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वार्षिक प्रतिवेदन

ANNUAL REPORT 2010-11

मूंगफली अनुसंधान निदेशालय

पो. बो. नं. ५, जूनागढ - ३६२ ००१, गुजरात, भारत

Directorate of Groundnut Research

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

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Directorate of Groundnut Research
(Indian Council of Agricultural Research)
P. B. No. 5, Ivnagar Road, Junagadh, Gujarat, India

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Groundnut crop in farmers' field

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CONTENTS

	Page
Executive Summary	1
Project 01: Breeding and genetic studies on tolerance of biotic and abiotic stresses in groundnut	7
Project 02: Integrated pest management (IPM) in groundnut based production system	27
Project 03: Physiological studies on environmental stresses in groundnut	35
Project 04: Microorganisms in relation to soil health and plant nutrition in groundnut	39
Project 05: Management of mineral nutrition and associated stresses in groundnut	44
Project 06 : Development of sustainable packages of practices for groundnut based cropping systems	54
Project 07: Management of existing and emerging problems of soil and water salinity for groundnut production	66
Project 08: Management of germplasm of cultivated groundnut (*A. hypogaea* L.) and its wild relatives	69
Project 09: Biotechnological approaches to the characterisation and genetic enhancement of groundnut	76
Project 10: Assessment and enhancement of quality in groundnut and its value added products	79
Project 11: Biotransformation of groundnut by-products into useful products	82
Project 12: Prevention and management of mycotoxin contamination in groundnut	83
Project 13: Impact assessment of improved groundnut prouction technologies: sustainable livelihood analysis	86
Project 14: Breeding for large-seeded and confectionery groundnut	91
Project 15: Utilization of wild *Arachis* gene pools for groundnut improvement	95
Project 16: Economic analysis of production, processing and utilization of groundnut in major groundnut growing states of India	105
Project 17: Breeding for improved fodder quality traits in groundnut	113
Project 18: Development of statistical models for evaluation of field trials on groundnut crop and determination of scenarios in area, production and yield	116
All India Coordinated Research Project on Groundnut	119
Achievements of externally funded projects	128
Publications	137
Human resource	145
Meetings	150
General information	151
Finance and Accounts	153

PROJECT 09: BIOTECHNOLOGICAL APPROACHES TO THE CHARACTERISATION AND GENETIC ENHANCEMENT OF GROUNDNUT

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

Genetic transformation

Transformation of groundnut (*A. hypogaea* L. cv. GG 20) with mannitol-1- phosphate dehydrogenase (*mtlD*) gene construct

From the PCR-confirmed T_0 plants, 469 T_1 plants were grown in PII glass house. Among these 264 plants have so far been analyzed for the segregation of the transgene using PCR and gene specific primers and 160 plants were identified to have the transgene (Figure 1).

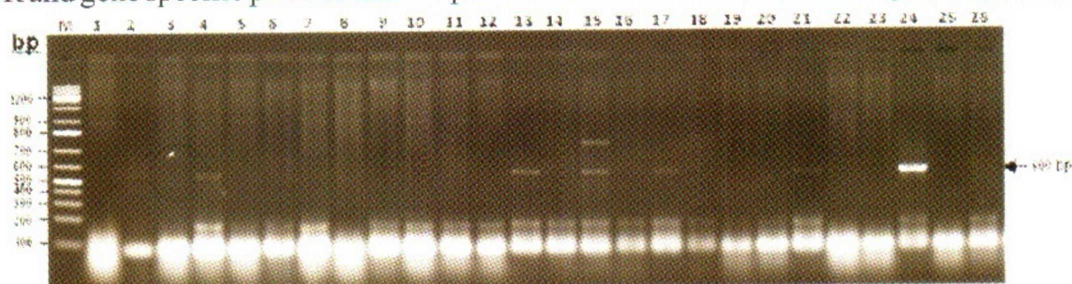

Figure 1. Screening of *mtl*D T_1 transgenics by agarose gel electrophoresis using gene-specific primers

Physiological analysis:

The T_1 generation of *mtlD* transgenics was evaluated for their response to varying levels of salinity (0, 100, 200, and 300 mM NaCl) using leaf disc assay. The leaf discs were floated for 72 h under continuous white light (30 mmol m^{-2} s^{-1}) at 28±1°C. Data are the mean of three independent experiments. The total leaf chlorophyll content was also estimated. It was observed that the chlorophyll content of T_1 plants was lower than that of wild type (untransformed groundnut variety GG 20). The chlorophyll content may show improvement in the T_2 generation homozygous plants.

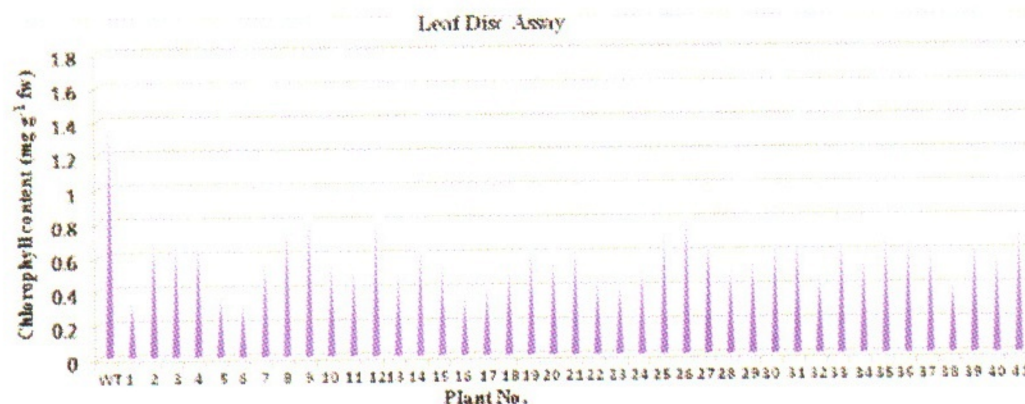

Figure 2. Total chlorophyll content in the leaf-discs from wild and transgenic plants

