INDUCTION OF SALT TOLERANCE FOR RADICLE GROWTH IN GROUNDNUT THROUGH GAMMA RAY MUTAGENESIS

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Abstract

Groundnut is an important oilseed, food and feed crop of our country whose productivity is curtailed due to biotic and abiotic stresses. Salinity is one of the important abiotic stresses which affects all stages of groundnut growth and finally the yield. Soil amelioration against salinity is a costly and time-consuming procedure. Alternative strategy is to develop salinity-tolerant groundnut genotypes to overcome salinity problem, towards which, success has so far not been attained in groundnut, due to lack of efficient and simple screening protocol for salt tolerance. In the present study, we have developed a simple, cost effective and repeatable protocol for screening large number of mutant/segregating populations for radicle growth. Genetic variability for germination under 400mM NaCl stress was observed, among established TAG 24 mutants. Superior radicle growth in six of the TAG 24 mutants was noted by screening at 125mM and 150mM NaCl. Further, three mutants showed increased tolerance due to greater radicle length and lesser radicle reduction at 125mM and one mutant at 150mM NaCl. Induction of genetic variability for radicle growth under saline conditions in large seed groundnut variety TPG 41 was carried out, through gamma ray mutagenesis. By screening large number of seeds at 100mM NaCl during M_3 generation followed by screening plant wise during M_4 and M_5 generations, 91 true breeding mutants having salt tolerance for radicle growth were isolated. As a result, by screening from F3 to F5 generations, 114 true breeding lines were identified having salt tolerance for radicle growth. These mutants and breeding lines will be evaluated in the salt-affected soils for their salinity tolerance and yield performance.
Introduction

In India, groundnut is an important oilseed, food and feed crop grown in an area of 6.45 million ha with a total production of 6.57 million tons based on an average of the last five years (FAO, 2005). This contributes to 26.6% of world’s groundnut area and 18.5% of world’s groundnut production. Groundnut occupies nearly 28.3% of the cultivated area and contributes 31.7% of the production of the total oilseeds in the country. It is widely used as cooking oil, digestible protein, minerals and vitamins in many countries and contributes significantly to food security and alleviating poverty. About 80% of India’s groundnut production is crushed for oil, 12% for using as seed, 5% for food and 2% for export.

Among many reasons ascribed for the lower productivity of groundnut, salinity is an important abiotic stress which significantly affects seedling, vegetative and reproductive growth, seed quality and yield. Root zone salinity increases as a result of continuous use of saline water for irrigation because of limited or non-availability of good quality water in majority of groundnut growing areas. It can rapidly inhibit root growth and in turn their capacity to uptake water and essential mineral nutrients from the soil (Neumann 1995). Groundnut yields were severely affected with an increase in soil and water salinity (Singh and Abrol, 1985; Lauter and Meiri, 1990; Patel et al, 1993; Girdhar et al, 2005).

Materials and Method

Standardization for salt screening

Development of salt-tolerant plant materials, will require selection at several stages of plant growth. Dewey (1962) proposed a breeding scheme to improve salinity tolerance in perennial grasses that included selection during germination with subsequent selection at later crop growth stages. Selection for salt tolerance in the field has proven to be very difficult and often not effective, because of lack of uniformity of most salt affected fields.

In order to develop simple screening methodology for salt tolerance, initially, dry seeds of two popular groundnut varieties TAG 24 (Patil et al, 1995) and TG...
26 (Kale et al, 1997) were treated with 100 to 1000 mM NaCl in laboratory at BARC, Mumbai (Fig. 1). Each treatment contained 20 seeds in petri plates lined with filter paper and moistened with 20 mL NaCl solution in three replications and incubated at 28° ± 2°C. For control, 20 mL of distilled water was used. LD50 for germination was analyzed in TAG 24 and TG 26 by Probit analysis based on germination % at different NaCl concentrations. Seeds of both varieties were germinated up to 400 mM NaCl and hence this concentration was taken for screening seed germination.

In another experiment, to determine effective lower NaCl concentration for screening large mutant/segregating populations so as to have radicle growth as a criterion in addition to germination %, TAG 24, JL 24, SB XI and

**Induced mutagenesis**

TAG 24 was irradiated with 150, 250 and 350 Gy gamma rays and 83 true breeding mutants for various morphological and biochemical traits were isolated (Badigannavar, 2007) and were studied for salt tolerance.

