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Genotypic Variability in Soybean [*Glycine max* (L.) Merrill] through *Agrobacterium*-Mediated Transformation

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Abstract

Embryonic tip explants of 92 Indian soyabean and 7 advanced breeding lines derived from soaked mature seeds were inoculated and co-cultivated for 5-day with *Agrobacterium* strain EHA105 carrying the binary vector pCambia1305.1 containing a hygromycin and kanamycin resistance gene as plant and bacterial selectable markers, respectively. Transient expression of transgene was monitored by histochemical localization of β-glucouronidase (GUSPlus) reporter activity in transformed ET tissues. A high genetic variability for *Agrobacterium*-infection ranging from 3.8 to 100% was observed in the form of transient GUS expression. Five highly efficient genotypes, namely DS-228, JS 335, JS 72-44, KHSb2, and JS 72-280 with transient GUS expression of 100, 98.1, 96.5, 96 and 92%, respectively were identified. In addition, various infectivity patterns in these genotypes were observed. Genotypes with very high transient GUS expression identified in this study may improve success rate of development of transgenic soybean.

Introduction

In 2018, the global area planted to soybean [*Glycine max* (L.) Merrill] was 123.5 million hectares, out of which 78% was biotech soybeans. The production area of biotech soybeans was 95.9 million hectares in 2018, comprising 69.3 million herbicide tolerance (HT) and 26.6 million hectares stacked insect resistance/HT.

The increment in income for farmers growing biotech soybean during 1996 to 2016 was US$ 59.7 billion in which 2016 alone produced US$ 6.9 billion (Brookes and Barfoot 2018).

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Development of transgenic crop by transforming pre-existing genotypes with desired traits, such as high yield, insect, disease and herbicide resistance is the most important achievement of biotechnology.The most commonly applied methods for plant transformation are *Agobacterium tumefaciens* and microprojectile bombardment-mediated transformation. Over more than 20 years, both methods have been progressively improved and developed but *A. tumefaciens*-mediated transformation is preferred for transformation since it is less expensive easy to manipulate and produces lower transgene copy number with higher stable gene expression. Soybean transformation using *A. tumefaciens* was first time achieved by Hinchee et al. (1988). *Agrobacterium*-mediated method is primarily used for soybean transformation, but the transformation efficiency is still relatively low (Chen et al. 2018) due to a number of factors affecting the efficiency of T-DNA delivery into the plant cell. These factors include plant and strain genotypes, explant types, explant damage, biotic shock, antibiotic stress and inoculation time (Mamidala and Nanna 2009, Wagiran et al. 2010).

Soybean transformation is genotype-dependent (Jia et al. 2015). Non availability of highly efficient genotype amenable to transformation is one of the major obstacles in the development of transgenic soybean as the susceptibility of soybean genotype to *Agrobacterium* infection plays a key role for the high level of genetic transformation efficiency. Genetic variability for susceptibility of soybean genotype to *Agrobacterium* infection exists in soybean gene pool as observed by Meurer et al. (1998). Therefore, screening soybean genotypes from the available germplasm resources suitable to *Agrobacterium*-mediated genetic transformation has become the focus for optimizing the soybean transformation system and improving transformation efficiency (Song et al. 2013) that offers a significant advancement for soybean breeding programs allowing the production of novel and genetically diverse plant materials. Genotypic dependency restricted the application of this method in the routine production of elite and commercially valuable soybean cultivars (Jia et al. 2015) as it needs time consuming process for transfer of transgene in commercial genotypes. If high transformation efficient genotypes are available in elite and commercially valuable cultivars, the success rate of transgenic soybean production would be improved considerably (Jia et al. 2015, Verma et al. 2014). Moreover, function of the most of soybean genes is not clear despite the availability of whole genome sequence. Availability of soybean genotype with high transformation efficiency will help in studying the function of gene response for various metabolic and physiological process of soybean plant.