Of the PCR-positive plants, nine were confirmed with southern hybridization of genomic DNA for the integration of the transgene and the copy number. These plants were tested for the expression of the transgene using RT-PCR and in 8 events were confirmed.

Transgenics (*A. hypogaea* L. cv. GG 20) using annexin gene constructs

In 20 sets, 774 de-embryonated cotyledon-explants were co-cultured and 652 explants were regenerated, 1561 well developed shoots were obtained, and eventually 1023 rooted and healthy plants were transferred to glass house. PCR analysis of these plants using gene-specific primers is in progress and so far 8 PCR positive plants have been identified.

Transformation of groundnut (*A. hypogaea* L. cv. GG 20) using the plant defensin Rs-AFP2 (*Raphanus sativus* Anti fungal protein 2) and Tfgd2 (*Trigonella foenum gracecum* defensin 2) genes

Out of 794 explants were co-cultured with *Agrobacterium* in 20 batches, 672 explants regenerated and 1617 shoots were regenerated. Of these, 1135 shoots were established in glasshouse after hardening. PCR analysis of these shoots by using gene-specific primers revealed the presence of the transgene in 14 putative transgenics.

Development of mapping populations and assessment of molecular diversity

Genotyping of the parental lines and populations

Eight parental genotypes viz. TAG 24, R9227, JL 24, ICGV 86590, GG 20, CS 83, CS 75, and CS 19 which were used for developing mapping populations, were analysed for their marker polymorphisms. The PCR products of 250 markers were separated on an on-chip microfluidic electrophoresis system and the profile (Figure 3) revealed 64 markers to be polymorphic between the two parents JL 24 and ICGV 86590.

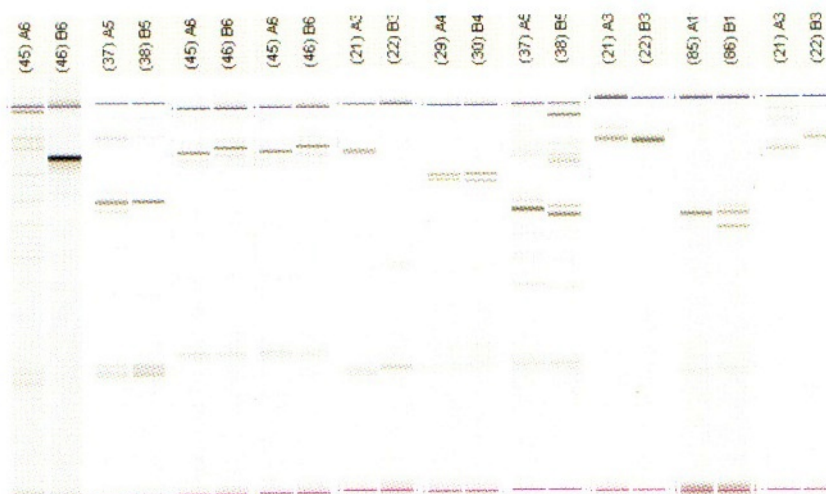

Figure 3. Electropherogram of ten cultivars showing polymorphic amplicons as revealed by automated microfluidic chip electrophoresis

Hybridisations for development of mapping populations

The number of recombinant progenies obtained from four different crosses (three for tolerance of stem rot and one for collar rot) which were in generation F_1 and the three crosses (two for tolerance to stem rot and one for LLS and rust) in F_2 generation produced are given in Table 1 and Table 2.

Table 1. F1s confirmed in crosses for developing mapping population (RILs)

S.No.	Crosses	F_1s confirmed
For tolerance of stem rot:		
1	GG 20 x CS 19	44
2	GG 20 x CS 75	59
3	GG 20 x CS 83	45
For tolerance of collar rot:		
4	GG 20 x SGL 4233	48

Table 2. Number of RILs (F_2s) in different crosses meant for developing mapping populations

S.No.	Crosses	No. of RILs obtained
Tolerance of stem rot:		
1	GG 20 x CS 19	137
2	GG 20 x JSP39	129
Tolerance of rust, LLS:		
3	JL 24 x VG 9816	82

Cloning of oxalate oxidase gene from wild species of groundnut

PCR amplification of oxalate oxidase gene from 18 wild species of groundnut was attempted using gene-specific primers designed from the sequence available for barley (from the NCBI database). Efforts aimed at PCR optimization including designing alternative primers failed to give the desired results. In contrast, the amplification obtained from the barley DNA as template gave excellent amplification at approx. 700bp, as expected. Hence, RD2660 variety of *Hordeum vulgare* was used for isolating the cds (coding sequence) of oxalate oxidase gene. An amplification of 684 bp was obtained. The cds has been cloned into pDrive cloning vector and mobilised into *E. coli* strain XL-1blue. The sequence has been submitted to GenBank (Accession no. HQ634345).

