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ORIGINAL ARTICLE

Optimization of Factors Affecting *Agrobacterium*-Mediated Transformation Efficiency in Soybean

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ABSTRACT
The objective of this study was to observe the major factors affecting Agrobacterium-mediated transformation efficiency in soybean. In this present study, several factors such as plant genotype, explant type, antioxidant, and phenolic compound were investigated. To see the effect of explant type and plant genotype, 11 Indian soybean cultivars (DS 228, JS 335, KHSb2, JS 72-44, JS 72-280, NRC 149, Punjab 1, NRC 37, MAUS-32, NRC 138, and SL 525) were selected, inoculated and co-cultivated for 5 d in the dark with Agrobacterium strain EHA105 carrying the binary vector pCAMBIA1305.1 containing the hptII and GUSPlus genes. The GUSPlus reporter gene-based assay demonstrated that different genotypes displayed varying susceptibilities to agro-infection. The transient expression was compared on 3 different explant types: cotyledonary nodes (cot-nodes), half seeds, and embryonic tips in these 11 cultivars. The embryonic tip was found to be more susceptible to transformation followed by the half-seed and cot-nodes. Further, to optimize the concentration of antioxidant and phenolic compound in the transformation of the embryonic tip, 7 different concentration combinations of dithiothreitol (DTT) and cysteine, and 3 different concentrations of acetosyringone (AS) were assessed on 2 soybean cultivars, JS 335 and JS 72-280. The lowest necrosis and a high transient expression rate were observed in co-cultivation media (CCM) containing 200 mg/L DTT and 262 mg/L cysteine. Similarly, in CCM containing 300 µM AS transformation efficiency was found to be high. This study offers a more effective protocol for Agrobacterium-mediated transformation in soybean using embryonic tips.
Keywords: *Transient expression, Explant type, Genotype, Antioxidant, Phenolic compound*

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INTRODUCTION

Transgenic soybean has been the predominant commercialized biotech crop over the past decade. Genetic engineering provides us with an alternative tool to introduce valuable agronomic traits into the conventional breeding program. Among the various transformation methods such as the biolistic method, *Agrobacterium tumefaciens*-mediated transformation is becoming more popular nowadays in crops. An efficient transformation method is essential for the genetic improvement of soybean. Therefore, developing an efficient transformation system is a prerequisite for both cultivar improvement and functional gene studies. The transfer of T-DNA from *Agrobacterium* to the host cell is a highly complex process where genetic determinants from both plant and bacterial cells affect the transformation efficiency [1–3]. The development of an effective *Agrobacterium* transformation protocol for soybean has shown dependence on several factors, including plant genotypes, explant type, seed soaking period, vector type, the virulence of *Agrobacterium tumefaciens* strains, *Agrobacterium* inoculation density and period, selection systems, explant vigor, the addition of thiol and phenolic compounds, culture conditions, wounding, etc. [4]. Although the effect of various factors on transformation and regeneration frequency has already been investigated in soybean, it is less amenable to genetic modification because each of the factors has only been explored individually, which limits their applicability. Thus, an efficient transformation protocol focuses on the identification of the right combination of factors that affect transformation efficiency. To optimize and increase the success rate of transgene delivery, multiple

