence of moderate variability in respect of these traits.

ANOVA for Parbhani professed that the genotypic differences for almost all the characters were found highly significant except germination per cent indicating the presence of lot of variability among the genotypes for those characters.

ANOVA for Akola environment, revealed that the genotypic differences expressed in terms of genotypes mean sum of squares are found highly significant for characters viz. days to 50 % flowering, days to maturity, plant height (cm), effective tillers plant^{-1}, tillers sq.meter^{-1}, spikelets spike^{-1}, spike length (cm), grains spike^{-1}, test weight (g), grain yield kg plot^{-1} and straw yield kg plot^{-1}, indicating the sufficient amount of variability for those characters. Shirpurkar *et al.* (2006) and Shirpurkar *et al.* 2007 also reported the similar results.

Mean performance:

The three environments (Niphad, Parbhani, and Akola) revealed highly significant differences for almost all the characters studied except straw yield kg plot^{-1} at Niphad location. Since all these three locations differed from each other in respect of climatic conditions. Implies that there is a scope for selection of better genotypes at all the locations. Genotype NIDW-295 was found most superior amongst sixteen genotypes since it has recorded highest mean for five traits over three environments, while other genotypes viz. PBN-4876, AKAW-4739 and PBND-4825

exhibited high mean only for two or three characters. Since NIDW-295 followed by PBN-4876, AKAW-4739 and PBND-4825 found most stable genotypes. Present findings are in agreement with the results reported by Kota *et al.*(2013), Singh *et al.* (2013).

On the basis of magnitude of mean 12 traits studied over environments; Niphad location was proved to be the best environment as compared to Parbhani and Akola environments since, Niphad location produced significantly higher grain yield than Parbhani and Akola.

Stability performance of genotypes

In the development of stable varieties, main objective is to produce maximum yield per unit area and consistency in productivity over the environments. A genotype is stable, which can adjust its genotypic and phenotypic state in response to environmental fluctuations in such a way that it gives high and stable performance for yield and its contributing traits can be termed as well buffered. Stability performance is one of the most desirable attribute of a genotype to be released as an improved variety for multi environments. Methods have been developed which could be used to provide reliable estimates of G x E interactions. Finlay and Wilkinson (1963) and Eberhart and Russell (1966) proposed stability models which gives better amount of stability of different genotypes.

Analysis of Variance for Stability

The ability of a genotype to produce a narrow range of phenotype in different environments can be called as stable (Lewis 1954). The genotype will be stable in the absence of influence by environment as well as genotype X environment interaction effects.

Eberhart and Russell Model of stability analysis was used for the assessment of influence of environment and genotype X environment interaction effects on genotypes for each character. When genotype X environment interactions were significant for the characters, then partitioning of total sum of squares due to genotype X environment

interaction into predictable and unpredictable sources of variation was done using the procedure provided in Eberhart and Russell model.

Analysis of Variance for stability proffessed that the genotypic differences pooled over three environments were significant for germination per cent, days to 50% flowering, days to maturity, plant height (cm), effective tillers plant^{-1}, tillers sq.meter^{-1}, spikelets spike^{-1}, spike length (cm), grains spike^{-1}, test weight (g), grain yield kg plot^{-1}, straw yield kg plot^{-1}, indicating the presence of sufficient amount of variability for those characters and there is scope for selection of genotypes having desirable characters.

Mean sum of squares due to Environment and (G × E) interactions are highly significant for germination per cent, days to 50 per cent flowering, days to maturity, spike length (cm), grains spike^{-1}, test weight (g), grain yield kg plot^{-1} and straw yield kg plot^{-1} indicating that the environments and their interaction with genotypes played an important role in determining these traits. While for character tillers sq.meter^{-1}, environment and (G × E) interaction mean sum of squares are moderately significant. Similar results were reported by Javed *et al.* (2007), Singh and Pathania (2008) and Mondal *et al.* (2010)

Mean sum of squares due to environment alone are highly significant for germination percentage, days to 50 per cent flowering, days to maturity, tillers sq.meter^{-1}, spike length (cm), grains spike^{-1}, grain yield kg plot^{-1} and straw yield kg plot^{-1} depicting the presence of variability and chances of good selections for above mentioned traits under the environments selected for study (Kamal Tripura *et al.*, 2011). While mean sum of squares due to environment for plant height (cm), effective tillers plant^{-1} and test weight (g) were found moderately significant and for character spikelets spike^{-1} was non-significant there by indicating the absence of variability for this character under environments selected for study.

While G × E interaction source is highly significant for days to maturity and grain yield kg plot^{-1} and moderately significant for test weight

(g) and straw yield kg plot^{-1}, depicting the differential response of genotypes for these traits in three environments for these traits. Present results are in agreement with Naser Sabaghania *et al.* (2013) and Singh *et al.*(2013)[a]. The genotype X environment (G × E) interaction was further partitioned into linear and non-linear components. The environment linear component was found highly significant for 10 characters out of 12 which includes germination percentage, days to 50 per cent flowering, days maturity, plant height (cm), tillers sq. meter^{-1}, spike length (cm), grains spike^{-1}, test weight (g), grain yield kg plot^{-1}, straw yield kg plot^{-1} and for two characters viz. effective tillers plant^{-1} and spikelets spike^{-1} (Kota *et al.*, 2013, Singh *et al.* 2013[b]), the environment linear component was found moderately significant, thereby indicating the differences between environments and their considerable influence on all the characters studied. Present findings are in agreement with the results reported by Parveen *et al.* (2010), Gowda *et al.* (2010) and Patel *et al.* (2014).

