Advances in Genetic Engineering of Primary Grain Legume Crops: a Review Study

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Abstract

In general, the seeds of many legumes are rich in both oil and protein; therefore, these grain legumes are commonly used as an important staple food worldwide. Transgenetic manipulation or production of transgenic crops has been needed for genetic enhancement as part of plant breeding programmes. This study is an attempt to evaluate the advancements in technology based on genetic transformation in major primary grain legume crops such as; chickpea, pigeon pea, garden pea, mung bean and lentil. Moreover, this study also involves to analyzing the screening of the competent explants as target tissues for gene delivery, mode of gene transformation, and further selection of transgenic tissues followed by regeneration of transgenic plantlets. Although most reports on introducing genes into grain legumes are based upon the use of Agrobacterium tumefaciens during transformation experiments, however, there have been also parallel efforts involving the use of biolistics and electroporation methods to produce transgenic grain legume crops with essential improved traits.

Keywords*: Grain Legume, Agrobacterium, Biolistics, Electroporation, gus expression, Transgenics.*

1. INTRODUCTION

Primary grain legumes or pulses provide protein, complex carbohydrates, dietary fiber, vitamins and dietary minerals. Since, these grain legumes are essential for humans as well as animal consumptions, therefore, improvement in any manner could be a meaningful approach to meet the global demands as good source of food and fodder.

Unfortunately, conventional methods of pulse improvement have been proved little beneficial in developing resistant varieties because of unavailability of suitable donor parents and lack of proper screening methods. Additionally, with conventional methods, the available gene pool is further restricted by the sexual incompatibility of many inter-specifics and inter-generic crosses **[1].** Moreover, grain legumes are mainly self-pollinated and have a narrow genetic base, so, there is need to widen the genetic base and incorporate desirable characters. Hence, use of transgenic technologies for any qualitative and quantitative improvements in the grain legume crops has been always a meaningful alternative.

It is suggested that Agrobacterium-mediated gene transformation method has been proved as the most successful method of gene transfer into plant cells but generally grain legumes are one of the least amenable groups for gene transformation amongst dicotyledonous crops, however, legumes are usually susceptible to Agrobacterium infection **[2]**. Moreover, two main species of Agrobacterium (A. tumefaciens and A. rhizogenes) are known and have been used for gene transfer technology in plants.

Direct DNA transfer through physical or chemical methods provides an alternative of Agrobacterium-mediated transformation method to introduce genes into the chloroplast genome [3]. Biolistic method is less genotype dependent and is generally more efficient. Additionally, there have been also efforts to develop the procedures by which plants could be efficiently and successfully regenerated from single cell or protoplast and organized tissues. Therefore, single cell or protoplast was also treated as the target site for electroporation-mediated protoplast transformation during grain legume improvement programme [4].

Thus, with the realization of the significance of primary grain legumes and its possible biotechnological improvements in recent past, this study deals with the evaluation of the available reports based on genetic transformations in some of the primary grain legume crops such as; chickpea, pigeon pea, garden pea, mung bean and lentil.

II. CHICKPEA (*Cicer arietinum* L.)

Chickpea is a self-pollinating diploid primary grain legume and unfortunately, gram pod borer, Helicoverpa armigera is the most devastating insect pest to reduce the chickpea production caused by severe pod damage up to 90% [5, 6]. Hence, advances in biotechnology of grain legumes could lead to introduction of novel traits through genetic transformation into chickpea.

A. Agrobacterium-Mediated Gene Transformation

In general, Agrobacterium tumefaciens-mediated transformation has been achieved successfully in grain legumes and also it could be

equally possible in chickpea grain legume [7, 8, 9, 10, 11, 12]. Transgenic calli were initially established through culture of 12-15 days old leaf and stem explants with wild as well as disarmed strains of Agrobacterium tumefaciens LBA4404 carrying pBI121 vector with nptII and gus genes [7]. Transgenic shoots raised from these transformed calli were gradually selected on medium containing kanamycin (50 mg/1) and gus expression was also evident in leaves and roots of transformed plants.