To induce genetic variability for salt tolerance, 500 seeds each of a large seed variety TPG 41 were irradiated with 200 and 300 Gy of gamma rays during rainy season (June-September), 2004. The M2 generation was grown in summer, 2005 by advancing M1 plants as plant to row progenies. M2 plants were harvested individually to obtain M3 seeds and were bulked dose-wise.

To incorporate salt tolerance in recently released varieties TG 37A (Kale et al, 2004a) and TPG 41 (Kale et al, 2004b) they were hybridized with salt-tolerant accessions, NRCG 2419 and NRCG 7548 (Rajgopal and Bandyopadhyay, 1999) during rainy season, 2004. F1 plants were grown in summer, 2005 and crosswise harvested individually.

**Screening mutant/segregating populations for salt tolerance**

Using protocol for germination, 83 mutants of TAG 24 were screened with 400 mM NaCl and germination % was recorded after 48 h based on radicle appearance. In another study under the same experimental settings, 20 seeds from 43 mutants of TAG 24 grown in summer and rainy seasons were treated with 20 mL each of distilled water (control), 125 and 150 mM NaCl in two replications for radicle growth.
growth. Radicle length from the collar region to radicle tip was measured from the germinated seeds after four days and mean radicle length was estimated. Percent reduction in radicle length from 125 and 150 mM NaCl treatments in comparison with control was estimated.

M<sub>3</sub> seeds of TPG 41 from both 200 and 300 Gy treatments were subjected to NaCl treatment. In each plastic tray (54 cm X 34 cm X 5 cm) lined with blotting paper, around 300 g (~500 seeds) of M<sub>3</sub> seeds were put and 600 mL of 100 mM NaCl was added. Similarly, 20 trays per day were treated with NaCl to cover — 10,000 seeds (Fig. 2). Two controls, one with 100 unirradiated seeds of TPG 41 and 200 mL distilled water and another one with 100 unirradiated seeds of TPG 41 and 200 mL 100 mM NaCl were maintained at each time of testing. Treated seeds were incubated for four days at 30° ± 2°C and germination percent was taken. NaCl tolerant M<sub>3</sub> seeds were selected by comparing radicle growth with that of the distilled water control (Fig. 3). M<sub>3</sub> seeds having radicle growth similar to the radicle growth of the unirradiated seeds in distilled water were considered as tolerant ones. Such tolerant seeds were transferred to distilled water for 45 min and then to thermocel cups (9 cm X 6 cm) having soil and farm yard manure (3:1) mixture (Fig. 4). Established seedlings were transplanted in the field after 10 days. Similarly F<sub>3</sub> seeds (~13,000) from four crosses were screened for NaCl tolerance. M<sub>3</sub> and F<sub>3</sub> plants were harvested individually.

M<sub>4</sub> and F<sub>4</sub> seeds were screened for NaCl plant wise. In each plastic tray, plant wise M<sub>4</sub> and F<sub>4</sub> seeds from 15 plants were kept separately after adding 600 mL NaCl. Through out the experimentation, two controls were maintained. Tolerant M<sub>4</sub> and F<sub>4</sub> progenies were selected based on 1) all the seeds in each plant should have been germinated in NaCl solution and 2) radicle growth of these seeds should be similar to radicle growth in distilled water (Fig. 5). Five such germinated seeds from each tolerant plant were directly sown in the field and maintained progeny-wise, through out the crop cycle. In each M<sub>5</sub> and F<sub>5</sub> progeny, one out of five plants having good pod setting was selected and harvested separately, dose-wise and cross-wise, respectively. Similarly, progeny screening for NaCl tolerance for M<sub>5</sub> and F<sub>5</sub> seeds was undertaken in the next season.
Results and discussion

**Screening TAG 24 mutants for salt tolerance**

Based on the germination %, LD_{50} for germination in TAG 24 and TG 26 was 416.9 mM and 331.1 mM of NaCl, respectively when they were initially subjected to 100 mM to 1000 mM NaCl under laboratory conditions. Percent reduction was arrived at by comparing radicle length in control. Further at 100, 200, 300, and 400 mM NaCl, mean reduction in radicle growth was 40.4, 70.0, 84.4 and 93.3% for TAG 24 and 44.8, 68.4, 88.4 and 95.0% for TG 26, respectively.

