REVIEW OF LITERATURE

The literature review was carried out related to the topic of research, in terms of the importance of legumes and groundnut in particular, the significance of mutation and other related aspects like, induced mutation, plant tissue culture, *in vitro* mutagenic studies in higher plants, role of biochemical constituents like, proteins, amino acids and isoenzymes with *in vitro* culture were critically examined and presented in the form of review of literature.

Mandal and Mandalm, (2000) reported that major part of the human diet all over world consists of cereals and legumes. Boudoin and Maquet, (1999) viewed that legumes are considered as the major source of protein and dietary amino acids for man and farm animals. FAO/IAEA, (1970) reported 70% of human food comprises cereals and legumes and the remaining 30% comes from animals. Bolbhat, (2011) reported that pulses are basic ingredient in the diet of a vast majority of Indian populations as they provide a perfect mix of high biological value, when supplemented with cereals.

Bouchenak and Lamri-Senhadji, (2013) reported that legumes are economically cheaper and a vital source of nutrients such as protein, dietary, fiber, carbohydrates, minerals and vitamins which represent a significant food components of the human diet in various areas of the world particularly in the developing countries.

Krapovickas and Gregory, (1994) reported that the genus *Arachis* is exclusively of South American origin with about 69 described species. Kocher, (1996), noted that the current commercial peanut cultivars are allotetraploids, apparently derived from a single hybridization event between diploid peanut, *A. hypogaea* and *A. ipaensis*. Giller *et al*., (2002) and Smatt, (1994) suggested that groundnut improves soil fertility by fixing nitrogen and increases productivity for smallholder farmers of the semi-arid cereal cropping system.

Smatt, (1994) explained that groundnut is an important food security crop for household consumption and also a source of family cash income. This is due to its

adaptability to adverse environmental conditions such as drought, ability to grow well in marginal soils and relatively short growing season that allows it to be harvested throughout the year. In addition, as a leguminous plant the harvested leaves and stems can be incorporated into the soil as low input fertilizer.

Naidu *et al*., (1999) showed that groundnut (*Arachis hypogea* L.) is an important oil, food and fodder crop. It plays an important role in the agricultural economies of countries of the semi-arid tropics. It contributes significantly to food security and alleviates poverty and as a legume, improves soil fertility by fixing nitrogen and increases productivity simultaneously. FAO, (2011) reported that groundnut is grown on 26.4 million hectare worldwide, with a total production of 37.1 million metric tons and average productivity of 1.4 metric tons/ha. Developing countries constitute 97% of the global area and 94 % of the global production of this crop.

Singh and Singh, (1992) proposed that the domesticated groundnut (*A. hypogaea*) is currently the fourth most important oilseed worldwide and is considered a key crop in subsistence agriculture in several countries due to the high oil and protein content of its seeds. Mukhtar , (2009) noted that groundnut (*Arachis hypogaea* L.) is the 6^{th} most important oil seed crop in the world . It contains 48-50% oil, 26-28% protein and 11-27% carbohydrate, minerals and vitamins.

Ramawat *et al*., (1997); Mukhopathyay and Bhojwani, (1978); Gosal and Bajaj, (1979); Bharal and Rashid, (1990) reported the legumes have high nutritional value hence they have been subjected to many studies for potential improvement through cell and tissue culture. Jacobsen, (1991); Mohamed *et al*., (1996) ; Zhang *et al*., (1997) reported regeneration ability depends on the genotype, physiological state of the explants, tissue and cell specialization of the culture and the cultivation conditions.

Sugiyama, (1999) suggested that the regeneration of whole organisms depends upon the totipotency of plant cells, in that all plant cells can express the total genetic potential of the parent plant given the correct stimuli. Chaleff and Parsons, (1978)

reported that tissue and cell culture techniques are being increasingly exploited in the selection for agronomically useful variants and in varying disease free lines.

Nakamura and Maeda, (1989) viewed that plant biotechnology, a modern technique is mainly based on plant cell culture. Regeneration of plants from cells or tissues is an important and essential component of biotechnology, which is required for the genetic manipulation of plants. Plant cell culture has become an excellent method for plant cell differentiation as well as a supplementary technique for plant breeding programs through the uses of new and expanded genetic variability.