The factors responsible for effect of genotypes on transformation efficiency are not clear. Shan et al. (2008) reported that some genetic factors are responsible for the susceptibility of genotypes to *Agrobacterium* infection in the transformation process. Genetics behind genotypic variability to transformation efficiency is defined by chromosomal and plasmid genomes of *Agrobacterium* strains which encode all the machinery necessary for attachment and T-DNA transfer. Explants of different plant genotypes produce inducer molecules varying in their inducing ability and cellular

concentration which leads to different level of *vir* gene expression, thereby affecting their sensitivity to *Agrobacterium*-infection (Karami 2008). Bacterial ability and inability to synthesize and transfer sufficient T-DNA essential for a successful transformation is affected by high and low level of *vir* gene expression, respectively. Low susceptibility of genotype to *Agrobacterium* may be a result of the presence of inhibitors of the *Agrobacterium* sensory machinery. Zhang et al. (1999) noted that 2-hydroxy-4,7-dimethoxybenzoxazin-3-one (MDIBOA) inhibits induction of *vir* gene expression by an unknown mechanism. MDIBOA is the major organic exudates present in maize seedling roots. Other than MDIBOA, IAA was also shown an inhibition mechanism to *vir* gene induction (Liu and Nester 2006).

A number of reports are available on differential susceptibility of various plant species to *Agrobacterium* infection (Porter 1991, Cheng et al. 2004). Genotypic variability have also been noted in various legumes (Atif at al. 2013, Hood et al. 1987, Owens and Cress 1984), maize (Ritchie et al. 1993), aspen (Beneddra et al. 1996), *Pinus* species (Bergmann and Stomp 1992), tomato (Van Roekel et al. 1993), Arabidopsis (Nam et al. 1997), and grape (Lowe and Krul 1991). Very few reports are available on genotypic variability to susceptibility of *Agrobacterium* infection in soybean (Song et al. 2013, Jia et al. 2015). The present study was undertaken to assess the genotypic variability of commercial cultivars and elite lines of soybean available in India.

Materials and Methods

A total of 92 diverse Indian soybean cultivars and 7 advanced breeding lines were tested for genotypic variability in the present study. Healthy and bold seeds were surface-sterilized by exposure to chlorine gas. The chlorine gas was prepared using a mixture of 5 ml HCl (39.6%) and 100 ml sodium hypochlorite (4%) and kept for 16 - 18 hrs (Liu and Wei 2002).

*Agrobacterium tumefaciens*s train EHA105 (Hood et al. 1993) containing binary vector pCambia1305.1 (CAMBIA, Australia; Fig. 1) was used for genetic transformation. pCambia1305.1(https://www.markergene.com/pcambia-vectors) contained hygromycin phosphotran-ferase (*hpt*) as the plant selectable marker, Kanamycin resistance gene as the bacterial selection marker and an intron containing GUSPlus gene from *Staphylococcus* as the reporter gene.

A. tumefaciens strain EHA105 was transformed with binary vector pCambia1305.1 by preparing heat-shock agro-competent cells of strain EHA105 following the protocol of Höfgen and Willmitzer (1988). The presence of pCambia1305.1 plasmid was confirmed in the antibiotic resistant bacteria colonies by colony PCR using 35 S promoter, nptII and GUS gene specific primers. Primer sequence and amplicon size is given in Table 1.

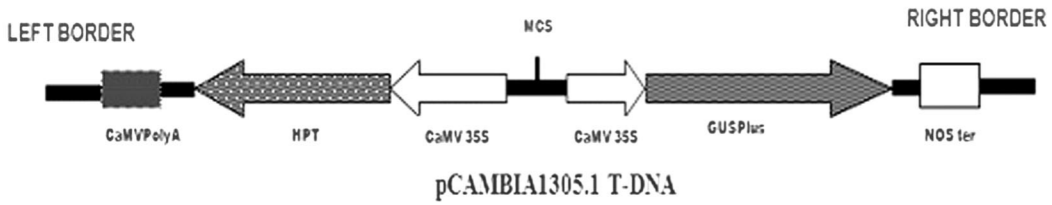

Fig. 1. Schematic diagram of T-DNA construct of binary vector pCAMBIA13051.1 used for genetic transformation of soybean, NOS ternpaline synthase terminator, GUSPlus coding region of the β–glucuronidase reporter gene, cauliflower mosaic virus polyadenylation signal.

Table 1. Sequence and product amplicon size of primers used for confirmation of transformed bacterial colonies.

