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Combining ability for stem borer resistance and heterotic orientation of maize inbred lines using CIMMYT single cross testers under Chilo partellus infestation

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The spotted stem borer, Chilo partellus, Swinhoe (Lepidoptera, Crambidae) is one of the most destructive insect pests of maize in tropical environments. However, the combining ability and heterotic orientation of the germplasm for grain yield and borer resistance is limited. The objective of this study was to determine combining ability and heterotic orientation of new maize inbred lines under C. partellus infestation. Sixty six (66) inbred lines were crossed to two single cross testers in a line x tester mating scheme. The 132 testcross hybrids and four checks were evaluated at three locations in Kenya under C. partellus infestation. General and specific combining ability effects were significant for C. partellus stem borer resistance traits and grain yield, suggesting the importance of both additive and non-additive gene effects for these traits. Heterotic classification of lines was done based on both heterosis and specific combining ability data. Based on heterosis for grain yield data at Embu, 15 lines were allocated to group A, 18 to group B and 12 to group AB. At Kakamega, 26 lines were oriented towards group A, 19 to group B and 9 to group AB. At Kiboko, 15 lines were inclined towards group A, 18 to group B and 11 to group AB, whilst the remainder could not be classified. Based on the SCA estimates, at Embu, 10 lines revealed positive SCA effects with both testers and were considered to be AB-oriented while 8, and 1 lines were oriented towards A, and B, respectively. A similar trend was detected at Kakamega and Kiboko. The identified lines and heterotic groups would be used by hybrid maize programs where *C. partellus* stem borers occur exclusively or in league with other stem borers.

Key words: Tropical maize, maize breeding, biotic and abiotic stresses tolerance.

INTRODUCTION

Maize is the principal crop grown by the mainstream of rural families in Sub-Saharan Africa (SSA). Maize is both

a staple food and a cash crop through consumption and income generation for small scale farmers (Brooks et al.,

*Corresponding author. E-mail: mwimali@yahoo.co.uk. Tel: +254722915500. Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> 2009; Sasson, 2012), respectively. The progress made in breeding plants for improved quality and tolerance to both biotic and abiotic stresses has led to development of new maize hybrids with better agronomic characteristics. Despite these advances farmers in SSA continue to suffer huge crop losses from high insect pest incidences which occur in several generations per year due to friendly environmental conditions that enable insect development (Kfir et al., 2002; Tefera, 2012). Stem borers attacking cereal crops are considered one of the devastating biotic stress factors limiting production of maize in tropical Africa.

The spotted stem borer, Chilo partellus, Swinhoe (Lepidoptera, Crambidae), is one of the serious borer species affecting maize in SSA. Chilo partellus occupied Africa from Asia before 1930's and accounts for 90% of the stem borers in the lowland tropics, mid altitude and the moist transitional areas of East Africa (Ong'amo et al., 2012). The distribution and occurrence of C. partellus stem borers in different crop ecosystems is affected by host availability, location and suitability, mate location, success of oviposition, larval survival and establishment, temperature and altitude (Mailafiya et al., 2011; Ong'amo et al., 2012). Although C. partellus is absent in the highland tropics, it is progressively intensifying its range to higher altitudes, and currently, it is the most widely distributed stem borer in the maize growing zones in Kenya (Kfir et al., 2002; Tefera et al., 2011).

Chilo partellus in combination with other stem borer species greatly reduce maize grain yield in tropical environments ranging from 10% to total loss (Ajala et al., 2010). In Kenya, grain yield loss due to stem borers in maize is estimated annually at about 400,000 metric tonnes or about \$72 million (De Groote et al., 2003). This amount represents approximately 13.54% of the farmers' total annual harvest of maize and prompts breeding investigations.

Various management options exist for alleviating the damaging effects due to maize stem borers, but each opportunity has its own limitations and should be employed in an integrated pest management program. Host plant resistance is an important part of the management since it provides inherent control without adverse effects to the environment and is also compatible with other pest control options (Mugo et al., 2005). Currently, however there are few if any commercial varieties with imbedded resistance to stem borers in tropical environments. Therefore, for effective control of borers by host resistance, efficient breeding methods to better the use of new and existing sources of resistance. A better understanding of the genetic basis of the resistances among the germplasm used may contribute towards the development of effective approaches against these C. partellus borers.

Hybrid maize breeding programmes focusing on stem borers must encompass and exploit the knowledge of general combining ability (GCA) of lines and specific combining ability (SCA) of their testcrosses, heterosis and heterotic orientation. The knowledge of combining ability, type of gene action controlling economic traits, and heterosis is useful in fixing the appropriate parent lines, and in designing successful hybrids (Liberatore et al., 2013). Maize inbred lines with a high genetic diversity, strongly determine the levels of heterosis exhibited by the single cross hybrids and vice versa (Hallauer et al., 1988), and may be useful in hybrid development including breeding for resistance to stem borers.

The line x tester mating design provides reliable information on the general and specific combining ability effects of parents and their hybrid combinations (Kempthorne, 1957), and has been effectively applied in various previous quantitative genetic investigations in maize (Kanagarasu et al., 2010). The line x tester mating scheme is mainly used to generate data on the nature and magnitude of gene action, combining ability effects. heritability and nature and extent of heterosis for different traits (Sanghera et al., 2012). This mating scheme has been applied for determining gene action configuration for stem borer resistance in maize (Sharma et al., 2007). Populations and inbred lines or single cross hybrids have been used as testers in the identification of hybrids for yield performance (Sanghera et al., 2012). This mating design continues to be applied in determination of the maize heterotic orientations using different testers (Morais and Pinheiro, 2012). Single cross testers were used in the current study because the end product would be a three way cross. The three ways cross is the most appropriate for majority of the farmers in SSA due to the low price of seed compared to the single cross hybrids. The objective of this study was to determine combining

ability and heterotic orientation of maize inbred lines under *C. partellus* infestation. The information generated would be important in the allocation of inbred lines and testcrosses into heterotic clusters as a basis for possible exploitation in a hybrid breeding program with focus on *C. partellus* stem borer resistance.

