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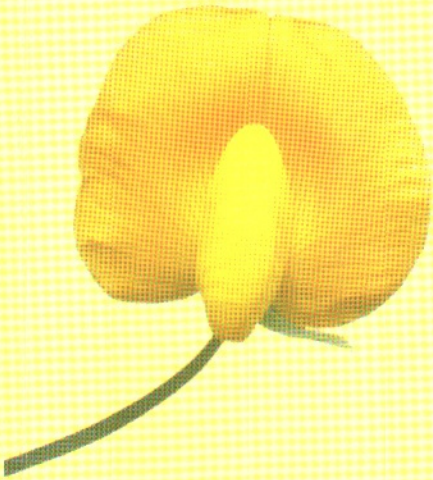
Annual Report

2009-10



मूँगफली अनुसंधान निदेशालय

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Directorate of Groundnut Research

P. B. No. 5, Jnuagadh - 362 001, Gujarat, India

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Directorate of Groundnut Research
(Indian Council of Agricultural Research)
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PROJECT 09: BIOTECHNOLOGICAL APPROACHES TO THE CHARACTERISATION AND GENETIC ENHANCEMENT OF GROUNDNUT

(RADHAKRISHNAN T., A.L. RATHNAKUMAR, CHUNI LAL, S.K. BERA, MANJUNATHA, T. AND ABHAY KUMAR)

Genetic transformation

Confirmation of putative transgenics from *mtlD* gene construct

An attempt was made to transform groundnut cultivar GG 20 with *mtlD* gene. In all 636 de-embryonated cotyledons were co-cultured with *Agrobacterium* containing the gene construct *mtlD*. Multiple shoots could be induced only in 380 explants. From these explants 207 shoots were isolated and grown on medium with selection pressure of antibiotic kanamycin. Only 84 (40.6%) putative transgenics were identified of which only 16 were PCR positive with the expected fragment of length of 400 bp.

The nine transgenics identified earlier as positive by PCR based amplifications of the candidate gene as well as by the southern hybridisation for confirmation of integration, were confirmed further by RT-PCR for the expression of the *mtlD* gene sequence.

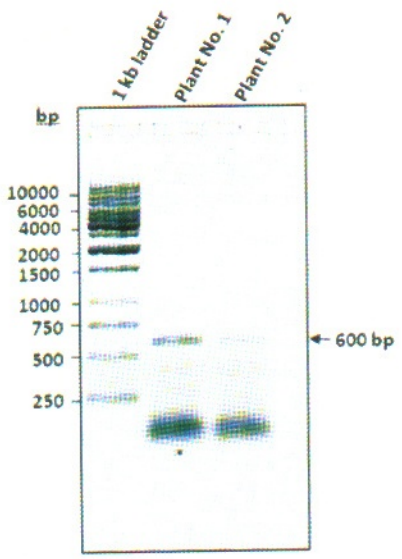


Figure 1. RT-PCR amplification of the *mtlD* gene showing the expected amplification of 600bp

Rapid multiplication of transgenics from *mtlD* gene construct

Vegetative multiplication of the T_0 plants was done to increase the plant population required for the characterisation. From 9 confirmed plants, about 40 plants were produced

which were grown and the pods were harvested. About 100 pods each from the nine confirmed T_0 plants are now available for further studies.