Primer	Sequence	Amplicon size (bp)	Reference
35S promoter Specific primer	'F' GCTCCTACAAATGCCATCA 'R'GATAGTGGGATTGTGCGTCA	213	Raharjo and Surajiman 2017
NPT I	'F'TGCGCTGCGAATCGGGAGCG 'R'GAGGCTATTCGGCTATGACT	710	Liu et al. 2004
GUS	'F'CGACGGCCTGTGGGCATTCA 'R TGGTCGTGCACCATCAGCAC	900	Rani et al. 2012

For infection *Agrobacterium* strain EHA105 containing pCambia1305.1 was grown on Luria agar (LA) plates (10 g/l casein enzymichydrolysate, 5 g/l yeast extract and 10 g/l NaCl, 1.5% agar; Himedia, India) containing 50 mg/l kanamycin and rifampicin each at 28°C for 2 days. Single colony of *Agrobacterium* were obtained from the plate and inoculated into 50 ml Luria broth (LB, liquid LA) containing 50 mg/l of both kanamycin and rifampicin (primary culture) for 6 hrs at 28°C in 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 in 200 rpm using a shaker incubator. On the day of infection, bacterial pellet was obtained by centrifuging the overnight culture at 4000 rpm for 10 min and re-suspended in infection medium containing 1/10 Gamborg's B5 medium (Gamborg et al. 1968) supplemented with 2.3 g/l2-[N-morpholino] ethanesulfonic acid, 3% (30 g/l) sucrose(pH 5.4), filter sterilized 1.67 mg/l 6-N6-benzylaminopurine, 0.25 mg/l gibberellic acid and 50 mg/l acetosyringone. Bacteria cell density was adjusted to 0.7 OD_{600} using a nanodrop spectrophotometer (Denovix) before infection of explants and incubated at room temperature for 30 min before infection.

One day before infection the sterilized seeds were soaked in autoclaved distilled water overnight in the dark at 24°C. ET explants were prepared by making a longitudinal cut along the hilum to separate the cotyledons, and the seed coat was removed. The ET

was then excised from the junctions of the hypocotyls and primary leaves on ET were removed to expose the meristem (Liu et al. 2004).

After explants preparation ET explants were immersed in *A. tumifecience* EHA105 suspension (0.7 OD_{600}) harboring the binary vector pCAMBIA1305.1 for 10 min, at room temperature. After inoculation, 10 - 15 ETs (apical regions directed upwards) were placed in sterile Petri dish (90 mm) containing semi-solid co-cultivation medium (CCM), which is composed of infection medium additionally with 0.06% agar-agar (Himedia, India), filter sterilized cystein (200 mg/l) and dithiothreitol (154.2 mg/l) with a piece of Whatman filter paper and then incubated at 24°C in dark for 5 days.

After 5 days of co-cultivation, histochemical GUS assay was performed following the method of Jefferson et al. (1987). Transformed ET explants were incubated overnight at 37°C in a solution containing 200 mmol/l sodium phosphate buffer (pH 7.0), 500 mmol/l ethylenediaminetetraacetic acid, 0.5% v/v Triton, 20 mmol/l K-ferricyanide, 20 mmol/l K-ferrocyanide, 20 mmol/l 5-bromo-4-chloro-3-indoxyl-β-D-glucuronide-cyclohexy-lammonium salt (X-Gluc) (Himedia, India) and 20% v/v methanol. After incubation, tissues were washed several times with 70% ethanol until the complete removal of chlorophyll. The tissues showing blue color after the removal of chlorophyll was scored and GUS stained transformed ETs were counted as GUS-positive transformants.

Transient GUS expression (%) in 99 soybean cultivars was determined in three biological replications and each biological replication was measured in triplicate, the means and standard deviations were calculated. The data were statistically assessed using the one-way ANOVA. The comparison of the variation between means was performed via Fisher least significant difference (LSD) value through DMRT at a significance level of $p < 0.05$.

Results and Discussion

A total of 92 Indian soybean cultivars and 7 advanced breeding lines were transformed with the *Agrobacterium* strains EHA105 carrying pCAMBIA1305.1vector expressing the GUSPlus reporter gene to see the effect of genotypic variability on *Agrobacterium*-mediated transformation efficiency. Transient GUS expression was observed in transformed ET tissues (Fig. 2) after 5 days in co-cultivation media.

