

SUMMARY

- The optimum surface sterilization treatment for best explant survival and initiation of regeneration was standardized for groundnut seeds explants. The treatment of 0.1% HgCl_2 for 7.5 min followed by three double distilled water wash gave around 80% sterile explant initiation without any aberrant effect.
- Direct organogenesis was achieved from the young, immature leaflets on MS medium with 13.32 μM BAP and 2.68 μM NAA which gave 70 % direct shoot formation within 30 days of incubation.
- Direct organogenesis was achieved from the cotyledon explants on MS medium with 17.76 μM BAP and 2.68 μM NAA which gave 65 % direct shoot bud initiation within 30 days of incubation.
- Direct organogenesis was achieved from the embryo axis explants on MS medium with 17.76 μM BAP and 0.53 μM NAA which gave 76 % direct shoot formation within 30 days of incubation.
- Indirect organogenesis was achieved from the mature leaf explants on MS medium with 2.32 μM kinetin after 4-5 sub cultures on the same medium within 5-6 months.
- *In vitro* rooting of the plantlets produced from various explants was achieved on MS medium with 4.30 μM NAA within 30 days of transfer.
- In the pot experiments, it was observed that groundnut plants could withstand salinity up to 50 mM NaCl concentrations beyond which all the morphological growth parameters were observed to be affected.

- In the *in vitro* seed germination experiments also it was observed that the seeds could germinate normally in the medium containing 50 mM NaCl without delay compared to control.
- The release of H₂O₂ was observed to be increasing with increase in exposure of NaCl stress.
- The catalase activity was observed to be increasing with increasing level of NaCl up to 75mM. However, there was no significant change in activity at NaCl concentration of 75mM and 100mM indicating that catalase activity was not adequate for the complete scavenging of H₂O₂ and thus to combat salt stress. Despite its increased activity compared to control.
- The peroxidase activity was observed to be increasing with increasing level of NaCl stress up to 100 mM NaCl linearly. Hence at very high salt concentration peroxidase rather than catalase plays major role in combating salt.
- Transformation of the binary vector **pHS724** containing gene of interest i.e COX was carried out through CaCl₂ mediated transformation in order to amplify the plasmid using the vector *E.coli* DH5 α . The presence of the plasmid in *E.coli* DH5 α was checked by restriction digestion pattern as well as phenotypic expression (kanamycin resistance).
- The expression of COX gene in the vector *E.coli* DH5 α could not be observed. This is due to the absence of inducible promoter which can be expressed in the bacteria *E.coli*. Since the gene choline oxidase is under the control of viral promoter 35S, no difference in the salt tolerance capacity was observed in *E.coli* DH5 α transformants as compared to *E.coli* DH5 α (control).

- *Agrobacterium tumefaciens* LBA4404 was transformed with the COX gene through triparental mating using *E.coli* DH5 α with pHS724 (kan^R) as the donor strain, *E.coli* DH5 α with pRK2013 (kanR) as the helper strain under appropriate antibiotic selection.
- Confirmation of *A.tumefaciens* transformants was carried out through ketolactose test (also known as Benedict's test).
- The phenotypic expression of COX gene in *A.tumefaciens* was carried out by growing in various NaCl concentration where transformants were able to survive in presence of 300mM NaCl.
- Co-cultivation of *A.tumefaciens* containing COX gene with different groundnut explants was carried out for 5 days under dark condition. After co-cultivation, the explants were washed and inoculated on MS medium containing respective growth hormones for shoot induction.
- The cultures were sub cultured on respective medium containing (cefotaxime 200 mg/l) and kanamycin (60 mg/l) for selection.
- The transgenic plants so raised were rooted on MS medium with 4.30 μ M NAA.
- The transformation success was confirmed through GUS assay.
- The transgenic plants so raised were shown to tolerate higher salt levels. However, plants could not be grown beyond 150mM NaCl concentration.