Tropentag 2012, Göttingen, Germany
September 19-21, 2012

Conference on International Research on Food Security, Natural Resource Management and Rural Development organised by:
Georg-August Universität Göttingen and University of Kassel-Witzenhausen

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

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Introduction

Pea (*Pisum sativum* L.) is one of the economically important legume crop cultivated worldwide for different purposes such as human consumption and animal feed (Oelke et al., 1991). However, pea production and storage is constrained by different species of insect pests such as pea moth (Legowski and Gould, 1960) and weevil (Clement et al., 2002). Worsening the problem, trait for resistance is lacking in the species' gene pool for most of the problematic pests (Clement et al., 2002; Keneni et al., 2011). Thus, development of insect resistant varieties is one of the main goals of breeding and improvement efforts in many producing countries. In line with this, genetic engineering can complement the conventional breeding strategy through widening access to resistance genes beyond the species gene pool. During the last few decades, a number of reports on pea transformations were published (Schroeder et al., 1993; Richter et al., 2006; Hassan et al., 2009). However, little attention was given to the development of insect resistant pea varieties. Hence, this study was conducted with the objective of developing insect resistant transgenic pea line. The result from this study would be useful both from breeding and production point of view.

Material and Methods

In this study, *in vitro* putative transgenic pea plants transformed with *Agrobacterium* strain EHA105 harboring binary vectors pSoup-pGIICry1Ac (Aftabi, 2011) were micro-grafted on etiolated seedling rootstock and used for PCR detection of transgene integration.

DNA was isolated from leaves using CTAB method (Doyle and Doyle, 1990) and PCR analysis of putative transgenic plants and subsequent filial generations was conducted using transgenes (*cry1Ac* and *bar* genes) specific primers, as well as HMG primers as internal control. Leaf paint functional assay (Schroeder et al., 1993) was used to detect *bar* gene activity in the segregating progenies of transgenic plants by applying 600 mg/l BASTA® herbicide solution.

Results and Discussion

The molecular analysis of successfully grafted *in vitro* putative transgenic plants showed the stable integration of the transgene into the analyzed clones i.e., A2, B3, BR, C, C1, D4R, DA and DqR (Figure 1 A, B, C). Further molecular analysis of the filial generations (T1-T4) from confirmed transgenic clones showed the inherence of the introduced transgenes to the next generations (Figure 1 D).The result of RT-PCR analysis and immunostrip assay of selected lines showed the expression of the introduced *cry1Ac* gene at RNA and protein level.

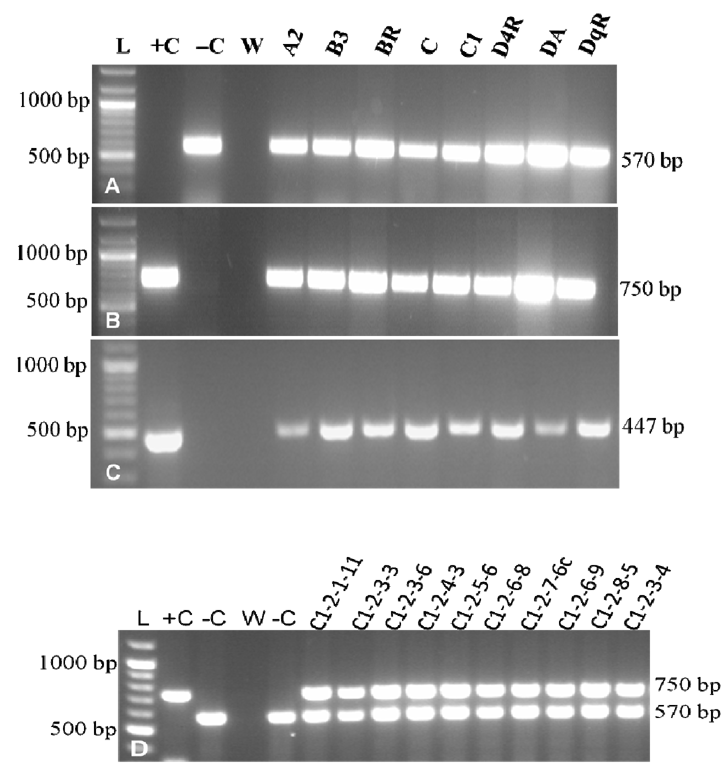

Figure 1. The genomic integration of T-DNA in the developed transgenic pea plants. PCR analysis of putative transgenic clones using genes specific primers: A) *HMG* (product size of 570 bp), B) *cry1Ac* (product size of 750 bp) C) *bar* (product size of 447bp). D) Multiplex PCR detection of *cry1Ac* and *HMG* genes in T4 generation plants indicating the stable integration and inheritance of the introduced transgene. L: GeneRuler™100 bp plus DNA ladder, +C: plasmid DNA (pGII35S-cry1Ac) as positive control, -C: Non-transgenic plant as negative control, W: water control.

Leaf paint functional analysis showed a clear difference between transgenic and non-transgenic control plants (Figure 2). The herbicide treated leaves of non-transgenic control plants showed a clearly observable necrosis. However, the leaves of progenies from transgenic clones showed both tolerance and susceptibility to herbicide application. Based on the state of herbicide treated leaf, individual plant was classified as *bar* gene positive, partially positive and negative plants. Similar observation was reported in transgenic pea expressing antifungal gene (Richter et al., 2006).This result is in line with the expectation due to segregation when the parental line is not homozygous.

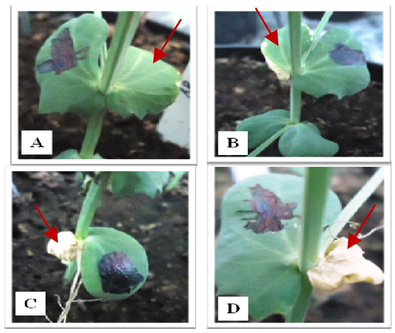

Figure 2. Leaf paint functional assay result indicating herbicide tolerance difference between transgenic and non-transgenic control plants. A) *Bar* gene positive transgenic plant, B) Partially *bar* gene positive transgenic plant, C) *Bar* gene negative transgenic plant, and D) Non-transgenic control plant. The arrows indicate herbicide treated leaf and the mark indicates the control leaf.

Conclusions and Outlook

In general, the molecular and functional analysis from this study has confirmed the genomic integration and heritance of the introduced GOIs. The developed transgenic lines can be considered for further studies such as transgene stacking with already developed transgenic lines as well as feeding tests.

Acknowledgement

Alemayehu Teressa Negawo is thankful to DAAD for the scholarship award.

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