factors need to be examined. Jia et al. [5] and Li et al. [6] various parameters of *Agrobacterium*-mediated transformation for cot-nodes and reported a maximum of 10% transformation efficiency. Likewise, Hada et al. [7] assessed 8 different parameters for half-seed and reported 14% transformation efficiency and 45% regeneration efficiency. However, there are few reports on transformation-affecting factors in the embryonic tip [8, 9]. Therefore, it is required to investigate the effect of various parameters on the improvement of soybean transformation using the embryonic tip as an explant. Explant selection is critical for efficient transformation efficiency because explants are the starting tissue material for the entire transformation mechanism. Explants should be highly transformable with the great recovery of whole transgenic plants. However, a variety of explants have been used for transgenic soybean production using *Agrobacterium* and biolistic methods, including shoot meristems [10], half seeds [11], embryonic tips [8, 12], split-seed explants with an attached partial embryonic axis [13], embryogenic suspension cultures [14], immature cotyledons [15], and axillary meristematic tissue located in seedling cotyledons [16]. Although, the varying responses of plant tissues to *Agrobacterium* infection are determined by the ability of *Agrobacterium* to adhere to plant tissue and the T-DNA transfer mechanism [17]. Moreover, various tissues of a plant show different susceptibilities to *Agrobacterium* transformation. Thus, selecting a highly infectious explant type is necessary for successful transformation efficiency. Genetic transformation efficiency is also affected by the genotype of the recipient plants. The transformation efficiency varied among different soybean genotypes [12, 18]. Thus, to improve the soybean transformation system and increase transformation efficiency, the focus has shifted to screening soybean genotypes for germplasm resources suited for *Agrobacterium*-mediated genetic transformation. Large numbers of wound-adjacent cells that are capable of transformation, only develop in plants that have a proper wound response [19]. Enzymatic browning of explant tissues negatively affects regeneration frequency and, consequently, transformation efficiency and remains a significant limitation. Various antioxidants, such as cysteine, DTT, glutathione, tocopherol, selenite, and ascorbic acid, have also been reported to help reduce tissue browning and improve transformation efficiency [20]. The phenolic compound is another important factor influencing transformation efficiency. According to Owens et al. [21], the inclusion of phenolic compounds in inoculation media boosts transformation efficiency. The host range of *Agrobacterium*-mediated transformation has been increased in many crops with the addition of a phenolic compound, such as AS, into the CCM [22–25], as these compounds help in *vir* gene-induction by making plant tissues more susceptible to agro-infection, resulting in improved transformation efficiency [26]. Thus, optimizing the concentration of AS is a prerequisite for improving transformation efficiency. In this study, we assessed the conditions affecting *Agrobacterium*-mediated soybean transformation. Here we designed experiments to find out the highly susceptible explant type to *Agrobacterium* infection and optimized the concentrations of antioxidants (cysteine and DTT), and phenolic compound (AS) on the *Agrobacterium*-mediated transformation of embryonic tip explant type.

MATERIAL AND METHODS

Plant materials

A total of 11 Indian soybean cultivars, namely, DS 228; JS 335; KHSb2; JS 72-44; JS 72-280; NRC 149; Punjab 1; NRC 37; MAUS-32; NRC 138; and SL 525, were selected to see the effect of genotype and explant type on transformation efficiency. Healthy and bold seeds of the selected cultivar were surface-sterilized by exposure to chlorine gas for 16–18 h. The chlorine gas was prepared by mixing 5 mL HCl (39.6%) and 100 mL sodium hypochlorite (4%) [27].

Agrobacterium strain and vector

The binary vector pCAMBIA1305.1 (CAMBIA, Australia) containing hygromycin phosphotransferase (hpt), Kanamycin Resistance (KanR), and GUSplus as a plant selectable, bacterial selectable, and reporter gene, respectively were utilized in the current investigation.

Agrobacterium culture and infection medium

Agrobacterium strain EHA105, harboring pCAMBIA1305.1, was grown on Luria Agar (LA) plates (Himedia, India) containing 50 mg/L kanamycin and rifampicin each at 28 °C for 2 days. A single *Agrobacterium* colony was picked from the plate and inoculated into 50 ml of Luria Broth (LB, liquid LA) containing 50 mg/L of both antibiotics (primary culture) for 6 h at 28 °C and 200 rpm. Subsequently, 500 µl of the 50 ml primary culture was mixed in a 200 ml LB culture and grown overnight at 28°C at 200 rpm using a shaker incubator. The overnight culture was centrifuged at 4000 rpm for 10 minutes to obtain a bacterial pellet that was then resuspended in an infection medium containing 1/10 of Gamborg's B5 medium [41], 3% (30 g/L) sucrose, filter-sterilized 1.67 mg/L 6-N6-Benzylaminopurine (BAP), 0.25 mg/L gibberellic acid (GA3), DTT, cysteine, and (concentration according to experimental design). Before

infecting explants, OD_{600} of the bacteria was adjusted to 0.7 using a Nanodrop Spectrophotometer (Denovix) and cultured at room temperature for 30 min.

