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STUDY OF CHİCKPEA GENOTYPES (*CICER ARIETINUM* L.) RESISTANCE TO FUSARIUM DISEASE WITH DNA MARKERS

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The study was conducted to investigate the resistance of 65 chickpea genotypes (*Cicer arietinum* L.) introduced from ICARDA to Fusarium wilt both under field conditions and through DNA markers (ISSR and RAPD). As a result, 44 points were synthesized with both markers, 38 of which were polymorphic. DNA marker analysis showed high genetic variation (GDI=0.45; PIC=0.21) between native and introduced chickpea samples. The value of genetic distance index was 0.134-0.241. 417 n.c. of A7C RAPD primer, 600 n.c. of OPJ20 primer connected with H2 locus. and ISSR primers 1250 n.c. of UBC-811 primer, 1200 n.c. of UBC 825 primer. long fragments were recorded in most genotypes. 33.8% of the samples were evaluated as fusarium sensitive and 66.2% as resistant samples. Flip 13-123, Flip 13-28, Flip 13-109, Flip 13-75, Flip 13-79c, Flip 13-80c, Flip 13-161, Flip 13-52, Flip 13-33, Flip 13-35, Genotypes Flip 13-47, Flip 13-54, Gusar 44, Jalilabad 11, Ordubad 39, Ordubad 41, Flip 13-102, Flip 13-105, Flip 13-106 according to the phytopathological assessment carried out in field conditions and the results of molecular analyzes were evaluated as fusarium resistant samples. The information we obtained as a result of the research can be effectively used in the cultivation of disease-resistant chickpea samples.

Keywords: Chickpea, Fusarium wilt, molecular markers, resistance, electrophoresis, cluster

In Azerbaijan, there is a great need to create new pea varieties that meet modern requirements, are resistant to stress factors, diseases and pests, have high productivity and technological indicators for different regions of the Republic. As in other countries of the world, the main biotic factor that reduces the productivity of chickpeas in Azerbaijan is fungal diseases (ascochitosis, fusarium, olive mold, etc.). As a result of the lack of a gene resistant to all of these in plants, the resistance of plants decreases during the time.

Chickpeas ranks third in the world among leguminous plants by the size of cultivated areas [1]. The world average annual yield of chickpea is estimated to be about 105,78 kg/ha, which is lower than expected [2]. Low productivity is caused by biotic (Fusarium wilt, Aschochyta, nematodes, etc.) and abiotic stress factors together with a narrow genetic base [3-5]. Especially Fusarium oxysporum f. sp. is one of the most dangerous diseases that reduces productivity by 10-90% [6]. Cultivation of disease-resistant pea genotypes is the most effective method in the fight against Fusarium [7]. Using DNA markers closely linked to wilt resistance genes, it is possible to convert the genes into agronomically superior cultivars without actually exposing the genes to the pathogen. Marker-based sampling is an accurate, easy, and less time-consuming process than conventional methods. It has also been confirmed in previous studies that ISSR markers are more effective than RAPD markers [8, 9] Genetic studies confirm that resistance to race 4 is monogenic recessive [10]. A number of studies have been conducted to decipher the molecular marker closely related to Foc-4 resistance, and RAPD, SCAR, ISSR, STMS, etc. markers have been reported to be closely related to foc-4 [11]. In another study, it was found that most of the fusarium-resistant genotypes have the Foc01 resistance gene and the OPJ20 600 bp fragment [12]. Ratnaparkhe et al. (1998) associated disease resistance gene of UBC-825 ISSR primer and Tullu et al. (1999) reported that CS-27 and UBC-170 RAPD marker were associated with disease resistance.

The present study was conducted to investigate fusarium wilt resistance of 65 chickpea genotypes introduced from ICARDA both under field conditions and through RAPD and ISSR markers.

Material and methods of research

58 of the 65 chickpea genotypes selected for molecular characterization against Fusarium were introduced from the ICARDA genebank, and 8 samples were collected from different regions of Azerbaijan. Samples were grown in field conditions for 3 years and the response to Fusarium disease was determined, resistant, highly resistant, sensitive and highly sensitive samples were selected. Disease incidence and persistence were measured according to the IBU scale.

Immune (no fungus on plants)

Up to 10% – highly resistant

Up to 11-25% – moderate resistant
Up to 26-50% – moderate susceptible
More than 50% – susceptible

In order to group cultivars according to resistance to Fusarium disease, cluster analysis was performed based on the UPGMA (Unweighted Pair Group Method Using Arithmetic Average) method based on the Euclidean genetic distance.