Embryo axes without root and shoot-meristems were also used to inoculate with A. tumefaciens strain LBA 4404 strain. Multiple-shoots were obtained and putative transgenic shoots were further selected on kanamycin (50 mg/1) supplemented medium. Moreover, leaves and roots of these transformants were found to be positive for gus expression during GUS assay experiments and also for nptII gene positive during Southern blot tests [13].

In additional experiments, embryonal-axis explants obtained from four accessions of chickpea were treated with A. tumefaciens strains (C58C1/p35SGUSINT and GV2260/ pIBGUS/EHA101) and kanamycin (50 mg/1) or phosphinothricin (10 mg/1) were further employed for multiple-shoot formation and selection of transgenic shoots. T_0 (parent) plants exhibited PCR and Southern tests positive for both nptII and GUS whereas T_1 plants showed PCR positive for nptII but not for GUS. It could be probably due to improper integration of T-DNA into the plant genome [8].

Furthermore, longitudinal slices from embryonal axis of imbibed mature seeds were also used for Agrobacterium-mediated transformation with vector LBG66 (pPBI3008) containing binary vector pPBI3010 [14]. Molecular analyses of T_0 and T_1 by GUS activity, MUG assay and Southern blots revealed the presence of nptII gene with single insert which showed the 3:1 Mendelian inheritance pattern in T_1 population and, therefore, it was confirmed that T-DNA was stably inherited to plant genome [9].

B. Influencing Factors in Chickpea Transformation

Studies have been also undertaken to evaluate the effect of various promoters on expression of marker genes nptII and gus employing Agrobacterium-mediated gene transfer into zygotic embryos of chickpea. It was suggested that gus gene under control of Actin promoter is more effective for expression as compared to CaMV35S and Win promoters. Further, it was also observed that Agrobacterium strains A281 was found to be more virulent than C58 strains [15].

In another experiment, four different chickpea genotypes were transformed with three different strains of Agrobacterium (EHA105, AGL1 and LBA4404) carrying the binary vector pCAMBIA1301 with reporter genes (gus, hpt) driven by CaMV35S promoter. Further, T_2 progeny of efficiently regenerated rooted plants expressed both reporter genes in the expected 3:1 inheritance [16].

Among other factors, Sonication-Assisted Agrobacterium-mediated Transformation (SAAT) method [16, 17, 18] and vacuum infiltration [19, 20, 21] methods have been reported to enhance the efficiency of Agrobacterium-mediated transformation of plant species [22]. The transformation efficiency was almost 60% higher in the SAAT-mediated method than a simple Agrobacterium infection without sonication and sonicated wounding of the plant tissue [23]. Further, the suitable conditions for efficient delivery of Agrobacterium T-DNA, harboring cry1Ac gene, along with selectable marker nptII and reporter gene uid A into chickpea was also optimized [22].

C. Biolistic-mediated Gene Transformation

An agronomically important *cry1Ac* gene providing resistance towards pod borer *Heliothis armigera* and *Helicoverpa armigera* has been reported to be transferred to chickpea [24, 25, 26]. Embryo or embryonal-axes without root and shoot apices were co-transformed with *cry1Ac* and *nptII* gene using a biolistic 1000/He particle gun. Explants gave rise to transformed multiple-shoots and gradually selected on kanamycin (50 mg/1) supplemented medium. Molecular analyses based on Southern and Northern blots further revealed the presence of *cry1Ac* gene and its expression was confirmed by inhibition of larval development on feeding transgenic shoots. Even the insect bioassay for pest resistance was also performed using stem of the plant instead of pods/seeds [24].

In general, the transformation frequency in chickpea was found to be very low and thus, an efficient method of gene transformation is required to achieve high frequency of stable transformation events in chickpea grain legume crops.

