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Soybean: Plant Manipulation to *Agrobacterium* Mediated Transformation

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1. Introduction

Revolution in plant biotechnology can be categorized into in vitro culture and genetic transformation. Plant regeneration was successfully achieved in 1950's while production of transgenic plant was accomplished in 80's. For production of transformants, in vitro culturing strategies are prerequisite.

Soybean has been cultured through organogenesis and embryogenesis but still it is considered recalcitrant. Many explant types has been subjected for shoot induction but immature cotyledons and cotyledonary node of mature seeds got attention in recent years due to high number of shoot production in less time period. But still nature of culture media, application of plant growth regulator and environmental conditions affect on regeneration efficiency. If all constrains are consistent, genotype dependency along with age of explants can not be neglected.

2. In vitro manipulation of plants

Plant cell and tissue culture or in vitro manipulation of plant is the key of modern plant biotechnology. Whole plant can be regenerated under aseptic conditions (in glass vessels) using tissues and even cell when provided balanced nutritional conditions. This technology successfully lead to production of elite cultivars, conservation of endangered plant, production of virus free plant, safeguarding of germplasm and production of secondary metabolites. Beside all these, establishment of culturing protocol is main principle in near about all transgenic plant production strategies. Ability of cell to generate into whole organism is attributed to totipotency and plant cells are unique in this case. However, understanding culture conditions with regard to plant species and explant type is critical for development of reliable system. The physiology of explant is more important because stage and age of explant respond differentially under same conditions. While, some plant species can be easily propagated and some species demand variability in growth regulator(s) concentration(s).

The development of successful tissue culture procedure demand appropriate physiological and chemical conditions. Physiological settings include temperature, pH, light and humidity. As a matter of concern, plant cells and tissues have capability to accommodate minor variations in these parameters. However, regarding chemical environment, that include growth medium and hormone, a little variation may wrench the ability of

regeneration. Growth medium consists of appropriate level of essential minerals (major, minor and trace elements), vitamins, carbon source (monosaccharide or disaccharide) and in some specific cases additives such as charcoal, amino acids, specific chemical etc. Now a number of media are commercially available for plant tissue culture such as MS (Murashige & Skoog, 1962); B5 (Gmaborg et al., 1968); SH (Schenk & Hildebrandt, 1972); LS (Linsmaier & Skoog, 1965); White, 1963 and many more. The choice of suitable media depends upon a number of factors such as plant specie, explant type, explant age, geographical distribution of plant and even season if explant is picked from in vivo condition. These basal medium are designed to keep the plant tissue alive and somewhat proliferative. However, for callus induction, shoot and root differentiation, plant growth regulators are required for these developmental programs. Most common classes of growth regulators include auxins, cytokinins and gibberellins either natural or synthetic. For all the stages of development from a cell to whole plant, appropriate type and concentration of these hormones is required that is selected only on hit and trial basis.

2.1 Callogenesis to organogenesis

Callus is mass of undifferentiated cells that develop when explant is grown on appropriate medium. Callogenesis is basically absence of organogenesis. Callus often produces organs and in this situation callus proliferation is halted. Organ production is dependent upon level of cytokinin in the medium. Such differentiation that lead to bud or shoot formation is also termed as direct organogenesis. However, depending upon hormone type and concentration, callus may undergo different developmental stages that lead to somatic embryogenesis (indirect organogenesis). Organ formation is hooked on the balance of auxin and cytokinin and even ability of cell to develop shoot or root. During culture in the presence of suitable phytohormones, cell become competent that leads to differentiation and lastly morphogenesis occurs.

Sometime cell irrespective to plant tissue or callus may undergo embryo formation. These somatic embryos like zygotic embryos pass through different developmental stages as bipolar, globular, torpedo and cotyledonary. These somatic embryos can be successfully bred into whole plant even in the absence of growth hormones.

