The present investigation was carried out to study plantlet regeneration, root induction, meristem culture, *in vitro* germplasm conservation, microtuber induction and induced mutations in potato (*Solanum tuberosum* L.). The *in vitro* regeneration is a complex phenomenon influenced by large number of factors like genotype, explant, added hormones and culture conditions etc. Hence, the search for specific genotype capable of better plantlet regeneration and microtuber induction is an important step towards the application of tissue culture techniques to potato improvement. There is also need of standardizing growth regulator levels for better *in vitro* response comprising an important step for the application of tissue culture to agriculture.

The potato is world’s fourth important food crop after wheat, rice and maize. It is most important among root crops that give high yield per acre many times more than that of any grain crop. It has many table, processed and industrial uses. It is also extremely important for its high nutritive value rich in proteins, carbohydrates, minerals and vitamins. The present study comprised seven potato genotypes belonging to different phenotypic groups for plantlet regeneration and microtuber induction, while two genotypes were studied for induced mutational aspect. The relation among genotype, explant and different growth regulators has been critically studied. The plantlet regeneration from shoot tip and axillary node explants was studied.

The shoot tip and node meristems have many applications in crop improvement. It has proved to be very useful tool in eliminating viral pathogens from infected plants also (Hooker, 1981; Khurana, 1992; Salazar, 1996). Plant meristems and shoot tips have been identified as excellent materials for germplasm conservations of not only vegetatively propagated crops like potato (Westcott, 1981; Pruski *et al.*, 2000), but also in seed propagated crops like legumes (Kartha *et al.*, 1981) and fruit crops like strawberry and raspberry (Lisek and Orlikowska, 2004). The mass production of genetically stable multiple shoots from shoot tip and axillary node explants and complete plantlet formation is essential to establish a system for *in vitro*...
clonal propagation (Griga et al., 1986). The higher regenerative potentials of shoot tips and nodal explants can be further exploited for clonal propagation (Sounder Raj et al., 1991) as plants regenerated from these explants are true-to-type (Murashige, 1974). In potato, it is alternative to conventional propagation (Chandra and Birhman, 1994) and used for commercial production of seed material (Wang and Hu, 1982). The microtubers are small in vitro tubers, which are physiologically and morphologically identical to conventional tubers (Chandra et al., 1992c; Randhawa and Chandra, 1990). As produced under pathogen free conditions, they are disease free and used for production of breeders’ seed (Chandra and Dhignra, 1990; Addy, 1988).

The shoot tip and axillary node explants were taken from glasshouse-grown plants and inoculated on MS and B5 media containing four levels of BAP for plantlet regeneration. The explants of about 0.5-1.0 cm long were taken, as size of the explant is also important. The percent regeneration was directly proportional to size of explant (Bajaj and Dhanju, 1979). The genotypic variability for days to shoot regeneration, percent regeneration, plantlet height and number of leaves per plantlet was studied in relation to explant and BAP levels. The genotypic variability for days to root initiation, percent root induction, percent mortality of plantlets, root length and number of roots per plantlet was also studied on MS and B5 medium supplemented with four NAA levels. In the present investigation, the four BAP levels standardized were 0.25, 0.50, 0.75 and 1.00 mg/l for plantlet regeneration and four NAA levels were 0.10, 0.25, 0.50 and 0.75 mg/l for root induction. The BAP at low levels was found more suitable for large number of crops including legumes like Phaseolus vulgaris (Allavena et al., 1983), Dolichos biflorus (Soundar Raj et al., 1989), field bean (Kale, 2004); commercial crops like sugarcane (Kale et al., 2004; Bhagade et al., 2005) and also in solanaceous crops like tomato (Bhagade and Kale, 2004) and Capsicum (Kale, 2005). Though the plantlet regeneration was observed with both the cytokinins (BAP and Kinetin), the BAP was found more suitable than Kinetin which was also observed in other crops like Capsicum (Christopher and Rajam, 1994; Kale, 2005), tomato (Bhagade and Kale, 2004) and sugarcane (Kale et al., 2004). The root induction with NAA has been reported by many workers in other
crops like tomato (Patade and Kulkarni, 2004), Capsicum (Wankhede and Kale, 2004) and field bean (Kale, 2004). The plantlets after rooting were hardened in glasshouse and planted in nethouse for minituber production.

The plantlets were multiplied on multiplication medium, elongated on liquid multiplication medium and stored at 18-20°C in dark after replacing liquid multiplication medium with microtuber induction medium. Thus microtuber induction is three steps process (Tovar et al., 1985; Safadi et al., 2000 and Kale and Kothekar, 2004). The microtuber induction was studied in seven potato genotypes on MS and B₅ medium containing two sucrose concentrations (75 and 85 gm/l) and four BAP levels (6.00, 8.00, 10.00 and 12.00 mg/l). The genotypic variability for days to initiation of microtubers, percent microtuber induction, maturity duration of microtubers, weight of microtubers, storage loss of microtubers and percent germination of microtubers was studied. The microtubers were stored at 4-6°C for breaking dormancy and planted in nethouse for minituber production.

