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RESEARCH ARTICLE

Novel and stable QTL regions conferring resistance to MYMV disease and its inheritance in blackgram (*Vigna mungo* (L.) Hepper)

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Abstract. Mungbean yellow mosaic virus (MYMV) disease is a significant constraint for blackgram production. The present study employed a mapping population derived from a cross between susceptible (MDU 1) and resistant (TU 68) genotypes to identify quantitative trait loci (QTL) associated with MYMV disease resistance in addition to bruchine resistance loci identified from the previous study. Phenotyping was carried out in F_2 generation under the disease spreader row method at field condition. Disease score observations were carried out 60 days after sowing (DAS). The chi-square goodness of fit test revealed inhibitory gene action with two genes controlling the expression of resistance to MYMV disease. However, QTL analysis revealed one major QTL region, i.e. *qMYMVD_60* at LG 10 responsible for MYMV disease score at 60 DAS, accounted for 21 per cent of variation. The identified QTL has the flanking markers as CEDG180 and CEDG116. Hence, the QTL, *qMYMVD_60* may be utilized in the breeding of MYMV disease resistance. Further, the marker-assisted introgression of both the MYMV and bruchine resistance QTLs can be performed in the near future.

Keywords. blackgram; inheritance; mungbean yellow mosaic virus; quantitative trait loci; simple-sequence repeats.

Introduction

Blackgram [*Vigna mungo* (L.) Hepper] is an important food legume crop of Asian countries. It is referred to as the 'king of the pulses' due to its delicious taste and numerous other nutritional qualities. In India, it occupies 4.50 million hectares with a production of 2.83 million tonnes (MULLaRP 2019). Both biotic and abiotic constraints limit grain production across southern Asia and most parts of the world. Viral diseases are the major biotic causes responsible for production losses (Ilyas *et al*. 2009).

This disease has seriously increasing because of lack of genetic resistance in most cultivated high-yielding varieties. The transmission of the virus has been carried by whitefly (*Bemisia tabaci*). The symptoms appear in tiny irregular yellow specs and spots along the veins, which enlarge later until leaves completely yellowed under severe infestation. Diseased plants also have very few flowers and pods; pods were curled and reduced in size, and the percentage of shrivelled seeds increased (Shukla *et al*. 1978). MYMV disease infection may cause yield losses up to 85–100% in blackgram. Since vector whitefly attributes the virus transmission, controlling MYMV disease limits the vector population by using insecticides. However, it is ineffective under severe whitefly infestations and also not an eco-friendly approach. The most effective way to prevent this disease is to develop genetically superior resistant cultivars for blackgram.

Improved resistance to MYMV disease is now the primary goal of blackgram breeding programmes. However, breeding for MYMV disease resistance in blackgram is difficult with field screening. It is hampered by nonuniform development of the disease due to the fluctuation of the whitefly population in different locations and at different seasons. Hence, understanding the genetic resistance mechanisms and discovering genes or QTLs are essential for

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developing MYMV disease resistance in blackgram. SSRs as DNA markers have many advantages over other markers and have been used to assess genetic variation (Morgante and Olivieri 1993). Much work has not been done towards the development of genomic resources in this crop, but by using genomic resources from adzuki bean, common bean and mungbean, genetic maps were constructed (Chaitieng *et al*. 2006; Gupta *et al*. 2008; Isemura *et al*. 2012). By using SSR primers, a low level of polymorphism has been observed in cultivated blackgram (Chaitieng *et al*. 2006).

Previously, an attempt was made in the same mapping population to identify the QTL responsible for bruchine resistance and got two major QTL regions with a high PVE % of 17.01 on LG 5 and LG 8, respectively (Subramaniyan *et al*. 2021). In the present study, an attempt was made to impart multiple resistances in the MDU1 × TU 68 mapping population in addition to pest resistance by identifying the genomic regions responsible for MYMV resistance. Hence, the objectives of the present study were (i) to assess the genetic variation for MYMV disease resistance among the mapping population. (ii) To understand the inheritance pattern of the MYMV disease resistance among the mapping population. (iii) To identify the QTL region responsible for the MYMV disease resistance.