The result showed that transformed ET explants of some soybean genotypes stained intensely with the substrate X-Gluc (Fig. 2a,b), while transformed ETs of some genotypes stained weakly (Fig. 2c,d). Transient GUS expression calculated by dividing number of stained ETs by total number of ETs infected by *Agrobacterium* in the experiment and is listed in Table 2. Soybean genotypes showed wide variations for transient GUS expression ranging from 3.8 to 100%. Twenty genotypes showed > 70% transient GUS expression indicating high susceptibility to *Agrobacterium* infection, 48 genotypes showed moderate expression ranging from 30 to 70% and 31 genotypes were weakly susceptible showing < 30% *Agrobacterium* infection. Five genotypes, DS -228 (100%), JS 335 (98.1%), JS

72 - 44 (96.5%), KHSb 2 (96%), and JS 72-280 (92%) were highly sensitive to *Agrobacterium* infection. In contrast, five genotypes, namely Pusa 98 - 14 (3.8%), Davis (7%), MACS-58 (7), PK 1092 (7.2%), and Palam soya (8.3%) showed the lowest transformation efficiency.

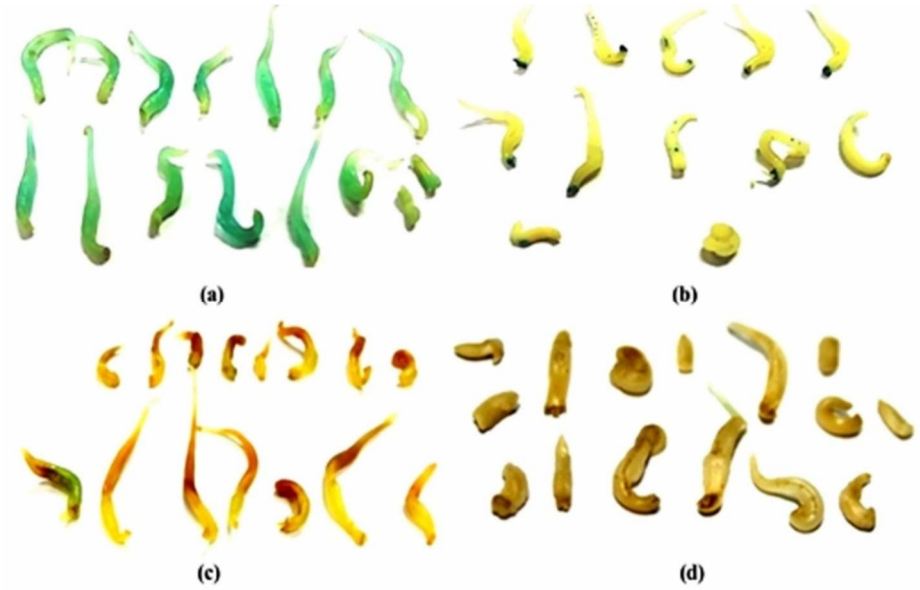

Fig. 2. Histochemical GUS staining of transformed ET explants infected with *Agrobacterium* EHA105 containing pCambia1305.1 vector. (a) Highly susceptible genotype KHSb 2 showing GUS expression covering all parts of explant, (b) highly susceptible genotype JS 72-280 showing GUS expression at shoot meristem only, (c) weakly susceptible genotypes ACS-58 and (d) showing GUS expression in a few explants with very weak blue color intensity.

Present study also proved that *Agrobacterium*-transformation efficiency is highly affected by plant genotypes. The effect of cultivars on transient GUS expression was found to be highly significant. The transient GUS expression of DS228 was found be the highest (100%) and transient GUS expression of JS 335 (98.1%) was not significantly lower than DS 228 but significantly higher than all the other varieties examined under the study (Table 2).

It was also observed genetic relatedness among genotypes showed high susceptibility to *Agrobacterium* infection. All the genotypes, which have JS 335 (98.1%) as one of its parent showed high transient GUS expression (> 70%), suggesting the role of genetic factors. DS - 228 (100%) and MAUS - 81 (73.1%) showing high transient GUS expression are evolved from the cross JS 335 × DS 181 and KB - 74 × JS 335, respectively. Similarly, NRCSL1 and NRCSL2, advanced breeding lines both developed from cross between JS335 and SL525 showed 86 and 72% transient GUS expression, respectively. Though Co 3 developed from UGM 69 × JS 335 did not show high transient GUS expression (52%).

