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Development of Insect Resistant Transgenic Pea (*Pisum sativum* L.): Molecular and Functional Characterisation of Putative Transgenic Pea Plants

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Abstract

Pea (*Pisum sativum* L.) is one of the economically important legume crop cultivated worldwide. However, its production and storage is constrained by different species of insect pests. Thus, development of insect resistant variety is one of the main goals of breeding and improvement efforts in many producing countries. In line with this, gene transformation can complement the conventional breeding strategy through widening access to resistance genes beyond the species gene pool.

In this study, putative transgenic pea plants developed *via Agrobacterium*-mediated transformation using a construct harboring *cry1Ac* gene for insect resistance and *bar* gene for herbicide resistance were characterised using molecular and functional analysis. The *in vitro* putative transgenic shoots were micro-grafted on a 5–7 days old seedling rootstock germinated under laboratory condition and leaf samples were collected for DNA isolation from successfully grafted and well grown plants. Then, molecular analysis of the isolated DNA samples was done for *cry1Ac* and *bar* genes using their respective primers. Functional analysis based on leaf paint was done to detect *bar* gene activity in the segregating progenies of transgenic plants. Filial generation of confirmed transgenic plants were raised and analysed for the inheritance of the transgene.

The molecular analysis of successfully grafted *in vitro* putative transgenic plants showed the stable integration of the transgene construct in the analysed clones. Further molecular analysis of the filial generations from confirmed transgenic clones showed the inheritance of the introduced transgenes to the next generations (T1, T2 and T3). So far, T4 generation was obtained and further analysis to select homozygous transgenic plants is ongoing. Leaf paint test as functional analysis using herbicide solution, showed a clear difference between transgenic and control non-transgenic plants. The herbicide treated leaves of non-transgenic control plants showed necrosis after 5–7 days of herbicide application while the herbicide treated leaves of transgenic plants showed no sign of necrosis. In general, the molecular and functional analysis from this study confirmed the integration and inheritance of the introduced GOIs. Any confirmed homozygous *cry1Ac* transgenic lines from this study would be very valuable in the effort of gene stacking in pea improvement.

Keywords: *cry1Ac* gene, *Pisum sativum*, functional analysis, insect resistant, molecular analysis, transgenic pea