CHAPTER VI

6. SUMMARY AND CONCLUSION

6.1 In vitro plant regeneration from seedling explants of Cucumis sativus L.

The present study describes an efficient method for induction of multiple shoots and in vitro flowering from shoot tip explants of cucumber (Cucumis sativus L,). In vitro flowering is an alternative breeding tool for generating hybrid Cucumis spp. as it is able to overcome limitations caused by interspecific incompatibility. Shoot tip explants were excised from 7-day-old seedlings and cultured on Murashige and Skoog (MS) medium fortified with different concentrations of 6–benzylaminopurine (BAP; 0.5–2.5 mg/L) alone or in combination with 0.5 mg/L kinetin (KIN). The highest frequency (93.1%) of multiple shoot formation with maximum number of shoots (15.2 shoots/explant) was achieved on MS medium supplemented with 1.0 mg/L BAP. For in vitro flowering, shoots were cultured on MS medium supplemented with 0.5 mg/L BAP and different concentrations of sucrose. Flowering occurred on about 95% of in vitro shoots cultured on MS medium fortified with 6% (w/v) sucrose and 0.5 mg/L BAP after 15 days of culture. For rooting, shoots (>2 cm) were cultured on MS medium augmented with various concentrations of indole–3–butyric acid (IBA; 0.5–2.5 mg/L) alone or in combination with 0.5 mg/L KIN. Among the combinations tested, supplementation with IBA (1.5 mg/L) and KIN (0.5 mg/L) induced maximum rooting rates (95.4%) with 7.8 roots/shoot. Rooted plantlets were successfully transferred into plastic cups containing a mixture of soil and sand (1:1), established in the greenhouse, and subsequently acclimatized in the field. The in vitro flowering reported in this study may facilitate rapid hybridization in Cucumis species and offers a model system for studying the physiological mechanisms involved in flowering.

The cotyledonal node explants of Cucumis sativus L., cv. ‘Green long’ collected from 5-day-old seedlings were cultured on MS medium augmented with various concentrations of BAP and KIN (0.5–2.5 mg/L) for shoot bud induction. The highest frequency of shoot bud initiation (100%) was observed on a medium containing 2.0 mg/L BAP, however, BAP at 1.5 mg/L was found to be best.
concentration for multiple shoot bud development (88.4%) with 19.85 shoots/culture. An increased percent of multiple shoot regeneration (100%) as well as number of shoots (44.6 shoots/culture) were achieved on MS medium containing different concentrations of silver nitrate (AgNO₃) along with 1.5 mg/L BAP. It is interesting to note that addition of silver nitrate in the medium not only enhanced multiple shoot bud regeneration but also elongation of shoot buds was observed. Elongated shoots were transferred to half–strength MS medium containing different concentrations of IBA and NAA (0.5–2.0 mg/L) in combination with 0.5 mg/L KIN for root induction. The highest percent of rooting (96.2%) was noticed on a medium containing the combination of IBA (1.5 mg/L) and KIN (0.5 mg/L) followed by NAA (1.5 mg/L) and KIN (0.5 mg/L). Rooted plantlets were successfully transferred into plastic cups containing sand and soil (1:1) and maintained in the controlled environment for a week. After acclimatization, the plantlets were transferred to the green house and subsequently established in the field where the survival rate observed was 72%. The genetic fidelity analysis of in vitro regenerated plantlets of cucumber was performed by RAPD fingerprinting analysis. The RAPD profiles generated from in vitro regenerated plants were found to be monomorphic and identical banding pattern which is similar to the control plant. DNA fingerprinting results confirmed that the in vitro raised plantlets were found to be genetically identical and true–to–type in nature. In conclusion, an efficient and high frequency plant regeneration system using silver nitrate was established and this protocol could be used to transform agronomically important genes into cucumber plants in the future.