Non-linear component (G×E interactions) are highly significant for days to maturity, test weight (g), grain yield kg plot^{-1}, straw yield kg plot^{-1} and moderately significant for grains spike^{-1}. These results indicated that the relative ranks of the genotypes differed from one environment to another. Non-significant of non-linear interaction, indicated that there was no influence of G X E interaction on the genotypes. Similar results were reported by Yadav and Sharma (2008), Yadav *et al.* (2009) and Patel *et al.* (2014).

Pooled deviation was found highly significant for days to 50 % flowering, plant height (cm), effective tillers plant^{-1} and grains spike^{-1} while only significant for test weight (g), indicated that the genotypes differed considerably with respect to their stability for all studied traits. These results are in line with those obtained by Singh *et al.* (2013[b]), Patel *et al.* (2014).

The magnitude of non-linear component (pooled deviation) was found higher than the magnitude of linear component (G X E linear) for the characters plant height (cm) and effective tillers plant^{-1}. While the magnitude was lower than for characters days to 50 % per cent flowering,

grains spike^{-1} and test weight these results are in agreement with Yadav *et al.* (2009), Gowda *et al.* (2010). Singh *et al.* (2013[b]) and Patel *et al.* (2014).

Ashraf *et al.* (2001) also reported significance of both linear and non-linear components and indicated the presence of both predictable and unpredictable components of G × E.

Jena *et al.* (2005) has reported the predominance of linear and non-linear components for plant height, spike length and number of grains per spike, whereas predominance of non-linear components for all most all the characters except spike length which are in agreement with the present findings.

Stability parameters of genotypes

Finlay and Wilkinson (1963) considered linear regression slope as a measurement of stability. However, Eberthart and Russell (1966) emphasized the need of considering both linear (bi) and non-linear (S^2di) components of G x E interaction in judging the stability of genotype.

An ideal genotype is defined as, one possessing high mean performance, with regression coefficient around unity (bi=1) and deviation from regression (S^2di) close to zero however, for the characters viz. days to 50 % flowering and days to maturity had lowest mean performance with regression coefficient around unity (bi=1) and deviation from regression (S^2di) close to zero considered as ideal genotype. The linear regression is regarded as the measure of linear response of a particular genotype to the changing environments. If the regression coefficient (bi) is greater than unity, the genotype is said to be highly sensitive to environmental fluctuations but adopted to high yielding environments i.e. it exhibits below average stability. If the regression coefficient (bi) is equal to unity, it indicates the average sensitivity to environmental fluctuations and adaptable to all kinds of environments i.e. it exhibits average stability. If the regression coefficient (bi) is less than unity, it indicates less sensitivity to environmental changes and if this is accompanied by a high mean value,

then the genotype is said to be better adapted for poor condition i.e. it exhibits above average stability.

In the present study, stability parameters such as mean (X), regression coefficient (bi) and deviation from regression (S^2di) as suggested by Eberhart and Russell (1966) were considered to explain and discuss the stability of different genotypes for various characters under consideration.

In present investigation on the basis of grain yield kg $plot^{-1}$ parameter only genotypes NIDW-0950 was exhibited most stable performance since it exhibited better grain yield as compared to the mean over the environments (2.74 kg). Similarly this genotype exhibited non-significant regression coefficient value nearer to unity (bi = 0.926). Further the deviation from regression value (S^2di=0.034) was also close to zero. There by indicating its highly stable performance for grain yield over the environments tested.

Present findings are in close agreement with Shirpurkar *et al.*2006, Yadav *et al.*2008, Gohil and Jadeja 2009, Gowda *et al.*2010 and Kamal Tripura *et al.*2011.

High yielding genotype NIDW-0950 also exhibited above average stability for the traits days to 50% flowering, days to maturity, tillers $plant^{-1}$, test weight (g), indicating that it may perform well in different environments for these characters also. Whereas, it showed below average stability for the traits germination per cent, plant height (cm), tillers $sq.meter^{-1}$, $spikelets\ spike^{-1}$, spike length (cm), $grains\ spike^{-1}$ and straw yield k $plot^{-1}$

Such kind of performance for various yield contributing characters by the stable genotypes are also reported by Graussgruber *et al.*2006, Yadav *et al.*2008 and Parveen *et al.* (2010), also observed wide range of stability statistics among 12 wheat cultivars for productive tillers $sq.m.^{-1}$, 1000 grain weight (g) and grain yield, which are in close agreement with present findings

Gohil and Jadeja (2009) performed stability analysis and reported that none of the genotype was stable for evaluated traits; however genotypes depicting stable performance for yield per plant, offered the possibilities of exploitation for varietal improvement program in durum wheat. Since, segregates combining high mean and stability of performance could be expected in the advance generations.

