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Exciting Times for Cowpea Genetic Transformation Research

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Abstract: Cowpea represents a major food crop for the poor in Asia and especially in Africa. However, its production is constrained principally by insect pests as well as diseases. Attempts to improve cowpea insect resistance have not yielded significant result till date. This paper reviews biotechnological approaches that have been employed to transfer foreign genes into cowpea with a view to conferring desirable traits on it. The recent advances made in generating cowpea transformants with stable inheritance of trangenes by the progenies heralds exciting times for the genetic transformation research on this erstwhile recalcitrant food crop. As such, this genetic transformation approach could be used to transfer insect resistance traits to the crop species.

Key words: cowpea, genetic transformation, developing countries, food security

INTRODUCTION

These are exciting times to study genetic transformation of cowpea, using genes that confer desirable agronomic traits to it. For two decades, scientists in this field made great effort with little success to develop reliable transformation systems for the crop that is very important as a good source of protein and energy for people in developing countries of Africa and Asia. In Africa, it is estimated that over 200 million people consume cowpea daily. Worldwide, cowpea is cultivated on a total area of over 10.5 million hectares, with a total production of 3.9 million tons (FAOSTAT, 2006). In spite of the great importance of this crop to the people where it is grown, its productivity is very low due to many biotic and abiotic stress factors. These factors include attack of insect pests and disease pathogens, as well as drought and heat. Although, considerable progress has been made, through conventional genetics and breeding, on cowpea improvement over the decades with respect to resistance to most of these stress factors (Singh, 2007), however, the problem of insect pests still remains largely unresolved. Hence genetic engineering approaches stand out as the only effective alternative means of transferring genes that confer desirable agronomic traits like insect resistance to cowpea. The success of these approaches, however, depends largely on the availability of reliable and efficient methods of transformation. This review concentrates on advances in cowpea genetic transformation over the past two decades. It also highlights some implications for food security in the developing countries of sub-Saharan Africa.

Early Work on Cowpea Transformation:

Garcia's group in the Netherlands pioneered study on genetic transformation in cowpea. They used an *Agrobacterium*-mediated system to transform cowpea leaf disc explants (Garcia *et al* 1986a, 1986b). Although stable transformation was achieved in both investigations, as confirmed by Southern analysis, however, transgenic calli failed to regenerate into mature plants. Similarly, Perkins *et al.* (1987) and Filippone (1990) were not able to regenerate their transgenic calli that were generated from different explants co-cultivated with *Agrobaterium tumefaciens*. Grain legumes generally have been regarded as recalcitrant to transformation because of poor regeneration ability (especially via callus), and also because *in vitro* regeneration is genotype specific. Worst still, most cowpea cultivals are infrequently amenable to regeneration. There is also the problem of compatible gene delivery system. Excellent reviews on legume transformation are also available (Somers *et al*., 2003, Chandra and Pental, 2003, Popelka *et al.*, 2004).

Owing to the failure of callus to regenerate plants, focus was shifted to organogenic regeneration for cowpea transformation. Using longitudinal embryo slices co-cultivated with *A. tumefaciens*, Penza *et al.* (1991)

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reported only transient expression but no evidence of stable integration of the introduced genes. Penza's group and another group in the US later separately resorted into exploring the transformability of intact cowpea embryonic tissues using the electroporation method, using naked DNA in the presence of protectants such as spermine but it was merely transient gene expression that was achieved for all the efforts (Penza *et al*., 1992, Akella and Lurquin, 1993). Muthukumar's group also avoided the callus regeneration route by co-culturing de-embryonated cotyledons with *A. tumefaciens* and selected four plants on hygromycin. Stable transformation was confirmed by Southern analysis in only one of the transgenic plants, whose seeds unfortunately failed to germinate (Muthukumar *et al*., 1996). Hence, there was no evidence of stable transformation. Similarly, the work of Sahoo *et al*. (2000) only produces transgenic shoots but did not provide evidence of stable integration. Furthermore, Ikea (2003) using an entirely different method of DNA delivery known as particle bombardment for cowpea transformation, observed transformation in cowpea. However, further molecular evidence of stable transformation with Mendelian transmission of the transgenes to progeny was not provided.

