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EXTENDED ABSTRACTS

Protocol for Direct Transformation through Gene Gun in *Capsicum annuum* L.Cv. Mathania

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ABSTRACT

protocol for direct transformation through biolistic gun has been established for chilli (*Capsicum annuum* L. cv. Mathania). A disarmed strain of *Agrobacterium tumefaciens* EHA 105 carrying a binary vector plasmid p35SGUSINT has been used for transformation. This vector contains neomycin phosphotransferase gene (npt II), whose expression confirms Kanamycin resistance in transformants. additionally to npt II, plasmid encodes -glucuronidase, reporter enzyme used for studying the expression of foreign genes in plants. We report for the primary time, the utilization of Nitrogen gas for the biolistic transformation of chilli. We found physical parameter like 6 cm target distance with 900 psi rupture disk for partical gun experiment the foremost efficient for transient GUS expression. just in case of nitrogen gas the frequency of transient GUS expression was better both in leaves (67.74%) and hypocotyl (69.18%) and frequency of conversion of transient to stable transformation was 3.4% in leaves and 4.0% in hypotocyls as against 63.91 % transient GUS expression showed by leaves and 63.77% showed by hypocotyls when helium gas was utilized in biolistic transformation and stable transformation frequency was 3.0% in leaves and 4.0% in hypocotyls. The transgenic nature of the regenerated plants was confirmed by the histochemical staining of GUS, and polymerase chain reaction (PCR) analysis of npt II gene. *Capsicum annuum* (red pepper) is a crucial spice cum vegetable crop in tropical and subtropical countries. Here, we report an efficient and reproducible auxin free regeneration method for 6 different red pepper cultivars (ACA-10, Kashi Anmol, LCA-235, PBC-535, Pusa Jwala and Supper) using hypocotyl explants and an efficient *Agrobacterium*-mediated transformation protocol. The explants (hypocotyls, cotyledonary leaves and leaf discs) collected from axenic seedlings of six red pepper cultivars were cultured on either hormone free MS medium or MS medium supplemented with BAP alone or together with IAA. Inclusion of IAA within the regeneration medium resulted in callus formation at the cut ends of explants, formation of rosette leaves and ill defined shoot buds. Regeneration of shoot buds might be achieved from hypocotyls grown in MS medium supplemented with different concentrations of BAP unlike other explants which did not respond. Incorporation of GA3 in shoot elongation medium at 0.5 mg/l concentration enhanced the elongation in two cultivars, LCA-235 and Supper, while other cultivars showed no significant response. Chilli cultivar, Pusa Jwala was transformed with β C1 ORF of satellite DNA β molecule related to Chilli leaf curl Joydebpur virus through *Agrobacterium tumefaciens*. Transgene integration in putative transformants was confirmed by PCR and Southern hybridization analysis. *Capsicum annuum*, a member of the Solanaceae, is a crucial crop grown worldwide. it's used as a spice, a source of red pigment and as a vegetable in many countries. additionally to its use as a food, its powder disinfects the oral and gastric mucous membranes and destroys the pathogenic bacteria within the intestine. Red pepper is vulnerable to many pathogens including viruses, fungi, bacteria and nematodes. The approaches aiming for genetically engineered red pepper plants for creating resistant varieties or value addition are protracted thanks to the shortage of highly efficient regeneration method. due to lack of efficient transformation method for the cultivated species of red pepper,

understanding of host virus interaction is restricted to the model plant like *Arabidopsis thaliana* or in transient system using leaf disc assay. Pepper (*Capsicum annuum* L.) is an economically important vegetable and spice crop. In our laboratory we've established a regeneration and transformation protocol for the sweet red pepper type 'Florinis' and for 2 pepper hybrids PO1 and C using hypocotyl explants. the speed of plant regeneration was found to depend upon the kinds of explants cultured and therefore the media used. In our regeneration protocol shoot bud initiation is simpler on MS media supplemented with IAA and BAP and shoot bud development is promoted with addition of GA 3. Rooted shoots are successfully established in soil. so as to realize the transformation of pepper we applied two different methods, using *Agrobacterium* and therefore the particle gun. Following the primary method fertile transgenic pepper plants were regenerated from hypocotyl explants that were co-cultivated with *Agrobacterium tumefaciens* strain LBA4404 harboring a plasmid that contains the gus reporter gene and therefore the nptII selection gene or a plasmid with the Cu/Zn SOD gene of tomato, that's expressed in chloroplasts. Transgenic pepper plants were developed, verified and characterized but the share of transformed plants obtained using *Agrobacterium* is quite small which is why we've applied as alternative the biolistics method. consistent with the tactic pepper hypocotyls as explants were bombarded by the hand gene gun of Bio-Rad. The plasmid that utilized in this transformation contains the gus reporter gene driven by the CaMV-35S promoter. The reporter gene facilitates the comparison of the 2 transformation methods, and indeed the amount of the kanamycin-resistant plants that were produced through the particle gun seem to be quite large. INTRODUCTION Pepper (*Capsicum annuum* L.) is a crucial crop plant grown worldwide for its use as a spice, vegetable or ornamental plant. Pepper is very vulnerable to fungal and viral pathogens, also on the environmental stresses, and these cause considerable damage to the crop. one among the solutions to the present problem is that the gene-splicing of sweet pepper for useful traits, which depends on an efficient and reliable transformation and regeneration protocol. While many members of the Solanaceae family are facile with reference to cell culture and regeneration, pepper (*Capsicum annuum* L.) is taken into account to be recalcitrant to regeneration. So far, the foremost successful method of regeneration involves direct organogenesis from cotyledons and hypocotyls and recently from young leaves (Zhu et al., 1996). Pepper cultivars differ markedly in their regeneration requirements. the main problem during the in vitro regeneration process is shoot elongation. Regeneration is additionally severely limited thanks to the formation of ill-formed buds or shoot-like structures which either resist elongation or produce rosettes of distorted leaves that don't produce normal shoots (Husain et al., 1999). the foremost recent report for the regeneration of cayenne (*Capsicum annuum*) from cotyledon explants was developed by Husain and may be a highly efficient three-stage protocol.

Keywords: npt II; Biolistic gun; Nitrogen gas; p35SGUSINT