Therefore it is concluded that the genotype NIDW-0950 can be included in the hybridization program to converge the stability for grain yield and development of stable cultivar adapted to a wide range of environments.

The genotype NIAW-301 exhibited above average stable performance for days to 50% flowering, days to maturity, effective tillers plant^{-1} and spike length (cm) with better mean performance and based on the value of regression coefficient for days to 50% flowering and spike length (cm), this genotype NIAW-301 was found most stable in favorable environment, while for days to maturity and effective tillers plant^{-1} having the regression coefficient values less than unity indicating it's adaptability to the unfavorable (poor) environments.

Another genotype PBN-4881 showed above average stability for the traits germination per cent, spikelets spike^{-1} and grains spike^{-1} with better mean performance and values of regression coefficient, indicating that it performs well in poor environments. It showed below average stability for the traits spike length (cm), days to 50% flowering, days to maturity, plant height (cm), effective tillers plant^{-1}, tillers sq. meter^{-1}, spike length (cm), grain yield kg plot^{-1} and straw yield kg plot^{-1} indicating that genotype is suitable only in favorable environments for these characters.

Another genotype NIAWS-2595 have shown above average stability for the traits germination per cent, tillers sq. meter^{-1} and grains spike^{-1} with better mean performance and values of regression coefficient, indicating that for germination per cent it performs well in poor environments whereas for tillers sq. meter^{-1} and grains spike^{-1} this genotype performs well in desirable environments.

While the genotype NIAW 2495 showed above average stability for the traits days to 50 per cent flowering and days to maturity indicated it's well performance in favorable (rich) environment. It also showed below average stability for the traits germination per cent, plant height (cm), effective tillers plant^{-1}, tillers sq. meter^{-1}, spikelets spike^{-1}, spike length (cm), and grains spike^{-1}, test weight (g) grain yield kg plot^{-1} and straw yield kg plot^{-1}.

The genotype NIAW-2539 exhibited above average stable performance for days to 50% flowering and test weight (g) with better mean performance and based on the value of regression coefficient for days to 50% flowering this genotype NIAW-2539 was found most stable in undesirable environment, while for test weight (g) having the regression coefficient values less than unity indicating it's adaptability to the favorable environments.

As regards the AKAW-4800 have shown above average stability for the traits effective tillers plant^{-1} and spike length (cm), with better mean performance and values of regression coefficient, indicating it's better performance in poor environments for the trait effective tillers plant^{-1} while for spike length (cm), this genotype performed well in rich environments.

The genotype PBND-5175 exhibited above average stable performance in poor environment for germination per cent and spikelets spike^{-1} based on mean performance, regression coefficient and deviation from regression line as stated by Eberthart and Russell (1966).

The genotype PBN-4876 have shown above average stability for the trait days to 50% flowering indicating it's better stability in poor environment. It also showed below average stability for the traits days to maturity, plant height (cm), effective tillers plant^{-1}, tillers sq.meter^{-1}, spikelets spike^{-1}, spike length (cm), number of grains spike^{-1}, test weight (g) and grain yield kg plot^{-1} and straw yield kg plot^{-1} indicating that these genotype are suitable only in favorable environments for these characters.

The genotype MACS-6478 has shown above average stability for the traits effective tillers plant^{-1} in unfavorable environment.

The genotypes PBND-4825 and AKAW-4798 have shown above average stability for the traits spikelets spike^{-1} showed better adaptability of this genotype in favorable environment only.

Thus any generalization regarding stability of genotypes for all characters it is too difficult since the genotypes may not simultaneously exhibit uniform responsiveness and stability for all the characters.

Several authors have reported stability for various characters. Some of them were Gohil and Jadeja (2009); Shah *et al.* (2009) for the traits spike length, spikelets per spike, grains per spike and maturity days. According ti Tyagi *et al.* (2016), promising genotypes identified for different environments could serve as donars to develop multi-parent advanced generation integrated cross populations to stack genes/ alleles.

4.5 Molecular diversity

In any crop improvement programme, genetic diversity plays an important role. In order to make best utilization of genetic potential of genotypes for improvement of traits and for adaptation to various stress conditions, genetic study is very crucial (Apoorva Arora *et al.*2014) Continuous genetic diversity assessment helps to maintain the diverse species more stable against environment fluctuation, across diverse environments and for crop improvement. The results also help the breeders for effective selection of the parents leading to progenies with high differentiation among them.