Materials and methods

The present field experiment was carried out at National Pulses Research Centre (NPRC), Tamil Nadu Agricultural University, Vamban, India, located at the latitude of 10.363505°N longitude of 78.902283°W. The NPRC, Vamban, is the best hotspot to assess MYMV disease incidence during June–July. Hence, the MYMV disease screening was performed following the infector row method. The molecular analysis was performed at the Pulses Marker Assisted Selection Laboratory, NPRC, Vamban.

Plant material and development of mapping population

MDU 1 (female parent) and TU 68 (male parent) were used to develop an F_2 mapping population. MDU 1 is a high yielding popular blackgram variety of Tamil Nadu which was developed at Agriculture College and Research Institute, Tamil Nadu Agricultural University, Madurai and released in 2014. It is recommended for commercial cultivation in MYMV disease free period, namely October to November season of Tamil Nadu. This variety (MDU 1) is highly susceptible to MYMV disease. TU 68 is a genotype developed from Bhabha Atomic Research Centre, Trombay. It is a wild cross derivative obtained from the cross TU 94-2 × *Vigna mungo* var. *silvestris* and resistant to MYMV disease. TU 68 was selected as a male parent and crossed with female parent MDU 1 during January–March 2019 to produce F_1 seeds. Polymorphic SSR markers between the parents were employed to find true F_1s. Further, selected true F_1s were selfed during April–June 2019. The F_2 generation was evaluated during July–September, 2019 season.

Phenotyping of MYMV disease resistance

All the 108 single plants of the mapping population and the parents were screened for MYMV disease resistance under the infector row method at NPRC, Vamban, during July–September 2019. Susceptible genotypes CO 5 and MDU 1 were sown as disease spreader rows after every eight rows and around the plots. No insecticide was sprayed to maintain the natural whitefly populations. The MYMV disease incidence was recorded on all plants of F_2 population and parents based on the visual scores on the 60th day. The MYMV disease score was recorded using the phenotype rating scale as suggested by Singh *et al*. (1995) (table 1). Based on the disease grade, two phenotypic classes were formed among F_2 plants with the scales of 1 to 3 as resistant and 4 to 9 as susceptible phenotype. The goodness of fit for MYMV

Table 1. Phenotypic rating of MYMV disease score.

Grade	Description	Reaction
1	No visible symptoms on leaves	Free
2	Small yellow specks with restricted spread covering 0.1–5% leaf area	Highly Resistant (HR)
3	Mottling of leaves covering 6–10% leaf area	Resistant (R)
4	Yellow mottling covering 11–15% leaf area	Moderately resistant (MR)
5	Yellow mottling and discolouration of 15–20% leaf area	Moderately susceptible (MS)
6	Yellow colouration of 21–30% leaves and yellow pods	Susceptible (S)
7	Pronounced yellow mottling and discolouration of leaves and pods, reduction in leaf size and stunting of plants covering 30–50% of foliage	
8	Severe yellow discolouration of leaves covering 50–75% of foliage, stunting of plants and reduction in pod size	Highly susceptible (HS)
9	Severe yellowing of leaves covering above of foliage, stunting of plants and no pod formation	Highly susceptible (HS)

Table 2. Phenotypic distribution of the MYMV disease resistance trait at 60 DAS in MDU 1 × TU 68 mapping population.

	Parents		Mapping population					
Trait name	MDU 1	TU 68	Minimum	Maximum	Mean	Variance	Skewness	Kurtosis
MYMV disease score @ 60 DAS	8	1	1	7	2.0	2.5	1.68	1.81

SE, standard error; DAS, days after sowing.

disease resistance in the segregating population was tested by the chi-square test (Stansfield 1991).

