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Segregation of pulp pigments and seed related traits in biparental guava progenies

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ABSTRACT

The current research was conducted to investigate segregation of pulp pigments and seed related parameters in the full-sib progenies derived from 'Allahabad Safeda' (♀) and 'Arka Kiran' (♂) cross. Fifty, four-year-old full-sib progenies confirmed through SSR markers along with their parental genotypes were evaluated. Significant variability was observed for seed related parameters including seed length, width, and seed weight per fruit, seed hardness as well as for pigments like lycopene and β-carotene contents, indicated wide genetic diversity and segregation. Pulp colour revealed predominance of Red group pulp colour progenies (45 Nos) over White group (5 Nos). Lycopene content ranges from 0.31 to 23.21 mg/100 g and β-carotene from 0.08 to 1.95 mg/100 g, with several progenies surpassing values in both the parents, indicating transgressive segregation and strong potential for nutritional improvement. Correlation analysis revealed significant positive association among seed parameters, particularly between seed weight per fruit and seed number, while seed parameters showed no significant correlation with lycopene and β-carotene contents, suggesting their independent inheritance. Principal component analysis indicated that first four components (PC 1-4) accounted for 81.88% of the total genetic variation, with carotenoids loading independently from seed morphology. Hierarchical clustering grouped the full-sibs into five distinct clusters, reflecting substantial genetic divergence for selecting superior genotypes with desirable traits.

Key words: Pulp pigment, lycopene, β-carotene, HPLC, full-sib progenies, genetic variability.

INTRODUCTION

Guava (*Psidium guajava* L.) is a tropical fruit crop, valued not only for its economic importance but also for its remarkable nutritional and therapeutic attributes. It is native to tropical America and belongs to the family Myrtaceae having a basic chromosome number of n = 11 (Nakasone and Paull, 10). Owing to its abundant fruit production and suitability for growth in marginal areas with little maintenance, guava is viewed as remunerative crop in the country. Presently, India is one of the leading producers of guava globally, with cultivation spanning 358 thousand hectares and yielding 5.35 million metric tons (MoA& FW, 3).

Guava, popularly known as the "Apple of the Tropics," is also regarded as a "superfruit" in light of its nutrient-dense composition, notable antioxidant potential, and widespread accessibility (Sanda *et al*., 13). Fruits are rich in vitamins, antioxidants, and bioactive compounds, so it has gained significant attention in both food and pharmaceutical sectors (Naseer *et al*., 11). Additionally, guava seeds possess approximately 5-13% oil, which is a significant source of omega-3 and omega-6 fatty acids (Adsule and Kadam, 1). The pulp of guava fruits showed considerable variation in colour including yellow, pink, white and purple (Thakre *et al*., 16). Coloured pulp guava genotypes are preferred over white pulped for fresh as well as processing purposes since they have bioactive health-promoting properties (Bhuvaneswari and Nagini, 5), and are also rich source of lycopene, β-carotene and anthocyanins.

Fruit pulp colour in guava is regulated by a polygenic inheritance pattern, where white is homozygous recessive and pink/red often comes from heterozygous parents (Thakre *et al*., 16). Pulp colour correlates with several fruit attributes, Subramanyam and Iyer, 15 demonstrated that red-pulped guavas are typically associated with bold, hard seeds, in contrast to white-pulped guavas, which commonly contain softer seeds. While some researchers have also reported no correlation between seed hardness and pulp colour (Bishnoi *et*

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al., 6). So, the present study was aimed to analyse the segregation for pulp pigments and seed related parameters among the biparental guava progenies derived from the hybridization of Allahabad Safeda and Arka Kiran.

MATERIALS AND METHODS

The present study consists of 4-year-old, 50 hybrids confirmed through SSR markers, along with their parental genotypes 'Allahabad Safeda' (♀) and 'Arka Kiran' (♂) that had been evaluated for pigments and seed related parameters, during the *Mrig Bahar* of 2024. The hybrid progenies and the parental genotypes were planted at a spacing of 4 m × 3 m and maintained under uniform standard horticultural practices at High Density Orchard, ICAR-IARI, New Delhi (28°40′N, 77°12′E; 228.60 m above the mean sea level).

