Salinity is a severe problem at world level. It have adverse effect on plant growth (Shahid et al 2011). Numerous studies have demonstrated improvement in seed germination of different plant species under both normal and stress conditions in response to priming with plant growth hormones or other organic substances (Ashraf and Foolad 2005). Tissue culture technique has emerged as a feasible and cost-effective alternative tool for developing stress-tolerant plant in recent years. Many plant species for the development of stress tolerant plants and considerable efforts have been made to produce stress tolerant plants using this technique (Borsani et al. 2003, Yamaguchi and Blumwald 2005). Generally several stresses are the silencing of transgene, consequent reduction of gene expression and low transformation frequency (Mondal et al. 1997). But present study is undertaken to analyse that phytohormones treatment can overcome the effect of salt stress or a salt tolerant plant may develop through these experiment.

**MATERIALS AND METHODS**

All the glassware were washed with detergent under running tap water. After washing, these were rinse and clean with distilled water and dry in oven at 150°C for 2hr. Forceps and scalpels were wiped thoroughly with absolute alcohol and wrapped in aluminium foil. Thereafter, forceps, scalpels, petriplates, mask and beakers were wrapped with paper. These were then autoclaved for 20 minute at 15 lb psi. All of these were autoclaved on same day of inoculation. Prior to the day of inoculation of plant material, the laminar air flow (LAF) was saturated with alcohol vapours. The inoculation room was fumigated by burning potassium permanganate and formaldehyde. On the day of inoculation, the LAF chamber was wiped with alcohol. Thereafter, all the material (except the living tissue) were placed in the chamber and exposed to UV rays for 15-20 minutes. While working with LAF chamber the sterile filtered air was passed continuously. Inoculation, transfer etc. were done over the gas flame.

MS medium (Murashige and Skoog 1962) was employed in the experiment. MS medium without any plant growth regulator served as the basal medium. Sterilization of seeds was done by washing of seeds by submerging in water with a few drops of detergent (mild) in a beaker and shaking by hand. Now cover the beaker with two layer of cheese cloth and put it under running tap water. Submerge the seeds in 70% alcohol for 30-60 seconds and then decant the alcohol. Transfer the seeds to a flask or beaker containing 20-40% hypochlorite for 15-20 minute. Rinse 2-3 times with sterile distilled water. Place 2-3 seeds per culture vessel on the surface of simple agar media without growth regulators. Incubate the culture at 25°C in dark for 72 hours.

The leaves of *Phaseolous aureus* was obtained from 15 days old in-vitro seed
developed plant. The leaves were surface sterilised with 0.1% Mercuric chloride (HgCl₂) solution and were given three changes of sterile distilled water to get rid of trace of mercuric chloride. The leaves thus obtained were inoculated on different MS medium containing the different growth hormones e.g. IAA, GA and cytokinin (1×10⁻⁹ M IAA, 10⁻¹⁰ M Kn and 1×10⁻¹⁰ M GA) and sodium chloride (1×10⁻¹ M) also. They were allowed to grow in the medium for 24 days for the production of salinity tolerant plant. The effect is presented in the form of Plate-1.

RESULTS AND DISCUSSIONS

The results are shown in Plate-1 and Table 1. The leaf of *P. aureus* cv. K-851 are placed into the MS medium treated with the 1×10⁻⁹ M NaCl, inhibited callus formation, shoot growth and root growth are observed at treatment of NaCl. Callus formation initiation after 9 days of inoculation and callus is not well developed after 17th days. Demirkiran *et al.* (2013) found that all the concentration of NaCl inhibit the shoot growth, decreased fresh weight and protein in plant tissue when MS medium containing 0. 50, 100 mM NaCl with mature embryo.

MS medium having leaf when treated with 1×10⁻¹⁰ M IAA and 1×10⁻¹ M NaCl, it is observed that IAA reduces the inhibitory effect of NaCl in the growth medium. After 4 days of inoculation, the callus initiation occurs and after 9 days it shows the chlorophyll development i.e. well developed green callus is formed. The root initiation is started after 12 days and multiplication shoot formation is occurred after 17 days.

The MS medium treated with 1×10⁻¹⁰ M Kn and 1×10⁻³ M NaCl shows that cytokinin also decreases the inhibitory response of NaCl in the medium. Meristem proliferation and multiple shoot initiation are best in this medium after 4 days of inoculation, callus initiation occurs and after a week and shoot formation is visible. The root initiation occurs after 2 days of regeneration.

Similarly, when the Murashige and Skoog medium having leaf treated with 1×10⁻¹⁰ M GA and 1×10⁻¹⁰ M NaCl, the colourless callus formation occurred after 3 days of inoculation and after 8 days chlorophyll development occurred in callus. After 17 days multiplication of shoot formation occurred. The root initiation occurred after 15 days of shoot initiation. It is showed that GA affect the negative response of NaCl.