Explant preparation

Three different explant types, embryonic tip, half seed, and cot-node were selected for this study. Further to prepare the former two explants type; sterilized seeds were soaked in autoclaved distilled water overnight in the dark at 24°C.

To prepare the embryonic tip explants, a longitudinal cut along the hilum was made to separate the cotyledons. Then the seed coat was removed. Afterward, the primary leaves on the embryonic tip were excised in order to expose the meristem, and the embryonic tip was separated from the junctions of the hypocotyls.[8].

To obtain the half-seed explants, the imbibed seeds were longitudinally sliced along the hilum using a scalpel, and the seed coats were scraped off [28].

To prepare the cot-node explants, soybean seeds were sterilized as described above and germinated for 5 d on half-strength MS-B5 medium (pH 5.8) supplemented with 3% sucrose and 0.6% agar. A horizontal slit was made through the hypocotyl regions of germinated seeds, about 3–5 mm below each cotyledon. A subsequent vertical slice was made between the cotyledons, and the primary leaves were removed [29].

Infection of explant and culture conditions

Prepared explants were infected with an *Agrobacterium tumefaciens* EHA105 suspension (0.7 OD600) containing the binary vector pCAMBIA1305.1 for 10 min. at room temperature. After inoculation, 10-15 explants (apical regions directed upwards) were placed in the 90 mm sterile petri dish containing semi-solid CCM, which is composed of infection medium additionally with 0.6% agar-agar (Himedia, India), filter sterilized cysteine and DTT with a piece of Whatman filter paper and then incubated at 24°C in dark for 5 days.

Experimental design for optimization experiments

Various parameters such as genotype, explant type, antioxidants, and phenolic compounds influencing transformation efficiency were assessed to establish a high *Agrobacterium*-mediated transformation efficiency in soybean. For each variable studied, experiments were repeated three times with 100 explants per treatment.

Culture Condition

Some modification was done in culture conditions to optimize the concentration of antioxidant and phenolic compounds on the transformation of embryonic tip explants. To assess the effect of various concentrations of antioxidants on ***Agrobacterium***-mediated transformation of the embryonic tip, 7 different combinations of DTT and cysteine (category 1: 200 mg/L + 262 mg/L; category 2: 200 mg/L +200 mg/L; category 3: 154.2 mg/L+262 mg/L; category 4: 154.2 mg/L + 200 mg/L; category 5: 154.2 mg/L +140 mg/L: category 6: 120 mg/L + 200 mg/L; category 7: 120 mg/L +140 mg/L) were taken in which category 4 (154.2 mg/L DTT and 200 mg/L cysteine) is already being used in our laboratory for half seed and cot-node transformation system. Similarly, to confirm the effect of the phenolic compound, 3 different concentrations of AS (100, 200, 300 µM) were added in CCM. Antioxidants and phenolic compounds were added to the medium when the temperature dropped to 50-60°C.

Histochemical GUS assay

Post 5-d co-cultivation, a histochemical GUS assay was performed following the method of Jefferson et al. [30]. Transformed explants were incubated overnight at 37°C in a solution containing 200 mM/L sodium phosphate buffer (pH 7.0), 500 mM/L Ethylenediaminetetraacetic acid, 0.5% v/v Triton, 20 mM/L K-ferricyanide, 20 mM/L K-ferrocyanide, 20 mM/L 5-Bromo-4-chloro-3-indoxyl-β-D-glucuronide cyclohexyl ammonium salt (X-Gluc) (Himedia, India) and 20% v/v methanol. Following incubation, tissues were repeatedly rinsed in 70% ethanol to completely remove the chlorophyll. After the chlorophyll was removed, tissues that had blue spots were scored and counted as GUS-positive transformants.

Transient GUS expression was calculated using the formula;

Transient GUS expression (%) = the number of positive transformants / the total infected explants × 100.

After infection and staining different *Agrobacterium* infectivity pattern was seen in transformed embryonic tip explants. The blue color was observed either on stem meristem (SM), coleoptiles region (CL), SM, and CL both or the entire part of the stained transformed embryonic tips. GUS staining was noted from any of the parts (either CL or SM or CL and SM part both) of stained embryonic tips in the case of the genotypic effect on transformation efficiency. However, the transient GUS expression was solely recorded from the SM part of the transformed explant in order to optimize antioxidant and phenolic compounds concentration for embryonic tip transformation.