Guava pulp pigments were estimated using fully mature fruits collected in triplicate from each hybrid and parental genotype. The fruit pulp of the hybrids and parents were lyophilised till the pulp was fully air-dried and stored at -80°C deep freezer until analysis. Lycopene and β-carotene contents were estimated through HPLC following modified protocols expressed as mg/100g fresh weight (Liu *et al*., 9; Stoll *et al*., 14) and. For HPLC analysis, 1 g of lyophilised sample was extracted using a 1:1 mixture of acetone and hexane, followed by dilution with methanol, filtration, and analysis using a Shimadzu UFLC system fitted with a C8 column and methanol-acetonitrile (80:20, v/v) as mobile phase. Pigments were identified by comparison with the authentic standards.

Similarly, seed morphological parameters of hybrids and their parents were evaluated using guava descriptors prescribed by PPV&FRA (12) and UPOV (17). Fully mature, healthy fruits were harvested in three technical replications (five fruits per replication) from each plant. Seeds were extracted, washed thoroughly, and air dried at room temperature. Seed length and width were measured among the hybrids and parental guava genotypes using digital vernier calliper (Themisto, Model: TH-M61, India) and expressed in millimetres. Seed weight per fruit was recorded using electronic balance (Citizen Scale Pvt. Ltd, India) and expressed in grams, while the number of seeds per fruit was assessed by manually counting the seeds from the selected fruits. The genotypes were grouped into three categories, *i.e.* few (< 200 seeds), moderate (200-300 seeds) and high (> 300 seeds). Seed hardness was measured performing a single compression test for 3 seeds per fruit using texture analyser (Model: TA + HDi, Stable Micro Systems, UK). The seed hardness values were obtained from the force-time curve and reported as peak force (N) in the compression test.

For statistical analysis, ANOVA was determined using the SAS statistical software (version 9.3; SAS Institute Inc., Cary, NC, USA). Further, the datasets were also analysed for Pearson's correlation coefficient, principal component analysis, cluster analysis using R Studio (Ver. 4.5.0) employing R packages.

RESULTS AND DISCUSSION

Seed parent 'Allahabad Safeda' has white pulp colour, while pollen parent 'Arka Kiran' had red pulp colour. The segregation of pulp colour among progenies (predominantly pink/ red pulped) reflects polygenic inheritance of pulp, consistent with earlier findings (Thakre *et al*., 16). Lycopene content exhibited extremely high variation (0.31–23.21 mg/100 g), with GH20_1D (23.21 mg/100 g) recording the highest value followed by GH20_3B (22.12 mg/100 g) and GH20_9B (20.74 mg/100 g) having statistical difference between them, significantly exceeding both the parents (Fig. 1). Donor parent 'Arka Kiran' (15.51 mg/100 g) had markedly higher lycopene content than 'Allahabad Safeda'. β-carotene content ranged from 0.08 to 1.95 mg/100 g, with GH20_3D (1.95 mg/100 g), GH20_8C (1.63 mg/100 g), and GH20_11D (1.54 mg/100 g) showing superior values. 'Arka Kiran' (0.54 mg/100 g) again recorded higher β-carotene levels than 'Allahabad Safeda'. These finding were in line with the earlier findings of β-carotene and lycopene content in guava genotypes and hybrids (Thakre *et al*., 16; Chandana *et al*., 7). High variability for carotenoid contents suggests independent genetic regulation and strong selection potential for nutraceutical improvement.

Significant variability was observed among the hybrid progenies and parental genotypes for the studied seed related parameters, indicating wide genetic diversity and pronounced segregation (Table 1 & Fig. 2). Seed length ranged from 2.63 to 4.58 mm, with GH20_8D (4.58 mm) and GH20_12E (4.42 mm) recording the highest values, while GH20_2E (2.63 mm) exhibited the lowest. Among parents, 'Arka Kiran' (4.34 mm) had longer seeds than 'Allahabad Safeda'. Seed width varied from 2.23 to 3.59 mm, with 'Arka Kiran' (3.59 mm) showing broader width whereas GH20_8B (2.23 mm) and GH20_6E (2.29 mm) showed the lowest values. Moderate coefficients of variation for seed length (12.53%) and width (12.56%) indicate reliable measurements and moderate genetic variability. Similar patterns of segregation for seed related traits have been reported in guava hybrids and germplasm collections across diverse agro-climatic regions (Azam *et al*., 4; Bishnoi *et al*., 6; Gethe *et al*., 8).