Leaf samples for DNA extraction were taken 20 days after sowing. Genomic DNA was obtained from leaf tissue (2g) according to the CTAB method. The quality and quantity of extracted DNA was determined using a spectrophotometer. For the PCR mix, a 25 µl reaction volume contained 2.5 µl 10 X PCR buffer, 2 µl dNTP (5 mM), 2 µl primer (10 µm), 1.5 µl MgCl2 (50 mM), 0.2 µl Tag polymerase, and 20 The extracted DNA was used. PCR was performed under the following conditions: initial denaturation at 94 °C for 2 min, 40 cycles at 94 °C for 1 min, annealing at 50–55 °C for 45 s, annealing at 72 °C for 1 min, and a final denaturation at the same temperature for 7 min. PCR products were stained with ethidium bromide, electrophoresed on a 1.8% agarose gel, and documented using the BIO-RAD gel-documentation system. A molecular size standard of 1000 bp was used to measure the length of the fragments. The presence or absence of fragments synthesized with RAPD and ISSR primers was coded as (1) or (0), respectively (table 1).

Table 1

Name of DNA primers used in the study

RAPD primers	Primer sequence	Expected fragment length (bp)
UBC 170	ATC TCT CCT G	550
OPJ 20	AGT GGT CGC G	855
A_7C_{417}	TAC TTA TAT CAT G	417
R2609	AGAGAGAGAGAGAGAGG	1600

USSR primers	Primer sequence	Expected fragment length (bp)
UBC 811	GAGAGAGAGAGAGAGAC	1250
UBC 825	ACACACACACACACACT	1200
UBC 864 ACTG 4 UBC 855	ATGATGATGATGATGATG AGAGAGAGAGAGAGAGC ACACACACACACACACYT	400 650 500

Results of the research and discussion

In this research work, the resistance of new cultivars of chickpea (Cicer arietinum L.) introduced from ICARDA to Fusarium diseases was studied based on structural analysis and phytopathological assessment (table 2). RAPD and ISSR primers were also used to distinguish between resistant and susceptible genotypes against fusarium.

In the present study, RAPD (UBC-170550, OPJ-20855, R26091600, A7C417) and ISSR primers (UBC-8251200, UBC-8111250 and ACTG4, UBC-864400, UBC-855500) previously reported to be associated with a disease susceptibility gene against fusarium used to distinguish resistant and susceptible genotypes [12, 6, 10]. The specific fragment expected with the primers used was synthesized only in susceptible genotypes. Thus, 417 n.c. with A7C RAPD primer, 550 n.c. with UBC-170 RAPD primer, 855 n.c. with OPJ-20 RAPD primer, 600 n.c. with R26091 RAPD primer attached to H2 locus. length fragment was synthesized. UBC-825 ISSR primer 1200 n.c., UBC 864 primer 400 n.c., UBC-855 primer 500 n.c., and UBC 811 primer 1250 n.c. in susceptible and moderately susceptible genotypes. gave a clause in length. These fragments were not observed in resistant and highly resistant accessions. (Figures 1, 2 and 3).

Using RAPD and ISSR primers, 44 points were synthesized for 65 chickpea samples, of which 38 points (86.3%) were polymorphic (table 3). On average, a total of 4.9 points were recorded with each primer, of which 3.3 points were polymorphic. The highest polymorphism was recorded with primer UBC-825, UBC-811 and A7C (100%), and the weakest polymorphism was recorded with primer R2609 (75%). The highest value of GMI was calculated with primer UBC 825 (GMI=0.95), and the lowest value was calculated with UBC 855 (GMI=0.33).