The present study was undertaken to study the diversity among 16 wheat genotypes based on SSR markers polymorphism and their relation with stability. In present study out of twenty one SSR primers six viz., Xgwm-130, Xgwm-136, Xgwm-193, Xgwm-493, Xgwm-610 and XPSP-2999 amplified total of 93 allels and ranged from 13 to 20 per primer, with an average of 15.5 allels per primer. Salah El-Din El- Assal and Gaber (2012), Mustafa Erayman *et al.* (2015) indicated that these SSR primers

were efficient for discrimination of wheat genotypes. These primers showed greater level of polymorphism, ranged from 92.31 to 100 per cent. Similar level of polymorphism reported by Arslan Sheikh Sehgal *et al.* (2012), Ratiba Bousba *et al.* (2012) and Emon *et al.* (2010) and concluded that these findings provide basis for future efficient use of these markers in genetic analysis of wheat. It was noted that a marker detecting a lower number of alleles also showed lower genetic diversity, compared to markers detecting a higher number of alleles, which revealed higher levels of genetic diversity. The maximum number of repeats within the SSR_s was also positively correlated with the genetic diversity.

In present study, PIC values ranged from 0.774 for primer Xgwm-136 to 0.924 for primer Xgwm-130 with an average of 0.871 per primer. Such greater level of PIC values also reported by Ratiba Bousba *et al.* (2012) and Saina Ahmed *et al.* (2014) indicating sufficient discrimination by these SSR markers. Primers Xgwm-130 and XPSP-2999 found most suitable for molecular polymorphism study (Meriam Nefzaoui *et al.*2014) because these two primers had showed 100 per cent polymorphism and highest PIC values 0.924 and 0.918 respectively. High PIC value indicated high degree of polymorphism among the genotypes which in turns helps to estimate genetic distance with more precision. In present study all six primers showed considerable level of PIC values indicating their utility for assessment of genetic diversity. It is necessary to obtain information about genetic diversity from polymorphic primers only so that genetically divergent genotypes can be effectively identified.

Present investigation indicated that SSR primer system is very efficient system for molecular diversity studies. Several other reserchers viz., Roder *et al.*(2002), Wang *et al.*(2007), Strelchenko *et al.* (2008), Uddin and Borner (2008), Bahadar Zeb *et al.* (2009), Tahir (2010),Emon *et al* (2010), Gorji and Zolnoori (2011), Mardi *et al.* (2011), Khavarinejad and Karimov (2012), Islam *et al.* (2012), Valentia *et al.* (2012), Hanna Mahdy Abouzied *et al* (2013) and Apoorva Arora *et al.* (2012) had reported the efficiency of SSR markers in molecular diversity analysis in wheat.

On the basis of calculated similarity matrix, the similarity and dissimilarity between two genotypes can be predicted. The genotypes showing similarity index of 1 are presumed to be 100 per cent similar, while that of 0 are said to be 100 per cent dissimilar from each other. In present study 16 wheat genotypes showed similarity coefficient value ranged from 0.634 to 0.882 indicating more variation in respect of genetic similarity at studied loci across sixteen genotypes. This ultimately means that large range of genetic diversity exists among the studied genotypes.

The sixteen genotypes studied were diverged into three main clusters based on similarity index values and dendrogram. In this study, it was observed that genotypes AKDW-4525 and PBND-4825 showed 88 per cent similarity. They also grouped in the same sub-ciuster and cluster, respectively. Islam *et al.* (2012) also studied genetic diversity of wheat with the dendrogram analysis. Maximum diversity between three main clusters across 16 genotypes was found between NIAW-2539 and AKAW-4739 genotypes.

Thus, as similarity index goes on decreasing, the degree of divergence goes on increasing. The degree of divergent or similarity helps to identify genetically diverse genotypes. Thus this information would be helpful to identify genetically diverse genotypes and high stable performance of genotypes.

4.7 Correlation between genetic diversity and stability performance of genotypes

Heterogenity, heterozygosity and mode of pollination are major factors to affect the adaptability of the genotype over a series of environments. The heterogeneous populations have broad genetic base. Such populations have greater capacity to stabilize productivity over a wider range of changing environments because the broad genetic base provides a higher degree of population buffering across the environment. In my research following genotypes, NIDW-0950 showed highest stability across the tested environment. This genotype found to most diverse from other 15 genotypes. Similarly NIAW-301 for characters days to 50%

flowering, days to maturity, effective tillers plant^{-1} and spike length (cm) with similarity index value (0.677), PBN-4881 for characters germination per cent, spikelets spike^{-1} and grains spike^{-1} with similarity index value (0.699) and NIAW-2595 for traits germination per cent, tillers sq. meter^{-1} and grains spike^{-1} with similarity index value (0.720) found most stable across three locations (Niphad, Parbhani and Akola) and exhibited more genetic diversity while, genotypes NIAW-2495, NIAW-2539, AKAW-4800, PBND-5175, PBN-4876, MACS-6478 and PBND-4825 exhibited stable performance merely for two or three traits. Genetically, genes are monomorphic and polymorphic ("monomorphic" genes are defined as those for which the frequency of the most common allele is > 99%; thus, some variation may be present even at these loci). The monomorphic proportion of the total genome varies among taxonomic groups, 50% in plants. The remainder of loci (on average 50% in plants) is polymorphic varying among individuals in a population, and among populations within a species. These loci are of most concern to restoration, adaptability, yield, resistance to biotic and abiotic stresses and buffered the population having this polymorphism or diversity, against diverse and fluctuating environments and give the stable performance across environments.

