CHAPTER 2
REVIEW
OF
LITERATURE

REVIEW OF LITERATURE

The present review cites some of the important work done in the regeneration aspect of in vitro cultures, primary and secondary metabolites obtained from the in vitro cultures and in vitro mutagenesis.

Plant tissue culture is a technique which has a potential to produce millions of clones by inducing morphogenesis from plant tissues and organs. It is commonly utilized for mass propagation of crop species and ornamental plants (Fiuk and Rybczynski 2008). These days the technique has been extensively used for the conservation and propagation of medicinal plants which has been obtained in species such as *Gentiana* (Fiuk and Rybczynski 2008); *Glinus lotoides* (Teshome and Feyissa 2015); *Gymnema sylvestris* (Roy et al. 2008); *Ophiorrhiza japonica* (Kai et al. 2008); *Physalis peruviana* (Otroshy et al. 2013) etc. Successful application of plant tissue culture technique depends on a number of factors such as explants type, developmental age, plant growth regulators and genotype (Brown 1995). Regeneration from a number of explants have been achieved such as node (*Spilanthes acmella*, Singh et al. 2009), leaves (*Uraria picta*, Ahire et al. 2011); stem (*Cyclea peltata*, Bhagya and Chandershekar 2013); cotyledon (*Astragalus sinicus*, Cho et al. 1995), rachis (*Sutherlandia frutescens*, Dewir et al. 2010); petiole (*Anthurium andreanum*, Raad et al. 2012) etc.

Genotype of the plants also affects the regeneration potential of plants as was demonstrated in carnation cultures with cv. Coral, Jaaguar, Salome and Sarinah (Kallak et al 1997). In the *Gentiana* spp., the leaf explants of *G. kurro, G. cruciata, G. tibetica, G. lutea* and *G. pannonica* were cultured on BAP/TDZ and NAA. Optimum somatic embryo formation was achieved on *G.kurro* (Fiuk and Rybczynsk (2008)

2.1 PLANT REGENERATION STUDIES

The technique of plant tissue culture has been extensively employed in the regeneration of shoot and callus cultures

2.1.1 Role of plant growth regulators in regeneration of shoots

PGR's are a determining factor to induce morphogenesis in plants and the response varies according to the type and concentration of the PGR utilized. These PGR's are used singly or in synergistic combination. In *Anthemis xylopoda* (Erdag and Emek 2009) single concentration of BA was effective in inducing adventitious shoots and flower buds were

induced when the shoot was rooted in IBA. The studies also concluded that IBA was more effective in flowering as compared to IAA. Similarly the studies of Kasprzyk-Pawelec (2015) also indicated that single concentration of BA was sufficient to induce regeneration in *Citrus.* BA also proved to be effective in inducing regeneration on *Origanum sipyleum* (Oluk and Cakir 2000). The efficiency of BA to induce shoots as compared to Kin has been demonstrated in a number of studies such as *Crataeva magna* (Benniamin et al. 2004); *Leptadenia reticulata* (Rathore et al. 2013) and *Arnebia hispidissima* (Shekhawat and Shekhawat 2011).

Synergistic combination of PGRs had also induced regeneration within the explants. Combined effect of cytokinin BA and Kin has been reported to be effective in a number of explants like the studies of Sujatha and Kumar (2007) on *Carthamus* sp have reported maximum shoot formation in presence of BA and Kin from nodal explants. In *Passiflora foetida* (Shekhawat et al. 2015) the shoot buds originated on the medium fortified with BA and Kin with individual concentration but the multiplication of the shoots was achieved on a synergistic combination of BA and Kin. Sen et al. (2014) in *Achyranthes aspera* has also demonstrated that the synergistic combination of BA and Kin evoked optimum number of in vitro shoots.