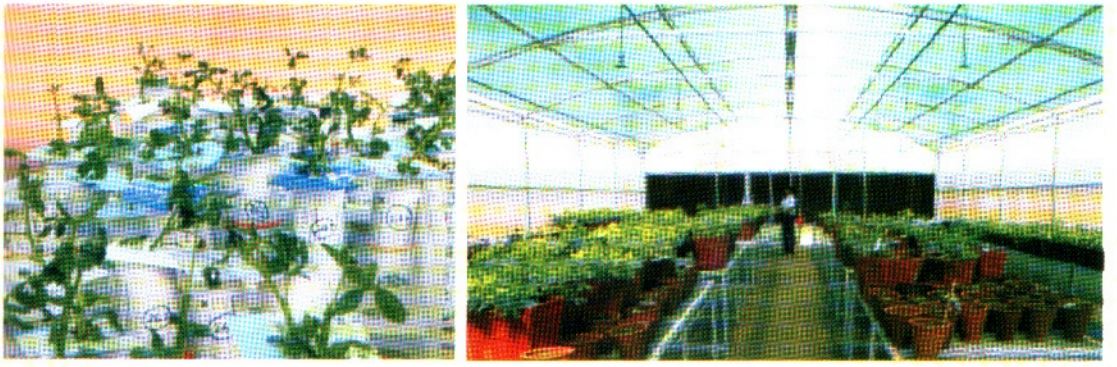


Figure 2. Rapid multiplication of the transgenics under greenhouse conditions

Confirmation of putative transgenics from *defensin* gene constructs

Using de-embryonated cotyledons as explants, 1191 co-cultures were made. Only 869 explants regenerated. From the regenerated shoots, out of 112 shoots transferred for rooting under selection pressure only 33 shoots produced roots.

Though several putative transgenics, which passed the antibiotic selection were grown. All these plants, however, failed the PCR test for the presence of the transgene. A second lot of plants is now being PCR tested for confirmation.

Development of mapping populations and assessment of molecular diversity Fresh hybridisations for developing mapping populations

Three crosses were made for developing mapping populations for tolerance of stem rot. Probable hybrid pods were harvested for confirmation and isolation of true hybrids. The particulars of the crosses made are given in table 1. Three crosses were made for developing mapping populations for tolerance of foliar fungal diseases and another two crosses for tolerance of stem rot and confirmed F_1 hybrids were isolated. The particulars of these five crosses are given in table 2.

Table 1. Probable hybrid pods harvested

No.	Cross	No of pods harvested
1.	GG 20 x CS 75	91
2.	GG 20 x CS 83	91
3.	GG 20 x CS 19	71

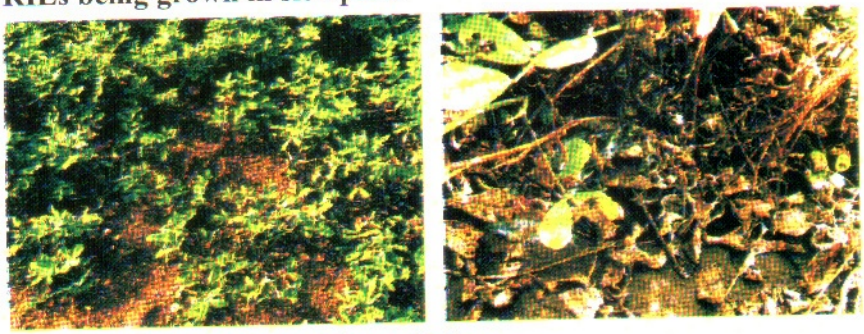
Table 2. Number of confirmed hybrids isolated from the crosses

No.	Cross	Pods harvested	Confirmed hybrids
	Foliar fungal diseases		
1.	TG 37A x VG 9816	67	16
2.	JL 24 x VG 9816	71	30
3.	GAUG 10 x VG 9816	23	07
	Stem rot		
4.	GG 20 x CS 19	36	23
5.	GG 20 x JSP 39	57	29

Phenotyping of the mapping populations in sick plot

The mapping population of recombinant inbred lines from the cross TAG 24 x R 9227 was screened in a sick plot for stem rot. The extent of infection, among the 316 inbred lines, was in the range of 0 to 100%.

Figure 3. RILs being grown in sick plots



Genotyping of the parental lines and populations

The genotypes *viz.* TAG 24, R 9227, JL 24, ICGV 86590, GG 20, CS 83, CS 75 and CS 19 which were used for developing mapping populations were analysed for DNA polymorphisms. Out of 54 primers already screened, only 5 primers were polymorphic and several primers did not even produce amplicons.

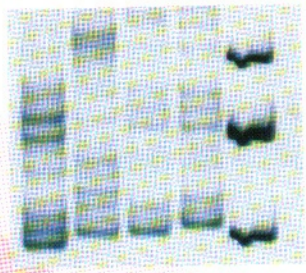


Figure 4. Electrophoregram of four cultivars showing polymorphic amplicons with PM 30

Fingerprinting of extant cultivars

The 26 extant cultivars selected for fingerprinting were tested with the available primers and then 18 primers were short listed for developing the fingerprints. A dendrogram (fig. 5) was developed to represent, the composite differentiation patterr of the cultivars using these primers. Of these primers, PM 15, 32, 35, 36, 45, 50 and 238 could individually distinguish the varieties VRI 4, LNG 2, ALR 3, VG 9251 and JL 220.