The present study is focused on adventitious shoot regeneration from mature leaf explants of cucumber. Mature leaf explants were cultured on MS medium fortified with different concentrations of BAP and KIN alone (0.5–2.5 mg/L) for shoot bud regeneration. The highest number of multiple shoot formation was noticed on MS medium supplemented with 0.4 mg/L BAP. The regenerated shoot buds were cultured on different concentrations of ADS (0.5–3.0 mg/L) and GA₃ (0.5–2.0 mg/L). Among the different concentrations used for shoot bud elongation, the maximum shoot length (4.2 cm) was noticed on 0.4 mg/L GA₃. The elongated shoots were excised and cultured on MS medium containing different concentrations of
IBA/NAA/IAA for root induction. The highest percent of root induction (88.7%) with 5.0 roots/shoot was observed on MS medium fortified with 1.0 mg/L IBA. Rooted plantlets were successfully transferred into plastic cups containing a mixture of soil and sand in the ratio of 1:1 and established in the greenhouse and subsequently acclimatized in the field.

6.2 Agrobacterium–mediated genetic transformation and plant regeneration from cotyledonary node explants of Cucumis sativus L.

In the present study, cotyledonary node explant was used to develop an efficient plant transformation protocol for cucumber (Cucumis sativus L.) via Agrobacterium–mediated genetic transformation. Development of this protocol was based on the systematic evaluation of factors that influence transformation efficiency including Agrobacterium strain, precultivation period, co–cultivation period and different concentrations of kanamycin. The cotyledonary node explants from 5-day-old in vitro grown seedlings were pre-cultured for 3 days, and co-cultivated with Agrobacterium strain EHA 105 for 10 min, immersion in the bacterial culture containing 25 mg/L rifampicin and 50 mg/L kanamycin. Further, 3-days co-cultivated explants produced the highest efficiencies of transformation. The putative transgenic plants were subjected to histochemical GUS assay and molecular analysis to assess the transgene incorporation into plant genome and its expression. A blue colouration was observed in the putatively transformed plantlets indicates that the transgene was successfully transferred and the control plantlets did not show any blue coloration which indicates the presence of gus gene. The presence of npt II gene in the putative transgenic lines was confirmed by PCR analysis. The present plant transformation system of Agrobacterium–mediated genetic transformation will be used to introduce genes of interest into the cucumber genome for the purpose of crop improvement.

6.3 Green synthesis of silver nanoparticles using callus and leaf extracts of Cucumis sativus L., and its antimicrobial activity as well as wound healing activity.

The present study reports an environmentally friendly and rapid method for synthesis of metallic silver nanoparticles. A silver nanoparticles synthesis using
callus and leaf extracts and its wound healing activity in rat model was reported. The prepared silver nanoparticles were characterized by UV-Visible spectroscopy (UV), Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), Field emission scanning electron microscopy (FE-SEM) and X-ray energy dispersive spectrophotometer (EDX) analysis. The UV-visible spectroscopy analysis was performed in the range of 200-700 nm to determine the silver nanoparticle from 0-5 hrs. The FTIR spectroscopy measurements were carried out to identify the possible biomolecules present in leaf and callus extracts that bound specifically on the silver surface. X-ray diffraction studies were performed to confirm the crystalline structure of synthesized silver nanoparticles. FE-SEM images were measured and topographical analysis was performed based on the surface study. The FE-SEM studies provide the information on the nanoparticle morphology and particle size. The EDX attachment present with the FE-SEM is known to provide information on the chemical analysis of the fields that are being investigated or the composition at specific locations and to find out the metal signals. The nanoparticle synthesized using leaf extracts by centrifugation method showed the best antimicrobial activity as well as wound healing activity. Furthermore the synthesized AgNPs were found to show potential antimicrobial activity against Gram-negative (Escherichia coli and Pseudomonas aeruginosa) and Gram-positive (Bacillus subtilis and Staphylococcus aureus), among these organisms Bacillus subtilis was found to be more susceptible to AgNPs synthesized using centrifuged leaf extracts of cucumber. Also, the results strongly reveal the enhanced wound healing potential of formulating silver nanoparticles based ointment that was found to be effective in wound repair as well as softens the skin in the experimental rats.

