Advance in Grain Legumes Genetic Transformation: The case of GM Pea and Cowpea

Alemayehu Teressa Negawo[1,2*], Fathi Hassan[2], Edgar Maiss[3] and Hans-Jörg Jacobsen[2]

[1]International Livestock Research Institute. Box 5689, Addis Ababa, Ethiopia;

[2]*Gottfried Wilhelm Leibniz Universität Hannover, Institute for Plant Genetics, Section of Plant Biotechnology, Herrenhäuser str.2, 30419 Hannover, Germany.*

[3]*Gottfried Wilhelm Leibniz Universität Hannover, Institute of Horticultural Production Systems, Section of Phytomedicine, Herrenhäuser Str. 2, D-30419 Hannover, Germany.*

Introduction

Grain legumes are agronomically and socio-economically important crops playing substantial roles in providing dietary protein for millions of households in the world (Gepts et al., 2005). They also fix atmospheric nitrogen, thus contributing to the sustainability of farming systems by enriching soil fertility and maintaining the productivity of agricultural land (Ferguson et al., 2010). However, different production factors have limited the productivity of grain legumes and are impacting their contribution to food security and poverty alleviation. Furthermore, in the current trend of climate change, there is an increasing pressure on plant breeders to develop climate-smart crop varieties. To enhance the economic contribution of grain legumes, genetic transformation approaches have been used to develop transgenic lines with new traits such as resistance to insects and disease and tolerance to drought. In this paper, the experience and result of pea and cowpea *Agrobacterium*-mediated transformation will be presented. Especially emphasis will be given to the success and challenges of transgenic insect resistance and its importance in these two important grain legumes. Finally, recommendation will also be discussed for future genetic transformation to develop climate-smart variety of transgenic grain legumes.

Genetic transformation of grain legumes

Overview of pea transformation steps (modified from Schroeder et al., 1993) is shown in Fig 1.

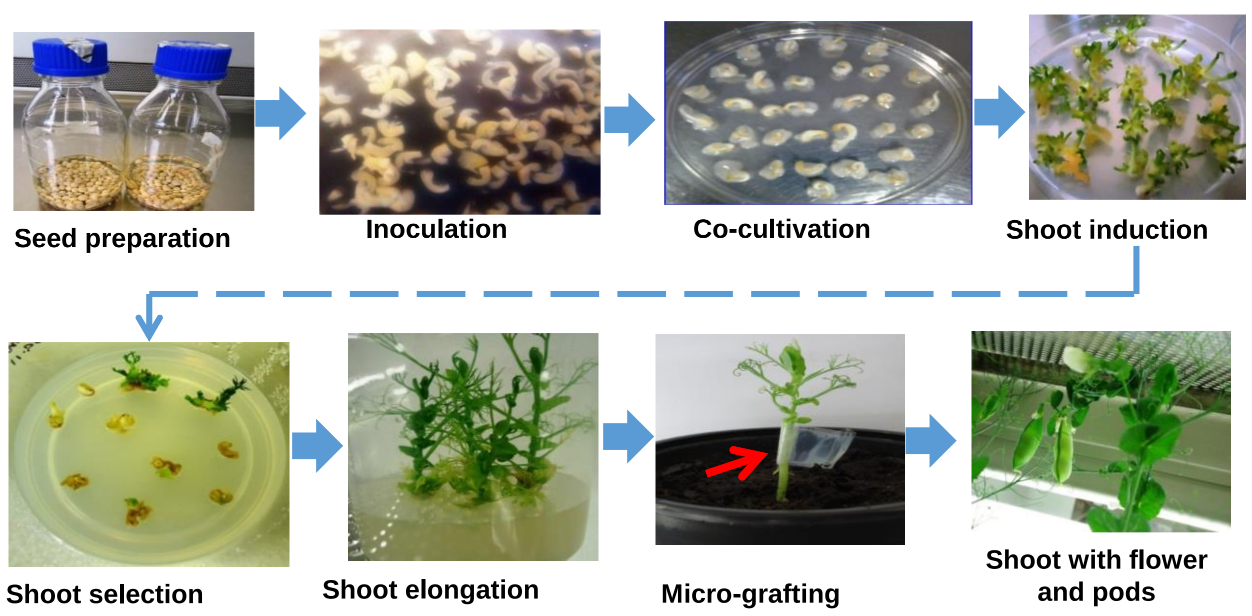
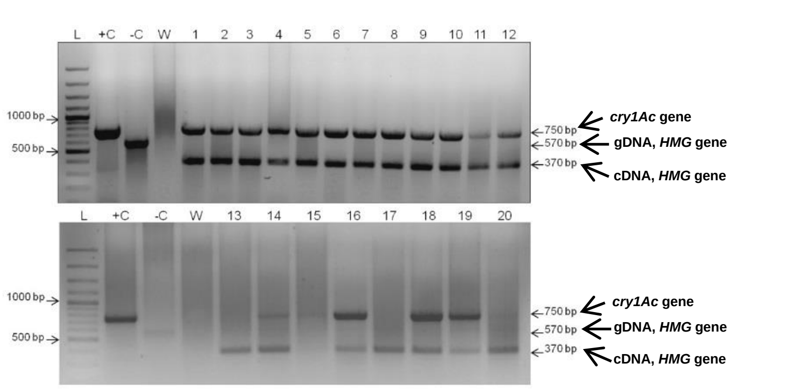


