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Title: Regeneration and transformation studies in Indian maize (*Zea mays* L.)

ABSTRACT

Efficient methods for *in vitro* propagation, regeneration and transformation in temperate maize are available; however, very little information is available on tropical maize in this regard. The present investigation was conducted to develop a reproducible regeneration and transformation protocol for Indian maize grown in tropical environment. The study was conducted using five elite maize inbred lines with established *per se* and hybrid performance in India, namely: CM-111, CM-117, CM-124, CM-125 and CM-300. Different sources of ex-plants, viz. immature and mature embryos, split seeds and nodal segments were used for establishing the *in vitro* regeneration and genetic transformation protocol. In case of nodal segment additional two lines, namely: LM-6 and LM-13 were also used. In order to identify the best media for *in vitro* callusing and plant regeneration a set of different callusing and regeneration media were tested. Two methods of genetic transformation of viz. particle bombardment (biolistic method) and *Agrobacterium*-mediated transformation were attempted to standardise and optimize the transformation protocol for Indian maize.

Findings of the present investigation showed that immature embryo was the best ex-plant in terms of both callusing and plant regeneration in comparison to other ex-plants, viz. mature embryo, split seed and nodal segments from mature seed. Among various ex-plants derived from mature seeds, nodal segment gave relatively better response in terms of both callusing and plant regeneration. Significant genotypic variability exists in Indian maize with regards to *in vitro* callusing and plant regeneration from various ex-plants. CM-300 and CM-124 gave best response with immature embryo as ex-plant, while LM-13 was best in case of nodal segment.

Highest *in vitro* callusing from immature embryo was obtained with the media NDA2 (N6 medium supplemented with dicamba 2.0 mg l^{-1} and $AgNO_3$ 15.0 mg l^{-1}), while in case of nodal segment callusing media C1 (N6 medium supplemented with 2,4-D 4.0 mg l^{-1}) and C3 (MS medium supplemented with 2,4-D 3.0 mg l^{-1}) gave better response. Among various regeneration media used, MIB (MS with BAP 1.0 mg l^{-1} and IAA 0.5 mg l^{-1}) gave best response in terms of *in vitro* shoot induction, elongation and whole plantlet development. In genetic transformation studies, in general, biolistic method showed relatively better results in terms of transformation efficiency. Among various treatment combinations used in biolistic method, particle size of 1.0 µm bombarded at rupture pressure 1100 psi from the distance 6.0 cm gave highest number transients *gus* expression in CM-300. The same treatment combination resulted in maximum number of shoot development under selection of *basta* herbicide.