Training Report

Application of molecular markers for crop improvement

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ABSTRACT:

Characterization of cereal cultivars by DNA-markers provides powerful tool to precise the identification and quantify the genetic diversity and to estimate the phylogenetic relationship among genotypes in many crop species. The results of the present study realized between Jull and Sep 2018 in biotechnologie unit (ICARDA, Morocco) which aimed to characterize a subset of 10 Algerian selected Chickpea cultivars (*Cicer arietinum* L) , using 15 SSR (Single Sequence Repeat) indicated the presence of a total of 61 alleles. The genetic diversity at the 15 microsatellites loci was varied from 0,32 for *TA22* to 0.78 for *TA72* and *TA117* with an average of 0.66 . Polymorphic information content (PIC) values ranged from 0.27 to 0.74. Clustering analysis showed that the studied varieties were grouped according to their population of origin, suggesting a provenance effect in their ordination. In fact the most similar varieties were those introduced from Algerien fields , which may have common parents in their pedigree.

Keywords: Chickpea , SSR , genetic diversity

1. INTRODUCTION:

Chickpea (*Cicer arietinum* L , 2n=2x=16) is an important diploid self pollinated pulse crop of the semi arid tropics and ranks third in worldwide cultivation after pea and common bean. It is mainly cultivated in Indian subcontinent, Mediterranean region, West Asia , America and recently Australia . The genome size of chickpea is estimated to be 740Mb which is 1.5 times that of *Medicago truncatula* , a model legume . Chickpea (*Cicer arietinum* L) , also commonly known as ''garbanzo beans'' or ''bengal gram'' . Two main types are widely accepted by chickpea breeders: ' Kabuli' (white flower, larger and cream coloured seeds) and '' Desi'' (purple flower, small angular and dark seeds) . Chickpea is being valued for its high dietary protein content, absence of specific major anti-nutritional factors and its ability to fix atmospheric nitrogen, making it an important component of the cropping system. It is mostly used for human consumption and to a lesser extent for animal feed .

India is the largest producer of chickpea, accounting for 66% of the world production. Despite its agronomical importance the seed productuvity of chickpea is quite low (~700Kg/ha;. Chickpea has been recognized as a crop with minimal genetic variation . The yield is adversely affected by various biotic and abiotic stresses, including cold drought, insects and nematode pests and fungal infection Two fungal disease, Aschochyta Blight (caused by *aschochyta rabiei* ; syn Phome *rabei* and Fusarium Wilt (caused by *Fusarium oxysporum* f.sp *ciceri*) are important limiting factors for yield worldwide accounting 10% to complete failure of the crop. Improvement of historic low yield and increasing tolerance to biotic/abiotic stresses are the major key parameters of breeding. However , the wild annual species of chickpea have drawn the attention of breeders since they posses many agronomically desirable traits .

In order to improve the productivity of chickpea, the use of DNA based molecular markers has been proposed for marker assisted selection (MAS) , mapping of QTL (Quantitative

Trait Loci) and positional cloning of genes in chickpea . Biochemical and DNA based molecular markers like RFLP , RAPD were unable to adress thee genetic variation within chickpea . The low polymorphic ability of isozymes, RAPD and RFLP markers may be due to lesser polymorphism in structural genes in chickpea genome . have also reported low genetic diversity within *Cicer arietinum* using amplified fragment length polymorphisms (AFLPs).

Simple sequence repeats (SSRs) are short tendem repetitive DNA sequence with a repeat length of few (1-6) base pairs . These sequences are abundant, dispersed throught the genome and are highly polymorphic in comparaison with other molecular markers . Due to therir short repear length and limited interaction at individual loci, SSR markers were used in the present study to investigate the genetic polymorphism among chickpea cultivars .

2.MATERIALS AND METHODS

2.1 Plant Material

Ten local chickpea varieties (names as names as ILC482 ,Garbansa ,FlipR1 ,FlipR2 , Bled ,ILC212 , Flip8492c ,ILC223 ,Flip9393c ,F1038) were grown in the randomized blocks at the research farms of the department of plant breeding of the International Center for Agricultural Research in the Dry Areas - Morocco " ICARDA " , the leaves of three to four weeks old seedlings were used for the present study .