2.2 Soybean tissue culture strategies

Meristemic tissue formation from cotyledons of immature embryos of *Glycine max* through somatic embryogenesis first time was observed by Lippmann & Lippmann, 1984. Age of explant and concentration of auxin in the medium strongly affect the development of somatic embryos. However, addition of cytokinin along with 2,4-dichloro phenoxy acetic acid (2,4-D) and higher concentration of sugar inhibited embryo formation. Li and co-workers (Li et al., 1985) obtained thousands of plantlets and somatic embryoids from single cell of young embryo when cultured on Murashige and Skoog (MS) medium containing 6-benzyl amino purine (BAP) and indole acetic acid (IAA) under low light conditions. Single cells obtained in this case converted into proembyos in liquid medium leading to somatic embryos formation and hence plantlet on agar containing medium. Further, Lazzeri et al. (1985) presented a reliable system for the regeneration from somatic tissues of soybean. They predicted that formation of somatic embryos from immature cotyledons of soybean looks imitated process that occurs over a range of culture conditions; and the efficiency of embryogenesis depends upon physiological and chemical conditions mostly plant growth regulators. Surface and subsurface cells of cotyledons can be converted into somatic

embryos at high concentration of auxin (Naphthalene acetic acid; NAA), however, the germination of soybean somatic embryos usually do not require exogenous growth regulators and young immature cotyledons have great tendency to give rise somatic embryos. After embryo development and in secondary stage of plantlet formation desiccation perform positive role for better recovery (Parrott et al., 1988; Finer, 1988). Lazzeri et a*l*. (1987) furhter reported that embryo initiation in soybean system is predominantly multicellular and 2,4-D plays a major role in it. However, efficiency of process can be enhanced by NAA and these induced embryos were closely related to zygotic embryos. Subculturing also influence frequency of normal embryo development during somatic embryogenesis. Although, complete cotyledon is considered to produce embryos, Hartweck et al. (1988) reported that epidermal and sub-epidermal cells at distal periphery of cotyledon and geterogeneous embryogenic tissues in central region of cotyledons can produce embryos in the presence of NAA and 2,4-D, respectively. Later on Liu et al. (1992) stated that epidermal cells produce somatic embryos without intervening callus phase (direct organogenesis) and presence of 2,4-D and NAA play major role in this histo-differentiation. Different developmental stages of somatic embryo formation initiate from proembryo while secondary embryogenesis and chimeric embryo development occur during differentiation (Gyulai et al., 1993). The differentiation process takes place in 4-6 weeks, initiated by three and four cell embryo leading to development of globular and heart shape embryo. Abaxial side of explant facing the medium resulted in faster formation of somatic embryos from subepidermal tissue in the presence of silver nitrate, irrespective to pH conditions and high light intensity causes faster production of somatic embryos (Santarem et al., 1997; Hofmann et al., 2004). Meurer et al. (2001) & Fermando et al. (2002) worked on soybean somatic embryogenesis from immature zygotic cotyledons from different locations. They found that genotype and location strongly affect soybean primary embryo development and to develop somatic embryos one should be able to realize acceptable level of embryo initiation of each cultivar. Influence of genetic variations in soybean on embryo initiation from immature cotyledons has been well established but upturn in weight, volume, embryo developmental stages and plant recovery can partially be overcome by modifying protocols. The use of ethylene inhibitor, low concentration of nitrogen and sucrose, desiccation, spermidine and alteration in nitrogen source, polyethylene glycol and sorbitol, reported by different researchers, significantly enhanced embryoid formation and their maturation to plant. Conclusively, in addition to above mentioned factors, breeding line; immature embryo age, quality and appropriate choice and concentration of hormone is essential for significant results.