MATERIALS AND METHODS

Germplasm

The experimental material used in the study comprised of three-way cross hybrids derived from crosses of 66 stem borer resistant lines (as female parents) with two single cross testers (as male parents) (CML312/442 and CML395/444), and four commercial check varieties. The sixty six inbred lines were selected from a wide genetic base for resistance to two stem borers *B. fusca* and *C. partellus* from various nurseries at CIMMYT, Kenya, and the Kenya Agricultural and Livestock Research Organization (KALRO) breeding programmes. Elite stem borer resistant and susceptible maize lines from CIMMYT and KALRO were included as checks. Orientations of lines into heterotic group A (CML312/CML442) and B (CML395/CML444) depended on the sign of SCA effect such that lines exhibiting positive SCA with tester A were allocated to the opposite heterotic group B, and vice versa, whereas lines

Table 1. List of pedigree information of maize inbred lines used in the study.

Line no.	Pedigree code	Line no.	Pedigree code	Line no.	Pedigree code
1	CKSBL10105	29	CKSBL10286	57	CKSPL10086
2	CKSBL10108	30	CKSBL10170	58	CKSPL10087
3	CKSBL10138	31	CKSBL10168	59	CKSPL10088
4	CKSBL10073	32	CKSBL10178	60	CKSPL10089
5	CKSBL10107	33	CKSBL10307	61	CKSPL10090
6	CKSBL10195	34	CKSBL10154	62	CKSPL10136
7	CKSBL10194	35	CKSBL10153	63	CKSPL10146
8	CKSBL10196	36	CKSBL10158	64	CKSPL10212
9	CKSBL10197	37	CKSBL10155	65	CKSPL10229
10	CKSBL10201	38	CKSBL10321	66	CKSBL10060
11	CKSBL10202	39	CKSBL10160	67	MBR C5 BC F1-13-3-2-1-B-4-2-B-resistant check
12	CKSBL10200	40	CKSBL10155	68	CML395-susceptible check
13	CKSBL10203	41	CKSBL10157		
14	CKSBL10204	42	CML442		
15	CKSBL10205	43	CKSBL10020		
16	CKSBL10206	44	CKSBL10082		
17	CKSBL10209	45	CKSPL10256		
18	CKSBL10208	46	CKSPL10273		
19	CKSBL10207	47	CKSPL10280		
20	CKSBL10210	48	CKSPL10309		
21	CKSBL10213	49	CKSPL10028		
22	CKSBL10250	50	CKSPL10035		
23	CKSBL10254	51	CKSPL10036		
24	CKSBL10165	52	CKSPL10042		
25	CKSBL10169	53	CKSPL10070		
26	CKSBL10171	54	CKSPL10074		
27	CKSBL10150	55	CKSPL10080		
28	CKSBL10212	56	CKSPL10081		

displaying positive SCA to both testers were designated as AB group, classified according to the heterotic group system at CIMMYT (CIMMYT, 2001). Single cross testers were used because the programme aims at releasing three-way cross hybrids that can be nominated directly into the national performance trials for additional evaluation and use (Table 1).

Experimental sites

Experiments were established at KALRO Kakamega, KALRO Embu and KALRO Kiboko in Kenya (Figure 1). KALRO Kakamega (37°75'E 2°15'S, 1585 masl) centre is located in the moist transitional-mid altitude agro-ecological zone of western Kenya and experiences mean annual temperatures of 25°C. Kakamega lies within a high potential agro-ecological zone and receives a bimodal mean annual rainfall of approximately 1850 to 1916 mm. The soils in Kakamega are well drained, moderately deep to very deep, red to dark in colour and in some places shallow over petroplinthite (Jaetzold and Schmidt, 1982).

KALRO-Embu centre (03°56' 44'S and 39°46' 00'E, 1510 masl) is located in the moist transitional mid altitude agro-ecological zone of eastern slopes of Mt. Kenya and experiences mean annual temperature ranges of 14 to 25°C. Embu lies within a high potential agro-ecological zone. Rainfall received is bi-modal ranging between 800 to 1400 mm annually. The soils are deep (about 2 m); well weathered Humic Nitisols with moderate to high inherent fertility (Jaetzold and Schmidt, 1982).

Experimental design and infestation

The 66 three way testcross hybrids along with 4 checks were evaluated in a 10 × 7 α -lattice design with three replications in each location for two seasons. Each testcross hybrid was sown in one row plot of 6.75 m. Two seeds were sown per hill and later thinned to one. Inter-row spacing of 0.75 m and inter-hills spacing of 0.25 m within the rows was used.

Recommended fertilizer application of nitrogen (60 kg N ha⁻¹) and phosphate (60 kg P_2O_5 ha⁻¹) and irrigation were applied as recommended for each location to ensure healthy and vigorous plants. Nitrogen was applied in two splits, while supplementary irrigation was applied when needed. The fields were kept free of weeds by hand weeding throughout the growth cycle.

Artificial infestation with insects

Insect larvae were obtained from the International Centre for Insect Physiology and Ecology (ICIPE) and the KALRO Katumani stem borer insect pests mass rearing facility. At KALRO Kakamega, KALRO Embu and KALRO Kiboko, eight plants per plot were



Figure 1. Map of Kenya showing Kakamega and Kiboko locations of the studies. Source: KARI Land Resources and Analytical Services (KARI Land Resources and Analytical Services, 2013).

artificially infested in a controlled and uniform manner with 10 first instar larvae of *C. partellus* stem borers by placing in the maize whorl using a camel brush at 14 days after planting.

