the fungus-resistant chickpea plants have an inherent capability to inactivate/degrade the phytotoxic compounds induced by the invading fungi or if the fungus is unable to induce the synthesis of the phytotoxic compounds. In any event the results of analysis of artificially-infected chickpea plants (Latif et al. 1996) suggest that the defence mechanisms of fungus-resistant chickpea cultivars may inactivate the phytotoxic compounds.

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Biotechnology

Random Amplified Microsatellite Polymorphism (RAMPO) Analysis of Chickpea Accessions

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Analysis of genetic diversity in chickpea using restriction fragment length polymorphism (RFLP) technique has shown little molecular diversity (Udupa et al. 1993). Alternatively, random amplified polymorphic DNA (RAPD) markers are frequently used for fingerprinting due to simplicity of this technique. Since it is less reproducible than other DNA markers (Winter and Kahl 1995) a combination of RAPD amplification of DNA and subsequent hybridization using microsatellites, known as random amplified microsatellite polymorphism (RAMPO) has recently been reported to be efficient for detecting genetic variability in plant and fungal DNA samples (Richardson et al. 1995). We evaluated this technique to study genetic diversity in five chickpea accessions.

DNA was isolated from five different chickpea accessions (four cultivated *Cicer arietinum* accessions and one wild *C. echinospermum* accession; Table 1) using the CTAB method (Saghai-Maroof et al. 1984) and performed RAPD analysis (Williams et al. 1990) using 12 random primers [(OPF-2 to OPF-12, and OPF-14) Operon Technol. Inc, USA]. Polymerase chain reaction (PCR) was carried out in a thermocycler (Perkin Elmer PCR system, GeneAmp 9600) programmed for 40 cycles (94°C for 1 min for template denaturation, 40°C for 1 min for primer annealing and 72°C for 2 min for primer extension) using the fastest possible temperature transitions. The amplified DNA fragments were resolved in either 2.5% Nusieve 3:1 agarose gels (FMC Biotech., USA) or 1.5% agarose gel (Sigma, USA), stained with ethidium bromide and photographed under UV illumination. For RAMPO analysis, the RAPD amplified DNA fragments separated on agarose gels were blotted onto Hybond N^+ (Amersham Intl, UK) nylon membrane

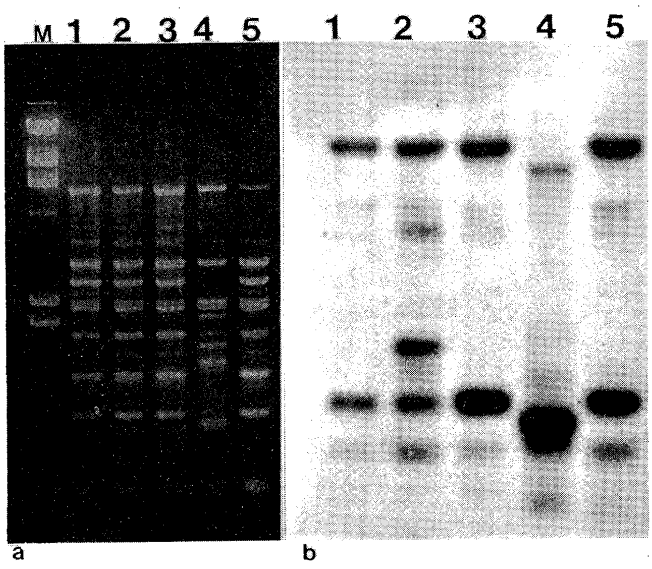

Figure 1. (a) RAPD amplification profile obtained using primer OPF-2 among the various *Cicer* accessions; (b) RAMPO analysis of the OPF-2 amplified RAPD products using (γ-^{32}P labeled probe $(TG)_{10}$. Lanes 1–5 = chickpea accessions ICCV 92504, ICCV 88202, Pant G114, ICCW 44 (wild), and PBG 1. Lane M = λ DNA *Hind* III digest.

Table 1. Comparison of RAPD diversity across different chickpea accessions.

S. No.	Accessions	Total number of amplified bands	Polymorphic bands	Average number of bands per primer	Unique bands
1.	ICCV 92504	85	0	7.08	0
2.	ICCV 88202	86	2	7.16	0
3.	Pant G 114	85	3	7.08	0
4.	ICCW 44 (wild)	85	34	7.08	30
5.	PBG 1	83	2	6.9	0

Table 2. Comparison of RAMPO diversity across different chickpea accessions.

S. No.	Accessions	Polymorphic bands with $(TG)_{10}$	Polymorphic bands with $(GATA)_4$	Polymorphic bands with $(CAC)_5$	Unique bands
1.	ICCV 92504	2	0	0	0
2.	ICCV 88202	4	1	2	4
3.	Pant G 114	6	0	1	5
4.	ICCW 44 (wild)	20	7	4	27
5.	PBG 1	5	0	0	0

and hybridized to γ-^{32}P labeled microsatellite oligonucleotide probes [$(TG)_{10}$, $(GATA)_4$, and $(CAC)_5$] as described for chickpea (Sharma et al. 1995).