Genotyping

The genetic linkage map of MDU 1 × TU 68 mapping population was constructed using polymorphic SSR markers between the parents. The linkage map construction, QTL analysis and other standard methods involved are described below.

DNA isolation and SSR-based genotyping

Total genomic DNA of each parental and F_2 plants were extracted from fresh young leaf tissue using the CTAB method (Lodhi *et al*.1994). The polymerase chain reaction (PCR) mixtures contain 2 μL of 10 ng template DNA, 1.0 μL of 10 × Taq buffer + $MgCl_2$ (15 mM), 1.0 μL of dNTPs (2 mM), 1.0 μL of forward and reverse SSR primers (0.5 μM), 0.3 μL of Taq polymerase (3 IU) and 4.7 μL of sterile double distilled water. The DNA was amplified in a thermo cycler (M/s Eppendorf, Germany, model AG 6325) under the following conditions: 94°C for 4 min followed by 30 cycles of 94°C for 30 s, 55°C for 45 s, 72°C for 1 min, with a final extension step of 72°C for 20 min. The PCR products were separated on 3% agarose gel and photographed using GELSTAIN 4× advanced gel documentation unit (M/s Medicare, India).

Linkage map and QTL analysis

Linkage analysis was carried out using QTL IciMapping (v. 4.1.0.0) software (Wang *et al*. 2016). Linkage groups were constructed using a minimum LOD threshold of 3.0 and a maximum distance of 50 cM. Map distance in centimorgan (cM) values were calculated using the Kosambi mapping function (Kosambi 1944). Further, QTL analysis was employed using QGene4.4.0 software (Joehanes and Nelson 2008). Composite interval mapping (CIM) was carried out with automatic cofactor selection. LOD threshold significance for each QTL was calculated with 1000 runs with a permutation test.The study performed is true with novelty in the interpretations.

Results and discussion

Parents and mapping population were evaluated for MYMV disease resistance. Resistant (TU 68) and susceptible (MDU 1) parents showed resistant (score 1) and highly susceptible (score 8) reactions for the MYMV disease at 60 DAS, respectively (table 2). The parents were more variable for MYMV disease resistance. In the present study, mean and variability parameters were estimated for MYMV disease scores and are presented in table 2. The mean and variability calculated for MYMV disease reaction show much variation among the population. The MYMV disease score at 60 DAS had positive skewness and leptokurtic nature (figure 1). Kang *et al*. (2005) reported that monogenic host resistance factors control more than 80% of viral resistance in plants. The digenic recessive nature of resistance was also stated by some authors (Singh 1980; Verma and Singh 1980; Vadivel *et al*. 2021). The inheritance pattern of MYMV disease resistance in F_2 generation was studied, and the results are presented in table 3. The nonsignificant chi-square value (0.034) indicates digenic inhibitory gene action with an 80–90% probability at 5% for MYMV disease.

Further, this study involved 625 SSR primers in identifying the polymorphism between parents, i.e. MDU 1 and TU 68. The SSR primers and its sequence information were

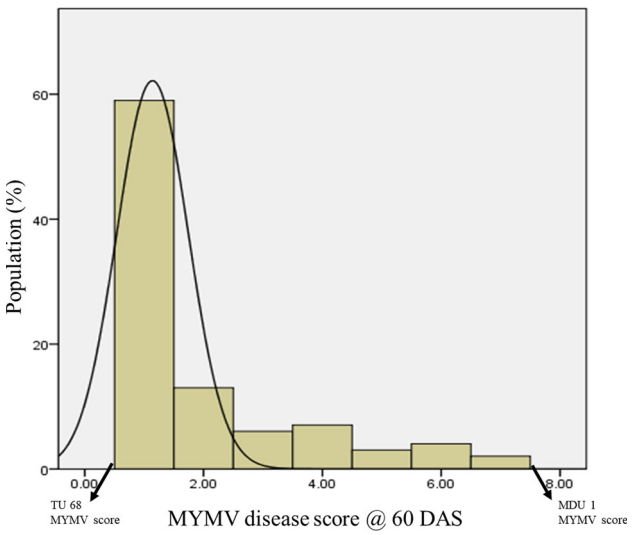

Figure 1. Frequency distribution for MYMV disease resistance trait at 60 DAS.