Data collection and analysis

Plants were evaluated for leaf damage (LD) scores using a scale of 1 (resistant) to 9 (susceptible) (CIMMYT, 1989). Other plant damage parameters were measured at harvest namely; cumulative tunnel length (TL) (measured as the total length (cm) of tunneling along the maize stalk), tunnel length to plant height ratio, number of exit holes (EXH), and number of larvae recovered per plant. Agronomic traits (plant height, ear height, number of days to anthesis (AD), number of days to silking (SD) etc) were measured following standard protocols used at CIMMYT (CIMMYT, 1989). Grain yield (kg plot⁻¹) was obtained as grain weight adjusted for moisture content at 13%, and converted to tha⁻¹. Data on cumulative stem tunnel length were transformed into arcsine values before subjecting them to analysis of variance (ANOVA).

The analysis of variance (ANOVA) across environments for all data was performed using PROC GLM procedures in SAS

computer package, version 9.2 following a linear model:

$$\begin{array}{l} Y_{ijk} = \mu + r(e_k) + e_k + l_i + t_j + (l \ x \ t)_{ij} + (l \ x \ e)_{ik} + (t \ x \ e \)_{jk} + (l \ x \ t \ x \ e)_{eijk} \\ + \ \varepsilon_{iik} \end{array}$$

Where: Y_{ijk} is measured trait of the genotype of ith line crossed to jth tester evaluated in r replications across k environments; μ is grand mean; r (e_k) = effect of replication nested within the *k* environments; I and t represent average effects of lines and of testers, respectively, which is equivalent to GCA effects of lines and testers, respectively; I x t = line x tester interaction effects that is equivalent to the SCA effects of the crosses; *e* is the environmental main effects; I x e, t x e and I x t x e are the interactions of the lines, testers and the lines x testers with the environments, and e_{ijk} = random experimental error.

Relative standard heterosis (SH) was calculated for each testcross relative to the two testers according to Fehr (1987): SH = ((F1- Mean of tester)/ Mean of tester) * 100, where: F1 = F1 hybrid mean performance; MoT = mean of tester (A), mean of tester (B); SE for heterosis was calculated as $\sqrt{\sigma_e^2}/2$.

Clustering of lines into heterotic group A (CML312/CML442) and B (CML395/CML444) depended on the direction of the specific

Source	DF	GY	TL	EXH	LD	EA	PA	AD	SD
Rep	2	0.37	139.48	29.73	8.92	2.35	1.40	7.44	3.52
Env.	5	280.46**	304.16**	451.42**	144.31**	23.21**	61.37**	11652.58**	11876.82**
Line	65	6.77**	69.24**	33.68**	6.38**	1.84**	1.39**	64.64*	59.71*
Tester	1	0.13	11.68	54.31	11.48	5.42**	2.10	3.97	9.80
Line * Env	325	4.79**	46.27**	15.23**	4.04	0.95	1.62**	63.76**	66.25**
Tester * Env	5	1.50*	34.76	6.17	5.51	0.45	2.33**	8.82	7.70
Line*Tester	65	1.55**	34.57	20.99**	4.64	0.85	1.29*	9.57	10.97
Line * tester *Env	325	0.83**	25.65	9.24	2.13	0.79	0.71	10.62	10.89
Error	1581	0.56	30.54	11.05	3.75	0.80	0.91	47.46	48.24
Cp Mean		1.17	9.06	3.99	2.21	2.53	2.46	74.02	75.13
(%) R ²		80.96	39.94	44.32	37.14	40.70	46.52	53.76	54.02

 Table 2. Mean squares of combined analysis for selected stem borer resistance and agronomic traits for hybrids over six environments under C. partellus infestation.

DF, degrees of freedom; GY, grain yield; TL, cumulative stem tunneling; EXH, number of exit holes; LD, leaf feeding damage; EA, ear aspect; PA-Plant aspect; AD-days to anthesis; SD-days to silking; *; ** = significant (p≤0.05); highly significant (p≤0.01).

combining ability such that lines exhibiting positive SCA with tester A were allocated to the opposite heterotic group B, and vice versa, whereas lines displaying positive SCA to both were designated as AB group.

RESULTS

Genotype x environment interactions

There was a highly significant genotype x environment interactions for grain yield, therefore the three test locations were treated as representative environments. Consequently, the results are presented on a site by site basis.

Trait variations under Chilo partellus infestation

The mean squares of the test crosses from the combined analysis of selected stem borer resistance and agronomic traits for C. partellus at Embu, Kakamega and Kiboko were significant (p≤0.05) for most traits (Table 2). The site and the lines showed highly (p≤0.01) significant differences for all traits. The testers showed highly significant (p≤0.05) differences for grain yield and ear aspect. The line x tester interaction effects were highly significant (p≤0.05) for grain yield, cumulative stem tunneling, leaf feeding damage and plant aspect (Table 2). The line x site interaction effects were significant (p≤0.05) for all traits except leaf feeding damage. The tester x site, and the line x tester x site interaction effects were not significant for all traits except for grain yield (p≤0.05) (Table 2). The mean for the various traits under C. partellus infestation were as follows: Grain yield (1.2 t ha⁻¹), cumulative stem tunneling (9.1 cm), number of exit holes (4.0), leaf feeding damage score (2.2) and ear aspect (2.5), plant aspect (2.5), days to anthesis (74.1), and days to silking (75.1) (Table 2).

General combining ability effects

Results of general combining ability effects of top 20 lines and their corresponding two testers for *C. partellus* stem borer resistance traits and grain yield are presented for Embu, Kakamega and Kiboko (Table 3).

At Embu, Kakamega and Kiboko, for grain yield, positive significant ($p\leq0.05$) GCA effects were detected for all top 20 lines. Negative significant ($p\leq0.05$) GCA effects were detected for cumulative stem tunneling ranging from -7.47 to -4.69 and number of exit holes from -3.04 to 2.25. However, no significant GCA effects were found for leaf feeding damage for *C. partellus* in all environments. The number of days to anthesis and days to silking showed negative significant ($p\leq0.05$) GCA effects for all top lines across locations. The testers; CML395/444 and CML312/442 had varied trends for GCA effects for the various stem borer resistance and agronomic traits for *C. partellus* at the three locations (Table 3).