Besides using immature cotyledons and embryos, a lot of work has also been carried out using cotyledonary node explants from seeds or plantlets after few days of germination. First report of plant regeneration from soybean cotyledonary node segment of seedlings grown in the presence of BAP was by Cheng et al., 1980. They obtained multiple shoot bud formation on medium containing high concentration of BAP but better bud growth was noticed when cultures were transferred to low concentration of BAP. Wright and co-workers also reported that BAP is an essential component of media for shoot induction from cotyledonary node explants. Carbon source (sucrose or fructose) and salt concentration (full MS, ½ MS or 1/4MS) have different effects even hormone concentration is kept constant. They further reported that seedlings germinated on water agar medium were not so responsive for shoot induction (Wright et al., 1986). BAP treatment to embryonic axes does not allow the cell to remain quiescent and cells are reprogrammed to produce multiple somatic foci (Buising et al., 1994). Presence of cytokinins (BAP) interrupts chromosomal

DNA replication in large number of cells in shoot apex that ultimately leads to formation of multi cell loci leading to shoot development. Thidiazuron (TDZ) induce adventitious shoots more efficiently than BAP and hypocotyls proved better than cotyledonary nodes for multiple bud formation while plating method (hypocotyls ending in contact to media) and cutting of explant also effects adventitious shoot formation from mature soybean seed hypocotyl (Zia et al., 2010a). However, after shoot bud induction, placement of explant on zeatin riboside containing medium allow the shoots to increase in length more as compared with other cytokinins. Sairam et al. (2003) developed an efficient protocol for callogenesis and embryogenesis from cotyledonary node explant on MS medium containing 2,4-D and BAP. According to them regeneration efficiency was genotype dependent and the best choice of carbon source might be sorbitol for callus induction and maltose for organogenesis. Addition of other growth regulators such as TDZ and Kinetin in MS or B5 medium varied embryo or shoot formation in different soybean genotypes from mature half seed's nodal segment. However, different stages of proliferation and regeneration also vary depending upon genotype. Such variability's can partially be overcome by some modifications in embryogenesis and regeneration protocols (Bailey et al., 1993). Recently Loganathan et al. (2010) reported the somatic embryogenesis from immature embryonic shoot tips on MS medium containing 6% sucrose, 2,4-D and amino acids. The embryos efficiently regenerated into shoots on hormone free MS medium containing charcoal. While, 72-96hr desiccation positively influenced on plantlet formation.

There are very few reports of soybean regeneration from other explants. In 1977 Beversdorf & Bingham reported callogenic response from hypocotyls and ovaries as explant on semi solid and liquid medium. They failed to regenerate shoots; however, they observed structures similar in appearance to embryos in liquid medium. Primary leaf explant turned into callus when cultured on B5 medium. Indirect organogenesis was successfully achieved when callus was further cultured on modified medium containing pyroglutamic acid that greatly enhanced regeneration capability (Wright et al., 1987). Kim et al. (1994) stated that addition of proline in the medium increased the number of shoots but decreased the length of generated shoots. They also reported that cobalt and zinc also play an effective role in shoot induction from primary leaf nodes. Droste et al. (1993) cultured primary leaf less meristem on organic enriched medium and find microscopic bud like structure within two weeks; however, very few plants were developed from these buds. Reichert et al. (2003) and Tripathi & Tiwari (2003) demonstrated that regeneration efficiency from hypocotyls, epicotyl and primary leaf explants is also genotype maturity dependent. The shoots regenerate from acropetal end and/or central region of cotyledonary node tissue. They further concluded that explant, inoculation medium and appropriate concentration/ combination of growth hormone are also essential for better regeneration efficiency. Stem node segments were also cultured on different basal medium for shoot bud formation (Saka et al., 1980). Combination of MS salts and B5 vitamins supplemented with BAP was found better choice to produce shoot buds. However, bud growth stimulated on medium containing low BAP concentration and replacement of sucrose with fructose.

