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Research review paper

Advances in development of transgenic pulse crops

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Abstract

It is three decades since the first transgenic pulse crop has been developed. Todate, genetic transformation has been reported in all the major pulse crops like *Vigna* species, *Cicer arietinum*, *Cajanus cajan*, *Phaseolus* spp, *Lupinus* spp, *Vicia* spp and *Pisum sativum*, but transgenic pulse crops have not yet been commercially released. Despite the crucial role played by pulse crops in tropical agriculture, transgenic pulse crops have not moved out from laboratories to large farm lands compared to their counterparts – 'cereals' and the closely related leguminous oil crop – 'soybean'. The reason for lack of commercialization of transgenic pulse crops can be attributed to the difficulty in developing transgenics with reproducibility, which in turn is due to lack of competent totipotent cells for transformation, long periods required for developing transgenics and lack of coordinated research efforts by the scientific community and long term funding. With optimization of various factors which influence genetic transformation of pulse crops, it will be possible to develop transgenic plants in this important group of crop species with more precision and reproducibility. A translation of knowledge from information available in genomics and functional genomics in model legumes like *Medicago truncatula* and *Lotus japonicus* relating to factors which contribute to enhancing crop yield and ameliorate the negative consequences of biotic and abiotic stress factors may provide novel insights for genetic manipulation to improve the productivity of pulse crops.

Keywords: Commercialization; Genetically modified crops; Transgenic pulse crops

Contents

1. Introduction

Despite significant political and regulatory barriers, genetically modified crops represent one of the most rapidly adopted technological innovations to have been commercialized in the history of agriculture (Dunwell, 2000; Fernandez-Cornejo, 2005). It is almost a decade since the first transgenic crop moved from laboratory to large farm lands. Pulse crops, well known for their inherent ability to fix nitrogen and the pivotal role they play as the major source of protein for the population of developing countries, ranking third in world food production

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after cereals and oil seed crops need further genetic improvement by incorporation of alien genes using transgenic technology to meet the increase in demand for food along with improved quality (Christou, 1997; Popelka et al., 2004). Crop yields can be improved by manipulating the physiological processes and the ability to withstand biotic and abiotic stresses. Although conventional plant breeding methods in the past have contributed to the improvement of pulse crops, transgenic technology has immense potential to achieve this objective as an additional/ supplementary technology. Despite the development of transgenic technology in 1980's and the first report on development of a transgenic pulse crop-*Vigna aconitifolia* in the same decade (Eapen et al., 1987; Kohler et al., 1987a,b), the progress achieved is not significant compared to their counterpart crops-namely cereals. Cereals were considered to be recalcitrant for regeneration in 1970's and for transformation in 1980's. However, concentrated efforts and free flow of funding support for cereal transformation have pushed transgenic cereals to the forefront of transgenic success stories (Shrawat and Lorz, 2006), while transgenic pulse crop research ,although have moved forward, is still confined to the four walls of the laboratory. Since these tropical grain legumes are of prime importance to developing countries, less efforts have been put forward compared to cereals. Besides, different factors like recalcitrance of pulses for regeneration, low competency of regenerating cells for transformation and lack of a reproducible *in planta* transformation system have been pointed out as reasons for non-development of transgenic pulse crops with high efficiency (Somers et al., 2003; Popelka et al., 2004; Dita et al., 2006). However — soybean, a leguminous oil crop and a source of protein is a successful example in commercialization of transgenics.

The major pulse crops of the world are bean (*Phaseolus vulgaris* L.), pea (*Pisum sativum* L), broadbean (*Vicia faba* L), chickpea (*Cicer arietinum* L), pigeonpea (*Cajanus cajan* L Millsp), blackgram (*Vigna mungo* L), green gram (*Vigna radiata* L Wilczek), grasspea (*Lathyrus sativus* L), lupin (*Lupinus* spp), lentil (*Lens culinaris* Medik L Walsp), cowpea (*Vigna unguiculata*) and winged bean (*Psophocarpus tetragonolobus* L) and majority of them are grown in tropical and subtropical regions of the world.

