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Diversity analysis by using molecular marker in wheat (*Triticum aestivum* L.)

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Abstract
The Molecular experiment is put out in the biotechnology laboratory of department of genetics and plant breeding at College of Agriculture, S. D. Agricultural University, Sardarkrushinagar. A total of seventeen SSR primers were used for the study of thirty genotypes, of which ten primers showed amplification, among ten primers six were polymorphic amplifying *viz.* barc 186, CFA 2114, GWM 148, CFD 71, Xgwm 11, Xgwm484. The PIC values for these six primers ranged from 0.14 (Xgwm484) to 0.36 (CFA 2114) with an average of 0.29. The PIC values provide information about amount of variability for a primer among the genotypes studied. The dendrogram generated through sub-programme cluster tree analysis of the NTSPYC-pc software divided thirty genotypes into two major clusters A and B. Cluster A is subdivided into two sub clusters A1 and A2. Sub cluster A1 includes one genotype VA2020-15 whereas sub cluster A2 consists of one genotype VA2020-06. Similarly, Cluster B is subdivided into four sub clusters *i.e.*, B1, B2, B3 and B4. Sub cluster B1 consists of one genotype VA2019-06, likewise sub cluster B2 also included three genotypes, B3 consist fifteen genotypes and B4 consist nine genotypes.
These primers are use in further molecular breeding programmes for improving the ability of particular trait and the information are important for future as gene cloning and also provide support for the implementation of marker-assisted selection (MAS).

Keywords: Wheat, primer, genotypes, polymorphic, monomorphic

1. Introduction
Wheat (*Triticum aestivum* L.) is a cereal grass from the family *Gramineae* and also known as the *Poaceae* and from the genus *Triticum*. *Triticum aestivum* L., an allohexaploid and an important world's largest growing and consuming cereal crop for the majority populations of world. On a global basis, wheat provides more nourishment than the any other food crop (Breiman and Graur, 1995) [2].
The global wheat production area about 2190 lakh ha and production is about 760.92 million MT and at 3474 kg/ha stands its global wheat utilization (Anonymous, 2020-21). India's wheat growing area in 2020-2021 was 311.25 lakh ha with production 109.58 million MT and productivity 3521 kg/ha and Gujarat's wheat growing area is about 10.17 lakh ha with production 3.25 million MT and yield is 3205 kg/ha. (Anonymous, 2020-21). India is the world's second largest producer of wheat accounted production of wheat during the year 2021 rose by 4.11% from 103,600 thousand tonnes in 2019 to 107,860 thousand tonnes in 2020, while in 2021 109,520 thousand tonnes. Since the 9.73% slump in 2015, wheat production soared by 25.60% in 2021. India are the Uttar Pradesh is the largest producer of wheat in India. wheat cultivation in India started 5000 years ago (Feldman, 2001) [3].
Method for estimating genetic diversity and the relationship among groups of plants by using morphological characters, agronomic information and biochemical variations. DNA molecular markers are an attractive alternative (Slorfor, 1996) [14]. The molecular marker reveals genetic diversity at the molecular level (Hill *et al*., 1998) [7]. The use of molecular markers for the evaluation of genetic diversity is very common. Molecular markers are a relatively new technology of breeding and are widely used by breeders to select variations in the selected all genotypes. The leading marker technologies with the markers like RFLP, RAPD, AFLP, SSR, ISSR etc. are becoming determinant for cultivar identification and diversity analysis (Patterson, 1996) [12]. Simple Sequence Repeats (SSRs) provide an efficient tool in diversity studies for identifying the degree of genetic similarity.

Due to their high rate of polymorphism, co-dominant character, selective neutrality, distribution across the genome and cost and labour efficiency the SSR markers are suitable for detecting allele frequency within the population and for assessing population structure (Khaled *et al*., 2015) [8]. Simple sequence repeats (SSRs).
(Tautz and Renz, 1989) [15] has been widely exploited in wheat due to its high level of polymorphisms, co-dominant inheritance and equal distribution in the wheat genome.
(Roder *et al*., 1995; Parker *et al*., 2002) [13, 11]. SSR is more abundant, ubiquitous in presence, hypervariable in nature and has high polymorphic information content (Gupta *et al*., 1996) [6].

2. Materials and Methods

The isolation of genomic DNA of wheat genotypes was carried out as per cTAB method of Doyle and Doyle (1987) [4]. Various stock solutions and buffers were prepared and used during isolation of genomic DNA and during gel electrophoresis.