Table 3. Chi-square test for the inheritance of MYMV disease resistance trait at 60 DAS in MDU 1 × TU 68 mapping population.

		F_2 phenotype					
		Observed values					
Generation	F_1 phenotype	Resistant	Susceptible	Expected ratio	χ^2 values	Probability %	Gene action
MDU 1 × TU 68	Resistant	87	21	13:3	0.034 ns	80–90	Inhibitory gene action

Ns, not significant at 5% probability.

utilized from the maps of related *Vigna* species, namely mungbean (Gwag *et al*. 2006; Somta *et al*. 2008; Seehalak *et al*. 2009; Tangphatsornruang *et al*. 2009; Isemura *et al*. 2012; Chotechung *et al*. 2016) and adzuki bean (Wang *et al*. 2004; Chankaew *et al*. 2014). These SSR markers were cross-species amplification in nature. Among the 625 SSR markers, 49 showed polymorphism between parents. Genotyping was carried out with 49 polymorphic SSR markers for the F_2 mapping population of the cross between MDU 1 × TU 68. A linkage map was constructed using QTL IciMapping (v. 4.1.0.0) (Wang *et al*. 2016). Among the polymorphic markers obtained, 46 SSR markers were found to be linked with 10 linkage groups, and three primers were left unlinked. These markers covered a total map length of 454.7 cM.

QTL analysis was employed to locate QTL for MYMV disease resistance on the linkage map. The LOD threshold for MYMV disease resistance was determined by a permutation test with 1000 runs (figure 2). In the present study, a QTL was found for MYMV disease score at 60 DAS on LG 10 and designated as *qMYMVD_60* (figure 3; table 4), respectively. Markers CEDG180 and CEDG116 flanked the *qMYMVD_60* with a LOD score of 4.56 and significant at $P = 0.01$. This QTL explained 21% of variation for the MYMV disease score at 60 DAS. Alleles from the resistant parent of TU 68 contributed towards an increase in the resistance against MYMV disease. It was supported by the previous reports of Vadivel *et al*. (2021).

However, the presence of another gene that alters the gene expression is still unknown. Hence, the obtained information can be improved further through fine mapping in the near future. The large PVE explains the major gene linked with the MYMV disease resistance on the LG 10. Further, SSR marker CEDG180 was frequently reported as associated with a major gene controlling MYMV disease resistance in blackgram (Gupta *et al*. 2013; Vadivel *et al*. 2021). Markers flanking the QTL can be used in marker-assisted selection for MYMV disease resistance in blackgram. Based on the previous research, the mapping population possessed bruchine resistance QTL regions on the LG 5 and LG 8. In addition, the present study identified the major QTL region linked with MYMV disease resistance on LG 10. Chen *et al*. (2013) reported QTL regions for bruchine pest (LG 7 and LG 9) and mungbean yellow mosaic India virus (MYMIV)