Rey *et al*., (2000) reported that groundnut has narrow germplasm base without satisfactory resistant to major pathogens and viruses. The development of suitable protocol for genetic improvement of the plant using biotechnological methods is the pre-requisite.

Evans *et al*., (1984) explained that progress in techniques for plant cell, tissue, and organ culture makes it possible to introduce genetic variability and to more easily select for desirable traits. Skoog and Miller, (1957) reported the essentiality of plant growth regulator concentrations in culture media. Gagliardi *et al*., (2000) noted that the standardization of *in vitro* plant regeneration protocols with intervening callus phase would certainly help in the mass scale propagation of the wild species and also facilitate germplasm conservation *in vitro*.

Nickell and Heinz, (1993); Engler and Grogan, (1982) suggested that tissue culture techniques may be utilized conveniently to overcome incompatibility barrier through fusion of vegetative cells of interspecific, intraspecific, intergeneric and interfamilial group. Scowcroft *et al*., (1987) focused on tissue culture techniques can play a significant role for the enrichment of genetic variability giving rise to variations/mutations at an unexpectedly high rate and may be a novel source of genetic variability in many plant species.

Ahloowalia, (1998) opined that the rapid development of biotechnology in recent years accelerated the studies on development of modernized improvement

methods and increased the genetic variability. Anuradha *et al.,* (2006). Opined that legumes are the most important group of crop plants next to cereals and they are very recalcitrant to tissue culture regeneration. Therefore, many efforts have been devoted to develop efficient *in vitro* regeneration system.

Parrott *et al*., (1992) described that the legumes the most important group of plants, are difficult to regenerate from tissue cultures. In order to facilitate development of tissue culture based crop improvement for these species. Considerable effort has been devoted in developing and optimizing *in vitro* protocols.

Iqbal *et al*., (2011) stated that the groundnut regeneration is genotype dependent and therefore optimization of an efficient *in vitro* regeneration protocol for specific genotypes is imperative to their micropropagation and transformation. Heatley and Smith, (1996); Ponsamuel *et al*., (1998) observed that groundnut has proven to be difficult crop to manipulate in *in vitro* and only a limited success of whole plant regeneration has been achieved in some cultivars.

Vajranabhaiah *et al*., (1993) ; Venkatachalam *et al*., (1994); Venkatachalam *et al*.,(1998) plantlet regeneration has been achieved both seed and seedling explants via organogenesisin groundnut. Mckently *et al*.,(1990) reported that groundnut plantlets have been directly regenerated using de-embryonated cotyledons.

Cheng *et al*., (1992) viewed that *Arachis hypogaea* is recalcitrant by tissue culture. Mroginski *et al*., (1981); Atreya *et al*., (1984); Diamon and Mii, (1991); Vajranabhaiah *et al*., (1993); Palanivel *et al*.,(2002) successfully achieved de novo organogenesis from seed and seedling explants. Pierik, (1987) reported that micropropagation is suitable method for obtaining a large quantity of genetically homogenous and healthy plant material which can be used for planting.

Arcioni *et al*., (2001) reported that the tissue culture protocol can be exploited for generating new genetic variability in groundnut by somatic hybridization through protoplast fusion. Mroginski *et al*., (1981) achieved organogenesis in immature

leaflets collected from young seedlings of groundnut. Cheng *et al*., (1992) reported bud primordial development that failed to regenerate normal plants.

Radhakrishnan, (1996); Pawar *et al*., (2012) reported that differences in regeneration frequencies among different explants are due to their difference in the physiological state, endogenous level growth regulators and /or in their response towards growth regulators. Banerjee *et al*., (1988) reported that BAP and NAA combination was very effective in inducing multiple shoots from cotyledonary explants of groundnut. Multiple shoot formation by application of cytokinins have been reported in some other legumes such as *Vigna radiata* Mathews, (1987), *Phaseolus vulgaris* Franklin *et al*., (1991)

Palanivel and Jayabalan, (2000, 2002); Palanivel *et al.,* (2001); Palanivel *et al.,* (2002) reported both direct and indirect organogenesis in two important groundnut cultivars such as VRI-2 and VRI-3. Mrogenski *et al*., (1981); Kartha *et al*., (1981) have studied regeneration potentiality of different explants of peanut.

McKently *et al*., (1995) emphasized the use of zygotic embryo axes of explants but cotyledons have several comparative benefits like robustness, time saving and cost effectiveness. Venkatachalam *et al*., (2000) also used cotyledons as an explants to produce fertile plants via embryogenesis in groundnut.