PROJECT 15 : UTILIZATION OF WILD ARACHIS GENE POOLS FOR GROUNDNUT IMPROVEMENT

(S. K. BERA, P. C. NAUTIYAL, A. L. SINGH, RADHAKRISHNAN T., CHUNILAL, P. P. THIRUMALAISAMY AND ABHAY KUMAR)

Hybridization

As the climate at Junagadh during summer is not congenial, hybridization was carried out at RRS, Virdhachalam, Tamil Nadu. The following 12 back-crosses were attempted and more than 100 probable cross-pods were harvested for each cross combination.

GG 20//GG 20/CS 19,
GG 20//GG 20/OG52-1,
GG 20//GG 20/ICGV86590,
CS 19//CS 19/OG 52-1,
ICGV 86590//ICGV 86590/CS 19,
OG 52-1//OG 52-1/ICGV 86590 and
CS 19// GG 20/CS 19,
OG52- 1//GG 20/OG52-1,
ICGV86590//GG 20/ICGV86590,
OG52-1//CS 19/OG 52 -1,
CS 19//ICGV 86590/CS 19,
ICGV 86590//OG52-1/ICGV86590)

Back crosses were made again under field conditions during rainy season (*kharif*) at Junagadh. Out of 13 back crosses, 7 were with inter-specific hybrids aimed at introgression of tolerance of biotic stresses while 6 were inter-varietal crosses aimed at improving kernel size and crop duration. Approximately, 500 pollinations were attempted for each combination. Probable cross-pods collected at harvest will now be used in further back-crossing during *kharif* 2011. Among the inter-specific back crosses, largest number (220) of probable cross pods were harvested from the combinations involving interspecific hybrids of *A. appresipilla* while smallest probable cross pods were harvested from the combination involving interspecific hybrids of *A. kempfmarcadoi*. Among intervarietal back crosses, the largest number of probable cross-pods was harvested from the combination involving hybridization between Chico and CS-148 while smallest number of probable cross pods was harvested from the combination involving hybridization between CS-148 and CS-281.

Identification of hybrids

The seeds from probable cross-pods harvested during *kharif* 2009 from nine interspecific and two back crosses, were sown in the field during *rabi* 2010 for identification of true hybrids. Hybrid plants were identified and tagged at 45-50 days after emergence on the basis of runner growth habit which is a dominant character from wild male parent over bunch growth habit of cultivated female parent. Six out of nine crosses, produced hybrid plants. For three cross combinations viz., 'J11/ *A. helodes*', 'J11/ *A. rigonii*' and 'J11/ *A. correntina*' attempts to produce hybrid pods did not succeed. In six crosses, the number of hybrid plants were in the range of 2 to 7, and the largest number of hybrid plants were identified from the cross 'J11/ *A. pussilla*'. The back cross 'J11//J11/*A. pussilla*' produced 3 hybrid plants while 'GG 20//J11/*A. pussilla*' did not produce any hybrid plant.

The hybrid plants of interspecific and back crosses were maintained in the field due to their perennial growth habit, while the selfed plants were rouged out. The mature pods were harvested from the perennial hybrid plants at regular intervals for further use.

Initial varietal trial

Evaluation of Spanish bunch genotypes

During summer season, 40 Spanish bunch inter-specific advanced breeding lines along with 2 checks (TAG 24 and TG 26) were evaluated in initial yield trial in a randomized block design with three replications. Each genotype was sown in a single row of three-meter with a plant-to-plant spacing of 10 cm and row-to-row spacing of 60 cm. Recommended crop management practices were followed to raise the crop. Observations on specific leaf area and SCMR were recorded 65 days after germination while biological yield, pod yield, shelling out-turn, hundred-kernel mass and percentage of sound mature kernels were recorded after harvest. Out of 40, although eight genotypes (NRCG-CSs 351, 353, 354, 358, 362, 405, 406 and 408) produced numerically higher pod yield than the best check TAG 24, the differences were not significant. These eight genotypes also showed higher values for shelling out-turn (65 to 71%) and SCMR (34 to 42) and lower values for SLA. These genotypes may have good potential for pod yield as well as drought tolerance.

During rainy season, the initial yield evaluation trial for Spanish bunch genotypes was repeated with 37 genotypes along with four checks (TG 26, GG 7, TG 37A and TAG 23). The crop was raised by following standard crop management practices. Observations were recorded on pod yield and related traits at harvest. Due to excessive rainfall in the season there was stagnation of water in the fields and consequently the pod yield levels were too low hence the data was not statistically processed.

Evaluation of Virginia genotypes

Advanced yield evaluation trial for Spanish genotypes was conducted in RBD with 3 replications during rainy season. Forty-three genotypes along with two checks (Somnath and GG 20) were included in the trial. Each genotype was sown in a single row of three-meter with a plant-to-plant spacing of 10 cm and row-to-row spacing of 60 cm. The crop was raised by following standard crop management practices. This trial was also spoilt by the excessive rainfall in the season resulting in stagnation of water in the fields and consequent low levels of pod yield.

