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Screening of soybean [*Glycine max* (L.) Merrill] genotypes for somatic embryogenesis and plant regeneration potential

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Abstract

Twenty two soybean genotypes were evaluated for the capacity of embryo initiation, differentiation, maturation and plantlet regeneration using standardized protocols. Among the genotypes, Bragg was to have high response to induction with 91.11% and average 38.40 embryos/callus mass), proliferation (89.47%) and differentiation (78.95%) however, SL 688 gave the highest maturation frequency (91.57%). Highest somatic embryogenesis germination was recorded in SL 525 (85.67%) whereas, highest survival was observed in JS 335 (82.22%). Genotype Pusa 37 gave the poor response. Fifteen genotypes which were identified as responsive were Bragg, Bragg-Palampur, Pusa 5, Pusa 12, Pusa 14, Pusa 16, Pusa 24, Pusa 40, Pusa 9814, SL 688, SL 525, SL 979, SL 958, DS 2706 and DS 2708. The study thus open up vistas for efficient transgenic plant development in soybean.

Key words: *Glycine max* L., immature cotyledon, somatic embryogenesis, regeneration.

Introduction

In soybean, Christianson et al. (1983) demonstrated somatic embryogenesis from excised zygotic embryos. Later, Lazzeri et al. (1985) reported the use of immature cotyledons and, since then, immature cotyledons have been used as the sole explant system capable of regenerating into plantlets *via* somatic embryogenesis (Ko and Korban 2004; Hiraga et al. 2007; Klink et al. 2008). Somatic embryogenesis from immature cotyledons is highly genotype-dependent (Meurer et al. 2001; Ko et al. 2004). Several studies have shown significant differences among soybean genotypes in their capacity to respond to the different steps of somatic embryogenesis (Hiraga et al. 2007; Yang et al. 2009). The efficiency of regeneration and transformation of soybean is genotype-dependent and remains effective mainly for the variety 'Jack' and a few other genotypes in the USA (Walker and Parrott 2001; Schmidt et al. 2005).

The potential for embryogenesis can be improved to a certain extent by modification of tissue culture protocols for specific genotypes. The present investigation was undertaken to screen number of Indian soybean genotypes for their regeneration potential through somatic embryogenesis using protocol standardized in our laboratory (Huynh et al. 2015).

Materials and methods

Plant materials

Total 22 soybean genotypes were screened for their somatic embryogenesis response (Table 1). The experiments were carried to refine the earlier standardized protocol (Huynh et al. 2015) for somatic embryo initiation to germination and hardening of plantlets. The seeds were collected from the Soybean Laboratory, Division of Genetics, IARI, Pusa, New Delhi. The genotypes were raised in polyhouse at Division of Genetics, IARI, New Delhi. The temperature conditions maintained were 25^{o}C day and 20^{o}C night under 16/8 h photoperiod with 97 µmol.$m^{-2}s^{-1}$ light intensity and relative humidity maintained at 60-65%. After fruit set, the immature pods (3.0-4.0 cm) of all these genotypes were harvested after 1-2 weeks of flowering. These immature pods were collected in polybags and taken to laboratory for culture.

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The immature seed-derived explants were prepared for culture as described by Huynh et al. (2015).

SE initiation, proliferation and differentiation

The explants were cultured on embryo induction medium comprising MS salts (Murashige and Skoog 1962), B5 vitamins (Gamborg et al. 1968), 3% sucrose, 40 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D), gellan gum (0.2%, Gelrite™, Sigma Chem Co. St. Louis), with pH adjusted to 7.0. The cultures were maintained at 26±1^{0}C with 16/8 h light and dark photoperiod (40µmol.m^{-2}s^{-1}) and sub-cultured onto the same fresh medium at 15-day interval until induction of somatic embryos. The percentage of embryogenesis was calculated after 6 weeks. The primary somatic embryos were transferred onto varied proliferation medium comprising MS salts, B5 vitamins supplemented with 20 mg l^{-1} 2,4-D and 13 mg l^{-1} ABA; 3% sucrose and 0.2% gellan gum, and pH = 5.8. Sub-culture was done at monthly interval. Cultures were maintained at 26±1^{0}C with 16/8 h photoperiod (40µmol.m^{-2}s^{-1}) in culture room. Globular stage embryos from above step gave rise to cotyledonary stage embryo by transferring to medium comprising of MS salts, B5 vitamins, 6% maltose, 0.5% activated charcoal and 0.2% gellan gum, and pH = 5.8. Embryos were kept on this medium for one month and transferred for maturation.