Table 2

The name of the samples used in the study and resistance to Fusarium

Specimen name	Fusarium Continuity	Specimen name	Fusarium Continuity	Specimen name	Fusarium Continuity
Flip13-24c	Moderate resistant	Flip13-55c	Resistant	Flip13-81c	High durable
Flip13-26c	Moderate resistant	Flip13-56c	Susceptible	Flip13-83c	Susceptible
Flip13-28c	Resistant	Flip13-57c	Susceptible	Flip13-86c	Susceptible
Flip13-30c	Moderate resistant	Flip13-58c	Resistant	Flip13-89c	Resistant
Flip13-31c	Resistant	Flip13-59c	Resistant	Flip13-93c	Susceptible
Flip13-32c	Susceptible	Flip13-64c	Resistant	Flip13-98c	Resistant
Flip13-33c	Moderate resistant	Flip13-65c	Susceptible	Flip13-102c	Resistant
Flip13-35c	Resistant	Flip13-66c	Susceptible	Flip13-105c	Resistant
Flip13-36c	Resistant	Flip13-67c	Susceptible	Flip13-106c	Resistant
Flip13-39c	Resistant	Flip13-69c	Susceptible	Flip13-108c	Susceptible
Flip13-43c	Resistant	Flip13-72c	Resistant	Flip13-109c	Resistant
Flip13-47c	High durable	Flip13-74c	Resistant	Flip13-120c	Susceptible
Flip13-48c	Susceptible	Flip13-75c	Resistant	Flip13-122c	Moderate susceptible
Flip13-50c	Moderate susceptible	Flip13-76c	Resistant	Flip13-123c	Resistant
Flip13-52c	Resistant	Flip13-78c	Moderate susceptible	Flip13-128c	Resistant
Flip13-53c	Moderate resistant	Flip13-79c	Resistant	Flip13-161c	Resistant
Flip13-54c	Resistant	Flip13-80c	Resistant	Qusar44	Resistant
Flip06-8c	Moderate resistant	Flip 06-161	Moderate resistant	Ağstafa42	Moderate susceptible
Flip06-133c	Moderate susceptible	Flip 05-169c	Moderate resistant	Flip03-22	Susceptible
Flip06-61c	Moderate susceptible	Ordubad 39	Moderate resistant	Bakı30	Moderate resistant
Abşeron34	Moderate resistant	Ordubad 41	Moderate susceptible	Cəlilabad11	Moderate resistant
Flip 06-33c	Moderate susceptible	Qusar 43	Moderate resistant	Nərmin	Moderate susceptible

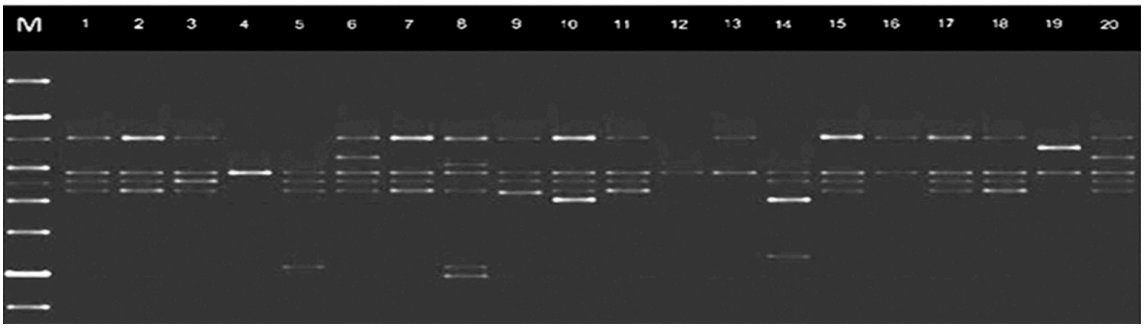

Fig. 1. Distribution of alleles synthesized by primer CS-27 among chickpea genotypes

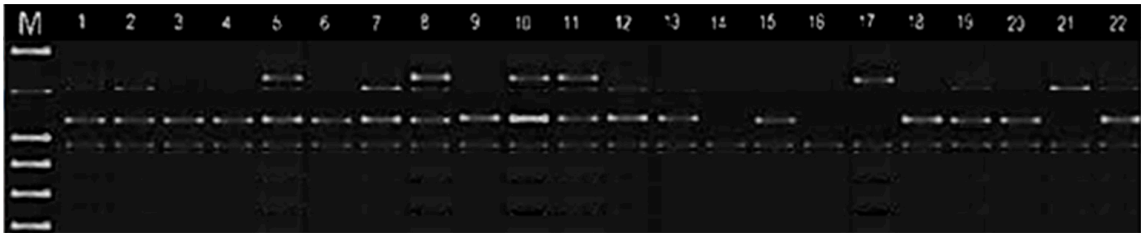

Fig. 2. Distribution of alleles synthesized by primer UBC 170 among chickpea genotypes

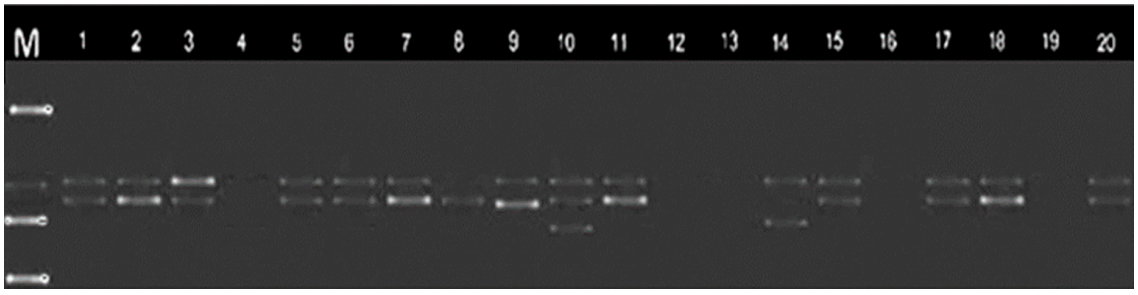

Fig. 3. Distribution of alleles synthesized by primer UBC 811 among chickpea genotypes