Advanced varietal trial

Evaluation of Spanish bunch genotypes

Advanced yield trial was conducted comprising 12 interspecific Spanish advanced breeding lines and 2 checks (TAG 24 and TG 26) during summer season. Experiment was laid out in Randomized Block Design with three replications. Each genotype was sown in five rows of three meter each with a plant-to-plant spacing of 10 cm and row-to-row spacing of 60 cm. The recommended crop management practices were adopted to raise the crop. Observations on specific leaf area and SCMR were recorded in 65 days after germination

while biological yield, pod yield, shelling out-turn, hundred-kernel mass and percentage of sound matured kernel were recorded at harvest. The pod yield of genotype NRCGCS-401 was significantly higher than that of the best check TG 26 while those of six other genotypes (NRCGCSs-360, 361, 368, 369, 389 and 400) were only numerically higher than that of TG-26. The shelling out-turn (65 to 69%) and SCMR (34.7 to 44) of these six genotypes was high while the SLA was low (115-227). Moreover, the values for HKM and SCMR of NRCGCS-401 were higher than those of TG 26. Performance of these genotypes needs to be confirmed.

During the rainy season, the advanced yield evaluation trial of Spanish bunch genotypes was repeated in RBD with 3 replications. Twelve test genotypes along with four checks (TG 26, GG 7, TG 37A and TAG 23) were evaluated. Each genotype was sown in five rows of three meter each with a plant-to-plant spacing of 10 cm and row-to-row spacing of 60 cm. Crop was raised by following standard crop management practices. Observations were recorded on pod yield and the related traits at harvest. The performance of all the check varieties and test entries was poor due to prolonged stagnation of water in the experimental field because of excessive and continuous rainfall during the season. Among the four checks, GG 7 recorded highest pod yield per plot (374 g), shelling outturn (55%), sound mature kernel (21%) and hundred kernel mass (30 g). The values of pod yield per plot (475 g) and percentage of sound mature kernel (46%) of genotype NRCGCS-401 were significantly higher than those of the best check GG 7 while the values of SPAD reading and shelling out turn were at par. In summer season too, the pod yield of genotype NRCGCS-401 was higher than that of the best check (GG 7). The pod yields of NRCGCS-360 and NRCGCS-391 were only numerically higher than that of GG 7.

Evaluation of Virginia genotypes

During rainy season, the advanced yield evaluation trial for Virginia bunch genotypes was conducted in RBD with 3 replications. Three genotypes along with two checks (GG 20 and Somnath) were included in the trial. The plot size was of 5 rows of 3 meter each. The spacing between plants in a row was 10 cm while the inter row spacing was 60 cm. Crop was raised by following standard crop management practices. Observations were recorded on pod yield and the related traits at harvest. Of the two checks, GG 20 was superior in respect of pod yield per plot (493 g), shelling out turn (59%), percentage of sound mature kernel (35%) and hundred-kernel mass (45.7 g) and none of the test genotypes could outperform GG 20 in terms of pod yield. Among the three test genotypes, NRCGCS-385 was the best with a pod yield of 552.8g per plot, 22% sound mature kernel, 45.0 g hundred kernel mass and 37.2 SPAD reading i.e. a performance nearly at par with GG 20.

Genotypes identified for inclusion in AICRP-G trials

Two large-seeded Spanish groundnut genotypes (NRCGCS-268 and NRCGCS-281) developed at DGR and identified earlier for inclusion in AICRP-G trials in *rabi*-season were evaluated during *rabi*-2009 and *rabi*-2010 seasons at five AICRP-G centres. Although the pod yield of both these genotypes was not significantly higher than that of national check

(TPG 41) yet on the basis of numerically higher pod yield/ha (3650 kg), kernel yield/ha (2569 kg) and significantly higher hundred kernel weight (76 g) than the national check, NRCGCS-268 has been promoted to AVT.

Freshly developed breeding lines at DGR, two Spanish bunch (NRCGCS-264 and NRCGCS-369) and two Virgina bunch (NRCGCS-424 and NRCGCS-425) have been recommended for AICRP-G testing. The Virginia bunch genotypes recorded 15 to 34% yield advantage over the best check and also showed resistance to rust and late leaf spot.

Novel germplasm developed and registered

Although the breeding lines developed through interspecific hybridization possess high level of tolerance/resistance to abiotic and biotic stresses, they can not be promoted directly as cultivars because of tight-linkage between desirable and undesirable agronomical traits. Thus these interspecific breeding lines are used as pre-breeding material in the crop improvement programme. Eight interspecific breeding lines (NRCGCS-21, NRCGCS-77, NRCGCS-83, NRCGCS-85, NRCGCS-86, NRCGCS-124, NRCGCS-180, NRCGCS-22) developed at DGR were characterized over locations and years and were found to possess multiple disease resistance (late leaf spot, rust, stem rot, PBND and Alternaria blight). These interspecific multiple disease resistant breeding lines were submitted to the NBPGR for registration. The Plant Germplasm Registration Committee approved eight multiple disease resistant groundnut germplasm for registration. These multiple disease resistant genotypes would help in broadening the genetic base of cultivated groundnut.