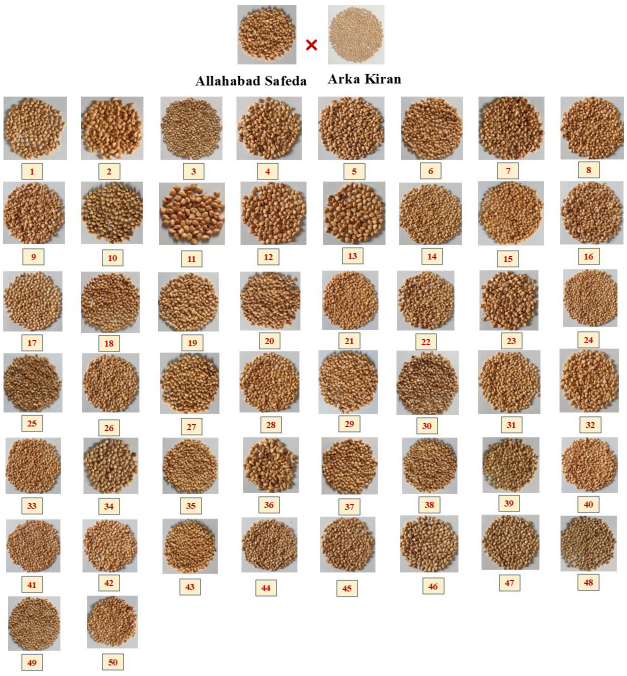

Fig. 1. Variations in the seed parameters among the guava hybrid progenies and parental genotypes evaluated in present study. (1. GH20_1A, 2. GH20_1B, 3. GH20_1C, 4. GH20_1D, 5. GH20_10D, 6. GH20_2B, 7. GH20_2C, 8. GH20_2E, 9. GH20_3A, 10. GH20_3B, 11. GH20_3C, 12. GH20_3D, 13. GH20_4B, 14. GH20_5A, 15. GH20_5C, 16. GH20_5D, 17. GH20_5E, 18. GH20_6A, 19. GH20_6B, 20. GH20_6D, 21. GH20_6E, 22. GH20_7C, 23. GH20_7E, 24. GH20_8A, 25. GH20_8B, 26. GH20_8C, 27. GH20_8D, 28. GH20_8E, 29. GH20_9A, 30. GH20_9B, 31. GH20_10A, 32. GH20_11B, 33. GH20_11C, 34. GH20_11D, 35. GH20_12B, 36. GH20_12E, 37. GH20_13B, 38. GH20_13E, 39. GH20_14D, 40. GH20_15B, 41. GH20_15E , 42. GH20_18B, 43. GH20_16D,44. GH20_17B, 45. GH20_17E, 46. GH20_5B, 47. GH20_11A, 48. GH20_12C, 49. GH20_18A, 50. GH20_20B).

Seed weight per fruit showed wide variation (1.40-4.50 g), with GH20_11A (4.50 g) and GH20_8B (4.36 g) recording significantly higher values than both parents, indicating transgressive segregation. Conversely, the minimum seed weight per fruit was observed in GH20_10D (1.40 g), GH20_12E (1.70 g), GH20_5E (1.74 g) and GH20_7C (1.79 g) representing approximately 69% reduction compared to the highest value. Among the parental genotypes 'Allahabad Safeda' (2.38 g) had higher seed weight per fruit than 'Arka Kiran'. The high CV (27.80%) suggests strong genetic control and suitability of this trait for selection. Similar variability for seed weight per fruit has been documented by Azam *et al*. (4) in 22 guava lines. Genotypes with lower seed weight per fruit (GH20_10D, GH20_12E) are desirable for fresh consumption.

The number of seeds per fruit ranged from 134.67 to 519.67, with parental genotypes having high in 'Allahabad Safeda' (317.67) and moderate seed number per fruit in 'Arka Kiran' (244.33), whereas hybrid progenies grouped into three different categories, *i.e.* low (5 Nos), moderate (19 Nos) and high (26 Nos) for seed number per fruit. GH20_12E (134.67) recorded lowest seed number per fruit followed by GH20_11D (153.33) and GH20_5D (175) statistically significant, whereas GH20_16D (519.67) had the highest, which is in agreement with the previous reports (Bishnoi *et al*., 6; Ahir *et al*., 2). The CV (31.14%) reflects substantial genetic diversity and hybrid guava progenies with fewer seeds are of high commercial relevance due to improved consumer acceptability.