SE maturation, germination and plantlet conversion

Cotyledon stage embryos from the preceding step were separated individually and transferred onto medium containing MS salts, B5 vitamins (MSB5) supplemented with 6% maltose + 0.2% gellan gum, pH = 5.8 for one month to achieve somatic embryo maturation. After one month, cream coloured cotyledonary stage embryos were separated into individual embryos and desiccated. In this stage, several embryos were placed in a sterilized empty petri dish, sealed with Parafilm® wrap and incubated at 26±1^{0}C and 80% relative humidity for a week period (Parrott et al. 1988). Thereafter, the embryos were transferred onto hormone-free medium containing MS salts, B5 vitamins, 1.5% sucrose and 0.2% gellan gum, pH = 5.8 for germination. At this stage, a 23/1 h light & dark photoperiod was maintained.

Acclimatization of plantlets

After somatic embryos developed into complete plantlets, they were transplanted into glass jar with PP cap containing peat, perlite and vermiculite (2:1:1; v/v). The hardened plants were maintained at 26±1^{0}C with 16/8 h photoperiod (97µmol.m^{-2}s^{-1}) and maintained under high relative humidity condition for two weeks. The hardened plantlets were then transferred to glasshouse under natural light conditions.

Statistical analysis

All the experiments were carried out in completely randomized design with three replications. Each treatment comprised of 25-30 units. The percentage data was subjected to Arc Sine $\sqrt{}$percentage transformation before calculating the ANOVA.

Results

Somatic Embryo (SE) initiation

Total 22 soybean genotypes were screened for their somatic embryogenesis capacity. In the culture initiation stage, the medium MSB5 supplemented with 40 mg l^{-1} 2,4-D was used. Pusa 5 registered the shortest duration to somatic embryo initiation (16.40 days), while in Pusa 37 it was most delayed (27.30 days). Bragg was most responsive genotype, which gave the highest somatic embryo induction frequency (91.11%), while the lowest frequency was noted in genotype Pusa 20 (28.33%). Similarly, the highest number of somatic embryos per cotyledon was observed in genotype Bragg (38.40), followed significantly by Pusa 9814 (26.60) and SL 979 (23.40), while the lowest number of embryos per cotyledon was noted in genotype Pusa 37 (2.0) (Table 1).

SE proliferation

After initiation stage, somatic embryos were transferred to the MSB5 medium supplemented with 20 mg l^{-1} 2,4-D and 13 mg l^{-1} ABA. At this stage, Bragg was most responsive genotype to proliferation with 89.47%, while lowest frequency was noted in Pusa 37 (19.45%) (Fig. 1).

SE differentiation

After proliferation of somatic embryos, they were subjected to MSB5 medium supplemented with 6% maltose and 0.5% activated charcoal for further differentiation. Again Bragg was found to be the most responsive genotype with 78.95% of embryos got differentiated, while the lowest frequency was noted in Pusa 37 (25.0%) (Fig. 2).

Table 1. Effect of genotype on somatic embryos initiation

Genotype	Day to SE initiation (days)	SE frequency (%)	No. of SE/ callus mass
Pusa 5	16.40	62.32 (52.12)*	14.60
Pusa 12	19.10	65.06 (53.79)	9.80
Pusa 14	20.90	62.11 (52.00)	13.00
Pusa 16	20.60	63.54 (52.83)	19.80
Pusa 20	21.10	28.33 (32.14)	5.60
Pusa 22	17.10	66.67 (54.76)	2.60
Pusa 24	18.40	34.92 (36.21)	6.80
Pusa 37	27.30	31.67 (34.27)	2.00
Pusa 40	22.70	57.89 (49.54)	13.00
Pusa 9814	22.60	77.54 (61.68)	26.60
Pusa 9712	22.40	38.76 (38.53)	8.00
JS335	24.70	37.25 (37.64)	3.80
PS-1347	25.60	61.90 (51.88)	3.00
SL-525	24.70	51.24 (45.69)	17.80
SL-688	23.30	72.73 (58.50)	15.20
SL-958	23.60	47.62 (43.62)	18.40
SL-982	21.20	39.51 (38.94)	10.60
SL-979	23.90	64.93 (53.67)	23.40
Bragg	23.70	91.11 (72.64)	38.40
Bragg -Palampur	17.60	84.31 (66.66)	13.60
DS 2706	20.30	66.36 (54.57)	8.00
DS 2708	20.20	54.76 (47.75)	13.00
LSD (*P* d™ 0.05)	3.74	6.78	7.71
CV (%)	19.60	7.20	47.00