III. PIGEON PEA (*Cajanus cajan* L.)

Pigeon pea is the main food legume of the semi-arid tropics and among many insect pests, the pod borer Helicoverpa armigera causes significant damage to this crop. It is also susceptible to the wilt disease caused by Fusarium udum, particularly in the humid regions. As pigeon pea is a self-pollinated plant with narrow genetic base, genetic transformation plays an important role in the incorporation of agronomically important traits.

A. *Agrobacterium-mediated Gene Transformation*

The first study on *A. tumefaciens*-mediated gene transformation in pigeon pea using shoot-apices and cotyledonary-nodes as explants was reported in 1999, and integration of T-DNA into the genome of transgenic plants was further confirmed by Southern hybridization [27], however, the frequency of transformation was found to be very low.

Studies on gene transformation in pigeon pea indicate that the rate of *A. tumefaciens*-mediated transformation is based on the efficiency of reproducible regeneration of the target tissues. Direct organogenesis from mature embryo-derived explants was obtained using *nptII* as selectable marker and *GUS* and *GFP* as reporter genes. Southern hybridization experiments exhibited stable integration of *GFP* gene in transgenic plants, however, calli induced from agrobacterium-infected mature embryo-derived explants showed GFP and GUS expression but these transgenic calli were unable to differentiate plants [28].

In pigeon pea, organogenesis-mediated plant regeneration has been the most adopted technique for genetic transformation experiments due to high regeneration frequency whereas somatic embryogenesis has been the least preferred pathway due to poor embryo germination rate. Moreover, cotyledonary-node was proved highly responsive over other tissues for regeneration [29].

B. *Transgenic Pigeon pea for Pest-Resistance*

In pigeon pea, gene transformation was conducted to improve resistance against insects, fungal diseases and nutrient quality. Various genes such as Bacillus thuringiensis cry1Ab, cry1 E-C, cry1AcF and cowpea protease inhibitor were used for conferring insect resistance [30, 31, 32, 33, 34]. Further, gene transformation in pigeon pea with rice chitinase (Rchit) gene was also reported for improved fungal resistance [35].

Transgenic pigeon pea plants resistant to chewing insects, expressing the cowpea protease inhibitor gene, were successfully obtained. Agrobacterium strain GV2260 was used to recover transgenic plants carrying the cowpea protease inhibitor gene [30]. Transgenic nature of regenerants was characterized by Northern blotting to confirm the presence of mRNA but unfortunately, the transformation frequency was found to be very low (~1%).

However, the first record on the successful production of pest-resistant pigeon pea was reported in 2006 and transgenic pigeon pea was obtained using axillary-meristem explants with the Bt cry1Ab gene driven by double-enhanced CaMV35S promoter along with fused uidA and nptII genes driven by CaMV35S promoter as selectable marker genes [33].

C. *Transgenic Pigeon pea for Nutritional Improvement*

Pigeon pea is considered as one of the nutritionally poorest among the grain legumes due to low amount of sulphur-containing amino acids. The key enzyme of the lysine biosynthetic pathway, dihydrodipicolinate synthase (DHDPS), is inhibited by lysine through feedback regulation in pigeon pea. The transgenic pigeon pea with dhdpr-r_1 was developed for enhancement of lysine content in the seed protein [36, 37].

Besides these, edible vaccine genes such as haemagglutinin gene of rinder pest virus and haemagglutinin neuraminidase gene of peste des petits ruminant's virus (PPRV-HN) were also integrated into pigeon pea to immunize goat and sheep to render pest virus and peste des petits ruminants virus, respectively [38, 39]. Further, an effort was also undertaken to develop bio-fortified pigeon pea for the enhancement of β-carotene (pro-vitamin A), a precursor of vitamin A and success in producing transgenic pigeon pea plants with high-levels of β-carotene would be significantly helpful to the malnourished population in the dry lands of the world [40].

