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Effect of Kanamycin and Cefotaxime on cultured tissues of Broccoli (*Brassica oleracea* L. var. *italica*) and its role in genetic transformation studies

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Broccoli (*Brassica oleracea* L. var. *italica* cv. Solan green head) is an important nutritionally rich vegetable cole crop grown in the world. The production of broccoli is challenged by many stresses including infestation of insects. The conventional insect control method is mainly dependent on the intensive and extensive use of chemical pesticides, which have drawbacks such as damage to the ecological system and residual poisoning to humans and animals. Therefore, it is desirable to develop insect resistant plants through plant genetic engineering. Among insecticidal genes, *Bacillus thuringiensis* crystal protein genes have been proven effective in controlling insect larvae in many crop plants. Genetically engineered disarmed *Agrobacterium tumefaciens* strain containing binary vector pBin-1Aa with *cryIAa* (insect resistance gene) and *npt-II* (neomycin phosphotransferase-II) genes was used for genetic transformation studies. Successful plant transformation requires efficient high frequency shoot regeneration protocol and suitable selection system. In this regard, the effect of kanamycin and cefotaxime was studied on cultured hypocotyl, cotyledon, leaf and petiole tissues of broccoli to explore the aptness of kanamycin resistance as a selectable marker and cefotaxime in controlling excessive bacterial growth during genetic transformation studies. Explants showed decrease in fresh weight as concentration of the kanamycin increased resulting in full or partial inhibition of shoot regeneration. A negative correlation was observed between the concentration of kanamycin and fresh weight of the explants at different intervals of time. Effect of different concentrations of cefotaxime was studied on the regeneration potential in cotyledon and hypocotyl explants of broccoli and found no much effect of cefotaxime on regeneration potential. Effect of different concentrations of cefotaxime and kanamycin (50 mg/l) were studied on the growth of agrobacterial cells and regeneration potential of cotyledon and hypocotyl tissues after cocultivation. In both the explants, the growth of agrobacterial cells were controlled at concentration of 400 mg/l cefotaxime and maximum per cent shoot regeneration in hypocotyl (44.88 %) and cotyledon (36.29 %) was obtained on MS selective medium supplemented with 400 mg/l cefotaxime respectively. PCR analysis of genomic DNA using specific designed primers was done to detect the presence of the *cryIAa* and *npt-II* genes in kanamycin resistant shoots of broccoli (*Brassica oleracea* L. var. *italica* cv. Solan green head). Out of randomly selected five putative transgenic shoots, three shoots were found to be positive for the presence/integration of transgene i.e., *cryIAa* and *npt-II* during T-DNA transfer and integration into the plant genome. Thus the results indicated that kanamycin and cefotaxime as an effective selective agent during genetic transformation studies.

Biography

Pankaj Kumar is a PhD Research Scholar in the Department of Biotechnology, Dr. Y S Parmar University of Horticulture and Forestry, India. He is awarded with DST/INSPIRE-SRF for pursuing full-time Doctoral (PhD) program and qualified ICAR ASRB National Eligibility Test (NET) in 2013 and ICAR AICE-SRF (PGS) in 2014. He has attended many workshops, trainings, international & national conferences, seminars, symposium and published research papers.

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