Scenario of in Vitro Regeneration and Transformation Studies in Multipurpose Sesbania Species

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ABSTRACT

Sesbania species grow mainly in tropical and subtropical regions of the world as multipurpose legume crops. These are very important part of sustainable agriculture and also for diverse industrial or other uses. But their production has compromised due to lack of interest among farmers and presence of constraints, rendering it appropriate to use as a food crop. Improvement has lagged in Sesbania species because of the unavailability of the gene pool, along with self-incompatibility in closely related species. Lacunae in improvement of its species could overcome with the help of biotechnological tools that could transfer genes in distantly related species, too. Mostly, an efficient and reproducible regeneration system is a prerequisite for efficient transgenic plant production. However, progress has made towards in vitro regeneration in various Sesbania species such as S. bispinosa, S. grandiflora, S. sesban using hypocotyl explants and using cotyledonary node explants in S. drumondii and S. rostrata. Considerable success has also been obtained in plant regeneration via somatic embryos in S. sesban and via protoplasts in S. bispinosa. But, till date only in S. drumondii one report on generation of transgenic plants is available, where T0 plants have been obtained using cotyledonary node explants. In this regard, the current paper highlights the in vitro regeneration and genetic transformation protocols developed so far in Sesbania species along with future directives towards improvement of this important genus.

Keywords

multipurpose legume, regeneration, Sesbania, transformation

1. Introduction

The genus Sesbania belongs to the Leguminosae family and comprises 60–85 species of herbs, shrubs and trees. Of all the species, about 22 species have been evident in use widely by humans in tropical and subtropical regions of the world. These are in cultivation mainly for soil improvement as bio-fertilizers and agroforestry as well as also grown to provide us food, medicines, wood, fibre, dyes and fuels [1]. Different Sesbania species had reported to possess different pharmacological properties like antibacterial properties in S. aculeata [2], anti-inflammatory properties in S. bispinosa [3], anticancer and antidiabetic activity in S. grandiflora [4,5]. In India S. rostrata is the most widely used Sesbania species that possess various medicinal properties and is used to treat various ailments such as rheumatism, gout, leprosy, inflammation, bronchitis, anaemia, headaches, fevers, anxiolytic [6]. Species of Sesbania also have the metal accumulation competence and used in wastewater treatment [7]. Despite of such myriad uses of Sesbania species, much attention has not given by the researchers. So, there is need to develop a long-term goal for the improvement of the Sesbania plants for better attributes such as increase in biomass, secondary metabolites content, higher accumulation of heavy metals and better acceptability as food crop. Such improvements through conventional breeding methods are not only very time consuming but sometimes totally not feasible either due to lack of germplasm or inability to transfer genes from non-compatible distant species. So, genetic engineering
with the help of in vitro regeneration and genetic transformation is the one option to further improve these economically important crops in less time along with maintaining their genetic integration.

Like other grain legumes where genetic manipulation is very difficult and challenging because of recalcitrance towards in vitro regeneration and transformation [8], Sesbania species are no exception to that. There is not so much of progress has made in the last few decades for transformation of the Sesbania species. For genetic manipulation via gene transfer methods, the pre-requisite is a robust regeneration system that is compatible for routine transformation experiments. The regeneration system should be quick that avoid somaclonal variations, and an efficient selection system must be there to recover the transgenic fertile plants in appropriate numbers. So, this review first time cumulates and highlights the current status of in vitro regeneration and genetic transformation studies that have developed for the improvement of Sesbania species along with outlining the future perspectives.