Fig 1. Pea transformation step (Negawo et al., 2013)

- The same transformation procedure has used to introduce a number of transgenes into pea for enhancing disease resistance and abiotic stress tolerance (Richter et al., 2006; Hassan et al., 2009).

Molecular Characterizations of Cry1Ac Transgenic Pea Lines and Their Progenies

- Stable integration and expression of the cry1Ac gene has been demonstrated(Fig 2 and 3).



L: GeneRulerTM 100 bp plus DNA ladder, +C: plasmid (pGII35S-cry1Ac) DNA as a positive control, -C: genomic DNA of non-transgenic pea plant as a negative control; W: water control, lane 1-12 and 14-20: cDNA from different transgenic lines and lane 13: cDNA from non-transgenic control plant.

Fig 2. Expression of *cry1Ac* gene in transgenic pea lines

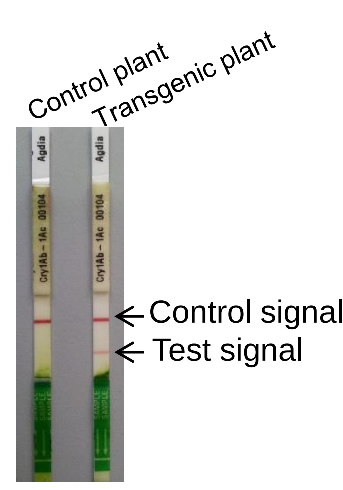


Fig 3. Immunostrip detection of Cry1Ac protein

Insect bioassay:

- High level of larvae mortality and reduced feeding damage on transgenic plants (Fig 4)

Fig 4. larvae Feed damage on control and transgenic pea plants

Control plants **Transgenic plants**

Achievement in pea transformation:

- ✓ Transgenic pea lines expressing *cry1Ac* gene were successful developed which
  - could play a vital role in pea production and improvement program and
  - can also be used in gene pyramiding with other transgenic type.

Overview of Cowpea transformation

Overview of the transformation procedure is shown in Fig 5. Using the transformation procedure, a number of putative transgenic lines were regenerated (Fig 6).