2.2DNA extraction:

DNA was extracted from the fresh leaves of the plants using the CTAB method of Murray and Thompson 1980 . For DNA extraction , about Four grams of the leaves from each variety were ground on a mortar and pestle with the extraction buffer (Tris-HCl; 1M,Na2EDTA; 0.5M,NaCl; 5M, CTAB; 2%, βME; 0.2%) which was then incubated at 65° C for 1 hour in a water bath with manual hand shaking each 15 minutes followed by the addition of 0.5ml of chloroform isoamyl alcohol (24:1) , the content was vigorously mixed then centrifuged at 13000 rpm for 15 minutes at 25 C° , the supernatant was transferred to fresh tubes and then added 0.75ml of ice chilled isopropanol and kept for more than 1 hour in room temperature then centrifuged at 13000 rpm for 10 minutes at 4C° . DNA was accumulated down tubes and given washing in 1ml of 70% ethanol then centrifuged at 13000 rpm in 4C° and dissolved in TE buffer over a night .

Quality and purity of the isolated DNA was determined by agarose gel electrophoresis and spectrophotometry respectively , samples that showed a single band on agarose gel and had a 260nm/280nm ratio of 1.8 ~ 2 were used for further analysis . DNA was quantifed by lambda DNA of known concentrations in 1 % agarose gel .

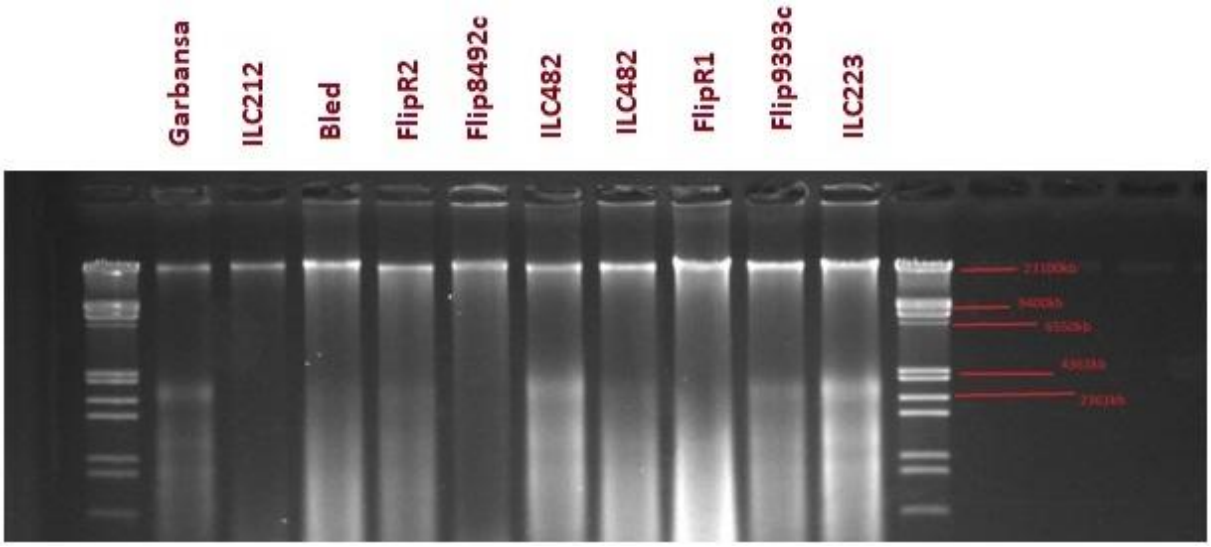

Figure 1: Agarose gel pattern of DNA extraction products of 10 Chickpea cultivars

2.3 PCR AMPLIFICATION :

PCR amplification was carried out in thermal cycler (BIORAD) using SSR molecular markers. The PCR reaction was carried out in a reaction volume of 10µl which consists of 2µl of Bioline 5x PCR buffer with dNTPs , 1µl of primer, 0.05µl of Taq DNA polymerase and about 1µl of template DNA and 5.95µl of Ultra pure water . The PCR was carried for 35 cycles consisting of : initial denaturation of 94°C for 5min , 35cycles of 94°C for 1 minute, 55°C for 1 minute, 72°C for 2 minutes and final extention at 72°C for 10 minutes . The amplified products were separated on 40% Polyacrylamide gel electrophoresis using TBE buffer and the amplification pattern was visualized using BET under UV light of (BIORAD GelDoc XR+) .

3.DATA ANALYSIS:

Power Marker software, Ver. 3.0, was used to calculate genetic diversity, number of alleles and the shared allele genetic distance. A dendrogram was constructed based on genetic distance by using the Neighbor-joining (NJ) method and visualized using MEGA5 software (Tamura et al., 2011). Principal Coordinates Analysis (PCoA) was undertaken using GenAlEx 6.5 software.