D. *Biolistic-mediated Gene Transformation*

An efficient regeneration protocol for pigeon pea based on callus induction and differentiation from seed explants was developed and transformed pigeon pea was obtained using biolistics methods **[36]**. Stable transmission and expression of transgenes in the progeny was also confirmed through GUS assays, PCR and Southern hybridization.

Moreover, a significant improvement in gene transformation frequency could be possible by establishing an efficient plant regeneration method and more than 90% of transgenesis in pigeon pea using biolistics was obtained from leaf explants. During the study, 90% of the bombarded explants exhibited transient expression of the uidA gene and 50% of the selected plants that were transferred to the glass house showed positive gene integration **[41]**.

E. *In Planta–Gene Transformation in Pigeon pea*

Of late in 2008, *In planta*-gene transformation method was developed to avoid the recalcitrant nature of the target transforming tissue [42]. It was a novel technique being first reported in pigeon pea, where sewing needles were used to prick the meristematic-regions of 2-day-old seedlings. This procedure avoids the selection strategy and primary transformants were chimeric in nature and molecular analysis was further carried out to confirm gene

integration and 13.7% of plants were found to be positive for marker and reporter genes both. Transgenic nature of these plants was further confirmed by dot blot and Southern hybridization techniques [43].

F. Influence of Vector and Promoter on Transformation

An important aspect of binary vectors is the usage of constitutive promoters, which provide transgene expression in most of the plant tissues. Majority of the studies conducted on pigeon pea have largely based on the use of CaMV35S constitutive promoter and a CaMV35SDE double enhanced promoter [33]. Additionally, Phaseolin (Phas), Arabidopsis thaliana 2S2 (2S2) and dhdps promoters were also used in Agrobacterium-mediated as well as the biolistic-mediated gene transformation in pigeon pea [37].

Expression levels of the insecticidal cry1Ab gene associated with CaMV35SDE were 0.10% and 0.025% of total soluble protein (tsp) in flowers and leaves, respectively [33] whereas, the expression levels of RVPH with CaMV35S were found to be relatively higher (0.12–0.49%) of tsp [38]. Similarly, seed-specific promoters such as bean phaseoline and 2S2 showed as high as 400–600-fold higher expression of dhdps-r_1 at late stages of seed development in comparison to its non-transgenic counterparts [37].

IV. GARDEN PEA (Pisum sativum L.)

During the last two decades, a number of studies on pea gene transformations were undertaken [44, 45, 46] and many potential transgenes have been identified and selected as target genes to generate transgenic improved pea.

Agrobacterium-mediated Gene Transformation

Studies of the interactions between Agrobacterium tumefaciens and pea started in the late 1980s and early 1990s [47, 48, 49]. Historically, the first complete transgenic pea plants were regenerated from transformed protoplast cultures in 1989 and soon it was followed by regeneration of transgenic pea plants from epicotyls and cotyledonary-nodes cultures [50, 51]. Furthermore, a number of protocols are available for Agrobacterium-mediated gene transformation in pea [52, 53, 54, 55, 14, 56, 57]. During these studies, various explants were used as the target tissues such as; segments of the embryogenic axis [14], cotyledonary-nodes [53, 55] and immature cotyledons as starting material [58, 59] for A. tumefaciens-mediated gene transformation in peas.

A. Insect Resistant Transgenic Pea

Production of insect resistant transgenic pea plants was first time reported in 1990 using Agrobacterium as a vector [60]. Transgenic plants were produced for enhanced resistance against predators by expression of enzyme inhibitors. Bean α-amylase inhibitors derived from Phaseolus vulgaris was found to be effective against Callosobruchus maculatus in pea. Later this enzyme inhibitor was also proved to be effective against Bruchus pisorum and Callosobruchus chinensis [61]. Similarly, tobacco proteinase inhibitor also showed enhanced resistance against Helicoverpa armigera in transgenic peas [62].

