Genetic Transformation of *Vigna* Species: Current Status and Future Perspectives

Darshna Chaudhary[1], Manish Sainger[1], Lingaraj Sahoo[2] and Pawan K. Jaiwal[1]*

[1]*Advanced Centre for Biotechnology, M. D. University*, Rohtak-124001, India,
[2]*Department of Biotechnology*, *Indian Institute of Technology*, Guwahati, India
* Corresponding author, e mail: jaiwalpawan@rediffmail.com

Abstract

Vigna species form an important component for vegetarian diet and sustainable agriculture. During the last four-five decades their production is reducing or stagnating, which has resulted in escalation of their market price. To meet the demand of bourgeoning population, their yield is to be enhanced and stabilized in their traditional growing areas where they are exposed to numerous abiotic and biotic stresses. Their improvement through conventional breeding is low or often not possible due to the lack of sufficient and satisfactory level of genetic variability within the germplasm. Biotechnological approaches are required to widen the gene pool beyond sexual boundaries. Much progress has been made in *in vitro* regeneration of sexually mature plants, in majority of these recalcitrant species, from seedling explants such as cotyledonary node and shoot tip via direct shoot organogenesis in *V. mungo*, *V. radiata* and *V. unguiculata* and considerable success in generating plants from callus in *V. angularis* and from protoplast in *V. aconitifolia*. These protocols are used for generation of transgenics in major cultivated *Vigna* species. This paper provides an overview of *in vitro* regeneration, state of the art, current status and critical analysis of the genetic transformation in major *Vigna* species along with future directions for their improvement.

Introduction

The genus *Vigna* has 150 species, only seven of them are cultivated, six Asian of subgenus *Ceratotropis*, *V. radiata*, *V. mungo*, *V. angularis*, *V. aconitifolia* and *V. umbellata* , and two African of subgenus *Vigna*, *V. unguiculata* and *V. subterranea*. These species are grown for their protein rich edible seeds and sprouts which are rich in minerals and vitamins. The plants are also used as fodder. All *Vigna* species fix atmospheric nitrogen and are of short duration, therefore, they are grown as mixed/ intercrop or in rotation to improve nitrogen status of soil and to break disease/pest cycles. The production of *Vigna* species has not improved significantly due to their prominent susceptibility to abiotic stresses such as salinity and drought etc and to several fungal, bacterial or viral pathogens and insect pests (see Jaiwal and Gulati, 1995, Sahoo and Jaiwal, 2008) The other yield limiting factors are their indeterminate growth habit, defective plant type, low harvest index, sensitivity to photoperiod and temperature, asynchronous flowering and shattering pods and sprouting of seeds *in situ* following rains. Genetic improvement of these species through conventional breeding has been slow due the lack of desirable and satisfactory levels of variability in germplasm. Genes conferring resistance to biotic and abiotic stresses are found in wild or related species (Tomooka *et al*., 2004, Vaughan *et al*., 2004) which are sexually incompatible with the cultivated species. The only option left is to transfer desirable genes from other sources for their quantitative and qualitative improvement through *in vitro* regeneration and genetic transformation techniques in more precise and faster ways as compared to traditional Mendelian methods. Besides crop improvement, these approaches also help in studying gene function. The present review provides the current status of genetic transformation undertaken for the improvement of *Vigna* species and outlines the future directions.

Genetic transformation of *Vigna* species

Like the large-seeded legumes, the genetic transformation of *Vigna* species have been difficult and challenging because of their recalcitrant nature to *in vitro* regeneration and genetic transformation (Sahoo and Jaiwal, 2008). During the last almost three decades significant progress has been made to transform grain legumes including *Vigna* species (Gulati and Jaiwal, 1995, Somers et al., 2003; Popelka *et al*., 2004; Eapen *et al*., 2008, Sahoo and Jaiwal, 2008). Gene transfer in these species requires a robust plant regeneration system from tissues amenable to routine transformation method, efficient selection system for the recovery of viable and fertile transgenic plants from transformed explants at a reasonable frequency and a time frame in culture to void somaclonal variation and possible sterility (Sahoo *et al*., 2003).

In vitro regeneration

A fast, reliable and efficient plant regeneration system is essential for the development of a robust

genetic transformation procedure. However, grain legumes in general and *Vigna* species in particular are highly recalcitrant to *in vitro* regeneration. In recent years significant progress has been made to regenerate them through proliferation of apical and axillary meristems, *de novo* organogenesis and somatic embryogenesis (see Jaiwal and Singh, 2003). Direct multiple shoot organogenesis from cotyledonary node and shoot tip of embryonal axis or seedling has been found more suitable for transformation as in *Vigna radiata* (Jaiwal *et al*., 2001; Sonia *et al*., 2007), *V. mungo* (Saini *et al*., 2003, Saini and Jaiwal, 2005) and *V. unguiculata* (Chaudhary *et al*., 2007).

