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MOLECULAR DIVERSITY ANALYSIS IN WHEAT (*TRITICUM DURUM* L.) GENOTYPES USING SSR MARKERS

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ABSTRACT

The genetic diversity provides an opportunity to plan breeder to identify a novel genotype. Assessment of genetic diversity is a key factor in germplasm characterization and conservation. The present investigation was carried out to study the nature and magnitude of genetic divergence with twenty-one wheat genotypes constituting checks collected from different sources and twenty SSR markers. The results showed that, all 20 makers showed amplification and 12 markers showed polymorphism. Among all the primers amplified 8 unique loci in 8 genotypes were identified. Each primer had produced on an average of 1.66 polymorphic bands. Among the SSR primers Wmc-294, Barc-24 and Barc-170 produced maximum number of 4 loci while Barc-12, Barc-120, Gwm-312, Wmc-420, Barc-108 and Wmc-46 produced minimum number of two loci. Maximum PIC value of 0.73 was observed in SSR primer Wmc-24 followed by 0.72 for marker Gwm-294 which indicates the high discrimination capacity of these markers. Thesemarkers can be used for evaluation of genetic diversity and molecular study in macaroni wheat. Based on cluster analysis, two major clusters I and II were formed. Cluster II consist of only one genotype NIDW-1576 which found completely diverse from other genotypes highlights uniqueness this genotype to be utilized in further breeding programmes of wheat improvements.

***Key words*: SSR Maker, polymorphism, Genetic diversity, PIC Value**

Introduction

Wheat is the foremost and strategic cereal crop of the world. Wheat is the most important and major staple food of more than thirty-five percent of worlds' population. Globally, it is the most crucial oldest and edible grain cereals. It is worldwide cultivated and domesticated grass. Wheat is the 'King of cereals' due to its area, productivity and the conspicuous position in the international food grain trade. India is second largest producer of wheat in the world after china. According to the 3rd advance estimates of production of wheat for 2024-25 released by the Department of Agriculture, Cooperation and Farmers Welfare, the estimated production of wheat during 2024-25 is 117.51 million tonnes from the area of 32.76 million hectare. In India Uttar Pradesh, Madhya Pradesh, Punjab, Hariyana, Rajasthan are the leading states in Wheat production in India during the year 2024-25 Anonymous 2024.

The domestication process in history of evolution of wheat crop has contributed to increased yield but this has reduced the genetic base of crops. So, it is necessary to broaden the genetic base of crops. The genetic diversity is base for biodiversity. Genetic diversity is essential for conservation and utilization of genetic resources of targeted species and population. It is necessary to study and understand genetic diversity of germplasm for their utilization to widen genetic base for crop improvement. With the advancement in recent genetics and genomics tools and techniques, the purpose of revealing genetic diversity is served by the use of molecular markers.

Microsatellites or simple sequence repeats (SSRs)

Table 1: Composition of SSR -PCR reaction mixture.

PCR reaction component	SC	FC	Volume for one tube
Taq buffer B	10 X	1 X	2.0 µl
$MgCl_2$	25 mM	1 mM	1.2 µl
dNTP mix	10 mM	3.2 mM	1.6 µl
Primer (F)	0.2 picomole/µ	0.32 picomole	1.6 µl
Primer (R)	0.2 picomole/µ	0.32 picomole	1.6 µl
Taq DNA polymerase	3 U	1 U	0.33 µl
Sterilized distilled water	-	-	9.67 µl
Template DNA	25 ng/ µl	50 µl	2.0 µl
Total volume			20.00 µl

SC: Stock concentration; **FC:** Final concentration

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 labor efficiency, microsatellites markers are suitable for detecting allele frequency within the population and for assessing population structure Khaled and Hameed 2015, Kumar *et al.*, 2015, Kumari *et al.*, 2019.

Durum or macaroni wheat accounts for about 3-5 % of total area sown under wheat and 6-8% of the total wheat production. Madhya Pradesh has largest area under durum wheat cultivation. It has yellow colour grains and it contain stronger gluten. Additionally, its good texture, resistant surface disintegration, retained structure and longer durability makes it more suitable for pasta making.