Cytokinins in combination with auxins have also proved to be beneficial in regeneration of shoots. *Bacopa monnieri* is an important medicinal plants and its regeneration has been attempted by a number of workers. Ceasar et al. (2010) devised a two stage culture procedure for its regeneration in presence of BA. Similarly in *Plumbago zeylanica* (Lubaina and Murugan 2012) indirect organogenesis was achieved in presence of BA and NAA. *Swertia chirata* is an important medicinal plant and Indian Medicinal Plant Board has placed it as a highly prioritised plant (Chaudhuri et al. 2008). Direct organogenesis in this plant was achieved in presence of BA, Kin and NAA. Chen et al. (2014) in their studies on *Glossogyne tenuifolia* demonstrated that regeneration was better in the combination of BA and NAA as compared to Kin and NAA. In *Catharanthus roseus* (Verma and Mathur 2011) the leaf explants were incubated in BA and NAA resulting in 75% shoot bud differentiation. In *Anthmis nobilis* (Echeverrigaray 2010) adventitious shoot buds were induced in BA and NAA and their proliferation and multiplication was achieved on BA and IAA. The plants so formed depicted a high rate of uniformity with no somaclonal variations. Similarly in *Scoparia dulcis* (Sakthi and Mohan 2012) maximum number of shoots were produced in BA and IAA. Similarly Liberman et al. (2010) in their studies on *Brunfelsia calycina* also depicted maximum regeneration in presence of BA and IAA. *Coleus forskohlii* is a medicinal

endangered plant and its regeneration was obtained on BA followed by its multiplication on BA and IAA (Krishna et al. 2010).

2.1.2 Role of plant growth regulators in regeneration of callus

Besides organogenesis, callus formation is another response which is elicited from the explants. These callus cultures can be utilized to produce cell suspension or for producing metabolites of commercial interest (Sharmila et al. 2013). In *Achyranthes aspera* (Sen et al. 2014) internode, leaf and root were subjected to callus induction and plantlet formation. It was reported that maximum callus induction was obtained from leaf explants in presence of 2,4D and NAA and plantlets from this callus was obtained on a synergistic combination of BA and Kin. In another species the two cultivars of *Anthurium andreanum* Casino and Antadra were investigated for shoot induction utilizing petiole and lamina explants. It was observed that proliferation of shoot from callus was obtained in presence of BA and NAA combination and among the genotype Antadra gave better response as compared to Casino (Raad et al. 2012). In *Celosia argentea* (Abu Bakar 2014) greenish red callus induction was achieved on BA and NAA which subsequently gave rise to in vitro shoots in two combinations. The leaf explants of *Crepis novena* (Corral et al. 2011) in presence of BA and NAA induced callus which formed shoots. Similarly in *Ophiorrhiza japonica* (Kai et al. 2008) callus induction was achieved on either BA or NAA from leaf explants and the callus regenerated shoots in presence of BA and NAA. In *Glinus lotoides* (Teshome and Feyissa 2015) the leaf explants induced optimum callus in presence of BAP and 2,4 D while highest number of shoot and shoot length was obtained with BAP and Kin. The studies also suggested that 2,4D alone could not induce appreciable callus and it needed to be supplemented with a cytokinin.

Besides indirect organogenesis single callus mass has also been achieved in a number of species mostly under the influence of 2,4D. Tissue culture studies on *Allium ampeloprasum* (Monemi et al. 2014) revealed that, combination of BA+2,4D and Kin+2,4D both induced callus however optimum callus induction was achieved on BA and 2,4D. Contrarily to this study, Kin and 2,4D was optimum for callus induction in *Gymnema sylvestris* (Roy et al. 2008) and in three genotypes of *Gentiana* spp. (Kallak et al. 1997). In *Andrographis paniculata* (Sharmila et al. 2013) callus was induced in presence of cytokinin (BA, Kin and TDZ) and auxins (NAA, 2,4D, IAA, IBA) and good callus growth was observed in 2,4D, BAP+2,4D, NAA+2,4D and Kin+2,4D. The studies of Tamilselvan and Rajeswari (2014) in *Asystasia gangetica* elaborated on the morphology of the callus as they reported creamish

yellow friable profuse fast growing callus with low regenerative ability with cytokinin (BA/Kin) and NAA and sticky white fragile callus with 2,4D. A similar morphology of callus was reported in *Cyclea peltata* (Bhagya and Chandrashekar 2013) in presence of 2,4D alone or in combination with BA and Kin while with NAA/IAA alone or in combination with BA/Kin it was green and compact. Studies of *Uraria picta* (Ahire et al. 2011) indicated that auxins NAA and IAA formed callus interspersed with root.

Thus these studies indicated that PGR's are an important factor which determines the type of response elicited by the explants.