2. In vitro regeneration

The most essential thing required for genetic transformation is efficient and reliable in vitro regeneration system but being a legume, Sesbania species are also recalcitrant towards it. In past few decades, some progress has made towards regenerating them from pre-existing meristems and de novo organogenesis either directly or indirectly via the callus phase. Hypocotyls and cotyledons are the most commonly used explants for regeneration. De novo shoot organogenesis has been obtained either directly or via callus phase by using hypocotyl explants [9]. Almost every part of the plant can produce callus, but with variation in time period of formation, amount and nature of it [10]. The shoot bud regeneration from the callus varies with the explant type and concentration of growth regulators. Shoot organogenesis had reported from the callus derived from the leaves, cotyledons and hypocotyls [9,11] but not from the roots of the plants [10]. Direct shoot organogenesis with no intervening callus phase has reported using explants such as cotyledons, cotyledonary node, stem node and internodes [12–14]. This method of regeneration involves a single step and hence is a quick method of regeneration. Another way of producing a large quantity of propagules in short time is by somatic embryogenesis, which also has the potential for the non-chimeric transgenic plants production. S. sesban had reported for the production of somatic embryos from the cotyledon explants up to cotyledonary stage [15]. The embryos so formed developed into complete plantlet successfully. Somatic embryogenesis offers a good potential for the synthetic seeds production and also a potential way to genetic improvement using gene transfer techniques. Another method is regeneration through the isolated proplasts. Protoplasts are required for a range of plant genetic manipulations, production of somatic hybrids, cybrids and transgenic plants. In Sesbania, protoplasts have been obtained from stems in S. rostrata [16] and cotyledons in S. bispinosa [17]. The isolated protoplasts divided to form callus. The callus either changed into pro-embryo [16] or gave rise to shoots directly with no embryo like structure formation [17]. There is a need for development of protoplast regeneration system in Sesbania species so that somatic hybrids could develop in between wild and related species. The regeneration studies in different Sesbania species have summarized below (Table 1).
Table 1: Current status of *in vitro* regeneration studies in *Sesbania* species

| *Sesbania* species | Basal Medium* | Growth regulators | Explant | Morphogenetic responses | References |
|--------------------|---------------|-------------------|---------|--------------------------|------------|
| *S. aculeata*      | MS            | 10 µM BAP + 1 µM IBA | Hypocotyl | Shoot buds | [9] |
|                    |               | 0.1 µM BAP + 0.1 µM NAA | Leaves | Shoot buds |             |
| *S. bispinosa*     | B5            | 10 µM BAP | Hypocotyl Cotyledon | Shoots | [10] |
|                    | MS            | 9 µM 2,4-D + 2.22 µM BAP | Cotyledon Leaves | Callus | [11] |
|                    | MS            | 8.88 µM BAP | Callus | Shoots |             |
|                    | MS            | 8.88 µM BAP + 0.25 µM IBA | Hypocotyl | Shoots | [12] |
|                    | MS            | 8.88 µM BAP + 0.25 µM NAA | Cotyledon | Shoots |             |
|                    | MS            | 4.52 µM 2,4-D + 8.88 µM BAP | Protoplast derived from cotyledons | Callus | [17] |
|                    | MS            | 4.92 µM IBA + 4.44 µM BAP | Callus derived from protoplasts | Shoots |             |
| *S. cannabina*     | MS            | 4.44 µM BAP + 0.49 µM IBA | Hypocotyl | Shoots | [12] |
|                    |               | 4.44 µM BAP + 0.49 µM IBA | Cotyledon | Shoots |             |
| *S. drummondii*    | MS            | 22.50 µM BAP | Cotyledonary node | Shoots | [14] |
|                    |               | 13.30 µM BAP | Axillary node | Shoots |             |
|                    |               | 2.27 µM TDZ | Stem node | Shoots |             |
|                    | MS            | 11 µM BAP + 2.5 µM IBA | Cotyledonary node | Shoots | [18] |
| *S. furmosa*       | MS            | 4.44 µM BAP + 0.25 µM IBA | Hypocotyl | Shoots | [12] |
|                    |               | 4.44 µM BAP + 0.25 µM IBA | Cotyledon | Shoots |             |
| *S. grandiflora*   | B5            | 1 µM BAP + 1 µM NAA + 1 µM GA3 | Hypocotyl Cotyledon | Shoot buds | [19] |
|                    | MS            | 1 µM BAP + 0.1 µM GA3 | Shoot buds | Shoots |             |
|                    | MS            | 4.44 µM BAP + 5.7 µM NAA | Cotyledon | Shoot buds | [20] |
|                    | MS            | 3.4 µM Kin + 0.57 µM IAA | Cotyledon | Shoot buds | [21] |
| *S. rostrata*      | B5            | 4.44 µM BAP + 5.7 µM IAA | Hypocotyl | Shoots | [22] |
|                    | MS            | 200 mg/L Claforan | Tumour | Shoots | [23] |
|                    | MS            | 0.88 µM BAP | Stem | Shoots | [26] |
|                    | MS            | 0.22 µM BAP + 10.74 µM NAA | Stem | Callus |             |
|                    | MS            | 4.46 µM zeatin + 0.53 µM NAA | Callus | Shoots |             |
|                    | N             | 4.44 µM BA | Internode Node | Shoot buds Callus | [27] |
|                    | N             | 4.44 µM BA | Internode Node | Shoots | [28] |
| *S. sesban*        | MS            | 8.88 µM BAP + 0.25 µM IBA | Hypocotyl | Shoots | [12] |
|                    | LS            | 0.54 µM NAA | Cotyledon | Shoots | [15] |
|                    | N             | 7 µM Kin | Node | Shoots |             |
|                    | N             | 2.32 µM Kin | Internode | Shoots |             |