2.1 DNA isolation from wheat leaves

1. The fresh leaves were taken from 20-25 days old seedlings and were made to grind to a fine powder using liquid nitrogen in a pestle and mortar and were filled into 2 ml centrifuge tube.
2. 1 ml of cTAB extraction buffer and 10 µl β-mercaptoethanol was added into the tubes and kept in a water bath for 45 minutes to 1 hour, where tubes are given a jerk for every 10-15 minutes interval.
3. After incubation the tubes were centrifuged at 12000 rpm for 12 minutes at 24 °C.
4. The supernatant was carefully transferred to a fresh tube and an equal volume of chloroform: isoamyl alcohol (24:1) was added and again centrifuged for 12 minutes at 12000 rpm.
5. The supernatant was transferred to a fresh tube and given a RNase A treatment and kept in water bath at 37 °C for 45 minutes.
6. Equal volume of chloroform: isoamyl alcohol (24:1) was added after the RNase A treatment and centrifuged at 12000rpm for 12 minutes.
7. The supernatant was taken and equal volume of chloroform: isoamyl alcohol (24:1) was added and again centrifuged at 12000 rpm for 12 minutes.
8. After centrifugation the supernantant was transferred to 1.5 ml centrifuge tube and 0.6 volume of chilled isopropanol was added and stored overnight at -20 °C for better precipitation of DNA.
9. Next day morning, the fine pellets of DNA will be obtained after it has been given a centrifugation at 4 °C for 10 minutes at 10000 rpm.
10. The pellets obtained was washed with 70% ethanol, twice and kept in the oven for the evaporation of ethanol.
11. Later, the pellets were made to dissolve in 100 µl of 1X TE buffer by tapping on the tubes and stored at -20 °C.

The quantification of nucleic acid was performed using UV spectrophotometer. The good quality DNA will have the O.D. in the range of 1.8-2.0. The O.D. below 1.8 or 1.7 indicates protein contamination and O.D. above 2.0 will be indicating contamination of RNA. The 0.8% agarose gel run will show bands with less shearing indicates good quality and amount of DNA. For the PCR based amplification total seventeen number of SSR primers were used for the diversity analysis of heat tolerance. PCR reactions were performed in the reaction volume of 15 µl containing 1.5 µl of Taq buffer B (10X) with $MgCl_2$, 0.5 µl dNTP mix (10 mM), 1.0 µl of each primer (5 p mol), 0.1 µl of Taq DNA polymerase and 1.0 µl of genomic template DNA(50 ng/ml)

3. Result and Discussion

The fresh leaves were taken from 20-25 days old seedlings. A total of seventeen heat tolerant specific SSR primers were used for the study of thirty genotypes, of which ten primers showed amplification, among ten primers six were polymorphic amplifying a total of 16 alleles with an average of 2 alleles per locus, the primer Xgwm190, GWM397, GWM407, Xgwm133 was monomorphic. The polymorphic amplified product was recorded for primer barc 186,
CFA 2114, GWM 148, CFD 71, Xgwm 11, Xgwm484. The PIC values for the six polymorphic primers ranged from 0.14 (Xgwm484) to 0.36 (CFA 2114) with an average of 0.29. The PIC values provide information about amount of variability for a primer among the genotypes studied. The PIC value was highest for CFA 2114 (0.36) followed by
barc 186 (0.35), CFD 71 (0.35) and Xgwm 11 (0.35), whereas lowest PIC were recorded for Xgwm 484 (0.14). Similar findings were obtained by Mwale *et al.* (2016) [9]. molecular band size range between 180bp to 500bp Maximum band size obtained in primer Xgwm133 (498bp-500 bp) and minimum band size obtained in primer Xgwm484 (180 bp-205 bp).
The similarity matrices were developed using SIMQUAL sub-programme of NTSPYC-pc software for thirty genotypes by data generated from six polymorphic SSR primers. The allelic diversity was used to produce dendrogram by the sub-programme cluster tree analysis of the same NTSPYC-pc software which revealed the genetic relationship among the genotypes studied, represented in the Figure 3. Based on the dendrogram, two clusters were formed for thirty genotypes *i.e.*, cluster A and cluster B which is formed at the similarity co-efficient of 0.80. Cluster A is subdivided into two sub clusters A1 and A2 includes genotypes VA2020-15 and VA2020-06. Similarly, Cluster B is subdivided into four sub clusters *i.e.*, B1, B2, B3 and B4. Sub cluster B1 consists of one genotype VA2019-06, likewise sub cluster B2 consists of three genotypes VA2020-8, 21th ESBWYT-21, 28th SAWYT-321 and B3 consists of genotypes HI 1544, 28th SAWYT-328, VA2019-14, GW451, VA2020-14, 28th SAWYT-324, VA2020-04, 21th ESBWYT-23, VA2020-11, VA2020-34, 21th ESBWYT-8, 28th SAWYT-310, VA2019-10, VA2020-19, VA2020-02 and B4 consists of genotypes 21th ESBWYT-20, 28th SAWYT-320, VA2019-02, 28th SAWYT-347, 28th SAWYT-305, GW 322, VA2020-10, VA2020-13, VA2020-16. Mwale *et al.* (2016) [9] also reported the similar findings. Based on the primers used, genotypes VA2019-10 and VA2020-19, 21thESBWYT-8 and 28thSAWYT-310, 21th ESBWYT-23 and VA2020-11, VA2019-14 and GW 451 recorded highest similarity of 1.