For identifying salt-tolerant mutants, 83 TAG 24 mutants along with parents were screened at 400 mM NaCl and germination % recorded (Fig. 6). Wide variability for germination % in the mutants was observed, indicating their differences in genotypic responses to salinity stress. Parent, TAG 24 itself exhibited considerable tolerance to NaCl by scoring 80% germination. Though, none of the mutants had significantly superior germination, TGM 22, TGM 25, TGM 28, TGM 32, TGM 35, TGM 51, TGM 52, TGM 75, TGM 79 and TGM 94 scored higher germination (82.5%-90.0%) as compared to TAG 24. Earlier varietal differences for germination and seedling growth due to salinity stress were noted in groundnut (Nautiyal et al, 1989; Girdhar, 2004; Girdhar et al, 2005; Vadez et al, 2005). Understanding genotypic variability would offer good scope for identifying tolerant types.
In order to find out effective lower NaCl concentration for screening for radicle growth, TAG 24, JL 24, SB XI, Girnar 1 and TPG 41 were treated with 25, 50, 75, 100, 125 and 150mM NaCl. Pooled mean over the varieties indicated that there was a significant reduction in the radicle growth from 13.0% in 25mM to 76.3% in 150mM as compared to control (Fig. 7). Among the varieties screened, the least reduction of 57.4% was noticed in JL 24 at 150 mM and for the rest of the varieties it was over 80%. By 100mM NaCl concentration, radicle reduction was more than 60% in SB XI, Girnar-1 and TPG 41 while in TAG 24 and JL 24 it was around 35%. In order to ascertain the influence of environmental conditions on the response of the variety to NaCl treatment, seeds of TPG 41 grown at Kolhapur, Solapur, Parbhani and Trombay were treated with 25, 50, 75, 100, 125 and 150mM NaCl. There was a difference in the response to different locations, for lower NaCl concentration. However, higher concentration drastically affected the radicle growth of TPG 41 irrespective of the location (Fig. 8). Based on these studies, 100-150mM NaCl was found to be the ideal concentration for radicle growth. Vadez et al (2005) also showed that 100-125 mM range of NaCl treatment was suitable for salinity screening, based on biomass parameters in groundnut.

Using radicle growth as the criteria for salt tolerance, 43 mutants and TAG 24, grown during both rainy and summer seasons were screened at 125 and 150 mM NaCl. In both the seasons, significant differences were observed among TAG 24 mutants for radicle growth. In summer, five mutants had longer radicle (2.7 – 3.0cm)
than TAG 24 (2.3cm) while, 13 mutants had reduced length (0.9 - 1.8cm) with 125mM NaCl treatment. In two mutants, the radicle reduction was comparatively less (22.1 to 24.1%) as compared to parent (40%), while in four mutants, it was more (54.2 to 70.6%). Following 150mM NaCl treatment, nine mutants recorded significantly superior radicle length (2.3 – 2.6cm) than TAG 24 (1.9cm) while in 17 mutants, it was reduced (0.6 – 1.5cm). Due to 150 mM NaCl treatment, the radicle reduction was relatively less (26.7 to 37.2%) in six mutants as compared to parent (50.6%) while in other six mutants, it was more (65.0 to 81.3%). TGM 64 and TGM 93 exhibited higher tolerance by having longer radicle both at 125 and 150mM NaCl levels than TAG 24. Further, TGM 2 and TGM 67 also showed increased tolerance due to greater radicle length and lesser radicle reduction at 125mM NaCl and TGM 77, TGM 79 and TGM 93 at 150mM NaCl.

During rainy season, following 125mM NaCl treatment, eight mutants recorded significantly greater radicle length (2.2 – 2.9cm) than TAG 24 (1.8cm) while, 19 mutants had reduced length (0.3 – 1.5cm). The radicle reduction in six mutants was comparatively less (13.0 to 32.1%) than parent (42.6%) while in 17 mutants, it was more (54.9% to 88.6%). With 150mM NaCl treatment, six mutants recorded superior radicle length (2.2 – 2.6cm) than TAG 24 (1.8cm) while, 27 mutants had reduced length (0.3 – 1.5cm). In four mutants, the radicle reduction was relatively less (23.5 to 37.0%) than parent (44.1%) while in 28 mutants, it was more (52.4 to 92.4%). TGM 16, TGM 53, TGM 64, TGM 81, TGM 82 and TGM 93 exhibited higher tolerance by having longer radicle both at 125 and 150mM NaCl levels than TAG 24. Further, TGM 53, TGM 64 and TGM 81 showed increased tolerance due to greater radicle length and lesser radicle reduction at 125mM NaCl and TGM 53 and TGM 81 at 150mM NaCl.