Statistical Analysis

Statistical analysis was done on the average numbers of GUS transient expressions for each treatment over three replications. Standard deviations and means were calculated. Using the one-way analysis of variance (ANOVA), the results were statistically evaluated. The comparison of the variation between means was performed via Fisher's least significant difference (LSD) value through Duncan's multiple range test (DMRT) at a significance level of $p < 0.05$.

RESULTS

Effect of Different Explant Types

Transient expression (%) was compared between 3 different explant types: embryonic tip, half seed, and cot-nodes. Embryonic tips and half seed explants were prepared by overnight imbibition, and cot-nodes were prepared post-5-d germination on MS, using seeds from 11 randomly selected Indian soybean cultivars: DS 228; JS 335; KHSb2; JS 72-44; JS 72-280; NRC 149; Punjab 1; NRC 37; MAUS-32; NRC 138; and SL 525. The ***Agrobacterium*** was collected at an OD_{600} of 0.7 and re-suspended in the liquid CCM containing 1.67 mg/L BAP, 0.5 mg/L GA3, 154.2 mg/L DTT, 200 mg/L cysteine, and 200 µM AS to infect the explants (previously optimized culture condition in our laboratory). Then the explants were subjected to GUS staining after 5 days of co-cultivation. The result showed that the rate of total transient expression in the embryonic tip explants was highest and significantly ($P<0.05$, DMRT) higher than the other two types of explants, half seeds and cot-node explants, for all soybean cultivars except one; NRC 138 (Fig. 1).

Effects of genotypes

Our research has shown that genotypic variability in *Agrobacterium infection* exists in soybean cultivars. Some cultivars were highly susceptible to ***Agrobacterium*** infection, while some cultivars showed less or no susceptibility. To see the effect of genotype, 11 soybean cultivars (DS 228; JS 335; KHSb2; JS 72-44; JS 72-280; NRC 149; Punjab 1; MAUS-32; NRC 138; and SL 525) were taken for the study with all the other factors previously optimized (which was collecting the ***Agrobacterium*** when OD_{600} = 0.7, then resuspended in the liquid CCM with 1.67 mg/L BAP, 0.5 mg/L GA3, 154.2 mg/L DTT, 200 mg/L cysteine, and 200 µM AS and co-cultured for 5 days). We found that different genotypes have different susceptibilities to agro-infection, and these were classified as highly, moderately, and weakly susceptible genotypes based on the rate of transient GUS expression. The results demonstrated that in embryonic tip explants, the rate of transient GUS expression (>80%) was considerably ($P< 0.05$, DMRT) greater in DS 228; JS 335; JS 72-44; KHSb2; NRC 149 and JS 72- 280 than others. Four cultivars, DS 228; KHSb2; NRC 149; and JS 72-280, were shown to be very susceptible to agro-infection in half seed, however, only two cultivars, DS 228 and KHSb2 were found to be highly susceptible in cot-node explants. While MAUS-32 and SL525 were observed as weakly susceptible, DS 228 and KHSb2 were noted to be significantly highly susceptible for all 3 types of explant types (Fig. 1).

