https://doi.org/10.56093/ijas.v90i4.102146

Research Articles

Indian Journal of Agricultural Sciences **90** (4): 675–80, April 2020/Article

Micronutrient specific SSR marker based diversity analysis for developing short duration wheat (*Triticum aestivum*)

DIVYA PHOUGAT* and S K SETHI

Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana 125 004, India

Received: 15 March 2017; Accepted: 30 August 2019

ABSTRACT

Malnutrition leads to high mortality especially in developing countries. Present study comprised 11 high yielding wheat (*Triticum aestivum* L.) genotypes sown during *rabi* 2014-15 and 2015-16 and crosses attempted during 2014-15 and sown at Research Field of CCS Haryana Agricultural University, Hisar, Haryana. Forty nine microsatellite markers linked with identified QTLs for micronutrient were used for the genetic diversity assessment among bread wheat parents their F_1s. Out of 49, 38 SSRs were found polymorphic which amplified a total of 76 alleles with an average of 2.00 alleles per locus. The range of amplified PCR products ranged from 100 to 480 bp. Estimates of similarity coefficient among parents and F_1s suggest enough divergence and averaged to 0.71. Enough genetic variability was observed for all the 14 agronomic traits in all genotypes. Selection of genotypes from different clusters helps in making proper choice of breeding programme. In the present study, the information generated about genetic diversity will be useful for selection of parents for breeding including gene mapping and ultimately for marker assisted selection (MAS) for micronutrient content improvement worldwide.

Key words: Diversity, Malnutrition, Micronutrient, Microsatellites markers

Wheat is the most widely grown cereal grain, occupying 17% of the total cultivated land and it alone makes up two-thirds of the world's food energy intake, provides one-fifth of the globally required calories (Reynolds *et al*. 2011, Friedrich *et al*. 2014). Two billion people are estimated to be deficient in one or more micronutrients leading to micronutrient deficiency. Over 30% of the world's population – are anemic. In many micronutrient-deficient regions, >50% of the food in the diet was wheat based (Cakmak *et al*. 2010). Iron (Fe) and zinc (Zn) are the two mineral elements mostly found deficient in human diets (White and Broadley 2009, Stein 2010). Current level of Zn and Fe in the cereal grains is only 10-30 mg/kg; to meet RDA of Zn or Fe for human beings, it should range between 40-60 mg/kg. The nutritional composition of staple crops, especially micronutrients and protein quality, is important but often overlooked. Generation of micronutrient-dense wheat varieties through the use of biotechnology or the breeding for wheat with high grain Zn and Fe concentration is a promising and cost-effective strategy to alleviate micronutrient malnutrition (Phougat and Sethi 2019). Emphasis has been laid on the screening of genotypes having Fe and Zn content by making use of molecular markers as they are independent of the influence of environment, do not exhibit epistatic interactions, provides more reliable estimates of genetic distances (Tatikonda *et al*. 2009).

Microsatellite markers, term coined by Litt and Lutty (1989), also known as Simple Sequence Repeats (SSRs), are sections of DNA, consisting of tandemly repeating mono-, di-, tri-,tetra- or penta-nucleotide units distributed unevenly in the genomes of prokaryotes and eukaryotes (Asp *et al.* 2007). These co-dominant markers are excellent for studies of population genetics and mapping (Goldstein and Schlotterer 1999). The present study was done on bread wheat genotypes and biofortified F_1s derived from them to assess genetic diversity at morphological and molecular level by screening micronutrient specific SSR markers and to identify most diverse donors for their utilization in hybridization programme aiming to improve yield and micronutrient content simultaneously.

MATERIALS AND METHODS

Present study involved genetic diversity assessment of 11 high yielding bread wheat (*Triticum aestivum* L.) genotypes whose pedigree has been mentioned in Table 1 and 22 biofortified F_1s derived from them. The parents were grown during *rabi* 2014-15 and 2015-16 under late sown environments in 2.5 m long paired rows in Randomized Block Design (RBD) in three replications with row to row distance of 25 cm while plant to plant distance was 15 cm at Research Field of CCS Haryana Agricultural University,