CHAPTER V

SUMMARY AND CONCLUSION

The present investigation entitled as "Stability and Molecular Diversity Studies in Wheat and It's Contributing Traits" was conducted with a view to study the stability of genotypes for yield parameters over diverse environments, to know the effect of morphological parameters for imparting stability and to characterize the genotypes for genetic diversity and to identify the variation among them using SSR markers. The experiment comprising sixteen genotypes including released varieties.

The experiment was conducted during *rabi* 2014-2015 using randomized block design with three replications. observations were recorded on randomly selected five plants in each treatment for twelve different characters *viz.*, germination percentage, days to 50% flowering, days to maturity, plant height (cm), effective tillers plant^{-1}, tillers sq. m^{-1}., spikelets spike^{-1}, spike length (cm), grains spike^{-1}, test weight (g), grain yield kg plot^{-1} and straw yield kg plot^{-1} .

Mean performance for three environments viz. Niphad, Parbhani and Akola recorded highly significant differences for almost all the characters studied except straw yield kg plot^{-1} at Niphad location. Implies that there is a scope for selection of better genotypes having desirable characteristics. Genotype NIDW-295 was found most superior amongst sixteen genotypes since it has recorded highest mean for more number of traits over three environments, while other genotypes viz. PBN-4876, AKAW-4739 and PBND-4825 exhibited high mean only for two or three characters.

On the basis of magnitude of mean 12 traits studied over environments Niphad location was proved to be the best environment among all the environments since, Niphad location produced significantly higher grain yield than other environments.

Analysis of Variance for stability proffessed that the genotypic differences pooled over three environments were significant for germination per cent, days to 50% flowering, days to maturity, plant height (cm), effective tillers plant^{-1}, tillers sq.meter^{-1}, spikelets spike^{-1}, spike length (cm), grains spike^{-1}, test weight (g), grain yield kg plot^{-1}, straw yield kg plot^{-1}, indicating the presence of sufficient amount of variability for those characters and there is scope for selection of genotypes having desirable characters.

Mean sum of squares due to Environment and (G × E) interactions are highly significant for germination per cent, days to 50 per cent flowering, days to maturity, spike length (cm), grains spike^{-1}, test weight (g), grain yield kg plot^{-1} and straw yield kg plot^{-1} indicating that the environments and their interaction with genotypes played an important role in determining these traits. While for character tillers sq.meter^{-1}, environment and (G × E) interaction mean sum of squares are moderately significant. Mean sum of squares due to environment alone are highly significant for germination percentage, days to 50 per cent flowering, days to maturity, tillers sq.meter^{-1}, spike length (cm), grains spike^{-1}, grain yield kg plot^{-1} and straw yield kg plot^{-1} depicting the presence of variability and chances of good selections for above mentioned traits under the environments selected for study. While mean sum of squares due to environment for plant height (cm), effective tillers plant^{-1} and test weight (g) were found moderately significant and for character spikelets spike^{-1} was non-significant there by indicating the absence of variability for this character under environments selected for study.

While G × E interaction source is highly significant for days to maturity and grain yield kg plot^{-1} and moderately significant for test weight (g) and straw yield kg plot^{-1}, depicting the differential response of genotypes for these traits in three environments for these traits. The genotype X environment (G×E) interaction was further partitioned into linear and non-linear components. The environment linear component was found highly significant for 10 characters out of 12 which includes germination percentage, days to 50 per cent flowering, days maturity, plant

height (cm), tillers sq. meter^{-1}, spike length (cm), grains spike^{-1}, test weight (g), grain yield kg plot^{-1}, straw yield kg plot^{-1} and for two characters viz. effective tillers plant^{-1} and spikelets spike^{-1}, the environment linear component was found moderately significant, thereby indicating the differences between environments and their considerable influence on all the characters studied. Non-linear component (G×E interactions) are highly significant for days to maturity, test weight (g), grain yield kg plot^{-1}, straw yield kg plot^{-1} and moderately significant for grains spike^{-1}. These results indicated that the relative ranks of the genotypes differed from one environment to another. Non-significant of non-linear interaction, indicated that there was no influence of G X E interaction on the genotypes. Pooled deviation was found highly significant for days to 50 % flowering, plant height (cm), effective tillers plant^{-1} and grains spike^{-1} while only significant for test weight (g), indicated that the genotypes differed considerably with respect to their stability for all mentioned studied traits. Remaining traits viz. germination per cent, days to maturity, tillers sq.meter^{-1}, spikelets spike^{-1}, spike length (cm), grain yield kg plot^{-1} and straw yield kg plot^{-1} (kg) were found non-significant.

On the basis of stability parameters NIDW-0950, NIAW-301, PBN-4881 NIAW-2595 were promising genotypes for majority of characters with higher mean performance across the environments.

In present study genotype NIDW-0950 recorded most stable performance across the environments, NIAW-301, PBN-4881 found stable to unfavorable environment, while NIAW-2595 performed well in desirable environment. While, genotypes NIAW-2495, NIAW-2539, AKAW-4800, PBND-5175, PBN-4876, MACS-6478 and PBND-4825 exhibited stable performance merely for one or two traits.