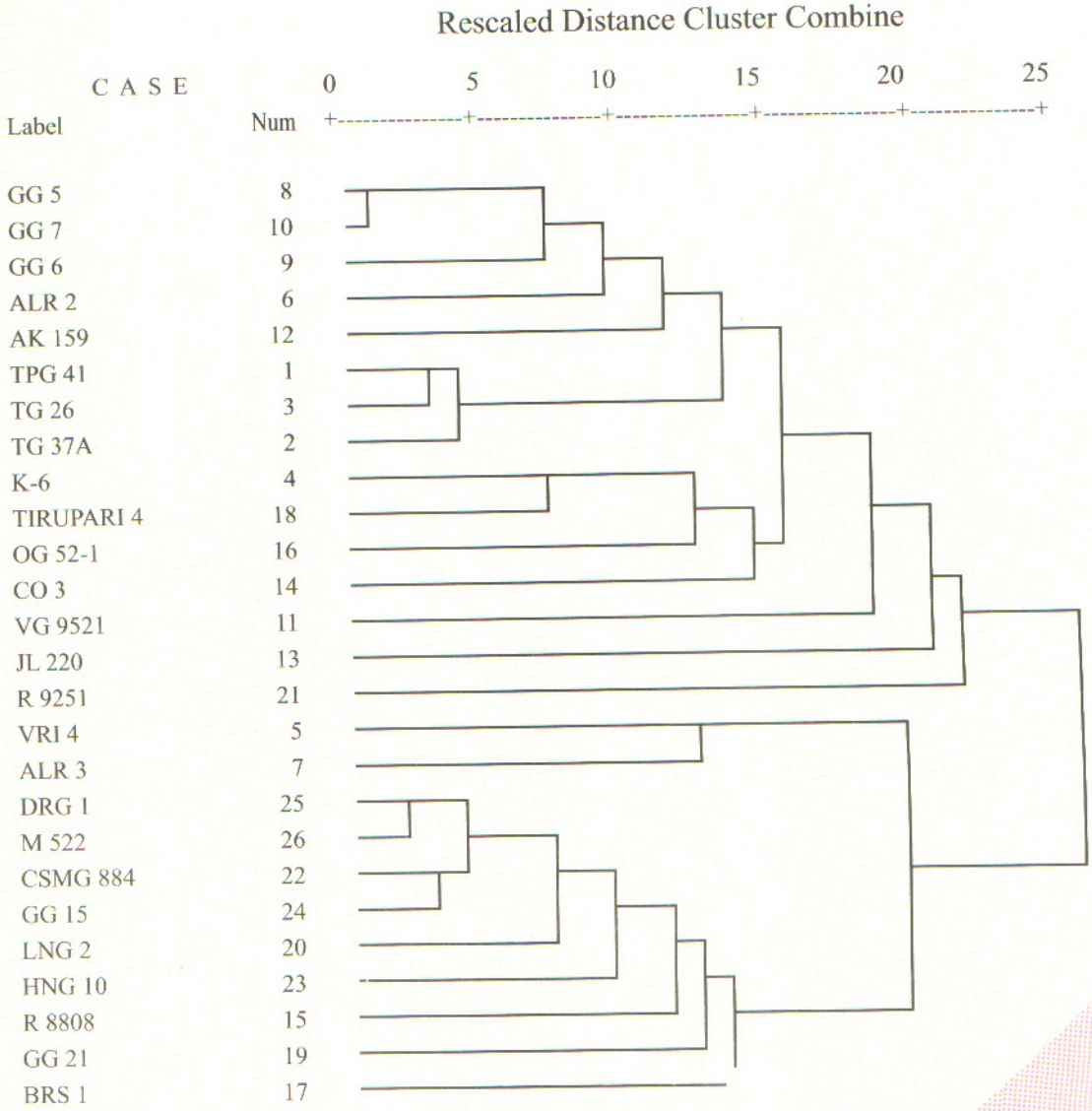


Figure 5. Composite dendrogram depicting the differentiation of the cultivars on the basis of DNA polymorphism elicited by using 18 SSR primers

Transformation work has been initiated with three gene constructs and good number of shoots has been regenerated. These shoots are now being selected in the antibiotic containing selection medium.