Reinert and Bajaj, (1976) reported the callus culture is one of the most important plant science techniques for developing clonal populations, plant regeneration and genetic manipulation in both monocotyledons and dicotyledons. Kaeppler *et al*., (2000) reported that the somaclonal variation caused by the process of tissue culture induced variation to more specially define the inducing environment.

Kanyand *et al.,* (1994) review that induction of multiple shoots from various parts of the Valencia type peanut seedling in different concentration of TDZ and observed that hypocotyl and cotyledon explants produced higher number of shoots in the medium containing 30 mg/l TDZ. Those shoots rooted on the MS basal medium and gave rise to plantlets.

Li *et al.,* (1994) demonstrated the ability of TDZ in induction of adventitious shoots from hypocotyl region of cultured seed explants of peanut. An exposure of one week in 10 μM TDZ was sufficient to stimulate initiation of adventitious shoots that subsequently developed into normal and fertile plants. Hoque *et al*., (1992) proposed that the best callus growth was observed for groundnut when supplemented with 2,4-D (2mg/l) and kinetin (0.5mg/l).

Victor *et al*., (1999); Little *et al*., (2000) noticed that plant regeneration until the recent cultivated and in wild groundnut has been achieved either directly via organogenesis or indirectly through somatic embryogenesis. Jain, (2001), explained that induced somaclonal variation can be used for genetic manipulation of crop with polygenic traits. Biswas *et al*., (2009) noted the new varieties derived from *in vitro* tissue culture could exhibit disease resistance and improvement in quality as well as quantity.

Narasimhulu and Reddy, (1983) reported the callus mediated plant development is only 19 % of the explants in groundnut. Akasaka *et al*., (2000) reported various abnormalities in shoot development and low conversion rate (34.7%) from shoot buds to shoots. Orton, (1985) and Evans, (1989) achieved that plant tissue culture has been proposed alternatively as a tool for propagation of useful genotype.

Chengalrayan *et al*., (1995) reported that leaflet explants derived from mature embryos of peanut induced caulogenic buds in the combination of NAA and BAP showed active cell divisions at the leaf base of peanut.

Popescu *et al*., (1997); Letham, (1974) reported that successful callus culture depends on the type of plant growth regulators. Akiyoshi *et al*., (1983) reported the cytokinins and auxins are known to promote callus formation in tissue culture. Dietz *et al*., (1990) reported a natural auxin of higher plants involved in regulating cell elongation, cell division and differentiation. Rayle *et al*., (1982) suggested that cytokinin can promote cell elongation in certain tissue. Rani and Reddy, (1996) who

obtained higher percentage of embryogenic callus from cotyledons and seedling explants of groundnut.

Mckently *et al*., (1991) and Pacheco *et al*., (2008a) reported the occurrence of indirect organogenesis from leaflets of *A. glabrata* and *A. stenosperma*, respectively, in the combinations of NAA and BAP. On the other hand, when NAA was used as the sole of growth regulators, leaflets of *A. stenosperma* showed direct organogenesis, with subsequent shoot formation from adventitious roots, thus resulting in an additional multiplication system that can be used for conservation Pacheco *et al*., (2008a).

Fratini and Ruiz, (2003) reported that the recalcitrant nature of legumes towards rooting has slowed down the application of biotechnological tools in legume crops. Mroginski *et al*., (1990) reported that IBA is a potent auxin to induce rooting.

Gagliardi *et al*., (2000) noted that NAA has been shown to be effective in rooting of wild *Arachis species*. Venkatachalam *et al*., (1996) reported higher concentration of NAA produced callusing at the cut end of shoots kept for rooting in contrast. Asylin *et al*., (2005), reported that Virginia type peanut shoots have a natural capacity to form adventitious roots in medium without plant growth regulators.

Bhanumathi *et al.*, (2005) suggested that genetic improvement of crop plants using biotechnological methods required development of suitable protocols. Plants regenerated through somatic embryogenesis are more useful than plants generated through organogenesis.