Screening of interspecific derivatives at Raichur, Karnataka

Over the years, several interspecific genotypes have been developed at DGR using various wild *Arachis* species. Most of these interspecific genotypes possess resistance to one or more biotic stresses. Interspecific genotypes available (437 lines) with DGR were evaluated at RRS, Raichur (a hot spot) for identification of genotypes resistant to LLS, rust, PBND and stem rot. Each interspecific genotype was sown in one row of five-meter each. Single row of susceptible check (KRG 1) was sown in every four lines of interspecific genotypes for increasing disease pressure. The genotypes were scored for occurrence of PBND 60 days after germination as well as at harvest and the incidence was expressed as per cent. Scoring for LLS and rust was done at harvest on standard 1-9 scale. The scoring for stem rot was done at harvest and was expressed as per cent. For stem rot and PBND, 77 and 111 genotypes, respectively scored less than 5% incidence. For LLS and rust, in 26 and 25 genotypes, respectively the disease score was below 3. Besides, as many as 55 genotypes showed multiple diseases resistance (stem rot, PBND, rust and LLS). The resistance in these genotypes needs further confirmation in the ensuing rabi season.

Molecular diversity analysis of *A. glabrata* accessions

Wild species of *Arachis glabrata* is grouped into section rhizomatosae under tertiary gene pool of genus *Arachis. A. glabrata* propagates through rhizome and not cross compatible with cultivated groundnut. Most of *A. glabrata* accessions have been reported to possess high degree of resistance to various biotic and abiotic stresses.

Somehow, these accessions have so far not been used effectively in the groundnut improvement programme because of cross incompatibility.

Of late, due to availability of molecular tools, the chances of incorporation of genes present in *A. glabrata* into cultivated background have gone up. More than 50 accessions of *A. glabrata* have so far been reported which need to be systematically evaluated for tapping the potential of genetic variability. Hence, RAPD and SSR markers were used to study the genetic diversity of 34 accessions of *A. glabrata* available at DGR. Amplification of genomic DNA of these 34 accessions using RAPD primers comprising 4 of D series, 3 of OPT series, and 3 of OPI series yielded 242 fragments. Out of 242 bands, 178 were polymorphic, with an average of 17.8 polymorphic fragments per primer. Primers D5, D6, D7, D9, OPT5, OPI5 and OPI6 produced more than 70% polymorphism. The PIC value for 10 RAPD primers varied from 0.966 (D5) to 0.969 (D6, D9, OPI5, and OPI7), with a mean of 0.968. The marker index (MI) value for 10 RAPD primers varied from 44 (OPT7) to 93.3 (D6), with a mean of 70.96. Amplification of genomic DNA of 34 *A. glabrata* accessions using 15 SSR primer pairs yielded 148 fragments. Out of which 148 bands 133 were polymorphic, with an average of 8.86 polymorphic fragments per primer. All the SSRs used in the study produced more than 70% polymorphism except PM15 and PM402 primers which produced 62.5% and 53.8% polymorphism, respectively. The polymorphic information content (PIC) value for 15 primers varied from 0.933 (TC1A02) to 0.968 (PM32), with a mean of 0.958. The marker index (MI) value for 15 SSRs varied from 52 (PM402) to 96.7 (PM188), with a mean value of 87.23. The SSRs used in this study were highly polymorphic and efficient in revealing the extent of genetic diversity present in the populations studied. High levels of genetic diversity in *A. glabrata* accessions indicated that these populations are not experiencing genetic drift.

The dendrogram constructed on the basis of band pattern generated by using RAPD and SSR primers were close representation of the values of similarity matrix. Seven polymorphic RAPD primers discriminated 30 *A. glabrata* genotypes and divided them into two major clusters where first major cluster contained 13 *A. glabrata* accessions (code Nos. R, AF, Y, AE, AD, AA, AB, AC, X, Z, T, V and U; denoted as cluster-I) and second major cluster contained 17 *A. glabrata* accessions (code No. A, G, H, F, B, C, D, E, L, N, K, M, O, Q, I, J and P; denoted as cluster-II) (Fig.-1). These two major clusters shared 50% similarity. In cluster-I, the minimum similarity (68%) was observed between accessions U and R, while the maximum (83%) was observed between accessions Y and AE. Likewise in cluster-II, the minimum similarity (60%) was observed between accessions P and A while the maximum (82%) was observed between accessions A and G. Among 30 *A. glabrata* accessions subjected to diversity analysis using RAPD, the accessions U and P were most diverse and can be used for tapping the maximum genetic diversity for improvement of groundnut. Thirteen polymorphic SSRs discriminated 30 *A. glabrata* accessions and divided them into two major clusters in which the first cluster contained 13 accessions (code Nos. R, T, U, S, V,

W, X, Z, Y, AA, AD, AB and AC; denoted as cluster-I) and the second cluster contained 17 accessions (code No. A, K, F, G, B, L, C, N, D, I, E, H, J, O, M, Q and P; denoted as cluster-II) (Fig.-2). These two clusters exhibited 37% similarity between them. In cluster-I, the minimum similarity (42%) was observed between accessions AC and R while the maximum similarity (78%) was observed between accessions R and T. Similarly, in cluster-II, the minimum similarity (52%) was observed between accessions P and A while the maximum (82%) was observed between accessions F and G. Accessions AC and P were most diverse among 30 *A. glabrata* accessions subjected to diversity analysis using SSR primers and both of these could be used for tapping maximum diversity for groundnut improvement.