Because of tetraploid inheritance and complex breeding system, the potato breeding is considered very difficult and the constraints on conventional breeding are significant. The high degree of hetero-zygosity, self-incompatibility, male sterility pose difficulty in improvement of genotype by incorporation of useful characters. The modern plant breeder has a wide range of variability including induced mutations for introduction of profitable genes which should open a new insight for potato improvement. Through induced mutations, the broadening of genetic base can be quickly achieved. In the present investigation, EMS and SA mutagens were used. For any mutation programme, selection of effective mutagen is very important to get more frequency of desirable mutations. The tubers of two potato genotypes (MF-II and TPS-67) were treated with three concentrations each of EMS (0.03, 0.05 and 0.07 %) and SA (0.02, 0.04 and 0.06 %). The various biological parameters were studied in M₁ generation and mutants for desirable parameters were evaluated in M₂ generation. The mutagenic effects of EMS and SA have been reported earlier by other workers in cotton (Muthusamy and Jayabal, 2002) and lentil (Gaikwad and Kothekar, 2004).
The variation in the regeneration frequency with genotype has been reported by Martins and Sondhal (1984ab) while studying shoot apex cultures of *Phaseolus vulgaris*. The studies on plantlet regeneration from shoot tip and axillary node explants in seven potato genotypes revealed that the genotypes differed significantly for days to shoot regeneration and percent plantlet regeneration. Similar results were also observed in legume crop like field bean (Kale, 2004). In the present investigation, the mean variation ranged from 5.83 to 8.21 days on MS medium and from 6.25 to 8.58 days on B5 medium for days to shoot regeneration. The minimum and maximum days for shoot regeneration were required in genotypes MF-II and TPS-67, respectively on both the media. In field bean, the genotypes differed significantly for days to shoot regeneration on B5 medium also (Kale, 2005).

The percent plantlet regeneration ranged from 89.10 to 91.95 on MS medium and 79.17 to 88.75 on B5 medium. On both the media, the genotypes MF-II and TPS-67 recorded maximum and minimum plantlet regeneration, respectively. In cotton, several workers reported the response for plantlet regeneration to be genotype dependent (Gupta *et al*., 1977; Bajaj and Gill, 1986). There was considerable genotypic variability for multiple shoot formation especially in different legume crops like *Phaseolus vulgaris* (Allavena *et al*., 1983) and field bean (Kale, 2005).

A wide range of variability is on record for plantlet height and number of leaves per plantlet in potato (Kale and Kothekar, 2005). The mean height ranged from 3.65 to 4.60 cm on MS medium and from 3.52 to 4.42 cm on B5 medium, while number of leaves per plantlet ranged from 5.90 to 10.40 on MS medium and 6.37 to 8.79 on B5 medium. The maximum height and number of leaves per plantlet were recorded in genotype MF-II, while minimum height and number of leaves per plantlet were recorded in genotype TPS-67 on both the media. The genotypic variability for both the plantlet characters regenerated from shoot tip and cotyledonary node explants was also observed in legume crop like field bean (Kale, 2004). In *Capsicum*, genotypes differed significantly for plantlet height, while they displayed non-significant effect on number of leaves per plantlet (Kale, 2005).
EFFECT OF EXPLANT ON PLANTLET REGENERATION

The effect of explant on plantlet regeneration was studied by Kalantidis and Griga (1993) and stated that the explants had significant effect on plantlet regeneration. The further development of shoot tips was strongly determined by size of explant and there was a direct correlation between size and percent regeneration (Bajaj and Dhanju, 1979). The size of shoot tip explant was a critical factor for complete plantlet development affecting regeneration potentials (Griga et al., 1986). In potato, shoot tip explant was found more suitable than axillary node explant for days to shoot regeneration and percent plantlet regeneration. In legume crop like field bean, minimum days for shoot regeneration were required in shoot tip than cotyledonary node and also had greater regeneration potentials (Kale, 2004). The fewer days for shoot regeneration (MS = 5.87 and B₅ = 6.33) were required in shoot tip explant than axillary node explant (MS = 8.03 and B₅ = 8.39) on both the media in potato. Also more regeneration (MS = 91.86 % and B₅ = 85.95 %) was recorded by shoot tip explant than axillary node explant (MS = 88.12 % and B₅ = 81.85 %) on both the media.

The explant had significant effect in regard to plantlet height, while it displayed non-significant effect on number of leaves on MS medium (Kale and Kothekar, 2005). On B₅ medium, explant had significant effect on both plantlet characters. The increased height (4.14 cm) and number of leaves per plantlet (8.20) were recorded in shoot tip regenerated plantlets than axillary node regenerated plantlets (Height = 3.88 cm and leaves = 7.80) on MS medium. On B₅ medium also, more height (3.99 cm) and leaves (8.01) were recorded in shoot tip regenerated plantlets than axillary node regenerated plantlets (Height = 3.75 cm and leaves = 7.33). The same observations were also recorded in Capsicum (Kale, 2005) and field bean (Kale, 2004).

EFFECT OF BAP LEVELS ON PLANTLET REGENERATION

The regeneration response varied with the growth regulators and also at different levels of same growth regulator (Soundar Raj et al., 1991). In
potato, four different BAP levels created variable responses for days to shoot regeneration and percent plantlet regeneration, which was also observed in sugarcane (Kale et al., 2004). The mean variation ranged from 6.07 to 8.28 days on MS medium and 6.52 to 8.78 days on B₅ medium for days to shoot regeneration. The minimum and maximum days were required with BAP levels of 0.25 and 1.00 mg/l, respectively for days to shoot regeneration on both the media. The percent plantlet regeneration ranged from 77.33 to 100.00 and 77.02 to 92.59 on MS and B₅ medium, respectively. The BAP levels of 0.25 and 1.00 mg/l recorded maximum and minimum regeneration, respectively on both the media. It was observed that increase in BAP level decreased the response for plantlet regeneration, which was also reported by Sounder Raj et al. (1989) in Dolichos biflorus.