* B5: Gamborg [32]; MS: Murashige and Skoog [33]; N: Nitsch [34]; LS: Linsmaier and Skoog’s medium [35]
2.1 *Sesbania aculeata* (Schreb.) Pers.

There is only one report of *in vitro* regeneration in *S. aculeata* where differentiation of shoot buds occurs directly and indirectly on the same medium from hypocotyl and leaf explants [9]. The organogenesis depended on the combination of auxin and cytokinin in the medium. The best response reported on MS medium supplemented with a combination of BAP (8.88 µM) and IBA (1.0 µM) with hypocotyl explants regenerating about 24 shoot buds in 100% of culture. In comparison, leaf explants were less responsive and regenerates only 3–4 shoot buds per explant in 25% of cultures on MS medium supplemented with 0.1 µM BAP and 0.1 µM IBA. The shoot buds grew normally and developed into shoots in about 4 months in culture on the same medium, though how many buds converted into shoots was not mentioned. The same shooting medium also resulted in the root formation in the shoots formed.

2.2 *Sesbania bispinosa* (Jacq.) W.Wight

*In vitro* regeneration of *S. bispinosa* reported first time by Kapoor and Gupta [10] using cotyledon and hypocotyl explants. They got multiple shoots (10–12 shoots per explant) directly on the B5 medium containing cytokinin (10.0 µM BAP) in 86% of cotyledon explants and 65% of hypocotyl explants. Later, Sinha and Mallick [11] developed the shoots indirectly through a callus phase using cotyledons and leaves as explants. Callus induction had achieved by using the combination of auxin (9.0 µM 2,4-D) and cytokinin (2.22 µM BAP). So developed callus then transferred to auxin free medium containing a high dose of cytokinin (8.88 µM BAP) for shoot development. Callus got from both the explants found equally responsive, giving rise to around 6 shoots in 100% of culture. According to Yan-Xiu [12], shoots were obtained via direct and indirect regeneration on MS media, supplemented with different concentration of auxin and cytokinin using hypocotyl and cotyledon explants. Shoots regenerated directly on medium containing 8.88 µM BAP with 0.25 µM IBA and 4.44 µM BAP with 0.49 µM IBA in 50% and 40% of hypocotyls and cotyledons cultures, respectively. For indirect shoot organogenesis, initially callus raised from hypocotyls on medium fortified with NAA (5.37 µM) and then cultured it on MS medium supplemented with BAP (4.44 µM) and IBA (4.92 µM) where 1.5 shoots/explant had regenerated in 60% of cultures. The shoots rooted in rooting medium containing auxin (0–2.46 µM IBA) and had successfully transplanted to soil. Shoots have also developed through the protoplast cultures [17]. The protoplasts derived from the cotyledons gave rise to the callus in the presence of auxin (4.52 µM 2,4-D) and cytokinin (8.88 µM BAP). The callus thus formed, then produced the shoots in presence of IBA (4.92 µM) and BAP (4.44 µM). Complete plantlets were obtained from the developed shoots.

