SUMMARY AND CONCLUSIONS

The present study attempts to evaluate the effect of mutagenic agents on direct, indirect organogenesis and somatic embryogenesis in groundnut cultivar TMV-7. In addition, the SDS-PAGE analysis, Amino acids profiling, Isoenzyme analysis and Biochemical characteristics were also carried out in mutagen treated *in vitro* cultures of groundnut.

The two mature seed explants such as whole embryonated cotyledon (WEC) and Whole embryonal axes (WEA) were separated from the whole seed and subjected to direct multiple shoot induction KIN and IAA/BAP and IAA. Among the different concentrations of KIN and IAA/BAP and IAA combinations 25.0mg/l of KIN/BAP with 1.0mg/l of IAA was found to be the most suitable for multiple shoot induction.

Of the two cytokinins tested, BAP was better than KIN in terms of percentage of response, number of shoots per explant and fresh and dry weight production. Among the two explant types WEC responded well than WEA. Both KIN/IAA and BAP/IAA combinations produced multiple shoots at varying frequencies. The shoots obtained via direct organogenesis from WEC were inoculated on root induction medium containing different concentrations of NAA and WEA on IBA individually. The NAA/IBA concentration of 3.0 mg/l produced maximum number of roots/shoots.

The SDS-PAGE analysis was carried out in multiple shoots derived from different concentrations of KIN and BAP. Based on densitometric pattern a minimum of 8.0 peaks were noticed in 5.0mg/l of KIN and 15 peaks in BAP. But it was increased up to 17.0 peaks in 25.0mg/l of KIN and 25.0 peaks in BAP. The visible protein bands were calculated based on Rf value. The number of protein bands with kDa also varied in different concentrations of KIN and BAP.

The isolated mature embryos were cultured on callus induction medium containing different concentrations of NAA + KIN and NAA+ BAP. Maximum percentage of callus formation was noticed in 3.0 mg/l of NAA with BAP. But the highly compact nodular calli were observed in 2.0 mg/l of NAA with 1.0 mg/l of

KIN/BAP. For shoot induction 3.0 mg/l of BAP with 0.5 mg/l of NAA or KIN with NAA. The embryonal leaflets excised from mature embryos were subjected to callus induction and plantlet regeneration. For callus induction, the maximum percentage was obtained in 3.0 mg/l of IAA with BAP and highly organogenic nodular calli were found in 2.0 mg/l of IAA combination with 1.0 mg/l of KIN/BAP. The highest shoot bud differentiation was found in 3.0 mg/l of BAP or KIN.

The seedling explants such as hypocotyl, cotyledonary segments and immature leaflets were collected from 7 day old aseptically grown seedlings were cultured on callus induction medium with IAA with KIN/BAP, NAA with KIN/BAP and 2,4-D with KIN/BAP respectively. The percentage of callus induction fresh and dry weight production were enhanced with addition of auxins.

Somatic embryogenesis was carried out epicotyl portion of the mature embryo/apical portion. The somatic embryo induction medium containing 2,4-D or NAA (10.0 to 50.0 mg/l). Of the two concentrations tested 2,4-D (30.0mg/l) recorded the highest percentage of response followed by NAA (30.0mg/l). But the highest number of somatic embryo were recorded in 30.0mg/l of 2,4-D followed by NAA. The apical portion of the mature embryo formed direct embryos without any intervention of callus.

The maximum percentage of embryogenic cultures were noticed in 30.0mg/l of 2,4-D followed by NAA at 30.0mg/l. for the differentiation of somatic embryos, the embryogenic masses were transferred to medium without any growth regulator. The maximum number of somatic embryos per culture was recorded in 30 mg/l of 2,4-D followed by 30.0 mg/l of NAA.

The *in vitro* mutagenic studies were carried out with different mutagenic agents like gamma rays, EMS and SA. In general the mutagenic agents influenced the cultural characteristics at varying extent.

The seeds were treated with different doses of gamma rays (10Kr -50Kr) and chemical mutagens (10mM-50mM) and cultured *in vitro* for direct organogenesis.

The WEC and WEA explants collected from mutagen treated seeds responded differently depending upon the dose/concentrations of mutagenic agents.