2.1.3 Elongation of the in vitro shoots

The explants in presence of cytokinin or a synergistic combination of cytokinin and auxin induce shoot buds which form cluster of shoots. In many experiments it has been observed that the shoots do not attain appreciable length and their elongation needs to be achieved by transferring them to a new medium fortified with other PGR's or additives. Usually the elongation of micro shoots is achieved in presence of GA_3.Studies of Geetha et al. (1998) in *Cajanas cajan* demonstrated that in vitro shoot induction was achieved in presence of BAP. The shoot cultures when transferred on BA and NAA medium multiplied the shoots and still further elongation was observed in presence of GA_3. Golegaokar and Kantharajah (2006) in the studies on *Capsicum annuum* indicated that shoot elongation was obtained on BAP and GA_3 while Siddique and Anis (2006) achieved elongation of this plant on hormone free media. Saini and Gill (2010) in *Citrus jambhiri* have reported the effectiveness of BA and GA_3 in shoot elongation. Besides BAP and GA_3, individual concentration of GA_3 has also proven to be effective in facilitating elongation of the shoots. Individually GA_3 elongated shoots in *Pothomorphe umbellate* (Pereira et al. 2000) and *Andrographis neesiana* (Karuppusamy and Kalimuthu 2010). Synergistic effect of cytokinin and auxin also elongated the shoots as was observed in *Clitoria ternatea* (Anand et al. 2011) in presence of BA and IAA. Similarly coconut water has also proved to be effective in elongation of shoots as in *Asparagus racemosus* (Thakur et al. 2014) and *Ananas comosus* where elongation of shoots was achieved in 15% coconut water (Akbar et al. 2003). Concentration of sucrose is yet another factor which can affect the elongation of shoots e.g. elongation of shoots from flax anther culture was obtained on increase level of sucrose concentration (Chen et al. 2002). Similarly half strength MS medium without plant growth regulators was suitable for elongation in *Solanum donianum* (O'Connor-Sánchez 2009).

2.1.4 Rooting of in vitro shoots

The in vitro shoots formed are subjected to rooting in presence of auxins such as IBA, IAA and NAA of which IBA is most frequently utilized. Individual concentration of IBA induced rooting in half strength media in a number of plants like *Plumbago zeylanica* (Lubaina and Murugan 2012); *Catharanthus roseus* (Verma and Mathur 2011); *Celosia argentea* (Abu Bakar et al. 2014); *Anthemis nobilis* (Echeverrigaray et al. 2010);*Eryngium foetidum* (Arockiasamy et al. 2002);*Achyranthes aspera* (Sen et al. 2014) and *Ophiorrhiza japonica* (Kai et al. 2008). Rooting from full strength MS medium along with IBA has also been reported such as in *Spilanthes acmella* (Pandey and Agarwal 2009) *Physalis peruviana* (Otroshy et al. 2013). Similarly IBA in combination with PGR's or additives have also reported to be effective like rooting was obtained in IBA and Kin in *Anthurium andreanum* (Raad et al. 2012) and in *Bacopa monnieri* (Ceasar et al. 2010) in presence of IBA and Phloroglucinol.

Thus the review clearly highlights the important work done in the regeneration of the explants to in vitro shoots.

2.2 METABOLITE STUDY

2.2.1 Metabolites from in vivo plants and in vitro cultures

Plants produce chemicals which are categorized as primary or secondary metabolites. Primary metabolites are widely distributed and are found in seeds and vegetative organs because they are generally utilized in basic cell metabolism. Such metabolites include vegetable oil, fatty acids and carbohydrate. They are pollinator attractants, represent chemical adaptations to environmental stresses, or serve as chemical defences against microorganisms, insects and higher predators, and even other plants (Balandrin and Klocke 1988).

Andrographolide has diverse pharmacological properties and is obtained from leaves of *Andrographis paniculata*. In this plant the callus was induced in presence of cytokinin and auxins and andrographolide content was evaluated in different combinations of callus cultures(Sharmila et al. 2013). Plumbagin content was evaluated in *Plumbago zeylanica* (Lubaina and Murugan2012) and it was concluded that the content was effected by the PGR's in the medium as maximum content of plumbagin was reported in BA+2,4D followed by Kin +2,4D and then in vivo plants. In *Pothomorphe umbellate* (Pereira et al. 2000) micropropogated plants produced 4-nerolidylcatechol. The yield of this catechol was 26.1 mg/g dry weight while in vivo plants accumulated 32.2 mg/g dry weight.