* Figure in parentheses is Arc Sin √% transformed data; MS salts + B5 vitamins + 40 mg/l 2,4 D + 3% sucrose + 0.2% gellan gum

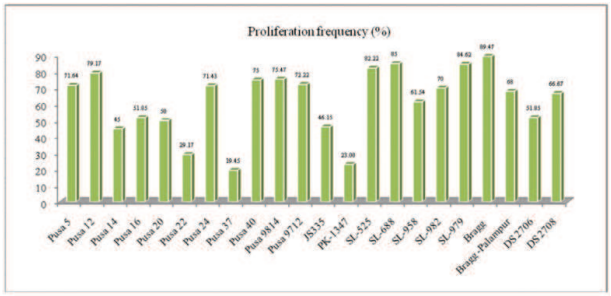

Fig. 1. Effect of genotype on somatic embryo proliferation in soybean

SE maturation, germination and plant conversion

Differentiated embryos were then transferred to MSB5 medium supplemented with 6% maltose for maturation.

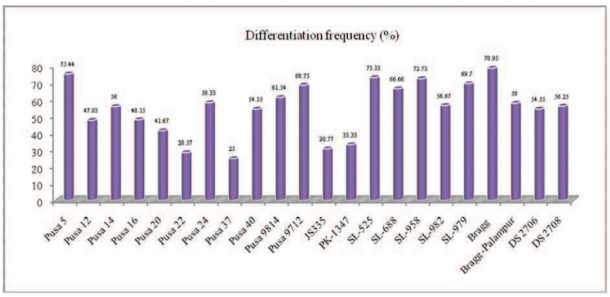

Fig. 2. Effect of genotype on somatic embryo differentiation in soybean

During this stage, SL 688 was identified as the most responsive genotype (91.57%), while lowest maturation frequency was recorded in genotype Pusa 37 (51.07%) (Fig. 3).

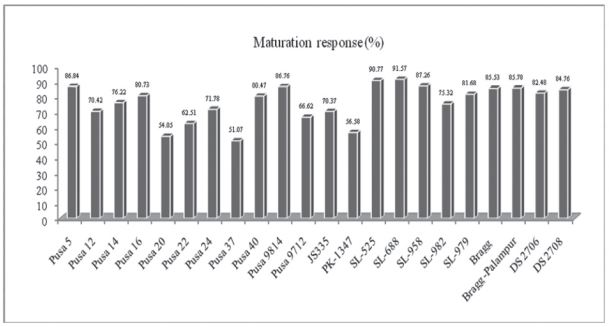

Fig. 3. Effect of genotype on somatic embryos maturation in soybean

Matured embryos were subjected to desiccation treatment by air-drying method for 5-7 days before they were transferred to germination medium. MSB5 medium containing 1.5% sucrose was used for embryo germination. In this medium, genotype Pusa 14 gave the earliest germination in 6.33 days, while SL 982 was the most delayed genotype (10 days). In contrast, highest germination was recorded in genotype SL 525 (85.67%), while lowest germination was noted in Pusa 37 (15%) (Table 2).

Shoot and root length of plantlet were also recorded (Fig. 4). Maximum shoot length was observed in genotype SL 958 (8.72 cm), while shortest shoots were noted in Bragg with 1.56 cm. However, SL 688 gave the longest roots (9.70 cm), while the shortest roots were observed in Pusa 20 (2.38 cm). Genotype Pusa 24 gave the highest number of roots per plantlet (9.60) while lowest number of roots per plantlet was observed in Pusa 20 and Pusa 37 (2.8) (Fig. 5).