Specific combining ability effects

Results of SCA effects of top 20 lines and their corresponding two testers for *C. partellus* stem borer resistance, grain yield and agronomic traits are presented for Embu, Kakamega and Kiboko (Tables 4 and 5). The SCA data was averaged over seasons at each site.

At Embu, all the testcrosses revealed significant and desirable SCA effects ($P \le 0.05$) for grain yield except testcrosses with CML395/CML444. However, the same testcrosses had significant and desirable SCA effects ($P \le 0.05$) for the following traits; cumulative stem tunneling ranging from -7.77 to -2.26, number of exit holes from -2.91 to -1.03 and leaf damage score from -2.75 to -1.43. Similarly, there were significant and desirable SCA effects ($P \le 0.05$) for the following

	Site	Line	GY	Site	Line	TL	Site	Line	EXH	Site	Line	LD	Site	Line	SD	Site	Line	AD
	EMB	1	0.99**	EMB	30	-6.24**	EMB	41	-2.85**	EMB	14	-1.16	EMB	13	-2.68**	EMB	13	-2.31**
	EMB	2	0.75**	EMB	31	-6.34**	EMB	42	-2.71**	EMB	34	-1.29	EMB	16	-1.68*	EMB	16	-2.14**
	EMB	3	0.72**	EMB	36	-6.69**	EMB	43	-2.73**	EMB	35	-1.29	EMB	17	-1.35*	EMB	28	-1.48*
	EMB	10	0.32*	EMB	37	-4.87**	EMB	44	-3.04**	EMB	41	-1.29	EMB	28	-2.02	EMB	38	-1.48*
	EMB	18	0.79**	EMB	38	-5.07**	EMB	45	-2.41**	EMB	54	-1.14	EMB	38	-1.52*	EMB	45	-2.98**
	EMB	19	0.89**	EMB	40	-5.92**	EMB	49	-2.36**	EMB	55	-1.13	EMB	45	-1.68*	EMB	53	-1.31*
	EMB	20	0.93**	EMB	42	-5.94**	EMB	60	-2.71**	EMB	57	-1.12	EMB	50	-1.52*	EMB	66	-2.31**
	EMB	21	0.74**	EMB	46	-5.79**	EMB	63	-2.54**	EMB	58	-1.13	EMB	59	-1.85*	KAK	28	-1.75*
	EMB	22	0.91**	EMB	48	-6.12**	EMB	66	-2.26**	EMB	59	-1.19	EMB	60	-1.52*	KAK	56	-1.25*
	EMB	23	0.92**	EMB	50	-6.39**	KAK	42	-3.39**	EMB	61	-1.13	EMB	66	-2.85**	KAK	66	-1.38*
	EMB	24	0.48	KAK	36	-7.09**	KAK	44	-2.89**	EMB	62	-1.13	KAK	63	-1.47*	KIB	4	-1.44*
	EMB	25	0.76**	KAK	37	-6.40**	KAK	45	-2.34**	EMB	63	-1.16	KIB	9	-1.99*	KIB	11	-2.28*
	EMB	26	0.39*	KAK	38	-5.87**	KAK	46	-3.29**	EMB	64	-1.15	KIB	11	-2.66**	KIB	24	-2.28**
	EMB	28	0.40*	KAK	43	-7.47**	KAK	47	-2.75**	EMB	65	-1.10	KIB	16	-2.16**	KIB	33	-2.44**
	EMB	30	0.17	KAK	44	-6.24**	KAK	49	-2.25**	EMB	66	-1.17	KIB	18	-2.16**	KIB	39	-1.61*
	EMB	40	0.29*	KAK	46	-5.35**	KAK	51	-2.47**	KAK	34	-1.13	KIB	24	-3.33**	KIB	40	-1.28*
	KAK	8	0.27*	KAK	49	-5.99	KAK	54	-2.33**	KAK	35	-1.12	KIB	32	-1.49*	KIB	43	-2.11**
	KAK	29	0.24*	KAK	50	-5.79**	KAK	55	-2.59**	KAK	53	-1.08	KIB	33	-1.83*	KIB	48	-3.11**
	KAK	37	0.19*	KAK	51	-4.69**	KAK	59	-2.49**	KAK	54	-1.01	KIB	39	-1.66*	KIB	58	-1.31*
	KAK	47	0.25*	KAK	52	-5.67**	KAK	60	-2.74**	KAK	55	-1.01	KIB	48	-2.99**	KIB	62	-1.28*
	KIB	25	0.15*	KIB	3	-5.19**	KAK	63	-2.55**	KAK	58	-1.02	KIB	58	-1.56**	KIB	63	-2.44**
	KIB	53	0.15*	KIB	63	-6.30**	KAK	64	-2.29**	KAK	61	-1.02	KIB	62	-1.76*	KIB	66	-2.91**
Standard Error- EMBU			0.23			2.19			1.24		-	-			1.06			0.94
Standard Error- KAK			0.23			2.25			1.51		-	-			0.82			0.88
Standard Error –KIB			0.23			2.50			1.24		-	-			1.81			1.71
Site	Те	ster	G	ſ	TL		EXH		LD	A	D	SD		PH		PA		EA
Embu	CML395	5/CML444	0.2	4	7.37		0.05		0.01	0	.99	-0.0	8	-3.56		0.53	-	0.07
	CML312	2/CML442	-0.2	24	-7.37		-0.05		-0.01	-0	.99	30.0	3	3.56		-0.53	().07
Kakamega	CML395	5/CML444	-0.1	7	8.05		0.26		-0.12		-	1.36	6	-0.24		-0.09		-
	CML312	2/CML442	0.1	6	8.05		-0.26		0.12		-	-1.3	5	0.24		0.09		-
Kiboko	CML395	5/CML444	-0.2	25	11.00		-0.94		1.19		-	1.71	1	-1.88		-0.59		-
	CML312	2/CML4A2	0.2	5	-11.00)	0.94		-1.19		-	-1.7	1	1.88		0.59		-
Standard error			0.0	3	0.64		0.12		0.08	0.	.50	0.22	2	1.57		0.05	().12

Table 3. General combining ability estimates of top 20 maize inbred lines for selected stem borer resistance traits and grain yield under *C. partellus* infestation at Embu, Kakamega and Kiboko.