2. Development of transgenic pulse crops

Transgenic pulse crops have been produced using *Agrobacterium* — mediated (Eapen et al., 1987; Krishnamurthy et al., 2000; Sharma et al., 2006), by particle gun bombardment (Kamble et al., 2003; Indurker et al., 2007), by electroporation of intact axillary buds (Chowrira et al., 1996) and by electroporation and PEG mediated transformation using protoplasts (Kohler et al., 1987a,b). Of all the methods, *Agrobacterium* mediated transformation of explants is the most popular method for development of transgenics in pulse crops (Table 2). While antibiotic marker genes were introduced to test the feasibility of transformation (Eapen et al., 1987), other genes of economic importance such as Cry genes (Sanyal et al., 2005; Indurker et al., 2007), sunflower albumin gene (Molvig et al., 1997), α — amylase inhibitor gene (Sonia et al., 2007) and chitinase gene (Kumar et al., 2004) have also been introduced into major pulse crops (Tables 1, 2). Since most legumes regenerate from young embryonic tissues, embryonic axes (Krishnamurthy et al., 2000) and cotyledonary nodes (Kumar et al., 2004) are the most preferred explants for transformation.

3. Competent cells for regeneration

Plant regeneration in pulses like in other plants can occur through three pathways namely *de novo* organogenesis, somatic embryogenesis or through proliferation of shoot meristems from areas surrounding a shoot bud. (See Jaiwal and Singh, 2003). Among the three modes of regeneration as target cells for transormation, meristematic areas of cotyledonary nodes are the most preferred explant source as in *C. cajan* (Dayal et al., 2003; Thu et al., 2003), *C. arietinum* (Sarmah et al., 2004), *V. mungo* (Saini et al., 2003) and *P. sativum* (Pniewski and Kapusta, 2005). In some cases, *de novo* organogenesis from leaf (Dayal et al., 2003) and epicotyl (Barik et al., 2005; Indurker et al., 2007) was utilized for developing transformants, while somatic embryogenesis was employed for development of transgenic plants as in case of *Vicia narbonensis* (Pickardt et al., 1991). Introduction of genes which will induce somatic embryogenesis into grain legumes may further improve the target source with competent cells capable of regeneration and transformation. Introduction of genes such as Wuschel (Zuo et al., 2002), Baby boom (Boutilier et al., 2002) and *esr*-1(Bano and Chua, 2002) into pulse crops may enhance their regeneration potential and the regenerating tissues can subsequently be used as targets for introduction of transgenes.

4. Improving the efficiency of gene transfer

A quick method of delivering foreign gene with high transformation efficiency is required for the development of transgenic pulse crops. *Agrobacterium* mediated gene transfer is the most common method of transformation of tropical grain legumes, although particle gun bombardment is also used to develop transgenic plants. Among the pulse crops, *V. aconitifolia* is the only pulse crop reported to have regeneration of complete plants from isolated protoplasts (Eapen et al., 1987) which was exploited for genetic transformation using *Agrobacterium* (Eapen et al., 1987) and direct DNA transfer using PEG and electroporation (Kohler et al., 1987a,b). However, these methods of gene transfer into protoplasts/cells cannot be applied to other pulse crops, where totipotency of protoplasts has not yet been demonstrated. *In planta* infection/electroporation of axillary meristems of pea, cowpea and lentil for transformation, although reported by Chowrira et al. (1996, 1998) needs to be repeated by other laboratories and also extended to other pulse crops. In lentil, vacuum infiltration with *Agrobacterium* was found to enhance transient gene expression (Mahmaudian et al., 2002). Although *Agrobacterium* is the most commonly used vector for transformation, it is worthwhile trying other vectors such as *Rhizobium* (Broothaerts et al., 2005) for genetic transformation of pulse crops, since *Rhizobium* is a natural agent, which causes nodulation