Effects of antioxidants

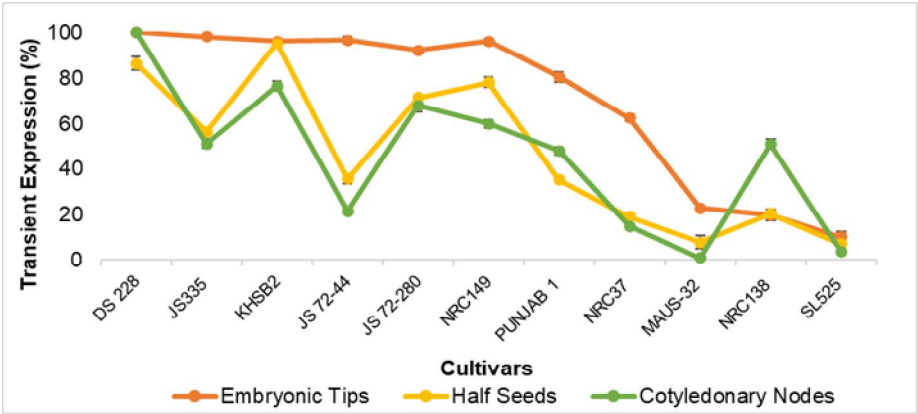

Fig. 1 The effect of genotype and explant type on transient GUS expression. The results are expressed as mean ± standard error. Hundreds of explants were stained for each treatment and the experiments were repeated thrice. The transient GUS expression was calculated as follows: Transient GUS expression (%) = The number of positive transformants/the total infected explants × 100. NRC numbers are advanced breeding lines.

In the current study, we found that after co-cultivation, the embryonic tip explants showed signs of enzymatic browning and tissue necrosis at the wounded area, which was likely to hinder the effective transformation. The most effective anti-necrotic agents are DTT and L-cysteine. Therefore, to see the effect of various concentrations of antioxidants on the reduction of tissue browning and necrosis, 7

different combinations of DTT and cysteine (Category 1: 200 mg/L + 262 mg/L; Category 2: 200 mg/L +200 mg/L; category 3: 154.2 mg/L+262 mg/L; category 4: 154.2 mg/L + 200 mg/L; category 5: 154.2 mg/L +140 mg/L: category 6: 120 mg/L + 200 mg/L; category 7: 120 mg/L +140 mg/L) were taken. We chose JS 335 and JS 72 280 in the further study due to their higher susceptibility to agro-infection in the embryonic tip. To see the individual effects of both antioxidants, one antioxidant was kept constant and the other was variable. Then embryonic tip explants of both cultivars were subjected to GUS staining after 5 days on modified CCM. After infection and staining, different *Agrobacterium* infectivity pattern was seen in stained embryonic tip explant. The blue spot was either on SM, CL, SM, and CL both or an entire part of the stained transformed explant. In the case of genotypic variability, the GUS infectivity pattern was recorded by counting blue spots from the all part of stained embryonic tip explant, whereases to see the effect of antioxidants, this was only recorded from the SM part as shoot induction takes place in the SM part only. In embryonic tip explants of JS 335, the rate of transient GUS expression was observed to be high in categories 1 (85.2%) and 2 (75.3%) with no necrosis; moderate in categories 3 (66.5%) with 6.5% necrosis and category 4 (55%) with 5% necrosis; and low in categories 5 (21%), 6 (10%), and 7 (7.1%) with 20%, 5.2%, and 27.6% necrosis, respectively (Fig. 2a). In contrast, a higher transient GUS expression was noted in categories 1 (98.9%), 2 (93.3%), 3 (92.4%), and 4 (92%) with 1.2%, 8.3%, 15.3%, and 17.7% necrosis, respectively and moderate or low was noted in category 5 (50%), 6 (26.7%), and 7 (24.4%) with more than 20% necrosis in the JS 72-280 soybean variety (Fig. 2b). Moreover, the rate of total transient GUS expression was highest in category 1, followed by categories 2, 3, 4, 5, 6, and 7 in both soybean cultivars JS 335 (85.2%) and JS 72-280 (98.9%). Therefore, after adding a high concentration of both antioxidants in the medium, transient expression was increased by 3.6 to 85.2% for JS 335 and 18.5 to 98.9% for JS 72-280, compared with the control, and the rate of strong transient GUS expression was significantly improved by 23.7 and 5.3-fold for JS 335 and JS 72-280, respectively. Meanwhile, the best combination of DTT and cysteine for embryonic tip is 200 mg/L and 262 mg/L (category 1), respectively, to obtain better transformation efficiency with no or less necrosis (Fig. 2).