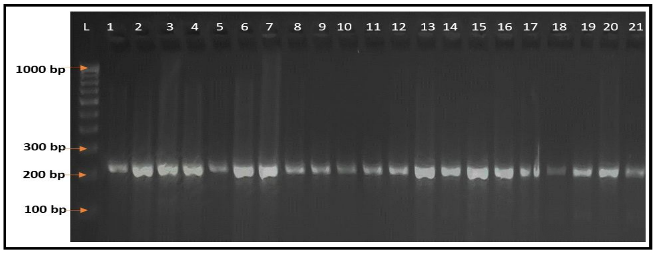

Fig. 1: PCR amplification of macaroni wheat genotype by using marker Wmc-596.

Lane of macaroni wheat genotypes L: Ladder (100 -1000 bp)

S	Genotype	S.	Genotypes	S.	Genotypes
1	Panchavati (C)	8	NIDW-1573	15	NIDW-1561
2	Godavari (C)	9	NIDW-1574	16	NIDW-1542
3	MACS-3949 (C)	10	NIDW-1576	17	NIDW-1556
4	NIDW-1149 (C)	11	NIDW-1578	18	NIDW-1527
5	AKDW-2997-16 (C)	12	NIDW-1589	19	NIDW-1520
6	NIDW-1569	13	NIDW-1582	20	NIDW-1534
7	NIDW-1572	14	NIDW-1555	21	NIDW-1499

Table 2: Analysis of wheat genotypes with SSR primers.

Sr.	SSR Analysis	Observations
1	Total number of primers pairs used	20
2	Number of primers amplifying DNA	20
3	Number of primers not amplifying DNA	00
4	Total number of polymorphic primers	12
5	Total numbers of bands amplified	32
6	Total number of polymorphic bands	32
7	Total number of monomorphic bands	00
8	Total number of unique bands	08
9	Percent polymorphism	60%
10	Average number of bands primers	1.66
11	Average polymorphic bands produced per primer	1.66
12	Amplified products size	95 bp -290 bp

Keeping in view the importance of macroni wheat and advantage of SSR markers in genetic studies, the present investigation was carried out to study molecular diversity among various wheat genotypes using SSR markers and to find genetically most diverse genotypes of wheat which can further be used in future breeding programs.

Material and Methods

The present investigation of molecular diversity in macaroni wheat genotypes (*Triticum macaroni* L.) using the SSR marker was carried out at Department of Biotechnology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri (Maharashtra) during year 2022-23. Experiment was conducted in potculture at Biotechnology laboratory. The experimental material comprised of twenty one genotypes, which were collected from Agriculture Research Station,Niphad, District Nashik, Maharashtra. The SSR primers used in the

Table 3: Information contents of SSR primers used for wheat divergence analysis.

Sr.	Primer	NAA	PA	UA	PSR	PIC
1	Wmc-617	3	3	01	95-105	0.31
2	Barc-09	3	3	01	130-150	0.27
3	Gwm-294	4	4	01	210-260	0.72
4	Barc-120	2	2	00	165-170	0.41
5	Wmc-24	4	4	00	120-135	0.73
6	Gwm-312	2	2	01	200-230	0.09
7	Wmc-596	2	2	00	210-220	0.50
8	Barc-12	2	2	01	195-200	0.09
9	Wmc-170	4	4	01	265-290	0.40
10	Barc-108	2	2	00	150-155	0.09
11	Wmc-420	2	2	01	145-150	0.37
12	Wmc-46	2	2	00	185-190	0.30

NAA: No. of alleles Amplified; **PA:** Polymorphic Alleles; **UA:** Unique alleles*; **PSR:** Product Size range (bp)
*Unique allele are also counted under polymorphic

Table 4: Unique SSR fragment amplified in wheat genotypes.