3.1Microsatellite polymorphism :

A total of 61 alleles were detected, The number of alleles per locus was ranged from **2** for *TA22 , TA76s* to **5** for *TA27 , TA130, TA200 , TA72 , TA58 , TA117* , the average number of alleles was 3.93 . The genetic diversity (*Hep*) was varied from 0,32 for *TA22* to 0.78 for *TA72* and *TA117* with an average of 0.66 . The Polymorphic Information Content (PIC) was varied from 0.27 for *TA22* to 0.74 for *TA72* and *TA117* with an average of 0.60 (Table1) .

Table2 : Number of alleles and genetic diversity in ten chickpea genotypes using 15 markers .

Markers	Sample Size	Alleles Number	Genetic Diversity (H)	PIC
TA113	**10**	**3**	**0.62**	**0.55**
TA27	**10**	**5**	**0.74**	**0.70**
TA64	**10**	**4**	**0.69**	**0.64**
TA22	**10**	**2**	**0.32**	**0.27**
TA130	**10**	**5**	**0.72**	**0.68**
TA135	**10**	**4**	**0.70**	**0.65**
TA200	**10**	**5**	**0.74**	**0.70**
TA46	**10**	**4**	**0.70**	**0.65**
TA72	**10**	**5**	**0.78**	**0.74**
TA142	**10**	**4**	**0.70**	**0.65**
TA58	**10**	**5**	**0.76**	**0.72**
TA76s	**10**	**2**	**0.48**	**0.36**
TA117	**10**	**5**	**0.78**	**0.74**
TA116	**10**	**3**	**0.56**	**0.50**
TA118	**10**	**3**	**0.58**	**0.49**
Total	**-**	**61**	**-**	**-**
Mean	**-**	**3.93**	**0.66**	**0.60**

3.2 Genetic relationships among varieties:

At a genetic distance of 0.25, the 10 Chickpea genotypes were grouped into 2 main clusters (Figure 1). one cluster contain basically the variety F1038 the other contain all the rest of varieties , at 0.20 another important separation between the rest of varieties giving result of 2 other culsters separating betwen Fl9393c , FI8492c , ILC223 and Bled , FlipR1 , ILC482 , ILC212 FIR2 ,GARB respectively , Principal-coordinate analysis (PCoA) was chosen to complete the information coming out from cluster analysis .