Both these explants have pre-existing meristems which give rise to multiple shoots on induction by cytokinins and are transformation competent. In *V. angularis*, *de novo* organogenesis from epicotyl has been used for developing transformants. Regeneration via somatic embryogenesis is preferred for transformation in order to homogenously (non-chimeric) transformed plants as somatic embryos develop from single cells. Although somatic embryogenesis has been obtained from cell suspension cultures established either from primary leaf-derived embryogenic calli in *V. unguiculata* (Kulothungan *et al*., 1995; Ramakrishnan *et al*., 2005) or hypocotyl derived calli in *V. mungo* (Saini *et al*., 2004) and through embryogenic calli obtained from immature cotyledons (Girija *et al*., 2000) or primary leaf segments (Devi *et al*., 2004) in *V. radiata*, it has been very difficult to convert somatic embryos into plantlets at a high frequency, therefore, organogenesis pathways are to be followed. Further, *in vitro* plant regeneration in *Vigna* species is dependent on genotype, explant type, media composition and culture conditions. These parameters are to be optimized for establishing an optimal regeneration protocol.

Recent knowledge on in vitro regeneration will soon allow introduction of genes that promote somatic embryogenesis such as Wuschel (Zuo *et al*., 2002), *Baby boom* (Boutilier *et al*., 2002) and *esr-1* (Bano and Chuo, 2002) into grain legumes to enhance their embryogenic response and improve their regeneration potential.

Reporter and selectable marker genes

Screenable markers or reporter genes code for products that detoxify selective agents or catalyze specific reactions whose products are detectable. The β-glucuronidase (*uidA*/*gusA*) gene is widely employed as reporter for transient and stable transformation studies in legumes including *Vigna* species (see Sahoo *et al*., 2003). Localized gus activity in intact tissues can be visualized as blue-spots or -areas within the tissue. However, GUS assay is destructive in nature. The assay of green fluorescent protein (GFP) gene of the jellyfish, a reporter gene is non-destructive and do not require any cofactor or external substrate. In plants, GFP accumulation does not have any toxic effect. Use of GFP protein gene has been beneficial for screening of transformed shoots at an early stage to avoid the chimeras. None of methods developed so far for *Vigna* species, except in *V. angularis* (Yamada *et al*., 2001), explored this possibility.

Selectable marker genes are useful in selection and maintenance of transformed cells/plants as they allow the transformed cells to proliferate and grow in the presence of a selective agent while the non-transformed cells either do not grow or multiply at a slow rate. The most commonly used selectable marker gene in legumes is neomycin phosphotransferase (*nptII*) which imparts resistance to kanamycin or its analogues, geneticin or paromomycin by inactivating them through phosphorylation. Kanamycin has been used for the selection of transformants in *V. radiata* (Jaiwal *et al*., 2001), *V. mungo* (Saini et al., 2003, Saini and Jaiwal, 2005), *V. unguiculata* (Chaudhary *et al*., 2007) and *V. angularis* (Yamada *et al*., 2001). It neither impairs the normal shoot regeneration nor shoot vigor but helps in the early identification of green transformed shoots as the shoots emerging from non-transformed cells are bleached (albino). However, recovery of a large number of escapes or chimeric shoots in the presence of kanamycin is the main problem. Geneticin in conjunction with *nptII* has been found to be more effective in selection of transformed shoots in cowpea (Solleti *et al*., 2008). The hygromycin phosphotransferase (*hpt*) gene which confers resistance to hygromycin has also been used in *V. radiata* (Mahalakshmi *et al*., 2006) and *V. angularis* (El-Shemy *et al*., 2002) for the recovery of transformants with minimum escapes. The efficiency of transformation has been improved by replacing antibiotic resistance genes with genes for herbicide tolerance, as selectable marker. Bialaphos resistance gene (*bar*) which acetylates and detoxify phosphinothricin has been efficiently used for selection of transformants in *V. radiata* (Sonia *et al*., 2007), *V. mungo* (Muruganantham *et al*., 2007), *V. unguiculata* (Popelka *et al*., 2006) and *V. angularis* (Khalafalla *et al*., 2005). PPT selection is more efficient than kanamycin. Delayed application of selective agent after coculture was found to be more efficient for the recovery of transformants in *V. unguiculata* (Popelka *et al*., 2006). The concentration of selective agent (le-

thal or sublethal), time of its application (i.e., more precisely immediately after coculture or at a much delayed stage), and increments (gradual increasing or decreasing increments) need to be carefully assessed for a particular species and an explant (Sahoo and Jaiwal, 2008). Judicious choice of selective levels has remained as an important criterion for the recovery of transformed cells at initial stage of screening. Studies on the use of positive selectable markers such as phospho-mannose isomerase (*pmi*), xylose isomerase (*xyl* A) etc and the elimination of marker genes that are considered environmentally safe, are to be taken up in *Vigna* species (Eapen *et al*., 2008).

