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Mapping of genes for leaf and stem rust resistance in bread wheat genotype Selection 212

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Abstract

Resistance genes for leaf and stem rusts in bread wheat line Selection212 are recessive in nature. Both leaf and stem rust resistance genes, named tentatively as *LrSel212* and *SrSel212*, have been mapped to the short arm of chromosome 2B separated by genetic distance of 16.4 cM. *Xwmc474* was the closest marker located between two genes, 5.6 cM proximal to *LrSel212* and 10.8 cM distal to *SrSel212*. Leaf rust pathotype 77-5 is virulent to leaf rust resistance genes located on chromosome 2B *viz*., *Lr13, Lr16, Lr23, Lr35* and *Lr73*, but avirulent to Selection212, suggesting that *LrSel212* is distinct from these genes. Six stem rust resistance genes have been assigned to chromosome 2B *viz*., *Sr19, Sr20, Sr23, Sr36, Sr39* and *Sr40*. Stem rust pathotype 40A used in genetic analysis was virulent to *Sr19* and *Sr20,* but avirulent to Selection212; and the latter showed a significantly lower infection type in comparison to *Sr39*. *Sr23* and *Sr36* showed susceptibility to few other stem rust pathotypes to which Selection212 was resistant. While the response of *Sr40* to Indian pathotypes of *Pgt* is not known, differences in the genetic distance and nature of inheritance between Selection212 and *Sr40* indicate their distinct identity. However, test of allelism with *Sr40* is required to confirm whether *SrSel212* represents a different locus. Selection212 may be useful in broadening the genetic base of rust resistance in wheat.

Keywords: Wheat (*Triticum aestivum*), leaf rust, stem rust, double inoculation, recessive class analysis, molecular mapping

Introduction

Wheat rusts are the most important fungal diseases worldwide with potential to cause severe damage to crop (Figueroa et al. 2018; Sharma et al. 2013). There are three rust diseases in wheat, namely, leaf rust, stem rust and stripe rust caused by *Puccinia triticina* Eriks., *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn. and *Puccinia striiformis* Westend, respectively. Development of resistant varieties is the most effective and environmentally safe method of managing rust diseases. So far, 79 leaf rust resistance genes and 59 stem rust resistance genes have been designated (McIntosh et al. 2017; Qureshi et al. 2018). Being obligate parasites, wheat rusts have intrinsic capacity to evolve quickly into new pathotypes rendering many resistance genes ineffective. In India, from 1931 to 2015, nearly 120 different pathotypes have been reported for the three wheat rusts. Leaf rust resistance genes commonly postulated in Indian cultivars are *Lr1, Lr3, Lr9, Lr10, Lr13, Lr14a, Lr17, Lr19, Lr23, Lr24, Lr26, Lr28* , *Lr34* and *Lr46* (Bhardwaj et al. 2010a; Tomar et al. 2014). Similarly, stem rust resistance genes *Sr2, Sr7a, Sr8a, Sr9b, Sr11, Sr13, Sr14, Sr21, Sr23, Sr24, Sr25, Sr28, Sr30, Sr31* and *Sr36* have been identified in Indian bread wheat cultivars. Pathotype 77-5 is most predominant in India and is virulent to most of the *T. aestivum* derived seedling leaf rust resistance genes (Tomar et al. 2014; Prasad et al. 2017). Virulent pathotypes have also evolved against several alien genes such as *Lr9* (Nayar et al. 2003), *Lr19* (Bhardwaj et al. 2005), *Lr2*6 (Nayar et al. 1991; 1993) and *Lr28* (Bhardwaj et al. 2010b). *Lr24* is the still effective in India, however, virulence for *Lr24* has already been reported in several parts of the world (Singh, 1991). Recently leaf rust resistance genes in synthetic hexaploid wheat 45 was also found to be effective against most of the prevalant races of leaf rust pathotypes (Gyani et al. 2017). Of the stem rust resistance genes, *Sr31* is effective against all the pathotypes in India, though emergence of Ug99 and its variants in several countries has made this gene

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vulnerable (Pretorius et al. 2000). *Sr2* confers wide spectrum but moderate level of resistance at adult plant stage (Mishra et al. 2011; Tomar et al. 2014).