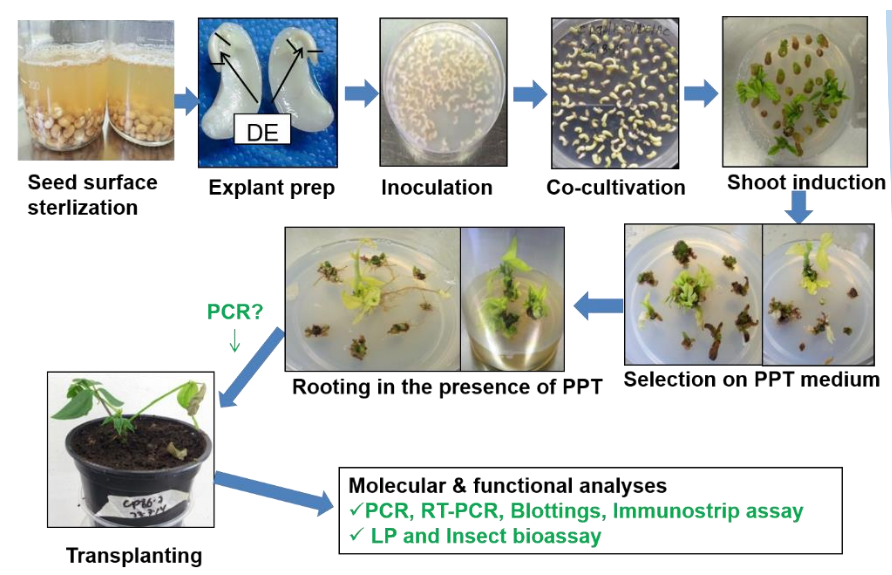


Fig 5. Overview of the optimized cowpea transformation step

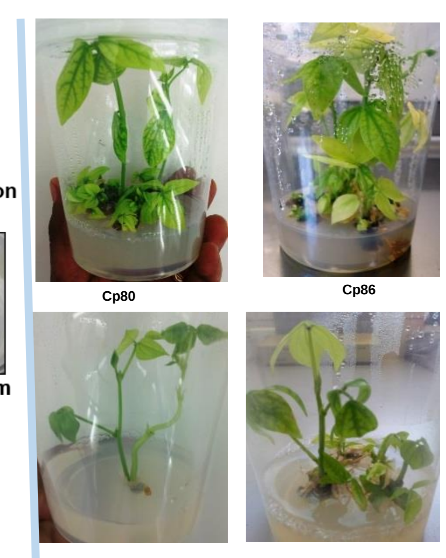
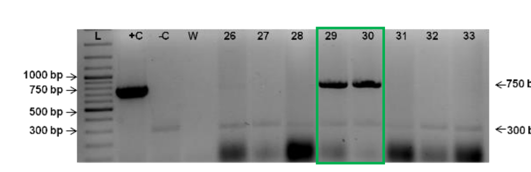


Fig 6. *In vitro* putative transgenic shoots

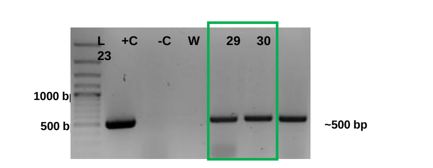
Molecular analysis of putative transgenic cowpea shoots

The result of PCR analysis showed the genomic integration of Cry1Ac gene in two putative lines (Fig 7). RT-PCR and immunostrip assays showed the expression of the introduced *cry1Ac* gene at mRNA and protein levels, respectively. Seeds were collected from the transgenic lines for further molecular, segregation and functional analyses.

Primers for *cry1Ac* gene



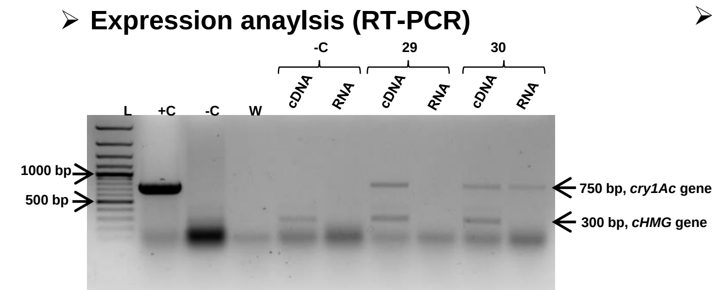
Primers for *bar* gene



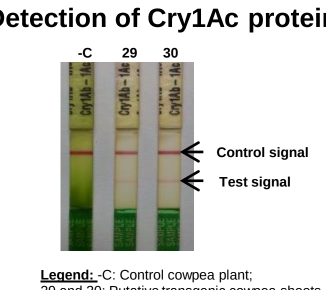
L: GeneRuler™ 100 bp plus DNA ladder, **+C:** plasmid (pGII35S-cry1Ac) DNA as a positive control, **-C:** genomic DNA of non-transgenic cowpea plant as a negative control, **W:** water control and **Lane 26-33:** genomic DNA from putative transgenic shoots of cowpea

→Transformation efficiency: 2 out of 246 (0.81 %)

Expression anaylsis (RT-PCR)



Detection of Cry1Ac protein



Legend: -C: Control cowpea plant; 29 and 30: Putative transgenic cowpea shoots

Fig 6. Expression analysis of transgene *cry1Ac* gene in the putative transgenic shoots of cowpea.