The leaf of *Phaseolus aureus* cv. K-851 is inoculated in the Murashige and Skoog medium treated with 1×10⁻¹⁰ M GA + 1×10⁻¹⁰ M IAA + 1×10⁻¹⁰ M Kn and 1×10⁻¹⁰ M NaCl. It is found that the selected concentration of growth hormone reduce the effect of NaCl. The early callus initiation is observed after 2 days of inoculation and chlorophyll development occurred after 5 day of it. The multiplicatons of shoot formation are also triggered after 8 days and the early root and shoot initiation and the elongation of shoots occurs after 12 days. As compared to above responses growth is very fast and earliest and adverse effect of NaCl is maximally lowered.

It is very interesting to note that mixture of plant hormone i.e. IAA, GA and Kn stimulate the salt stressed plant growth *in vitro*. Patil *et al.* (2012) reported that when seeds were cultured *in vitro* on MS medium, addition of all types of concentration of cytokinin and auxin stimulated the rate and percentage of seed germination. Priyanka *et al.* (2013) also reported that the best callus formation is observed on 1mg/L 2, 4D. Mature embryos are cultured in Murashige and Skoog medium containing 0 (control), 50 and 100 mM NaCl for 20 days. Both concentrations, inhibited shoot growth, decreased fresh weight and protein content, and increased SOD activity in a dose-dependent manner. The lower concentration increased root growth. Salinity caused nucleotide variations in roots, but did not affect shoot DNAs. The higher concentration caused methylation changes, mainly hypermethylation in shoots. This was the first study on genetic and epigenetic
Plate 1: Showing the effect of $1 \times 10^{-1}$ M NaCl and growth hormone, $1 \times 10^{-6}$ M IAA + $1 \times 10^{-10}$ M Kn + $1 \times 10^{-10}$ M GA on in vitro callus culture of *P. aureus* cv. K-851 using MS medium.

Table 1: *In vitro* callus culture of *P. aureus* cv. K-851 using MS medium containing $1 \times 10^{-4}$ M NaCl and growth hormone, $1 \times 10^{-6}$ M IAA + $1 \times 10^{-8}$ M Kn + $1 \times 10^{-10}$ M GA.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Medium</th>
<th>4th day</th>
<th>9th day</th>
<th>12th day</th>
<th>17th day</th>
<th>24th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MS medium + $1 \times 10^{-4}$ M NaCl + leaf</td>
<td>Callus initiation</td>
<td>Initiation of callus formation and leaf formation.</td>
<td>Callus development and shoot formation.</td>
<td>Callusing and development of short leaf.</td>
<td>Elongation of shoot which is fully developed.</td>
</tr>
<tr>
<td>2</td>
<td>MS medium + $1 \times 10^{-6}$ M IAA + $1 \times 10^{-8}$ M NaCl + leaf</td>
<td>Callus initiation and browning of callus in medium.</td>
<td>Root initiation in callus and leaf formation.</td>
<td>Callus development and shoot formation.</td>
<td>Multiple shoot formation and root are fully developed.</td>
<td>Elongation of shoot and root which is fully developed in medium.</td>
</tr>
<tr>
<td>3</td>
<td>MS medium + $1 \times 10^{-6}$ M GA + $1 \times 10^{-4}$ M NaCl + leaf</td>
<td>Green Callus initiation and browning of callus in medium.</td>
<td>Yellowing of callus and shoot formation initiation.</td>
<td>Root initiation and shoot formation in medium.</td>
<td>Multiple shoot formation that are fully developed.</td>
<td>Leaves fully developed in medium. But the roots are not well developed.</td>
</tr>
<tr>
<td>4</td>
<td>MS medium + $1 \times 10^{-8}$ M Kn + $1 \times 10^{-6}$ M NaCl + leaf</td>
<td>Callus initiation and browning of callus in medium.</td>
<td>Root initiation and shoot formation in callus.</td>
<td>Proliferation of shoot in medium.</td>
<td>Multiple shoot formation that are fully developed.</td>
<td>Elongation of shoot which is fully developed in medium while the roots are not well developed.</td>
</tr>
<tr>
<td>5</td>
<td>MS medium + $1 \times 10^{-6}$ M GA + $1 \times 10^{-10}$ M IAA + $1 \times 10^{-8}$ Kn + $1 \times 10^{-4}$ M NaCl + leaf</td>
<td>Green Callus formation in medium.</td>
<td>Shoot formation in callus.</td>
<td>Multiple shoot formation that are fully developed and root initiation</td>
<td>Elongation of shoot.</td>
<td>Leaves and roots are fully developed in medium.</td>
</tr>
</tbody>
</table>