B. Transgenic pea for Proteinase Inhibitor

Gene transformation with a target gene encoding multi-domain proteinase inhibitor precursor was performed and was expressed in transgenic pea under the control of Rubisco small subunit promoter [62]. Insect-feeding traits trials have shown that the mortality of Helicoverpa armigera larvae was high as compared to controls. Protease inhibitors from insects have also been expressed in plants.

Furthermore, Agrobacterium rhizogenes is a promising alternative to A. tumefaciens for obtaining transformed pea plants. The host range of several A. rhizogenes strains on a range of pea genotypes has been evaluated and only a strain of A. rhizogenes, A_4T, gave the typical hairy root response while the other strains gave tumors or no response. It is reported that the A_4T strain of A. rhizogenes containing the binary vector pKIWI110 to produce transformed hairy root cultures of the cultivar Pania [62].

Moreover, the modified T-DNA of pKIWI110 contains genes for kanamycin resistance and β-glucuronidase. It was, therefore, selected the transformed hairy roots on medium with kanamycin and further confirmed their transformed nature by detecting expression of β- glucuronidase using a simple histochemical test.

C. Biolistic-mediated Gene Transformation

This approach involves the DNA coated particles (tungsten or gold) is accelerated into plant tissue. Transgene delivery into pea plant cells by the biolistic approach was initially reported by several groups [63, 64]. Moreover, a series of experiments were carried out with Biolistic (PDS-1000/He Particle Delivery System) method to check the mean frequency of transformation. Alternatively, integrated transformation by using particle bombardment in combination with *Agrobacterium*-mediated approach was also tested. These approaches have been successfully used to transform other grain legumes also [65, 66].

Transient *gus* gene expression was exhibited by 6% of explants after biolistic experiment and by 33% after composite approach. Moreover, in case of

apical-meristem cultures where the regeneration frequency appeared to be limited up to 5%, and a further decrease of explants viability was caused by application of the transformation protocol which led further to reject use of the biolistic method for mass production of transformants.

D. Electroporation-mediated Gene Transformation

In pea, the major limitation of recovering stable transformants by protoplast electroporation was the requirement for an efficient protoplast-to-plant regeneration scheme. In a study, stable transformation of protoplasts from two different pea cultivars (Belman and Filby) by using electroporation method and recovery of transgenic calli could be possible when hygromycin resistance was used as the selective trait. But in contrast of it, no transformants were obtained when kanamycin resistance was used as selective marker [67].

Furthermore, *gus* gene was used to test transformation efficiency using histochemical staining, and the transgenic nature of the calli selected for resistance against antibiotics was confirmed by DNA analysis. Unfortunately, plants could not be regenerated from these transformed calli [67].

V. MUNG BEAN (*Vigna radiata* L.)

Mung bean production is limited due to certain undesirable agronomic traits and its susceptibility to biotic stresses like diseases caused by fungi, bacteria, viruses and insect pests. Genetic improvement of mung bean has been possible by transfer of agronomically important genes through genetic transformation techniques.

A. Agrobacterium-mediated Gene Transformation

Historically, the establishment of optimized conditions necessary for regeneration and efficient A. tumefaciens-based transformation of V. radiata was documented first time in 2001 [68]. During the study, hypocotyl and primary leaves were co-cultivated with A. tumefaciens strains LBA4404 (pTOK233), EHA105 (pBINGUSINT) and C58C1 (pIG121Hm). Moreover, these constructs were contained with nptII and gus marker genes under CaMV35S promoter and stable fertile transformants were identified within 4–6 weeks from kanamycin-resistant GUS positive calli and node explants.

In additional experiment, transgenic calli and shoots were obtained from primary leaves and cotyledonary-nodes respectively [68]. Transformation frequency of up to 50% was reported using strain EHA105 (pBINGUSINT) and hypocotyls explants; however, such transformed calli failed to regenerate shoots. Moreover, cotyledonary-nodes infected with strain LBA4404 (pTOK233) gave rise to transgenic plants at an overall efficiency of 0.9%.