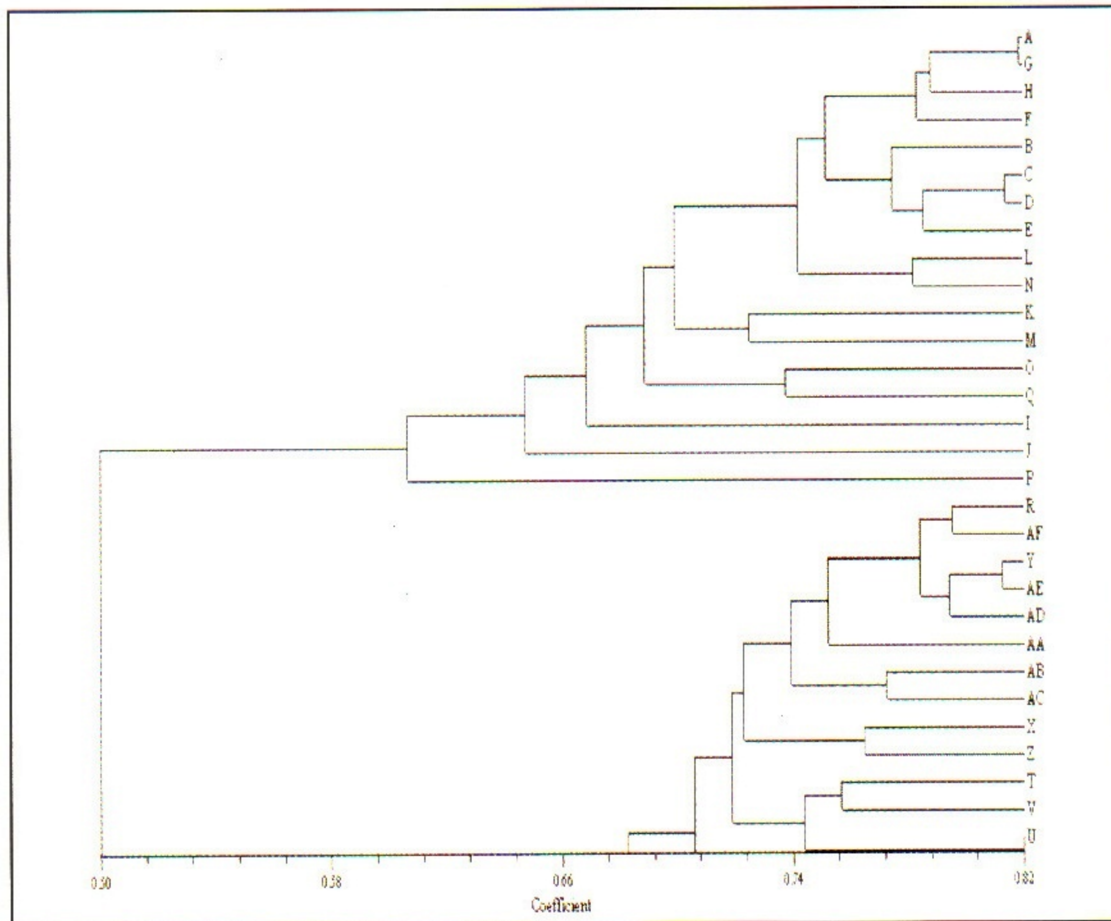

Figure 1. UPGMA cluster analysis showing the relationship and diversity among 30 accessions of *A. glabrata* as revealed by data using RAPD markers.

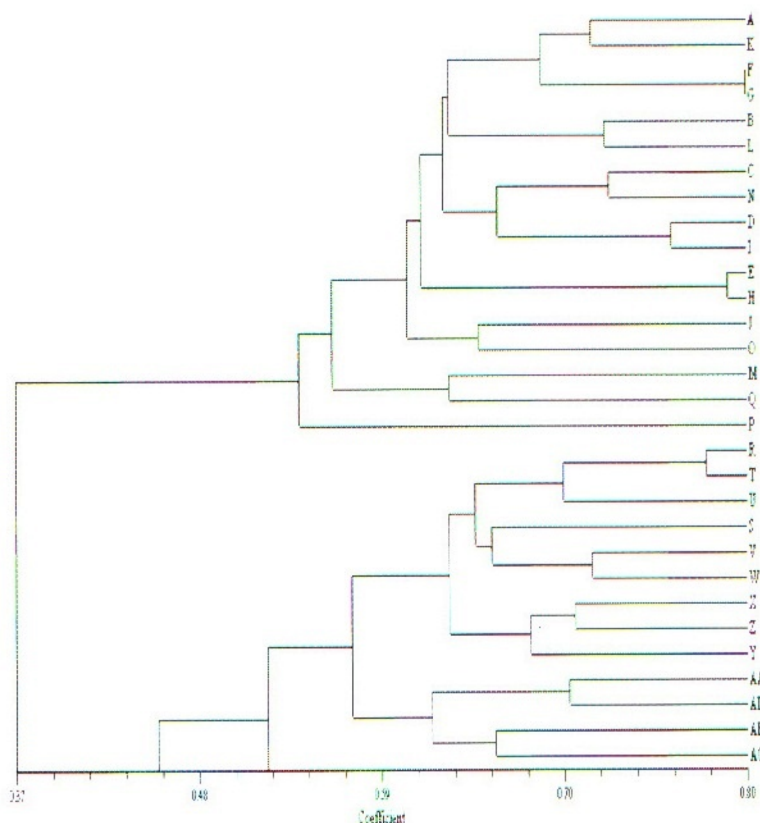

Figure 2. UPGMA cluster analysis showing the relationship and diversity among 30 accessions of *A. glabrata* as revealed by data using SSR markers.