Sites EMB=Embu; KAK=Kakamega and KIB=Kiboko; GY, grain yield; TL, cumulative stem tunneling; EXH, number of exit holes; LD, leaf feeding damage; PA, plant aspect; EA, ear aspect; AD, days to anthesis; SD, days to silking; *, ** = significant (p<0.05), highly significant (p<0.01), SE for heterosis for grain yield = 0.33.

		Embu					Kakamega					Kiboko		
тс	GY	TL	E×H	LD	тс	GY	TL	E×H	LD	тс	GY	TL	E×H	LD
11 × 2	-0.02	-7.77**	-2.91**	-2.75**	4 × 1	-0.01	-3.63**	-1.54	-1.43	3×2	0.02**	-3.46**	-1.19	-0.71
11 x 1	0.02**	-5.56**	-2.87**	-2.58**	4 × 2	0.01**	-2.57	-1.56	-1.58*	3 × 1	-0.02	-2.45	-2.18*	-0.48
16 × 2	-0.04	-5.09**	-2.73**	-2.50**	11 x 1	0.01**	-2.70	-1.71	-1.69*	5×2	0.02**	-2.57	-2.38*	-1.17
16 × 1	0.04**	-4.94**	-2.35	-2.19**	11 x 2	0.01**	-2.81	-1.56	-1.84*	5 × 1	-0.02	-2.68	-2.87*	-0.60
26 × 2	-0.04	-4.74**	-2.08	-2.06**	15 × 1	-0.01	-5.60**	-1.94	-1.67*	13 × 2	0.05**	-5.33**	-0.92	-0.55
26 × 1	0.04**	-4.32**	-1.87	-1.80**	15 × 2	0.01**	-4.36**	-4.10**	-1.90*	13 × 1	-0.05	-4.15**	-1.61	-0.48
28 × 2	-0.05	-4.29**	-1.80	-1.68**	19 × 1	-0.04	-4.18	-8.04**	-2.17	14 × 2	0.03**	-3.98**	-2.39*	-0.95
28 × 1	0.05**	-4.17	-1.79	-1.68**	21 x 1	-0.04	-2.49	-2.22	-1.46	31 × 2	0.05**	-2.37	-2.47*	-0.95
39 × 2	-0.06	-4.08	-1.74	-1.63**	26 × 1	0.01*	-5.88**	-1.59	-2.61*	31 × 1	-0.05	-5.60**	-2.59*	-0.72
39 × 1	0.06**	-3.99	-1.64	-1.60**	26 × 2	-0.01	-3.31	-2.28	-2.71*	39 × 2	0.05**	-3.15**	-0.87	-0.63
46 × 2	0.06**	-3.51	-1.62	-1.60**	30 × 1	-0.03	-2.94	-2.28	-1.55	39 × 1	-0.05	-2.80	-1.01	-0.60
46 × 1	0.06**	-3.36	-1.60	-1.55**	43 × 1	-0.01	-3.02	-1.74	-1.33	43 × 2	0.05**	-2.88	-0.92	-0.65
9×2	-0.07	-3.19	-1.54	-1.52**	43 × 2	0.01*	-2.71	-3.61**	-1.36	43 × 1	-0.05	-2.58	-1.14	-0.61
9 × 1	0.07**	-3.01	-1.39	-1.51**	46 × 1	-0.04	-2.57	-2.66	-1.40	60 × 2	0.05**	-2.45	-1.01	-0.56
40 × 2	-0.1	-2.88	-1.38	-1.50**	50 × 1	-0.04	-2.70	-3.24**	-1.32	60 × 1	-0.05	-2.57	-0.98	-0.62
40 × 1	0.11**	-2.66	-1.37	-1.49**	51 × 1	-0.03	-2.92	-1.51	-1.38	61 × 2	0.05**	-2.78	-0.89	-0.48
29 × 2	0.10**	-2.44	-1.20	-1.48**	51 × 2	0.03*	-5.93**	-3.01*	-1.38	61 × 1	-0.05*	-5.65**	-2.74*	-0.76
29 × 1	0.11**	-2.44	-1.10	-1.45	64 × 1	-0.03	-6.21**	-1.34	-1.64*	65 × 1	0.05**	-5.91**	-0.91	-0.57
60 × 2	-0.11	-2.36	-1.08	-1.44	64 × 2	0.03**	-4.00	-1.91	-1.42	65 × 2	-0.05	-3.81	-1.22	-0.49
60 × 1	0.11**	-2.26	-1.03	-1.43	66 × 2	0.01**	-4.12	-1.26	-1.56	66 × 2	0.06*	-3.92	-1.21	-0.86
SE	0.23	2.18	1.24	0.74		0.13	2.25	1.51	0.68		0.14	2.50	0.97	0.71

Table 4. Specific combining ability effects of top 20 testcrosses for selected stem borer resistance traits and grain yield under *C. partellus* infestation at Embu, Kakamega and Kiboko (averaged over 2 seasons per site).

TC=testcross; GY, grain yield; TL, cumulative stem tunneling; EXH, number of exit holes; LD, leaf feeding damage; *; ** = significant (p≤0.05); highly significant (p≤0.01); 1=CML395/CML444 and 2=CML312/CML442; SE for heterosis for grain yield = 0.33.

Table 5. Specific combining ability effects of top 20 testcrosses for selected agronomic traits under *C. partellus* infestation at Embu, Kakamega and Kiboko (averaged over 2 seasons per site).