6.4 Hyperaccumulation of Chromium and Lead in in vitro regenerated plants of Cucumis sativus L.

Phytoextraction is a remediation technology with a promising application for removing chromium (Cr) and lead (Pb) from soils and waters. The present study was conducted to evaluate the in vitro grown Cucumis sativus L., for the uptake of Lead and Chromium metals. In vitro plants were grown on MS basal medium containing different concentrations of chromium (0, 5, 10, 20, 30 and 40 mg/L) and lead (0,
100, 200, 300, 400 and 500 mg/L). After 15 days of heavy metal treatment, the regenerated shoots were harvested and used for antioxidative enzyme activities and isoenzyme studies. The antioxidative enzyme activities varied accordingly with different concentrations of heavy metal (chromium and lead) exposure. Isoenzyme band intensity was more consistent with respective changes in antioxidative enzymes activities. The in vitro method was found to be a valuable tool in identifying prospective phyto remediation candidates to reduce environmental toxic metals. The present results concluded that heavy metal exposure in growth medium, which alter the physiological and biochemical profiles of the regenerated shoots of Cucumis sativus and confirmed that, in growth medium cucumber tolerate up to the 5 mg/L of Cr and 300 mg/L of Pb exposure. The present results supported that, in future, the in-vitro grown hyper accumulator plants can be used as an effective and better tool of phyto remediation for the removal of heavy metals through their rhizosphere scavenging action, from the contaminated lands on a wider scale.

In conclusion, in the present study, in vitro regeneration of cucumber plant was achieved using different explants viz., shoot tip, cotyledonary node, and mature leaf tissue. Efficient plant regeneration and in vitro flowering was also achieved using shoot tip explants of cucumber. Genetic fidelity of the in vitro regenerated plantlets using cotyledonary node explants was confirmed by RAPD–PCR analysis. In vitro regeneration protocol could be useful for introduction of agronomically important genes for crop improvement in cucumber plant.

Cotyledonary node explants were co–cultivated with Agrobacterium strain EHA 105 harbouring binary vector pBI121 having uid A (gus) gene and npt II gene and selection of transformed shoots was performed on medium with 1.5 mg/L BAP, 50 mg/L kanamycin and 300 mg/L cefotaxime. Transformants showed histochemical GUS expression and untransformed shoots did not show GUS expression and it confirms the presence of gus gene. PCR analysis using specific primers for npt II gene showed the expected band size in putative transformants whereas no amplified fragment was observed in the untransformed (control) plants. The present protocol could be used for production of transgenic plants with agronomically important genes.
A simple green method for extracellular synthesis of highly stable bioactive molecules loaded metallic silver nanoparticles using callus and leaf extract was described and AgNPs showed promising antibacterial activity as well as wound healing activity. The synthesized AgNPs using centrifuged leaf extracts by this green chemistry approach had shown best efficacy against Bacillus subtilis bacterium. Topical application of ointment prepared from silver nanoparticles of cucumber were formulated and evaluated in vivo using the excision wound healing model on wistar albino rats. The measurement of the wound areas were taken on 3rd, 6th, 9th, 12th, 15th, 18th and 21st days and the percentages of wound closures were calculated. By the 21st day, the ointment containing 5% w/w of silver nanoparticles in ointment base showed 100% wound healing activity compared with that of the standard as well as control bases. The results suggested that the silver nanoparticles based ointment was found to be effective in wound repair activity.

Effects of Pb and Cr metal ions on plants growth, antioxidative enzyme activities and isoenzymes were studied. Accumulation of chromium and lead metal ions from the growth medium confirmed that cucumber has the ability to accumulate the heavy metals.