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Allelic and bio-chemical characterization of Indian wheat (*Triticum aestivum*) varieties and breeding lines for quality traits

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ABSTRACT

Thirteen released and two elite genotypes of wheat (*Triticum aestivum* L.) from North-West Plain Zone of India were subjected to biochemical and molecular characterization to study the genetic variability, their grouping and identification using a combination of biochemical and molecular markers during 2014–16 at Department of Genetics and Plant Breeding, COA, CCSHAU, Hisar. HD 2967, WH 1142 and WH 1080 were overall better performing for protein content, sedimentation value, hectolitre weight and grain appearance. A total of 99 and 117 polymorphic bands were detected using ISSR and SSR primers, respectively. The similarity indices for SSR ranged from 0.64 to 0.77 while ISSR showed the range of 0.63–0.92. Present study could be effectively utilized in DNA fingerprinting, identification of wheat varieties and elite lines for bio-chemical traits and determination of seed purity.

Keywords: Allelic diversity, Biochemical traits, Characterization, Markers, wheat

Wheat (*Triticum aestivum* L.) is one of the most important nutrient-rich cereal, covering around 270 million ha world-wide. In India, wheat is cultivated on around 31.45 mha and has reached a record production of 107.59 mt with average national productivity of 3421 kg/ha (DES, MoA & FW, India 2020). Bread wheat is used for making chapati and its variants, bread, biscuit, naan, kulcha, buns, cake, pastry etc. Factors influencing wheat grain quality have been classified in two groups: Physical characters including grain appearance score, grain hardness, 1000-kernel weight, hectolitre weight (test weight), kernel size and shape and Chemical characters including protein content, protein quality and sedimentation test. Today, the most challenging task for breeders is not only to increase grain yield but also to improve the grain quality for end products to meet the requirements of ever increasing population.

The wheat varieties released for the different agro-ecological regions of the country have played an important role in enhancing production. However narrow genetic base of these varieties has led to genetic vulnerability to various biotic and abiotic stresses. Nonconventional approaches - Biochemical and DNA markers have been commonly in use. The limitation of characterization of wheat germplasm using biochemical parameters is that they are tissue and developmental stage specific (Sammour 1991) which can be overcome by molecular marker analysis. Polymerase chain reaction (PCR) based molecular markers such as RAPD, AFLP, ISSR, SSR and STS etc. have been developed into powerful tools to analyse genetic relatedness. ISSR analysis involves amplification of regions between adjacent and inversely oriented microsatellites using di, tri, tetra and Penta-nucleotides SSR primers, with the advantage that knowledge of the DNA sequence of the target regions is not needed. Co-dominant SSRs (also known commonly as microsatellites) are preferred for genotyping because of their reproducibility, abundance and amenability to high throughput screening. So, in the present investigation, characterization of wheat varieties and elite lines has been carried out by collective approach of biochemical and molecular markers for cultivar identification and to establish the genetic relationship among superior genotypes which will accelerate the future crop improvement program of wheat.

MATERIALS AND METHODS

The present study was carried out at Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar situated at the latitude of $29^{0}10'$N, the longitude of $75^{0}46'$E and altitude of 215.2 m amsl in the semi-tropical region of NWPZ. The genotypes comprised of 13 most popular bread wheat varieties (covering the majority of the wheat cultivated area of NWPZ) and two elite lines (Table 1). These 13 varieties were selected from three groups, viz. rainfed/restricted irrigation (WH 1025, WH 1080 and WH 1142); normal sown irrigated condition

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Table 1 Wheat varieties and elite lines along with their source and year of release

Genotype/ Advance line	Origin	Pedigree	Year of release
WH 542	CCS HAU, Hisar	JUP/BJY'S'//URES	1992
Raj 3765	RAU, Durgapura	HD2402/VL639	1996
PBW 343	PAU, Ludhiana	ND/VG1944//KAL//BB/3/YACO's'/4/VEE5's'	1996
WH 711	CCS HAU, Hisar	ALD'S'/HUAC//HD2285/3/HFW17	2002
DBW 17	IIW&BR, Karnal	CMH79A.95/3*CN079//RAJ3777	2007
PBW 550	PAU, Ludhiana	WH594/RAJ3856//W485	2008
WH 1021	CCS HAU, Hisar	NY0T95/SONAK	2008
HD 2967	IARI, New Delhi	ALD/CUC//URES/HD2160/HD2278	2010
WH 1025	CCS HAU, Hisar	C591/PBW231	2010
DPW 621-50	IIW&BR, Karnal and PAU, Ludhiana	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/ HUITES	2011
WH 1080	CCS HAU, Hisar	PRL/2*PASTOR	2011
WH 1105	CCS HAU, Hisar	MILAN/S87230//BABAX	2013
WH 1142	CCS HAU, Hisar	CHEN/Ae.Sq.(TAUS)//FCT/3/2*WEAVER	2015
WH 1081	CCS HAU, Hisar	PBW65/2*PASTOR	Elite line
WH 1164	CCS HAU, Hisar	RL6043/4*NAC//2*PASTOR	Elite line