Frequent evolution of virulent pathotypes necessitates the search for new sources of resistance. To enhance the diversity of resistance in cultivated varieties, secondary and tertiary gene pools of wheat are being exploited. Rye (*Secale cereale*), a member from tertiary gene pool is donor of several resistance/ tolerance genes against biotic and abiotic stresses. The leaf rust resistance genes like *Lr25* (Driscoll & Anderson 1967), *Lr26* (McIntosh 1988a) and *Lr45* (McIntosh 1995), and stem rust resistance genes *Sr27* (Acosta 1963), *Sr31* (McIntosh 1988a) and *Sr50* (Mago 2002) have been introgressed from rye. The use of diverse rye accessions can serve as means of broadening genetic diversity in wheat. Rye derived leaf rust resistance genes like *Lr25* (Procunier et al. 1995), *Lr26* (Mago et al. 2005), *Lr45* (Naik et al. 2015) and stem rust resistance genes namely *Sr31* (Das et al. 2006) and *Sr50* (Anugrahwati et al. 2008) have been mapped using molecular markers.

A wheat-rye recombinant named Selection212 developed at Division of Genetics, Indian Agricultural Research Institute, New Delhi (Singh 1991) was found to be resistant to leaf and stem rusts. Sharma and Singh (2000a) assessed and identified a leaf rust resistance gene (*LrSel.212*) and a stem rust resistance gene (S*rSel.212*) in Selection212 (2000b & 2000c). Both leaf and stem rust resistance genes were located on chromosome 2B by monosomic analysis (Sharma and Singh 1999; Sharma and Singh 2001). The genes showed broad spectrum resistance against leaf and stem rust diseases. The present paper describes the molecular mapping of linked leaf and stem rust resistance genes in Selection212.

Materials and methods

Plant materials and pathotypes

Leaf and stem rust resistant line 'Selection212' and susceptible cultivar Agra Local were used for genetic analysis and mapping of leaf and stem rust resistance genes. Selection212 was used as female parent and crossed with Agra Local to produce F_1 generation. F_1 plants were selfed to produce F_2. *Puccinia triticina* (*Pt*) pathotype 77-5 (121R63-1) and *Puccinia graminis* f.sp. *tritici* (*Pgt*) pathotype 40A (62G29) were used for genetic analysis and mapping of resistance genes. In addition, *Pt* pathotypes 77-2, 77-4, 162, 162A and 104B were used to discriminate the leaf rust resistance gene in Selection212 from other leaf rust resistance genes on chromosome 2B. Initial pure inoculum of rust pathotypes used in the study were procured from ICAR-Indian Institute of Wheat and Barley Research, Regional Station, Flowerdale, Shimla (H.P.) and multiplied on susceptible cultivar Agra Local under glasshouse conditions at Division of Genetics, Indian Agricultural Research Institute, New Delhi.

Rust phenotyping by double inoculation method

Selection212, Agra local, F_1 and F_2 seeds were sown in small paper cups and each cup carried a single seedling. After 10 days of sowing, the seedlings were inoculated in glass house with *Pt* pathotype 77-5 and *Pgt* pathotype 40A. The lower half of primary leaf of each seedling was inoculated with leaf rust and upper half with stem rust pathogen. The fresh inoculum of each pathotype was mixed with non-toxic talcum powder and applied on 10 days old seedlings by hand. Care was taken to leave some uninoculated region in the middle of leaf. The double inoculated seedlings were kept in humidity chambers for 48 hours after which they were transferred to benches in glass house under ambient conditions of light and humidity. Temperature in the glasshouse was recorded regularly which varied from minimum of 10^0C to maximum of 28^0C during the duration of experiment. Infection types (IT) were recorded after 10-12 days of inoculation using 0-4 scale described by Stakman et al. 1962. The infection type '0', ';' , '1' and '2' were considered as resistant reaction while infection types 3 and 4 were classified as susceptible. Individual plants of F_2 population were scored for the leaf and stem rust reactions.