The BAP levels had significant effect on plantlet height and number of leaves per plantlet regenerated from shoot tip and axillary node explant. The mean height ranged from 3.35 to 4.55 cm on MS medium and 3.22 to 4.39 cm on B₅ medium, while number of leaves per plantlet ranged from 6.98 to 8.95 on MS medium and 6.64 to 8.43 on B₅ medium. The maximum height and number of leaves per plantlet could be observed with 0.25 mg/l BAP, while minimum could be noted with 1.00 mg/l BAP. It was observed that at higher BAP concentration, plantlet height and number of leaves per plantlet were reduced as compared to low concentration. Increase in BAP concentration resulted in decrease in plantlet height in legume crop like Dolichos lablab (Sounder Raj et al., 1991). In sugarcane also, BAP levels created variable responses for these plantlet characters (Kale et al., 2004).

**EFFECT OF GENOTYPE X EXPLANT INTERACTION ON PLANTLET REGENERATION**

The interactions of genotype and explant were highly significant for days to shoot regeneration and percent plantlet regeneration. These results were also observed in legume crop like filed bean (Kale, 2004). In genotype X explant interaction in potato, the mean variation ranged from 4.75 to 9.25 days on MS medium and 5.16 to 9.58 days on B₅ medium for days to shoot regeneration, while percent plantlet regeneration ranged from 86.60 to 93.30 on MS medium and from 76.25 to 91.25 on B₅ medium. The minimum days were required in shoot tip explant of genotype MF-II, while maximum in
axillary node explant of genotype TPS-67 for shoot regeneration on both the media. The genotype MF-I recorded maximum regeneration on MS medium, while it was MF-II, which demonstrated it on B₅ medium with shoot tip explant. The genotype TPS-67 recorded minimum regeneration with axillary node explant on both the media. The shoot tip explant gave better response in most of the genotypes for both the parameters.

The genotype X explant interactions also had significant effect for plantlet height and number of leaves per plantlet in potato (Kale and Kothekar, 2005). The mean variation ranged from 3.50 to 4.80 cm on MS medium and 3.40 to 4.62 cm on B₅ medium for plantlet height, while 5.50 to 10.70 on MS medium and 6.08 to 9.17 on B₅ medium for number of leaves per plantlet. The maximum height was recorded by genotype MF-II with shoot tip explant on both the media. The genotype TPS-67 recorded minimum height on MS medium, and the genotype EX/A-680-16 on B₅ medium with axillary node explant. Maximum number of leaves per plantlet could be noted in shoot tip regenerated plantlets of genotype MF-II, while the minimum response was in axillary node regenerated plantlets of genotype TPS-67 on both the media. More height and number of leaves per plantlet were recorded in shoot tip regenerated plantlets of most of the genotypes in potato. Similar results were also observed in tomato as well (Bhagade and Kale, 2004). The pronounced effects of genotype X explant interactions on both these plantlet characters have been observed earlier in field bean (Kale, 2004).

**EFFECT OF GENOTYPE X BAP LEVELS INTERACTION ON PLANTLET REGENERATION**

The interactions of genotype and BAP levels were highly significant for days to shoot regeneration and percent plantlet regeneration. The mean variation ranged from 5.00 to 9.83 days on MS medium and 5.67 to 10.33 days on B₅ medium for days to shoot regeneration. The percent plantlet regeneration ranged from 75.00 to 100.00 and 71.67 to 97.50 on MS medium and B₅ medium, respectively. The minimum days were required with 0.25 mg/l BAP in genotype MF-II on MS medium and in genotypes MF-II and TPS-7 on B₅ medium for shoot regeneration. The maximum days were required with 1.00 mg/l BAP in genotype TPS-67 on both the media for shoot regeneration. The maximum plantlet regeneration was observed with 0.25 mg/l BAP in all
the genotypes on MS medium, while minimum regeneration was observed with 1.00 mg/l BAP in genotype JTH/C-107. The maximum and minimum regeneration could be noted with 0.25 mg/l BAP in genotype MF-II and with 1.00 mg/l BAP in genotype TPS-67, respectively on B5 medium.

The interactions between genotype and BAP also had significant effect on plantlet height and number of leaves per plantlet. The mean plantlet height ranged from 3.00 to 5.10 cm and 2.78 to 4.83 cm on MS and B5 medium, respectively. The maximum plantlet height was observed with 0.25 mg/l BAP in genotype MF-II on both the media. The minimum plantlet height was observed with 1.00 mg/l BAP in genotypes TPS-67 and EX/A-680-16 on MS medium and in genotype EX/A-680-16 on B5 medium. The number of leaves per plantlet ranged from 4.80 to 11.50 on MS medium and 5.17 to 9.50 on B5 medium. The maximum leaves per plantlet were recorded with 0.25 mg/l BAP in genotype MF-II, while minimum leaves per plantlet were recorded with 1.00 mg/l in genotype TPS-67 on both the media. The BAP level of 0.25 mg/l was found more suitable than other three levels for most of the parameters studied in potato regeneration.

**GENOTYPIC VARIABILITY FOR ROOT INDUCTION**

The genotypes differed significantly for days to root initiation, percent root induction and percent mortality of plantlets. The minimum days were required by genotype MF-II on both media (7.30 on MS and 7.83 on B5 medium), while maximum days were required by genotype EX/A-680-16 on MS (11.91 days) and B5 (12.83 days) medium for root initiation. The percent root induction ranged from 91.83 (in genotype EX/A-680-16) to 97.67 (in genotype MF-I) on MS medium, while it ranged from 84.41 (EX/A-680-16) to 94.91 (MF-I) on B5 medium. The minimum mortality has been observed in genotype TPS-7 (8.17 %) on MS medium and in MF-I (9.83 %) on B5 medium. The maximum mortality was observed in genotype JTH/C-107 on both the media (MS = 11.58 % and B5 = 12.91 %).

