Novel approach in screening rice genotype for tolerance to salt stress under hydroponic culture

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ABSTRACT
This study was undertaken to standardize an efficient and effective protocol for callus induction, subsequent growth and regeneration of ADT 43 genotype and hydroponic solution was used for salinity screening (NaCl) in regenerated plants of ADT 43. Proline content was analyzed in salinity screened plants. The medium used for callus induction was MS medium, with six different concentrations of 2,4-D were used for callus induction viz., 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l. In this 2.0 mg/l 2,4-D having higher callus induction frequency (98.33%). For regeneration of callus eight combinations of BA and IAA were also used in MS medium. In this MS+3.0 mg/l BA+2.0 mg/l IAA having maximum regeneration frequency (71.67%). The well regenerated plantlets were transferred to Yoshida’s solution for hydroponic salinity screening. In that, III-7 (113.66 µg/g) and VIII-2 (117.66 µg/g) somaclone lines had high proline accumulation. Hence, the salt tolerant somaclones produced higher proline content than the other somaclones. These lines can be exploited further to get salt tolerant varieties. These lines can be used to adopt in salt affected soil environment.

Key words: Callus induction, Callus regeneration, Rice, Salt tolerance, Somaclone.

INTRODUCTION
Rice (Oryza sativa L.) is a top five world carbohydrate crop, especially in Asia. The world’s projected demand of rice by 2020 is 880 million tons in proportion to the increased population (Anbazhagan, et al., 2009). Although with the increasing population and decreasing land availability, food grain production reduced by the severe damage of biotic and abiotic stresses. So need to develop plants with resistance to abiotic stresses. Conventional breeding is essential to improve rice but progress is slow due to several barriers, According to Shavindra et al., (2005), these challenges can be met by using advanced biotechnologies as a result of improved stress resistance with a high stable yield potential and good grain quality. Tissue culture technique can be used as a source for genetic variability by means of genetic modifications through the process of in vitro cultures. This technique has been widely used for breeding purposes, especially for stress tolerance selection, which severely limits rice production (Htwe et al., 2011).

Proline is an osmoprotectant use to conserve osmotic stability and prevent damage. Plants cultured under salt stressed show high proline accumulation (Praderm et al., 2003). It is an extensive phenomenon, whereby the proline accumulation in plants exposed to salt stress has been correlated in many species with their adaptation to osmotic stress. The objective of this study was identification of somaclones with salinity tolerance through tissue culture and hydroponic screening.

MATERIALS AND METHODS
Callus induction: ADT 43 rice seeds were manually dehusked and washed with sterile water, and then the seeds were transferred to the laminar airflow chamber. The seeds were kept in 70 per cent ethanol for one minute. Then seeds were washed with sterile distilled water three times and soaked with 0.1 per cent mercuric chloride for 15 minutes. Again the seeds are washed thoroughly three to four times with sterilized distilled water to remove all the trace of mercuric chloride and were blot dried using sterilized tissue paper. Surface sterilized seeds were cultured with the help of sterilized forceps into the test tube containing callus induction medium. Callus induction medium supplemented with different concentrations of 2,4-D viz., 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l. Cultures were incubated in dark at 25±1°C. Callus induction frequencies were recorded and calli were sub cultured at 15 days intervals to obtain embryogenic callus.

Callus regeneration: After one month, calli were transferred to regeneration medium having different concentrations of BA and IAA viz., 5.50 and 2.0; 5.0 and 2.0; 5.0 and 2.0; 4.50 and 2.0; 4.0 and 1.0; 4.0; 3.0 and 3.0; 3.0 and 2.0; 3.0 and 1.0 for shoot development and MS + 1 mg/l of IBA for root development. Well regenerated plantlets with sufficient root and shoot system were transferred into Yoshida’s nutrient culture solution for salinity screening.

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**Handling of somaclones and salinization:** The Yoshida’s nutrient solution (Yoshida et al., 1976) was salanized by adding NaCl with constant stirring until desired EC was obtained. The nutrient solution gives an EC of 2 and 4 dS/m. ADT 43 derived somaclones were placed on Styrofoam seedling floats having hydroponic solution and was taken to screen house. The roots should be inserted through the nylon mesh. After three days, when seedlings were well established the nutrient solution was salanized by adding NaCl. Proline was extracted the method suggested by Bates et al., 1973. The frequency of callus induction was calculated according to the following formula:

\[
\text{Callus induction frequency (\%) = \frac{\text{No. of seeds produced calli}}{\text{No. of seeds inoculated}} \times 100}
\]

Plant regeneration from plated calli was calculated with the following formula:

\[
\text{Plant regeneration (\%) = \frac{\text{No. of calli produced plants}}{\text{No. of calli inoculated}} \times 100}
\]

The experiment was laid out following complete randomized block design (CRD) was laid out (Panse and Sukatme, 1967) with three replications and the data analyzed using Agres Statistical package.

**RESULTS AND DISCUSSION**

In tissue culture, somaclonal variation is produced while culturing explants on synthetic media with varied levels of phyto-hormones under in vitro condition (Larkin and Scowcroft, 1981). Such variation obtained in de novo during the process of de-differentiation of ex-plants into unorganized cell masses (calli) and re-differentiation of calli into plantlets (Chopra et al., 1989).