2.2.2 Fatty acid profile in plants and in vitro cultures

Fatty acids are primary metabolites and their content has been evaluated in a number of plants. In *Linum usitatissimum* (Friedt et al. 1995) the fat content and Linolenic acid content of the plants was determined in haploid plants after anther culture. Results indicated the development of plants where there was an increase in fat content by 1.9% and Linolenic acid by 4.5%. The essential oils were estimated in the plant and callus cultures of *Allium ampeloprasum* (Monemi et al. 2014) where the studies suggested that the essential oil constituents were affected by the culture medium composition. Synthesis of fatty acid was studied in vitro by (Lem and Stumpf 1984) in *Anabaena variablis* and it was concluded that fatty acid synthesis was dependent on acyl carrier proteins and required NADPH and NADP.

PGR's have the potential to modify the fatty acid content in plants and it was validated by several reports. The studies on *Jatropha curcas* (Hernandez et al. 2015) suggested that PGRs modify the fatty acid profile in callus cultures and BAP induced a higher content of Linoleic and Linolenic acid. Similarly in *Simmondsa chinensis* (Aly et al. 2008) the PGR's affected the fatty acid synthesis of somatic embryos. The seeds of this plant have long chain fatty acids such as Behenic acid while the leaf derived somatic embryos reported abundant Linoleic acid, Linolenic acid, Palmitic acid and Oleic acid.

The type of explant also influences the fatty acid profile which was elaborated by the studies of Haldar and Gadgill (1984).They induced callus from root, hypocotyls, cotyledon, stem and leaf explants of *Cucumis melo* and the fatty acid content of different calli from each explants was evaluated. It was concluded that the callus from root, stem and leaves were rich in Linolenic acid while cotyledon has Linoleic acid as the most predominant acid. Studies on 6 species of Cucurbitaceae suggested that the callus cultures induced from cotyledon were rich in Linolenic acid as compared to Linoleic acid. Further in *Momordica charantia* Oleic acid was the major unsaturated fatty acid Haldar and Gadgill (1983).

Callus has been frequently studied for fatty acid content. In *Ibervillea sonorae* (Estrade-Zuniga et al. 2012) the callus was induced in presence of Kin and 2,4D and the fatty acid content was evaluated in the callus. It was revealed that there was a direct relationship between fatty acid content of callus and growth index which was significantly higher than the wild tuber root. The evaluation of fatty acid in callus cultures of *Cereus peruviana* (Machado et al. 2006) showed that Linoleic acid was the major fatty acid present in the callus and unusual fatty acids such as Pentadecenoic acid, Palmitolenic acid and Heptadecanoic acid were also detected. In Parsley plant (*Petroselinum crispum*, Lopez 1999) Palmitic acid,

Stearic acid was the dominant fatty acids in callus cultures and cell suspension cultures. In *Wedelia prostrate* (Abd El-Mawla 2011) methyl esters of fatty acids such as methyl stearate and methyl palmitate were detected from callus cultures. In cell suspension cultures of *Lesquerella fendleri* (Kharenko et al. 2011) abscisic acid was responsible for accumulation of very long chain fatty acids.

Fatty acids from in vitro shoot cultures was reported in *Ajuga multiflora* (Sivanesan et al. 2016) where Linolenic acid was the major fatty acid followed by Linoleic acid and Palmitic acid. Somatic hybrids were also evaluated for fatty acid content e.g. somatic hybrid between *Brassica napus* and *Camelina sativa* (Jiang et al. 2009) depicted high Linolenic acid content.

2.2.2.1 *P oleracea*- a sourceof Fatty acid

P oleracea is a leafy vegetable which is rich in omega fatty acids and it has been evaluated for is fatty acid content under different parameters. In this plant the fatty acid content in three stages of harvest (6, 10 and 14 true leaf stage) was examined by Palaniswamy et al. (2009). The 14 leaf stage was ideal as at this level there was maximum vegetative growth and PUFA content was also highest. Similarly the influence of planting date on chemical composition of *Portulaca* accessions was determined by Ezekwe et al. (1999) They reported that total lipid varied from 4- 5.8% and 3.7- 5.15% in first and second planting dates and fatty acid content with respect to Linoleic and Linolenic acid was highest in Dutch garden accession as compared to other varieties. Literature survey did not reveal the content of fatty acids in callus cultures and in vitro plants therefore the present study aims to determine its content within in vitro cultures.