Recent Advances in Cowpea Transformation:

Cracking the hard nut of cowpea transformation is critical to the maximal deployment of recombinant DNA technology for genetic improvement of this very important crop of the poor. Popelka *et al.* (2006) is the first report on cowpea transformation where transgenic cowpeas obey the Mendelian rule of transmitting the transgenes to their progeny. The workers have really used brute force to ensure stable integration of the transgene. They used a hypervirulent Agrobacterium strain, AGL1 that habours a binary vector pBSF16 that contains a reporter gene (*uid*A), encoding the β-glucuronidase (GUS) and a selectable marker gene (bar), which encodes phosphinothricin acetyl transferase (PAT) – a highly reliable selectable marker. Furthermore, the co-cultivation was extended to 6 days, coupled with the addition of thiol-compounds during infection and co-cultivation with *Agrobacterium*. Additionally, they cautiously employed a lengthy regeneration procedure that ensured there were no escapes from the selection regime, but which took between 5 to 8 months from explants preparation to harvest of T_1 seeds. Slightly improved transformation efficiency from 0.05% to 0.15% (Popelka *et al.,* 2006) to 0.76% was reported by Chaudhury *et al.* (2007).They used cotelydonary nodal explants as Popelka's group but they inflicted wounds on the nodal cells by stabbing with sterile needle prior to *Agrobacterium* infection. Additionally, they introduced a second selection regime at the rooting stage, the procedure that has been found to be critical in the transformation of other related species (Saini and Jawail, 2005). Employing the biolistic method of gene transfer, Ivo *et al*. 2008, reported 0.9% transformation efficiency from bombarded embryonic axes, thereby presenting the first work on the use of this approach for generating transgenic cowpea plants that obey the Mendelian law. Yet, much higher transformation efficiencies from 1.64% to 1.67% have been reported recently by Sahoo's group in India. This remarkable increase in the transformation efficiency was attributed to the constitutive expression of additional virulence genes in the LBA4404 *Agrobacterium* strain coupled to a regimen of geneticin selection at 45 mg/l (Solleti *et al*. 2008a), a more effective selective agent than phosphinothricin and kanamycin that were used by Popelka *et al*. 2006 and Chaudhury *et al*. 2007, respectively. The group also reported heterologous expression of the common bean α-amylase inhibitor 1 gene in the transgenic seeds (Solleti *et al*. 2008b), which is probably the first report on the expression of gene that confers desirable agronomical trait on the cowpea plant. These recent successes in cowpea genetic transformation have therefore pave way for the introduction of more agronomic traits to cowpea, thereby enhancing the genetic diversity of the crop, and consequently complementing existing breeding programmes.

Implication for Food Security in Developing Countries:

The world population is growing at a fast pace, and it is projected to be well over 7 billion by 2020. It is pathetic to note that the developing world, which is under pressure with the challenge of food insecurity, is contributing most to this increase (Pardey and Wright, 2002). This paints a bleak picture of the future's food security, especially in the developing countries of the sub-Saharan Africa; as food production is currently not matching up with the population growth. With over 200 million Africans depending on cowpea daily, as the major source of protein and vitamins, common sense suggests that the current production level of the crop should be increased dramatically in order to prevent a major future crisis. Attack of insect pests remains the major constraint to cowpea production and storage. The recent advances in cowpea transformation have sparked new hope that the menace of insect pests can be dramatically reduced by introducing more resistance genes into cowpea. This would hopefully enhance crop production and seed quality in storage, and consequently provide higher incomes for the poor farmers.

Directions:

Since a good number of insect-resistance genes, such as those that encode plant lectins, α-amylase inhibitors, protease, chitinases, as well as *Bacillus thuringiensis* crystal proteins are available, an important immediate challenge is to aggressively transform these genes into elite cowpea lines. Consideration could also be given to gene pyramiding strategies in order to accumulate insect resistance in the crop, as a way to ensure effective resistance to most of the insect pests. Hence, collaborative efforts should be facilitated among scientists working in this field, particularly in the concerned regions, to generate tailor-made cowpea plants that can now successfully challenge these ravaging pests in their respective agro-ecological zones.

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