A wide range of variability was observable for root length and number of roots per plantlet. The root length ranged from 7.00 to 10.00 cm on MS medium and from 5.67 to 8.25 cm on B5 medium. The number of roots per plantlet varied from 5.08 to 12.83 and 4.50 to 10.58 on MS and B5 medium,
respectively. Both maximum height and number of leaves per plantlet could be recorded in genotype MF-II, while the minimum became evident in EX/A-680-16, on both the media.

**EFFECT OF NAA LEVELS ON ROOT INDUCTION**

The NAA levels created variable responses with mean variation from 8.50 to 10.70 days on MS medium and 9.38 to 11.14 days on B₅ medium for days to root initiation, from 87.47 to 100.00 % on MS and 85.90 to 92.42 % on B₅ medium for percent root induction, while from 6.95 to 12.19 % on MS and from 8.28 to 13.61 % on B₅ medium for percent mortality of plantlets. The minimum and maximum days were required with NAA levels of 0.25 and 0.75 mg/l, respectively for root initiation on both the media. The maximum root induction was observed with 0.25 and 0.50 mg/l NAA on MS medium, while with 0.50 mg/l NAA on B₅ medium. The minimum root induction was observed with 0.75 mg/l NAA on both the media. The minimum and maximum mortality could be recorded with 0.25 and 0.75 mg/l NAA, respectively on both the media.

The NAA levels also had significant effect on root length and number of roots per plantlet. The mean length ranged from 6.95 to 10.09 cm (on MS medium) and from 5.28 to 8.33 cm (on B₅ medium), while number of roots per plantlet ranged from 6.95 to 9.95 (on MS medium) and from 6.05 to 8.43 (on B₅ medium). The maximum length and number of roots per plantlet were recorded with 0.25 mg/l NAA, while minimum length and roots per plantlet could be seen with 0.75 mg/l NAA on both the media.

**EFFECT OF GENOTYPE AND NAA LEVELS INTERACTION ON ROOT INDUCTION**

The genotype X NAA levels interactions created variable responses for days to root initiation, percent root induction and percent mortality of plantlets. The minimum days were required with 0.50 mg/l NAA on MS medium (6.30) and with 0.25 mg/l NAA on B₅ medium (7.00) in genotype MF-II for root initiation. The maximum days were required with 0.75 mg/l NAA in genotype EX/A-680-16 on both the media (MS = 14.60 and B₅ =
15.67). The percent root induction ranged from 83.00 to 100.00 and 81.00 to 97.00 on MS and B₅ medium, respectively. The maximum root induction was observed with 0.25 and 0.50 mg/l NAA in all the genotypes on MS medium, while with 0.25 mg/l NAA in genotypes MF-I and MF-II on B₅ medium. The minimum root induction was observed with 0.10 mg/l NAA in genotype EX/A-680-16 on both the media. The percent mortality ranged from 6.00 to 15.33 % on MS medium and 6.67 to 15.00 % on B₅ medium. The minimum mortality was observed with 0.25 mg/l NAA in genotype MF-II and with 0.25 and 0.50 mg/l NAA in genotype MF-I on MS and B₅ medium, respectively. The maximum mortality was observed with 0.75 mg/l NAA in genotype JTH/C-107 on MS medium and with 0.75 mg/l NAA in genotypes MF-I and JTH/C-107.

The genotype X NAA level interactions also had significant effect on root length and number of roots per plantlet, with mean variation from 5.00 to 11.33 cm (MS medium) and 3.33 to 9.67 cm (B₅ medium) for root length, while from 4.00 to 15.00 (on MS medium) and 3.33 to 12.00 (on B₅ medium) for number of roots per plantlet. The maximum root length was observed with 0.25 mg/l NAA in genotype MF-II on both the media. The minimum root length was observed with 0.75 mg/l NAA in genotype JTH/C-107 on MS medium and in genotype EX/A-680-16 on B₅ medium. The maximum roots were recorded with 0.25 mg/l NAA on MS medium and with 0.25 and 0.50 mg/l NAA on B₅ medium in genotype MF-II. The minimum roots were recorded with 0.10 and 0.75 mg/l NAA on MS medium and with 0.75 mg/l NAA on B₅ medium in genotype EX/A-680-16.

**STUDIES ON MERISTEM CULTURE**

In the present investigation, the four viruses were removed with meristem culture only but the size of meristem is most important. The elimination of PVA, PVY, PVM and PVX was observed with meristem without any leaf primordia indicating the effect of size of meristem on virus elimination. Among different factors studied by several workers it has been found that the size of meristem is the most important factor than other factors.
for removal of viruses at high frequency in potato (Hayami et al., 1984; Sajid, et al., 1986 and Abukhes et al., 1991).

The PVS and PLRV viruses were not removed with meristem culture for which heat treatment was given which was found most effective before meristem culture for removal of these two viruses. In the present study, more elimination of these two viruses was observed at 35-40°C. Thermotherapy before meristem culture was found an effective technique for removal of the viruses which are present in small meristems also and not removed by meristem culture only which was reported previously be several workers (Hollings, 1962; Dhingra, et al., 1988). Kassanis (1950) observed that PLRV can be inactivated by giving heat treatment to tubers at 37°C, while Hamid and Locke (1961) reported that PLRV can be removed by giving heat treatment to tubers at 40-45°C.