Achievement in cowpea transformation :

- ✓ *In vitro* conditions for *Agrobacterium–mediated* transient transformation has been optimized.
- ✓ Limited transformation success was obtained with the GOI.
- ✓ The result of this study highlights the recalcitrance of cowpea to *in vitro* conditions and the need for further study to optimize a more efficient protocol for cowpea transformation.

Conclusion:

- While recent achievements in a few legume species are encouraging, genetic transformation in many pulses is still difficult due to their recalcitrance.
- Experience from successfully transformed legumes (e.g., pea, soybean, etc.) could help to address some of the challenges for difficult-to-transform legume species to develop climate-smart varieties.

References

Chaudhury, D., Madanpotra, S., Jaiwal, R., Saini, R., Kumar, P. A. & Jaiwal, P. K. 2007. *Agrobacterium tumefaciens*-mediated high frequency genetic transformation of an Indian cowpea (*Vigna unguiculata* L. Walp.) cultivar and transmission of transgenes into progeny. *Plant Science,* 172**,** 692-700.

Diouf , D. & Hilu, K. W. 2005. Microsatellites and RAPD markers to study genetic relationships among cowpea breeding lines and local varieties in Senegal. *Genetic Resources and Crop Evolution,* 52**,** 1057-1067.

Ehlers, J. D. & Hall, A. E. 1997. Cowpea (*Vigna unguiculata* L. Walp.). *Field Crops Research,* 53**,** 187-204.

Fatokun, C. A. 2002. Breeding cowpea for resistance to insect pests: attempted crosses between cowpea and *V. vexillata*. In: Fatokun CA, Tarawali SA, Singh BB, Kormawa PM, Tamo M (eds) *In:* C.A, F., S.A, T., B.B, S., P.M, K. & M., T. (eds.) *Challenges and opportunities for enhancing sustainable cowpea production. Proceedings of the world cowpea conference III, held at the International Institute of Tropical Agriculture, IITA, Ibadan, Nigeria.*

Ferguson, B.J.; Indrasumunar, A.; Hayashi, S.; Lin, M.H.; Lin, Y.H.; Reid, D.E.; Gresshoff, P.M. Molecular analysis of legume nodule development and autoregulation. *Journal of Integrative Plant Biology*, 2010 *52*(1), 61-76.

Gepts, P.; Beavis, W.D.; Brummer, E.C.; Shoemaker, R.C.; Stalker, H.T.; Weeden, N.F.; Young, N.D. Legumes as a model plant family. Genomics for food and feed report of the cross-legume advances through genomics conference. *Plant Physiology*, 2005,*137*(4), 1228-1235.

Hassan, F., Meens, J., Jacobsen, H.J. and Kiesecker, H., 2009. A family 19 chitinase (Chit30) from Streptomyces olivaceoviridis ATCC 11238 expressed in transgenic pea affects the development of *T. harzianum in vitro*. *Journal of biotechnology*, *143*(4), pp.302-308.

Negawo, A.T., Afatbi, M., Jacobsen, H.J., Altosaar, I. and Hassan, F.S., 2013. Insect resistant transgenic pea expressing *cry1Ac* gene product from Bacillus thuringiensis. *Biological Control*, *67*(3), pp.293-300.

Richter, A., de Kathen, A., de Lorenzo, G., Briviba, K., Hain, R., Ramsay, G., Jacobsen, H. J. & Kiesecker, H. 2006. Transgenic peas (*Pisum sativum*) expressing polygalacturonase inhibiting protein from raspberry (*Rubus idaeus*) and stilbene synthase from grape (*Vitis vinifera*). *Plant Cell Reports,* 25**,** 1166-1173

Schroeder, H.E., Schotz, A.H., Wardley-Richardson, T., Spencer, D. and Higgins, T.J., 1993. Transformation and regeneration of two cultivars of pea (*Pisum sativum* L.). *Plant Physiology*, *101*(3), pp.751-757.

Solleti, S.K., Bakshi, S. and Sahoo, L., 2008. Additional virulence genes in conjunction with efficient selection scheme, and compatible culture regime enhance recovery of stable transgenic plants in cowpea via *Agrobacterium tumefaciens*-mediated transformation. *Journal of biotechnology*, *135*(1), pp.97-104.

***For corresponding: alemayehu_teressa@yahoo.com**

BMZ Federal Ministry for Economic Cooperation and Development
giz Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH
ILRI INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE better lives through livestock
CGIAR science for a food secure future
ILRI is a member of the CGIAR Consortium