Molecular characterization of J11, *A. diogoi* and their F_1 hybrids

A set of 31 SSR primers was screened using cultivar J11, wild species, *Arachis diogoi* and their hybrids to identify markers for use in screening of F_2 populations. As many as 21 SSRs produced polymorphism among the parents and their F_1 hybrids. Out of 21 polymorphic primers only two SSRs (PM32 and PM188) produced co-dominant alleles in the hybrids which could be useful in marker assisted selection for tolerance of LLS and rust in J 11x *A. diogoi*.

Identification of useful salinity stress responsive transcripts from *A. glabrata* accession (tolerant to NaCl induced stress)

Transcriptional analysis through DDRT-PCR was continued to understand tolerance to NaCl induced salinity stress in *Arachis glabrata* accession NRCG-11832. Some of the bands (transcripsts) were either suppressed or induced due to NaCl-induced stress for 7 and 14 days. These differentially expressed bands were eluted from agarose gel and sequenced. The differentially expressed transcripts were named 'groundnut transcript responsive to salt stress' (GTRS). Upon BLAST search for GTRS sequences (Table-1), similarity with the following important salt tolerance cds, mRNAs, nucleotide sequences and related proteins were found.

Table 1. Particulars of 20 differentially displayed bands eluted for sequencing

Primer used	Accession No.	Sample identity	Approx. size	Assigned code
OPT 13	NRCG 11832	1S (induced)	800bp	GTRS-6
OPT 18	NRCG 11832	1S (induced)	300bp	GTRS-7
OPT 18	NRCG 11832	1S (induced)	400bp	GTRS-8
OPT 20	NRCG 11832	1C(suppressed)	300bp	GTRS-9
OPT-7	NRCG 11832	1S(induced)	500bp	GTRS-10
OPT-7	NRCG 11832	1S(induced)	300bp	GTRS-11
OPT-6	NRCG 11832	1C(suppressed)	500bp	GTRS-12
OPT-6	NRCG 11832	1C(suppressed)	700bp	GTRS-13
D-8	NRCG 11832	2C(suppressed)	200bp	GTRS-14
D-8	NRCG 11832	2C(suppressed)	300bp	GTRS-15
OPT-18	NRCG 11832	2C(suppressed)	700bp	GTRS-16
OPT-19	NRCG 11832	2S(induced)	200bp	GTRS-17
OPT-19	NRCG 11832	2S(induced)	300bp	GTRS-18
OPT-8	NRCG 11832	2S(induced)	400bp	GTRS-19
OPT-8	NRCG 11832	2S(induced)	500bp	GTRS-20
OPT-7	NRCG 11832	2C(suppressed)	400bp	GTRS-21
OPT-7	NRCG 11832	2C(suppressed)	500bp	GTRS-22
OPT-10	NRCG 11832	2C(suppressed)	500bp	GTRS-23
OPT-12	NRCG 11832	2S(induced)	500bp	GTRS-24
OPT-11	NRCG 11832	2C(suppressed)	400bp	GTRS-25

GTRS-10 :

1ZCD_A Chain A, Crystal Structure of the Na+H+ ANTIPORTER NHAA>pdb|1ZCD|B Chain B, Crystal Structure of the Na+H+ ANTIPORTER NHAA.

100% similarity with DQ071264.1 *Pennisetum glaucum* Na/H vacuolar antiporter (NHXI) mRNA, complete cds. This plays important role during salt stress condition.

100% similarity with DQ228817.1 *Pennisetum glaucum* Na/H antiporter gene, complete cds.

GTRS-12:

Up to 75% similarity with Q9SY59.1 RecName:Full=NF-X1-type zinc finger protein NFXL1;Short=AtNFXL1. It mediates E2-dependent ubiquitination (by similarity). It confers resistance to osmotic stress such as high salinity and promotes H_2O_2 production. Negative regulator of some defense-related genes via a salicylic acid (SA)-dependent signaling pathway.

Up to 50% similarity is found with EF576564.1 *Oryza sativa* (indica cultivar-group) clone OSS-385-480-H11 alpha-glucan phosphorylase, h isozyme mRNA, partial cds. It is given in a comparative transcriptome map of early and late salinity stress response.

80% similarity is shown with NM_122333.4 *Arabidopsis thaliana* SOS3 (SALT OVERLY SENSITIVE 3); calcium ion binding/calcium-dependent protein serine/threonine phosphatase (SOS3) mRNA, complete cds.

Up to 60% similarity is found with GR405355.1 ICCV2_CAAF_Z71TFICCV2 Salinity-stressed chickpea root cDNA library *Cicer arientium* cDNA clone ICCV2_CAAF_Z71TF 5', mRNA sequence.

Up to 55% similarity is found with GR405029.1 ICCV2 Salinity-stressed chickpea root cDNA library *Cicer arientium* cDNA clone ICCV2_CAAF_W06TF 5', mRNA sequence.

Up to 52% similarity is found with GR404643.1 ICCV2_CAAF_R54TFICCV2 Salinity-stressed chickpea root cDNA library *Cicer arientium* cDNA clone ICCV2_CAAF_R54TF 5', mRNA sequence.

Up to 52% GR404282.1 ICCV2_CAAF_N36TFICCV2 Salinity Salinity-stressed chickpea root cDNA library *Cicer arientium* cDNA clone ICCV2_CAAF_N36TF 5' , mRNA sequence.