The virus elimination also varied from genotype to genotype and from virus to virus (Resende and Paiva, 1985). For overall elimination of four viruses, maximum (11.50 %) and minimum (8.00 %) elimination was observed in genotypes TPS-67 and EX/A-680-16, respectively. In case of viruses, PVA was eliminated at maximum frequency (11.57 %), while PVX at minimum frequency (4.86 %). Matsumoto et al. (1989) also reported that elimination frequency is dependant on virus. The overall elimination of PVS and PLRV viruses ranged from 2.50 to 3.50 % in genotypes. Among seven genotypes, maximum and minimum elimination was revealed by genotypes EX/A-680-16 and TPS-7, respectively. The PVS was eliminated at a better frequency (3.71 %) than PLRV (2.14 %).

**STUDIES ON IN VITRO GERMPLASM CONSERVATION**

In the present investigation, it was observed that storage at 4-6°C was possible for 1.5 to 2.0 years. The storage of potato shoot tips at low temperature for long duration has been reported earlier by several workers. Westcott (1981) stored potato germplasm at 6-12°C for 12 months. Pruski et al. (2000) reported that at 4°C the storage without any subculture was possible for 12 weeks. Nyende et al. (2003) stored shoot tips of potato encapsulated in
calcium alginate beads at 4°C for 390 days and stated that though the re-growth ability decreased with more duration, the storage was possible and the method was suitable for storing potato germplasm. The suitability of in vitro cold storage is on record in other crops like strawberry and raspberry (Lisek and Orlikowska, 2004) and legume crop like field bean (Kale, 2004).

The intervals between transfers of shoot tip cultures was extended to long period by addition of growth retardant to medium (Westcott, 1981). In the present study, the plantlet growth was reduced and sub-culturing of shoot tips was avoided for one year. The plantlet regeneration varied from genotype to genotype and concentrations of cycocel used in medium. The plantlet height was also reduced gradually, which was also dependent on genotype and reduced with increase in cycocel concentration.

PERFORMANCE OF POTATO TISSUE CULTURE PLANTS

The studies on performance of potato tissue culture plants revealed that genotypes differed significantly for percent survival during hardening, percent survival at planting, percent flowering, weight of biggest tuber, weight of smallest tuber and total tubers produced per plant (Kale and Kothekar, 2004). The percent survival during hardening ranged from 71.66 to 83.30 and at planting from 68.33 to 80.00 %. The maximum survival was recorded by genotype JTH/C-107, while minimum by genotype EX/A-680-16 during hardening and at planting. The maximum (95.66 %) and minimum (8.33 %) flowering was observed in genotypes TPS-67 and JTH/C-107, respectively. The weight of biggest tuber ranged from 17.67 to 94.00 gm and of smallest tuber from 1.67 to 6.33 gm. The maximum weight of biggest and smallest tuber was recorded in genotypes MF-II and TPS-7, respectively. The minimum weight of biggest and smallest tuber was recorded in genotype EX/A-680-16. The maximum (6.33) and minimum (3.30) tubers were harvested in genotypes TPS-67 and MF-II, respectively.

GENOTYPIC VARIABILITY FOR MICROTUBER INDUCTION

The genotypes differed significantly for days to initiation and percent induction of microtubers (Kale and Kothekar, 2004). The mean variation ranged from 8.71 to 11.62 days on MS medium and from 9.37 to
12.37 days on B₅ medium for initiation of microtuber. The minimum and maximum days were required in genotypes MF-II and TPS-67, respectively for initiation of microtubers on both the media. The percent microtuber induction ranged from 86.25 to 92.08 and 81.87 to 88.96 on MS and B₅ medium, respectively. On both the media, maximum and minimum microtuber induction was recorded by genotypes MF-II and TPS-67, respectively. The genotypic variability for microtuber induction was reported previously by several workers (Hussy and Stacey, 1984; Estarda et al., 1986 and Gopal et al., 2004).

A wide range of variability was observed for maturity duration of microtuber with mean variation from 64.25 to 71.00 days on MS medium and 65.25 to 72.50 days on B₅ medium. The minimum and maximum days were required in genotypes MF-II and TPS-67, respectively on both the media for maturity of microtubers. The genotypes differed significantly for microtuber weight (Chandra et al., 1992 c; Randhawa and Chandra, 1990). The mean weight ranged from 0.42 to 1.02 gm and 0.41 to 1.00 gm on MS and B₅ medium, respectively. The maximum and minimum weight was recorded in genotypes TPS-13 and JTH/C-107, respectively on both the media.

The storage loss of microtubers also varied from genotype to genotype (Naik and Sarkar, 1997). The percent storage loss ranged from 7.46 to 13.08 and 8.25 to 13.00 on MS and B₅ medium, respectively. The minimum and maximum storage loss was recorded in genotypes MF-II and TPS-67, respectively on both the media. There was a wide range of variability for germination of microtubers with mean variation from 97.37 to 98.50 % on MS medium and 96.00 to 97.75 % on B₅ medium. The maximum germination was observed in genotype MF-II on both the media. The minimum germination was observed in genotype EX/A-680-16 on MS and in TPS-67 on B₅ medium.

**EFFECT OF SUCROSE CONCENTRATIONS ON MICROTUBER INDUCTION**

The sucrose concentrations had significant effect on days to initiation and percent induction of microtubers on MS medium, while significant effect on days to initiation and non-significant effect on percent microtuber induction on B₅ medium (Kale and Kothekar, 2004). The less days were required with 85 gm/l sucrose (MS = 9.83 and B₅ = 10.55) than 75 gm/l
sucrose (MS = 10.38 and B₅ = 11.00) on both the media. The more microtuber induction was also recorded with 85 gm/l sucrose (MS = 90.71 % and B₅ = 85.83 %) than 75 gm/l sucrose (MS = 87.86 % and B₅ = 85.18 %) on both the media. Hussy and Stacey (1984); Estarda et al. (1986) and Gopal et al. (2004) studied and reported that sucrose concentrations had pronounced effect on microtuber induction.