Table 2. Effect of genotype on somatic embryos germination

S.No.	Genotype	Day to germination (days)	Germination (%)
1	Pusa 5	6.67	77.33 (58.89)*
2	Pusa 12	7.67	61.11 (51.41)
3	Pusa 14	6.33	78.64 (62.44)
4	Pusa 16	7.00	38.27 (38.23)
5	Pusa 20	7.33	20.00 (26.56)
6	Pusa 22	8.33	28.33 (32.14)
7	Pusa 24	8.33	71.67 (57.86)
8	Pusa 37	9.00	15.00 (22.79)
9	Pusa 40	9.33	75.74 (60.47)
10	Pusa 9814	6.67	81.33 (64.37)
11	Pusa 9712	7.33	40.67 (39.64)
12	JS335	8.00	40.00 (39.23)
13	PS-1347	9.67	16.67 (24.12)
14	SL-525	6.67	85.67 (67.78)
15	SL-688	7.33	82.00 (64.90)
16	SL-958	8.33	72.00 (58.05)
17	SL-982	10.00	66.67 (54.76)
18	SL-979	7.00	71.67 (57.86)
19	Bragg	7.33	63.33 (52.71)
20	Bragg-Palampur	7.67	67.33 (55.12)
21	DS 2706	8.00	72.33 (58.24)
22	DS 2708	8.67	71.00 (57.42)
	LSD (*P* d• 0.05)	1.33	15.90
	CV (%)	10.30	16.40

*Figure in parentheses is Arc Sin √% transformed data

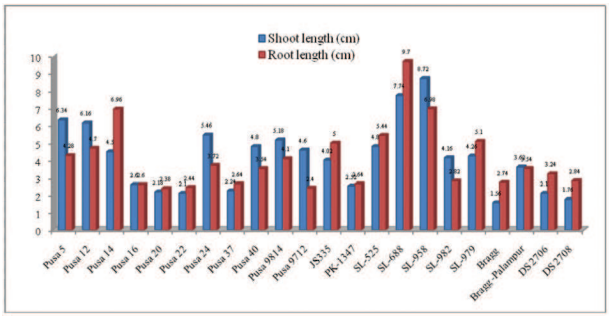

Fig. 4. Effect of genotype on shoot and root length of germinated embryos in soybean

After germination stage, plantlets were transferred to hardening medium filled in glass jar with PP cap containing peat, perlite and vermiculite (2:1:1; v/v). Earliest days to hardening was recorded in genotype JS 335 (16.40 days), while it was most delayed in Pusa 37 (20.40 days) (Fig. 6a). Similarly, highest plantlet survival was observed in JS 335 (82.22%), while lowest frequency was noted in Pusa 37 (39.76%) (Fig. 6b; Fig. 7a to 7h).

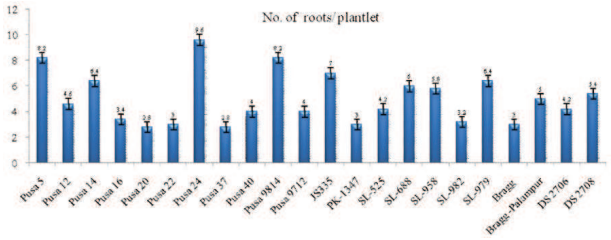

Fig. 5. Effect of genotype on number of roots on germinated embryos in soybean

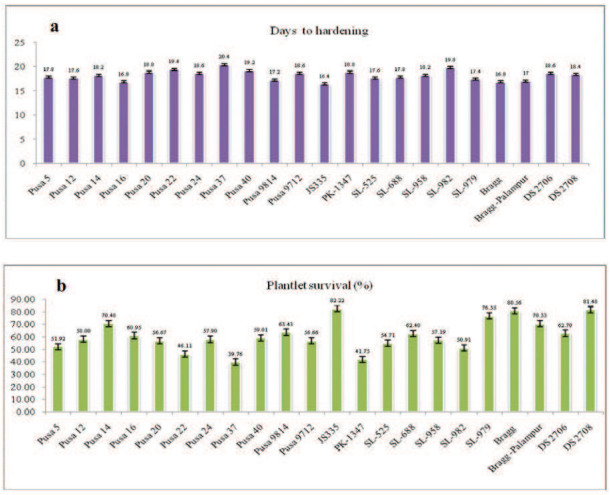

Fig. 6. Effect of genotype on hardening of plantlets. a = Day to hardening and b = Plantlet survival (%)

Discussion

In this experiment, 22 soybean genotypes were screened for their response to somatic embryogenesis using standardized protocol. There were significant differences among soybean genotypes screened in their response to the different stages of somatic embryogenesis.