Pooled analysis over both the seasons indicated that TGM 16, TGM 53, TGM 64, TGM 81, TGM 82 and TGM 93 showed enhanced tolerance by having longer radicle than TAG 24 at both 125 and 150mM NaCl. The reduction in radicle length was relatively less in TGM 10, TGM 29, TGM 53, TGM 64 and TGM 81 as compared to TAG 24 due to 125mM NaCl treatment and in TGM 29, TGM 53 and TGM 77 due to 150mM NaCl treatment. Further, TGM 53, TGM 64 and TGM 81 showed increased tolerance due to greater radicle length and lesser radicle reduction at 125mM NaCl and TGM 53 at 150mM NaCl.

Using sand culture experiments, Girdhar (2004) identified germplasm lines which are sensitive or tolerant to saline environments.

Screening mutant or segregating populations for salt tolerance

A large seeded variety, TPG 41 was irradiated with 200 and 300 Gy gamma rays to induce genetic variability for salinity tolerance. Around 45,775 $M_3$ seeds from 4,240 $M_2$ plants were screened with
100 mM NaCl during rainy season 2005. Of these, 55% of \( M_3 \) seeds germinated with NaCl treatment as compared to 60% in unirradiated NaCl control and 76% in unirradiated distilled water control (Table 1). Further, 1,790 seeds (1,155 seeds from 200Gy and 635 from 300 Gy) had radicle growth similar to unirradiated distilled water control and were considered as NaCl tolerant ones (Fig. 3). These germinated seeds were transplanted to thermocol cups and later to the field to ensure proper establishment of the seedlings. At the time of harvest, 631 plants from 200Gy and 390 from 300 Gy survived.

Plant-wise \( M_4 \) seeds from 631 plants from 200Gy and 390 from 300 Gy were screened without bulking with 100mM NaCl (Fig. 5). Using radicle growth criterion for all seeds from each plant, 181 \( M_4 \) plants from 200Gy (28.7%) and 390 from 300 Gy (31.0%) were identified as tolerant plants for radicle growth (Table 1). Since all the seeds in each of the tolerant plant were with radicle growth as that of unirradiated distilled water control, these plants were genetically true breeding for their response to NaCl treatment. In order to avoid escapes in these tolerant plants, plant wise \( M_4 \) seeds were screened with NaCl and 91 true breeding \( M_5 \) plants were isolated for radicle growth tolerance to NaCl (Table 1). As a consequence, only 0.2% of the \( M_3 \) seeds treated were true breeding tolerant mutants. In a preliminary yield trial of these 91 \( M_5 \) mutants during summer 2007 (Fig. 9), four mutants had significantly higher pod and seed yield and 10 mutants had larger seed size (> 88g/100 seeds) than parent TPG 41 (84g/100 seeds). Thus,

![Fig. 9](image_url)

**Fig. 9**: Preliminary field evaluation of \( M_5 \) mutants of TPG 41 groundnut variety for yield performance at Trombay

<table>
<thead>
<tr>
<th>Gamma ray (Gy)</th>
<th>No. of seeds/ plants treated</th>
<th>No. of true breeding tolerant seedlings/ plants transplanted</th>
<th>% of true breeding tolerant seedlings/ plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>29,635, 631</td>
<td>181, 1155, 161, 59</td>
<td>3.9, 28.7, 32.6</td>
</tr>
<tr>
<td>300</td>
<td>16,140, 390</td>
<td>121, 121, 32</td>
<td>3.9, 31.0, 26.4</td>
</tr>
<tr>
<td>Total</td>
<td>45,775, 1021</td>
<td>302, 1790, 302, 91</td>
<td>3.9, 29.6, 30.1</td>
</tr>
</tbody>
</table>

Table 1: Screening of mutant populations in groundnut for radicle growth tolerance to NaCl (100mM) treatment
following this simple screening technique, large seed groundnut mutants tolerant to salinity at radicle stage were identified. Salinity tolerance in these mutants at later plant phenophases is undergoing at salt affected soils, Agricultural Research Station, Digraj and Bheemarayanagudi.

\[ F_3 \] seeds from the crosses involving TG 37A, TPG 41, NRCG 2419 and NRCG 7548 were screened with 100mM NaCl during summer 2005. The mean germination % over these crosses was 90% as compared to 100%, 98%, 95% and 97% in TG 37A, TPG 41, NRCG 2419 and NRCG 7548 in distilled water control, respectively (Table 2). Further from all the crosses, 795 \[ F_3 \] seeds (6.1%) were found to be NaCl tolerant had radicle growth similar to distilled water control and one of these, 614 plants survived at harvest in the field (Table 2). Plant-wise \[ F_4 \] seeds from all the crosses were screened with 100mM NaCl and 266 plants (43.3%) were found to be tolerant to NaCl based radicle growth. Following the same protocol, 246 \[ F_5 \] plants were screened with NaCl and 114 true breeding \[ F_5 \] plants were isolated for radicle growth tolerance to NaCl (Table 2). Thus, 0.8% of the \[ F_3 \] seeds treated were true breeding tolerant genotypes and will be screened at salt affected soils to ascertain their salt tolerance.