The sucrose concentrations had significant effect on maturity duration of microtuber and microtuber weight. The less days (MS = 65.21 and B₅ = 66.61) were required with 85 gm/l sucrose than 75 gm/l sucrose (MS = 66.89 and B₅ = 68.25). The higher microtuber weight (0.61 gm on MS medium and 0.61 gm on B₅ medium) was recorded with 85 gm/l sucrose than 75 gm/l sucrose (MS = 0.58 gm and B₅ = 0.56 gm).

The sucrose concentrations also had pronounced effect on storage loss of microtubers and germination. The less storage loss was observed with 85 gm/l sucrose (MS = 9.59 % and B₅ = 10.18 %) than 75 gm/l sucrose (MS = 10.68 % and B₅ = 11.18 %). Gopal et al. (2004) stated that higher sucrose concentrations (60-80 gm/l) promoted not only in vitro microtuberization but also improved storage performance and tubers can be stored for 12 months with minimum storage loss. In the present study, more microtuber germination (MS = 98.75 % and B₅ = 97.25 %) was observed with 85 gm/l sucrose than 75 gm/l sucrose (MS = 97.18 % and B₅ = 96.28 %).

**EFFECT OF BAP LEVELS ON MICROTUBER INDUCTION**

In the present investigation, four BAP levels created variable responses with mean variation from 9.02 to 12.09 days (on MS medium) and from 9.86 to 12.76 days (on B₅ medium) for initiation of microtuber, while from 83.81 to 97.50 % (MS medium) and from 80.00 to 90.95 % (B₅ medium) for percent microtuber induction (Kale and Kothekar, 2004). The minimum and maximum days were required with 10.00 and 12.00 mg/l BAP, respectively on both the media for initiation of microtubers. The maximum and minimum microtuber induction was recorded with 10.00 and 12.00 mg/l BAP,
respectively on both the media. Previously, several workers suggested that BAP was one of the important factors in production of microtubers in vitro (Hussy and Stacey, 1984; Estarda et al., 1986).

The BAP levels also had significant effect on maturity duration and weight of microtubers. The maturity duration ranged from 62.43 to 71.43 days on MS medium and from 63.93 to 72.86 days on B5 medium, while microtuber weight ranged from 0.51 to 0.70 gm on MS medium and from 0.50 to 0.68 gm on B5 medium. The minimum and maximum days were required with BAP levels of 10.00 and 12.00 mg/l, respectively on both the media for maturity of microtubers. The highest and lowest microtuber weight was recorded with 10.00 and 12.00 mg/l BAP, respectively on both the media.

The BAP levels had pronounced effect on storage loss and germination of microtubers. The mean storage loss ranged from 8.52 to 12.43 % and germination from 96.50 to 99.14 % on MS medium, while on B5 medium storage loss ranged from 9.28 to 12.57 % and germination from 95.36 to 98.28 %. The minimum storage loss and maximum microtuber germination was observed with 10.00 mg/l BAP, while maximum storage loss and minimum microtuber germination was observed with 12.00 mg/l BAP on both the media.

EFFECT OF GENOTYPE X SUCROSE CONCENTRATION INTERACTION ON MICROTUBER INDUCTION

The interactions of genotypes with sucrose concentrations were highly significant with mean variation from 8.67 to 12.08 days (MS medium) and from 9.25 to 12.75 days (B5 medium) for days to microtuber initiation, while from 85.00 to 93.75 % (MS medium) and from 81.25 to 90.83 % (B5 medium) for percent microtuber induction (Kale and Kothekar, 2004). The minimum days were required with 85 gm/l sucrose in genotypes MF-I and MF-II on MS medium, while with 75 gm/l sucrose in genotype MF-II. The maximum days were required with 75 gm/l sucrose in genotype TPS-67 on both the media, for initiation of microtuber. The maximum microtuber induction was recorded with 85 gm/l sucrose in genotype MF-II, on both the media. The minimum microtuber induction was observed with 75 gm/l sucrose on MS medium and with 85 gm/l sucrose on B5 medium in genotype TPS-67. The pronounced interaction effects of genotype and sucrose concentration on
in vitro microtuberization were reported previously by several workers (Gopal et al., 2004).

The interactions also had significant effect on maturity duration and weight of microtubers. The mean maturity duration ranged from 63.25 to 72.00 days on MS medium and 64.50 to 73.50 days on B5 medium. The microtuber weight ranged from 0.41 to 1.03 gm on MS medium and 0.39 to 1.01 gm on B5 medium. The minimum days were required with 85gm/l sucrose in genotype TPS-13 on MS medium and in genotype MF-II on B5 medium. The maximum days were required with 75 gm/l sucrose in genotype TPS-67 on both the media. The maximum microtuber weight was recorded with 85 gm/l sucrose in genotype TPS-13, while minimum with 75 gm/l sucrose in genotype JTH/C-107 on both the media.