Achievements:

- For incorporating tolerance of abiotic stresses, especially drought and salinity two gene constructs *ZAT*12 TF and AtDREB1a were separately used for developing transgenic groundnut mediated through *Agrobacterium*. With *ZAT*12 TF gene, 1386 plants could be regenerated after co cultivation. The putative transformants are being selected in the antibiotic (kanamycin) medium. With the gene construct AtDREB1a, 1077 plants could be regenerated after co cultivation and are now being selected under antibiotic pressure (kanamycin).

- For developing groundnut having transgenic resistance to defoliating insect especially *Spodoptera litura*, the gene construct *cry1Fa1* was used. From the co-cultrues, 7974 plants are now in selection medium.

6. DEVELOPMENT OF TRANSGENIC RESISTANCE TO BUD AND STEM NECROSIS VIRUSES IN GROUNDNUT (a collaborative research project with IARI, New Delhi)

(PI: RADHAKRISHNAN, T.; Co-PI: P.P. THIRUMALAISAMI)

Funding agency: DBT

Objectives

- To develop transgenic plants of groundnut with nucleocapsid protein genes derived from PBNV and PSNV
- To characterize the putative transformants for integration, expression, and inheritance of the introduced gene(s)
- To carry out evaluation of the transgenic plants for resistance to PBNV and PSNV under glasshouse conditions

Achievements:

- Two gene constructs received from IARI were used for producing transgenics in the popular cultivars Kadiri 6 and Kadiri 136. Using the immature leaves and de embryonated cotyledons as explants several co-cultures were made, plants regenerated, selected under antibiotic pressure. The putative transformants are

Achievements:

For isolating fungi tolerant of desiccation, high temperature and salinity, 32 samples of saline soil and sediments were collected from man-made and natural salt pans from Kachchh of Gujarat and Jaisalmer and Pokhran of Rajasthan. From these samples, 19 different fungal isolates could be obtained. All the isolates were characterized for colony morphology, pigmentation, sporulation, and the levels of tolerance of NaCl. Most of the

Isolates belonged to the genus *Aspergillus*. Seventeen out these, could grow at 23.5% NaCl concentration. One fungal isolate BF5, could grow even at saturated NaCl concentration. Six fungal isolates MSPF 1, MSPF 2, MSPF 3, MSPF 4, MSPF 5, and NSPF 1 could grow not only at 23.5% NaCl but also at 50°C (Fig. 1). By improvising the composition of growth nmedium, isolate BF 5 could be successfully cultured *in vitro* at saturated NaCl conditions.

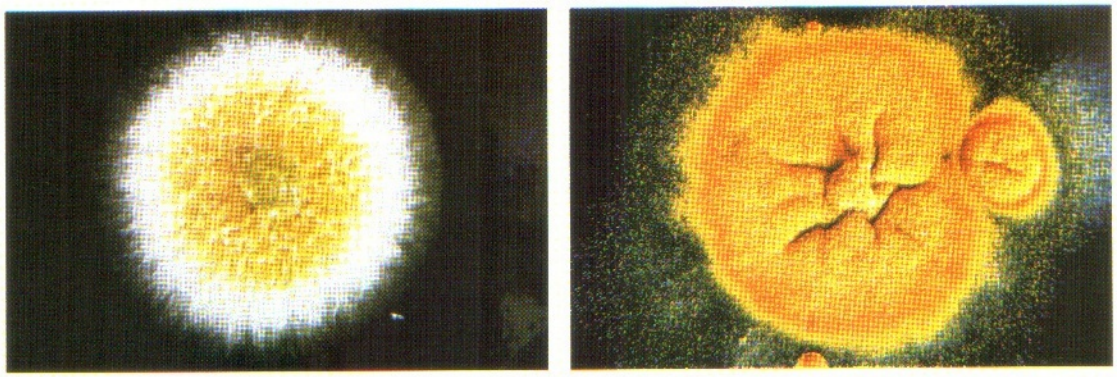


Figure 1. Mycelial growth of two isolates of *Aspergillus* sp. Growing in MEA medium containing 23.5% NaCl. A: MSPF1, and B: NSPF1

5. ICAR NETWORK PROJECT ON TRANSGENIC CROP

(PI: RADHAKRISHNAN, T.; Co-PI: ABHAY KUMAR)

Funding Agency: ICAR

Objectives:

- Development of transgenic groundnut tolerant to drought/salinity
- Development of transgenic groundnut tolerant to insect / pests