Transgenic Cowpea: Promising Solution for Cowpea Cultivation Challenges in Agriculture

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Abstract

Conventional breeding faces hurdles due to the apparent lack of resistant genes and cross-incompatibility with wild vigna species. Genetic engineering, specifically transgenic approaches using Agrobacterium tumefaciens, emerges as a promising solution. Successful attempts, spanning four decades, have demonstrated the feasibility of generating transgenic cowpea, with recent studies showcasing the utilization of the insecticidal protein Vip3Ba1 from Bacillus thuringiensis to confer pest resistance. This concludes that transgenic cowpea lines, such as TCL-709 and TCL-711, can be served as the potential and valuable sources of resistance against the maruca pod borer, offering hope for the development of maruca-resistant cowpea varieties to benefit African farmers under natural field conditions.

Introduction

Cowpea is a annual, herbecious, drought tolerant, grain legume. It is used for its grain, tender pod and it is also used as fodder for animals. Because of its great economic importance, cowpea has been the target of breeding programs for many years in different centers of research the world over. The ultimate goal of these programs is to address agronomic problems such as attack by virus, bacteria, pests and diseases. Unfortunately, the apparent unavailability of resistant genes in the plant has rendered the conventional breeding approach less attractive in strategies for producing pest-resistant genotypes of cowpea. This task is even more herculean given the cross-incompatibility between the cultivated and wild vigna species that may possess some inherent resistance. Genetic engineering offers important strategies with promising results in addressing not only problems caused by pests and diseases, but also by viruses.

In the last four decades, a number of attempts have been made to generate transgenic cowpea. The general approach in most cases has been through the employment of *Agrobacterium tumefaciens* as a gene delivery system via tissue culture.The first of such reports appeared in 1986, when Garcia transformed leaf discs from primary leaves of cowpea using *A. tumefaciens* designed with Ti plasmid harboring two identical chimeric genes for kanamycin resistance, it took close to a decade to produce the first transgenic cowpea plants when Muthukumar (1996) co-cultured explants of cotyledons with disarmed *A. tumefaciens* expressing the gene for resistance against hygromicin.

Bett *et al.* (2019) utilized Agrobacterium-mediated gene delivery to explants derived from the cotyledonary nodes of imbibed cowpea seed. The explants were regenerated following a sonication procedure and a stringent selection comprising alternating regimes of kanamycin and geneticin. The method was reproducible and led to the recovery of independent fertile transgenic plants in the greenhouse at a level of about one per cent of starting explants. A transgene encoding an insecticidal protein from *Bacillus thuringiensis* was used to demonstrate the efficacy of the system.

Bett *et al*. (2017) collected of 224 Bt isolates which were screened with gene-specific primers to identify those containing target vip genes namely vip3Aa35, vip3Af1, vip3Ag, vip3Ca2 and vip3Ba1. The coding sequences of the vip3 genes were cloned and over-expressed in *Escherichia coli* to produce Vip3 protein. The proteins were incorporated into Maruca artificial diets for use in insect bioassays with MPB larvae to screen for toxicity, of these, Vip3Ba1 protein was found to strongly inhibit larval growth and was selected as the candidate gene for cowpea transformation.

Cruz *et al.* (2014) produced ten cowpea transgenic lines presenting a normal phenotype and transferring the transgene to the next generation. Plants were tested for resistance to both Cowpea severe mosaic virus (CPSMV) and Cowpea aphid-borne mosaic virus (CABMV) by mechanical coinoculation. Seven lines presented milder symptoms when compared to the control and three lines presented enhanced resistance to both viruses. Northern analyses were carried out to detect the transgene-derived small interfering RNA (siRNA) in leaves and revealed no correlation between siRNA levels and virus resistance. Additionally, in the symptomless resistant lines the resistance was homozygosis-dependent. Only homozygous plants remained uninfected while hemizygous plants presented milder symptoms.

Mohammed *et al*. (2014) crossed two transgenic events TCL-709 and TCL-711 were crossed to three non-transgenic lines IT97K-499-35, IT93K-693-2 and IT86D-1010 to generate six set of F_1 hybrids. The study was designed and conducted to evaluate the performance of transgenic cowpeas and hybrids derived from them under natural field conditions and concluded that transgenic cowpea lines can be use as precious source of resistance to maruca pod borer in the development of maruca resistant cowpea varieties for the benefit of African farmers.

Kumar *et al*. (2021) developed transgenic cowpea plants expressing *Bacillus thuringiensis* Cry2Aa insecticidal protein using *Agrobacterium tumefaciens*-mediated transformation of cotyledonary explants. T_0 plants recovered from *Agrobacterium* cocultured explants on medium containing 120 mgl^{-1} of kanamycin were identified on the basis of the presence of transgenes by PCR, their integration into genome by Southern hybridization and expression of their transcripts by semi quantitative PCR (sqRT-PCR) and quantitative Real–time-PCR (qRT-PCR) and protein by Western blot analysis. The transformation efficiency obtained was 3.47% with 11 independent T_0 transgenic lines. The bio efficacy of Cry2Aa protein expressed in randomly selected four T_0 plant's leaves and pods was evaluated by feeding Maruca pod borer demonstrated a significant lower damage and a high level of Maruca mortality (more than 90%) for all these *Bt* lines. The inheritance of transgenes from T_0 to T_1 progeny plants was demonstrated by PCR analysis. The transgenic plants generated in this study can be used in cowpea breeding program for durable and sustainable legume pod borer resistance.

Current status and Future Prospects

Currently, various research groups in countries possess well established transformation systems which can be harnessed to improving cowpea. The recalcitrant nature of cowpea for in vitro regeneration has been tackled and cowpea carrying a gene of cry1ab has been commertialized. Recently in Nigeria (2019) the world's first genetically modified (GM) cowpea named Sampea 20-T was registered and approved for release to smallholder farmers in Nigeria. The new variety carries a microbial insecticidal gene making it resistant to a major pest that affects this crop.

The demand for genetically improved cowpea is increasing because of its immeasurable value for food, feed and soil improvement. However, new techniques are needed in pest management systems, due to continuous development of resistance against the existing control techniques. With the emergence of promising new tools like the CRISPR/Cas9 system, it is expected that the speed with which new and desirable traits are effectively inserted into cowpea genome has improved.

Conclusion

Currently, various research groups in countries including Australia, Brazil, India and Nigeria possess well established transformation systems which can be harnessed to improving cowpea, a hitherto unreachable goal by means of traditional breeding. Challenges posed by pests and disease can be tackled through systematic application and optimization of the protocols arising from the efforts of the last three decades. In the last few years, significant progress has been made to establish different protocols and their application in the development of transgenic cowpea with one type of characteristic or another. (Citadin *et al*., 2011)

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