SUMMARY

Plants are a rich source of compounds which can be utilized as medicine or in the synthesis of products which are used by people in their day to day lives such as food, fibre, wood and industrially important substances. Over exploitation of the plants for the yield of these compounds has lead to a sharp decrease in biodiversity and consequently many plants have become threatened or endangered. Development of techniques which could ease out the pressure on the natural population is the need of the hour. Plant tissue culture is a technique where economically important plants can be over exploited for the synthesis of these valuable compounds both from shoot or callus cultures. In vitro methods present new strategies for the development and improvement of the drug yielding plants by influencing the primary and secondary metabolites of plants. The level of these primary and secondary metabolites can be altered by manipulations such as precursor feeding, elicitors and mutation breeding. Mutation techniques have induced variability within the plants where the metabolites have been altered. EMS is the most commonly used mutagen which is extensively used in floriculture, horticulture and metabolites study. These days in vitro mutagenesis is preferred over conventional mutation breeding chiefly because of its precision and effectiveness. It also facilitates the study of a single cell as compared to the complex plant.

The present study revolves around three aspects of plant tissue culture (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. 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 were drawn based on the results obtained.

SECTION I- REGENERATION STUDIES

1.1 *P oleracea*

In *P. oleracea* the PGRs induced different responses which are summarized 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. Similarly 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.

The study was initiated with the objective of inducing large number of in vitro shoots and since single PGR's were unable to induce the desired response therefore synergistic combination of cytokinin was employed. 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 number of shoots (8.3±1.01) were induced. 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.

Since synergistic combination of cytokinin was beneficial in inducing shoots therefore synergistic combination of cytokinin and auxins were also evaluated for their role in regeneration. Combinations of BA and IAA largely induced roots and callus. At low concentration of BA (0.5-2.5 µM) and IAA poor callus and roots were initiated while at 5-10 µM BA and IAA formed profuse callus and roots. A similar response was also depicted by the combinations of BA and NAA. Combination of these two PGR's differentiated large number of roots and poor callus at 0.5 µM BA and NAA and their number decreased with increase in concentration of BA. Another auxin utilized in the study was 2,4D which is known to induce callus in a number of plants. 2,4D with BA induced white friable callus and optimum concentration of callus induction for fresh and dry weight was 2.5 µM BA and 2.5 µM 2,4D (20.2±0.70gm). Callus growth index revealed that 7^{th} week is the time duration when maximum

callus biomass is obtained. This protocol was employed for the establishment of callus cultures which were exploited for the extraction of fatty acids. 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.

The shoots obtained on the cytokinin rich medium (5µM BA and 10µM Kin) were multiplied and elongated in presence of coconut water (5%) where the number increased to 36.2±3.70 per explants. Histology of developing shoots confirmed direct organogenesis.

The protocol which was developed from in vivo leaf explant was employed for the regeneration from in vitro leaf explant. High rate of regeneration was depicted by in vitro leaf explants which had implication in further experiments. The shoots so formed were subjected to rooting in presence of IBA. A high rate of rooting potential (8.2±0.64 roots per shoot) in half strength MS medium fortified with 3 µM IBA was achieved.

1.2 *P grandiflora*

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

The leaf explant of *P grandiflora* in presence of individual concentration of cytokinin and auxins were ineffective in differentiating shoot buds. When synergistic combinations of cytokinin were employed it was noted that shoot buds were differentiate at 5-10 µM BA and Kin which failed to develop into shoots.

Since the individual PGR's and synergistic cytokinin were unable to differentiate shoots therefore synergistic concentration of cytokinin and auxins were employed which 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 callus which formed vitro shoots. 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. Similar to the response of BA and auxin, Kin when synergistically coupled with IAA or NAA induced callus and shoot buds and with 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.

The in vivo leaf explant depicted a low rate of regeneration and hence the in vitro leaf explant was assessed for its regeneration potential. In vitro leaf explants differentiated 4.6±0.50 shoots per explants which again indicated its low regenerative potential.

Nodal explant was also evaluated for its regenerative capacity and it 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). The shoots were obtained from nodal explant but they were associated with shoot tip necrosis which was alleviated in presence of 18 mM $CaCl_2$.

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:

2.1 *P oleracea*- Fatty Acids

P oleracea was evaluated for the fatty acid finger printing in the in vivo shoots, stem, leaves, in vitro shoots and callus cultures. Within the in vivo samples highest fat content was noted in leaves, and 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. Long chain fatty acids such as Docosanoic acid and Tetracosanoic was in less content (1%) while callus cultures 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.

2.2 *P grandiflora*- Betalains

Betalains are the pigments which are extensively utilized in the coloring of food stuff and the search for the better and novel source of this pigment is the recent trend. *P grandiflora* is a source of betalain and its 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. Stability analysis of the pigment revealed that addition of sodium ascorbate was also essential to the samples as it prevented its degradation even after 24 hours.

SECTION III- MUTATION STUDIES

EMS is a chemical mutagen which is known to induce variability in a number of crop or floriculture plants. Recent studies have shown that this mutagen can also influence the metabolite content in plants. Therefore studies were undertaken in *P oleracea* to evaluate the role of EMS on morphogenesis and fatty acid finger printing. 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%). For any successful mutation induction knowledge of LC50 dose is imperative. Thus Probit analysis was conducted to calculate the LC50 dose which revealed that 0.3% was the concentration at which 50% of the explants can survive. 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 in vitro shoots were formed at this concentration. In the callus inducing medium optimum callus weight of 18.61±2.68 gm 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 synthesize 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% EMS treated callus cultures. Tricosanoic content was also high at 0.1% EMS cultures.

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

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 similar in both in vivo and in vitro shoots so it can be a suitable candidate for different molecular studies.
- Within *P grandiflora* the content of betalain is highest in stem so it can be utilized for the extraction of the pigment.
- The EMS treated cultures depicted that both shoot and callus cultures have the capacity to synthesise long chain fatty acid.