Sr. No.	Wheat genotypes	Primer revealing unique SSR(Size of base pair of amplified fragments)
1	NIDW-1555	Wmc-617 (270 bp)
2	NIDW-1578	Barc-09 (150 bp)
3	NIDW-1520	Gwm-294 (95 bp)
4	NIDW-1576	Gwm-312 (200 bp)
5	NIDW-1561	Barc-12 (195 bp)
6	NIDW-1574, NIDW-1556	Wmc-170 (280 bp , 290 bp)
7	NIDW-1578	Wmc-420 (145 bp)

present investigation as below Xwmc-9, Xwmc-596, Xwmc-603, Barc-108, Xwmc-89, Xwmc-420, Xwmc-170, Xwmc-24, Xbarc-240, Xbarc-12, Xwmc-617, Xwmc-256, Xbarc-178, Xgwm-249, Xgwm-312, Barc-182, Xgwm-513, Xgwm-120, Xgwm-294,Xgwm-46.

Isolation of genomic DNA

Growing of seedlings and sample collection.

The clean and bold seeds of each genotype were planted in plastic plots in green house for 10 days and labelled properly. Young (15 DAS) healthy tender leaves were collected from individual plants for DNA extraction.

Isolation of genomic DNA from leaves

Genomic DNA was isolated from 21 wheat genotypes following CTAB (Cetyl Tri methyl Ammonium Bromide) extraction method with some modifications as described by Helguera *et al*., (2005).

DNA amplification by SSR markers

PCR amplification was carried out using Thermal Cycler PCR (Eppendorf, Master Cycler Gradient, Germany). Amplification reaction mixture was prepared in 0.2ml thin-walled flat capped PCR tubes, containing the following components. The total volume of each reaction mixture was 20 µl (Table 1).

Data Analysis:

Molecular data analysis

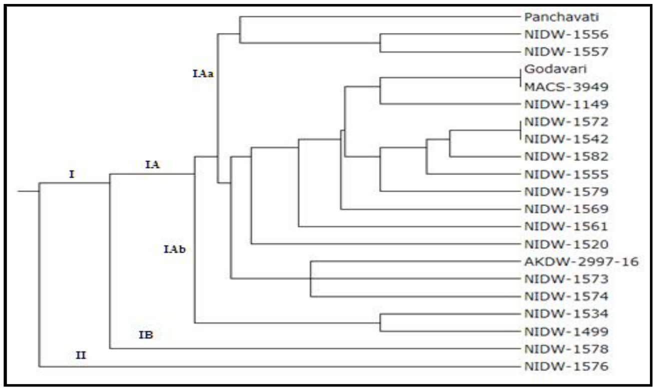

Fig. 2: Dendogram of 21 macaroni wheat genotypes using the marker data by using TASSAL 4.0 software.

The clearly resolved PCR amplified bands of wheat genotypes with different SSR primers were scored manually as binary matrix for their presence (1) and absence (0) in the data sheet. The polymorphism information content (PIC) value was calculated as:

$$\text{PIC} = 1 - n\,\frac{\Sigma Pi^2}{i = 1}$$

Where, n is the number of band positions analyzed in the set of accessions and Pi is the frequency of it pattern.

Cluster analysis

Data obtained from all the polymorphic markers were used to determine genetic relationships. The amplified products were scored for the presence or absence of each marker allele. Data were entered into a binary matrix and scored as '1' for the presence and 'o' for absence of the allele. Dendrogram was constructed through UPGMA based method to group individuals into different clusters making use of the archaeopteryx tree option as provided in TASSEL 4.0 (Bradbury *et al.*, 2007).

Results and Discussion

Molecular diversity study has been carried out by using molecular makers. Molecular makers are the complementary sequence of DNA which is lie close to the particular gene. In the present investigation SSR markers were used to assess the genetic diversity among 21 wheat genotypes collected from ARS Niphad. From the SSR analysis, it was observed that a total of 32 alleles were generated by amplification with 12 polymorphic primers. All 32 of them were polymorphic, from which 08 were unique. Average polymorphism was 60%. Each

Table 5: Grouping of twenty-one genotypes of wheat into two major clusters on the basis of dendogram analysis.

Sr.	Cluster	Sub cluster	Sub sub cluster	Genotypes
1	I	IA	IAa	Panchavati, Godavari, NIDW-1556, NIDW-1557, NIDW-1149, NIDW-1572, MACS-3949, NIDW1542, NIDW-1582, NIDW-1555, NIDW-1579, NIDW-1569, NIDW-1561, NIDW-1520, NIDW-1573, NIDW-1574, AKDW-2997-16.
			IAb	NIDW-1543, NIDW-1499
		IB		NIDW-1578
2	II			NIDW-1576

SSR primer pair thus produced on an average 1.66 polymorphic bands. (Table 2). Similar resultswere reported by Somers *et al*., 2004, Malik *et al.*, 2013, Kumar *et al*., 2015, Kabbaj *et al.*, 2017.