Williams and Maheswaran, (1986); Tautorus *et al*., (1991) stated that somatic embryogeneesis has been described for more than one hundred species and in most of them the presence of auxins in the culture medium, especially the synthetic auxin 2,4-D, is the factor that determines the induction of embryo functions. Filippov *et al*., (2006) suggested that the period of exposure to auxins had a significant effect on the efficiency of somatic embryogenesis. Thus optimum exposure to auxin is important for the development of somatic embryos and for their maturation.

Somatic embryogenesis has been reported from immature embryo axis Eapen and George, (1993); Baker *et al*., (1994), mature embryos Chengalrayan *et al*., (1994); Baker *et al*., (1995), mature embryo derived leaflets Venkatachalam, (1996), mature and immature cotyledons Gill and Saxena, (1992); Durham and Parrott, (1992); Wetstein and Baker, (1993), leaflet cultures McKently (1991); Gill and Saxena, (1992); Baker and Wetzstein,(1998), hypocotyl Venkatachalam *et al*., (1997) and epicotyl Little *et al*., (2000) in groundnut.

Radhakrishnan *et al*.,(2001) developed the methods for *in vitro* regeneration and somatic embryogenesis of peanut crop using various explants (cotyledonary nodes, de-embryonated cotyledons) and media combinations in different varieties. Tiwari and Tulli, (2009) developed a protocol using leaflets explants and obtained a higher shoot regeneration efficiency (80%).

Hazra *et al*., (1989); Ozias-Akins, (1989); Raja Rani and Padmaja, (2005) developed a protocol for somatic embryogenesis in groundnut (*Arachis hypogaea* L.) using various explants including immature embryo axes.

Ozias-Akins *et al*., (1992); Chengalrayan *et al*.,(1995,1997) attempted to obtain normal somatic embryos and improve the frequency of plant recovery in groundnut. Baker *et al*., (1995) developed a high frequency somatic embryogenesis system in peanut using embryo axes derived explants from harvested, dry and stored seeds.

McKently, (1990) stated that embryogenesis is induced by compounds such as 2,4-D and other components of auxinic effect such as picloram. Joshi *et al*.,(2008) recorded the effect of TDZ and 2,4-D on peanut somatic embryogenesis and *in vitro* bud development. They reported the appearance of bud-like projections in the embryogenic masses when these were cultured in media containing combinations of 2,4-D and TDZ. They found that the embryogenic mass was converting into organogenic mass in presence of TDZ.

Gill and Saxena, (1992) reported the influence of TDZ on direct somatic embryogenesis in peanut. They demonstrated the induction of somatic embryos from seedling explants of peanut e.g. cotyledons and juvenile leaves.

Saxena *et al.,* (1992) suggested the direct somatic embryogenesis from morphologically intact seedlings of peanut germinated on a medium supplemented with 10 μM TDZ. They observed somatic embryos were induced in the apical region and on the surface of cotyledon and hypocotyl of germinating seedlings, which eventually mature and develop into plants.

Murch and Saxena, (1997) reported the modulation of mineral and free fatty acid profiles during thidiazuron mediated somatic embryogenesis in peanut seedlings. Their observations suggested an alteration of nutrient availability and structural free fatty acid profiles, affecting both cellular functions and growth patterns. This seems to be part of the mode of action of TDZ and may play an important role in the induction of regeneration.

Murthy *et al.,* (1994) assessed the regulatory role of thidiazuron and explant factors in imparting somatic embryogenic potential in relation to endogenous growth regulator levels in peanut.

Hazra *et al*., (1989) reported somatic embryogenesis from immature zygotic embryos and achieved induction and maturation of somatic embryos in 2,4-D. Eapen and George, (1993) demonstrated that 2,4-D was most effective among the different auxins (NAA, 2,4-D and picloram) tested and resulted in higher number of somatic embryos per culture.

Baker *et al*., (1994) used immature cotyledonary explant in the presence of 2,4-D and NAA. The use of 2,4-D compared to NAA in the induction medium resulted in a greater percentage of embryogenesis and mean number of embryos. Venkatachalam *et al*. (1997, 1999b) reported somatic embryogenesis from peanut seedling derived leaflets and hypocotyl in presence of 2,4-D and NAA.

Roja Rani *et al*., (2005) observed the plant regeneration via somatic embryogenesis offers a particular advantage which consists to yield new plants with more stable genome. Silveira *et al*., (2008) noted that somatic embryo development based on biochemical and physiological principles that are essential to be understood. Banerjee, (2013) reported that somatic embrogenesis and plantlet regeneration using embryonic axes of *Arachs hypogaea* L.