2.3 *Sesbania drummondii* (Rydb.) Cory

First time regeneration in *S. drumondii* was reported by Cheepala [14], who used different explants having meristematic cells for direct multiple shoot regeneration. Cotyledonary node explants excised from seedlings, axillary node and stem nodal explants from mature plants were used for shoot organogenesis. All explants formed shoots on MS medium supplemented with cytokinin (BAP/TDZ) at different concentrations. The maximum regeneration potential had shown in cotyledonary nodes with 80% regeneration frequency and 7.75 shoots per explant at 22.50 µM BAP, 90% at 13.30 µM BAP for axillary nodes (4.08 shoots per explant) and 60% at 2.27 µM TDZ for stem nodes (6.2 shoots per explant). In another report also, cotyledonary node explants gave best response when auxin (2.5 µM IBA) added to the regeneration medium along with 11.0 µM BAP and produced multiple shoots directly in 90% of cultures [18]. The shoots rooted in medium supplemented with auxin (2.5 µM IBA) and
transplanted to soil. So in *S. drumondii*, cotyledonary node explant was most responsive towards shoot organogenesis and had used for transformation study.

2.4 *Sesbania grandiflora* (L.) Pers.

Shoot regeneration was obtained using different explants on B5 medium supplemented with different concentrations of growth hormones by Shankar and Mohan Ram [19]. Hypocotyl and cotyledon explants gave rise to shoot buds in the presence of a combination of BAP (1 µM), NAA (1 µM) and GA3 (1 µM) in medium. Hypocotyl explants were more responsive with production of 12 shoot buds per explant than the cotyledon explants (3–4 shoot buds per explant). The shoots buds elongated into shoots with subsequent sub-cultures onto the medium supplemented with 1 µM BAP and 0.1 µM GA3. The shoots rooted into medium supplemented with IBA. Detrez [20] obtained shoot buds from cotyledons on MS medium supplemented with a combination of BAP (4.44 µM) and NAA (5.37 µM) in 90% of culture. Regenerated shoots rooted in medium supplemented with NAA (5.37 µM) and then transplanted to soil. Shoots had successfully produced on MS medium supplemented with Kin (2.32 µM) and IAA (0.57 µM) using cotyledon explants [21]. The shoots rooted on medium supplemented with IAA (0.57 µM).

2.5 *Sesbania rostrata* Bremek. & Oberm.