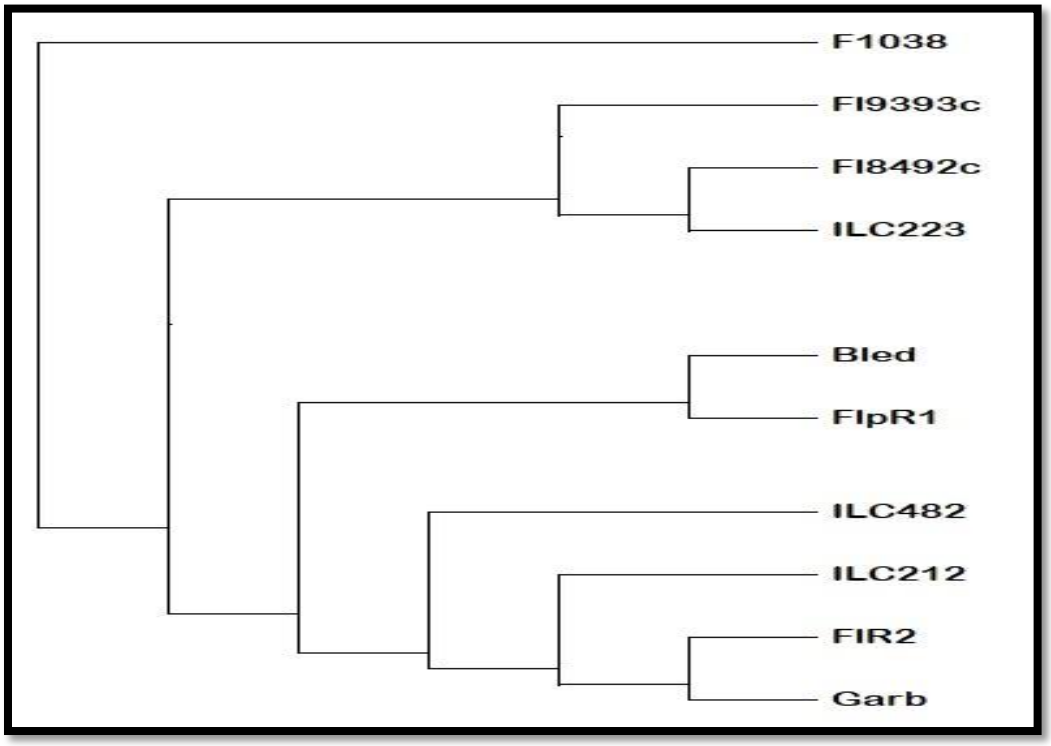

Figure2: Dendrogram showing the relationships among 10 Chickpea genotypes

The studied varieties are grouped according to their population of origin, indicating a provenance effect in their ordination. Along the first PCoA coordinate were opposed ILC223 , FLI8492c, FLI9393c (Group 1) to ILC212 and FLIR2, GARB, ILC482 (Group 2). While along the second PCoA coordinate FLIPR1,BlED (Group 3) were opposed F1038 (Group 4) , both axes contain about 38.67 % of the total explained variation (Figure 3).

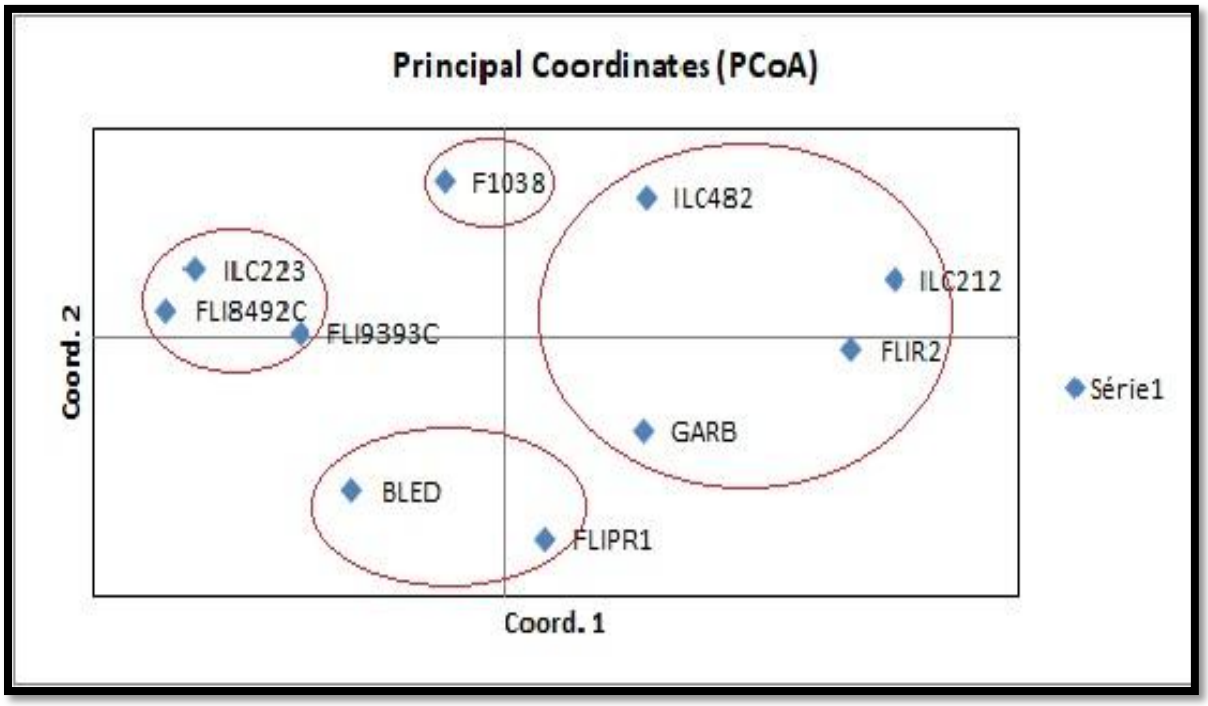

Figure3: Scatter plot of the first and second principal coordinates , after an analysis of genetic diversity derived from 15 microsatellite loce in 10 Algerian Chickpea

varieties .

4. CONCLUSION

Genetic diversity using 15 microsatellite loci has been successfully employed in the molecular characterization of 10 Chickpea varieties grown in Algeria. Genetic variation was very important and diversified . These results are also important for Algerian Chickpea breeding as the success of a breeding program depends largely on the availability of a wide genetic base. The selected set of SSRs has generated high polymorphism, showing its utility in the characterization of Chickpea cultivars. Landraces selections should be a good source of genetic diversity since they had a high polymorphism and they were distinguished from the CIMMYT-derived materials. This benefits Chickpea breeding programs to make best choice of varieties to be used in crosses which facilitates cultivars management.

5. ACKNOWLEDGEMENTS

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