GENETIC DIVERSITY IN FEW ROMANIAN ALFALFA GENOTYPES USED RAPD MARKERS

DIVERSITATEA GENETICĂ LA CÂTEVA GENOTIPURI ROMÂNEŞTI DE LUCERNĂ UTILIZÂND MARKERI RAPD

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Abstract:*In this study, five alfalfa genotypes, cultivated in Romania, were analyzed to verify genetic identity using RAPD primers. The aims of this project are to use molecular markers to assess the overall genetic diversity among Gloria, Sigma, Coral, Satelit and Super alfalfa genotypes. The approach developed here was based on individual specific bands so each population was represented by 10 individuals. However this study shows that RAPD markers could be used in variety distinction, as specific bands were found.*

Rezumat:*În această lucrare, s-a studiat diversitatea genetică la cinci genotipuri de lucernă, cultivate în România, utilizând primeri RAPD. Obiectivul central al acestui studiu constă în utilizarea markerilor RAPD pentru evaluarea diversităţii dintre genotipurile Gloria, Sigma, Coral, Satelit şi Super. Această lucrare urmăreşte benzile specifice, fiecare genotip fiind reprezentat de 10 indivizi. Markerii RAPD pot fi utilizaţi în distingerea variabilităţii intergenotipice.*

Key words: *alfalfa, molecular markers, RAPD, genetic variability*
Cuvinte cheie: *lucernă, markeri moleculari, RAPD, variabilitate genetică*

INTRODUCTION

Alfalfa, (*Medicago sativa* L., 2n = 4x = 32) is the most important forage legume in temperate climates (MICHAUD *et al*., 1988) and is the most cultivated forage legume due to its ability to fix atmospheric dinitrogen and its high protein content. The analysis of the genetic variability within and among populations of cultivated alfalfa can assess future risk of genetic erosion and help in the development of sustainable conservation and genetic improvement strategies. However, since alfalfa is autotetraploid (2n = 4x = 32), allogamous and seed propagated successful assessment of the genetic diversity of alfalfa has been hampered by the statistical methods available (STANFORD 1951, FLAJOULOT *et al.* 2005).

At the moment, its importance is raising with the increase of public interest in sustainable agriculture because, as reported by MCCOY and ECHT (1992), alfalfa is a low input energy efficient crop that helps improve soil tilth. Furthermore, it occupies a significant economic position in the animal feed market (i.e. hay, dehydrated forage, pellets and silage products).

The necessity for sequence information for PCR was circumvented using short primers of arbitrary sequences to amplify DNA segments, namely RAPD. The speed and efficiency of RAPD analysis encouraged scientists to perform high-density genetic mapping in many plant species such as alfalfa (KISS ET al., 1993), faba bean (TORRESS et al., 1993) and apple (HEMMAT et al., 1994) in a relatively short time.

The discovery that PCR with random primers can be used to amplify a set of randomly distributed loci in any genome facilitated the development of genetic markers for a variety of purposes (WILLIAMS et al., 1990, WELSH et al., 1990).

MATERIAL AND METHODS

Seeds of five alfalfa genotypes Gloria, Sigma, Coral, Satelit and Super cultivated in Romania, were disinfected by immersion in a 70^0 EtOH for 10 sec. followed by 0,1% $HgCl_2$ solution for 3 min, followed by 5 rinses in sterilized water. Seeds were placed on half-strength MS basal medium [5] to obtain plant material. We tested 10 individuals from each considered genotypes.

DNA fingerprinting protocols

Molecular analysis based on Random amplified polymorphic DNA (RAPD) markers was carried out to verify genetic identity, among five alfalfa genotypes.

We used five RAPD primers (G03, G06, G10, G17, B07) with following sequences: $^{5'}$ GAGCCCTCCA $^{3'}$, $^{5'}$ GTGCCTAACC $^{3'}$, $^{5'}$ AGGGCCGTCT $^{3'}$, 5' ACGACCGACA 3', 5' GGTGACGCAG 3'.

Total genomic DNA was extracted from the bulked plantlets leaf tissues using a modified CTAB method. DNA samples were diluted in TE buffer and submitted to electrophoresis (3V cm-1) in 0.7% agarose gels (w/v).