	Kakamega					Kiboko											
T/cross	AD	SD	PH	PA	EA	T/cross	AD	SD	PH	PA	EA	T/cross	AD	SD	PH	PA	EA
11 × 2	-2.29**	-3.08**	-15.60	-0.44*	-0.38	4 × 1	-0.82	-0.81	-14.41	-0.59	-0.90	3 × 2	-0.82	-0.81	-14.41	-0.59	-0.90*
11 × 1	-2.12**	-2.59**	-14.80	-0.32	-0.72**	4 × 2	-0.69	-0.65	-15.24	-0.42	-0.88	3 × 1	-0.69	-0.65	-15.24	-0.42	-0.88*
16 × 2	-2.06**	-2.09**	-28.11**	-0.35	-0.38	11 × 1	-0.69	-0.65	-39.76**	-0.76**	-0.76	5 × 2	-0.69	-0.65	-39.76**	-0.76**	-0.76
16 × 1	-2.05**	-1.91*	-13.60	-0.39	-0.28	11 × 2	-0.85*	-0.65*	-31.42**	-0.58	-0.73	5 × 1	-0.85*	-0.65*	-31.42**	-0.58	-0.73

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26 × 2	-2.05**	-1.91*	-49.41**	-0.32	-0.45	15 × 1	-0.82*	-1.36*	-13.09	-0.59	-0.73	13 × 2	-0.82*	-1.36*	-13.09	-0.59	-0.73
26 × 1	-1.88**	-1.91*	-12.70	-0.35	-0.37	15 × 2	-0.82*	-0.65*	-30.09**	-0.58	-0.72	13 × 1	-0.82*	-0.65*	-30.09**	-0.58	-0.72
28 × 2	-1.88**	-1.91*	-11.90	-0.40*	-0.30	19 × 1	-1.19*	-0.65*	-18.09	-0.66	-0.71	14 × 2	-1.19*	-0.65*	-18.09	-0.66	-0.71
28 × 1	-1.88*	-1.90*	-27.74**	-0.32	-0.28	21 × 1	-0.82*	-0.65*	-21.76	-0.51	-0.65	31 × 2	-0.82*	-0.65*	-21.76*	-0.51	-0.65
39 × 2	-1.79*	-1.76*	-28.30*	-0.52*	-1.03**	26 × 1	-0.69	-0.65	-20.76	-0.58	-0.61	31 × 1	-0.69	-0.65	-20.76*	-0.58	-0.61
39 × 1	-1.79*	-1.76*	-11.90	-0.60*	-0.47	26 × 2	-0.67	-0.65	-17.73	-0.76**	-0.6	39 × 2	-0.82*	-0.65*	-17.73	-0.76**	-0.60
46 × 2	-1.71	-1.74	-21.40*	-0.35	-0.55	30 × 1	-0.82*	-0.65*	-14.76	-0.66	-0.56	39 × 1	-0.82*	-0.65*	-14.76	-0.66	-0.56
46 × 1	-1.62	-1.59	-15.80	-0.60*	-0.33	43 × 1	-0.99*	-0.81*	-26.26**	-0.49	-0.52	43 × 2	-0.99*	-0.81*	-26.26**	-0.49	-0.52
9 × 2	-1.62	-1.59	-13.10	-0.40*	-0.45	43 × 2	-1.02*	-0.65*	-12.26	-0.99**	-0.52	43 × 1	-1.02*	-0.65*	-12.26	-0.99**	-0.52
9 × 1	-1.55	-1.59	-23.10*	-0.32	-0.30	46 × 1	-0.69	-0.56	-27.74**	-0.67	-0.48	60 × 2	-0.69	-0.56	-27.74**	-0.67	-0.48
40 × 2	-1.55	-1.58	-12.40	-0.51	-0.53	50 × 1	-0.99*	-0.48*	-23.57	-0.76**	-0.48	60 × 1	-0.99*	-0.48*	-23.57*	-0.76**	-0.48
40 × 1	-1.38	-1.58	-21.10	-0.35	-0.55	51 × 1	-0.69*	-0.81*	-11.91	-0.49	-0.47	61 × 2	-0.69	-0.81	-11.91	-0.49	-0.47
29 × 2	-1.38	-1.42	-23.90*	-0.43*	-0.45	51 × 2	-0.82*	-0.81*	-14.41	-0.49	-0.41	61 × 1	-0.82	-0.81	-14.41	-0.49	-0.41
29 × 1	-1.38	-1.42	-12.70	-0.57*	-0.37	64 × 1	-0.69	-0.65	-24.41	-0.84**	-0.4	65 × 1	-0.69	-0.65	-24.41*	-0.84**	-0.40
60 × 2	-1.29	-1.42	-31.44**	-0.52*	-0.30	64 × 2	-1.02	-1.48	-12.74	-0.83**	-0.38	65 × 2	-1.02	-1.48	-12.74	-0.83**	-0.38
60 × 1	-1.29	-1.42	-20.40*	-0.44*	-0.70	66 × 2	-0.69	-0.65	-11.41	-0.51	-0.38	66 × 2	-0.69	-0.65	-11.41	-0.51	-0.38
SE	0.93	1.05	12.66	0.42	0.30		0.87	0.82	12.61	0.35	0.40		1.71	1.80	12.56	0.41	0.29

T/cross = testcross; AD, days to anthesis; SD, days to silking; PH, plant height; PA, plant aspect and EA, ear aspect; *; ** = significant (p≤0.05); highly significant (p≤0.01); 1=CML395/CML444 and 2=CML312/CML442.

Table 6. Heterotic orientation of top lines based on specific combining ability effects for grain yield under *C. partellus* infestation at Embu, Kakamega and Kiboko (averaged over 2 seasons per site).