Table 2. Evaluation of genotypic variability of Indian soybean for *Agrobacterium*-mediated transformation efficiency.

Genotype number	Soybean genotype	Transient GUS expression (%)	Genotype number	Soybean genotype	Transient GUS expression (%)
1	DS-228	100 ± 0^{a}	51	JS93-05	42.3 ± 0.7^{uv}
2	JS 335	98.1 ± 1.0^{ab}	52	SL-96	42.3 ± 1.1^{uv}
3	JS 72-44	96.5 ± 1.6^{b}	53	Co 2	40.5 ± 0.6^{vw}
4	KHSb2	96.0 ± 1.0^{b}	54	Bragg	40.4 ± 1.4^{vw}
5	JS 72-280	92 ± 3.0^{c}	55	MAUS-57	40.2 ± 1.2^{vw}
6	MAUS61-2	89.4 ± 0.6^{c}	56	MACS-57	40.1 ± 1.0^{vw}
7	NRCSL1	86 ± 1.7^{d}	57	PK 1042	39.6 ± 1.4^{vw}
8	JS95-60	85.4 ± 0.9^{d}	58	Birsa Soya-1	38.3 ± 2.2^{wx}
9	PK 471	85.3 ± 0.8^{d}	59	MAUS-47	37.2 ± 2.1^{wxy}
10	PRS1	85.0 ± 0.8^{d}	60	JS 2034	35.5 ± 1.8^{xyz}
11	PK 564	84.9 ± 0.9^{d}	61	Monetta	35.3 ± 1.1^{xyz}
12	JS 76-205	83.8 ± 1.1^{de}	62	Indira Soya	35.1 ± 1.0^{xyz}
13	MACS-124	83.7 ± 0.5^{de}	63	Pratap Soya 2	34.3 ± 0.3^{yzA}
14	DSB1	82.6 ± 2.5^{de}	64	JS 79-81	33.6 ± 1.3^{zAB}
15	Punjab-1	80.4 ± 2.2^{e}	65	Lee	32.4 ± 0.6^{zABC}
16	Pusa 37	75 ± 1.6^{f}	66	PS 1347	31.2 ± 1.0^{ABCD}
17	NRC107	75 ± 1.1^{f}	67	Pusa 24	30.2 ± 1.2^{BCDE}
18	Gujarati Soya-2	73.4 ± 0.6^{fg}	68	Kb-79	30 ± 0.8^{CDEF}
19	MAUS-81	73.1 ± 2.1^{fg}	69	PS 1225	29.9 ± 2.1^{CDEFG}
20	NRCSL2	72 ± 2.8^{fg}	70	Shilajeet	28.2 ± 1.0^{DEFGH}
21	JS 80-21	$70.\pm1.6^{gh}$	71	PS 97-12	27.5 ± 1.5^{EFGHI}
22	Pusa 22	$68.\pm3.2^{hi}$	72	JS-2	27.3 ± 4.5^{EFGHI}
23	JS97-52	65.5 ± 0.8^{ij}	73	Type49	26.6 ± 1.8^{FGHI}
24	Pusa 16	65.3 ± 2.3^{ijk}	74	VLS47	26.4 ± 2.1^{GHIJ}
25	MAUS-61	65.1 ± 1.0^{jk}	75	MAUS-158	26.3 ± 1.1^{HIJ}
26	Kalitur	63.5 ± 0.5^{jkl}	76	JS 2029	25.2 ± 1.1^{HIJK}
27	NRC 37	62.3 ± 1.2^{jklm}	77	VLS2	24.3 ± 4.0^{IJKL}
28	Improved Pelican	61.9 ± 2.4^{klm}	78	MAUS-32	23.0 ± 1.0^{JKLM}
29	RKS-24	60.5 ± 1.8^{lmn}	79	PS 1024	23.0 ± 1.0^{JKLM}
30	PK 1029	60 ± 1.1^{mn}	80	Lsb1	22.1 ± 0.8^{KLMN}
31	JS 75-46	58 ± 4.0^{no}	81	NRC109	21.9 ± 1.6^{KLMN}
32	SL295	55.9 ± 1.3^{op}	82	VLS1	21.8 ± 1.7^{KLMN}
33	Alankar	55.8 ± 1.5^{op}	83	Hara Soya	21.1 ± 1.4^{LMNO}

(Contd.)