DNA was stained by gel immersion into ethidium bromide solution for 30 min. RAPD reactions were performed in a final volume of 25 µl in PCR buffer containing MgCl2, RAPD primers, dNTP, DNA template and Taq DNA polymerase. Reactions were submitted to the following PCR program: preliminary DNA denaturation for 3 min at 94°C, followed by 45 cycles consisting of denaturation (3 min, 94°C), primer annealing (1,5 min, 36°C), and extension (2 min, 72°C). A final extension for 2 min at 72°C was included. The RAPD products were separated by electrophoresis (3V cm-1) in 2% agarose gels, which run with 1 x TAE buffer. Photo documentation was performed under UV light using a photo imaging system.

RESULTS AND DISCUSSIONS

Concerning RAPD analysis, template DNA produced clear PCR profiles. As a result of UV light screening of agarose gels, it has been observed slight differences between the genetic fingerprints of the studied varieties induced by amplification using GO3 primer. GO6 primer found complementary sequences in the genome of the varieties taken into study.

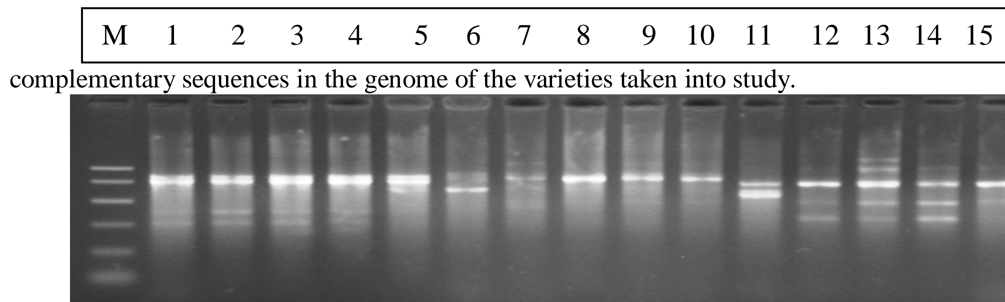

Figure 1. RAPD profiles (primers G03, G06, G10) by electrophoresis in 2% agarose gel
Legend:
M - PCR marker (1000, 750, 500, 300,150, 50bp);
Lane 1- 5 Gloria, Sigma, Coral, Satelit and Super alfalfa genotypes, primer G03;
Lane 6-10 Gloria, Sigma, Coral, Satelit and Super alfalfa genotypes, primer G06;
Lane 11-15 Gloria, Sigma, Coral, Satelit and Super alfalfa genotypes, primer G10;

The polymorphism was emphasized on Gloria genotype which showed a supplementary lane at middle distance between 750 and 500 bp. The amplification using G10 and G17 primers have led to satisfactory results, emphasizing strong polymorphism at molecular level. It has been observed that the use the BO7 primer did not revealed any variability between genetic fingerprints.

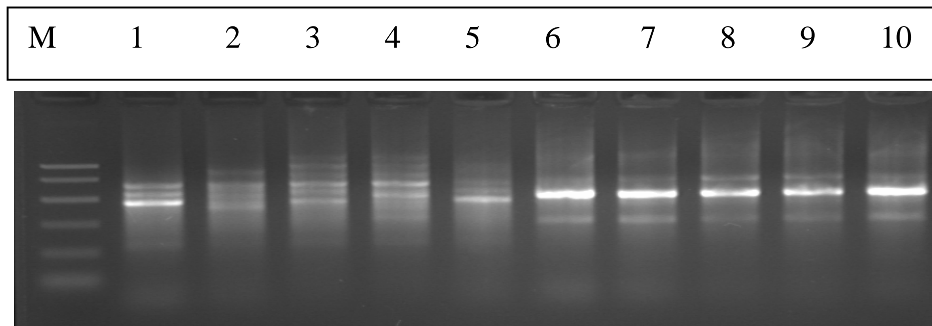

Figure 2. RAPD profiles (primer G17, B07) by electrophoresis in 2% agarose gel
Legend:
M - PCR marker (1000, 750, 500, 300,150, 50bp);
Lane 1- 5 Gloria, Sigma, Coral, Satelit and Super alfalfa genotypes, primer G17;
Lane 6-10 Gloria, Sigma, Coral, Satelit and Super alfalfa genotypes, primer B07;

CONCLUSIONS

- The evaluation of diversity between alfalfa studied genotypes using dominant RAPD markers allowed to draw the following conclusions:
- The best results were obtained by amplification using G10 and G17, that emphasized a strong polymorphism at molecular level;
- The amplification using G03 and GO6 primers showed small differences between genetic fingerprints of the studied varieties;
- The amplification using B07 primer has not detected the variability at molecular level.

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