The lower doses of gamma rays (10 and 30Kr) and lower concentrations of chemical mutagens (10 and 30mM) had stimulatory effect on percentage of response, mean number of shoots per culture, mean number of shoot length, fresh and dry weight of the shoots. The percentage of response was higher in explants from gamma ray treated seeds followed by EMS and sodium azide over control.

The explants derived from mutagen treated seeds produced shoots at varying frequencies. When compared to control, the two different explants produced more number of shoots upto 30Kr gamma rays 30mM of EMS and sodium azide. The reduction in number of shoots/explants was observed in 40 and 50Kr of gamma rays and 40 and 50mM of EMS and sodium azide. The reductions in all other characteristics were also observed in higher doses /concentrations.

The mutagenic agents influenced the protein content in multiple shoots derived from WEC. All the three mutagenic agents increased the protein content up to 30Kr/30mM treatments and decreasing trend in 40 and 50Kr/mM. Based on densitometric pattern, the number of peaks were influenced by the concentrations/doses of the mutagenic agent. Like that, the visible protein banding pattern also varied in multiple shoots with mutagenic treatment. Some protein bands are very specific to mutagenic treatment.

The isoenzymes like, Esterase, Alcohol dehydrogenase and Glutamate dehyndrogenas were analyzed in multiple shoots with mutagenic agents. The zymogram pattern exhibited bands with different Rf value. Both dark and light colored bands were recorded based on mutagenic treatments.The amino acids content in multiple shoots derived from mutagenic treatments showed both positive and negative trend. Some amino acid contents were increased up to 20Kr/20mM treatments and very few amino acid contents increased up to 30Kr/30mM. In general, there was a reduction of all amino acid contents 40 and 50Kr/mM.

Callus induction and plantlet regeneration was achieved in whole embryonal axes and embryonal leaflets obtained via callus treatment with mutagenic agents of 1.0 to 5.0 Kr/mM. The mutagenic treatments 3.0Kr/mM exhibited stimulatory effect on percentage of callus induction and callus growth. Then there was negative trend in 4.0 and 5.0 Kr/mM. The callus induction and callus growth of hypocotyl segments showed stimulatory effect upto 9.0 Kr/9.0 mM treatments of gamma rays, EMS and sodium azide. In 12.0 and 15.0 Kr /mM showed decreasing trend.

The SDS-PAGE analysis of hypocotyl calli with mutagenic agents exhibited varying in nature. The number of peaks based on densitometric analysis and calculated protein bands with Rf values were influenced by the mutagenic treatments. The mutagenic treatments such as 3.0, 6.0 and 9.0 Kr/mM showed positive tendency and 12.0 and 15.0 Kr/mM noticed negative tendency.

The biochemical constituents like proteins, amino acids, carbohydrates, RNA, phenol and total chlorophyll contents were significantly increased upto 30.0Kr/mM treatments and reduced in 40.0 and 50.0 Kr/mM in cotyledonary segments and immature leaflets calli derived from seedling treatment. The percentage of callus induction and callus growth also showed the same trend.

Both physical and chemical mutagens enhanced the percentage of embryogenic cultures as well as number of somatic embryos per culture. Upto 30.0 Kr of gamma rays and 30.0mM EMS and SA had stimulatory effect. But above 40.0Kr gamma rays and 40.0mM EMS and SA reduced the percentage of embryogenic cultures and number of somatic embryos per culture. When compared to gamma rays, the reduction was higher with chemical mutagens. The higher doses/concentrations (40-50Kr/ 40-50mM) produced abnormal somatic embryos. All the three mutagenic agents produced both morphologically varied and normal plantlets at varying frequencies.

CONCLUSIONS

Induced mutagenesis through irradiation or chemical mutagenic treatment has become a very important method for plant breeding. The mutation techniques combined with *in vitro* culture could be successfully applied for both seed and vegetative propagated crops. The combined effect *in vitro* culture and mutagenic treatment shorten the breeding period. The selection mutagenic dose is a critical for the success of mutation induction. Mutation induction aims to optimize genetic variation with minimal plant injuries meaning that a balance has to be found between achieving mutagenesis and maintaining the integrity of the majority of the genome constitution of the mutated material. The present study is useful to know the role of mutagenic agents on *in vitro* culture of groundnut and also a basis for achieving useful mutants of groundnut for crop improvement in future.