Seed hardness varied markedly among genotypes (60.04-182.33 N) and GH20_11C (60.04 N) exhibited the softest seeds, followed by GH20_1D (71.40 N) and GH20_14D (77.03 N), while GH20_12C (182.33 N) had the highest hardness. 'Allahabad Safeda' (156.85 N) recorded higher seed hardness than 'Arka Kiran' (138.63 N). High CV (26.89%) indicates substantial genetic variability. Softer seeds along with other desirable traits are preferred for

Table 1: Comparison of seed related parameters and pulp pigment content between parental genotypes and hybrid progenies.

Sl. No.	Parameter	Parental genotype		Biparental progeny				CV (%)
		Allahabad Safeda	Arka Kiran	Mean	Maximum	Minimum	Mean	
1	Seed length (mm)	3.92	4.34	4.13	4.58	2.60	3.52	12.53
2	Seed width (mm)	2.33	3.59	2.96	3.50	2.2	2.69	12.56
3	No. of seeds/ fruit	317.67	244.33	280.97	519.71	134.7	318.70	31.14
4	Seed wt.(g)	2.38	1.83	2.10	4.50	1.4	3.03	27.80
5	Seed hardness (N)	156.85	138.63	147.74	60.01	182.0	128.17	26.89
6	Lycopene (mg/100 g)	0.82	15.15	7.98	23.21	0.21	9.84	65.83
7	β-carotene (mg/100 g)	0.11	0.54	0.32	1.95	0.05	0.46	95.04

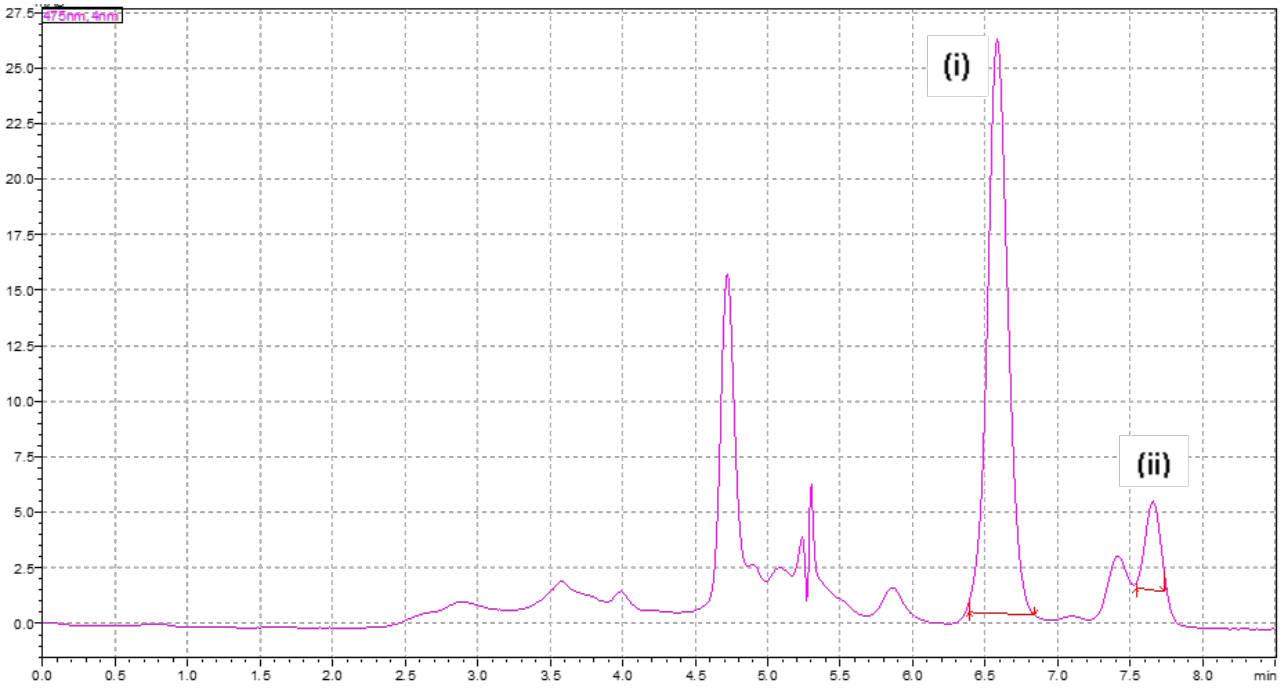

Fig. 2. HPLC chromatogram of (i) lycopene and (ii) β-carotene content in guava hybrid progeny GH20_8C.

fresh consumption due to improved palatability. The outcomes of the study are consistent with the results documented by Bishnoi *et al*. (6), who noted that most F_1 hybrids had soft seeds.