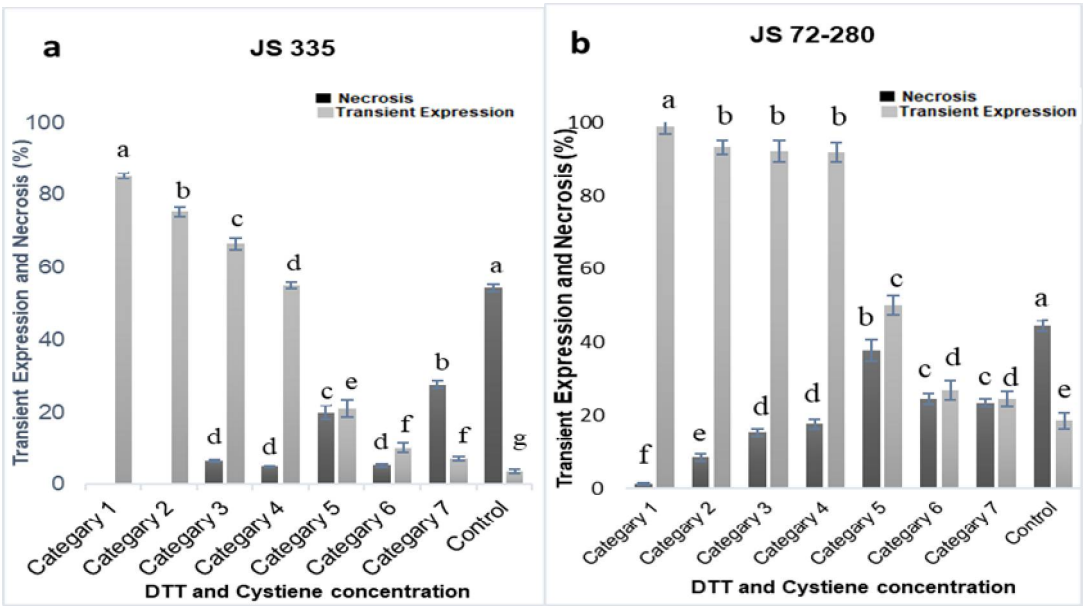

Fig. 2 The effect of different concentrations of antioxidants on necrosis and transient GUS expression in soybean embryonic tip explants. (a) Transient expression and necrosis in JS 335 and, (b) Transient expression and necrosis in JS 72-280. * Hundreds of explants of both cultivars were stained for each treatment and the experiments were repeated thrice. The results are expressed as mean ± standard error. Values followed with dissimilar letters differ significantly at $p = 0.05$ in accordance with LSD and DMRT. Means with the same letter above bars are not significantly different at 0.05 level according to DMRT.

Effects of different concentrations of phenolic compound

Phenolic compounds play an important role in the efficiency of transformation in the virulence system of *Agrobacterium*. As a result, a phenolic compound acts in a dose-dependent manner, and *vir* genes act as sensors for it. We analyzed the effective range of AS concentrations on increasing transformation by measuring GUS reporter gene activity. To gain insight into whether transformation efficiency exhibited differential virulence sensitivity towards a different level of AS in terms of transient GUS expression, we

set 3 different concentrations of AS, i.e., 100 µM, 200 µM, and 300 µM, of which 200 µM has already been used in our laboratory for half-seed transformation. We took one high (300 µM) and one low (100 µM) concentration of AS and compared transient GUS expression with 200 µM (control) to see which range is best for the embryonic tip transformation system. Embryonic tip explants of JS 335 and JS 72-280 were inoculated with *Agrobacterium* tumefaciens strain EHA105 harboring the pCAMBIA1305.1 vector and co-cultivated with all 3 concentrations of AS. After 5 days, the transient GUS expression was detected using a histochemical GUS assay. The staining pattern was recorded from the SM part only like antioxidants. As a result, in JS 335, we observed 68.7%, 55%, and 33.3% transient expression rates when CCM was supplemented with 300 µM, 200 µM, and 100 µM, respectively AS. Similarly, we found transient expression rates of 97.4%, 92%, and 60% with 300 µM, 200 µM, and 100 µM, respectively in JS 72-280. Transient GUS expression in embryonic tips was high when CCM was supplemented with 300 µM, followed by 200 µM and 100 µM. Conclusively, the higher the concentration of AS, the higher the transient expression in the embryonic tips. In addition, treatment with 300 µM and 100 µM AS concentrations had 1.2-fold higher and 1.6-fold lower GUS reporter activity, respectively than the control (200 µM) in JS 335. Similarly, a 1.0-fold increase and a 1.5-fold decrease change were observed with 300 µM and 100 µM AS, respectively, for the JS 72-280 variety (Fig. 3).