Gene transfer methods

The three methods, *Agrobacterium*-mediated, biolistics and electroporation have been used for genetic transformation of *Vigna* species. Among them, *A. tumefaciens* mediated transformation is the preferred method.

***Agrobacterium*-mediated transformation**

Agrobacterium T_i plasmid-based vector is the best system for plant transformation due to its simplicity, high frequency of precise transfer and integration of transgenes with single copy insertion, low incidence of transgene silencing and ability to transfer long stretches of DNA (Veluthambi *et al*., 2003).

Vigna radiata

Pal *et al*. (1991) were the first to recover primary transformants on kanamycin selection from cotyledons of *V. radiata* inoculated with *A. tumefaciens*. The transgenic nature of transformants was confirmed by dot blot and MUG assays. However, no evidence was presented for stabilization and inheritance of transgenes. Phogat *et al*. (1999) regenerated transgenic calli on kanamycin (100 mg/l) selection medium from primary leaf explants cocultured with *A. tumefaciens*. The transformed calli were found resistant up to 750 mg/l of kanamycin, exhibited β-glucuronidase activity and showed integration of *nptII* gene by Southern analysis. However, plants could not be recovered from transgenic calli. Jaiwal *et al*. (2001) successfully recovered fertile transgenic plants from agro-inoculated cotyledonary node explants on kanamycin selection medium at a frequency of 0.9 %. Molecular analysis of primary transformants revealed integration and expression of transgenes but no data for inheritance of transgenes to progeny was presented. Mahalakshmi *et al*. (2006) developed transgenic mungbean plants from primary leaf explant on hygromycin selection medium at a frequency of 2 %. Integration of marker gene in T_0 and its inheritance in T_1 was shown. Sonia *et al*. (2007) generated transgenic plants carrying *Phaseolus vulgaris* α-amylase inhibitor I gene, on PPT selection at a frequency of 1.5 %.

Vigna mungo

Karthikeyan *et al*. (1996) obtained transformed calli of *V. mungo* by cocultivating segments of primary leaves with *A. tumefaciens*. The stable integration of *nptII* in transgenic calli was confirmed by Southern hybridization and the expression was demonstrated by neomycin phosphotransferase assay. However, transgenic calli could not be regenerated into plants. Saini *et al*. (2003) developed fertile transgenic plants from cotyledonary node explants (without cotylendons) inoculated with *A. tumefaciens*. The transgenic nature was confirmed by stable GUS activity, integration of *uidA* gene by Southern analysis and inheritance of transgene to progeny in Mendelian fashion. Mechanical wounding of explants prior to inoculation with *Agrobacterium*, time lag in regeneration due to removal of cotyledons from explants and a second round of selection on kanamycin at rooting stage were found to be critical for transformation. However, the efficiency of transformation of meristematic cells in the axil of cotyledonary node was low (1%) which may be attributed to the presence of limited number of regenerable cells whose capacity for regeneration were short lived-in explant and inefficient T-DNA delivery to regenerable cells (Saini and Jaiwal, 2005). An *Agrobacterium*-mediated transformation of shoot apex explants was developed to overcome these limitations. A significant improvement in transformation efficiency from an average of 1% to 6.5% was obtained. Evidence for stable integration of transgenes and their inheritance to progeny were presented. Shoot apex explants are preferred because of their high regeneration potential with minimal tissue culture manipulations in a less genotype dependent fashion. Saini and Jaiwal (2007) optimized the conditions for enhanced transformation of cotyledonary node and generated stable transgenic plants at a frequency of 4.3%. Murugananthan *et al*. (2007) developed herbicide (Basta®) tolerant *V. mungo* plants using cotyledonary node and shoot tip explants (from seedlings germinated *in vitro* from immature seeds) and *A. tumefaciens* harboring a binary vector car-

rying *bar* and *uidA* genes. Transgenes were shown to be stably integrated and transmitted to progeny in Mendelian fashion. Immature cotyledonary node explants produced a high frequency of transformed plants (7.6%) than shoot tip explants (2.6%). Bhomkar *et al*. (2008) engineered salt stress tolerance in *V. mungo* by introducing glyoxalase I (*gly* I) gene under a novel constitutive *Cestrum* Yellow Leaf Curling Virus (CmYLCV) into regenerable cells present at cotyledonary node of embryonic axis using *A. tumefaciens*. The T_1 plants expressing glyoxalase I activity survived and set seeds under NaCl stress (100 mM).

