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**SSR-АНАЛИЗ МУТАНТНЫХ ЛИНИЙ НУТА
(*Cicer arietinum* L.), ПОЛУЧЕННЫХ В РЕЗУЛЬТАТЕ γ -ОБЛУЧЕНИЯ**

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Abstract

Molecular characterization of new chickpea mutants generated through γ -irradiation was carried out through SSR analysis. 16 chickpea accessions were used in the study, including 12 M5 lines and 3 M7 lines. SSR analysis was conducted with the use of 20 loci. The number of alleles per microsatellite locus ranged from none to 14, with a total of 90 bands being analyzed. The selected SSR markers successfully distinguished the genotypes under study.

Introduction

Chickpea (also known as gram or garbanzo), species *Cicer arietinum* L., is an annual, autogamous, diploid ($2n=2x=16$) plant, extensively grown for its nourishing seeds. Its genome is taxonomically proximal to that of the model legume *Medicago truncatula* [5]. Chickpea is the third most important grain legume after bean and pea [1, 7]. Gram is precious for its high protein content and capacity to fix atmospheric nitrogen in symbiosis with Rhizobiaceae [5].

For breeding purposes, chickpea is divided into two main types: Desi, with small, sharp, dark-coloured seeds and Kabuli, characterized by large, smooth, cream-coloured seeds [3]. As a valuable grain legume, chickpea is grown in more than 45 countries. In many areas of production though, the gram is vulnerable to biotic stresses (*Aschochyta* blight and *Fusarium* wilt), which severely reduce the yield [1, 2, 3]. Hence the identification of new high-productivity chickpea varieties resistant to biotic and abiotic factors is required. One of the techniques effective in producing crops with enhanced quality and novel properties is γ -induced mutagenesis. During the last decades physical mutagens, as sources of genetic variation, have already shown their potential for plant breeding purposes [8, 9]. The fast detection of mutants can be nowadays assured by the employment of DNA-based molecular markers.

Among the useful systems of molecular markers available nowadays are SSRs (simple sequence repeats or microsatellites). They are highly informative, co-dominant, could occur in non-coding regions, as well as in transcribed sequences and are widely used for many purposes in plant genetics and breeding such as: genetic diversity analysis, molecular mapping, etc. [2, 5, 6, 13]. A number of chickpea SSR (and EST-SSR) markers has been developed recently [2, 5, 6, 11].

This paper aims at providing the results of SSR analysis of newly-generated chickpea mutant lines obtained through gamma-irradiation.

Material and Methods

16 chickpea accessions were used in this study, including 12 M5 lines and 3 M7 lines. The genotypes were: 2/98 (control, parent line); M5 lines: F1, F3, F8, F9, F10, F15, F22, F25, F26, F30, F31, F44; and M7 lines: S3, S7, S9. The mutants were developed at the Institute of Genetics and Plant Physiology of the Academy of Sciences of Moldova. 3000 seeds of the parent line were irradiated, dose = 250 Gy, at the Co⁶⁰ source, which has the dose rate of 0.67 Gy/sec

DNA was extracted by the modified [10] CTAB method [4] from young leaves of individual seedlings. DNA quality and quantity was determined both spectrophotometrically and through 1% agarose gel electrophoresis. At least 3 individual plants of the same genotype served for DNA pooling. SSR analysis was performed at the Saraykoy Nuclear Research and Training Center, Ankara, Turkey. 20 primer pairs (developed by [6], employed and described [6, 10]) were used (table 1). All PCR amplifications of genomic DNA were carried out in a 20 µl reaction volume containing 200 pg/µl of genomic DNA, 1xPCR buffer, 1 µM of each primer, 0.2 mM of each dNTP, 1.5 mM MgCl₂, 0.5 U of Taq DNA polymerase (Sigma, Germany).