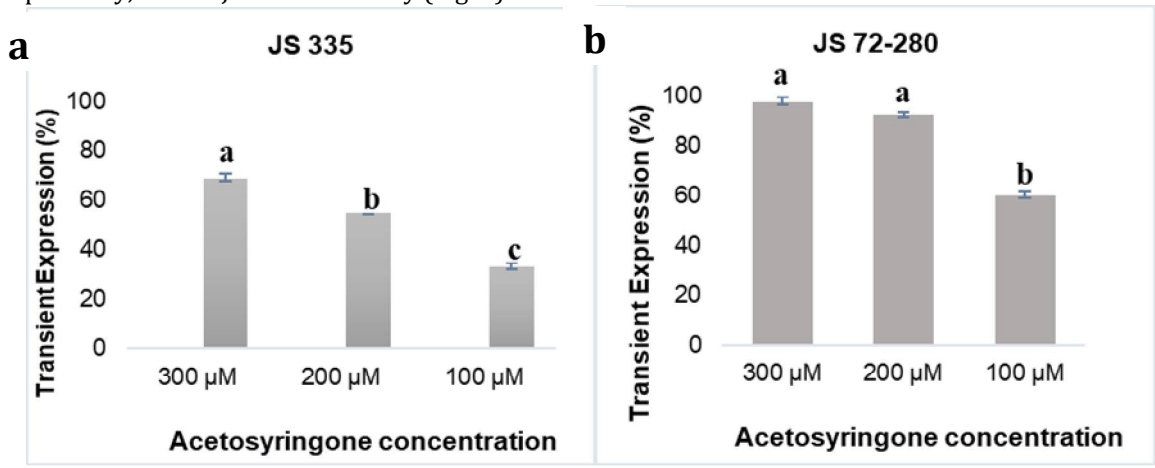

Fig. 3 The effect of different concentrations of phenolic compound (AS) on transient GUS expression of embryonic tip explants (a) Transient expression in JS 335 and, (b) Transient expression in JS 72-280. *Hundreds of explants of both cultivars were stained for each treatment and the experiments were repeated thrice. The results are expressed as mean ± standard error. Means with the same letter above bars are not significantly different at 0.05 level according to DMRT.

Therefore, 300 µM was the optimal concentration of AS for the embryonic tip transformation, giving high transformation efficiency in our study as compared to other concentrations. Shoot induction was also found to be higher (65.4%) with a 300 µM AS concentration, resulting in a positive correlation between shoot induction and transformation efficiency.

DISCUSSION

Since the first transgenic soybean plant was obtained by ***Agrobacterium***-mediated transformation using cot-node explants [16], this method has been modified and transformation efficiency fluctuates between 2% and 10% [8, 31, 32] which is still very low. The transfer of ***T-DNA*** from ***Agrobacterium*** to the host cell is a highly complex process where genetic determinants of plant and bacterial cells affect the transformation efficiency [1–3]. A lot of studies have been done to improve transformation efficiency [6, 31, 33]. However, this efficiency is not sufficient to screen enough transformation events. As a result, we attempted to improve transformation efficiency further by identifying the highly infectious soybean genotype, and explant type and optimizing culture conditions. In the present study, major transformation efficiency-affecting factors such as explant type, genotypes, the optimized concentration of antioxidants, and phenolic compounds were investigated. The effect of explant type was investigated by estimating the rate of GUS transient expression in 3 soybean explants: embryonic tip, half seed, and cot-nodes. Among these 3 explant types, higher ***Agrobacterium*** infection efficiencies were noted in embryonic tip explant followed by half seed and cot-node. The higher sensitivity to ***Agrobacterium tumefaciens*** may be due to the embryonic tip containing pro meristems and procambium [8]. Other than significantly higher sensitivity to *agrobacterium* infection, the embryonic tip system has the highest regeneration frequency (88.6%)[8]. Ahmed et al. [34], also found high transient GUS expression in embryonic tip explants of