(PBW 550, PBW 343, DPW 621-50, HD 2967, DBW 17, WH 542, WH 711 and WH 1105) and late sown irrigated condition (RAJ 3765 and WH 1021) sown in a randomized block design (RBD) in four rows of 2.5 m lengths and spaced 20 cm apart in *rabi* season of 2014-2015. Recommended tillage and plant protection practices were followed.

Biochemical and Allelic Characterization (2014–2016): Each grain sample was tempered to constant moisture content (14.0%) for 12 h before estimating the quality parameters. The parameters such as protein content, sedimentation value (ml), hectolitre weight (g) and grain appearance index (%) were estimated using standard procedures.

Protein content: It is an important quality parameter for making different products of wheat. More than 12.0% protein is required for making good quality pasta products. Grain Protein content analysis was done at IIBWR, Karnal at 14% grain moisture level by Near Infra- Red Spectrophotometer.

Sedimentation value (ml): The observation was recorded in triplicate manner for each genotype in each replication and averaged by using wheat flour. It was determined as (Axford *et al*. 1979);

(a) SDS/Lactic acid reagent was prepared by mixing 20% SDS (w/v), 80% lactic acid (v/v) and distilled water in ratio of 20:1:8.
(b) Whole meal (6 g) was added to 50 ml water in a 100 ml cylinder, a stopwatch was started and the material dispersed by rapid shaking horizontally for 15 sec. The contents of the material were again shaken for 15 sec at 2 min and at 4 min. immediately after the last shake, 50 ml of SDS lactic acid reagent was added and mixed by inverting the cylinder 4 times. Inversion (four times) was repeated at 6, 8 and 10 min. The contents of the cylinder were allowed to settle for 20 min. and the sedimentation volumes were read.

Hectolitre weight: Hectolitre weight (or weight per unit volume) is the weight of 100 litres of wheat and it is expressed as kg/hectolitre. Higher the hectolitre weight, the better is the flour yield. The factors affecting the hectolitre weight are kernel shape, uniformity of kernel size, density of the grain influenced by structure and its chemical composition.

Procedure; Scale-stand and pouring cylinder was fixed on the wooden casing; Pouring cylinder was filled with wheat grains; Slit was opened so that the filling cylinder gets filled with wheat grains; Wheat grains levelled using a scale; The filling cylinder containing wheat-grains hanged on the scale-stand; Balance the filling cylinder by moving the weight on scale beam; Reading noted on the scale beam; Test carried out in triplicates for each wheat sample; Average value expressed in kg/hl.

Grain appearance index: It is a subjective test and an important parameter in grain trade. For this grain size, shape, soundness, colour and lustre are collectively taken into consideration. We judge the grain appearance out of total score of 10.0.

Grain size: Bold and Plump

Grain colour: Amber to White

Grain texture: Hard, Vitreous, Lustrous

Molecular characterization: Genomic DNA of genotypes was isolated from young leaf tissues (at the seedling stage) using Cetyl-trimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1990). Quantity and quality of purified DNA were verified using spectrophotometry and 0.8% agarose gel electrophoresis respectively. Forty ISSRs and seventy SSRs primers were used for characterization of wheat varieties and elite lines. PCR amplified DNA products were resolved by submerged horizontal electrophoresis in 2.5% (w/v) agarose gels. The amplification profiles of ISSRs

& SSRs were scored visually based on presence (taken as 1) and absence (taken as 0) of bands of DNA on agarose gel. The 0/1 matrix was used to calculate similarity index and genetic distance using 'sim-qual' sub-program of software NTSYS–PC (Rohlf 1990). The dendrogram was constructed by using distance matrix by the unweighted pair-group method with arithmetic average (UPGMA) sub-programme of NTSYS-PC 2.0.