The interactions had pronounced effect on storage loss and germination of microtubers. The mean response ranged from 6.75 to 13.67 % on MS medium and 7.50 to 13.75 % on B5 medium for storage loss of microtubers. The percent germination ranged from 96.00 to 98.75 % and 95.25 to 98.00 % on MS and B5 medium, respectively. The minimum storage loss was observed with 85 gm/l sucrose in genotype MF-II, while maximum with 75 gm/l sucrose in genotype TPS-67 on both the media. The maximum microtuber germination was recorded with 85 gm/l sucrose in all the seven genotypes on MS medium, while with 85 gm/l sucrose in genotype MF-II on B5 medium. The minimum microtuber germination was observed with 75 gm/l sucrose in genotypes TPS-67 and EX/A-680-16 on MS medium and in genotype TPS-67 on B5 medium.

**EFFECT OF GENOTYPE X BAP LEVELS INTERACTION ON MICROTUBER INDUCTION**

The interactions of genotypes with BAP levels created variable responses for days to initiation and percent induction of microtubers (Kale and Kothekar, 2004). The mean variation ranged from 8.00 to 15.17 days and 8.67 to 15.67 days on MS and B5 medium, respectively for initiation of microtubers. The percent microtuber induction ranged from 80.83 to 97.50 and 76.67 to 92.50 on MS and B5 medium, respectively. The minimum days were required with 10.00 mg/l BAP in genotype MF-II, while maximum with 12.00 mg/l BAP in genotype TPS-67 on both the media. The maximum microtuber
induction was observed with 10.00 mg/l BAP in all the seven genotypes on MS medium and in genotype MF-II on B₅ medium. The minimum microtuber induction was observed with 12.00 mg/l BAP in genotype TPS-67 on both the media.

The interactions had significant effect on maturity duration and weight of microtubers. The maturity duration ranged from 61.00 to 75.50 and 62.00 to 77.00 days on MS and B₅ media, respectively. The minimum days were required with 10.00 mg/l BAP in genotypes TPS-7, MF-II and JTH/C-107 on MS medium and in genotypes MF-II and JTH/C-107 on B₅ medium. The maximum days were required with 12.00 mg/l BAP in genotype TPS-67 on both the media for maturity of microtubers. The highest microtuber weight (1.16 gm) was recorded with 10.00 mg/l BAP in genotype TPS-13, while lowest (0.30 gm) with 12.00 mg/l BAP in genotype TPS-67 on MS medium. The highest (1.17 gm) and lowest (0.19 gm) microtuber weight was recorded with 10.00 mg/l BAP in genotype TPS-13 and with 12.00 mg/l BAP in genotype JTH/C-107, respectively on B₅ medium.

The interactions also had pronounced effect on storage loss and germination of microtubers. On MS medium, minimum storage loss (5.83 %) was observed with 10.00 mg/l BAP in genotype MF-II, while maximum (15.83 %) with 12.00 mg/l BAP in genotype TPS-67. The minimum (6.00 %) and maximum (15.00 %) storage loss was observed with 8.00 mg/l BAP in genotype MF-II and with 6.00 mg/l BAP in genotype TPS-67 on B₅ medium, respectively. The percent germination ranged from 96.00 to 100.00 % on MS medium and 95.00 to 99.50 % on B₅ medium. The maximum germination was recorded with 10.00 mg/l BAP in genotype MF-II on both the media. The minimum germination was observed with 12.00 mg/l BAP in genotype TPS-67 on MS medium and in genotypes TPS-67 and JTH/C-107 on B₅ medium.

**PERFORMANCE OF MICROTUBERS**

The genotypes differed significantly for percent flowering, total tubers produced per plant, weight of biggest tuber and weight of smallest tuber (Kale and Kothekar, 2004). The maximum (97.33 %) and minimum (8.33 %) flowering was observed in genotypes TPS-67 and TPS-7, respectively. The maximum (6.67) and minimum (3.67) tubers were harvested in genotypes
TPS-67 and MF-II, respectively. The weight of biggest tuber ranged from 19.67 to 96.33 gm and of smallest tuber from 2.33 to 7.67 gm. The maximum weight of biggest and smallest tuber was recorded in genotypes MF-II and TPS-7, respectively. The minimum weight of both biggest and smallest tuber was recorded in genotype EX/A-680-16.

**STUDIES ON INDUCED MUTATIONS**

The germination, plant survival upto maturity and plant height showed gradual decrease, while abnormal plants increased with increase in concentration of EMS and SA in both genotypes. The dose dependant effects of EMS and SA i.e. increase in dose resulting, in corresponding increase in damage, were also reported in legume crop like lentil (Gaikwad and Kothekar, 2004). The germination ranged from 57.33 to 47.33 % after EMS treatment and 54.00 to 44.67 % after SA treatment in genotype MF-II. The germination ranged from 52.00 to 46.67 % and 52.00 to 42.00 % after EMS and SA treatments, respectively in genotype TPS-67.

In genotype MF-II, the highest survival (54.33 %) was recorded at 0.03 % EMS and lowest survival (41.00 %) at 0.06 % SA. In genotype TPS-67, the highest survival (49.00 %) was seen with low concentrations of both mutagens i.e. 0.03 % EMS and 0.02 % SA, while lowest survival (37.67 %) was seen with 0.06 % SA. In genotype MF-II, the maximum (74.00 cm) and minimum (66.67 cm) plant height could be recorded at 0.02 % SA and 0.07 % EMS, respectively. The maximum (96.33 cm) and minimum (90.00 cm) plant height was recorded at 0.02 % SA and 0.07 % EMS, respectively in genotype TPS-67.