Vigna angularis

Stable transformants of *V. angularis* were developed using *A. tumefaciens*-mediated gene transfer to epicotyl explants and subsequent selection on kanamycin (Yamada *et al*., 2001) or hygromycin (El-Shemy *et al*., 2002) or PPT (Khalafalla *et al*., 2005) containing medium. The transformation system based on Agro infection of epicotyl explants was also used to transfer *Phaseolus vulgaris* α-amylase inhibitor and D6 fatty acid desaturase genes into *V. angularis* to impart resistance against bruchids (Ishimoto *et al*., 1996, Yamada *et al*, 2005) and to produce poly unsaturated fatty acids (Chen *et al*., 2005) respectively.

Vigna unguiculata

Garcia *et al*. (1986) were the first to demonstrate susceptibility of cowpea to *A. tumefaciens* by inducing the formation of crown gall tumors (that produce opines) at the wound site. Primary leaf discs of cowpea plant were inoculated with *A. tumefaciens* strain C58C1 harboring a non-oncogenic T_i plasmid that contained two copies of a chimeric kanamycin resistant gene. By culturing the leaf discs on selective medium, kanamycin resistant calli were obtained. However, whole plants could not be regenerated from the transformed callus tissue. Southern blot hybridisation revealed the integration of kanamycin resistant gene into the plant genome. Muthukumar *et al*. (1996) obtained transformed shoots on hygromycin selection medium from the mature deembryonated cotyledons inoculated and cocultured with *A. tumefaciens* carrying pUCD 2340 that contained *hpt* gene. The shoots were rooted on B5 basal medium containing hygromycin and 17 rooted shoots (plantlets) were established in soil where only four of them survived and set seeds. Southern blot hybridization confirmed the integration of *hpt* gene in the genome of only one transgenic plant whose seeds failed to germinate. Hence transfer of transgene into progeny could not be confirmed. Sahoo *et al*. (2000) reported recovery of chimeric transgenic plants of cowpea under kanamycin selection using shoot apices and *A. tumefaciens* but did not provide evidence of stable transformation. Popelka *et al*. (2006) were the first to demonstrate the stable transmission and expression of two cointegrated genes, *bar* and *uidA* in the progeny of transgenic plants with a frequency of 0.001 to 0.003. They recovered transgenic plants by inoculating the longitudinally bisected embryonic axes with cotyledon attached without shoot and root apices with hyper virulent *Agro* strain AGLI harboring a binary vector that contained a selectable marker (*bar*) and a reporter gene (*uidA*). This protocol required 5-8 months from explant preparation to harvested T_1 seeds.

Chaudhary *et al*. (2007) improved the efficiency of transformation from 0.1 (Popelka *et al*., 2006) to 1.9%. Their transformation protocol is based on the direct shoot regeneration from the cotyledonary node explants without both the cotyledons. Removal of both the cotyledons not only reduces the size of the explants but also delayed the shoot regeneration response which was found to be effective for the selection of transformation events. Mechanical wounding of the explants at nodal regions prior to *Agro* infection and a second round of selection at rooting stage were found to be critical in *V. unguiculata* as in other *Vigna* species (Saini *et al*., 2003, Saini and Jaiwal, 2005, Sonia *et al*., 2007).

Solleti *et al*. (2008) used *Agrobacterium*-mediated transformation for the introduction of the bean (*Phaseolus vulgaris*) α-amylase inhibitor-1 (α AI-1) gene into cowpea to confer resistance to bruchids (*Callosobruchus* sps)- a storage pest. They enhanced the recovery of transgenic plants to an average of 1.67% by the use of additional copies of *vir* genes in resident pSB1 vector in *Agro* strain LBA4404, thiol compounds during cocultivation and a geneticin based selection.

Raji *et al*. (2008) demonstrated the compatibility of *in vitro* plant regeneration protocol from embryonic axes with *Agrobacterium*-mediated transfomation using *nptII* as a selectable marker gene and paromomycin as a selective agent. PCR, uniform expression of *uidA* gene and Southern hybridization analysis of primary transformants revealed the integration of *nptII* gene into the cowpea genome. However, transmission of transgene to progeny has not been demonstrated.

Nevertheless, these transformation systems are laborious, time consuming, likely to be genotype- dependent and present low frequency of germline transformation (Ivo *et al*., 2008).

Direct DNA transfer methods

Particle bombardment

Particle bombardment which relies upon the acceleration of DNA-coated particles into target cells is