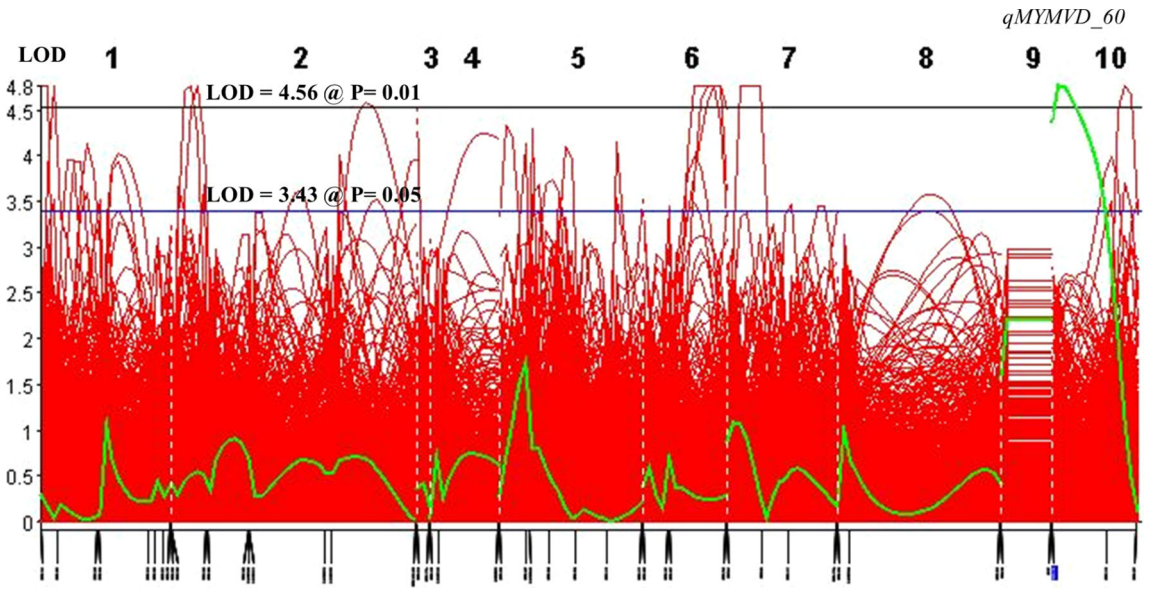

Figure 2. Permutation analysis on the MYMV disease resistance trait to identify QTL regions based on LOD threshold in MDU 1 × TU 68 mapping population.