Venkatachalam *et al*., (1999a) achieved direct somatic embryogenesis in cotyledon explants derived from mature, dry seeds of *Arachis hypogaea* L. According to histological observations, somatic embryogenesis was induced directly without any intervening callus on MS medium supplemented with different concentrations of BAP along with 2.68mM NAA after 4 weeks of culture. Mienie and Terblanche, (2013) reported the comparative somatic embryogenesis in four South African genotypes of groundnut (*Arachis hypogaea* L.)

Muller, (1927) opened a new vistas in plant breeding by introducing artificial induction of mutation. Later, the usefulness of micro mutations in plant breeding was established by Gregory, (1956, 1961). Subuthi *et al*., (1991) suggested that mutagenesis has been widely used as a potent method of enhancing variability for crop improvement. Mehandjiev *et al*., (2001) reported that induced mutations have great potentials and served as a complimentary approach in genetic improvement of crops.

Cove, (1993) explained that induced mutations are considerable value for comprehensive evolution and accelerating the process of plant improvement. Induced mutants constitute a value resource for research aimed at understanding the process in governing plant development. Ahloowalia and Maluszynski, (2001) stated that various mutagenic agents are used to induce favorable mutations at high frequency that include ionizing radiation and chemical mutagens.

Gottaschalk and Wolf, (1996) noted that mutation induction is an important complementary method of breeding crop species the utilization of induced mutations

for the improvement of crop plants have yielded several mutants which are used directly as new cultivars.

Khan and Siddiqui, (1995) isolated various types of morphological mutants by using chemical mutagens via ethyl methane sulphonate, methyl methane sulphonate and sodium azide. These mutants differ from control and also among themselves in height, growth and flowering habit. Breeding based on physical and chemical mutagenesis has been more effective than traditional plant breeding in producing cultivars with high resistance to biotic and abiotic stresses Lee and Lee, (2002); Dita *et al*., (2006); Fuller *et al*., (2006).

Mick *et al*., (1985) opined that mutation breeding is accomplished by chemical or physical treatments followed by selection for heritable changes of specific genotype and method has been successfully used in the genetic improvement of crop plants. Coe and Neuffer, (1977), Mashenkov, (1986); Ricardo and Ando, (1998) suggested that induced mutations have been used to improve major crops such as wheat, rice, barely, cotton, peanut and cowpea which are seed propagated.

Olejniczak and Patyna, (1985) reported on the role of chemical mutations in enhancing variability in higher plants. Ahloowalia and Maluszynski, (2001) observed mutants produced facilitate the isolation, identification and cloning of genes used in designing crops with improved yields, increase stress tolerance, longer shelf and induced agronomic input.

Lal *et al*., (2009) explained that induced mutations play important role in crop improvement and several popular mutant varieties have been developed in diverse crop plants including grain legumes.

Kodym, (2003) clearly showed that mutation by using both physical and chemical mutagens has successfully produced quite a large number of new and promising varieties in different seeds and ornamental plants and is considered to be a most successful tool for breeding ornamental plants .

Wan *et al*., (1991) found that the frequency of occurrence of mutation by the use of mutagen may as higher as 300 times than the occurrence of natural frequency seen. Jander *et al.,* (2003); Kim *et al*., (2006) stated that the frequency and saturation of mutations can be regulated by varying the mutagenic dose and mutagenic agents can induce different extensions of genomic lesions ranging from base mutation to larger fragments insertions or deletions Mackenzie *et al*., (2005).

Gilchrist and Haughn, (2005) revealed that plants response to physical and chemical mutagens is species - specific largely unknown for the majority of the species. Jain, (2005) opined that mutagens like gamma rays, ethyl methane sulfonate and methyl methane sulfonate are most frequently used for mutagenesis.

Broertjes and van Harten, (1988) described that mutation technology has been used to produce many cultivars with improved economic value. Ahloowalia and Malusznski, (2001) expressed that chemical mutation has been used to create and increase genetic variability in crop species and ultimately improves some plant traits.

Wongpiyasatid *et al*., (2000) described that induced mutation using physical and chemical mutagen one method to create genetic variation resulting to new varieties with better characteristics. Subuthi *et al*., (1991) noticed mutagenesis has been widely used as a potent method of enhancing variability for crop improvement. Solanki and Sharma, (1994) noted the selection of effective and efficient mutagens is very essential to recover a high frequency and spectrum of desirable mutations.