Table 3

Name of DNA primers used in the study

Primers	Total number of bands	Number of polymorphic bands	Percentage of polymorphic	Genetic diversity index	PIC	MI
RAPD primers						
UBC 170	5	4	80	0,64	0,31	0,13
OPJ 20	6	5	83,3	0,71	0,14	0,17
$A7C_{417}$	4	4	100	0,66	0,27	0,07
R2609	4	3	75	0,57	0,25	0,10
ISSR primers						
UBC 811	3	3	100	0,42	0,33	0,12
UBC 825	4	4	100	0,95	0,41	0,18
UBC 864	5	4	80	0,54	0,37	0,09
ACTG 4	6	5	83,3	0,63	0,39	0,11
UBC 855	7	6	85,7	0,57	0,22	0,16
Total	44	38	86,3	0,63	0,45	0,13

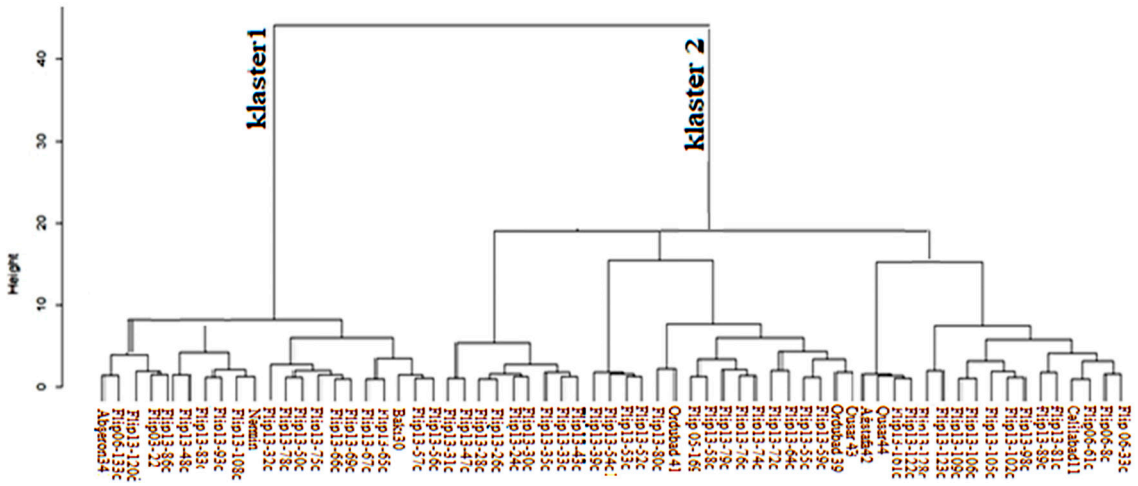

Fig. 4. Grouping of chickpea samples according to resistance to Fusarium disease as a result of RAPD and ISSR markers analysis

For comparison, let's note that in the previous research study, 77 points of which 76 were polymorphic with RAPD primer and 41 points of which 32 were polymorphic with ISSR marker were recorded in 62 pea genotypes [9].

Among the studied genotypes in this study, 86.3% polymorphism was recorded, which is higher than the value noted in previous studies [12]. However, the dendrogram based on the Nei similarity coefficient was only able to distinguish resistant and susceptible genotypes. Moderately resistant genotypes were grouped in separate clusters. Resistant genotypes are grouped in the first cluster. Sensitive genotypes are located in cluster II (Figure 4).

Thus, these primers, which were associated with susceptibility by other researchers, were also associated with susceptibility in our study. Among the studied genotypes in this study, polymorphism was recorded, resistant and susceptible genotypes were identified. However, in the dendrogram obtained as a result of the cluster analysis based on the Nei similarity coefficient, the genotypes were grouped into two main clusters, the first cluster contained susceptible and highly susceptible (22 samples), and the second cluster contained resistant and highly resistant genotypes (43 samples) (Figure 4).

Conclusion

Flip 13-123, Flip 13-28, Flip 13-109, Flip 13-75, Flip 13-79c, Flip 13-80c, Flip 13-161, Flip 13-52, Flip 13-33, Flip 13-35, Genotypes Flip 13-47, Flip 13-54, Gusar 44, Jalilabad 11, Ordubad 39, Ordubad 41, Flip 13-102, Flip 13-105, Flip 13-106 according to the phytopathological assessment carried out in field conditions and the results of molecular analyzes were evaluated as fusarium resistant samples. The information we obtained as a result of the research can be effectively used in the cultivation of disease-resistant chickpea samples.

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