---

*Corresponding author e-mail: divyaphougat786@gmail.com

Table 1 Pedigree of bread wheat genotypes

Genotype	Parentage Detail
WH 1080	PRL/2PASTOR
HD 2967	ALD/COC//URES/HD2160M/HD2278
WH 1063	BARBET 1 Selection
DPW 621-50	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES
WH 1127	RL6043/4/NAC//PASTOR/3/BABAX
WH 1136	NI 5663/RAJ 3765
WH 1142	CHEN AEGILOPS SQUARROSA(TAUS)//FCT/3/2*WEAVER
HD 3086	DBW 14/HD 2733// HUW 468
WH 1105	MILAN/S87230//BABAX
DBW 88	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES
WH 542	JUP/BJY“S”//URES

Hisar, Haryana situated at a latitude 29°10′N, longitude of $75^0$46′E and altitude of 215.2 m amsl in semitropical region of North Western Plain Zone of India. Total 22 F_1 crosses were attempted using the parents during 2014-15 which were sown during 2015-16. All recommended agronomic practices were followed to raise a good crop. Data was recorded for yield and attributing traits of parents for two consecutive years (2014-15 and 2015-16) and F_1s for 2015-16. For each entry, i.e. parents as well as F_1s, five competitive plants were randomly selected from each replication and observations were recorded for 14 metric traits, viz. days to heading, days to maturity, flag leaf length (cm), flag leaf breadth (cm), plant height (cm), peduncle length (cm), spike length (cm), tiller per meter, spikelet’s per spike, grains per spike, 1000-grain weight (g), biological yield per plant (g), harvest index (%) and grain yield per plant (g). Euclidean cluster analyses (Spark 1973) was applied to assess genetic diversity and grouping of parents on the basis of minimum genetic distance using Wards (1963) minimum method.

Young leaves from parents and individual crosses were collected, lyophilized and ground in liquid nitrogen using a pestle and mortar and genomic DNA was extracted using the modified CTAB method (Saghai-maroof *et al.* 1984). DNA was dissolved and diluted to a final concentration of 20 ng/µl with 1X TE buffer (10 mM/l Tris-HC1, *p*H 8.0; 1 mM/l EDTA, *p*H 8.0) and stored at -20ºC for further use. Forty-nine micronutrient specific SSR markers known to link with known QTLs synthesized from SIGMA OLIGA, India, were used for genetic diversity assessment (Sequence available at Grain gene 2.0e). Polymorphism information content (PIC) [$PIC=1-\Sigma Zi^2$] was calculated as per formula given by Nei *et al.* (1983). Resolving power (Rp) was determined as per formula by Prevost and Wilkinson (1999). The PCR protocol consisted of an initial denaturation at 94ºC for 4 min, followed by 35 cycles of 94ºC for 1 min, annealing at 50-66ºC for 60 sec, 72ºC for 1 min, and a final extension step of 72ºC for 10 min. All PCR reactions were carried out in Applied Biosystem Veriti Thermal Cycler. Amplification products were resolved by electrophoresis on 3% agarose gel (Genetix, Biotech Asia Pvt. Ltd.) at 90V for 2 h, visualized by ethidium bromide staining, and gel photograph taken by Gel documentation system (Syngene Ltd., USA). Bivariate data 1-0 (1-presence and 0-absence) were used to estimate genetic distances. Genetic similarity between two genotypes was calculated using the Jaccard similarity coefficient (Jaccard 1908) and dendrogram was obtained by clustering according to the un-weighted pair group method with arithmetic average algorithm using the NTSYS-pc software version 2.02 (Rohlf 1992). The analysis was performed using standard procedure.

RESULTS AND DISCUSSION

Genetic diversity analysis provides opportunity for plant breeders to develop new and improved cultivars with desirable characteristics. Thirty three genotypes (11 parents and 22 F_1s) were screened with different micronutrient specific SSR markers such as *xwmc, dupw, xbarc, xgdm, xcfd* and *xcfa*. Out of 49 SSRs used for genetic diversity study, 38 were found polymorphic and amplified a total of 76 alleles with an average of 2.00 alleles per locus. Six were not amplified at all while 5 SSRs gave monomorphic bands. The average number of fragments was 2.0 per marker with number of amplified bands ranging from 1 to 4. Similar results were also reported in previous studies (Drikvand *et al*. 2015 and Phougat *et al*. 2018). Banding pattern of bread wheat parents and their F_1s using primer *xbarc* 220 was observed. The size of PCR products ranged between100-480 bp with an average of 290 bp. The lowest size belonged to *xgdm, xcfd 22, xcfd* 211 (100 bp) and the biggest (480 bp) to *xbarc* 6. Similar PCR product range (100-475 bp) was obtained by Phougat *et al*. (2018) during genetic diversity evaluation of wheat cultivars and advance breeding lines using SSRs.