34	NRC 7	54.2 ± 0.8^{pq}	84	NRC138	19.9 ± 2.7^{MNOP}
35	PK 472	53.8 ± 1.4^{pq}	85	PK 327	19.2 ± 1.1^{NOP}
36	ADT-1	53.6 ± 2.3^{pq}	86	Pusa 40	18.6 ± 0.7^{NOP}
37	PS 1241	52.2 ± 0.8^{qr}	87	JS 71-05	18.1 ± 2.9^{OP}
38	Co 3	52 ± 2.6^{qr}	88	JS 90-41	17.2 ± 0.8^{PQ}
39	NRC 2	50.1 ± 1.1^{rs}	89	SL688	14.3 ± 2.0^{QR}
40	MAUS-2	50.1 ± 1.1^{rs}	90	NRC142	13.1 ± 0.7^{RS}
41	Pusa 20	49.9 ± 1.6^{rs}	91	NRC127	11.5 ± 2.4^{RST}
42	Tams-38	49.5 ± 2.4^{rs}	92	SL525	10.4 ± 2.1^{STU}
43	Hardee	49.4 ± 3.1^{rs}	93	Gujarati Soya-1	10.3 ± 1.9^{STU}
44	PK 308	49.0 ± 4.4^{rs}	94	NRC 12	9.6 ± 2.3^{TU}
45	MAUS-1	48.1 ± 0.9st	95	Palam soya	8.3 ± 1.2^{TU}
46	Ankur	45.6 ± 4.9^{tu}	96	PK 1092	7.2 ± 0.6^{UV}
47	NRC 86	44.4 ± 1.3^{u}	97	Davis	7 ± 1.7^{UV}
48	Co 1	44.3 ± 4.7^{u}	98	MACS-58	7 ± 0.3^{UVz}
49	PK 262	44.1 ± 0.8 u	99	Pusa 98-14	3.8 ± 1.2^{V}
50	Shivalik	42.7 ± 4.6^{uv}			
LSD (p = 0.05) 3.09					
Main effect***					

*The data presented here are the average values of three replicates ± Sd. Values followed with dissimilar letters differ significantly at p = 0.05 in accordance with LSD and DMRT. The transient GUS expression was calculated as follows: Transient GUS expression (in % = The number of positive transformants/the total infected explants × 100. NRC numbers are 7 advanced breeding lines.

After infection and staining different *Agrobacterium* infectivity pattern in transformed explants was observed. Blue color was either on stem meristem (SM, Fig. 3a), coleoptiles region (CL, Fig. 3b), SM and CL both (Fig. 3c) or all part of the stained transformed ET explants (Fig. 3d). Blue loci developed covering all area of the stained transformed ET explants in genotypes, KHSb 2 and SL 295 (Figs 2a, 3d) and both SM and CL in JS 335; whereas, in JS72-280 and MAUS-2, it was observed only in SM and CL region, respectively (Fig. 3a, b). In contrast, low transformation efficient genotypes do not or less developed blue color after GUS staining (Fig. 2c, d). It is worth noting that DS-228 (100%), JS 335 (98.1%, Fig. 3c), JS72-44 (96.5%), KHSb 2 (96%, Fig. 2a), and JS 72-280 (92%; Fig. 3a) showed high intense blue color with different infectivity patterns (SM, CL, both or all parts), especially at 5 days after co-cultivation, suggesting that *Agrobacterium*-mediated transformation was more acceptable by high-efficient genotypes. Consequently, variations in color formation and intensity among the tested genotypes indicated that these differences were completely dependent on genotypes.