much preferred option for gene delivery to recalcitrant grain legumes, *Vigna* species in particular, circumventing the host specificity as well as genotype dependence by *Agrobacterium*. To date, only a few reports are available on transformation of *Vigna* species using particle gun method. Kononowicz *et al*. (1993, 1997) demonstrated the possibility of transforming and obtaining cowpea plants with introduced genes. However, stable integration and expression of transgenes in progeny of cowpea was not shown. Bhargava and Smigocki (1994) observed transient expression of *gus* gene in the germinating embryos of *V. aconitifolia*, *V. mungo* and *V. radiata*, 18 to 24 h after particle bombardment. The raised plantlets were rooted on kanamycin selection medium but their transgenic nature was not confirmed. Ikea *et al*. (2003) optimized the conditions for delivery of plasmid that contained *uidA* and *bar* genes into embryonic axes explants of cowpea using biolistic PDS-100/ He particle delivery system. Putative transformed α were recovered from the bombarded explants on bialophas (3 mg/l) selection medium. Molecular analysis of T_1, T_2 and T_3 plants showed the transfer of genes into a small proportion of these progenies and there was no evidence for stable integration. Ivo *et al*. (2008) introduced the mutant *ahas* gene (coding for acetolactate synthetase) and *gus* gene into the shoot apical meristem of cowpea, which was fully exposed by removing primordial leaves, excised from mature seeds. The putative transformed shoot were selected on shoot regeneration medium containing 200μ-M imazapyr, a herbicide molecule capable of systemically translocating and concentrating in apical meristematic region of the plant. This selection coupled with a multiple shooting allowed for recovery of transgenic cowpea lines at a frequency of germ line transformation. The L_2 and L_3 layers of the meristem should receive the DNA coated particles as these layers divide in the anticlinal and periclinal plans to form the shoots. Southern analysis of T_0 plants confirmed the presence of both *ahas* and *gus* genes and their transmission to T_1 and T_2 progenies in Mendelian fashion.

Electroporation mediated transformation

Owing to the difficulties in regenerating *Vigna* species from protoplast and callus cultures, electroporation of intact tissues and organs has been attempted with promising results. Penza *et al*. (1991) showed that seed-derived embryos of *V. unguiculata* could take up and transiently express a chimeric gene after passive or electroporation-mediated DNA transfer. Akella and Lurquin (1993) demonstrated that seedlings generated from cowpea embryos after imbibition in the presence of plasmid DNA harbouring *gus* gene, expressed the *gus* transgene quite efficiently in a variety of tissues including apical meristem. Embryo electroporation in the presence of DNA and protectants such as spermine and cationic liposome increased both the proportion of embryo derived seedlings expressing the chimeric gene and the level of gene expression. However, they did not attempt to grow the seedlings into transformed plants.

Chowira *et al*. (1995) attempted electroporation of axillary nodal meristem of mature plants of pea, lentil, soybean and cowpea and produced the T_1 transgenic plants from the seeds developed on the chimeric branch grew out from the elctroporated nodal meristem. Although T_2 soybean and lentil plants were obtained, no transgenic T_2 cowpea plants were reported. Electroporation of whole plant tissue might results in unstable pattern of inheritence of the introduced trait. Subsequent attempts in electroporation of axillary buds of pea with a construct containing coat protein gene of Pea Enation Mosaic Virus (PEMV) lead to the generation of transformed plants resistant to PEMV (Chowira *et al*. 1996).

Adensoye *et al*. (2008) used electroporation method for introduction of an insecticidal *cry1Ab* and an antibiotic resistance *nptII* gene into the nodal buds of cowpea. T_1 seeds derived from electroporated branches were selected on a medium containing geneticin. Stable integration of *cry1Ab* gene into cowpea genome at T_1 was confirmed by PCR and Southern blot analysis. T_1 plant of cowpea also showed resistance to *Maruca vitrata* larvae and protection of cowpea from damage.

Other novel transformation technologies

Although *Vigna* species are susceptible to *Agrobacterium*, difficulties in regenerating whole plants especially from callus, cell suspension and protoplast necessitated the development of alternative transformation methods that essentially avoid tissue culture and regeneration procedures. Further the regeneration steps are time consuming, labour intensive and required skilled personnel and are associated with somaclonal variations or morphological abnormalities exhibited by the regenerated plants and reduced fertility. There are only a few species for which transformation system (like flower dip, vaccum infilteration of *Agrobacterium* etc.) avoiding tissues based regeneration system, has been developed which includes the modal plants *Arabidopsis thaliana*, *Medicago truncatula* and *Lotus japonica* and some legumes like soybean, peanut and pigeonpea. A non-tissue culture based approach should be followed for generating transgenics in *Vigna* species.

Improvement in transformation efficiency

The various approaches, such as mechanical wounding of explants prior to *Agrobacterium* inoculation, use of supervirulent plasmids carrying extra cop-

ies of virulence genes, presence of acetosyringone in bacterial inoculation and cocultivation medium, inclusion of thiol compounds (L-cysteine, DTT etc.) in cocultivation medium, lower pH (lower than 5.8-6.0) of cocultivation medium, low temperature (< 25° C) during infection and coculture, concentration of bacteria and duration of cocultivation have been used to improve transformation efficiency in *Vigna* species.