2.2.3 Betalains are a source of food colour

Betalains are natural colorant which is finding increased importance in food industry. Several studies have been done to increase the yield of this pigment such as aqueous two phase system was employed in beetroot for the removal of sugars which are responsible for its degradation and separation of polymers which increased its yield by 2.4 fold (Chethana et al 2007) In *Gomphrena globosa* and *Bougainvillea* sp the betalain content was evaluated (Kuglar et al 2007). The studies reported the presence of Histidine bx and a novel arginine bx- the betaxanthin in *Gomphrena globosa* while in *Bougainvillea* Dopa bx betaxanthin was reported and remarkable difference in betacyanin pattern of both the species was obtained. Comprehensive studies of Cai et al. (2005) on Amaranthaceae species outlined the importance of selection of betalain producing genotype and species, the method of extraction,

quantification of betalains and their antioxidant activities. The antioxidant potential of betalains was studied by Swarna et al. (2013) in *Talinum triangulare.*

In vitro cultures have also been evaluated for the betalain content. Betalain content was increased in *Beta vulgaris* L, var. 'Dark Detroit' callus cultures by 1.8 fold in 48 subcultures(Trejo- Tapia2008). Betalains were also quantified in callus cultures induced from stem explants of *Zaleya decandra* (Radfar et al. 2012) in presence of TDZ and 2,4D.

2.3 MUTATION STUDIES

Mutations are extensively employed for the generation of variability within plants.

2.3.1 Mutation in plants

Conventional mutation technique is used to improve yield quality, pest resistence in crops and to increase the attractiveness of ornamental plants (Maluszynski et al 1995). Mba et al. (2010) have opined that mutations are the most important factor in the process of evolution as the changes are passed to the offspring's. The technique has found extensive application in crop improvement such as in Kodomillet (Jency et al. 2017), *Cicer arietinum* (Kharakwal 2000); *Psophocarpus tetragonolobus* (Klu et al. 1997); *Brassica napus* (Rahimi and Bahrani 2011); sugarcane (Nikam et al. 2015) and in floriculture such as *Chrysanthemum* (Lee et al. 2008; Misra and Dutta 2007), *Anthurium* (Puchooa 2005)

The genetic variability in plants has been induced both in presence of physical mutagen and chemical mutagen.

2.3.2 Role of Physical mutagens in inducing mutagenesis within in vitro cultures

Physical mutagens are generally electromagnetic radiations such as gamma rays, X-rays and UV light, which are frequently used to induce mutations. In vitro mutagenesis has been employed with several explants to induce mutation via gamma irradiations and favourable traits have been achieved in plants e.g. streptomycin resistant mutant of *Solanum surattense* from cotyledon explants (Rama Swamy 2005 et al) and herbicide amitrole resistance in *Digitalis obscura* (Gavidia and Perez-Bermudez 1999). Shoot regeneration and mutation induction was achieved in *Etlingera elatior* (Jack) by gamma irradiation (Yunus et al. 2013) and protocorm like bodies and shoots were induced in *Dendrobium* cv. Sonia following gamma irradiation (Sheela et al. 2006). In vitro mutagenesis via gamma irradiation is also useful in developing new varieties and cultivars such as two new varieties of *Cryptocoryne willisii* (2011) were developed from shoot tip explants and new *Chrysanthemum* cultivars

(Albugo Sunny, Alchimist Tubular, Alchimist Golden Beet, Satinbleu Minty, Satinbleu Honey) were developed from *Chrysantheum×grandiflorum* (Ramat.) by in vitro mutagenesis on node and leaf explants (Zalewska et al. 2011).

The effect of gamma radiations has been reported to be both negative and positive to growth and development. Depletion in development of the cultures has been observed as somatic embryo development of Avocado cultures was inhibited in presence of gamma irradiation (Witjaksono and Litz 2004). Nodal explants of *Artemisia annua* (Inthima 2014) when irradiated with gamma rays depicted a decrease in survival % when irradiated for longer durations. Mutants such as plants with increased height and variation in leaf morphology were also obtained.

Besides the gamma irradiations X-rays have also been effective in inducing in vitro mutation such as *Rosa hydrida* (Ibrahim et al. 1998) and *Nelumbo nucifera* (Arunyanart and Soontronyatara 2002).

2.3.3 Role of chemical mutagens in inducing in vitro mutagenesis

Chemical mutagens include alkylating agents such as (EMS), Methyl methane sulphonate (MMS) Sodium azide, intercalating agents (ethidium bromide) and base analogue such as bromouracil (Mba et al. 2010). Alkylating agents are most commonly used to induce mutation in plant species such as EMS and MMS.