The maximum abnormal plants were seen at 0.07 % EMS in genotype MF-II (22.00 %) and at 0.06 % SA in genotype TPS-67 (25.00 %). The minimum abnormal plants in genotype MF-II (5.00 %) and TPS-67 (8.33 %) were seen at 0.02 % SA and 0.03 % EMS, respectively. The critical screening of M_1 generation demonstrated the induction of *chlorina* type of chlorophyll mutations at higher concentrations of both the mutagens in both the genotypes. The more frequency of chlorophyll mutations (3.67 %) was seen at 0.06 % SA and 0.07 % EMS (2.67 %) in genotype MF-II. In genotype
TPS-67 also, a similar trend with more chlorophyll mutation frequency (6.67 %) has been recorded at 0.06 % SA and 0.07 % EMS (4.67 %).

The frequency of plants for early flowering was maximum at the middle concentrations of both the mutagens in both the genotypes. The maximum frequency was observed with 0.05 % EMS in both genotypes (MF-II = 2.67 % and TPS-67 = 4.67 %). In genotype MF-II, the minimum (1.00 %) frequency was seen with 0.07 % EMS and 0.02 % SA, while in genotype TPS-67 minimum frequency (1.00 %) was seen with 0.03 % EMS and 0.06 % SA. The pollen sterility increased with an increase in concentrations of EMS and SA in both the genotypes. The similar results were also observed in cotton (Muthusamy and Jayabalan, 2002). In genotype MF-II, maximum (6.33 %) and minimum (1.33 %) pollen sterility was observable with 0.07 and 0.03 % EMS, respectively. The maximum (7.00 %) and minimum (1.33 %) pollen sterility could be recorded with 0.07 % EMS and 0.02 % SA, respectively in genotype TPS-67.

The frequency of plants for early maturity, increased number for tubers produced per plant, increased weight of biggest and smallest tuber was maximum at the middle concentrations of EMS and SA in both genotypes. In genotype MF-II, maximum (11.00 %) and minimum (1.00 %) frequency for early maturity was seen with 0.05 % EMS and 0.06 % SA, respectively. The maximum (6.67 %) and minimum (1.33 %) frequency was seen with 0.05 % and 0.07 % EMS, respectively in genotype TPS-67. In genotype MF-II maximum (4.67 %) and minimum (1.00 %) frequency for increased tubers per plant was observed with 0.05 % EMS and 0.02 % SA, respectively. In genotype TPS-67, maximum (9.33 %) and minimum (1.33 %) frequency was noticeable with 0.05 % EMS and two of the SA concentrations (0.02 and 0.06 %), respectively.

The maximum frequency for increased weight of biggest tuber was observed with EMS treatment of 0.05 % in both genotypes (MF-II = 12.33 % and TPS-67 = 14.00 %). The minimum frequency was observed with 0.02 % SA in genotype MF-II (1.00 %) and with 0.06 % SA in genotype TPS-67 (1.67 %). For increased weight of smallest tuber, maximum (11.33 %) and minimum (1.00 %) frequency was observed with 0.05 % EMS and 0.06 % SA,
respectively in genotype MF-II. In case of genotype TPS-67, maximum (13.33 \%) and minimum (1.00 \%) frequency was observed with 0.05 \% EMS and 0.02 \% SA, respectively.

The frequency for increased dry matter content was observed only with the low concentration of EMS in both the genotypes. The more frequency was seen in genotype TPS-67 (3.33 \%) than MF-II (1.67 \%). The desirable effects of EMS were also reported earlier by other workers and developed new cultivars as a result of such mutagenic treatment (Micke et al., 1985).

The evaluation and screening of mutants in M$_2$ generation revealed that positive response changed from parameter to parameter and had variable responses. Thus it was concluded that mutagens could have some temporary effect resulting in chimeras. The possibility of generating chimeras in vegetatively propagating crops like potato has been reported previously by other workers (Roest and Bokelman, 1980; Van et al., 1981). In the present study, the mutants for increased dry matter content had 100 \% positive response indicating the absence of chimeras for this character in both the genotypes. The minimum positive response was observed for early flowering mutants indicating more percentage of chimeras.

**IMPLICATIONS OF THE PRESENT INVESTIGATIONS ON FUTURE POTATO IMPROVEMENT**

In the present investigation, the protocol for plantlet regeneration and microtuber induction was standardized for seven potato genotypes. The highly culturable genotypes were identified which might be utilized to obtain reproducible results in further potato improvement. Although, the different factors had pronounced effect the maximum plantlet regeneration was observed with specific BAP level and the maximum response for microtuber induction was also observed with specific sucrose concentration and BAP level indicating the suitability of protocols for broad range of genotypes. There is need of testing these protocols in other genotypes and using these techniques for commercial seed production of potato.

In meristem culture studies, the effect of different factors on elimination of major potato viruses was studied and the protocol was standardized with high virus elimination frequency. This technique may be
used in other genotypes to remove viruses and improving the performance of commercial cultivars and potato germplasm, which were highly infected and saturated with viruses. During in vitro germplasm storage studies, it was observed that the germplasm may be stored at low temperature and using the growth retardant in multiplication medium and the period of subculture of shoot tip cultures may be extended from weeks to years. As the method is simple, low cost and efficient with better re-growth after storage, may be used for storing the potato germplasm collections.

The results in the present investigation revealed that there was considerable variability for plantlet regeneration, microtuber induction, virus elimination and re-growth after in vitro storage. Thus, this study needs to be intensified and may be extended to other genotypes.

In mutational studies, the desirable effects of EMS and SA were observed resulting in induction of mutants some of which were economically important. In $M_2$ generation, 100% positive response of mutants was not observed for all important parameters indicating the presence of chimeras. Hence, there is further need of clonal selection and further generations to isolate non-chimeric and solid mutants. The possibility to obtain induced mutations should open a new insight to the solution of breeding problems of potato.