CHAPTER6
CONCLUSION

CONCLUSION

The present study revolves around three aspects of plant tissue culture which are regeneration, metabolite studies and mutation studies within two species of *Portulaca* (*P oleracea* and *P grandiflora*).The regeneration of in vitro shoots and callus cultures was initiated with leaf explants because of its easy availability. Alternatively nodal explants was also tried to improve on the regeneration protocol of *P grandiflora*. Regeneration potential of in vitro leaf explants was assessed for further experiments and the shoots obtained in the regeneration studies were utilized for metabolite finger printing. The fatty acids in *P oleracea* and betalains in *P grandiflora* were evaluated in the in vivo and in vitro samples. Consequently the effect of a chemical mutagen EMS on the fatty acid profile of in vitro leaf explants of *P oleracea* was determined. The study was divided into three sections and following conclusions are drawn based on the results obtained.

6.1 SECTION I- PLANT REGENERATION STUDIES

In *P oleracea* the PGRs induced different responses which are summarised as follows:

- Individual concentration of BA differentiated shoots at 5µM (5.5±0.99) while at higher concentration of 15-20µM shoot buds and green compact callus was formed. Contrary to BA, Kin was ineffective in induction of shoots as only at a high concentration of 15 µM few shoots (1.1±0.34) were formed.
- Within the individual concentration of auxins IAA was able to differentiate roots and shoots in all the combinations however their number was less as 2-4 roots and 1-2 shoots per explants was formed. Profuse rooting was obtained in presence of NAA while 2,4D formed poor friable callus.
- Synergistic combination of cytokinin was effective in differentiating optimum number of shoots. At 0.5 and 2.5 µM BA and Kin (0.5-20 µM) shoot buds were differentiated while with 5 µM BA and 10 µM Kin optimum numbers of shoots (8.3±1.01) were obtained. Shoots were also induced with 10 µM BA and Kin but the percent response was low as compared to 5 µM BA and 10 µM Kin. Synergistic higher concentration of cytokinin induced stunted shoots and green callus.
- Synergistic combination of cytokinin and auxin was also evaluated. BA and IAA induced roots and callus. At low concentration of BA (0.5-2.5 µM) and IAA poor callus and roots were initiated while 5-10 µM BA and IAA formed profuse callus and

roots. The combinations of BA and NAA also formed large number of roots and poor callus at 0.5 µM BA and NAA and their number decreased with increase in concentration of BA. In presence of 2,4D and BA white friable callus was induced and optimum concentration of callus induction for fresh and dry weight was 2.5 µM BA and 2.5 µM 2,4D. The callus growth index revealed that in 7^{th} week maximum callus biomass is obtained.

- The response of Kin with different auxins was similar to BA and auxins. Kin +IAA and Kin + NAA both differentiated roots and poor to moderate callus while Kin and 2,4D induced callus only whose growth was less as compared to BA and 2,4D.
- Multiplication and elongation of shoots obtained on an optimum concentration of cytokinin (8.3±1.01) was achieved in presence of coconut water (5%) where the number increased to 36.2±3.70 per explants.
- Histology of developing shoots confirmed direct organogenesis.
- High rate of regeneration was depicted by in vitro leaf explants which had implication in further experiments.
- The regenerated roots depicted a high rate of rooting potential (8.2±0.64 roots per shoot) in half strength MS medium fortified with 1% sucrose and 3 µM IBA.

Regeneration in *P grandiflora* was initiated in presence of leaf and nodal explant. On the basis of the effect of PGR's in leaf explants the following results were obtained.

- Individual concentration of cytokinin and auxins were ineffective in inducing shoot buds.
- Synergistic concentration of cytokinin, BA and Kin were able to differentiate shoot buds at 5-10 µM which failed to develop into shoots.
- Synergistic concentration of cytokinin and auxins largely induced shoot buds and callus. Within the combination of BA and IAA callus and shoot buds were differentiated and at 10 µM BA and 5 µM IAA along with shoot buds; callus and shoots were also formed. Young leaf explants when incubated on the same combination depicted a faster response and the in vitro shoots were multiplied on 20 µM BA where the number of shoot increased to 6.25±0.85 per explants.
- Callus and shoot buds were formed at low concentration of BA (0.5-2.5 µM) and NAA while at higher concentration of BA (5-10 µM) and NAA profuse callusing was obtained. Poor callus induction was observed in the combinations of BA and 2,4D.
- Kin when synergistically coupled with IAA or NAA induced callus and shoot buds. Similarly Kin and 2,4D induced poor- moderate callus.