Although EMS is commonly used, successful in vitro mutagenesis induction has also been reported with other chemical mutagen as well. Vagera et al. (2004) demonstrated enhanced frequency of pollen embryogenesis in Barley cv. of Heris, Tolar, Granat and Novam under the influence of chemical mutagens N-nitroso-n-methylurea (MNU/MNH), N-nitroso-n ethylurea (ENU) Sodium azide (NaN_3) and EMS. The highest frequency of chimera plants was obtained at 0.4 mM ENU and lowest at 0.1 mM MNU. In vitro mutagenesis in *Gossypium hirsutum* (Muthusamy and Jayabalan 2011) was initiated in ovule culture in presence of Sodium azide, EMS and gamma rays where Sodium azide increased the percent response and induced a number of chlorophyll variants. The leaf explants of *Saintpaulia ionantha* (Gaj and Gaj 1996) were treated with MNH where in vitro variegated shoots with 100% survival rate were obtained at 5mM MNH. This study indicated that the chemical mutagen MNH has a high potential to induce variants both in leaf and flower with respect to shape and colour.

2.3.4 Role of EMS in tissue culture

EMS is the most commonly used chemical mutagen for inducing genetic variability in plants. This mutagen has been used in combination with other chemical mutagen as well as physical mutagen to induce variability.

In *Bacopa monnieri* (Naik et al. 2012) it was observed that single treatment of EMS or gamma rays to the leaf explants resulted in reduction of survival rate, number of adventitious shoots and their fresh and dry weight. The epicotyls of *Citrus jambhiri* (Sharma et al. 2013) were treated with mutagen EMS and MMS and it was reported that EMS was highly effective in reducing the number of days for regeneration as in EMS treated populations 50% regeneration was achieved in 60 days while in control 50% regeneration was obtained in 90 days. Morphological traits were studied in two varieties of *Solanum viarum* (Maruthi Kumar and Tejavathi 2011) in presence of EMS and gamma rays separately. A number of variations for plant height, plant spread, number of flowers and fruits per node and fresh fruit weight were obtained in presence of the mutagen EMS. Qualitative variations such as less spine plant, dwarf plant, sterile plant and pollen sterility were also observed. Similarly in vitro buds of Strawberry var. Akihimi (Murti et al. 2013) were treated with EMS and gamma rays and a decline in survival percent was noticed. A number of variants such as white streaked leaf, bigger petiole with distorted leaves, dwarf, variegated, heart shaped bright red fruit were obtained on EMS treatment.

EMS was also reported to give better results as compared to gamma rays. In tomato plant (Ahmed et al. 2017) it was observed that EMS gave better results as compared to physical mutagen UV light. EMS accelerated the plant growth with the formation of pale green leaves. *Bryonopsis laciniosa* (Caroline and Mallaiah 2011) is an endangered medicinal cucurbit. In this plant leaf, stem, node and cotyledon were treated with a physical mutagen gamma rays and EMS. It was noted that EMS was most effective in inducing direct shoot regeneration from the nodal explant.

Within different chemical mutagens EMS again depicted a better response which was illustrated by the studies of Agarwal et al. (2015). They studied the effect of three mutagens EMS, MMS and sodium azide on *Trigonella foenum-graecum* and *Trigonella corniculata*. An increase in growth index of the plant in presence of low concentration of all the three mutagens was observed with maximum enhancement at 0.2 M EMS.

A number of studies have indicated that EMS induced variability on callus as well as plants regenerated from it. Lee and Lee (2002) treated anthers of rice with EMS and the frequency of callus induction was highest in 0.5% EMS but the frequency of regeneration of in vitro

shoots was decreased in presence of EMS. A total of 14 mutants were obtained which were semi dwarf, grain shape and glabrous. The callus of Sugarcane (Mahmud et al. 2016) was treated with 0.1-0.3M EMS to evaluate the regeneration potential. The number of shoots regenerated after the treatment decreased as compared to control. Several mutant plants were obtained which showed variation in tiller number, internode, green leaf, millable cane and stalk color. Sadat and Hoveize (2012) induced mutations in callus cultures of two commercial varieties of sugarcane CP48-103 and CP57-614 with EMS. Variations in the callus were obtained which was confirmed with RAPD analysis. Maximum callus growth was obtained in *Cymopsis tetragonoloba* (Gulati et al. 2016) when the callus cultures were treated with 0.5% EMS at different time durations. Elhiti et al. (2016) treated the in vitro formed shoot tips of Peach (*Prunus persica*) with EMS and reported 0.2% EMS concentration to be optimum for generating variations in the tissues and the rate of mutation was reported to be directly proportional to the concentration of EMS.