PIC value provides information on allele diversity and frequency among different genotypes and can be evaluated for each SSR marker on the basis of number of alleles amplified (Table 2). PIC values showed variation for all the SSR loci tested. Eight genotypes generated single allele and their PIC value was zero. PIC value of remaining 30 markers ranged from 0.06 (*xbarc 184 and xbarc1055)* to 0.66 (*xcfa 2040)* with an average of 0.33 indicating enough genetic diversity among the genotypes. SSR markers have a high level of PIC in comparison to other types of molecular markers. Henkrar *et al*. (2016) in their study reported that the PIC values ranged from 0.07 to 0.84 which is slightly higher than the values obtained in this study. Botstein *et al*. (1980) reported that PIC value 0.5 > PIC > 0.25 is an informative marker. The Rp of the 38-microsatellite markers ranged from 0.16 (xcfa2158) to 6.32 (xcfa2040) (Table 2). The markers with the high Rp values were more informative as they were able to distinguish more number of genotypes. Sharma *et al*. 2010 reported that the resolving power (Rp) ranged from 0.16 to 0.7 (Xgwm165) with an average of 0.43 per primer while assessing genetic diversity

Table 2 Information on 38 micronutrient specific SSR markers found polymorphic

Primer	Chrom. Loc./ Motif	Anneal. Temp.	Allele	Range of allele sizes (bp)	P/M	PIC	RP
Barc 105	7D	58	2	150-160	P	0.44	2.24
Barc 1055	6A	59	2	170-180	P	0.06	2.56
Barc 108	7A	62	2	170-180	P	0.49	2.40
Barc 118	6A	61	2	120-130	P	0.47	1.28
Barc 114	2B	56	2	150-170	P	0.47	2.80
Barc 1150	(ATT)16	61	3	120-200	P	0.54	3.44
Barc 124	2A	53.3	2	220-280	P	0.49	2.96
Barc 141	5A	57	2	260-280	P	0.21	2
Barc 146	6A	58	2	150-160	P	0.33	2.72
Barc 165	5A	62	2	200-220	P	0.47	2.72
Barc 170	4A	60	2	180-190	P	0.50	2.24
Barc 180	5A	57.3	1	200	P	0.00	1.76
Barc 184	4A	63	2	200-220	P	0.06	2.40
Barc 186	5A	63	2	200-220	P	0.33	1.92
Barc 199	4B	56	2	240-280	P	0.50	2.00
Barc 201	6A	58	1	180	P	0.00	2.95
Barc 216	5B	54	1	170	P	0.00	1.84
Barc 220	2A	56	3	120-180	P	0.65	2.16
Barc 241	(ATT)17	59.3	2	140-160	P	0.50	4.40
Barc 008	1B	54	2	100-110	P	0.14	2.16
Barc 60	4B	56	2	250-300	P	0.41	1.92
Barc 48	4D	54	1	300	P	0.00	0.64
Barc 6	3D	54	2	450-480	P	0.24	2.32
Barc 90	2D	65	1	180	P	0.00	2.16
Barc 98	4D	52.5	1	170	P	0.00	2.40
Cfa2040	7B	54	3	180-220	P	0.66	6.32
Cfa2104	5A	58.1	2	300-320	P	0.50	4.00
Cfa2114	6A	54	2	210-220	P	0.19	2.24
Cfa 2158	1A	52	1	140	P	0.00	0.16
Cfa 2164	2A	61	3	220-290	P	0.47	3.20
Cfa 2262	3A	57.3	2	120-160	P	0.48	1.76
Cfd 19	1D	59	2	200-220	P	0.48	2.24
Cfd 211	6B	60	2	100-200	P	0.50	4.40
Cfd 28	3B	56	1	150	P	0.00	2.16
Cfd 267.1	2A	59	3	280-310	P	0.65	4.24
Cfd 80.2	6A	53	2	130-190	P	0.50	4
Dupw 08	-	53	4	190-310	P	0.60	2.72
Gdm 14.2	6D	59.3	3	100-145	P	0.65	5.20

using 10 microsatellite markers which is very low than the average RP value (3.24) obtained in present study. This shows that microsatellite markers used in present study are more informative and able to distinguish the wheat cultivars efficiently.