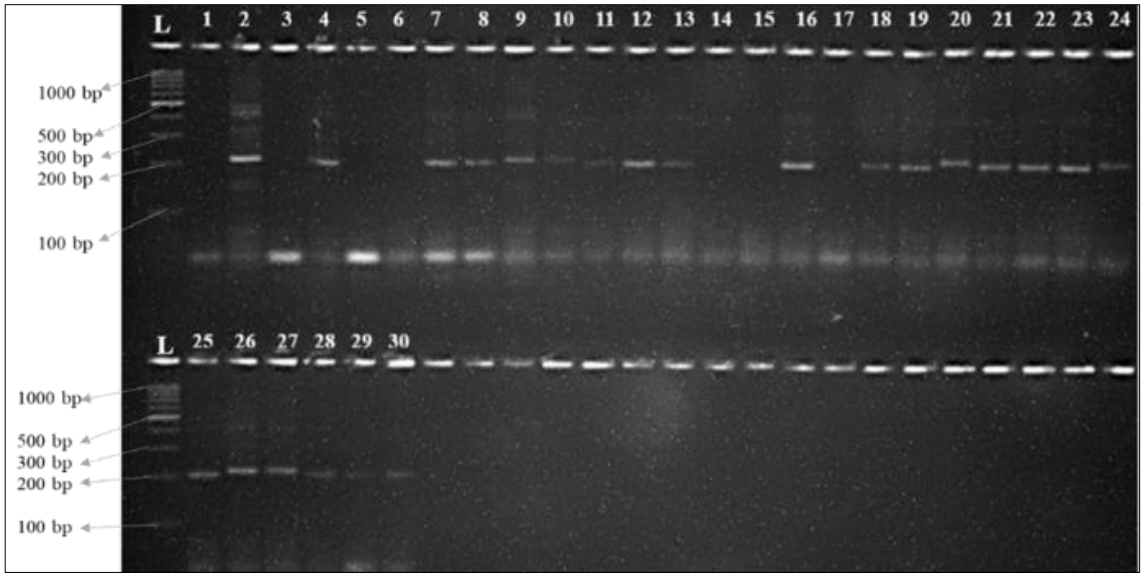

Fig 1: SSR amplification profile in wheat by primer barc186

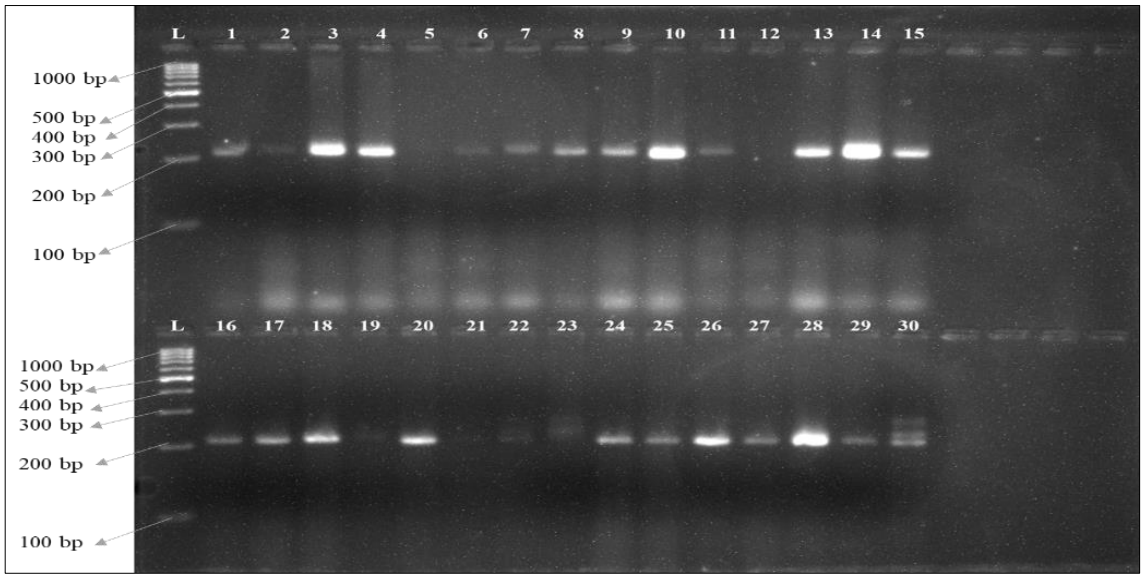

Fig 2: SSR amplification profile in wheat by primer Xgwm 11

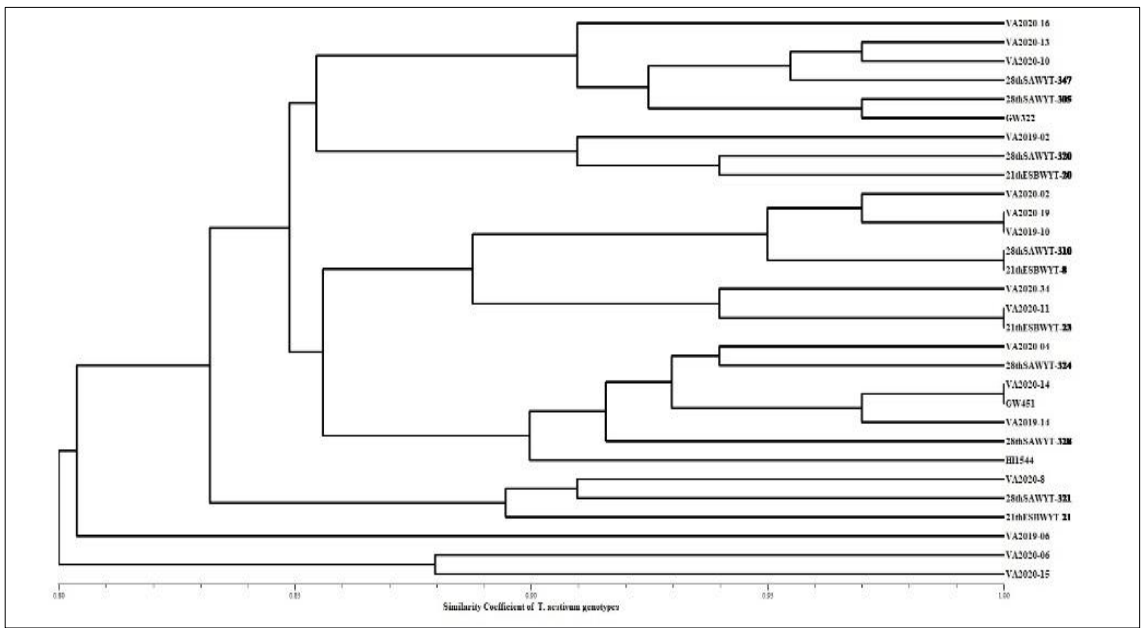

Fig 3: Dendrogram showing clustering of 30 wheat genotypes constructed using UPGMA based on Jaccard's similarity co-efficient obtained from SSR based PCR analysis

Table 1: Distribution of genotypes based on cluster from UPGMA dendrogram from SSR primers

Cluster	Subcluster	Genotypes	Genotypes
A	A1	VA2020-15	1
	A2	VA2020-06.	1
B	B1	VA2019-06	1
	B2	VA2020-8, 21th ESBWYT21, 28th SAWYT-321	3
	B3	HI 1544, 28th SAWYT-328, VA2019-14, GW451 VA2020-14, 28thSAWYT-324, VA202004, 21thESBWYT-23, VA2020-11, VA2020-34, 21thESBWYT-8, VA2020-02, 28th SAWYT-310, VA2019-10, VA2020-19	15
	B4	21th ESBWYT-20, 28th SAWYT-320, VA2019-02, 28th SAWYT-347, 28th SAWYT-305, GW 322, VA2020-10, VA2020-13, VA2020-16	9
Total			30

Table 2: List of SSR primers reported polymorphic for thirty wheat genotypes

Sl. No	Primer	Molecular band size (bp)	No. of alleles amplified	PIC
1	barc 186	~200-230	2	0.35
2	CFA 2114	~200-245	2	0.36
3	GWM 148	~190-235	2	0.22
4	CFD 71	~195-250	2	0.35
5	Xgwm 11	~190-220	2	0.35
6	Xgwm484	~180-205	2	0.14
7	Xgwm190	~203-205	1	-
8	GWM 397	~310-312	1	-
9	GWM 407	~205-208	1	-
10	Xgwm 133	~498-500	1	-
	Mean			0.29

4. Conclusion

These primers are use in further molecular breeding programmes for improving the ability of perticular trait and the information are important for future as gene cloning and also provide support for the implementation of marker-assisted selection (MAS).

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