Total 21 SSR primers used for genetic diversity study of wheat genotypes, six primers primers viz., Xgwm-130, Xgwm-136, Xgwm-193, Xgwm-493, Xgwm-610 and XPSP-2999 were found polymorphic for the set of sixteen genotypes while, 4 were found monomorphic and 11 primers did not show clear amplification.

In this investigation above mentioned six polymorphic primers were found most suitable for discrimination of 16 wheat genotypes included in this study. Marker detecting a higher number of allels which revealed higher levels of genetic diversity.

Primers Xgwm-136 and XPSP-2999 sowed great level of PIC values 0.924 and 0.918 repectively. High PIC values revealed high degree of polymorphism among the genotypes and indicating their utility for assessment of genetic diversity.

On the basis of similarity index and the dendrogram generated, the sixteen wheat genotypes were grouped into three main clusters at 75% cut off level. Main cluster 'I' subdivided into A, B, C and D sub-clusters at 80% cut level. Subcluster 'A' comprised of PBND-4825, AKDW-4525, PBN-4876 and AKAW-4798 genotypes while, sub-cluster 'B' comprised of NIAW-2595 and PBN-4881 genotypes similarly, sub-cluster 'C' comprised of NIAW-301 while, sub-cluster 'D' comprised of MACS-6478 and AKAW-4800. Main cluster 'II' comprised of NIDW-295, AKAW-4739 genotypes. Similarly main cluster 'III' sub-clustered into A anb B. Subcluster 'A' comprised of AKAW-3722 and PBND-5175 while, sub-cluster 'B' comprised of NIAW-2539 genotype. Genotypes NIDW-0950 and NIAW-2495 were remained single in dendrogram.

In my research following genotypes, NIDW-0950 showed highest stability across the tested environment. This genotype found to most diverse from other 15 genotypes. Similarly NIAW-301 for characters days to 50% flowering, days to maturity, effective tillers plant^{-1} and spike length (cm) with similarity index value 0.677, PBN-4881 for characters germination per cent, spikelets spike^{-1} and grains spike^{-1} with similarity index value 0.699)\ and NIAW-2595 for traits germination per cent, tillers sq. meter^{-1} and grains spike^{-1} with similarity index value 0.720 found most stable across three locations (Niphad, Parbhani and Akola) and exhibited more genetic diversity while, genotypes NIAW-2495, NIAW-2539, AKAW-4800, PBND-5175, PBN-4876, MACS-6478 and PBND-4825 exhibited stable performance merely for two or three traits.

CHAPTER VI

IMPLICATIONS

It is agreed that the more stable genotypes can somehow adjust their phenotypic responses to provide some measure of uniformity inspite of environmental fluctuations. The buffering ability of segregating populations seems to be directly related to the homeostatic responses of the parental lines. Therefore, it is feasible to develop phenotypically stable better performing genotypes by ordering homeostatic genotypes into a hybridization programme.

First High yielding genotype NIDW-0950 exhibited above average stability for the traits days to 50% flowering, days to maturity, tillers plant-1, test weight (g), grain yield kg plot-1 indicating that it may perform well in different environments for these characters.

Second genotype NIAW-301 exhibited above average stable performance for days to 50% flowering, days to maturity, effective tillers plant-1 and spike length (cm) with better mean performance and based on the value of regression coefficient for days to 50% flowering and spike length (cm), this genotype NIAW-301 was found most stable in favorable environment, while for days to maturity and effective tillers plant-1 having the regression coefficient values less than unity indicating it's adaptability to the unfavorable (poor) environments.

Third genotype PBN-4881 showed above average stability for the traits germination per cent, spikelets spike^{-1} and grains spike^{-1} with better mean performance and values of regression coefficient, indicating that it performs well in poor environments indicating that genotype is suitable only in favorable environments for these characters.

Fourth genotype NIAWS-2595 have shown above average stability for the traits germination per cent, tillers sq. meter^{-1} and grains spike-1 with better mean performance and values of regression coefficient, indicating that for germination per cent it performs well in poor

environments whereas for tillers sq. meter^{-1} and grains spike^{-1} this genotype performs well in desirable environments.

While, genotypes NIAW-2495, NIAW-2539, AKAW-4800, PBND-5175, PBN-4876, MACS-6478 and PBND-4825 exhibited stable performance merely for two or three traits.

In present study, the PIC value ranged from 0.774 (Xgwm-136) to 0.924 (Xgwm-130) with an average of 0.871 per primer in SSR primers. The highest PIC value was found in primer xgwm-130 (0.924) followed by XPSP-2999 (0.918). High PIC value indicates high degree of polymorphism among the genotypes which interns helps to estimate genetic distance with more precision. In present study six primers showed considerable level of PIC values indicating their utility for assessment of genetic diversity. It is necessary to obtain information about genetic diversity from polymorphic primers only so that genetically divergent genotypes can be effectively identified.