The estimates of a similarity coefficient ranged from 0.59 to 0.84 with an average of 0.71 which suggests that genotypes were diverse (Fig 1). NTSYS-UPGMA divides the 11 genotypes broadly into two clusters at similarity coefficient of 0.59. Highest similarity coefficient value (0.84) was between DPW 621-50 and WH 1105 and between DPW 621-50 and WH 1136 while the lowest (0.52) was observed between DPW 621-50 and DBW 88. Cluster I include HD 3086, HD 2967 and DBW 88 while cluster II contains all WH series varieties along with DPW 621-50. This may be ascertained that these genotypes

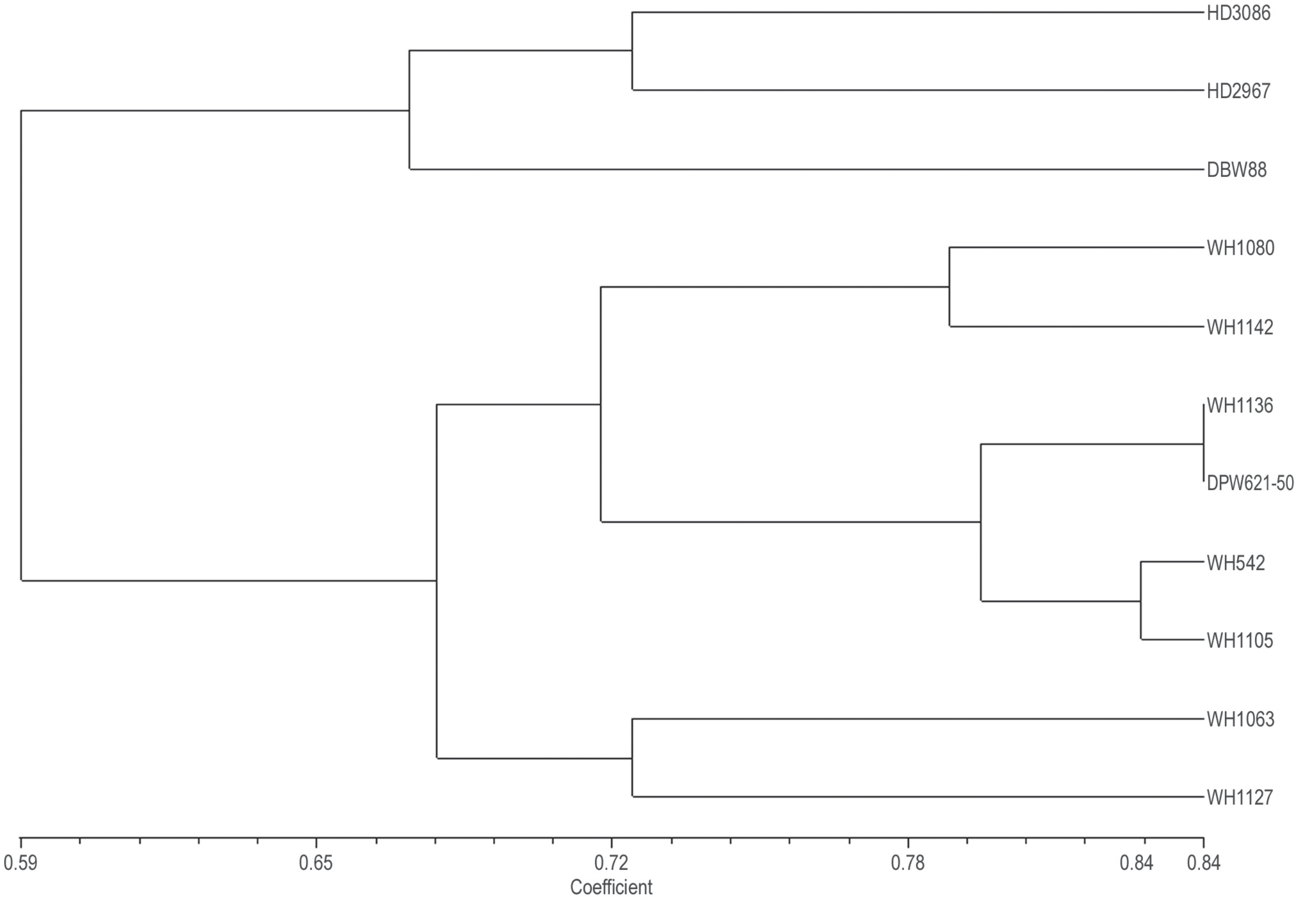

Fig 1 Dendrogram depicting genetic diversity among the parents using micronutrient specific SSR markers.

have some common parentage in the ancestors. Cluster analysis based on binary data obtained during molecular characterization of the genotypes revealed that HD 2967 and WH 1127 were most dissimilar genotypes. Cluster II was further divided at the similarity coefficient of 0.665 into two sub-clusters. Sub cluster IIA contains WH 1142, WH 1080, WH 1136, DPW 621-50, WH 542 and WH 1105 while sub-cluster IIB contains WH 1063 and WH 1127 which are high Fe and Zn content lines. Hence, the clustering pattern obtained by micronutrient specific SSR markers clearly puts the high micronutrient lines in separate cluster which proves the power of these SSR markers in diversity assays. Similar coefficient value 0.18 to 0.95 was observed by Drikvand *et al*. (2015). Utility of SSR markers for diversity assays has been reported by Drikvand *et al*. (2015) and Phougat *et al*. (2018). The estimates of a similarity coefficient between F_1s ranged from 0.36 to 0.86 with the average value of similarity coefficient around 0.66 (Fig 2). The similarity coefficient value obtained in present study for both parents