THE ASIAN JOURNAL OF HORTICULTURE
Volume 7 | Issue 1 | June, 2012 | 190-196

Research Paper

Article history :
Received : 05.03.2012
Revised : 08.06.2012
Accepted : 24.06.2012

Standardization of *Agrobactrium* mediated transformation in brinjal

SONIA KUMARI[1], CHHAYA SHARMA AND D.K. SRIVASTAVA[1]

Members of the Research Forum

Associate Author :
[1]Department of Biotechnology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, SOLAN (H.P.) INDIA

Author for correspondence :
CHHAYA SHARMA
School of Agtricultural Biotechnology, Punjab Agricultural University, LUDHIANA (PUNJAB) INDIA
Email : chhaya_uhf@yahoo.co.in

Abstract **:** Genetic transformation studies were carried out to standardize a protocol for *Agrobacterium*-mediated gene transfer technique in brinjal (*Solanum melongena* L. cv. Pusa purple long). High frequency shoot regeneration, 57.17% was obtained on MS basal medium (macro and micro nutrients) supplemented with 2.5 mg/l BAP + 0.5 mg/l IAA from hypocotyl explants and 77.46% was obtained on MS basal medium supplemented with 2.5 mg/l Kn + 0.5 mg/l IAA from cotyledon explants. High frequency of root regeneration from *in vitro* developed shoots was obtained on MS basal medium supplemented with 0.10 mg/l IAA. Kanamycin sensitivity (0-50 mg/1) was checked by the fresh weight of the explant/ callus which was measured at an interval of 7 days till 35 days. Fresh weight decreased with increase in kanamycin concentration. The fresh weight of explant/callus and kanamycin concentration was found negatively correlated (r=-0.88) at different intervals of time. Kanamycin concentration as low as 20 mg/l was toxic to the explants (hypocotyl) on selective shoot regeneration medium. For genetic transformation, disarmed *Agrobacterium tumefaciens* LBA4404 strain containing a reporter â-glucuronidase (*gus*) gene in binary vector pBI121 along with kanamycin resistance gene (*npt-II*) was used for selection in both bacteria and plant. Preincubation of 72 hrs and co-cultivation of 48 hrs was found optimum as it gave maximum transgenic shoot regeneration from hypocotyl explants (6.8%) and cotyledon explants (2.20%) on selective medium. The putative transformants were randomly selected for the amplification of *gus* and *npt-II* genes with specific designed primers and out of 8 randomly selected putative transgenic plantlets 5 have shown the amplification of *gus* and *npt-II* genes there by indicating the presence/ integration of *gus* and *npt-II* genes into the genome of transgenic brinjal. The expression of *gus* gene was studied by using biochemical and histochemical techniques of GUS assay. The *gus* gene was expressed in the PCR positive transgenic plantlets of brinjal. A protocol for genetic transformation in brinjal with *npt-II* and *gus* genes has been standardized.

Key words **:** *Agrobacterium*-mediated transformation, *Solanum melongena*, Hypocotyl and cotyledon explants, Polymerase chain reaction, Biochemical and histochemical assay, *gus* and *npt-II* gene

How to cite this article **:** Kumari, Sonia, Sharma, Chhaya, and Srivastava, D.K. (2012). Standardization of *Agrobactrium* mediated transformation in brinjal, *Asian J. Hort.*, **7**(1) : 190-196.

Brinjal or eggplant (*Solanum melongena* L.) is an agronomically important non-tuberous solanaceous crop grown primarily for its large oval fruit. It is a popular vegetable crop grown extensively in India along with other Asian, European and American countries (Chadha, 1993). China is the top producer (56% of world output) and India is second (26% of world output), Egypt, Turkey and Indonesia round out the top producing nations. In India total production of brinjal is 10,378 000 tones. Besides its high consumption as vegetable, eggplant is recommended for the ailment of diabetes, arthritis, asthma and bronchitis (Mgioli and Mansur, 2005). Genetic engineering offers a direct method of plant breeding that selectively targets one or a few traits for introduction into the crop plant such as disease and insect resistance and it depends on an efficient and reliable genetic transformation and plant regeneration protocol. *Agrobacterium tumefaciens*-mediated genetic transformation is an effective and widely used approach to introduce desirable genes into plants. There