This is an attempt to study the relationship between molecular diversity among sixteen wheat genotypes and its role in stability of genotypes. Following genotypes found stable across three locations (Niphad, Parbhani and Akola) viz. NIDW-0950, NIAW-301, PBN-4881, NIAW-2595 for more number of characters and exhibited more diversity in similarity matrix. While, genotypes NIAW-2495, NIAW-2539, AKAW-4800, PBND-5175, PBN-4876, MACS-6478 and PBND-4825 exhibited stable performance merely for two or three traits. Thus this information would be helpful to identify genetically diverse parents and high heterotic crosses for further breeding programme.

1 Thus these four genotypes (NIDW-0950, NIAW-301, PBN-4881, NIAW-2595) can be considered for cultivation in different environments.

2 Above four genotypes can be used as parents in hybridization programme for developing a high yielding stable hybrid/variety.

3 These six primers (Xgwm-130, Xgwm-136, Xgwm-193, Xgwm-493, Xgwm-610, XPSP-2999) can be used to study of polymorphism or molecular diversity.

CHAPTER VII

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VITA

1. Name of student : **Lokesh Kumar Verma**
2. Date of Birth : 21 August 1992
3. Name of the College : Post Graduate Institute,
   Dr. Panjabrao Deshmukh
   Krishi Vidyapeeth, Akola
4. Residential Address : VPO-Dei, The.-Nainwan, Dist.-Bundi
   (Rajasthan) Pin code-323802
5. Academic Qualification :

Sr. No.	Name of Degrees awarded	Year in which obtained	Division/ Class	Name of awarding University	Subjects
i)	B.Sc. (Agri.)	2014	First	SKRAU Bikaner (Rajsthan)	Agriculture and allied subject

6. Research papers published (if any) : - NIL
7. Field of Interest (in which you desire to work) : Research and Development

Place: Akola

Date: 21 / 06 /2016

Signature of Student

(**Lokesh Kumar Verma**)

Appendix I

Table A: Weekly Weather data for the year 2014-15 recorded at Meteorological Observatory Department of Agronomy Dr. PDKV., Akola

MW	Date	Rainfall (mm)	Rainy days	Wind Velo. (km/h)	Temperature (^{0}C)		Humidity (%)	
					Max.	Min.	Morn	Even
44	**29.10.2014**	2.3	0.2	4.1	32.7	16.0	73	32
45	5.11.	3.0	0.2	3.9	32.3	15.2	71	32
46	12.11.	5.3	0.2	3.9	31.6	14.6	73	32
47	19.11.	7.7	0.3	3.7	31.0	13.3	72	30
48	26.11.	5.5	0.3	3.6	30.5	12.8	71	32
49	3.12.	1.0	0.1	3.8	30.0	11.9	71	30
50	10.12.	0.8	0.1	3.6	29.6	10.9	71	28
51	17.12.	0.9	0.1	3.8	29.5	10.8	70	29
52	24.12.	2.6	0.2	4.5	29.1	11.1	71	30
1	**1.1.2015**	2.8	0.2	4.4	28.8	11.0	71	31
2	8.1.	3.3	0.2	4.4	29.3	11.7	71	30
3	15.1.	0.7	0.1	4.5	30.0	12.0	68	28
4	22.1.	0.9	0.1	4.6	30.6	12.0	65	26
5	29.1.	3.0	0.2	4.9	31.0	12.6	62	25
6	5.2.	3.7	0.3	5.0	31.4	12.7	59	23
7	12.2.	0.1	0.0	5.4	32.7	14.4	55	22
8	19.2.	2.5	0.2	5.7	33.4	14.5	54	21
9	26.2.	4.1	0.3	6.1	35.0	15.7	50	18
10	5.3.	5.2	0.3	6.1	35.9	17.3	46	20
11	12.3.	2.4	0.3	6.3	37.0	18.1	45	18
12	19.3.	0.6	0.1	6.4	38.4	19.3	39	15
13	26.3.	2.2	0.2	6.9	39.0	20.4	37	15
14	2.4.	1.0	0.1	7.3	40.0	21.7	37	14
15	9.4.	0.4	0.1	8.4	40.8	23.1	35	14
16	16.4.	0.5	0.1	8.6	41.6	24.1	36	14
17	23.4.	0.5	0.1	9.0	42.3	25.4	37	15
18	30.4.	0.8	0.1	10.5	42.6	26.6	39	15
19	7.5.	1.3	0.1	12.2	42.6	27.1	42	17
20	14.5.	2.8	0.4	14.2	42.5	27.7	47	19
21	21.5/27.5.15	3.8	0.4	15.1	42.1	27.8	50	20