**Table 2: Screening of segregating populations in groundnut for radicle growth tolerance to NaCl (100mM) treatment**

<table>
<thead>
<tr>
<th>Cross</th>
<th>No. of seeds/ plants treated</th>
<th>No. of true breeding tolerant seedlings/plants transplanted</th>
<th>% of true breeding tolerant seedlings/plants</th>
</tr>
</thead>
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<tr>
<td></td>
<td>[ F_3 ]</td>
<td>[ F_4 ]</td>
<td>[ F_5 ]</td>
</tr>
<tr>
<td>TPG 41 x NRCG 2419</td>
<td>3,115</td>
<td>131</td>
<td>59</td>
</tr>
<tr>
<td>NRCG 2419 x TPG 41</td>
<td>1,322</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>TPG 41 x NRCG 7548</td>
<td>2,361</td>
<td>110</td>
<td>30</td>
</tr>
<tr>
<td>NRCG 7548 x TPG 41</td>
<td>2,185</td>
<td>104</td>
<td>45</td>
</tr>
<tr>
<td>NRCG 2419 x TG 37A</td>
<td>1,401</td>
<td>96</td>
<td>37</td>
</tr>
<tr>
<td>TG 37A x NRCG 7548</td>
<td>886</td>
<td>57</td>
<td>30</td>
</tr>
<tr>
<td>NRCG 7548 x TG 37A</td>
<td>1,574</td>
<td>101</td>
<td>45</td>
</tr>
<tr>
<td>Total</td>
<td>12,945</td>
<td>614</td>
<td>246</td>
</tr>
</tbody>
</table>
Conclusion

This protocol provides a simple method to effectively screen and identify, potential genotypes from large populations in groundnut, whose screening is difficult in salt affected soils due to its heterogeneous nature. Furthermore, this protocol will be helpful as a selection tool since virtually any level of selection intensity can be obtained. But final evaluation and selection for high yielding tolerant genotypes in groundnut will require field evaluation in the salt affected soils.

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References


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**Dr Anand M. Badigannavar,** MSc (Agriculture) from University of Agricultural Sciences, Dharwad, joined BARC in 1997. He obtained his PhD (Botany) from University of Mumbai, Mumbai on the project “Genetic improvement for agronomical and biochemical traits in groundnut (*Arachis hypogaea* L.).” His field of interest is genetics, mutation and recombination breeding in groundnut leading to the development of improved groundnut varieties. Presently, he is involved in the pyramiding of disease resistant and salt-tolerant genes with largeseed trait in groundnut.

**Mr. Suvendu Mondal,** MSc (Microbiology) from Indian Agricultural Research Institute, New Delhi, joined BARC in 2003 through training school (46th batch, Bioscience) and is currently working in groundnut improvement group, Nuclear Agriculture and Biotechnology Division, BARC. He is currently pursuing his PhD on the project entitled “Induction of mutations and its morphological, biochemical and molecular characterization in groundnut.” His major research interest is to develop molecular markers for the biotic stress tolerance and characterization of mutants/mutations in groundnut. Besides he is involved in developing Trombay groundnut varieties and their dissemination.

**Dr G.S.S. Murty,** M.Sc. (Botany) from Andhra University, Waltair, joined BARC in 1971 and served up to January 2007. His interest was in mutation research of sesame and groundnut for which he received international acclaim. His significant contributions include inducing variability and discovering inter-mutant hybrid heterosis in sesame, leading Trombay Groundnut (TG) project for nearly two decades. Relentless efforts put in by him and his team, made TG project to attain a top position in India from the point of view of breeders as well as farmers, which increased BARC’s reputation. During his career in BARC, he produced more than 50 research papers; he is a co-breeder for 7 out of 12 TG varieties; he registered three sesame mutants with NBPGR; he was associated with many IAEA projects including undertaking Expert Mission.