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Genetic Diversity in Selected Maize Genotype of Pakistan by using Rapid Amplified Polymorphic DNA Technique

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Abstract—Maize is the 3rd most important agricultural crop in Pakistan with an annual production of 5,701,400 tons. Identification of genetically diverse maize genotype is valuable information which is efficiently used in upcoming maize breeding programs and acquiring specific traits with the modification of DNA for different conditions. In current study, the genetic diversity among twelve maize genotypes using Randomly Amplified Polymorphic DNA (RAPD) markers are reported. Four oligo decamer primers are used for the analysis. Young and fresh maize leaves are used for DNA extraction purpose followed by CTAB extraction procedure. The PCR results are checked on gel electrophoresis with 1.2% agarose having ethidium bromide where DNA bands were separated on the base of size. A total of 301 loci were obtained with the four primers. Bands were score with binary method, 1 for their presence and 0 for the absence. After statistical analysis of all loci, phylogenetic tree is constructed using computer program Dendro UPGMA. The results showed that genotypes FSH-810 and Karamat are the most diverse genotypes having maximum dissimilarity ratio of 66% to each other, while Islamabad Gold and Soan-3 are least diverse genotypes with 18% variation. Hence it is recommended that FSH-810 and Karamat genotypes are the ideal candidates for any breeding programs potentially generating maximum variation.

***Index Terms*—Polygenetic Tree, DNA, PCR, Genotypes, Genetic diversity**

I. Introduction

PAKISTAN is an agricultural country. Major crops produced in Pakistan include wheat, rice, cotton, sugarcane and maize. Maize is 3rd most important crop grown in world [1]. The botanical name of maize is Zea mays L, which belongs to the family Poaceae. In 94 developing countries with more than 4.5 billion people, maize contributes about 30% of food calories [2]. Major maize producing countries are Brazil, US, Indonesia, Italy, France, China, India, South Africa, Argentina and Mexico. They contribute about 79% of the production. Khyber Pakhtunkhwa (KP) and Punjab are main provinces in Pakistan for maize production. It is 2nd main crop in KP [3]. It has 3.5% agricultural value and grows on an area of 4.8% (GOP 2016). It has 10 pairs of chromosomes with 42000-56000 genes and was domesticated about 6000 years ago in mountainous region of America [4]. Maize is monoecious species, can be reproduce only by seeds and annually [5]. Normally it is a cross pollinated crop [6] with kernels have 40-50% oil content that is used in salads, cooking material and industries like soap making [7]. The products prepared from maize are bread, porridges, steamed products, beverages and snacks for human consumption. The maize kernels are rich source of multiple vitamins, folic acid, selenium, pyridoxine, triglycerides, lipids, sterols, monoglycerides diglycerides, carotenoids, tocopherols and waxes. High level of linoleic acid is present in the maiz oil which is polyunsaturated fatty acid [8].

Genomic difference among populations or genotypes is known as genomic diversity in germplasm [9]. This information is helpful for farmers and breeders to evaluate desirable (increase yield and quantity) and preferred traits (drought or pest resistance) [10]. Different maize varieties are selected and developed with respect to different environmental conditions and various morphological traits [11]. Genetic variations are important in biodiversity of maize. The genetic composition of maize varieties is changed with the use of different biotechnological application and hybridization that is suitable for existing environmental conditions. It is commonly estimated that in comparison to other domestic grass, crops maize has 2-5 folds higher molecular diversity [12]. Genomic variation achieved with the use of molecular markers is considered as best method because they can be used at any stage of plant development and environmental conditions does not influence marker interactions [13]. For genetic analysis of