RESULTS AND DISCUSSION

Biochemical parameters: The genotypes were grouped into three categories based on their protein (%) content. Three cultivars, namely, WH 1164, RAJ 3765, and PBW 550 had low, five namely, DBW 17, WH 711, WH 1025, WH 1105, and WH 542 had medium and remaining seven cultivars WH 1142, HD 2967, PBW 343, WH 1021, WH 1080, DPW 621-50 and WH 1081 had high protein content comparatively. Sedimentation values were high (>55 ml) for the genotypes WH 1080, WH 1105, WH 1164 and HD 2967. Based on hectoliter weight, genotypes were grouped into two categories, first group (<80 kg/hl) comprised RAJ 3765, WH 542, WH 711, WH 1025, WH 1080, DBW 17, HD 2967 and PBW 343 while the second group comprised WH 1021, WH 1081, WH 1105, WH 1142, WH 1164, PBW 550 and DPW 621-50 (>80 kg/hl) similar results reported in IIWBR Progress Report, 2016-17, Quality Vol. IV. Grain appearance was found to be better for the genotypes WH 1164, WH 542, WH 711, WH 1105, WH 1142, PBW 343 and HD 2967. Wheat differs for quality traits, which specify end-use. Development of cultivars with specialized end uses is of prime importance in the modern era of breeding.

Protein percent shows little variation in the genotypes, but enough to differentiate the genotypes into three major groups, viz. low (<11%), medium (11-12%) and high (>12%). The importance of wheat seed storage proteins (components of the gluten fraction which confers visco-elasticity to dough) in determining dough properties and bread-making quality has long been recognized (Shewry *et al*. 2003). Sedimentation value which measures gluten strength ranged from 34 ml to 57 ml. For making good quality bread, chapatti and biscuit, the required sedimentation values lie in the group with >60 ml, 30-60 ml and <30 ml respectively (Sheoran *et al*. 2015).

All the genotypes in the present study had sedimentation value between 30–60 ml, hence, fit for chapatti making. Grain appearance score was a subjective test to collectively rate size, shape, soundness, colour and texture out of total score 10. Grain appearance value ranged from 5.4–6.3. Two genotypes (WH 1025 and WH 1164) have long kernel length (>7 mm) and breadth (>3.5 mm), whereas, rest of genotypes had lesser values. In the low and medium protein group WH 1164 and WH 1105 had high sedimentation value, hectolitre weight and better grain appearance. HD 2967, WH 1142 and WH 1080 were overall better performing. Significant differences for quality traits were also earlier reported by Singh *et al.* (2018) in 35 wheat genotypes.

Molecular marker analysis: Forty ISSRs and seventy SSRs markers were used in the present study. Out of these seventeen and fifty markers were found to be polymorphic respectively. Both types of markers revealed high genetic diversity in the studied genotypes. Genetic similarity coefficients for ISSR markers ranged between 0.63–0.92 and averaged 0.77 while for SSRs, it ranged between 0.64–0.77 with an average of 0.70.

ISSR marker-based polymorphism: A total of 17 polymorphic ISSR markers were polymorphic in selected 15 wheat genotypes. These 17 markers generated 99 polymorphic alleles of different sizes. The number of alleles ranged from 3 (for ISSR 814) to 14 (for ISSR 844) with an average of 8.5 alleles per marker. The overall size of amplified PCR products ranged from 200-1500 bp. The PIC values varied from 0.34 to 0.88 with an average of 0.67. The MI values ranged between 1.02 and 10.5 with an average of 2.88. EMR varied from 2 to 12 with a mean value of 4.05. The primers which showed higher polymorphism had higher EMR values. The NTSYS-PC UPGMA cluster tree analysis led to the grouping of 15 genotypes at a similarity coefficient of 0.63 (Fig 1) in such a way that Group II contained only one genotype WH 1025 which falls alone to rest of 14 genotypes (Group I). The group I was broadly divided into two clusters at a similarity coefficient of 0.66. Cluster I included 12 genotypes (DPW 621-50, WH 1105, PBW 550, WH 1080, WH 1081, PBW 343, WH 1142, WH 711, DBW 17, HD 2967, WH 542 and RAJ 3765) while cluster II had only 2 genotypes (WH 1021 and WH 1164). Cluster I was further bifurcated into two sub-clusters at similarity coefficient 0.75. Sub-cluster 1 and sub-cluster 2 included 10 and 2 genotypes respectively. In sub-cluster-1, genotypes WH 1080 and WH 1081 were found to be highly similar toa similarity coefficient of 0.92. WH 1025 and DPW 621-50 were found to be the most diverse genotypes in the group.