The callus induction was the primary step in any in vitro screening technique. The significant differences were observed in different concentration of callus induction medium (Table 1). Among the callus induction media MS+2 mg/l 2,4-D having higher callus induction frequency of 98.33 percent and very early (7.3 days). Chen et al. (1974) reported that 2, 4-D concentration of 2.0 mg/l is suitable for callus induction and callus growth in rice. The similar reports also obtained by Jaseela et al. (2009). The role of 2, 4-D in cell division is to increase the rate of cell division and to the increased amount of callus. After transfer of embryogenic calli to the regeneration medium, green areas began to develop on callus surfaces within two-three weeks, and then the green plants developed from these green areas. The significant differences in callus regeneration frequency under different concentrations of BA and IAA were noticed (Table 2).

### TABLE 1. Callus induction frequency in ADT 43

<table>
<thead>
<tr>
<th>Media composition</th>
<th>No. of seeds inoculated</th>
<th>Days taken for callus induction</th>
<th>No. of seeds producing callus</th>
<th>Callus induction frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS+0.5mg/l 2,4-D</td>
<td>120</td>
<td>12.3c</td>
<td>96</td>
<td>80.00(63.45)b</td>
</tr>
<tr>
<td>MS+1.0mg/l 2,4-D</td>
<td>120</td>
<td>9b</td>
<td>84</td>
<td>70.00(56.80)c</td>
</tr>
<tr>
<td>MS+1.5mg/l 2,4-D</td>
<td>120</td>
<td>8.6ab</td>
<td>97</td>
<td>80.83(64.04)b</td>
</tr>
<tr>
<td>MS+2.0mg/l 2,4-D</td>
<td>120</td>
<td>7.3a</td>
<td>117</td>
<td>98.33(83.70)a</td>
</tr>
<tr>
<td>MS+2.5mg/l 2,4-D</td>
<td>120</td>
<td>8.3ab</td>
<td>99</td>
<td>82.50(65.29)b</td>
</tr>
<tr>
<td>MS+3.0mg/l 2,4-D</td>
<td>120</td>
<td>11.6c</td>
<td>85</td>
<td>70.83(57.05)c</td>
</tr>
</tbody>
</table>

*Values in parentheses indicate the transformed arc sin values
*The mean having the same letter following is not significantly different at 0.01 probability level by Least Significant Difference Test (LSD).

<table>
<thead>
<tr>
<th>Media composition</th>
<th>No. of calli inoculated</th>
<th>Regeneration frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS+5.50mg/l IAA+2.0mg/l</td>
<td>34</td>
<td>43.33(41.30)g</td>
</tr>
<tr>
<td>MS+5.00mg/l IAA+2.0mg/l</td>
<td>42</td>
<td>46.67(43.13)f</td>
</tr>
<tr>
<td>MS+4.50mg/l IAA+2.0mg/l</td>
<td>38</td>
<td>49.00(44.81)f</td>
</tr>
<tr>
<td>MS+4.00mg/l IAA+1.0mg/l</td>
<td>45</td>
<td>66.67(54.73)c</td>
</tr>
<tr>
<td>MS+4.00mg/l IAA</td>
<td>46</td>
<td>70.00(57.00)b</td>
</tr>
<tr>
<td>MS+3.00mg/l IAA+3.0mg/l</td>
<td>41</td>
<td>55.00(48.06)e</td>
</tr>
<tr>
<td>MS+3.00mg/l IAA+2.0mg/l</td>
<td>39</td>
<td>71.67(57.85)a</td>
</tr>
<tr>
<td>MS+3.00mg/l IAA+1.0mg/l</td>
<td>43</td>
<td>61.67(51.76)d</td>
</tr>
</tbody>
</table>

*Values in parentheses indicate the transformed arc sin values
*The mean having the same letter following is not significantly different at 0.01 probability level by Least Significant Difference Test (LSD).

The solution based screening is rapid and widely acceptable under controlled conditions instead of field screening (Gregorio et al., 1997). Saleem et al. (2005) used hydroponic solution for salinity screening in Basmati rice. For salt induction they used different concentrations of NaCl. The proline content was estimated in thirteen somaclones treated 4 dS/m and tested along with control (Figure 1.). Among the somaclones, the high proline accumulation was observed in the lines of III-7 and VIII-2 (87.17 µg/g) and low in ADT 43 control plants. There was significant difference between these two levels. Among the treatments, mean high proline accumulation was 83.31 µg/g. When interactions were worked out between genotypes and treatments,
the maximum proline content was observed in the line VIII-2 (117.66 µg/g) and minimum was observed in ADT 43 control plants (22.33 µg/g). There was a significant difference between these two levels. The proline content was high in salt treated plants (4 dS/m) and low in control plants. This conclusion correspond well with the report of Kumar et al. (2007) that the free proline content was significantly increased in the salt stressed plants over control plants. Pongprayoon et al. (2008) concluded that an increase in exposure period of salt stress directly enhanced the proline accumulation. Hence proline could be used as one of the differentiator of tolerant and susceptible genotypes. These salt tolerant lines can be exploited further to get salt tolerant varieties.

REFERENCES