and F_1s was found nearly similar. NTSYS UPGMA divides the 22 F_1s into two broad clusters, cluster I had 17 F_1s while cluster II had remaining 5 F_1s. WH 1080/WH 1142 and WH 1063/DPW 621-50 were the most diverse F_1s with maximum dissimilarity value of 0.53 while WH 1136/WH 542 and WH 1136/WH 1142 were the most similar F_1s, may be due to one common genotype WH 1136 involved in the cross. Hence, 38 polymorphic micronutrient specific microsatellite markers have clearly grouped the F_1s into different clusters based on the parent involved in the cross.

Quantification of genetic divergence between the parents and F_1s was performed using Euclidean cluster analysis and the constellation of clusters was done following Ward's (1973) minimum variance method. The 11 bread wheat parents were divided into 5 clusters. The cluster III and V were the largest cluster comprising 3 genotypes while cluster I and II had two genotypes and cluster IV had only one genotype. Cluster mean values of parents based on agronomic traits using pooled data (2014-15 and 2015-16) were calculated. Cluster V contained the genotypes which have higher cluster mean values for number of desirable traits, viz. flag leaf length (26.0), plant height (80.3) (dwarf wheat), spike length (12.3), spikelets/spike (20.7), biological yield per plot (5700), days to maturity (115.0) which gives longer grain maturity duration, 1000-grain weight (43.1), grain yield per plot (2398) and harvest index (42.0%). Cluster IV comprises of genotypes with above average cluster means for flag leaf length, plant height, peduncle length, harvest index and above average cluster mean values for tiller per plant, days to heading and maturity, 1000-grain weight and Cluster III comprised genotypes with highest cluster mean value for spikelets/spike, grains/ear, tillers/