Conclusions and future directions

Vigna species are cheap and rich source of protein for vegetarian diet and are important for sustainable agriculture. Their improvement through conventional breeding has hampered due to limited gene pool and sexual-incompatibility with wild and related species, the reservoir of desirable genes. Conventional breeding is to be supplemented by biotechnological means to transfer desirable genes from sources across the sexual boundaries. The recalcitrant nature of *Vigna* species has so far hindered the successful application of transformation technology. However, success has been achieved to regenerate them from seedling explants via adventitious or direct shoot organogenesis. These protocols are used for *Agrobacterium* -mediated transformation in all the cultivated *Vigna* species with low to moderate frequency in more or less genotype independent manner. Regeneration from callus, cell suspension and protoplast cultures except *V. aconitifolia* and *V. angularis* still remain problematic. Transgenics with integrated marker or reporter genes mostly up to T_0 or T_1 level have been developed. The stability and expression of transgene(s) in subsequent progenies have not been investigated. Agronomically desired genes in some cases have been introduced but these transgenics are so far confined to the research laboratories only. Transformation through bombardment of DNA-coated particle to regenerable tissue such as seedling meritems, electroporation of intact plants and in *planta* transformation are to be explored. Improvement in transformation frequency in *Vigna* species is required. A high throughput transformation system is not only essential for introduction of desirable genes for their better yield and nutritional quality but also required to explore gene functions through reverse genetic techniques. The new tools of genomics, proteomics and metabolomics would allow better understanding of vital processes of these food legumes for their improvement. In addition, for the sake of end user acceptance, the research should focus on (1) development of methods avoiding antibiotic or herbicide resistance genes as selectable marker and (2) selecting genes for the desirable traits for the transfer and (3)strategies for the seed distribution system, where the end user in the developing countries is benefitted and not only industries in developed countries. Further, there is a need of a sustained coordinated research with long term funding and involvement of private sector for generation of transgenics in these tropical food legumes so as to meet the daily protein requirement of millions of people of the developing countries.

Acknowledgement

Financial support from DBT, New Delhi to the laboratory of PKJ for the work on genetic improvement of legumes is gratefully acknowledged.

References

Adesoye A., Machuka J., and Togun A. 2008 CRY 1AB transgenic cowpea obtained by nodal electroporation Afr. J. Biotech. 7:3200-310.

Akella, V. and Lurquin, P. F. 1993 Expression in cowpea seedlings of chimeric transgenes after electroporation into seed-derived embryos. Plant Cell Rep. 12: 110-117.

Bhomkar P., Upadhyay C. P., Saxena M., Muthusamy A, Shia Parkash N. and Sarin N B 2008 Salt stress alleviation in transgenic *Vigna mungo* L.Hepper by overexpression of *glyoxalase* 1 gene using a novel *Cestrum* yellow leaf curling virus(CmYLCV) promoter . Mol. Breeding 22: 169-181.

Boutilier, K., Offiringa R., Sharma V K., *et al*. 200 Ectopic expression of Baby Boom triggers a conversion from vegetative to embryonic growth Plant Cell 14:1737-1748.

Bano H and Chua N. H. 2002 Esr 1-a plant gene that can promote plant regeration and transformation. US patent application number 20: 137-140.

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

Chen, R., Tsuda, S., Matsui, K., Fukuchi-Mizutani, M., Ochiai, M., Shimizu S., Sakuradani F., Aoki, T, Imaizumi, R., Ayube, S. and Tanaka Y 2005 Production of linolenic acid in *Lotus japonicus* and *Vigna angularis* by expression of D6-fatty acud desaturase gene isolated from Mortierella alpina. Plant Sci 169:599-605.

Chowrira, G. M., Akella, V. and Lurquin, P. F. 1995 Electroporation-mediated gene transfer into intact

nodal meristems *in planta*. Mol. Biotech. 3: 17-23

Chowrira, G. M., Akella, V., Fuerst, P. E. and Lurquin, P. F. 1996 Transgenic grain legumes obtained by *in planta* electroporation-mediated gene transfer. Mol. Biotech. 5: 85-96.

Devi, P., Radha, P., Sitamahalakshmi, L., Syamala, D. and Manoj Kumar S. 2004 Plant regeneration via somatic embryogenesis in mungbean (*Vigna radiata* L. Wilczek) Sci. Hortic. 99:1-8.

Eapen, S. 2008 Advances in development of transgenic pulse crops. Biotech. Ad. 26:162-168.

El-Shemy H. A., Khalafalla, M., Wakasa, K. and Ishimoto, M. 2002 Reproducible transformation in two grain legumes-soybean and azuki bean using different systems. Cellular and Mol. Biol. Lett. 7:709-719.

Garcia, J. A., Hille, J. and Goldbach, R. 1986 Transformation of cowpea (*Vigna unguiculata*) cells with an antibiotic resistance gene using a Ti-plasmid derived vector. Plant Sci.44: 37-46.

Girija, S., Ganapathi, A. and Ananthakrishanan, G. 2000 Somatic embryogenesis in *Vigna radiata* L. Wilczek. Indian J. Exp. Biol. 38:1241-1244.