*S. rostrata* has found to be regenerative using a variety of explants and on different mediums. Multiple shoot regeneration via direct organogenesis had reported using hypocotyl explants on B5 medium supplemented with a combination of BAP (4.44 µM) and IAA (5.7 µM) in 20% of cultures [22]. Cotyledon explants failed to produce shoots on the same medium. However, preconditioning of seedlings on medium with high dose of BAP (22.2 µM) produced shoots directly from cotyledons but at low frequency (6–8%). The shoots obtained were rooted in medium supplemented with NAA (0.53 µM). Mathews [23] obtained shoots from the tumor explants which in turn induced via infecting *in vivo* raised plants with *Agrobacterium tumefaciens* strain A208 carrying plasmid pTiT37 and then cultured on the MS medium supplemented with 200 mg/L claforan (cefotaxime). The cefotaxime has found to show a stimulatory effect on plant regeneration in different plant species [24, 25]. The obtained shoots then rooted in MS medium containing NAA (0.53 µM). Direct and indirect shoot organogenesis had reported from stem segments having one node and internode [26]. Multiple shoots induced directly on MS medium supplemented with 0.88 µM with an average number of 6 shoots per explants while only callus produced on medium supplemented with BAP (0.22 µM) and NAA (10.74 µM). More responsive callus produced when the explants inoculated, along with *Agrobacterium tumefaciens* strain 82.139. The callus thus formed regenerates shoots on medium supplemented with zeatin (4.46 µM) and NAA (0.53 µM). The shoots got rooted on basal medium using no growth hormone. Nodes and internodes explants were cultured on MS medium with no growth regulator, where aerial part of the internodal explants produced adventitious shoot buds [27]. However, addition of growth hormones, whether auxin or cytokinin didn’t produce shoots rather they resulted in callus formation. Part of the explant in contact with medium resulted in root formation. Multiple shoots (5.9 shoots per explant) had regenerated from hypocotyl explants on N medium supplemented with 4.44 µM BAP in 100% of cultures [28]. Similarly, multiple shoots (5.8 shoots per explant) also regenerated from cotyledonary node explants cultured on N medium supplemented with BAP (4.44 µM) in 100% of culture [29]. However, a number of shoots increased (9.8 shoots per explant) when explants were sub-cultured on MS medium with the same amount of growth regulator. The shoots got rooted in half strength MS medium supplemented with IBA (4.92 µM).
2.6 Sesbania sesban (L.) Merr.

Shoots had regenerated directly from hypocotyl and cotyledon explants in a single step on MS medium supplemented with 8.88 µM BAP and 0.25 µM IBA [12]. Indirect shoot organogenesis (7.8 shoots per explant) through callus derived from both explants had also observed on MS medium supplemented with a combination of BAP (2.22 µM) and IBA (0.49 µM) in 53% of cultures. Shahana and Gupta [15] successfully produced somatic embryos in *S. sesban* using cotyledon explants cultured on LS medium supplemented with various concentrations of NAA and 2,4-D with best response at 0.53 µM NAA concentration. The somatic embryos thus formed separated from the explants and transferred to the basal LS medium, where they formed shoots and roots from the plumular and radical end, respectively. Nodal and intermodal explants derived from mature plants produced shoots when cultured on N medium supplemented with cytokinin [13]. Nodal explants produced shoots at 7 µM KIN concentration, whereas intermodal explants showed maximum shoots development at lower concentration of KIN (2.32 µM). Kinetin found better than BAP for inducing a greater number of shoots. The developed shoots rooted in N medium supplemented with IBA (9.8 µM). Plantlets had acclimatized and transferred to the fields.

2.7 Other Sesbania species

Shoots had regenerated from cotyledon and hypocotyl explants of *S. cannabina* (Retz.) Pers. and *S. furmosa* (F.Muell.) N.T.Burb. on MS medium supplemented with various concentrations of BAP and IBA [12]. Shoot organogenesis was either direct in single step or indirect via callus formation. Direct shoot organogenesis had reported from cotyledon explants in 63% cultures of *S. cannabina* on medium supplemented with 4.44 µM BAP and 0.49 µM IBA, whereas in 87% cultures of *S. furmosa* on the medium contained 4.44 µM BAP and 0.25 µM IBA. In hypocotyls direct shoot organogenesis was in 60% and 80% cultures of *S. cannabina* and *S. furmosa*, respectively, on the same culture medium used for cotyledon explants. For indirect shoot organogenesis, initially callus raised from hypocotyls on medium fortified with NAA (5.37 µM) and then cultured it on MS medium supplemented with BAP and IBA. Callus derived from *S. furmosa* had resulted in 1.9 shoots per callus in presence of BAP (4.44 µM) and IBA (4.92 µM) in 90% cultures whereas in *S. cannabina*, 2.8 shoots per callus regenerated in 57% of cultures on medium supplemented with 2.22 µM BAP and 0.49 µM IBA. The shoots thus formed rooted on MS medium supplemented with IBA (0–4.92 µM).