Genetic mapping

Fresh and young leaves were collected for DNA isolation using CTAB protocol (Murray and Thompson 1980). DNA was quantified using λ uncut DNA (100ng, 200ng) on 0.8% agarose gel and diluted to 25 ng/μl concentration for working solution and stored at –20^{o}C. SSR primers were diluted to an initial concentration of 15 μM and further diluted to a working concentration of 5 ppm. The PCR reactions were performed in a reaction volume of 10 μl comprising 4 mM Tris-HCl (pH 8.0), 0.8 mM $MgCl_2$, 20 mM KCl, 40 mM of each dNTP (MBI Fermentas, Germany), Taq DNA polymerase of 1.0 unit (Bangalore Genei Pvt. Ltd., India), 1 μl of each primer (5pmol/μl), 1 μl of genomic DNA (25 ng) and 3 μl of water. The reaction was carried out in the 96-well PCR plates with thermal seal in

Eppendorf thermal cycler with the following thermal profile: initial denaturation step of 94°C for 4 min, followed by 45 cycles of 94°C for 1 min (denaturation), annealing at primer specific temperature for 1 min (annealing temperature depends on primers) followed by 72°C for 1 min (primer extension), and a final extension step of 72°C for 10 min followed by cooling at 4°C. Parental polymorphism between Selection212 and Agra Local was carried out with 128 SSR markers distributed across 2B chromosome. The polymorphic markers between two parents were subjected to Bulked Segregant Analysis (BSA) as described by Michelmore et al. 1991. Two bulks *viz*., resistant bulk and susceptible bulk were constituted separately by mixing equal amount of DNA from 10 resistant and 10 susceptible plants, respectively. For mapping of rust resistance genes, only resistant plants (recessive class and therefore homozygous for the gene) from F_2 population were genotyped rather than entire F_2 population as described by Zhang et al. (1994). Linkage map was constructed using software MAPMAKER version 3.0 (Lander et al. 1987) with a minimal LOD score of 3.0 and a maximal genetic distance of 30.0 centiMorgan (cM). The recombination frequencies were converted to map distances in cM using Kosambi's function (Kosambi 1943).

Results

Genetic analysis showed that leaf and stem rust resistance in Selection212 is governed by a single recessive gene. Two hundred and ninety four F_2 plants segregated for leaf rust into 75 resistant and 219 susceptible with $\chi^2_{(1R:3S)}$ value of 0.0408 (p-value 0.84). Similarly, F_2 population segregated for stem rust into 73 resistant and 221 susceptible plants with $\chi^2_{(1R:3S)}$ = 0.0045 and p-value 0.946 (Table 1). Selection212 and near-isogenic lines of Thatcher carrying *Lr13*, *Lr16* and *Lr23* were screened with discriminating races such as 77-2, 77-4, 77-5, 104B, 162 and 162A. Selection212 showed resistance against all these races while NILs Thatcher+*Lr13*, Thatcher+*Lr16* and Thatcher+*Lr23* were susceptible (Table 2). Additionally, some other genotypes carrying specific leaf rust (*Lr*) and stem rust (*Sr*) genes on chromosome 2B were screened with *Pt* pathotype 77-5 and *Pgt* pathotype 40A, respectively. Genotypes Manitou (*Lr13*), Exchange (*Lr16/Sr23*), IWP 94 (*Lr23*), Thatcher+*Lr35/Sr39*, Morocco (*Lr73*) and susceptible check Agra Local showed susceptible reaction with IT '3' or '3^+' against *Pt* pathotype 77-5. Similarly, genotypes Marquis (*Sr19+Sr20*), Exchange (*Lr16/Sr23*), CI12632 (*Sr36*), Thatcher+*Lr35/Sr39*, and susceptible check Agra Local were screened with *Pgt* pathotype 40A. Marquis (*Sr19+Sr20*) showed susceptible reaction along with check Agra Local while Exchange (*Lr16/Sr23*) and CI12632 (*Sr36*) showed resistant reaction. Thatcher+*Lr35/Sr39* showed a significantly higher IT ($3^=$), compared to Sel212 (;1) (Table 3).