At culture initiation stage, Pusa 5 was most responsive genotype which took shortest time to somatic embryo initiation with 16.40 days while Pusa 37 was most delayed genotype (27.30 days). Percentage of somatic embryo induction frequency ranged from 28.33 per cent in Pusa 20 to 91.11 per

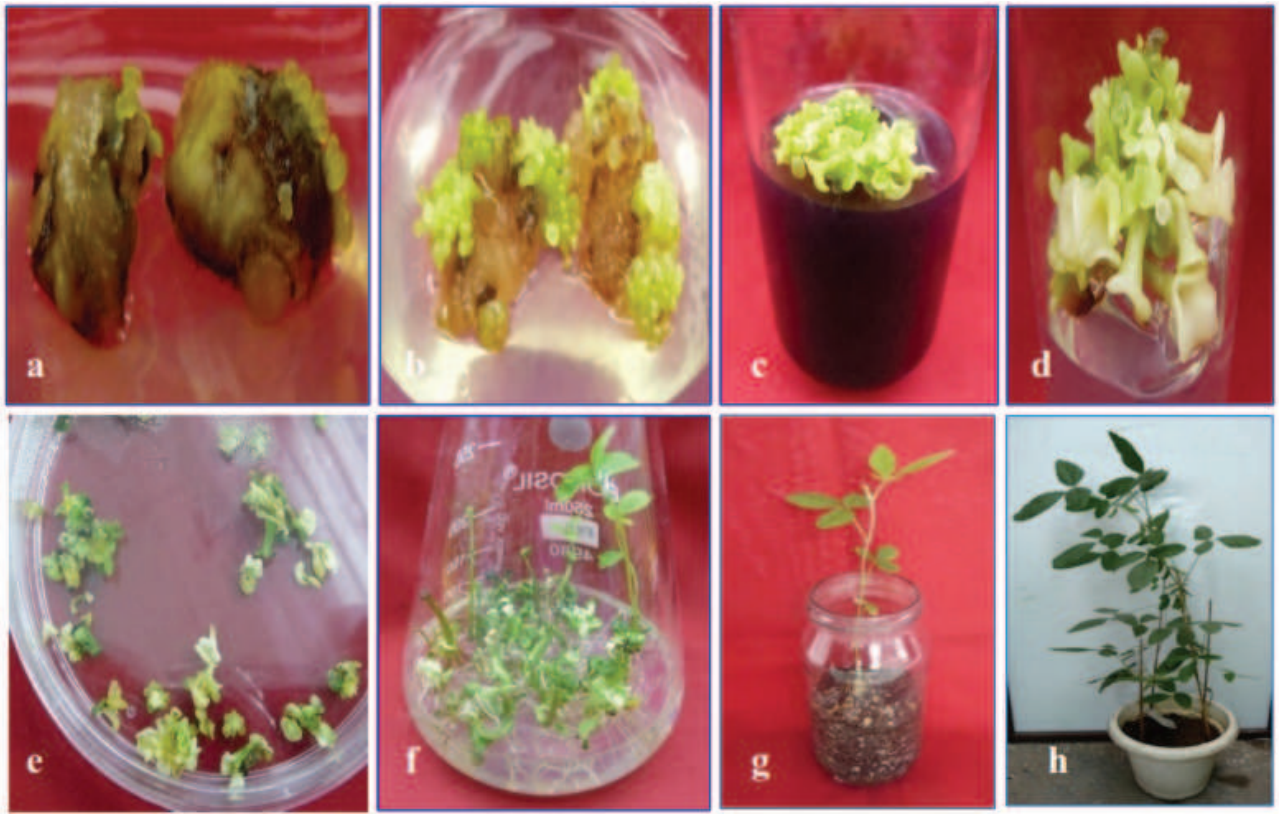

Fig. 7. Somatic embryo induction, proliferation, differentiation, maturation and plantlet regeneration in soybean genotype Bragg. a = Induction of embryogenenic calli; b = Multiplication of somatic embryo (SE); c = Differentiation of SE on callus mass; d = Maturation of SE; e = Desiccations of SE; f = Germination of SE and g & h = Hardening of plantlet