Significantly, in V. radiata, nearly 80–100% transformation frequency of cotyledon and hypocotyls explants has been observed with A. rhizogenes strain LBA9402, but transformed tissue gave rise to roots only [69].

B. Transgenesis against Insects/Pests

Mung bean production is commonly affected by different insect pests. An attempt was made to transform various mung bean tissue sources with A. rhizogenes K599 and A. tumefaciens EHA 105, harbouring plasmid pCAMBIA1301 containing cholesterol oxidase gene (choA). Moreover, gene choA codes a potent insecticidal protein which is active against boll weevil larvae [70]. GUS expressing transformed hairy roots developed from cotyledonary root explants could not regenerate into plants.

Significantly, successful production of insect resistant phenotypically normal and fertile mung bean expressing insect resistant gene was first time achieved in 2007 [71]. During the transformation experiments, cotyledonary-node explants were treated with A. tumefaciens strain EHA105 harboring α-amylase inhibitor gene of Phaseolus vulgaris with insecticidal nature, and bar as a selectable marker. Transgenic plants were also obtained via direct shoot organogenesis from the treated explants.

Furthermore, stable integration and expression of the bar gene in T_0 plants was shown by PCR-Southern analysis and PPT leaf paint assay, respectively. Presence of the α-amylase inhibitor gene was also confirmed by Southern blot analysis and inheritance of both transgenes to the progeny was evidenced by PCR [71].

C. Influencing Factors of Transgenesis in Mung bean

Among different conditions, pre-culture and wounding of the explants, use of acetosyringone during co-cultivation and PPT-based selection of transformants played vital role for achieving an enhanced transformation frequency [72]. In a study, different factors were considered to standardize the A. tumefaciens-mediated transformation protocol for V. radiata and A. tumefaciens strain C58C1 harboring a binary vector p35SGUSINT with NPTII gene as selectable marker and GUS as a reporter gene was used [72]. Moreover, higher transformation efficiency (80%) was achieved using primary leaves than hypocotyl (60%) or root (40%) explants but unfortunately, the calli induced from these transformed explants were unable to regenerate shoots.

Further, a comparative study was also undertaken using cotyledonary leaf and cotyledon attached with embryonic axis (CAEA) as explants for transformation studies of local mung bean varieties using A. tumefaciens strain LBA4404 [73]. Moreover, based on kanamycin selection and GUS assay, CAEA explants showed better response towards transformation than the cotyledonary leaf.

VI. LENTIL (*Lens culinaris* L.)

Lentil (Lens culinaris L.) is a grain legume produced in Asia, the Middle East and parts of North and South America as a source of protein in human diets. This crop has also been studied within the context of gene transfer by a number of groups.

Agrobacterium-mediated Gene Transformation

Agrobacterium-mediated transformation in lentil was reported first time in 1992 [74] and gene transformation for the improvement of lentil legumes has been based and also influenced by many factors like other primary legume crops.

Transgenesis against Abiotic/Biotic Stress

Production of disease-resistant lentil would help to increase its production as it is susceptible to many biotic stresses. Significantly, fungus-resistant lentil was developed by transforming decapitated embryos with one cotyledon with Ripgip gene [75]. This gene codes for polygalacturonase inhibitory protein which confers resistance against fungal pathogens and this procedure was followed by an optimized regeneration system which led to achieve very high (35%) transformation efficiency.

Further, micro-grafting was used for rooting transformants. It was among the first reports to develop a marker-free transformation system in legumes, by removing bar gene and PGIP gene was kept in T-DNA cassette prior to transformation experiment. Moreover, fungus-resistant, marker-free transgenic plants were demonstrated via semi-quantitative polygalacturonase-inhibition assay [75].