Joint segregation analysis for leaf and stem rust resistance

F_2 population of 294 plants derived from single F_1 plant of cross Selection212/Agra Local was tested against *Pt* pathotype 77-5 (leaf rust) and *Pgt* pathotype 40A (stem rust) at seedling stage using double inoculation method (Fig. 1). The joint segregation analysis of the two resistance genes showed significant deviation from the expected Mendelian segregation ratio for two independent recessive genes 1(RR): 3(RS): 3(SR): 9(SS) and exhibited segregation in the ratio of 65(RR): 10(RS): 8(SR): 211(SS) with compounded Chi square

Table 1. Joint segregation of leaf and stem rust resistance genes in F_2 population of cross 'Selection 212 x Agra Local' against pathotype 77-5 of leaf rust (*P. triticina*) and pathotype 40A of stem rust (*P. graminis* f.sp*. tritici*) at seedling stage using double inoculation at mean temperature range 20-28°C; (RR: Resistant to both leaf and stem rust; RS: Resistant to leaf and susceptible to stem rust; SR: Susceptible to leaf and to resistant stem rust; SS: Susceptible to both leaf and stem rust)

F_2 Population		Leaf rust		Total	$\chi^2_{(1R:3S)}$	P-Value
		Resistant (IT ;$1^=$ to 1^+)	Susceptible (IT: 3 to 4)			
Stem rust	Resistant(IT ;$1^=$ to $1^{+)}$	65	8	73	0.0045	1df, 0.946
	Susceptible(IT: 3 to 4)	10	211	221		
	Total	75	219	294		

χ^2 (1R:3S) = 0.0408, 1df, P= 0.840
χ^2 (Compounded) 1(RR):3(RS):3(SR):9(SS)= 208.11, 3df, P= < 0.00001
χ^2 difference (Linkage) = 208.074, 1df, P= < 0.00001

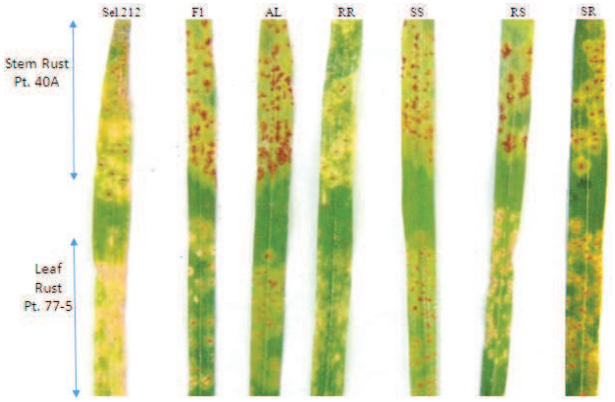

Fig. 1. Infection types on Sel.212, Agra Local and F2 population at seedling stage with double inoculation with pathotype 77-5 (lower half) and 40A (upper half)

value of 208.11 at 3df, P= < 0.00001. The linkage between leaf and stem rust resistance genes was suggested by highly significant calculated χ^2 (Linkage) value of 208.074, 1df, P= < 0.00001 over table value (Table 1).

Genetic mapping of linked leaf and stem rust resistance genes

Out of 128 SSR markers from 2B chromosome, 47 were polymorphic between Selection212 and Agra Local. The BSA suggested that four SSR markers *Xwmc474, Xgpw1148, Xgwm148* and *Xwmc770* located on chromosome 2BS were putatively linked to leaf and stem rust resistance genes *LrSel212* and *SrSel212* (Fig. 2).

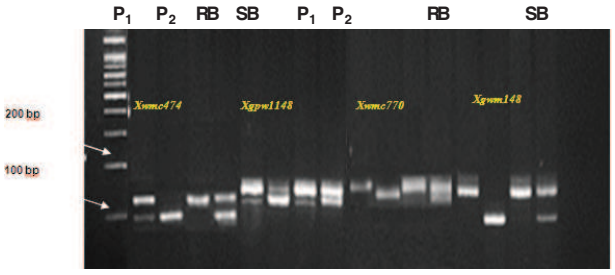

Fig. 2. Bulked Segregant Analysis (BSA) showing putatively linked marker

The genotyping of F_2 population was carried on leaf and/or stem rust resistant plants only. Total 83 F_2 resistant plants (65+10+8, Table 1) were genotyped with four putatively linked SSR markers *Xwmc474, Xgpw1148, Xgwm148* and *Xwmc770* on 2BS. Leaf and stem rust resistance genes *LrSel212* and *SrSel212* were 16.4 cM apart with *SrSel212* located proximally. Marker *Xwmc474* was located between *LrSel212* and *SrSel212* being 5.6 cM proximal to *LrSel212* and 10.8 cM distal to *SrSel212.* Marker *Xgpw1148* was placed 18.1 cM distal to *LrSel212.* The linkage map indicated the order of rust resistance genes and the markers as *#SrSel212-Xwmc474-LrSel212-Xgpw1148-Xgwm148-Xwmc770*. The genetic distances are shown in Fig. 3.