3. Genetic transformation

Cotyledonary node and cotyledon explants from the seedlings have been used for transformation in *S. drumondii* [18] and *S. rostrata* [22] (Table 2). The explants might be used because of presence of pre-existing meristems, which makes them amenable for transformation. The meristematic cells present at the sites divide rapidly in the presence of cytokinins to give rise to shoots. For the development of an efficient regeneration protocol, there are many factors like media compositions, explant type and other culture conditions that require optimization before it could used further for transformation studies. The selection of transformants depends upon screenable markers or reporter genes which are specified for catalysing a detectable reaction, thus confirming the transfer of gene in plant. The most widely used reporter gene is β-glucuronidase (*uidA/gusA*) gene employed for transient and stable transformation studies in legumes. Although this method is destructive and only localized gus activity can visualized in tissues. Another gene used that is non destructive is green fluorescent protein (GFP) gene isolated from jellyfish. The gene doesn’t require any cofactor or substrate, and early detection of the transgene in plants is also feasible with no toxicity to the plants, thus avoiding the chimeras. Both these reporter genes have been used in different *Sesbania* species. Selectable marker genes are the other set of gene required for the selection of transformed plants from the
non-transformed ones during the regenerative phase of the tissues. The selectable marker gene allows the transformed to grow and proliferate on the medium, while non-transformed will cease to grow or grow slowly on the medium containing selective agent. The most widely used selectable marker gene in legumes is neomycin phosphotransferase (nptII) gene which provides resistance against antibiotic kanamycin or its analogues by inactivation through phosphorylation. Another selectable marker gene used is Bialaphos resistance gene (bar) which acetylates and detoxify phosphinothricin and has used in various legume species. It is most efficient selectable marker gene for the selection of transformants. But in Sesbania, hygromycin phosphotransferase (hpt) gene has used that provides resistance against hygromycin to recover transformants [18]. Various parameters assessed such as selective agent, its concentration and the time of its use during selection process, so that chances of transformants selection increases. Also, the selective condition varies from species to species and from explant to explant thus parameters should be judiciously chosen for transformants recovery. Studies also require for marker free approaches or at least using positive markers such as phospho-mannose isomerase (pmi) that are considered safer and more efficient.

There are various gene transfer methods that have used in various legume species, but only Agrobacterium-mediated transformation has carried out in Sesbania species. Agrobacterium tumefaciens and Agrobacterium rhizogenes strains carrying Ti and Ri based vectors have been used for the transformation studies in Sesbania species. Vectors that are based on Ti plasmid of Agrobacterium tumefaciens are most widely used vectors because of the simplicity and efficiency of their use. Also, these vectors are useful for transfer of long stretches of DNA with a single copy number and lesser chances of transgene silencing [30]. Whereas Ri based vectors does not require any selectable marker, as the emergence of many roots shows the transfer of gene to the plants. But the drawback of the system is the requirement of shoot organogenesis from the root tissue, which is not feasible in most species.

An attempt to check the compatibility of the S. rostrata towards the Agrobacterium mediated transformation had checked by Vlachova [22]. In this report, two A. tumefaciens strains (GV 3101 and B6S3) and one A. rhizogenes strain (15834) were used to infect both in vivo and in vitro explants. The A. tumefaciens and A. rhizogenes, both results tumour formation in greenhouse raised plants, though tumours appeared different in morphology. However, when checked on in vitro explants, A. rhizogenes resulted in extensive rooting in explants though a pronounced infection rate had reported under in vitro conditions. These experiments resulted in tumours or roots formation, but no shoots produced and no molecular evidence of the transformation event had reported. Another attempt for transformation had carried out using embryonal axis explants [31]. The explants were co-transformed using A. rhizogenes strain 2659 carrying pRi2659 (pATAG3-35S: gusint) and A. rhizogenes strain 2659 carrying pRi2659 (pS800-35S: egfp) and produced transgenic roots that had vigorous growth. Transformation event had confirmed only by GUS analysis and GFP fluorescence. No transformed plantlets formed, and gene integration into the genome had not confirmed by any molecular studies. Among the Sesbania species transgenic plants production had reported first time in S. drumondii where transgenic plants had successfully recovered on selection medium containing hygromycin [18]. In this report, cotyledonal node explants had infected and co-cultured with Agrobacterium tumefaciens stain EHA101 carrying vector pCAMBIA 1305.1, for regeneration of transformants. Vector had hptII gene as a selectable marker gene along with gus gene containing an intron. Successful transformation event had confirmed by PCR using primers specific for both genes. Integration of the transgene confirmed by Southern analysis and showed transformation frequency of 4%. The inheritance pattern to the progeny had not studied.
Table 2: Current status of transformation studies in Sesbania species