Recently, in order to enhance drought and salinity tolerance, DREB1A gene driven by the rd29A promoter was introduced into lentil decapitated embryo explants followed by shoot regeneration from the apical-meristems and cotyledonary buds [76]. Subsequently, basta resistant putative transgenic explants were micro-grafted onto non-transformed rootstocks to establish transgenic plants. Further, transgene insertion and inheritance to the progeny were evaluated through PCR and Southern blot analysis. Moreover, expression of DREB1A gene in transgenic plants was induced by salt stress and was also confirmed through RT-PCR.

A. Influencing Factors on Lentil Transformation

To begin with, four strains of A. tumefaciens, i.e. C58, Achh5, GV3111 and A281 were considered in order to assess the susceptibility of lentil to crown gall transformation studies [77]. Significantly, all these strains were very much capable of inducing tumors at a high frequency when shoot apex explants were infected in vivo. However, when infected on excised shoot-apices in vitro, the treated explants were capable to grow on hormone-free medium, a typical feature of tissue transformed with oncogenic Agrobacterium strains [78].

Furthermore, for optimization of lentil transformation, a combination of several treatments with three A. tumefaciens strains, i.e. EHA105, C58C1 and KYRT1 was used to deliver T-DNA into cotyledonary-node tissues [79]. As compared to EHA105 and C58C1, KYRT1 was found to be about three fold more efficient for producing transient GUS expression on cotyledonary node tissues.

In another experiment, number of explants such as cotyledonary-nodes, decapitated embryos, immature embryos and epicotyls were tested for their regeneration ability following A. tumefaciens-mediated transformation [80]. Histochemical staining showed that epicotyl explants exhibited highest transgene expression followed by decapitated embryos, which were found to be more effective in formation of multiple-shoots and were thus suggested as suitable explants for lentil transformation. Unfortunately, root induction could not be possible in these transformed shoots.

B. SAAT-mediated Gene Transformation

Moreover, a first kind of study was undertaken to conduct the experiments on transforming lentil by using sonication-assisted A. tumefaciens (SAAT) transformation [76]. A super-virulent A. tumefaciens strain EHA105 was employed for transferring the T-DNA containing nptII and uidA genes into whole seeds using sonication and vacuum infiltration. Almost 40% of the kanamycin-resistant transfected shoots produced through direct shoot organogenesis were able to root on a medium with IBA and kanamycin. Further, transgene insertion and activity in leaves and roots were detected by PCR and GUS-histochemical assay, respectively [76].

C. Biolistic-mediated Gene Transformation

In order to establish an alternative approach, a reproducible system was developed for lentil transformation using highly regenerable cotyledonary-node meristems by biolistic-method [81]. Rooting of shoots was achieved through grafting. In an experiment, cotyledonary-node tissues of lentil were bombarded with herbicide resistance gene acetolactate synthase (*ALS*) and further, putative

primary transformants along with selfed progeny plants were screened by leaflet painting using metsulfuron herbicide while transgene insertion was confirmed through PCR and Southern hybridization.

VII. CONCLUSION

Although, the biolistic and electroporation-mediated gene delivery have been successful in producing transgenic lines in grain legume crops but Agrobacterium-mediated transformation is the most preferred method of gene transformation in primary grain legume crops. Also, Agrobacterium-mediated transformation of the explants or the callus initiated from the explants has been successful in many crop plants, but in the grain legumes these protocols exist for a few species as in groundnut and garden pea [2]. Further, the efficient recovery of transformed plants depends not only on the mode of regeneration and choice of transformation procedure but also on efficacy of selectable markers during screening of putative transformants. Though, kanamycin has been the most favored selectable agent but still it is not proved an efficient selectable marker for grain legumes.

Moreover, direct-DNA delivery methods can be extended to the genome modification of any plant species that is amenable to tissue culture and regeneration. This approach, combined with rapid advances in genome sequencing technologies and bioinformatics and the increasing efficiency of DNA delivery methods, establishes an efficient and precise strategy for plant genome engineering in legume crops [82].

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