All these reports suggested that EMS is effective in inducing variations within the callus and rate of mutation was also high.

Soil salinity is a serious problem which hinders the agricultural practices of many crops. EMS was utilized to develop salt tolerant mutants to increase the productivity of the crop. Salt tolerant mutants of sweet potato, *Ipomoea batata* L.(Luan et al. 2007) was developed using 0.5% EMS for 2 hours from somatic embryo obtained from calli. Strong resistance to salt stress was noted by Lu and Jia (1994) in millet where the plants regenerated from embryonic calli in 0.5% EMS and were tolerant to NaCl. Similarly Nobers et al. (1975) selected NaCl resistant cell lines from *Nicotiana tabacum* L. cell suspension cultures treated with EMS. Callus cultures of *Cymopsis tetragonoloba* (Gulati et al. 2016) was treated with 0.5% EMS at different time durations and it was revealed that EMS treated callus had a better tolerance to salt stress. Krupa-Malkiewiczi et al. (2017) studied the salt resistance pattern in callus culture of Petunia treated with EMS. The plants regenerated from such calluses have a higher tolerance to salt stress. Thus EMS can also be utilized to develop salt tolerant varieties in crop plants.

For commercial floriculture, development of new and improved varieties with respect to colour and shape of the flower is essential to meet the customers demand. In this respect EMS has been frequently used to introduce new traits within the plants. Mutations in Saintpaulia cv. Crystobal was initiated on leaf explants in presence of 0.1-0.6% EMS. A total of 10 mutants were recovered with variegated leaf chimeras and variation in flower colour and fringes. Rodrigo et al. (2004) obtained *Chrysanthemum* mutants with different petal

colour (pink, light pink, bronze, white, yellow and salmon) from immature floral pedicel. Similarly 7 leaf mutant and 13 flower mutant were obtained in *Asteracantha longifolia* (Bahera et al. 2012) when the leaf explants was treated with 25-200 mM of EMS. In *Tillandsia fasciculate* (Koh and Davies Jr 2001) leaf variegation an ornamental trait was induced in presence of EMS.

2.3.5 Effect on metabolite content by in vitro mutagenesis

Plants are a source of both primary and secondary metabolites; however their content is generally low in plants. A recent trend is to increase the content of these metabolites by in vitro mutations. Several papers have been published which report the increase of these metabolites by the use of physical and chemical mutagen.

Phytosterol is associated with gall stone dissolution and its content was evaluated in mutants developed from leaf explants of *Asteracantha longifolia* (Bahera et al. 2012) treated with EMS. The Phytosterol content ranged from 0.033- 0.0467 mg/g in the mutated population. Estimation of Bacoside A content in *Bacopa monnieri* (Naik et al. 2012) from the plant regenerated after gamma and EMS treatment revealed that the cell lines obtained from gamma treatment had a higher content of Bacoside A while the EMS treated leaf explant did not show any significant rise in Bacoside A content.

Besides the chemical mutagens metabolite content is also affected by the physical radiations such as gamma rays and X-rays. In *Wasabia japonica* (Hung and Johnson 2008) the use of X-rays and gamma rays on shoot tip explants altered the allyl isothiocyanate content. As compared to control there was a significant rise in allyl thiocynate content in gamma and X-rays treated shoot cultures.

A mutant obtained from the synergistic effect of a gamma radiations and EMS depicted higher thebaine content in *Papaver somniferum* (Chatterjee et al. 2010). The studies also depicted that the type of biomass also plays an important role in the content of secondary metabolite as it was observed that higher thebaine content was observed in stem followed by leaf callus, stem callus and cotyledons. In *Digitalis obscura* (Gavidia and Perez-Bermudez 1999) it was noted that the mutants developed after gamma irradiation had a similar or even higher content of cardenolide (878-3291 µg/g dry weight). *Artemisia annua* is a medicinal plant used for commercially producing Artemisinin an important antimalarial compound. The content of Artemisinin in the plant was increased by the ^{12}C beam irradiation on the nodal explants. Mutants were obtained where the Artemisinin content was 3.2 times higher than the control (Inthima et al. 2014). Similarly tannin content was found to be altered in mutants

(both increase and decrease in content) when winged bean *Psophocarpus tetragonolobus* (Klu 1997) was treated with Co^{60} gamma source.