Line	SCA effects for	grain yield with	- Hotorotic orientation
Line	CML312/CML442 (A tester)	CML395/CML444 (tester)	Heterotic orientation
Embu			
9	0.07**	-0.07**	A
11	0.02**	-0.02**	А
16	0.04**	-0.04**	А
26	0.04**	-0.04**	А
28	0.05**	-0.05**	А
29	0.10**	-0.10**	А
39	0.06**	-0.06**	А
40	0.10**	-0.10**	А
46	0.06**	0.06**	A/B
60	-0.11**	0.11**	В

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Kakamega			
4	-0.01**	0.01**	В
11	-0.01**	0.01**	В
15	-0.01**	0.01**	В
19	-0.04	0.04	В
21	-0.03	0.03	В
26	0.01*	-0.01*	А
30	-0.04	0.04	В
43	0.02*	0.02*	A/B
46	0.03	0.03	A/B
50	-0.03	0.03	В
51	0.03*	-0.03*	A
64	-0.03**	0.03**	В
66	0.01**	0.01**	A/B
Kiboko			
3	-0.02**	0.02**	В
5	-0.02**	0.02**	В
13	-0.05**	0.05**	В
14	-0.03**	0.03**	В
31	0.05**	-0.05**	А
39	-0.05**	0.05**	В
43	-0.05**	0.05**	В
60	-0.05**	0.05**	В
61	-0.05**	0.05**	В
65	0.05**	-0.05**	А
66	-0.06*	0.06*	В

*, ** = significant ($p \le 0.05$), highly significant ($p \le 0.01$).

agronomic traits and testcrosses; days to anthesis and days to silking, plant height, and plant and ear aspects (Tables 4 and 5).

At Kakamega, significant and desirable SCA effects ($P \le 0.05$) for grain yield were detected for the following 10 testcrosses. Six out of 10 testcrosses that showed desirable SCA effects

grain yield were crosses with CML312/CML442. These testcrosses had significant and desirable SCA effects ($P \le 0.05$) for the following borer resistance characters; cumulative stem tunneling ranging from -6.21 to -2.57, number of exit holes from -3.61 to -1.26 and leaf damage score from -2.71 to -1.32 (Tables 4 and 5). Twelve

testcrosses displayed significant and desirable SCA effects ($P \le 0.05$) for days to anthesis and days to silking and 5 testcrosses for plant height and 6 testcrosses for plant aspects. There were no testcrosses that showed significant SCA effects for ear aspects (Tables 4 and 5).

At Kiboko, significant and desirable SCA effects

Line	% yield relative to CML395/CML444	% yield relative to CML312/CML442	Heterotic orientation
40	116.4	103.5	AB
50	107.6	95.3	AB
47	103.3	91.2	AB
49	103.4	91.2	AB
48	95.4	83.8	AB
57	96.8	85.0	AB
1	82	71.2	AB
2	82	71.2	AB
3	82	71.2	AB
55	87	75.8	AB
CML395/CML444	1.24		
CML312/CML442		1.32	

Table 7. Percent grain yield of testcrosses relative to the testers and heterotic orientation under *C. partellus* infestation at Embu.

Z= inbred lines that were unclassified, SE of heterosis for grain yield = 0.33.

(P≤0.05) for grain yield were detected for all testcrosses with CML312/CML442. Only testcross 65 involving CML395/CML444 showed significant desirable SCA effects for grain yield at Kiboko (Tables 4 and 5). Similarly, these testcrosses had significant and desirable SCA effects (P≤0.05) for cumulative stem tunneling, number of exit holes, and leaf damage (Tables 4 and 5).

Heterotic orientations of lines based on specific combining ability

Results of the heterotic orientations of lines based on specific combining ability data for grain yield under *C. partellus* infestation are presented following. Heterotic orientation to CML312/CML442 and CML395/CML444 was determined according to the CIMMYT heterotic classification system as A and B, respectively (Table 6). Clustering of the lines into groups A, and depended on the direction of the SCA estimate such that lines displaying positive SCA with tester A were oriented towards the opposite heterotic group, and vice versa, whereas lines exhibiting positive SCA to both testers were elected as AB group.

At Embu, 8 lines showed significant ($p \le 0.05$) positive SCA effects for grain yield with CML395/CML444 therefore they were oriented towards heterotic group A. Lines 46 and 60 fitted into heterotic group AB and B, respectively (Table 6).

At Kakamega, 8 lines showed positive SCA estimates for grain yield with CML312/CML442, therefore they were oriented towards heterotic group B. Lines 26 and 51 were oriented towards heterotic group A and the remainder into group AB (Table 6).

At Kiboko, 2 lines (31 and 65) showed positive SCA estimates for grain yield with CML395/CML444, therefore they were allocated to heterotic group A, however, 9 lines displayed significant ($p \le 0.05$) positive SCA effects with

CML312/CML442 and were oriented towards heterotic group B. Lines 43 and 46 showed positive SCA effects for grain yield with both CML395/CML444 and CML312/CML442 in at least two locations, so they were consistently classified into AB group (Table 6).

Heterosis of maize inbred lines relative to testers

At Embu, 10 inbred lines showed positive heterosis with both testers for grain yield and were oriented towards heterotic group AB under *C. partellus* infestation (Table 7). In total 15 lines were allocated to A, 18 to and 12 to AB, while the remainder (*Z*) could not be classified with both testers (Figures 2 and 3).

At Kakamega, inbred lines 29, 37, 4, 5, 6, 12, 13, 14, 20, and 27 and 28 exhibited positive heterosis with both testers for grain yield and were allocated to heterotic group AB under *C. partellus*



Figure 2. Distribution of heterosis (%) for 132 testcrosses under C. partellus infestation at Embu.



Figure 3. Classes of the heterotic orientations for 66 maize inbred lines under C. partellus infestation at Embu.

Line	% yield relative to CML395/CML444	% yield relative to CML312/CML442	Heterotic orientation
29	64.6	54.8	A
37	60.5	51.0	A
4	58.7	49.2	A
5	58.7	49.2	A
6	58.7	49.2	A
12	58.7	49.2	A
13	58.7	49.2	A
14	58.7	49.2	A
20	58.7	49.2	A
27	58.7	49.2	A
28	58.7	49.2	A
CML395/CML444	0.59		
CML312/CML442		0.92	

Table 8. Percent grain yield of testcrosses relative to the testers and heterotic orientation under *C. partellus* infestation at Kakamega.