SSR marker analysis: Fifty polymorphic SSR markers generated 117 alleles of varying sizes in selected 15 wheat genotypes. The number of alleles ranged from one to four with an average of 2.34 alleles per locus. Drikvand *et al*. (2015) reported an average of 2.36 alleles per locus in a study of genetic diversity assessment in durum wheat using SSR markers. The overall size of amplified fragments ranged from 100-400 bp. The PIC values varied from 0.01–0.78 with an average of 0.41. The MI values ranged between 0.01 and 3.78 with an average of 1.25. EMR varied from 1.0 to 5.0 with a mean value of 2.60. The NTSYS-PC UPGMA cluster tree analysis led to the grouping of selected genotypes into two groups at a similarity coefficient of 0.64 in such a way that one group included 14 genotypes while second had only one genotype WH 1164 (Fig 2). Group one was further divided into two clusters. Cluster I included 12 genotypes while cluster II had only 2 genotypes DBW17 and PBW 343. Cluster I was further divided into two sub-clusters at a similarity coefficient of 0.66 in such a way that sub-cluster I-1 included 11 genotypes while sub-cluster I-2 had only one genotype WH 711. Sub-cluster I-1 further partitioned into two sub-clusters A and B. Sub-cluster A included 9

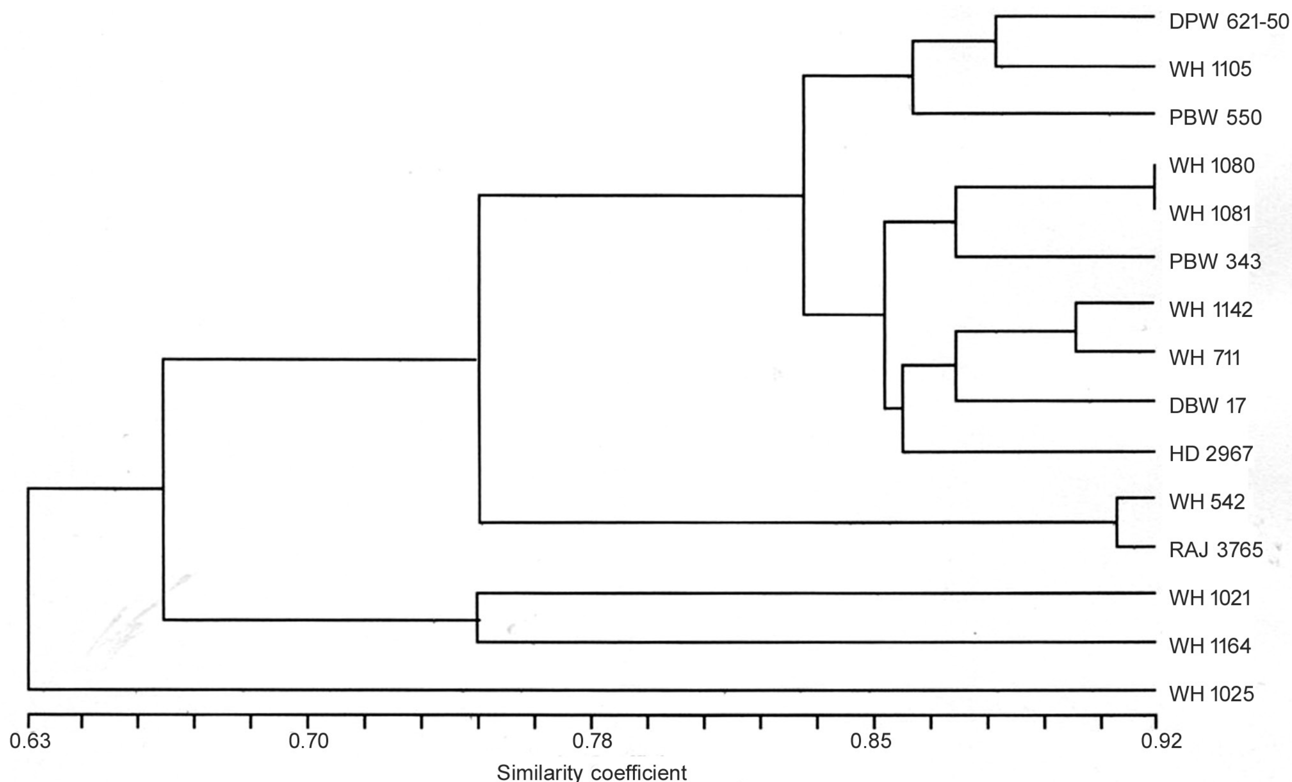

Fig 1 Dendrogram depicting molecular diversity using ISSR markers among 15 bread wheat genotypes.

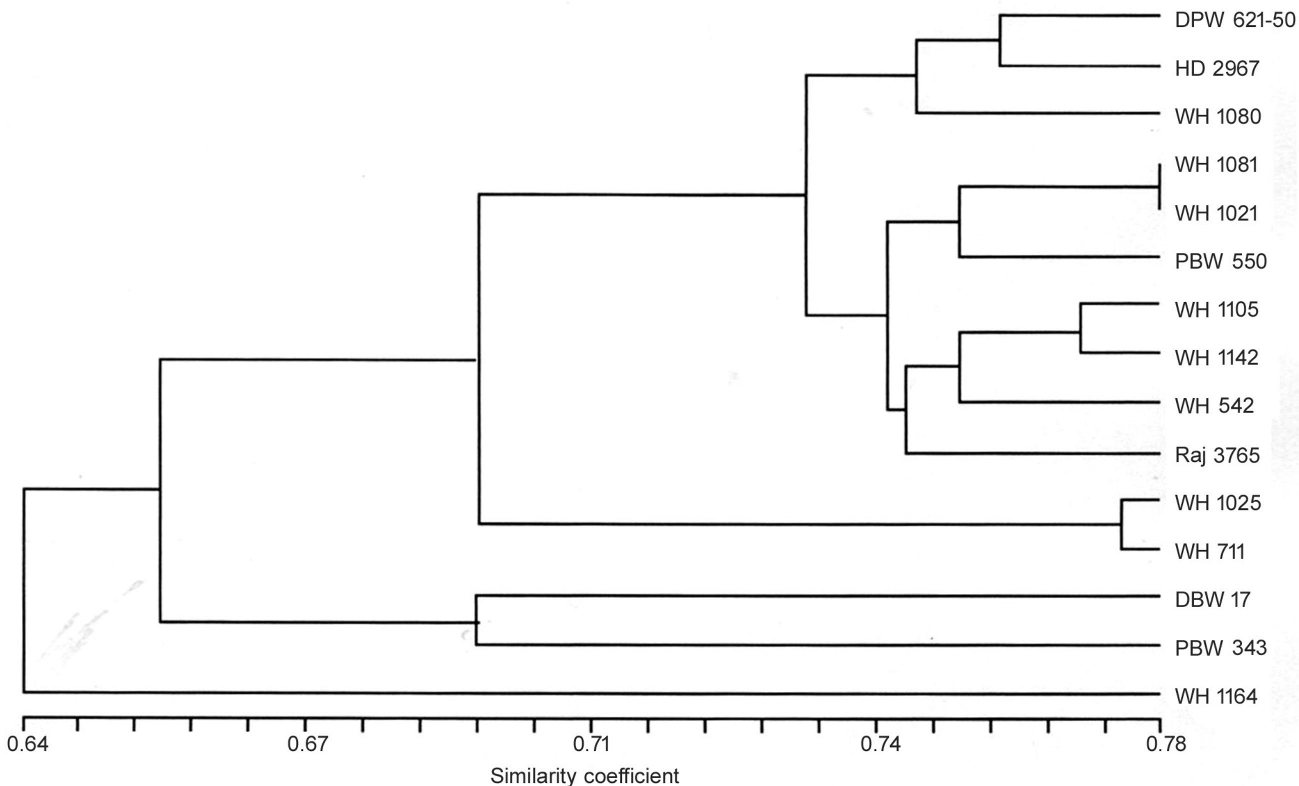

Fig 2 Dendrogram depicting molecular diversity using SSR markers among 15 bread wheat genotypes.