The effect of EMS has been evaluated in several reports. In vitro studies of Agarwal et al. (2015) on *Trigonella foenum-graecum* and *Trigonella corniculata* utilized three chemical mutagen, EMS, MMS and sodium azide to study their effect on steroidal sapogenins production. Sapogenins was depicted to increase in the presence of all the three mutagen however maximum effect was obtained at 0.1 M EMS. Kulkarni et al. (1999) isolated two semi dwarf and dwarf mutants in *Catharanthus roseus* var. Nirmal which had high alkaloid content in the roots and leaves. Das et al. (2010) treated two varieties of *Withania somnifera* Poshita and Jawahar 22 with different concentrations of EMS. Several mutants were obtained which reported a high content of alkaloids withanolides, withaferin A and withanolide A and high fibre content in the roots.

2.3.6 Effect on fatty acid content

Specifically fatty acid content have been altered in a number of studies by utilizing the mutagens. The Tamla variety of *Brassica napus* (Lee et al. 2014) was treated with 1% EMS to induce mutation in fatty acid biosynthetic pathway. Mutants were obtained where Oleic acid content ranged from 13.5-76.9%. The accumulation or reduction of Oleic acid was simultaneously accompanied with increase or decrease in Linoleic and Linolenic acid.

Rowland (1991) isolated a low Linolenic acid mutant in McGregor flax (*Linum usitatissimum*) which was controlled by recessive allele at two independent loci caused by a rare double mutation. Similarly Rowland et al (1995) utilized EMS to induce variation in fatty acid content of *Linum usitatissinum* var. Mc Gregor. Two independent dominant genes were identified which reduced the Linolenic content from 50-2% and increased the Linolenic content to 70%. Another gene was identified which increase the Palmitic acid content from 7-30% and Palmitolic acid to 4%. In (1997) Ntiamoah and Rowland isolated two low Linolenic acid McGregor mutant flax variety (*Linum usitatissinum*).

Mutations were induced in *Sesamum indicum* L (Savant and Kothikar 2011) in presence of EMS and Sodium azide and variation in oil content and fatty acid composition was noticed. Mutants were obtained which had high content of Oleic acid, enhanced seed oil content, higher saturated fatty acid content and low PUFA content. The fatty acid content of *Glycine max* cv. Century (Wilcox et al. 1984) was studied after the seeds were treated with EMS. There was an increase in fatty acid variability after EMS treatment as compared to control. A mutant with 3.4% Linolenic acid content was also isolated in the study.

In *Brassica carinata* the microspores were treated with EMS and a mutant was developed which had high erucic acid content. In these haploid plants the level of erucic acid was greater than 52%.

2.3.7 Mutation induction in *Portulaca*

The earliest record of mutation in *Portulaca* was described by Blakeslee (1920). He was the first to observe spontaneous mutation in *P grandiflora* which resulted in a dwarf plant with small pin cushion like growth pattern. Faberge and Beale (1942) studied the effect of temperature on mutation rate in *P grandiflora.* They reported reduced mutation rate with increase in temperature.

Gupta (1970) induced *P grandiflora* varieties with gamma radiations and reports a number of mutants in flower colour, shape and in number, shape and size of stamens and carpels. Meiotic behaviour and pollen fertility of these mutants were studied by Lata and Gupta (1971) which also revealed variations at the cytological level.

Raghuvanshi and Singh (1979) studied the mutation frequency in diploid and autotetraploid *P grandiflora* by irradiating them with 4 and 6 Krad gamma rays. They reported a number of variations in leaves and flower.

Wongpiyasatid and Hormchan (2000) irradiated the stem cuttings of two *P grandiflora* varieties “Double orange” and “Double Pink” with gamma irradiations. They obtained variations in phenotypic characters such as flower colour, form and size and were successful in establishing three mutant varieties of *P grandiflora*- “Chompoo Praparat”, “Pattik” and “Som Arunee”. Thus it was deduced that mutation in the plant *Portulaca* was induced for ornamental purpose and the metabolite content has largely been overlooked as no reports are available where the metabolite content of the plant has been evaluated in presence of mutagen. Thus the present investigation aims to fill in the gap in this area of research.