Jayabalan and Rao, (1983) pointed out using gamma irradiation caused different chromosomal aberrations in different plants. Kovacs and Keresztes, (2002) opined gamma rays are the most energetic form of electromagnetic radiation possesses the energy level from10keV to several hundred kilo electron volts and they are considered as the most penetrating in comparison to other radiation such as alpha and beta rays. Chaudhuri, (2002); Kiong *et al*., (2008) suggested that gamma radiation can be useful for the alteration of physiological characters.

Gunckel and Sparrow, (1961) opined that gamma rays are known to influence plant growth and development by inducing cytological, genetical, biochemical, physiological and morphogenetic chances in cells and tissue. Khatri *et al*., (2005) found that gamma rays and EMS could be fruitfully applied to develop new varieties with high yield and other improved traits. Shirsat *et al*., (2010). pointed out mutagenic effectiveness is measured in terms of mutagen and mutagenic efficiency represents the mutation frequency in relation to biological damage such as injury, sterility and chromosomal aberration etc. caused by mutagenic treatment.

Jabeen and Mirza, (2002); Kumar and Rai, (2005) revealed that EMS is an effective mutagen and has been used to induce genetic variability in a number of crop plants. Shah *et al*., (2008) noticed that mutagens may cause genetic changes in an organism, break the linkages and produce many new promising traits for the improvement of crop plants. Minocha and Arnason, (1962) stated that among the chemical mutagens, EMS is reported to be most effective and powerful mutagen. Freese, (1963) suggested that EMS induce a high rate of mutations in both micro and higher organisms and sometimes the mutation frequencies exceed those obtained by radiation Goud, (1967).

Salim *et al*., (2009) noticed that the main advantages of chemical mutation are the possibility for improving one or two specific characters without altering other. Adamu and Aliyu, (2007) reported that sodium azide is being used to create durability in different susceptible crops to improve their yield and quality characters in opposition to damaging pathogens.

Ahloowalia and Maluszynski, (2001) reported that the mutant plants formed by the application of sodium azide are able to withstand a range of unfavorable conditions and have enhanced yield, improved stress tolerance, longer shelf life and reduced agronomic input in comparison to normal plant.

Gao *et al*., (1992) showed that the combination of tissue cultures with mutation induction techniques may be an effective way to crop improvement. Micke *et al*.,

(1990) noted that experience in applying radiations or chemical mutagens to *in vitro* cultured plant material is limited and there are only few reports on successful selection of mutants after *in vitro* application of mutagens.

Wu *et al*., (2005) suggested that the combined use of mutagenesis and plant tissue culture can shorten the breeding cycle. Patade and Suprasanna, (2009) viewed that the combined application of mutagenesis and tissue culture is recognized as a valuable approach in crop breeding.

Ling *et al*., (2008) reported that *in vitro* mutagenesis is considered as a valid tool for the improvement of a crops, especially when we have to add one or more easily identifiable characters without changing the genotype of well developed variety and propagules are subcultured under sterile conditions.

Hell, (1983) reported that standardization of optimal doses for ionizing radiation and chemical mutagens to plant tissue culture and their response on *in vitro* mutation efficiency has been reported in many major crops such as tobacco, rice (Gao *et al*. 1992), groundnut (Venkatachalam and Jayabalan 1996), potato (Al-Safadi *et al*. 2000) and *Chrysanthemum* (Mandal *et al*. 2000).

Venkatachalam and Jayabalan, (1997b) suggested that γ-radiation and chemical mutagens have been used to induce mutations in callus cultures of groundnut. Venkatachalam and Jayabalam, (1997) noticed that *in vitro* mutagenesis in peanut has been reported but only a few mutants have been obtained after treating peanut calli with gamma radiation and ethyl methane sulfonate (EMS).

Melchers and Bergman, (1959) reported the use of cell culture for mutant selection as the easiest way of large scale screening of cell populations. Ahloowalia and Maluszynski, (2001) explained that plant breeders combine tissue technique for rapid multiplication of regenerants and mutation induction enhanced the variations and molecular marker methods to direct the genetic variation.

