

Optimizing In-planta transformation in *Brassica napus* L.

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Abstract

Brassica napus is an essential member of the *Brassicaceae* (*Cruciferae*) family, an important oilseed crop in Pakistan. Pakistan's annual oilseed import bill exceeds 2 billion USD. To reduce this bill, the emphasis must be put on developing new high yielding cultivars of oilseeds. Genetic engineering is one of the modern techniques for developing problem-free cultivars. In this research, optimization of in planta floral dip transformation was optimized in *B. napus*. *Agrobacterium* strain AGL-1 transformed with p7i-UG harbouring GUS (β -glucuronidase) as a reporter gene, and bar as a selectable gene was used in planta transformation. The efficiency of transformation was investigated under various transformation parameters, such as induction media and target tissues. 240 floral buds were treated through four induction media with *Agrobacterium* suspension. After treatment of the target tissues, racemes were enclosed with butterfly bags to ensure self-pollination. All the seeds were harvested and grown under controlled conditions. Upon developing 2-3 true leaves, Basta herbicide was sprayed @ 150 mg/L twice at an interval of 4 days. The 46 plants showed resistance against the selection pressure of Basta, which were declared as putative transgenic plants. These plants were subjected to genomic DNA extraction, and gene-specific PCRs were conducted using the DNA of each plant separately. Out of 46 phenotypically screened plants, just 20 of them revealed the existence of the GUS (β -glucuronidase) gene through gene-specific PCR. These plants have been extended to maturity, and seeds were harvested. The transformation effectiveness analysis showed that the highest transformation efficiency of 0.49 % is seen in "DUNCKLET" with 5% Sucrose + MS as Induction Medium and 0.54 % at 10 min with the aspect of dipping time of floral bud.

Biography

The coauthor has completed his Master of Philosophy in Biotechnology from the University of Agriculture Faisalabad, Pakistan. During his research worked on optimization of different transformation methods to develop transgenic plants and molecular

analysis. He published approximately 12 articles, including research and review papers in international and national journals related to Biotechnology, Plant Sciences and Bioinformatics