Protoplast culture; isolated from immature cotyledons has also been reported. Dhir et al. (1991) cultured these protoplast in the liquid medium in the presence of combination of cytokinins (BAP, Kinetin, Zeatin) and observed 21% multiple shooting response from compact calli. The regeneration efficiency increased upto 30% when glutamine, aspragine and Gibberellic acid (GA3) were added in the medium. However, medium supplemented with different amino acids and their derivatives as nitrogen source was found better for

plant recovery from protoplast derived calli. However, composition of medium varies embryogenic calli initiation and then somatic embryo differentiation (Zhang & Komatsuada 1993). Zhao et al. (1998) reported that TDZ plays an important role in embryo induction and germination during soybean anther culture but plant differentiation rate was quite low. Addition of 2,4-D in the medium and culturing in light significantly increased the morphogenic response of anther walls and connective tissues. No androgenic response was observed in anther culture of four soybean genotypes but somatic embryogenesis was observed from the epidermis and the middle layer (Rodrigues et al., 2004, 2005). Higher concentration of 2,4-D during anther culture results in plasmolysis of microspores. Time of culture was also found effective for induction of somatic embryos derived from anther culture. Frequencies of binucleate symmetrical grains and multinucleate / multicellular structure formation were also found significant in the day of culture and cultivar interaction (Cardoso et al., 2007).

3. Plant transformation: a prospective to revolution

Transformation is the alteration in genetic makeup of a cell due to incorporation of a foreign DNA fragment that expresses in the cell resulting variation in physiochemical properties. Plant transformation is now a routine practice and carried out through different approaches including *Agrobacterium* mediated, gene gun, electroporation, microinjection and few more. More than 120 diverse plant species have been transformed. Now in most of the developed countries transgenic crops are cultivated with improved nutritional quality and tolerance to biotic and abiotic stresses. This not only improved food quality and quantity for humans and animals but also somewhat has positive influence on environment. Even after a lot of advancement in transformation technologies, many plant species including soybean is considered recalcitrant to transformation.

Agrobacterium mediated transformation of soybean has shown significant improvement and enabled public and private sector for production of commercial cultivars with transgenic traits. A number of reports describe condition standardization for T-DNA delivery, effect of *Agrobacterium* strain and choice of cultivar and conditions to produce high yield of transformants. Beside all above mentioned conditions, soybean cultivar susceptibility to *Agrobacterium* can not be overlooked. Although, protocols for production of transgenic plant have been standardized but all seems ineffective. We are far away from getting transformants from a single experiment especially in case of soybean that is still considered obstinate to transformation.

3.1 Biological way to introduce DNA into plant cell

Nature has offered *Agrobacterium* the ability to transfer some part of DNA from plasmid to plant cell. This T-DNA (transfer DNA) naturally causes callus formation on plant's parts termed as crown gall disease. However, this is multifarious procedure that involves two biological systems; bacteria and plant cell and success is subjected to compatibility. Unsurprisingly virulence story of *Agrobacterium* is the key for tumor induction. This virulence provokes by simple carbohydrates and phenolic compounds that are released by injured plant tissue. After this initiative, vir genes activate and produce proteins. These proteins hold the charge of transformation that include scratch of T-DNA, carry, direct towards the plant cell and finally integrate into plant genome. Naturally this T-DNA contains genes that are involved in biosynthesis of plant hormones that are involved in uncontrolled proliferation of plant cell leading to callus formation.

Engineering technologies and molecular mindsets expiated asset of *Agrobacterium* to transfer the genes of interest into plant cell. This revolution lighted the pathway to break inert kingdom genetic exchange restrictions. They terminated the property of *Agrobacterium* to cause tumor but did not change the belongings that are involved in T-DNA transfer mechanism. Finally, plant biotechnological era came to revolution to produce transgenic plant species with desired characters. However, all the barriers could not be departed productively. Factors responsible for production of transformants have been studies worldwide and are found more or less same for all genotypes even plant or Agrobacterium. These factors, at *Agrobacterium* flank, include genotype, plasmid constrains, T-DNA length and signaling mechanism. While at plant cell side, the factors include type, age, genetic makeup and welcome address to T-DNA. The welcome discourse also depends upon physical and chemical conditions that finally lead to produce whole plant from a single transformed cell.

Although initially dicots were considered host for *Agrobacterium* but advancement in procedures commanded *Agrobacterium* to display same role in monocots as in dicots. The process of plant transformation is a routine matter in most of the labs but some plant species are still considered recalcitrant to transformation.