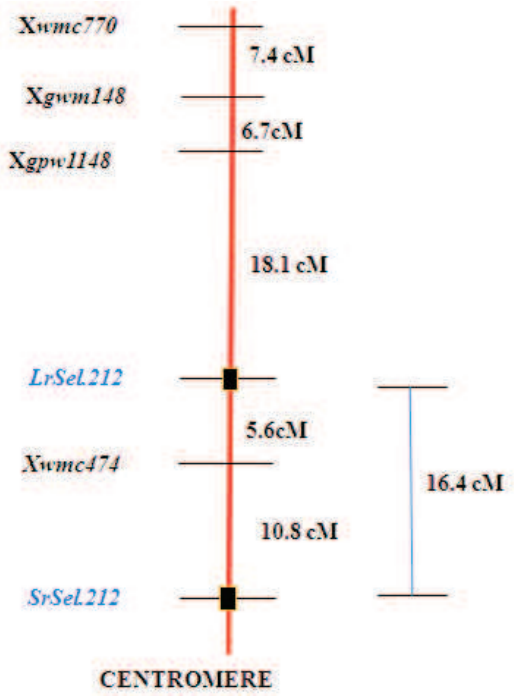

Fig. 3. Molecular map of *LrSel.212* and *SrSel.212*

Discussion

Genetic analysis of leaf and stem rusts resistance showed that resistance is governed by a single recessive gene for leaf rust as well as stem rust. The two resistance genes *LrSel212* and *SrSel212* were located on short arm of chromosome 2B at a distance of 16.4 cM. *Xwmc474* was the closest marker at a distance of 5.6 cM and 10.8 cM from *LrSel212* and *SrSel212*, respectively, and located between two rust resistance genes. Genetic analysis and molecular mapping validated the earlier report of recessive nature of these two linked rust resistance genes and their chromosomal location on chromosome 2B using monosomic analysis (Sharma and Singh 2001).

So far, five leaf rust resistance genes have been located on chromosome 2B *viz.*, *Lr13, Lr16, Lr23, Lr35* and *Lr73* (Dyck et al. 1966; Dyck and Samborski 1968; McIntosh 1975; Kerber and Dyck 1990; Park et al.

2014). The genotypes carrying these leaf rust resistance genes showed susceptible reaction when tested against *Pt* pathotype 77-5. On the other hand, Selection212 showed high degree of resistance against 77-5 (Tables 2 and 3). Selection212 also displayed resistance against other *Pt* pathotypes 77-2, 77-4, 162, 162A and 104B (Table 2). Leaf rust resistance genes *Lr13, Lr16, Lr23* are not effective against most *Pt* pathotypes in India (Tomar and Menon 2001; Tomar et al. 2014).

Table 2. Reaction of Selection 212, Thatcher NILs and AL against 3 differentiating races of leaf rust pathogen in 77 group (R: ;1 to 1^+, S: 3 to 3^+), and pathotypes 104B, 162 and 162A

	77-2	77-4	77-5	104B	162	162A
LrSel212	;	;1	$;1^+$	X^+	1^-	$;1^=$
Tc+*L13*	33^+	3^+	3^+	33^+	3^+	3^+
Tc+*L16*	33^+	3^+	3^+	33^+	3^+	3^+
Tc+*L23*	3^+	3^+	3^+	3^+	33^+	3^+
AL	3^+	3^+	3^+	3^+	3^+	3^+

AL = Agra Local

cultivar Marquis, showed susceptible reaction to *Pgt* pathotype 40A to which Selection212 is resistant (Table 3). Though, in the present study only one *Pgt* pathotype 40A was used, the response of Selection212 to different *Pgt* pathotypes is well characterized (Sharma and Singh 2000a). Stem rust resistance genes *Sr23* and *Sr36* showed resistance to pathotype 40A (Table 3), however, these genes are known to be susceptible to several *Pgt* pathotypes *viz*., 11, 11A, 21, 21-1 and 21A-2, respectively (Prasad et al. 2018) to which Selection212 showed resistant response in an earlier study (Sharma and Singh 2000a). It was therefore concluded that *SrSel212* is different from *Sr23* or *Sr36* genes. Stem rust resistance gene *Sr39* is derived from *Ae. speltoides,* and is unlikely to be present in Selection212. Moreover, in our study Thatcher+*Lr35/Sr39* showed distinctly different reaction to *Pgt* pathotype 40A with IT $3^=$ as against high degree of resistance in Selection212 (IT ;1). Stem rust resistance gene *Sr40* is also located on chromosome 2B, however, information is not available about response of *Sr40* to Indian pathotypes. *Sr40* was mapped on chromosome 2BS.