- Multiplication and elongation of in vitro shoots from young leaf explants in presence of 10 µM BA and 5 µM IAA was achieved on 5 µM GA_3 where the number of shoots increased to 8.2±0.37 per explants.
- Histology of developing shoots indicated indirect mode of organogenesis.
- In vitro leaf explants differentiated 4.6±0.50 shoots per explants which indicated its low regenerative potential.
- Regeneration from nodal explants indicated that individual concentration of BA responded better than Kin and a synergistic combination of 2-4 µM BA and 8 µM Kin formed optimum number of shoots per explants (4±0.63).
- Necrosis in the shoots obtained from nodal explants was alleviated in presence of 18 mM $CaCl_2$.

6.2 SECTION II- METABOLITE STUDIES

Plants are a rich source of primary and secondary metabolites and in the two species of *Portulaca* under study their content was evaluated. Fatty acid fingerprinting was done in *P oleracea* while the content of betalains was assessed in *P grandiflora*. The results obtained are briefly concluded as follows:

- Within the in vivo samples highest fat content was noted in leaves, while the in vivo shoot and in vitro shoots depicted a similar fat content.
- The fatty acid finger printing of all the samples revealed that Palmitic acid, Linoleic and Linolenic acid are the major fatty acids present in *P oleracea*.
- The content of Linolenic acid was highest (20.15±1.06) within in vivo shoots followed by leaves, stem, in vitro shoots and callus respectively.
- Stem is the major source of Linoleic acid followed by in vivo shoot and in vitro shoot.
- The content of Palmitic acid was relatively high in all the samples.
- Presence of long chain fatty acids such as Docosanoic acid and Tetracosanoic acid was reported in shoot cultures while callus cultures besides also synthesised odd chain fatty acid such as Tricosanoic acid.
- GCMS studies revealed that besides the fatty acid detected in GC studies long chain fatty acids such as Hexacosanoic acid, Octacosanoic acid were also present in different samples.

In *P grandiflora* betalain content was evaluated in different parts of the plant in presence or absence of sodium ascorbate.

- The content of betalain without sodium ascorbate was highest in stem followed by whole plant while in leaves and roots it was absent.
- Addition of sodium ascorbate was also essential to the samples as it provided stability to the pigment and prevented its degradation even after 24 hours.

6.3 SECTION III- MUTATION STUDIES

The in vitro leaf explants were treated with EMS and the morphological response and change in fatty acid content was evaluated.

- In vitro shoots were induced at low concentration (0.1-0.3%) while at higher concentration the response changed from shoot buds and callus (0.4-06%) to white callus (0.7-1%)
- Probit analysis revealed that 0.3% was the concentration at which LC50 value was obtained.
- Highest number of in vitro shoots/explant was achieved at 0.2% EMS (10.2±0.84).
- Some variations in the morphology of the shoots were obtained such as acicular leaves at 0.2% EMS and enlarged leaves at 0.1- 0.2%.
- It was noted that higher concentrations of EMS suppressed the organogenic potential of the leaf explant and shoot buds with stunted shoots were induced at 0.4% EMS. A variant shoot cultures were obtained at this dose where cluster of in vitro shoot was formed.
- At a high concentration of 0.6% EMS the leaf explant induced green nodular callus interspersed with shoot buds and stunted shoots. A variation in the culture was obtained where elongated well developed shoots were formed at this concentration.
- In the callus inducing medium optimum fresh callus weight of 18.61±2.68 gm/explant was induced at 0.2% EMS.
- Similar to the in vitro shoots the fatty acid profile of the treated shoot cultures depicted that Palmitic acid, Linoleic acid and Linolenic acid were the major fatty acids. Besides this the treated shoot cultures also had the capacity to synthesise Eicosanoic acid in high content (6.01±0.06%) and Tricosanoic acid and Tetracosanoic acid which were absent within in vitro shoots.
- The treated callus cultures depicted a decline in the variety of fatty acids with increase in concentration of EMS. At 0.1% EMS Linoleic acid was the major fatty acid (12.36±1.16%) while it was absent in 0.2-0.3%. In EMS treated callus cultures. Tricosanoic content was also high at 0.1%.

- Mass spectrometry studies revealed the presence of long chain fatty acids in both treated shoot and callus cultures.

6.4 OUTCOME OF THE STUDY

- A highly reproducible protocol for regeneration with enhanced number of shoots is reported in *P oleracea*
- In *P grandiflora* regeneration from leaf explants albeit with less number of shoots is reported.
- Fatty acid fingerprinting of *P oleracea* indicated that it is a good source of omega fatty acids and the content of Linoleic and Linolenic acid is almost similar in both in vivo and in vitro shoots.
- Within *P grandiflora* the content of betalain is highest in stem so it can be utilized for the extraction of the pigment.
- Both shoot and callus cultures of *P oleracea* have the capacity to synthesise long chain fatty acid.