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plants and manipulation of traits PCR-based markers are very useful in breeding programs as they involve in visual display of polymorphism and play important role in yielding stability and productivity. RAPD is first PCR-based markers that have ability to create many copies of DNA with the use of minute genetic material. This method is faster and easy to use than others [14]. RAPD markers are arbitrary or randomly markers consisting of oligonucleotides and amplified with suitable large number of loci for polymorphism [15]. This PCR based technique works with the single primer to produce final DNA loci. Polymorphic result is visualized with gel electrophoresis. Maize is model species for fundamental research to understand genetic basis of yield. For improving performance, it has wide range of biodiversity [16]. For any crop species, genetic diversity determination is necessary for their improvement, because, use of that genotype which is only successful when it has some genetic difference. More genetic diversity provides an opportunity to select and evolve new suitable varieties [17]. RAPD technique is widely used due to its efficiency, low cost, easiness, quickness with compare to other molecular markers and also no previous knowledge of genetic material is required. It is well applied to estimate genetic difference and relationship among crop genotypes or analysis of seed purity and identification of cultivars [18]. RAPD proved to be the best DNA fingerprinting technique for identification of different varieties [19].

The Amplified fragment length polymorphism (AFLPs), RAPDs, Restriction fragment length polymorphism (RFLPs) and Simple sequence repeats (SSRs) techniques have been used to identify the genetic variation/association among diverse genotypes of maize [20]. However RAPD is found very cost effective and efficient molecular method for estimation of genetic diversity among hybrid maize and analysis of somaclonal variations [21-22]. Maize hybrids cultivated in Southern highlands of Africa are studied to evaluate genetic diversity with the use of RAPD markers and results showed that there are an association between Dendrogram obtained by RAPD primers and morphological characteristics of the germplasm [23-24]. RAPD markers were considered as powerful tool for the analysis of genetic variation in maize genotypes for resource deficient scientist.

In this research work, the genetic variation in 12 (twelve) maize genotypes collected from National Agriculture Research Council (NARC) Islamabad are reported. The data gained from this study will provide a scientific insight for the selection of candidate parents containing maximum variation which will be necessary for a successful breeding program.

II. Material And Method

A. Plant Material (Maize)

The maize seeds for this research work, are provided by National Agricultural Research Centre (NARC) Islamabad Pakistan. The maize seeds are planted in pots for the collection of young leaves DNA extraction. The description of all maize genotypes are presented in Table I.

TABLE I.
TWELVE MAIZE GERMPLASM COLLECTED FROM NARC

Sr. No	Code	Name of Maize Germplasm
1	Z_1	Kashmir Gold
2	Z_2	Islamabad White
3	Z_3	Sarhad Yellow
4	Z_4	NARC 2704
5	Z_5	Karamat
6	Z_6	Chandni
7	Z_7	Soan-3
8	Z_8	FSH-810
9	Z_9	Babar
10	Z_{10}	Islamabad Gold
11	Z_{11}	Ghauri
12	Z_{12}	Yousafwala Hybrid

B. DNA Extraction

For research work, 12 (twelve) maize genotypes are grown and fresh leaves of these plants are collected after one month for genomic DNA extraction. First of all, fresh 2 to 3 leaves of maize are collected from the plant and cleaned properly with distilled water to make them free of dust and any foreign particles. The plant material was grounded to make homogenate mixture in the presence of CTAB-Buffer (0.8M Tris-Hcl, 0.5M EDTA, 4.5M Nacl, 2g CTAB and 2g PVP of 100 ml solution) with the help of mortar and pestle. CTAB Buffer is incubated at 65°C for about 10 minutes in water bath before use. After making paste, plant material was transferred in 2ml eppendorf tubes and incubated in water bath at 65°C for half an hour. Then 600 µl mixture of isoamyl-alcohol, phenol and chloroform (1:25:24), was added in tubes and centrifuged at 14,000 rpm for 5 minutes in centrifuge machine. After centrifugation, three layers are obtained. The top aqueous layer is poured into a fresh 1.5 ml tubes. $1/10^{th}$ of the volume of sample present in tubes, 3M sodium acetate and 600 µl chilled isopropanol are added in the tubes and centrifuged at 6,000 rpm for 3 minutes so that DNA could precipitate in the form of pellet. The supernatant is thrown carefully while pellet obtained is properly washed 2 to 3 time with the chilled 70% ethanol. The pellet is air dried for sometime to evaporate all moisture. After that, 100 µl TE-Buffer (0.5M Tris-HCL, 0.4M EDTA and dist. Water) is added in the tube to dissolve pellet in it. 1.5 µl of RNA degrading enzyme, is used to remove any RNA and kept in thermal incubator for an hour.