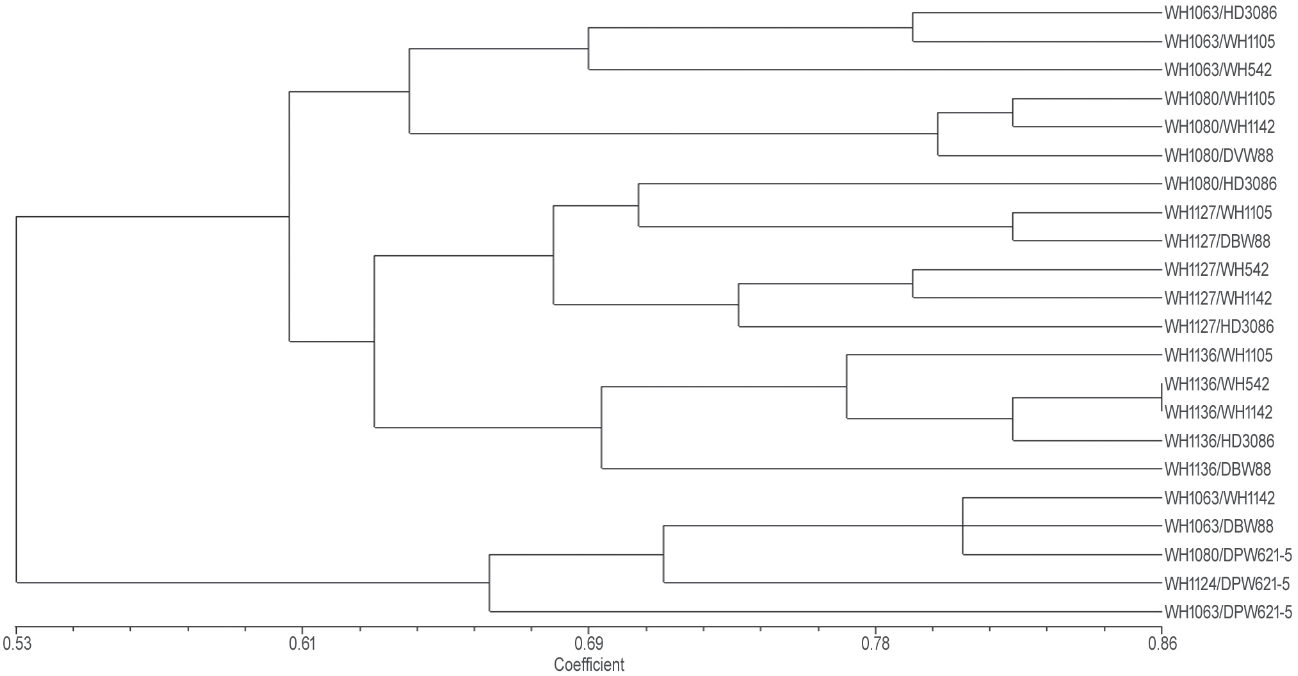

Fig 2 Dendrogram depicting genetic diversity among the biofortified F_1s using micronutrient specific SSR markers.

plant and appropriate harvest index. Selection of traits from these clusters could be helpful to breeder for success of a hybridization programme. Clustering pattern and grouping of F_1 crosses depicted that cluster I and V were the largest, each containing 6 genotypes while cluster IV contained 4 genotypes and both cluster II and III contained only 3 genotypes. Cluster means of F_1 crosses based on agronomic traits was determined. Cluster I comprised F_1crosses with highest peduncle length (14.9 cm) and 1000-grain weight (g) while cluster II had the crosses that could be utilized for traits like tiller per plant, days to heading and maturity, 1000-grain weight and grain yield/plot. Crosses from Cluster V could be selected for traits like flag leaf length and breadth, spike length, grains/ear, biological and grain yield per plot, heading and maturity days and harvest index. Selection of crosses from distinct clusters for specific traits could be helpful in getting transgressive segregants in advance generations and their incorporation in three way or double crosses can be useful in hybridization programme. It is clear from the phenotypic as well as from molecular profiling of genotypes using SSR markers that, there was sufficient diversity among the parents and F_1s used in this study.

Present results confirmed the hypothesis that the relationship of parents placed F_1s into the same group on the basis of common alleles. Further, the molecular genetics assay managed to throw more light on the inherent diversity among the studied bread wheat parents and F_1s which will be useful for identifying the genotype varieties having maximum variation for micronutrient concentration for short duration biofortified wheat and selected varieties/crosses will be useful for further studies aiming toward developing short duration biofortified wheat variety.

REFERENCES

Asp T, Frei U K, Didion T, Nielsen K K and Lübberstedt T. 2007. Frequency, type, and distribution of EST-SSRs from three genotypes of *Lolium perenne*, and their conservation across orthologous sequences of *Festuc arundinacea, Brachypodium distachyon,* and *Oryza sativa*. *BMC Plant Biology* **7**: 36.