Ikea, J., Ingelbrecht, I., Uwaifo, A. and Thottapilly, G. 2003 Stable gene transformation in cowpea (*Vigna unguiculata* L. Walp) using particle gun method. Afr. J. Biotechnol. 2: 211-218.

Ishimoto, M., Sato, T., Chrispeels, M. J. and Kitamura, K. 1996 Bruchid resistance of transgenic azuki bean expressing seed alpha amylase of common bean. Entomologia Experimentalis et Applicata 79:309-315.

Ivo, N. I., Nascimento C. P., Vieira, L. S. Campos, F. A. P., Arago, F. J. L. 2008 Biolistic mediated genetic transformation of cowpea (*Vigna unguiculata* L.) and stable inheritance of transgenes. Plant Cell Rep.DOI 10.1007/S00299-008-0573.2 .

Jaiwal, P. K. and Gulati, A. 1995 Current status and future strategies of in vitro culture techniques for genetic improvement of mungbean (*Vigna radiata* (L.) Wilczek) Euphytica. 86: 167-181.

Jaiwal, P. K. and Singh, R. P. (eds.) (2003) Focus on biotechnology, Vol 10A: Improvement strategies for leguminosae biotechnology. Kluwer Acad. Publ. The Netherlands.

Jaiwal, P. K., Kumari, R. Ignacimuthu, S., Potrykus,I. and Sautter, C. 2001 *Agrobacterium tumefaciens* -mediated gene transfer in mungbean- a recalcitrant grain legume. Plant Sci. 161:239-247.

Karthikeyan, A. S., Sharma, K. S. and Veluthambi, K. 1996 *Agrobacterium tumefaciens*-mediated transformation of *Vigna mungo* (L.) Hepper. Plant Cell Rep. 15: 328-331.

Kononowicz, A. K., Narasimhan, M. L., Reuveni, M., Moclatchey, G., Bressan, P. H., Zhang, Y., Larosa, P. C., Murdock, L. L., Chrispeels, M. J., Bressan, R. A., Hasegawa, P. M. 1993 Genetic transformation of cowpea (*Vigna unguiculata*) using microprojectile bombardment and *Agrobacterium tumefaciens* infection. Plant Physiol. 102, suppl. Abstract no. 945.

Kononowicz, A. K., Cheah, K. T., Narsimha, M. L., Murdock, L. L., Shade, R. F., Chrispeels, M. J.,Fillipone, E., Monti, L. M., Bressan, R. A. and Hasegawa, P. M. 1997 Developing a transformation system for cowpea (*Vigna unguiculata* L. Walp) Pages 361-371 in Advances in Cowpea Research, B. B. Singh, D. R. Mohan Raj, K. E. Dashiell, L. E. N. Jackai, IITA Ibadan, Nigeria.

Khalafalla, M. M., El-Shemy H. A., Mizanur, R. S., Teraishi, M.and Ishimoto, M. 2005 Recovery of herbicide resistant azuki bean (*Vigna angularis*) plants via *Agrobacterium* mediated transformation. Afr. J. Biotech. 4:61-67.

Kulothungan, S., Ganapathi, A., Shajahan, A. and Kathiravan, K. 1995 Somatic embryogenesis in cell suspension culture of cowpea (*Vigna ungiculata* L. Walp.) Israel J. Plant Sci. 43: 385-390.

Mahalakshmi, S. L., Leela, T. and Manoj Kumar, S. 2006 Enhanced genetic efficiency of mungbean by use of primary leaf explants. Curr. Sci. 91: 93-98.

Muruganantham, M., Amutha S., Selvaraj N., Vengadesan, G. and Ganapathi A (2007) Efficient *Agrobacterium* -mediated transformation of *Vigna mungo* using immature cotyledonary node explants and phophinothricin as the selection agent. In vitro Cell Dev. Biol. Plant 43: 550-557.

Muthukumar, B., Mariamma, M., Veluthambi, K. and Gnanam, A. 1996 Genetic transformation of cotyledon explants of cowpea (*Vigna unguiculata* L. Walp) using *Agrobacterium tumifaciens*. Plant Cell Rep. 15: 980-985.

Pal, M., Ghosh, U., Chandra, M., and Biswas, B. B. 1991 Transformation and regeneration of mungbean (*Vigna radiata*). Indian J. biochem and biol. 28: 449-455. . 38: 439-

Penza, R., Lurquin, P. F. and Fillipone, E. 1991 Gene transfer by cocultivation of mature embryos with *Agrobacterium tumefaciens*: Application to cowpea (*Vigna unguiculata* Walp) J. Plant Physiol. 138: 39-42.

Phogat S K, Kathikeyan A. S. and Veluthambi Generation of transformed calli of *Vigna radiata* by *Agrobacterium* mediated transformation, J Plant Biol. 26:77-82.