Similarity index matrices were generated based on the proportion of commonly amplified DNA bands between two accessions. The dendrogram was constructed using Euclidean distance matrix and centroid linkage method using SYSTAT, version 5.0 software package.

Comparison of diversity across different chickpea accessions. RAPD analysis with 12 primers revealed a total of 424 amplified DNA bands, amplifying an average of 35 DNA bands in all the accessions analyzed (Table 1). The wild species produced a maximum of 34 polymorphic bands in contrast to cultivars (Fig. 1 and Table 1). All accessions except ICCV 92504 produced either 2 or 3 polymorphic bands. RAMPO analysis of these accessions using microsatellite probes revealed microsatellite-complementary amplified bands not visible in ethidium bromide-stained gel. The combinations of RAPD analysis and probing with microsatellite repeats $(TG)_{10}$, $(GATA)_4$, and $(CAC)_5$ have revealed polymorphic and genotype-specific unique DNA banding patterns. Thus, all five chickpea accessions could be distinguished using RAMPO. ICCV 92504 revealed two polymorphic DNA bands in OPF 2-$(TG)_{10}$ and OPF 9-$(TG)_{10}$ combinations (Table 2). Accession-specific unique DNA bands were revealed in ICCV 88202, Pant G 114, and ICCW 44 (Fig. 1b), while ICCV 92504 and PBG1 did not reveal any such (accession-specific) bands in RAMPO analysis (Table 2). Phylogenetic analysis of the data derived from RAPD produced a tree where ICCV 92504, ICCV 88202, and Pant G114 grouped together while PBG1 was close to Pant G 114, and ICCW 44 (*C. echinospermum*) grouped separately. Accessions ICCV 92504 and ICCV 88202, PBG1, and Pant G114 formed two distinct subgroups belonging to a single major group, with ICCW 44 grouping separately in a tree derived from RAMPO data. The tree obtained based on combined (RAPD + RAMPO) data resembled the one obtained with RAMPO data since two new polymorphic bands were detected in ICCV 92504 (not detected in RAPDs), hence showing the advantage of RAMPO technique.

The RAMPO technique detected DNA bands homologous to microsatellite loci amplified in RAPD reaction. It also showed the DNA bands not visible in ethidium bromide-stained RAPD gels. Using microsatellites, Sharma et al. (1995) were able to fingerprint individual plants within a chickpea accession. As RAPD and microsatellite-based genome analysis identify different regions of the genome (differing in their degree of sequence variation; Rus-Kortekaas et al. 1994) their efficiency will vary. The RFLP using microsatellite

probes among the restriction digested DNA is time consuming and labor intensive. The use of microsatellite hybridization in combination with RAPD amplification or RAMPO technique will help to reduce the assay expenditure as a single RAPD blot can be reused for subsequent hybridization with many microsatellites. Also, for the initial RAPD reaction much less DNA is required. Thus, in the absence of sequence-characterized microsatellite markers for chickpea, the RAMPO technique will supplement RAPD analysis of chickpea genome and its characterization much faster.

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An Efficient In vitro Rooting Method for Chickpea

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In vitro micropropagation can be very useful in classical breeding, since it allows an increase in the number of individuals as well as maintenance of genotypes with poor reproduction. An efficient system should also be suitable for genetic transformation. In chickpea there are very few micropropagation protocols that are efficient and reproducible that can be used in the breeding of this species. Some regeneration methods by organogenesis have been reported in which the rooting of shoots were not satisfactory (Shri and Davis 1992). Recently rooting percentages of 50–65% have been reported using both α-naphthaleneacetic acid (NAA) and 3-indolebutyric acid (IBA) (Adkins et al. 1995; Barna and Wakhlu 1995). In this note we report an effective rooting medium for chickpea.

Seeds of genotype P-678 from ICRISAT and an F_2 population (CA-1938 × ICCL-81001) from our breeding program were sterilized by immersion in 10% sodium hypochlorite with a few drops of Tween-20 (Sigma Chemical Co, Madrid, Spain) for 10 min and rinsed three times with sterile deionized water. The seeds were aseptically germinated in MS medium (Murashige and Skoog 1962) with different concentrations of N^6-benzylaminopurine (BAP). Two successive experiments were performed.

In the first experiment, the shoots obtained from the seedlings were transferred to three solid rooting media (MS + 0.4% agarose) with 1, 2, or 4 mg NAA L^{-1}. For each genotype and NAA concentration, 43–59 shoots were cultured. We observed that the shoots rooted above the surface of the solid medium. For this reason the shoots that did not root after 4 weeks in culture were cultured in a liquid medium instead of solid medium with the same NAA concentration and with the shoot base on a filter paper that was in contact with the medium.

A second experiment to test the efficiency of the rooting in liquid medium was carried out. Shoots excised from seedlings were transferred to two rooting media, one solid (0.4% agarose) and the other one liquid, both with the same NAA concentration (1 mg L^{-1}). For each genotype and treatment, 58–160 shoots were cultured.