Table

List of SSR primers used [6] and the number of amplicons generated

№	primer	Expected Number of Amplicons	Maximal Number of Generated Amplicons
1	2	3	4
1	H1F14	1	1
2	H2A04	1	1
3	H4F09	1	3
4	H1B09	1	2
5	H1O10	2	9
6	H1O01	2	5
7	H4A09	1	6

Продолжение таблицы

1	2	3	4
8	H3G06	2	1
9	H1H20	1	4
10	H2I20	1	1
11	H4G08	1	5
12	H6B11	3	6
13	H5D02	1	2
14	H2J04	1	5
15	H6D02	2	2
16	H3B04	1	5
17	H1K18	2	14
18	H1E12	1	4
19	H5E05	1	8
20	H1P17	1	6
Total			90

The amplification programs followed the annealing and elongation temperature recommendations of the primer authors [6]. The analyses were repeated at least twice to ensure reproducibility of the results. Amplification products were separated in 4 % agarose (Invitrogen) gels in one-time Tris-borate-EDTA (TBE) buffer, stained with ethidium bromide. The fingerprints were examined under the ultraviolet light and photographed using the Vilber Lourmat Gel Documentation System. The SSR bands were scored in a binary matrix and analyzed using TreeCon version 1.3 b [12]. Genetic similarity estimations were used for the construction of UPGMA based dendrogram.

Results and Discussion

As ion particles induce different kinds of alterations of the DNA (both transmissible and non-transmissible), they could therefore produce various types of mutant alleles in plants [8, 9]. This is the time and the place when the molecular markers employed today could provide tools to assist the selection process. 15 mutant lines were generated as a result of γ -irradiation of 2/98, chosen as a parent line because of its high productivity.

In this study, the genetic fingerprinting of 16 chickpea genotypes was performed using 20 SSR primers (Fig.1). A total of 90 bands were amplified across the lines. The number of alleles per microsatellite locus ranged from none to 14. The maximal number of amplicons generated by each primer is presented in table.



Fig. 1. Example of gel patterns of SSR-PCR products amplified with H1H20. M: 2-Log Ladder (NEB); c: negative control. Lane identification refers to the genotypes as listed in Material and Methods.

The scoring and analysis of the bands resulted in the construction of an UPGMA-based dendrogram showing genetic similarities of genotypes under study, calculated using Nei and Li's coefficient (Fig.2).

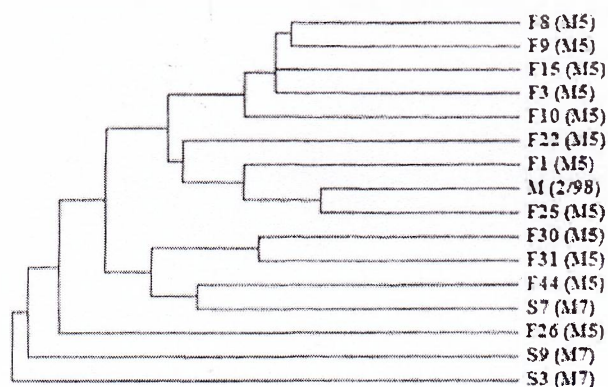


Fig. 2. UPGMA-based dendrogram showing genetic similarities among 16 chickpea lines

As shown in the dendrogram, the parent line 2/98 was grouped in cluster I, as well as all the M5 lines, except for F26. According to morpho-physiological characteristics, F26 differs from other lines through a very late flowering (more than 10 days later than the parent line), late and incomplete maturity and small

seeds. Among the M7 lines, S7 and S9 are considered very similar morpho-physiologically and are characterized through a high productivity and draught resistance (in 2007, under severe draught conditions, the lines' productivity was 50 % higher than in control). Molecular studies however discriminated these two lines, clustering S7 in group I. A third M7 line selected for analysis (S3) formed an outgroup. Morpho-physiological studies distinguished S3 as an early flowering and maturity line with big seeds (26g/100 seeds), medium protein content of 19,4 % and a productivity 20% higher than that of the parent line (being the highest among mutants).

Conclusion

The present study revealed the genetic diversity within a collection of new mutant lines obtained through γ -irradiation. The 20 SSR markers selected for analysis successfully discriminated the studied genotypes. The increase of the number of analyzed loci will though lead to a more precise molecular characterization of mutants and will permit a more accurate distinguishing of mutant lines.

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