Popelka, J. C., Terryn, N. and Higgins, T. J. V. 2004

Gene technology for grain legumes: can it contribute to the food challenge in developing countries. Plant Sci. 150: 41-49.

Popelka, J. C., Gollasch, S., Moore, A., Molvig, L., Higgins, T. J. V. 2006 Genetic transformation of cowpea (*Vigna unguiculata* L.) and stable transmission of the transgenes to progeny. Plant Cell Rep. 25: 304-312.

Raji, A. A. J., Oriero, E., Odeseye, B., Odunlami, T. and Ingelbrecht, I. 2008 Plant regeneration and *Agrobacterium* transformation of African cowpea genotypes using embryonic axis explants. J. Food, Agri. and Environ. 6:350-356.

Sahoo, L., Sushma, Sugla, T., Singh, N. D. and Jaiwal, P. K. 2000 *In vitro* plant regeneration and recovery of cowpea (*Vigna unguiculata*) transformants via *Agrobacterium*-mediated transformation. Plant Cell Biotech Mol Biol. 1: 47-54

Sahoo, L., Sugla, T. and Jaiwal, P. K. 2003 *In vitro* regeneration and genetic transformation of *Vigna* species. Pages 89-120 in P K Jaiwal and R P Singh (eds) Focus on biotechnology, Vol. 10B. Applied genetics of leguminosae biotechnology. Kluwer, Dordrecht, Netherlands

Sahoo, L. and Jaiwal, P. K. 2008 Asiatic Beans 115-132 in C. Kole and T. C. Hall (eds) A Compendium of Transgenic Crop Plants, Blackwell Publ, Oxford, UK.

Saini, R. and Jaiwal, P. K. 2005 Efficient transforma tion of a recalcitrant grain legume *Vigna mungo* L. Hepper via *Agrobacterium*- mediated gene transfer into shoot apical meristem cultures. Plant Cell Rep. 24:164-171.

Saini, R. and Jaiwal, P. K. 2007 *Agrobacterium tumefaciens*-mediated transformation of blackgram: an assessment of factors influencing the efficiency of *uidA* gene transfer. Biol. Plant. 51:69-74.

Saini, R., Sonia and Jaiwal, P. K. 2003 Stable genetic transformation of *Vigna mungo* L.Hepper via *Agrobacterium tumefaciens*. Plant Cell Rep.,21: 851-859.

Saini, R., Sonia, Madanpotra, S., Badola, A. and Jaiwal, P. K. 2004 An improved protocol for plant regeneration via somatic embryogenesis from cell suspension cultures of *Vigna mungo* L. Hepper. Physiol. Mol. Biol. Plants 10: 121-125.

Solleti, S. K., Bakshi, S., Purkayastha J., Panda S K. and Sahoo, L. 2008 Transgenic cowpea (V*igna unguiculata*) seeds expressing a bean amylase inhibitor 1 confer resistance to storage pests, bruchid beetles. Plant Cell Rep. DOI 10.1037/s00299-008-06-5-x.

Somers, D. A., Samac, D. A., Olhoft, P. M. 2003 Recent advances in legume transformation. Plant Physiol. 131:892-899.

Sonia, Saini, R., Singh, R. P. and Jaiwal, P. K. 2007 *Agrobacterium tumifaciens*-mediated transfer of *Phaseolus vulgaris* α-amylase inhibitor-1 gene into mungbean (*Vigna radiata* L. Wilczek) using bar as selectable marker. Plant Cell Rep 26:187-198.

Tomooka, N., Vaughan, D. A. and Kaga, A. 2004 Mungbean, Pages 325-345 in R J. Singh and P P Jauhar (eds). Genetic resources, chromosome engineering and crop improvement Grain Legumes, CRC Press Boca Raton, FL.

Vaughan D. A., Tomooka N, and Kaga A (2004) Azuki bean. Pages 341 - 352 in R. J. Singh and P P Jauhar (eds) Genetic resource, chromosome engineering and crop improvement. Grain legumes, CRC Press, Boca Raton, FL.

Veluthambi, K, Gupta, A. K. and Sharma, A.2003 The current status of plant transformation technologies. Curr. Sci. 84: 368-380.

Yamada, T., Teraishi, M., Hattori, K. and Ishimoto, M.2001 Transformation of azuki bean by *Agrobacterim tumefaciens*. Plant Cell Tiss. Org. Cult. 64: 47-54.

Yamada, T., Teraishi, M., Hattori, K. and Ishimoto, M.2005 Isolation of two α -amylase inhibitor genes of tepary bean (*Phaseolus acutifolius*) and their functional characterization in genetically engineered azuki bean. Plant Sci. 169:502-511.

Zuo I., Niu Q W , Frugi S. G. and Chua N H 200 The Wuschel gene promoter vegetative to embryonic transition in *Arabidopsis*. Plant J. 30:349-360.