genotypes while sub-cluster B had 2 genotypes Raj 3765 and WH 1025. Sub-cluster A was further bifurcated into two major parts. Part 1 of sub-cluster A comprised of 4 genotypes (DPW 621-50, HD 2967, WH 1080 and WH 1081) while part 2 of sub-cluster A included 5 genotypes (WH 1021, PBW 550, WH 1105 and WH 1142). Most

Table 2 List of SSRs producing unique bands in different genotypes

Genotype	Primer (s) and chromosomal location
DPW 621-50	wmc 517 (7B), gwm 133
WH 1080	psp 3000
DBW 17	barc 7, gwm 498, gwm 617.2
WH 1021	wmc 527 (3A), wmc 517 (7B)
Raj 3765	gwm 425
WH 711	wmc 517 (7B)
WH 1105	psp 3000
WH 1081	gwm 325, wmc 312 (1A)
WH 1025	gwm 413, wmc 312 (1A), gwm 368
PBW 343	wmc 312 (1A)
WH 1164	gwm 33

diverse genotypes were WH 1164 and HD 2967 with least similarity coefficient of 0.77. No parentage was found common in their ancestry which further provided enough evidence of their high divergence.

Principal component analysis (PCA) based on SSR data showed similar clustering of the 15 genotypes as was evident from cluster tree analysis. Since their introduction, ISSR and SSR markers, individually or in combination with other markers, have been broadly utilized for variability estimation of wheat genotypes (Teklu *et al*. 2007 and Prakash *et al*. 2015). Both SSR and ISSR markers were highly polymorphic and could reflect real genetic relationships among wheat accessions. These markers were potentially useful to identify cultivar particularly 18 SSRs which produced unique bands were able to discriminate 10 genotypes among the group. Both the markers used in the study revealed a high level of genetic diversity among the wheat genotypes. The PIC (Polymorphism Information Content) values for the ISSRs varied from 0.34 to 0.88 while for SSRs it varied from 0.01 to 0.78. The MI (Marker Index) could be considered as an overall measure of marker utility. EMR (Effective Multiplex Ratio) is the product of the fraction of polymorphic bands and the number of polymorphic bands and MI is the product of Polymorphism Information Content and Effective Multiplex Ratio, therefore, the higher polymorphism provides higher EMR. The values for these traits were high for ISSR than SSR. It may be due to that the number of polymorphic ISSRs and SSRs used in the study was highly different and it could be attributed that the ISSR markers used in this study were highly informative. Efficiencies of marker systems for detecting DNA polymorphism in wheat for different traits have earlier been reported by Ayala *et al*. (2016), Zhang *et al*. (2016) and Phougat *et al*. (2020). In most studies, it has been found that microsatellite markers are most used and popular for different applications in wheat breeding due to their high level of polymorphism and easy handling.

Unique bands in 15 wheat genotypes: Some SSR primers gave unique bands in specific wheat varieties (Table 2). These primers produced a specific allele which distinguished one genotype from the others. Out of 50 polymorphic SSRs used in this study, 18 SSR primers produced a total of 18 unique alleles. Unique alleles were observed in the genotypes WH 711, WH 1021, WH 1025, WH 1081, WH 1105, WH 1164, DBW 17, Raj 3765, DPW 621-50 and PBW 343. While, no unique allele was found for the variety PBW 550, HD 2967, WH 542, WH 1080 and WH 1142.The maximum number of unique alleles (3) were observed in the genotypes DBW 17 and WH 1025.

Biochemical and molecular markers successfully categorized the different wheat genotypes into various groups. HD 2967, WH1142 and WH 1080 were overall better performing for protein content, sedimentation value, hectolitre weight and grain appearance. A total of 99 and 117 polymorphic bands were detected using ISSR and SSR primers, respectively. Furthermore, SSR primers produced unique alleles in 15 genotypes and can be used to distinguish the genotypes. Clustering of genotypes was not according to their ecological geographical pattern. The results generated in the present study successfully categorizes wheat varieties and elite lines into distinct groups and also establishes relatedness among them based on the genetic diversity for evaluated quantitative characters but no correlation was found between the variations. A single marker system is not able to distinguish all the genotypes individually, but a combination of various markers provide important information for varietal identification, quality seed production, evaluating genetic diversity, parental selection will be helpful in further augmentation of wheat improvement programs of the country.

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