3.2 Susceptibility of soybean to *Agrobacterium*

Soybean genotype susceptibility for tumor induction was studied by Pedersen et al., 1983 and Owens & Cress in 1984 on infection with *Agrobacterium*. According to their reports, crown gall formation is dependent upon soybean genotype and *Agrobacterium* strain used as well as on environmental conditions. Physiological age of soybean cotyledons also exert great influence on tumor initiation and tumor morphology. Owens and Smigocki (1988) indicated that transformed soybean cells could be recovered by co-infecting with super-virulent strain and addition of phenolic compounds (Acetosyringone or Syringaldehyde) in inoculation medium increase transformation efficiency. It is also possible to produce tumorigenic genotype by crossing non-tumorigenic with highly tumorigenic genotype in soybean so conventional crossing may help to transform non-susceptible genotypes. Luo et al. (1994) observed production of transformed calli from mature seed cotyledons working on transformation friendly genotype "Peking" with *Agrobacterium* strain A281 harboring pZA-7 (UidA + nptII). They mentioned that production of transformed calli is a simple tool to test constructs designed for soybean transformation. Genotype of *Agrobacterium* (nopaline, agropine, octopine) also plays an important role in infection and T-DNA inheritance (Mauro et al., 1995). Acetosyringone may facilitate tumor formation significantly but not for all *Agrobacterium* strains. However, strain/genotype difference was observed significant while older plant parts showed less susceptibility to tumor formation. Transformation event occurs in number of cells but poor selection and non-regenerable callus formation attribute to poor recovery of transformed plants (Donaldson & Simmond, 2000). A new *Agrobacterium tumefaciens* strain KAT23 isolated from peach root also found effective to induce callus at soybean tissues (Yukawa et al., 2007). This nopaline type strain can transform T-DNA of Ti plasmid and of binary vector efficiently to many legumes including soybean.

3.3 Soybean *Agrobacterium* mediated transformation

Hinchee and his colleagues first time reported soybean transformation with *Agrobacterium* strain pTiT37-SE harboring pMON9749 (GUS + nptII) and pMON894 (nptII + glyphosate tolerance). They successfully regenerated plants on media containing kanamycin and

glyphosate (Hinchee et al., 1988). Modification to regeneration protocol is essential to get high level of transformants. Greater number of mitotic cycles are required before embryo initiation and production of plants with transformed germ lines cells. EHA101 was found more potent to transform soybean immature cotyledons and recovery of transformed plants over LBA4404 (Parrott et al., 1989). However, McKenzie & Cress (1992) were able to get transformed plants from cotyledon and hypocotyl explants from 10 days old seedlings working with LBA4404 harboring pBI121. Transformation efficiency is not dependent only on Agrobacterium genotype but soybean cultivar, age of explant and other conditions also influence. Trick & Finer (1997) introduced sonication assisted *Agrobacterium* mediated transformation (SAAT) system. SAAT permits efficient delivery of T-DNA to large number of plant cell in a variety of different plant tissues. In soybean, GUS expressing surface area increased upto 79.9% by SAAT treatment for 10 sec. Other tissues that are considered difficult to transform can be subjected to SAAT that permit Agrobacterium to infect deep within the plant tissue. While SAAT treatment was not found effective at post co-cultivation period with decreased shoot proliferation from cotyledonary node of some soybean genotypes (Meurer et al., 1998). They also reported that inoculum OD_{600} 1.0 gave better transient expression but no interaction was found between SAAT, *Agrobacterium* strain and soybean genotype. Micro-wound in plant tissues due to SAAT treatment release compounds that facilitate growth and accumulation of bacteria under aerobic conditions so facilitate transformation efficiency (Finer & Finer, 2000). However, longer sonication time may damage plant tissue (Santarem et al. 1998). Way of placement of explant (adaxial side incontact with medium) on medium (Ko et al., 2003); exposure of soybean explants to $AgNO_3$ throughout shoot induction and shoot elongation (Olhoft et al., 2004); explant preparation in the presence of *Agrobacterium* culture and varying level of kanamycin during selection and regeneration (Zia et al., 2010b) are important for better recovery of transformants.