C. Polymerase Chain Reaction

This technique is used to amplify the small section of DNA or gene into thousands or millions of copies in special machine known as Thermocycler. In the present study, four Randomly Amplified Polymorphic DNA primers were employed to estimate the level of genomic poly-morphism between the twelve maize genotypes. These RAPD primers were arbitrarily selected. The primers sequence and other information are present in Table II. The PCR reaction was prepared in 25 µl mix for each primer with specific

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genotype. This mixture contained 1 µl DNA, 1 µl dNTPs, 1 µl primer, 0.5 µl Magnesium chloride, 0.35 µl Taq Polymerase, 0.8 µl PCR-Buffer and 201 µl distilled water. DNA amplification settings are listed in Table II.

First step of denaturations for 5 minutes at 93 ℃ followed by 25 cycles each consisting of a 1 minute at 93 ℃, annealed step of 30 seconds on 37 ℃ and elongation step of 1 minute at 71.5 ℃. Lastly 8 minutes elongation step on 71.5 ℃ to make it sure that primer extension reaction has reached its completion. The PCR products were run on gel electrophoreses on 1.2% agarose gel and recorded in gel doc.

D. Data Analysis

After amplification of each primer various bands were obtained which were statistically analyzed. After Polymerase Chain Reaction for all primers a total of 301 bands were visualized on agarose gel with the illumination of UV light in gel doc. For analysis of data all intensity bands were used that were divided into groups and their presence or absence was represented by 1 (present) and 0 (absent) for each primer. Data was evaluated using Nei and Lei formula as shown in equation (1) for calculating genetic distance of maize genotypes and UPGMA Dendro, an online program was used for construction of Dendogram.

$$G.D = \frac{1 - d.(x,y)}{d.(x) + d.(y) - d.(x,y)}$$

Where,

G.D refers to Genetic Difference present among 2 germplasms

$d.(x,y)$ is the sum number of common bands (loci) present in 2 related germplasm,

$d.(x)$ is the sum of all bands (loci) present in first germplasms, and

$d.(y)$ is the sum of all bands (loci) present in second germplasm.

III. Results and Discussion

In the current study four RAPD markers were used to obtain the polymorphic pattern of DNA amplifications of twelve candidate maize genotypes. Multiple segregated loci were detected and visualized with four primers. The combined data analyzed from all RAPD markers amplification with maize germplasm used in this study is presented in the Table III as similarity matrix.

The phylogenetic relationship obtained with the use of similarity index [24] is grouped into four major clusters of all twelve maize genotypes as in *Fig. 1*. Each cluster ranges from single to multiple genotypes. Different genetic variation was estimated in various groups. The major groups were named alphabetically A, B, C and D. The A group contains genotype Chandni, B group has Kashmir Gold, Islamabad White, Sarhad Yellow, Karamat and NARC 2704, C group has Babar and D group has FSH-810, Islamabad Gold, Soan-3, Yousafwala Hybrid and Ghauri genotypes. Major groups have also some subgroups [18]. Placement of genotypes in the same group indicates the small genetic variation among those genotypes [9]. After statistical analysis of all loci, phylogenetic tree was constructed using program Dendro UPGMA.

IV. Conclusion

The results showed that genotypes FSH-810 and Karamat were the most diverse genotypes having maximum dissimilarity ratio of 66% to each other while Islamabad Gold and Soan-3 were least diverse genotypes with 18% variation. Therefore, FSH-810 and Karamat genotypes are the ideal candidates for any breeding programs potentially generating maximum variation.

