Genetic transformation of pigeonpea: An overview

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Abstract: Biotechnology over the years has emerged as a promising tool to overcome biotic and abiotic constraints in crop species that lack the required traits for crop improvement through conventional and molecular breeding approaches. New engineering tools are now available not only for single gene traits, but also to engineer multiple genes or plant regulatory machinery for driving the expression of different stress-responsive genes. Here, we discuss the recent progress and current status of transgenic technology in pigeonpea towards developing host plant resistance to various biotic and abiotic stresses and its use in the improvement of this important pulse crop.

Key words: genetic transformation, pigeonpea, trait, transgenic, tissue culture

Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is an important pulse or grain legume crop cultivated in semi-arid tropical and subtropical areas of the world that ranks third in Asia and sixth in the global pulse production after beans (*Phaseolus* spp.), pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), broad bean (*Vicia faba* L.) and lentil (*Lens culinaris* Medik.). Most of the differences in potential yields and the actual harvests by pigeonpea farmers have been attributed to several biotic and abiotic stress factors, besides the low productivity potential of marginal lands, where this crop is commonly grown.

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Genetic improvement of pigeonpea has been restricted due to the non-availability of suitable genetic resources and strong sexual barriers between the cultivated and wild species. Recombinant DNA and genetic transformation technologies can circumvent limitations due to taxonomic barriers and limited gene pool for resistance to pathogens, insect pests and tolerance to various abiotic stresses. Further, molecular biological techniques provide capabilities to engineer host plant resistance that is effective against both the specific and to a broad spectrum of pathogens and are genetically stable. However, for effective use of genetic engineering techniques, efficient regeneration and recovery of stable transformants is essential.

Tissue culture and transformation systems

Various laboratories have reported successful regeneration of pigeonpea by direct and indirect organogenesis from a variety of explants ranging from hypocotyls, immature embryos, leaves, cotyledons, nodal halves of cotyledons, and cotyledon-derived nodular callus (Fig. 1). Amongst the various explants used, direct organogenesis from cotyledonary node and leaf explants appear to be highly reproducible and efficient for use in genetic transformation studies. The type of genotype, physiological maturity and the nature of tissues (vegetative or reproductive) appear to be important factors

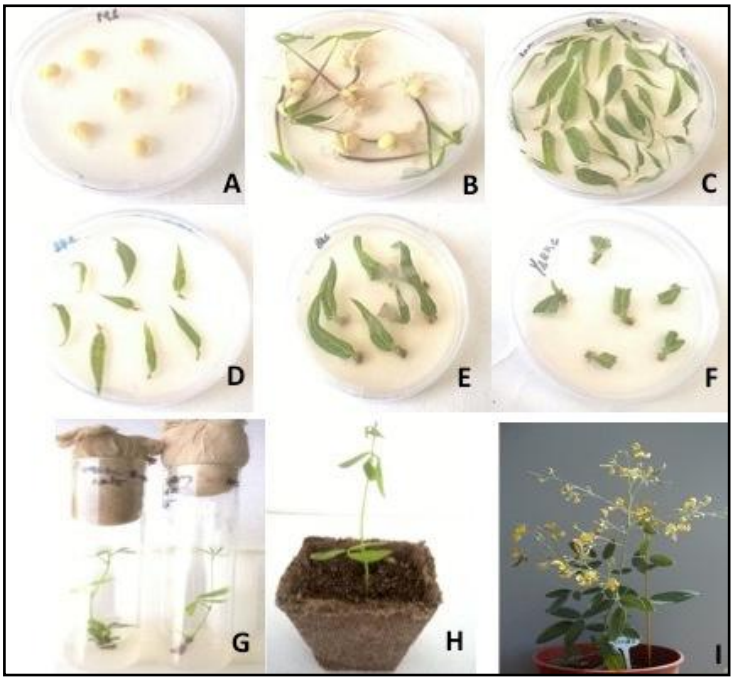

Figure 1. Regeneration of adventitious shoots and development of plants from leaf explants of *Cajanus cajan* L. (Millsp.): (C & D) leaf explants at 0 d on MS medium supplemented with 5 ⌠*M* BA and 5 ⌠*M* kinetin; (E) differentiation of shoot buds from petiolar cut end; (F) leaf explants with proliferating multiple shoots on shoot development medium with half-cut lamina; (G) individual shoot on MS medium supplemented with 0.58 ì*M* GA3 (elongation medium) after 7 d; (H) well-rooted putative transformant after hardening; (I) a well-established transgenic pigeonpea plant at flowering stage