cent in Bragg. Bragg also gave highest number of somatic embryos per callus mass (38.40), while lowest number was recorded in Pusa 37 (2.0 embryos). This wide variation in induction rate is similar to the results of Bailey et al. (1993), where induction rates ranged from 46 to 94%; and Tomlin et al. (2002) where induction frequencies ranged from 30.36 to 84.01%. The difference among cultivars for SE has been reported by several workers (Ko and Korban 2004; Hiraga et al. 2007; Yang et al. 2009; Droste et al. 2010; Texeira et al. 2011). In contrast to our results, Mariashibu et al. (2013) reported Pusa 16 to be most responsive cultivar for somatic embryo induction among Indian soybean cultivars but the media composition was different.

A clear influence of the genotype on the capacity for repeated embryogenesis was also detected. Proliferating cultures were established for all genotypes tested which ranged from 19.45% (Pusa 37) to 89.47% (Bragg). Depending upon the proliferation frequency, genotypes Bragg (89.47%), SL 688 (85%), SL 979 (84.62%), SL 525 (82.22%) and Pusa 12 (79.17%) were identified as more responsive genotypes to SE proliferation, despite some of them gave low response at induction stage. Genotype Pusa 37 was least responsive genotype.

At differentiation stage, response ranged from 25% in Pusa 37 to 78.95% in Bragg, which was followed by Pusa 5 (75.44%), SL 525 (73.33%), SL 958 (72.73%), SL 979 (69.7%), Pusa 9712 (68.75%) and SL 688 (66.66%)). This group of genotypes was considered as more responsive. Addition of activated charcoal to culture medium helped in enhancing the response frequency in large number of genotypes. Activated charcoal adsorbs auxins released from developing tissues and promotes a more normal morphology and increased germination ability (Merkle et al. 1995). During maturation stage, SL 688 was identified as the most responsive genotype (91.57%), while lowest was recorded in Pusa 37 (51.07%). The genotypes SL 688 (91.57%), SL 525 (90.77%), SL 958 (87.26%), Pusa 5 (86.84%), Pusa 9814 (86.76%),

Bragg-Palampur (85.78%), Bragg (85.53%), DS 2708 (84.76%), DS 2706 (82.48%), SL 979 (81.68%) and Pusa 16 (80.73%) were other responsive genotypes, though there were non-significant differences among genotypes for their SE maturation response.

It was found that low sugar concentration helped in improving somatic embryos germination frequency (Komatsuda 1992). Sucrose supplemented at 1.5% in the medium gave the earliest germination (6.33 days) in Pusa 14, while SL 982 had the most delayed response (10 days). In contrast, germination ranged from 15% in Pusa 37 genotype to 85.67% in SL 525. Group of genotypes consisting of SL 525 (85.67%), SL 688 (82.0%), Pusa 9814 (81.33%) 14 (78.64%), Pusa 5 (77.33%), Pusa 40 (75.74%), DS 2706 (72.33%), SL 958 (72.0%), Pusa 24, SL 979 (71.67%) and DS 2708 (71.0%) were considered as highly responsive to somatic embryo germination. Maximum shoot length was observed in genotype SL 958 (8.72 cm), however, longest root was noted in SL 688 (9.70 cm). Besides, genotype Pusa 24 gave the highest number of roots (9.60), while lowest number of roots per plantlet was in Pusa 20 and Pusa 37 (2.8 roots). Somatic embryogenesis has successfully been induced and applied in woody plants as well (Isah 2016; Guan et al. 2016).

Genotypes identified more responsive were Bragg, Bragg-Palampur, Pusa 5, Pusa 12, Pusa 14, Pusa 16, Pusa 24, Pusa 40, Pusa 9814, SL 688, SL 525, SL 979, SL 958, DS 2706, and DS 2708. It has been observed that somatic embryogenesis and maturation in soybean differed with genotype. The responsive genotypes can be successfully exploited for developing transgenic plants.

Authors' contribution

Conceptualization of research (SKL); Designing of the experiments (SKS, SKL); Contribution of experimental materials (SKL, AT); Execution of field/lab experiments and data collection (HNH); Analysis of data and interpretation (SKL, Vinod); Preparation of manuscript (SKL, AT).

Declaration

The authors declare no conflict of interest.

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