Donini and Sonnino, (1998) proposed that physical and chemical mutagens might be used together with *in vivo* or *in vitro* techniques to increase the mutation

frequency. Syano, (1974) demonstrated that protein synthesis and expression of specific protein occurs well in advance before any visual sign of anatomical as well as morphological events in *in vitro*.

Laemmli, (1970) achieved that acrylamide gel electrophoresis in the presence of sodium dodecyl sulfate has become one of the most broadly used techniques to separate and characterize proteins. Molecular markers have been used to study the extent of genetic variation.The protein profiling of germplasm and use of genetic markers have been widely and effectively used to determine the taxonomic and evolutionary aspects of several crops Nisar *et al*., 2007; Ladizinsky, (1979); Das and Mukarjee, (1995); Hameed *et al*., (2009).

Echart and Cavalli, (2000) reported this technique could also help to detect not only the "qualitative variability" through the presence or absence of bands but also "quantitative" variation in band intensities among genotypes.

Javid *et al*., (2004); Iqbal *et al.,* (2005) evaluated that electrophoresis of protein is a powerful tool for identification of genetic diversity and the SDS-PAGE is particularly considered as a reliable technology because seed storage proteins are highly independent of environmental fluctuations. Jha and Ohri, (1996) noted that seed protein patterns can also be used as a promising tool for distinguishing cultivars of particular crop species.

Ullah *et al*., (2010) noticed that technique of Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is commonly used for separation of seed storage proteins. Tanskley and Jones, (1981) suggested that banding patterns could be important supplemental method for cultivars identification particularly when there are legal disputes over the identity of a cultivar or when cultivars are to be patented.

Gebre *et al*., (1986) opined that the electrophoretic banding patterns of proteins have been found to be useful for the identification and characterization of particular genotypes and also establishing the predominance of one or the other parent in the

hybrids.Sethi and Guha, (1990); Lydon and Francis, (1992); Dey *et al*., (1998) achieved the growth and regeneration *in vitro* is a complex phenomenon and is influenced by a number of genetic and environmental factors.

Ronchi *et al*., (1984); John and Guha, (1997) reported that amino acids have been shown to be effective morphogenetic regulators in a number of species. Wetherell and Dougall, (1976); Kamada and Harada, (1984) reported that differences in endogenous amino acids pools have been found to affect the rate of protein synthesis during morphogenetic processes such as embryogenesis.

Chawla, (1989); Genkov and Ivanova, (1995) reported that the association between isoenzyme patterns on *in vitro* regenerated plants and different growth regulators. Huystee and Cairns, (1980) proposed that peroxidase involved in several physiological and biosynthetic functions.

Sounders *et al*., (1964) reported that peroxidase being one of the major metabolic catalysts utilize hydrogen peroxide to oxidize a wide range of organic and inorganic hydrogen donors such as cytochrome C-nitrites, leuco-dyes, ascorbic acid, indoles, amines and iodide ions. Talukdar, (2010) concluded that isoenzyme are widely used as molecular marker to distinguish mutants.

Arnison and Boll, (1974) studied the variation in isoenzyme pattern during growth of the callus cultures of root, hypocotyl and cotyledon derived from seedling of *Phaseolus vulgaris.* Hausman et al.,1993 and Kavers *et al*.,(1997) reported the role of auxin in relation to the peroxidase activity in rooting of various plant species. Wolter and Gorden, (1975) reported the changes in peroxidase activity precede the appearance of organs.

Scandalios, (1974); Carrillo-Cantaneda and Mata, (2000) opined that the biochemical attributes are indicators of morphogenetic potential, growth and differentiation, representing differential gene action/expression or change in endogenous level of growth regulators in cell cultures and are used for analysis of gene function and metabolic regulators.

Chawla, (1991); Verhese and Kour, (1991) reported that marked reductions in the number of biochemical attributes such as starch, protein, amylase, invertase, malate dehydrogenase, peroxidase and phosphorylase with a subsequent increase in soluble sugar and amino acid content precede in *in vitro* shoot differentiation process.

Thorpe, (1980) reported the biochemical changes that precede the onset of organogenesis/ embryogenesis can serve as markers of differentiation processes that bring about morphological developmental and functional specialization. Thorpe and Murashige, (1970) and Kevers *et al*., (1981 a,b) reported that the most of the study on biochemical aspects of organogenesis *in vitro* have been confined to the metabolism of auxin.