Correlation analysis revealed significant positive association between seed weight per fruit and seed number, while seed number showed significant negative correlations with seed length and width, indicating a trade-off between seed size and number. Seed length and width were positively correlated, and both traits showed positive associations with seed hardness. There were no significant correlations were observed between seed parameters and pigment content, suggesting independent inheritance of nutritional and seed related parameters (Fig. 3), similar finding was also reported by Bishnoi *et al.* (6) showing no correlation between seed hardness and pulp colour in guava.

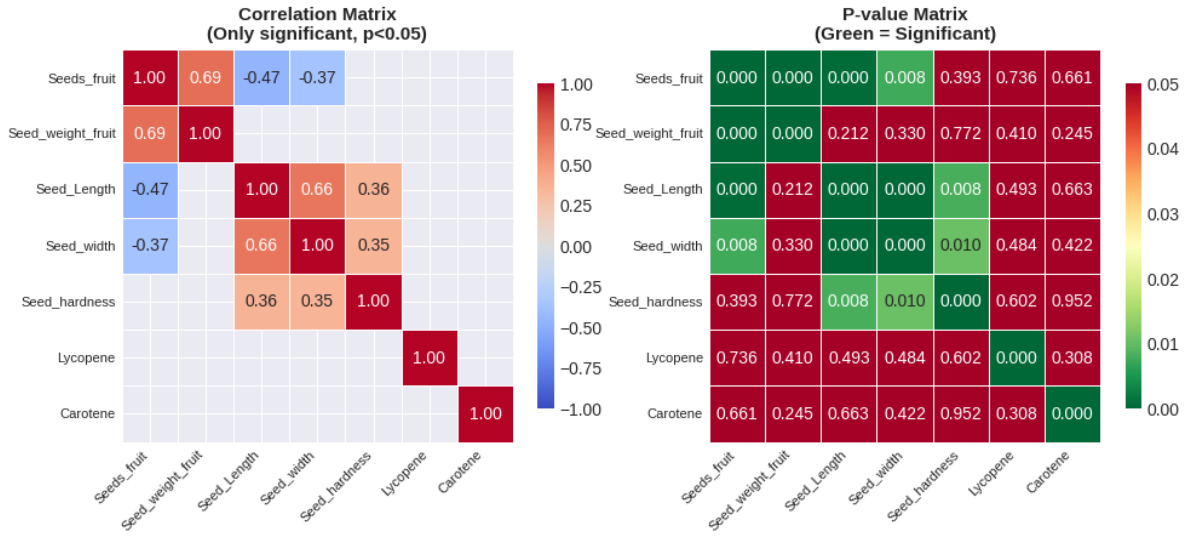

Fig. 3. Correlation matrix (left) displaying significant Pearson's correlation coefficients ($p \leq 0.05$), among seed parameters and pigment content; and corresponding p-value matrix (right), where green indicates statistically significant associations.

Principal component analysis (PCA) was performed to assess the relative contributions of the five seed morphological parameters and pigments towards overall variability among guava full-sib progenies and parental genotypes. PCA revealed that the first four components explained 81.88% of total variation. PC1 represented a contrast between seed size and seed number, PC2 was associated with seed weight and hardness, and PC3 was dominated by lycopene and β-carotene content, confirming the independence of pigment traits from seed morphology (Fig. 4 and Supplementary Table 1).

Hierarchical clustering divided the genotypes into five distinct clusters, placing the parental cultivars in

Fig. 4. Scree plot showing individual and cumulative variance for the studied seed parameters and pigments.