Up to 68% GR393446.1 CAABS71TFICC1882 field drought stressed root cDNA library *Cicer arientium* cDNA clone CAABS71TF 5' , mRNA sequence.

Up to 91% similarity with NC_003070.9 *Arabidopsis thaliana* chromosome 1, complete sequence 426 bp at 5' side: WRKY10 (WRKY DNA-BINDING PROTEIN 10); transcription factor. This protein is tolerant to abiotic stress condition.

GTRS-13:

Up to 90% similarity with NM_119745.3 *Arabidopsis thaliana* ATPLDDELTA; phospholipase D (ATPLDDELTA) mRNA, complete cds. It is involved in response to water deprivation, phosphatidic acid metabolic process, hyperosmotic salinity response, response to cold, programmed cell death.

GTRS-14:

Up to 64% similarity with Q9FFK8.2 RecName: Full=NF-X1-type zinc finger protein NFXL2; Short=AtNFXL2. It mediates E2 –dependent ubiquitination (By similarity), confers sensitivity to osmotic stress such as high salinity.

Up to 56% similarity with P17202.1 RecName: Full=Betaine aldehyde dehydrogenase, chloroplastic; Short=BADH.

Up to 89% similarity with GU252706.1 *Ageratina adenophora* high-affinity potassium transporter 1 (HAK1) mRNA, complete cds.

GTRS-18:

Up to 80% similarity with NM_104167.5 *Arabidopsis thaliana* ANAC019 (*Arabidopsis* NAC domain containing protein 19): transcription factor (ANAC019) mRNA, complete cds.

Up to 93% similarity with NM_201896.2 *Arabidopsis thaliana* SLT1 (Sodium-and lithium-tolerant 1) (SLT1) mRNA, complete cds.

GTRS 19:

Up to 68% similarity with P17202.1 RecName: Full=Betaine aldehyde dehydrogenase, chloroplastic; Short=BADH; Flags: precursor.

Up to 70% similarity with NM_201896.2 *Arabidopsis thaliana* SLT1 (sodium- and lithium-tolerant 1) (SLT1) mRNA, complete cds.

Up to 100% similarity with GR403408.1 ICCV2_CAAF_D12TF ICCV2 Salinity-stressed chickpea root cDNA library *Cicer arientinum* cDNA clone ICCV2_CAAF_D12TF 5', mRNA sequence.

GTRS 20:

Up to 73% similarity with 1ZCD_A Chain A, Crystal Structure Of The Na+H+ ANTIPORTER NHAA >pdb|1ZCD|B Chain B, Crystal Structure Of The Na+H+ ANTIPORTER NHAA.

Up to 68% similarity with NM_119212.4 *Arabidopsis thaliana* hydrophobic protein, putative / low temperature and salt responsive protein, putative (AT4G30660) mRNA, complete cds.

Up to 89% similarity with GR405344.1 ICCV2_CAAF_Z60TF ICCV2 Salinity-stressed chickpea root cDNA library *Cicer arietinum* cDNA clone ICCV2_CAAF_Z60TF 5', mRNA sequence.

Up to 58% similarity is found with A8CVF3.1 RecName: Full=Dehydrin DHN1; Short=AmDHN1.

Up to 70% similarity with NM_201896.2 *Arabidopsis thaliana* SLT1 (sodium- and lithium-tolerant 1) (SLT1) *Arabidopsis thaliana* SLT1 mRNA, complete cds.

GTRS 22:

Up to 70% similarity with GR403408.1 ICCV2_CAAF_D12TF ICCV2 Salinity-stressed chickpea root cDNA library *Cicer arietinum* cDNA clone ICCV2_CAAF_D12TF 5', mRNA sequence

Up to 100% similarity with EB710040.1 S7SLT_C62 *Lolium temulentum* salt stressed subtraction library *Lolium temulentum* cDNA, mRNA sequence.

GTRS sequences coding full length Cds, proteins or functional gene related to salinity stress could be useful further for cloning and use in improvement of salinity stress in groundnut.

Nucleotide sequence submitted to the GenBank:

i) bankit1384528HQ191219: Bera S K, N P Ved and Abhay Kumar (2010) Genomic DNA sequence obtained from RAPD analysis of a drought resistant *Arachis hypogaea* cultivar, TMVNLM-2.

ii) bankit1384538HQ191220: Bera S K, N P Ved and Abhay Kumar (2010) Genomic DNA sequence obtained from RAPD analysis of a drought resistant *Arachis glabrata* accession (ICG 8902).

5. ICAR NETWORK PROJECT ON TRANSGENIC CROP

(PI: RADHAKRISHNAN, T. CO-PI: ABHAY KUMAR)

Funding Agency: ICAR

Objective

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

  Transformation work was 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 ZAT12 TF and AtDREB1a were used separately for developing transgenic groundnut mediated through *Agrobacterium*.
- Of the 380 putative transgenics developed using the gene construct AtDREB1a, 159 were tested for the presence of the transgene using gene specific PCR and 10 were found positive
- Of the 290 putative transgenics developed using the gene construct *ZAT*12 TF, 103 were tested for the presence of the transgene using gene specific PCR and 20 were found positive.
- Of the 275 putative transgenics developed using the gene construct *cry1Fa1*, 165 were tested for the presence of the transgene using gene specific PCR and 22 were found positive.

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. THIRUMALASAMY)

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