PIC Value

Maximum scorable bands were observed using the primer Wmc-294, Wmc-24 and Wm-170 (4 loci each) followed by Wmc-617 and Barc-09 (3 loci) each. The least number of bands were reported in the primer Barc-120, Gwm-312, Wmc-596, Barc-12, Barc-108, Wmc-420 and Wmc-46 (2 loci each). Twelve primers showed 60 percent polymorphism. All the genotypes evaluated for the molecular diversity with 12 SSR primer showed variation in their banding pattern.

The Polymorphism Information Content values were calculated to find out the efficiency of primers in distinguishing individual genotypes. The PIC values of SSR primers ranges from 0.09 in primer Barc-12, Gwm-312 and Barc-108 to 0.73 in SSR primer Wmc-24. The SSR primers Wmc-596 showed PIC values, 0.50, more indicating this primer may be considered as more informative (Table 3). Similar results were reported by Mangini *et al.,* 2010, Emon *et al.,* 2010, Spanic *et al.,* 2012 and Akfirat and Uncuoglu 2013.

Amplification using SSR markers

Out of 12 polymorphic SSR markers, 7 markers showed unique amplification in 8 macaroni wheat genotypes. The details of these unique markers were presented in (Table 4). Among these primers evaluatedthe primer Wmc-596 showed amplification in all 21 genotypes with expected product size of 210 bp to 220 bp. The Wmc-596 marker showed amplification at 61°C as given by Somers *et al.,* (2004). Wmc-596 was found highly polymorphic between wheat genotypes. Total 21 bands were scored from Wmc-596 marker. Total two alleles were detected. The two alleles were observed at 210 bp and 220 bp. The PIC value of marker Wmc-596 was 0.50. (Fig. 1). Similar finding was reported by Malik *et al.*, 2013 and Dagnaw *et al.*, 2023.

Genetic diversity analysis by SSR markers

Genetic diversity detected using molecular marker in the present investigation indicates the high discrimination capacity of SSR marker. To visualize the genetic relationship among 21 wheat genotypes, a dendrogram was constructed based on the UPGMA using TASSEL 4.0 programme presented in plate 2. Based on cluster analysis using SSR markers, The twenty one wheat genotypes were grouped into two major clusters namely I (20 genotypes) and II (one genotype). The cluster I was again sub divided into 2 sub clusters namely IA and IB. The First sub-cluster was again divided into its subtypes that are IAa and IAb. First sub-cluster IAa comprised genotypes *viz.*, Panchavati, NIDW-1556, NIDW-1557, Godavari, MACS-3949, NIDW-1149, NIDW-1572, NIDW-1542, NIDW-1582, NIDW-1555, NIDW-1579, NIDW-1569, NIDW-1561, NIDW-1520, AKDW-2997-16, NIDW-1573, NIDW-1574. Second sub-cluster IAb comprised two genotypes NIDW-1534 and NIDW-1499.Second sub cluster IB comprised genotype NIDW-1578. Second major cluster II consisted of only single genotype NIDW-1576 (Table 5). Similar results were found by Islam *et al* 2012, Singh *et al*., 2015.

Conclusion

Genetic Diversity among 21 Wheat genotypes was determined using 12 SSR markers. The results reveal that genotypes have a great genetic diversity. SSR markers provided very useful data to exploit genotypes for future research on wheat breeding. Twenty one wheat genotypes were grouped into two main clusters *i.e.* Cluster I and Cluster II. Cluster I again divided into two sub-clusters *viz.,* IA and IB. Sub-cluster IA was again subdivided into sub sub-cluster IAa and IAb.The genotype of Cluster II consisted of only one genotype NIDW-1576 which was found be completely diverse from other genotypes and this genotype will be used in future breeding programmes.

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Conflict of Interest: None

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