Appendix II

Table B: Weekly Meteorological data of A.R.S., Niphad during the year 2014-15

MW	Date	Rainfall (mm)	Rainy days	Wind Velo. (km/h)	Sunshine (hrs)	Temperature (^{0}C)		Humidity (%)	
						Max.	Min.	Morn	Even
44	**29.10.2014**	0	0	4.2	9.2	31.9	14.9	75	47
45	5.11.	0	0	4.1	9.1	31.7	13.8	74	43
46	12.11.	17.2	2	3.2	5.6	29.8	19.3	80	66
47	19.11.	0	0	3	8.8	30.4	13.5	74	54
48	26.11.	0	0	3.4	8.4	30	12.8	72	42
49	3.12.	0	0	4.6	7.2	29.2	12	75	36
50	10.12.	1.2	0	3.8	7.2	28	11.7	84	30
51	17.12.	0	0	4.2	8.6	25	6.1	79	31
52	24.12.	0	0	3.8	8.6	26.2	7.5	75	31
1	**1.1.2015**	0	0	3.9	6.3	24.9	11.4	79	61
2	8.1.	0	0	3.3	9.5	27.4	5.5	68	27
3	15.1.	0	0	3.9	9.1	27.2	7.7	79	36
4	22.1.	0	0	3.6	6.8	27.1	11.3	80	40
5	29.1.	0	0	3.5	7.8	28.9	10.4	75	37
6	5.2.	0	0	3.5	7.8	28.9	10.4	75	37
7	12.2.	0	0	4	9	31.8	11	64	26
8	19.2.	0	0	5.5	8.7	33	11.1	68	31
9	26.2.	0	0	5.8	5.7	28.1	10.7	85	52
10	5.3.	0	0	4.1	8.7	31	10.7	76	48
11	12.3.	0	0	5.6	7.6	30.6	14.4	80	36
12	19.3.	0	0	4	9.7	34	15.1	72	30
13	26.3.	0	0	4.9	8.1	31.8	13.8	82	36
14	2.4.	0	0	8.6	9.4	37.6	20.2	68	28
15	9.4.	34	1	10.2	9	37.8	19.8	72	34
16	16.4.	0	0	9.8	9.6	38	20	66	24
17	23.4.	0	0	8.2	10.1	38.2	19.8	74	28
18	30.4.	0	0	7.5	9.8	38.4	20.6	78	26
19	7.5.	19	2	8.4	9.4	37.6	20.4	80	32
20	14.5.	0	0	7.2	10.2	39	21.8	64	28
21	21.5/27.5.15	0	0	3.8	9.6	37.8	22.2	68	34

Appendix III

Table C: Weekly weather data recorded at central meterology observation parbhani 2014-15

WK	Period	Rainfall (mm)	R.D.	Temperature °C		Humidity (%)		EVP	BSS (Hrs.)	W. V. (Kmph)
				Max.	Min.	AM	PM			
44	**29.10.2014**	0.0	0	34.2	13.0	74	31	7.2	9.0	2.8
45	5.11.	0.0	0	33.8	18.0	79	40	6.2	9.9	3.9
46	12.11.	0.0	0	32.0	15.4	75	18	4.7	7.9	3.3
47	19.11.	0.0	0	30.7	10.0	0	24	4.8	10.0	3.4
48	26.11.	0.0	0	31.5	11.5	84	28	4.8	0.0	2.6
49	3.12.	0.0	0	32.5	14.0	75	32	4.9	9.1	2.6
50	10.12.	0.0	0	27.5	8.3	77	27	5.5	9.3	4.7
51	17.12.	0.0	0	29.8	8.8	70	28	4.3	9.3	2.7
52	24.12.	0.0	0.0	0.0	0.0	0	0	0.0	0.0	0.0
1	**1.1.2015**	0.0	0	28.0	6.3	75	22	4.8	9.7	3.6
2	8.1.	0.0	0	29.0	8.4	76	22	5.1	9.4	5.1
3	15.1.	0.0	0	31.5	12.5	82	31	5.0	6.2	3.5
4	22.1.	0.0	0	31.0	14.0	78	38	6.0	9.2	4.1
5	29.1.	0.0	0	32.0	12.5	63	16	6.1	4.6	4.8
6	5.2.	0.0	0	33.0	14.0	72	22	6.6	9.5	3.5
7	12.2.	0.0	0	35.0	14.5	53	20	7.2	9.6	3.3
8	19.2.	0.0	0	34.5	14.9	68	19	8.0	9.6	3.4
9	26.2.	0.0	0	32.6	17.2	57	28	6.2	10.0	6.2
10	5.3.	0.0	0	31.5	19.0	63	28	6.8	9.5	5.6
11	12.3.	0.0	0	36.5	20.0	69	17	8.6	9.5	6.4
12	19.3.	0.0	0	38.5	18.5	79	18	10.0	8.1	4.0
13	26.3.	0.0	0	40.0	19.5	72	19	9.8	10.6	4.5
14	2.4.	6.0	1	38.0	22.9	98	36	9.8	9.5	5.3
15	9.4.	6.0	1	30.5	18.5	100	41	3.8	8.5	2.7
16	16.4.	0.0	0	40.0	22.0	68	20	9.8	9.6	4.0
17	23.4.	0.0	0	42.2	24.0	66	20	13.2	10.5	5.2
18	30.4.	0.0	0	40.3	22.5	65	13	9.1	10.5	3.7
19	7.5.	0.0	0	37.5	26.5	59	46	8.0	5.0	6.2
20	14.5.	0.0	0	45.0	27.0	51	11	16.0	10.9	7.9
21	21.5/27.5.15	0.0	0	44.0	27.0	45	11	16.3	10.4	7.4