cotton when compared with hypocotyl and plumule explants. Moreover, Pareddy et al. [13] used a split-seed explant with an attached partial embryonic axis taken from an imbibed seed to achieve an increased transformation efficiency (18.7%). A high transformation efficiency (9.84%) was also achieved by Paes de Melo et al. [35] using this explant type. The effect of genotype was checked on 11 soybean cultivars, and based on the rate of transient GUS expression, different cultivars showed varying susceptibility to agro-infection. Soybean cultivars, DS 228, and KHSb2 were found to be highly susceptible in all 3 types of explants; embryonic tip, half seeds, and cot-nodes. DS228 and KHSb2 cultivars were also found to be highly susceptible to *agrobacterium*-mediated transformation in our previous study [12]. There are many studies where many factors were optimized for half-seed and cot-node. However, there are few reports on transformation-affecting factors in embryonic tip explants. Although, an optimal embryonic tip regeneration system was established in some reports [8, 9]. This study mainly focused on cultivated cultivars of India. JS 335 and JS 72-280 were used for a further evaluation of factors affecting ***Agrobacterium***-mediated transformation efficiency because of their higher susceptibility using embryonic tip explant. We assessed other factors required for a high transformation success rate in embryonic tip explants by optimizing the right combination of antioxidants and phenolic compounds. In this study, after adding a high concentration of antioxidants (200 mg/L DTT and 262 mg/L cysteine) in the medium, transient expression was significantly improved by 23.7 and 5.3-fold, and necrosis was decreased completely in JS 335, and 43.4% in JS 72-280, when compared with control (Fig. 2). Many scientists worked on other crops and reported the importance of adding antioxidants to the CCM. A correlation between the reduction in cell death and the improved transformation frequency has been demonstrated in rice [36], sugarcane [37], sorghum [38], and maize [39]. Previous research has shown that combining L-cysteine, DTT, or Vc with AgNO3 in the solid CCM can significantly reduce the degree of tissue browning resulting in improved regeneration efficiency and the number of transgenic shoots [6, 32, 40, 41]. Addition of antioxidants in CCM results in a significant decrease in the browning and necrosis of hypocotyls and increased GUS expression [42]. Li et al. [6] got more than 96% infection efficiency in half-seed cotyledonary explants using 154.2 mg/L DTT in culture media. An experiment was set up based on an already established protocol aiming for better induction of the *vir* genes by the modification of one or more factors. In this study, a range of AS concentrations (100 µM, 200 µM, and 300 µM) was evaluated, where we noted 300 µM as the optimal concentration, giving maximal transformation efficiency in embryonic tip explants of both cultivars (Fig. 3). According to our findings, adding AS to the CCM significantly improved the transformation efficiency and is essential for the successful transformation of soybean. However, the AS concentration used for soybean transformation is 100–200 µM.[41, 43].

CONCLUSION

In this study, ***Agrobacterium***-mediated transformation efficiency in soybean was improved by selecting the highly susceptible genotype, and more infectious explant type to agro-infection and altering the concentration of antioxidants and phenolic compounds. We observed higher transient GUS expression in embryonic tip explants when compared with half-seed and cot-node explants, indicating that the embryonic tip may have morphogenetic potential and be a good source for agro-infection. We found more than 85% ***Agrobacterium*** infection efficiency when embryonic tip explants were co-cultured on CCM containing 200 mg/L DTT and 262 mg/L cysteine for 5 days, using the soybean cultivar JS 335 and JS 72 280. Moreover, embryonic tips were checked for a different range of AS concentrations (100 µM, 200 µM, and 300 µM). Transient expression was high when co-cultured with CCM containing 300 µM AS, which was proved to be the optimal concentration, giving maximal transformation efficiency. This study provides an optimized *Agrobacterium*-mediated transformation protocol for embryonic tips using Indian soybean cultivars of JS 335 and JS 72-280. The highly efficient explant type and genotypes with optimized culture conditions, identified in this present study can be used in the rapid development of transgenic soybean with an improved success rate. This research can also be helpful to investigate the functions of genes whose roles are unknown in order to enhance soybean performance under diverse biotic and abiotic conditions via virus-induced gene silencing or CRISPR-CAS9 approaches.

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COMPETING INTERESTS

The authors have declared that no competing interest exists.

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