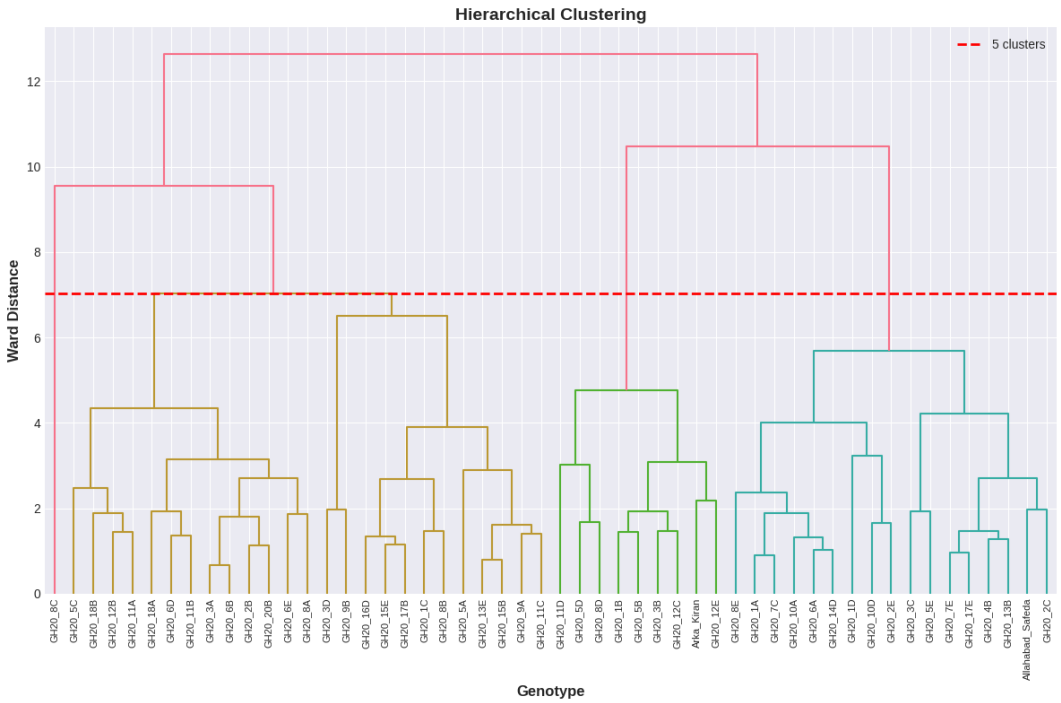

Fig. 5. Hierarchical clustering of hybrid progenies along with parental genotype based on the evaluated seed parameters and pigments.

separate clusters, reflecting their genetic divergence (Fig. 5). Cluster 5 was the largest group consisting of 17 genotypes, containing the parent variety 'Allahabad Safeda' along with 16 full-sib progenies. Clusters 1 (n = 13) and 2 (n = 12) represented medium-sized groups, while Cluster 4 contained nine genotypes including the male parent, 'Arka Kiran' and other 8 hybrid progenies showing similarities for the evaluated seed and pigment parameters with 'Arka Kiran'. Notably, Cluster 3 consisted of a single genotype (GH20_8C), indicating its extreme phenotypic distinctiveness from all other genotypes examined.

The present investigation revealed substantial genetic variability and pronounced segregation for seed parameters and pulp pigments among biparental progenies of 'Allahabad Safeda' and 'Arka Kiran'. The predominance of red pulp colour group and wide variations in lycopene and carotene content confirmed the polygenic control of pulp colour and carotenoid biosynthesis. The absence of significant associations between seed parameters and pigment content was found. Distinct guava hybrid progenies exhibiting extreme or lower values for desirable traits were identified like for small seed size GH20_2E, GH20_6E; for lower seed count GH20_12E, GH20_11D; for soft seed texture GH20_11C, GH20_1D; for enrichment of β-carotene and lycopene GH20_3B, GH20_3D. Multivariate analysis further substantiated the genetic divergence among hybrid progenies and highlighted distinct groups with superior attributes.

AUTHOR'S CONTRIBUTION

Research Conceptualization (CK, AKG, SKJ); Experimental Designs (CK, AKG, JP, SGR); Provision of experimental materials (CK, AKG); Execution of field and laboratory experiments and data collection (AK, PM, PK); Data analysis and interpretation (PC, NS); Manuscript preparation (AK, PM, CK, RMS).

DECLARATION

The authors confirm that there is no conflict of interest.

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