Table 3. Infection types on Selection 212, Agra Local and the near-isogenic line/varieties for leaf and stem rust resistance genes located on chromosome 2B or 2BS against leaf rust Pt.77-5 and stem rust Pt.40A

	Leaf rust Pt.77-5			Stem rust Pt.40A	
S.No.	NIL/variety	Infection type	S.No.	NIL/variety	Infection type
1	Manitou (*Lr13*)	3^+	1	Marquis (*Sr19+Sr20*)	33^+
2	Exchange (*Lr16/Sr23*)	3^+	2	Exchange (*Lr16/Sr23*)	1^+
3	IWP 94 (*Lr23*)	3^+	3	CI12632 (*Sr36*)	1
4	Thatcher+*Lr35/Sr39*	3^+	4	Thatcher+*Lr35/Sr39*	$3^=$
5	Morocco (*Lr73*)	3	5	Agra Local	4
6	Agra Local	3^+	6	Selection212 (*SrSel212*)	;1
7	Selection212 (*LrSel212*)	;1			

Selection212 also carried a recessive stem rust resistance gene on chromosome 2B located 16.4 cM proximal to leaf rust resistance gene. An effort was made to determine the identity of stem rust resistance gene *SrSel212*. Till date, six stem rust resistance genes have been assigned to chromosome 2B *viz*., *Sr19, Sr20, Sr23, Sr36, Sr39* and *Sr40* (Anderson et al. 1971; McIntosh and Luig 1973; McIntosh and Gyarfas 1971; Kerber and Dyck 1990; Dyck 1992). Among the already designated stem rust resistance genes on chromosome 2B, *Sr19* and *Sr20* present in

Several leaf and stem rust resistance genes are linked and the linkage information is also useful in identifying genes (Tomar and Menon 2001; Tomar et al. 2014). Leaf rust resistance genes *Lr13* and *Lr23* are linked in repulsion phase, while *Lr16* and *Sr23*, and *Lr35* and *Sr39* are tightly linked in coupling (McIntosh 1995). Rust resistance genes *LrSel212* and *SrSel212* in Selection212 were mapped 16.4 cM apart in our study. The Selection212 is a wheat-rye recombinant (Singh 1991) while rust resistance genes *Lr13, Lr16, Lr23, Lr73, Sr19, Sr20,* and *Sr23* are derived

from common wheat, *Sr36* is from *Triticum timopheevii* (McIntosh and Gyarfas 1971), *Lr35/Sr39* from *Aegilops speltoides* and *Sr40* is derived from *T. araraticum* (Kerber and Dyck 1990; Dyck 1992). *Sr40* inherited as a single dominant gene in F_2 population derived from RL6088/2174 and was closely linked to marker *Xwmc474* which was 2.5 cM distal to it (Wu et al. 2009). In our study, *SrSel212* behaved as a recessive gene and was mapped 10.8 cM distal to *Xwmc474*. Variation in genetic distances in different populations are not uncommon, therefore, test of allelism with *Sr40* is required to confirm whether *SrSel212* represents a different locus. Also, there is need to study the response of *Sr40* to Indian *Pgt* pathotypes. Based on the available evidence, Selection212 appears to be a novel source of leaf and stem rusts resistance which may be useful for broadening the genetic base of rust resistance in wheat.

Authors' contribution

Conceptualization of research (JBS, OML, V, SKJ); Designing of the experiments (OML, JBS, V, SKJ); Contribution of experimental materials (JBS); Execution of field/lab experiments and data collection (OML); Analysis of data and interpretation (OML, JBS, V, SKJ, NM, MN); Preparation of manuscript (OML, JBS, V, NM, SKJ).

Declaration

The authors declare no conflict of interest.

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