This assessment of genetic diversity among maize genotypes will be a helpful tool for the farmers and breeders to grow and breed the suitable variety in their breeding program.

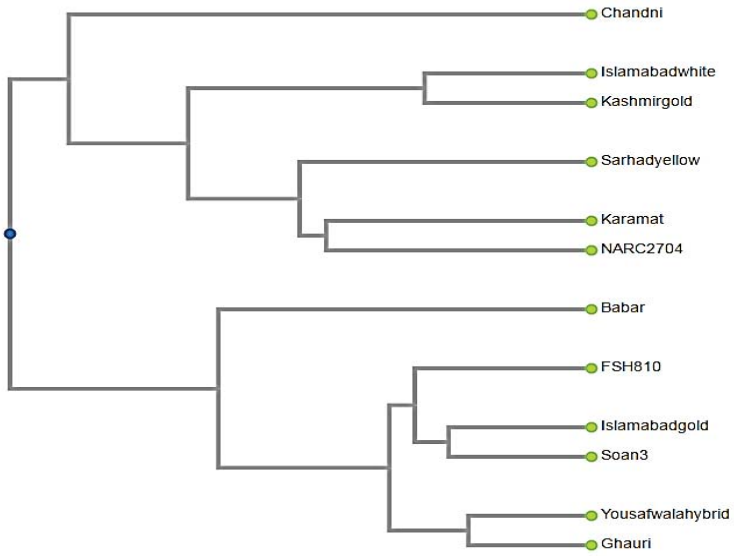

Fig. 1. Dendrogram of all twelve maize genotypes based on four RAPD markers that was obtained by clustering the similarity index data in computer program Dendro UPGMA.

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TABLE II
PRIMERS INFORMATION USED IN STUDY

Decamer-Primers	Primer Sequence	Molecular Weight (g/mol)	Mel. temperature in (^{0}C)	Nano mole	Size (bp)
GLDecamerAE-05	CCTGTCAGTG	3019	39.5	72.1	10
GLDecamerAE-06	GGGGAAGACA	3126.1	39.5	39	10
GLDecamerAE-07	GTGTCAGTGG	3099.1	39.5	52.1	10
GLDecamerAE-08	CTGGCTCAGA	3028	39.5	44.5	10

TABLE III
IDENTITY OF GENETIC SIMILARITY AMONG MAIZE GENOTYPES

	Z_1	Z_2	Z_3	Z_4	Z_5	Z_6	Z_7	Z_8	Z_9	Z_{10}	Z_{11}	Z_{12}
Z_1	1											
Z_2	0.763	1										
Z_3	0.668	0.663	1									
Z_4	0.633	0.6	0.678	1								
Z_5	0.685	0.685	0.738	0.738	1							
Z_6	0.515	0.55	0.55	0.575	0.748	1						
Z_7	0.61	0.585	0.668	0.665	0.83	0.603	1					
Z_8	0.603	0.645	0.598	0.593	0.345	0.61	0.795	1				
Z_9	0.518	0.585	0.64	0.658	0.653	0.58	0.695	0.635	1			
Z_{10}	0.555	0.68	0.7	0.705	0.648	0.618	0.82	0.815	0.728	1		
Z_{11}	0.58	0.617	0.564	0.584	0.627	0.577	0.764	0.74	0.667	0.77	1	
Z_{12}	0.539	0.6	0.574	0.55	0.5	0.557	0.67	0.724	0.607	0.807	0.784	1

Z_1=Kashmir Gold, Z_2=Islamabad White, Z_3=Sarhad Yellow, Z_4=NARC 2704, Z_5=Karamat, Z_6=Chandni, Z_7=Soan-3, Z_8=FSH-810, Z_9=Babar, Z_{10}=Islamabad Gold, Z_{11}=Ghauri, Z_{12}=Yousafwala Hybrid

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