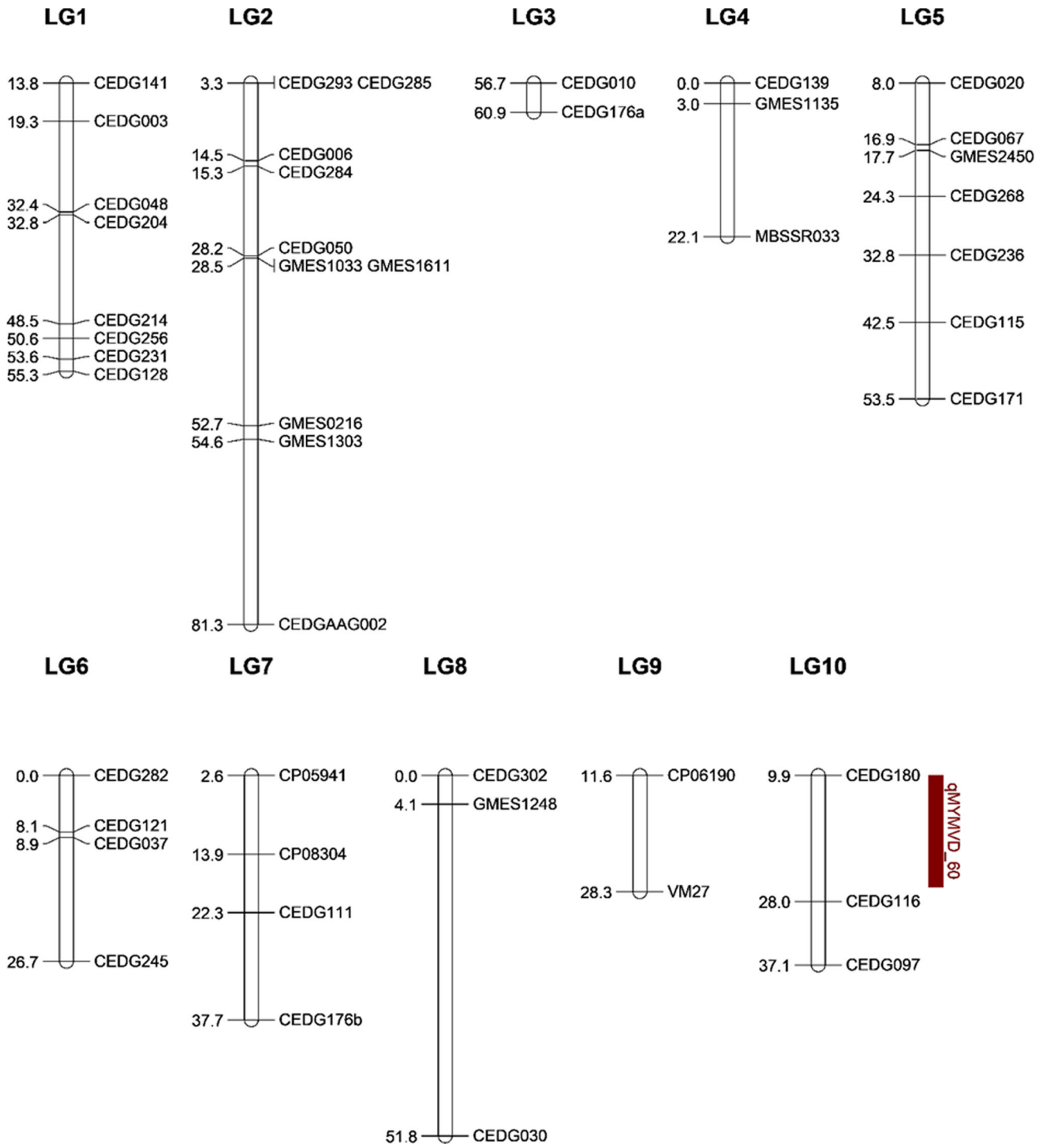

Figure 3. QTL regions linked with the MYMV disease resistance trait on the linkage group 10 in MDU 1 × TU 68 mapping population.

disease resistance (LG 7, LG 8 and LG 9) in blackgram. Hence, the resistance QTLs can be involved in the marker-assisted selection procedure to impart multiple resistances into the desired cultivar. Further, it is planned to perform additional research to reduce mapping distance and to be more precise in locating the genes through fine mapping. The cloning of the QTL region is also intended in the near future to identify the gene involved in the MYMV disease resistance.

In conclusion, based on the preceding discussion, a robust QTL, i.e. qMYMVD_60 was identified on LG 10, which accounted for a PVE of 21%. The goodness of fit ratio indicates inhibitory gene action in the expression of MYMV disease resistance. The flanking markers of the

Table 4. QTL regions associated with MYMV disease resistance trait at 60 DAS in MDU 1 × TU 68 mapping population.

Trait	QTLs	LOD (P=0.01) (1000 permutation)	LG	Left marker	Left marker with position (cM)	Right marker	Right marker with position (cM)	QTL region (cM)	PVE (%)	ADD
MYMV	*qMYMVD_60*	4.56	10	CEDG180	9.9	CEDG116	28.0	9.9–25.9	21.00	12.63 (TU 68)

LG, linkage groups; PVE, percent variation explained; LOD, logarithm of odds; *P*, probability; cM, centimorgan; *qMYMVD*, QTL for mungbean yellow mosaic virus disease; ADD, additive effect.

qMYMVD_60, namely CEDG180 and CEDG116 could be used in the MYMV disease resistance breeding programme. Further, the information on both the QTL regions responsible for storage pest bruchine and MYMV disease resistance can be utilized to impart multiple resistances into the cultivar through marker-assisted breeding and marker-assisted backcross breeding procedures.

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Authors' contribution

SR performed the field experiments, measurements, data analysis and drafted the manuscript. NM supervised the work, worked on the manuscript and aided in interpreting the results. NM, KI, NG and GK were involved in planning. All authors provided critical feedback on research, analysis and manuscript.

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