Botstein D, White R L, Skolnick M and Davis R W. 1980. Construction of a genetic linkage map in man using restriction Fragment Length Polymorphisms. *American Journal of Human Genetics* **32**: 314–31.

Cakmak I, Pfeiffer W and McClafferty B. 2010. Biofortification of durum wheat with Zn and Fe. *Cereal Chemistry* **87**: 10–20.

Drikvand R, Najafian G, Elham and Salahvarzi. 2015. Investigation of genetic diversity of some durum wheat genotypes using SSR markers. *Journal of Biodiversity and Environmental Sciences* **6**(3): 24–32.

Friedrich C and Longin HandReif C J. 2014. Redesigning the exploitation of wheat genetic resources. *Trends in Plant Sciences* **19**(10): 631–36.

Goldstein D B and Schlotterer C. 1999. *Microsatellites, Evolution and Applications,* p 368. Oxford University Press, New York.

Henkrar F, El–Haddoury J, Ouabbou H, Bendaou N and Udupa S M. 2016. Genetic characterization of Moroccan and the exotic bread wheat cultivars using functional and random DNA markers linked to the agronomic traits for genomics–assisted improvement. *Biotechnology* **97**(3): 1–11.

Jaccard P. 1908. Nouvelles recherché sur la distribution florale. Bulletin Society Vaud Science National. **44**: 223–70.

Litt Mand Luty J A. 1989. A hypervariable microsatellite revealed by *in vitro* amplification of a dinucleotide repeat within the cardiac muscle actin gene. *American Journal of Human Genetics* **44**: 397–401.

Nei M, Tajima F and Tateno Y. 1983. Accuracy of estimated phylogenetic trees from molecular data II. Gene frequency data. *Journal of Molecular Evolution* **19**: 153–70.

Phougat D, Panwar I S, Punia M S and Sethi S K. 2018. Microsatellite markers based characterization in breeding lines and cultivars of bread wheat (*Triticum aestivum* L. em. Thell). *Journal of Environmental Biology* **39**(3): 339–46.

Phougat Divya and Sethi S K. 2019. Wheat bioforticiation: Agricultural investment to refrain malnutrition especially in developing world. *Journal of Entomology and Zoology Studies* 7(4): 506–10.

Prevost A and Wilkinson MJ. 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theoretical Applied Genetics* **98**: 107–12.

Reynolds M, Bonnett D, Chapman S C, Furbank R T, Manes Y, Mather D E and Parry M A J. 2011. Raising yield potential of wheat. Overview of a consortium approach and breeding strategies. *Journal of Experimental Botany* **62**: 439–52.

Rohlf F J. 1992. NTSYS–pc: numerical taxonomy and multivariate analysis system. Exeter Software. New York. Retrieved from: http:/www.exetersoftware.com.

Saghai–Maroof M A, Soliman K M, Jorgensen R A and Allard R W. 1984. Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. (*In*) *Proceedings of National Academy of Sciences, USA* **81**: 8014–18.

Sharma J, Goyal N, Singh A, Pallavi J K, Sonah H and Gupta P. 2010. Assessment of genetic relationships among bread wheat (*Triticum aestivum* L. em. Thell) genotypes using microsatellite markers. *International Journal of Applied Agricultural Research* **5**: 575–82.

Spark D N. 1973. Euclidean Cluster Analysis.Algorithm As. 58. *Applied Statistics* **22**: 126–30.

Stein A J. 2010. Global impacts of human mineral malnutrition. *Plant and Soil* **335**: 133–54.

Tatikonda L, Wani S P, Kannan S, Beerelli N, Sreedevi, T K, Hoisington D A, Devi P and Varshney R A. 2009. AFLP–based molecular characterization of an elite germplasm collection of *Jatropha curcas* L., a biofuel plant. *Plant Science* **176**: 505–13.

Ward J H. 1963. Hierarchical Grouping to Optimize an Objective Function. *Journal of the American Statistical Association* **58**: 236–44.

White P J and Broadley M R. 2009. Biofortification of crops with seven mineral elements often lacking in human diets –iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytologist* **182**: 49–84.