Instead of kanamycin resistant plant, glufosinate resistant (bar gene) plants were produced by Zhang et al. (1999) and Clemente et al. (2000) using cotyledonary node explants of 5 days old seedlings. Glyfosinate selection regime is important to get rid of non-transformed plants and to minimize chimerism. Yan et al. (2000) analyzed that immature zygotic size 8-10mm and co-cultivation for short period increase transient expression while selection by direct replacement at low concentration of hygromycin also increase somatic embryo development and plant regeneration. Cystine present in co-cultivation medium increase transformation efficiency due to presence of thiol group and polyphenol oxidase and peroxidase inhibition (Olhoft & Somers, 2001; Olhoft et al., 2001). Copper and iron chelators were also found effective for better expression. Olhoft and his colleagues successfully transformed soybean by cot node method (Olhoft et al., 2003). High frequency upto 16.4% was observed due to presence of cystine, Dithiothreitol (DTT) and thiol compound in infection and co-cultivation medium. Beside this, addition of Silwet-77 as surfactant; co-cultivation at 22°C also played significant role in transformation (Liu et al., 2007). Donaldson & Simmonds (2000) demonstrated that competent cells, in the case of cotyledonary node transformation, are few so has low transformation competency therefore using cotyledonary nodes as explants present low transformation efficiency. Tight selection procedure (selection of explants on selective agent before infection) increases transformation efficiency and occurrence of less escape (Chen, 2004).

Xing and his colleagues produced marker free plants by introducing two T-DNA binary systems (Xing et al., 2000). Integration of two T-DNA followed by their independent

segregation in progeny is a viable mean to produce maker free soybean transgenic plants. Transformation efficiency was observed upto 15.8% using embryonic tips of soybean pre grown on MS medium containing BAP (Liu et al., 2004). They also observed that shoot regeneration and transformation efficiency increased using embryonic tips over hypocotyls and cotyledons. Embryonic tips were also found sensitive against kanamycin treatment at level higher then 10 mg/l. Addition of antioxidant in co-cultivation medium result in significant decrease in browning and necrosis of hypocotyls and increased GUS expression (Wang & Xu, 2008). Embryogenic tips showed better response for hypervirulent strain KYRT1 than EHA105 and LBA4404 when infected for 20 hours (Dang & Wei, 2007). While co-cultivation for 5 days in dark at 22°C in acidic medium (pH 5.4) also enhanced transformation efficiency. Paz et al. (2004) concluded that use of high vigor seed and minimum seed sterilization also raise transformation efficiency from cotyledonary node of 5-6 days seedling plants. Cystine and DDT during co-cultivation increase T-DNA delivery while glyfosinate selection over bialaphos during shoot induction and shoot elongation also increase transformation efficiency. Ko & Korban (2004) reported that size of immature cotyledon (5-8 mm in length), concentration of bacterial culture and co-cultivation for 4 days significantly increase transformation efficiency. However, they failed to get transformants in the presence of kanamycin during selection. Paz et al. (2006) used cotyledonary node of half seeds as an explant. Use of half seed explants ranged transformation efficiency 1.4 to 8.7% and this system is simple and does not require deliberate wounding of explants. Use of thin 30 fibers needle to wound cotyledonary node cells of half seeds also increased transformation efficiency up to 12% confirmed by gfp activity and L- Phosphinothricin (PPT) selection (Xue et al. 2006). Organogenic callus induced from axillary nodal tissue of soybean was also subjected for *Agrobacterium* mediated transformation (Hong et al., 2007). Moderate concentration of TDZ was required for induction of organogenic calli while low concentration of BAP proved best for organogenic response from callus. They also observed that young callus was more competent to T-DNA delivery and multiple shoot regeneration. Olhoft et al. (2007) tested two disarmed *Agrobacterium* strains for soybean transformation. Regeneration frequency was not significantly different when inoculated with *A rhizogenes* strain SHA17 and *A tumefaciens* strain AGL1 while infection with SHA17 increased transformation efficiency upto 3.5 folds.