Host plant genotype and *Agrobacterium* strain, both affect the level of *Agrobacterium* infection (Karami 2008). A number of studies have been reported genotypic variability in soybean (Song et al. 2013, Jia et al. 2015) and other crops like rice (Hoque et al. 2005), maize (Ritchie et al. 1993), various legumes (Hood et al. 1987; Owens and Cress 1984), aspen (Beneddra et al. 1996), *Pinus* species (Bergmann and Stomp 1992), tomato (van Roekel et al. 1993), Arabidopsis (Nam et al. 1997), and grape (Lowe and Krul 1991) for susceptibility to *Agrobacterium* infection. A variety of explants like cotyledonary node, half seed and ET have been used in development of transgenic soybean (Verma et al. 2011, Rani et al. 2012, Olhoft et al. 2003, Chen et al. 2018, Paz et al. 2006, Liu et al. 2004). ET was used for transient GUS expression in this study since it has many advantages over other explants. It is easier to obtain, shoot regeneration is direct, and is not prone to somaclonal variation and chromosomal abnormalities (Saeed et al. 1997). Moreover, the ET system has the highest regeneration frequency (Liu et al. 2004). This study was carried over to select genotypes of the most amenable to *Agrobacterium* infection as a first step to improve soybean transformation efficiency.

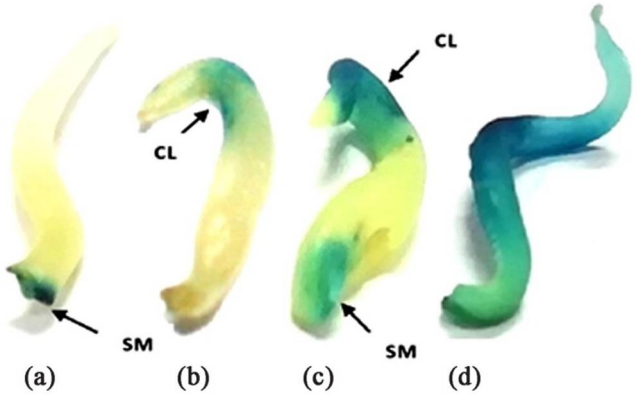

Fig. 3. Infectivity pattern of histocheical GUS expression in various regions of ET tissue observed after 5 days of co-cultivation when infected with pCambia 1305.1, (a) only stem meristem (SM) region stained in genotype JS72-280. (b) only coleoptiles (CL) region stained in MAUS-2, (c) both SM and CL regions stained in genotype JS 335, (d) complete tissue stained in SL 295.

This study mainly focused on cultivated variety and advanced breeding lines of India as identification of elite variety/breeding line with high transformation efficiency will help in rapid development of transgenic soybean variety and there will be no need to introgress transgene from primary transgenic with poor agronomic background into high yielding variety. Significant genotype variability for susceptibility to *Agrobacterium* infection was observed in Indian soybean genotypes. This study screened 5 high-efficient genotypes DS-228, JS 335, JS 72-44, KHSb 2, and JS 72-280 with transient GUS expression of 100, 98.1, 96.5, 96 and 92%, respectively and 5 weakly-susceptible genotypes Pusa98-14, Davis, MACS-58, PK 1092 and Palam soya with low transient GUS expression of 3.8, 7, 7, 7.2 and 8.3%, respectively (Table 2). JS335, a very high yielding Indian soybean

variety,was many times used in transformation protocols (Verma et al. 2009, Rani et al. 2012) in earlier studies. This genotype showed high transient GUS expression (98.1%) in this study, as well as four more genotypes, DS-228, JS72-280, JS 72-44, and KHSb2 also performed better (Table 2, Fig. 2). Five low-efficient genotypes, namely Davis, Palam soya, PK 1092, Pusa98-14, and MACS-58 displayed poor transient GUS expression. Jia et al. (2015) reported a strong defense response in the form of over expression of methyl jasmonate, polyphenol oxidase and peroxidase in weakly susceptible genotypes as compared to highly susceptible genotypes. Though these factors were not analyzed in this study, a mapping population has been developed from a cross of highly efficient genotype and genotype with very low efficiency to study the genetic factors responsible for the difference and map the genomic region regulating susceptibility to *Agrobacterium* infection. The highly efficient genotypes identified in present study can be used in development of transgenic soybean variety in India as all of the genotypes have been released for cultivation in India based on their yield performance in multi-location trial. Transgenics developed from these varieties can be directly released after legal clearance from the agencies authorized for release of transgenic crop varieties in India. These genotypes can also be used for studying function of genes with unknown roles to improve soybean performance under various biotic and abiotic stresses using virus-induced gene silencing (VIGS) or CRISPR-CAS9 techniques by silencing those genes.

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