besides the growth regulators to bring about the expected response in pigeonpea tissue cultures. Although pigeonpea was considered to be recalcitrant for long, recent reports on its genetic transformation with convincing molecular evidence indicate a feasibility of *Agrobacterium*-mediated genetic trans-formation. *Agrobacterium*-mediated gene transfer using leaf discs, shoot apics and cotyledonary nodal explants in pigeonpea was obtained (1, 2, 6, 7, 8), as well as a non-tissue culture-based method of generating transgenic pigeonpea using *Agrobacterium tumefaciens*-mediated trans-formation of injured embryonal axes (5).

Status of transgenic research in pigeonpea

Several transgenic traits have been incorporated in pigeonpea such as insect resistance, protein quality, and edible vaccines. Nevertheless, resistance to *Helicoverpa armigera* Hübner (legume pod borer) in pigeonpea is an ideal seed borne solution to enhance its productivity through an integrated pest management. *H. armigera* is the most important yield constraint in pigeonpea for which there is no absolute resistance available in the cultivated germplasm. Besides the genes producing insecticidal proteins from *Bacillus thuringienses* Berliner (Bt) and proteases, insecticidal chitinase is also important in controlling the devastating pod borer by dissolution of the chitin, an insoluble structural polysaccharide that occurs in the exoskeleton and gut lining of insects. Gene pyramiding with two different insecticidal genes and tissue-specific expression to reduce the risk of developing insect resistance isanother attractive option to combat this pest for durable resistance. Expression of a chimeric *cry1AcF* (encoding *cry1Ac* and *cry1F* domains) gene in transgenic pigeonpea has been demonstrated towards resistance to *H. armigera* (4). Research activities at ICRISAT involving *Bt cry* genes have yielded promising results where a large number of transgenic events are currently being evaluated for their efficacy. These transgenics not only showed high mortality of the larva but also resisted the damage caused by the larvae.

Pigeonpea transgenics carrying *dhdps-r1* gene for the overproduction of sulfur-containing amino acids, driven by a phaseolin or an *Arabidopsis* 2S2 promoter have been developed using *Agrobacterium* transformation and particle bombardment (10). A 2- to 6-fold enhanced DHDPS activity compared to the wild type was observed in the transgenic immature seeds which reflected a 1.6 to 8.5 times enhanced free lysine content compared to the wild types. Moreover, based on the lysine degradative enzyme, lysine ketoglutarate reductase (LKR) it was revealed that at later stages of seed development, LKR activity increased in the transgenic lines, that not only increased lysine levels but also resulted in conspicuous increase in free threonine. At ICRISAT, work is ongoing to develop biofortified pigeonpea for the enhancement of β-caretone (provitamin A), is a precursor of vitamin A. Success in producing transgenic pigeonpea plants with high-levels of β-caretone will have much to contribute to the malnourished population in the drylands of the world.

Besides, there have been efforts on developing pigeonpea as potential edible vaccines for goat and sheep to rinder pest virus and peste des petits ruminants virus, using haemaglutinin gene of rinder pest virus and haemaglutinin neuraminidase gene of *peste des petits ruminants virus* (PPRV-HN) respectively (3). Similarly, a constitutively expressed P5CSF129A gene in pigeonpea transgenics showed a higher proline accumulation and a lower lipid peroxidation than their non-transformed counterparts when subjected to 200 mM NaCl (9).This manifested in an enhanced growth, more chlorophyll and relative water content under high salinity, thereby suggesting that overproduction of proline could play an important role against salt shock and cellular integrity in pigeonpea.

Conclusions

In absence of availability of traits/genes-conferring resistance to biotic and abiotic constraints in the primary/cross-compatible gene pool, application of genetic engineering technology is a viable option to address complex problems in pigeonpea cultivation. Genetic transformation of pigeonpea can be seen as a logical extension of plant breeding research that has a considerable potential to benefit the global pigeonpea production systems. Since the implications of risk assessment studies of transgenics are dependent on the social context, a participatory approach is needed to determine the balance of benefits to risks. It is critical to determine baseline data on food safety, against which the safety of these transgenics can be evaluated. While this technology is critical in overcoming severe bottlenecks associated with conventional and/or molecular breeding approaches, a challenge will be to use this technology as part of a long-term strategy to improve nutrition, preserve biodiversity, and promote more sustainable agricultural practices.

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