3.4 Soybean transformation with *Agrobacterium rhizogenes*

Instead of *Agrobacterium tumefaciens*, soybean transformation also been studied by *Agrobacterium rhizogenes* to study efficiency of strain, properties of roots and resistance against nematodes. Cho et al. (2000) got transformed hairy roots by *A. rhizogenes* strain K599 harboring pBI121 (gus + nptII) and pBINm-gfp5-ER (nptII and gfp). They observed that cyst nematode may complete their life cycle in transformed hairy root cultures containing these genes but concluded that such system can be ideal for testing genes that might impart resistance to soybean against nematodes. RNAi silencing was also studied by *A. rhizogenes* mediated transformation to cotyledon explants of soybean (Subramanian et al., 2005). More than 50% roots were transformed with RNAi construct that exhibited more then 95% silencing. Kereszt et al. (2007) reported that infection of *A. rhizogenes* at cotyledonary node of few days seedling might produce 5-7 roots at infection site with 70-100% efficiency. These roots fully support the plants, are capable of nodulation, have phenotype as determined by genotype of shoot. This can further be used for high throughput transformation, to test high

number of genes, different biological processes and symbiotic relation etc. Klink et al. (2008) introduced a new soybean variety MiniMax with a rapid and short life cycle that produced hairy roots under non-axenic conditions when infected with *A. rhizogens* strains K599 harboring disarmed vector pKSF3. These transgenic roots were capable of compatible reactions with several *Heterodera glycines* races.

3.5 In planta *Agrobacterium* mediated transformation

The development of the in planta transformation system (Floral-dip method and Vacuum infiltration) radically accelerated research in basic plant molecular biology. These methods have been targeted mostly for meristems or other tissues that ultimately give rise to gametes.

Soybean transformation also has been subjected by infecting partially germinated seeds with *Agrobacterium* to vacuum infiltration with high frequency (de Ronde et al., 2001). In planta soybean transformation has also been carried out by Lei et al, (1991); Liu et al., (1996) and Hu & Wang (1999). They introduced foreign DNA by pollen tube pathway and by ovarian injection. Such procedures pass tissue culture steps but for routine transformation physiological conditions of recipient plant, type and concentration of DNA, location of ovary etc are critical factors. By such methods, they produced new varieties that yield batter protein and oil contents. But Li et al. (2002) were not able to produce positive results by pollen tube pathway. They reported that DNA was inside the cell but not integrated into soybean genome. Shou et al., 2002 also performed pollen tube pathway transformation procedure using different soybean cultivars. They observed that only 2% progenies were partially resistant to herbicide. However, no plant was confirmed by Southern blotting carrying transformed T-DNA as well as by histochemical GUS assay. They concluded that pollen tube pathway transformation technique is not reproducible for soybean.

4. Conclusions

Plant tissue culture has attained a lot of attention in recent years because it is a gateway to modern plant biotechnology including plant genetic transformation. Although soybean in vitro manipulation and transformation has passed more then thirty years but still establishment of acceptable protocol is far behind that could be used for all cultivars all over the word. The work is going on to overcome the limitations but soybean genotype could not be overlooked in all methodologies. Now destiny is near where new genetically modified varieties of soybean like Roundup ready will be produced globally by following the established protocols.

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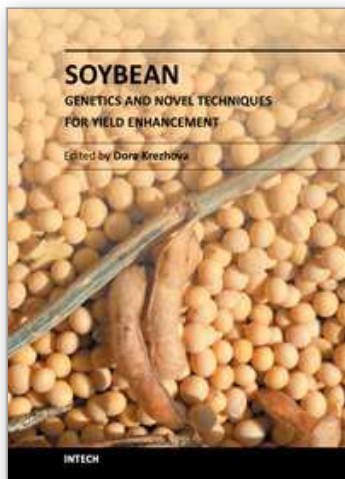
Soybean - Genetics and Novel Techniques for Yield Enhancement

Edited by Prof. Dora Krezhova

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