| Species     | Explant and mode of regeneration | Agrobacterium strain and Vector/ construct | Transgenic plant and progeny analysis | References |
|-------------|---------------------------------|-------------------------------------------|-------------------------------------|-------------|
| *S. rostrata* | Cotyledon, Hypocotyl, Stem; tumours | At GV3101, At B6S3, Ar 15834; pTiC58 | Not reported | [22] |
|             | Embryonal axis; Root organogenesis | Ar 2659; pRi2659, (with pATAG3-35S: gusint and pS800-35S: egfp) | Transgenic roots; No molecular analysis | [31] |
| *S. drumondii* | CN; Shoot organogenesis | At EHA 101 pCAMBIA 1305.1 (with hptII and GUS) | Integration of hptII gene confirmed by Southern analysis; PCR analysis revealed inheritance of both transgenes | [18] |

Ar: Agrobacterium rhizogenes; At: Agrobacterium tumefaciens; CN: cotyledonary node

4. Conclusions and future directions

To date, out of the 22 commonly used Sesbania species, *in vitro* regeneration has reported only in 8 species further these reports are very few whereas only two species have subjected to transformation studies. Cotyledons and hypocotyls explants are most extensively used for *in vitro* regeneration studies in every Sesbania species except *S. drumondii*. Cotyledons are capable of both direct and indirect shoot organogenesis and found more regenerative than hypocotyls in most cases. Cotyledons have also used for somatic embryogenesis in *S. sesban* and for isolation and culture of protoplasts in *S. bispinosa*. Shoot organogenesis from hypocotyl explants is *de novo* in nature and offer two modes of regeneration that are direct or indirect via the callus phase. Nodal explants have been used for direct organogenesis from the pre-existing meristem. Out of different media tested, MS medium has found more responsive than the rest of medium in all the species except *S. rostrata* and *S. sesban* where N medium has found more regenerative. Addition of cytokinins has found to promote shoot organogenesis. BAP has found more effective than the other cytokinins (Kin, zeatin, TDZ) in most of the reports. Combination of BAP with auxins (IAA, IBA, NAA) has found mostly promotive in shoot organogenesis in all the species studied. Rooting has observed in all species on the medium supplemented without or with auxins (IAA, IBA, NAA). Despite of the presence of regeneration protocols, transformation studies have not carried out in all the species but restricted to only Agrobacterium-mediated transformation in *S. drummondii* and *S. rostrata*. Transgenic plants have been obtained only in *S. drummondii* while in *S. rostrata* either showed the compatibility towards gene transfer or reported transgenic roots. The molecular characterization of transgenics developed in *S. drummondii* had limited to T0 level only, and inheritance of transgenes to the next progeny had not shown. The frequency of transformation is still low and needs further enhancement via any of the gene transfer methods so that transformation could achieved without use of markers for wider acceptance of transgenics of *S. drummondii* and the same could exploited in other *Sesbania* species. This enhancement could achieve with refinement of regeneration protocols either via high throughput shoot organogenesis or somatic embryogenesis. Till date, there is no report for the transformation of agronomical important trait in these species. So, in future there is also possibility for development of novel characteristics in these plant species either via tissue culture with production of haploids, somatic hybrids and selection of somaclones or through genetic engineering.
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