Z= inbred lines that were unclassified; SE of heterosis for grain yield = 0.33.

infestation (Table 8). In total, 26 lines were allocated to A, 19 to B and 9 to AB, and 12 lines were not be classified with both testers (Figures 4 and 5).

At Kiboko, similar heterotic orientations were detected (Table 9), where 15 lines were allocated to A, 18 to B and 22 to AB, while the remainder could not be classified with both testers (Figures 6 and 7).

DISCUSSION

Genetic variation

The highly significant differences detected among the lines and their testcrosses for the various *C. partellus* borer resistance and agronomic traits indicated the existence of considerable variation among the genotypes that allows for selection of preferred inbred lines and hybrids. There were revealed highly significant differences among lines, testers, lines x testers, and the line x tester x environment interactions for all the characters studied. The inferences that can be drawn from the findings are that additive effects were important for these characters. In addition, the results showed that the testers and the interaction lines x testers explained most of the variation in the expression of the stem borer resistance and agronomic traits. The study showed a large dissimilarity between lines and the testcrosses' for traits. Testers x site interaction with environment were not significant which shows that the testers chosen had stable performance across sites and would probably be most suited when choosing best lines for grain yield across different environments. The significance of the SCA effects suggested that the non-additive gene effects were crucial in influencing manifestation of stem borer resistance traits and yield. Additionally, the significance of the environment x line interactions for grain yield; environment x tester interactions implied that environmental influence is important in the expression of the characters. The separation of lines, testers and environment and their interactions into variances provided a better understanding of the different patterns among *C. partellus* and their response across locations. These findings corroborate with earlier studies on significance of genotype x environment interactions effects in maize (Fato et al., 2012; Morais and Pinheiro, 2012).

At Embu, Kakamega and Kiboko for all *C. partellus* treatments, the mean squares for lines, testers, and their interactions were highly significant for all the traits studied, indicating inconsistent ranking of the lines by the testers. However, the line x tester x site interactions were not significant for all traits except grain yield and leaf feeding damage. The highly significant differences among lines and testers for some traits may suggest that genotypes responded



Figure 4. Distribution of heterosis (%) for 132 testcrosses under *C. partellus* infestation at Kakamega.



Figure 5. Classes of the heterotic orientations for 66 maize inbred lines under C. partellus infestation at Kakamega.

Line	% yield relative to CML395/CML444	% yield relative to CML312/CML442	Heterotic orientation
25	39.6	31.3	AB
53	39.6	31.3	AB
9	36.9	28.7	AB
59	36.9	28.7	AB
10	31.5	23.7	AB
54	31.5	23.7	AB
8	28.9	21.2	AB
11	28.9	21.2	AB
CML395/CML444	1.24		
CML312/CML442		1.32	

Table 9. Percent grain yield of testcrosses relative to the testers and heterotic orientation under C. partellus infestation at Kiboko.

Z= inbred lines that were unclassified; SE of heterosis for grain yield = 0.33.



Figure 6. Distribution of heterosis (%) for 132 testcrosses under C. partellus infestation at Kiboko.

differently across locations. The significant differences for lines x testers' interaction for stem borer resistance traits and grain yield showed that specific combining ability is greatly attributed in the expression of resistance traits and shows the importance of dominance or non-additive variances. There were no significant differences detected in the site x tester, and the site x line x tester interactions for agronomic traits namely; days to anthesis, days to silking, plant and ear height. These may suggest a predominance of additive effect in the control of these traits for *C. partellus*.

General and specific combining ability

General and specific combining abilities in addition to gene action for different stem borer resistance and agronomic traits have been estimated by many researchers (Morais and Pinheiro, 2012; Sanghera et al., 2012; Wegary and Vivek, 2013). In the current study, the significant difference of mean squares between lines, testers, lines x testers for stem borer resistance traits and grain yield showed their suitability for combining ability studies.



Figure 7. Classes of the heterotic orientations for 66 maize inbred lines under C. partellus infestation at Kiboko.

Further, significant mean squares of lines, testers, lines x testers' revealed good possibility for manifestation of heterosis for all the traits studied. It is desirable that stem borer resistance traits namely; leaf feeding damage, cumulative stem tunneling, number of exit holes, and number of dead hearts to obtain negative GCA and SCA effects (Beyene et al., 2011; Morais and Pinheiro, 2012). Similarly, positive GCA and SCA effects are necessary for grain yield, number of plants and ears per plant (Morais and Pinheiro, 2012). The genetic variations due to lines and testers were significantly different in the Embu, Kakamega and Kiboko for C. partellus stem borer resistance parameters and grain yield, and other agronomic traits. These revealed a preponderance of the additive effects for these traits. Both additive and nonadditive gene effects have been reported in the literature for grain yield and yield components for various crops (Sanghera et al., 2012; Schnable et al., 2013).

Specific combining ability effects were significant for *C. partellus* resistance traits and grain yield signifying that non-additive effects were also crucial for borer resistance and grain yield. Maize inbred lines with high GCA effects also revealed hybrids with high SCA values for grain yield. For example, comparison of the lines and their responses to *C. partellus* at Embu, Kakamega and Kiboko, only lines 20, 28, 47 and 53 showed positive significant GCA effects for grain yield across locations for *C. partellus*. Different lines showed negative GCA and

SCA effects for cumulative tunneling, number of exit holes and the leaf feeding damage for C. partellus resistance. These may imply that in hybrid formation and deployment for the various mega-ecologies, there is a need to target *C. partellus* specific varieties that combine high yield and stem borer resistance. The lines that showed positive significant GCA effects for grain yield and negative GCA and SCA effects across locations and borer resistance may be subjected to further evaluations and probable exploitation as parents in hybrid pedigree breeding. The current study demonstrated that additive gene effects control cumulative stem tunneling, number of exit holes, leaf feeding damage and the related agronomic characters. These results corroborate with previous findings in studies on stem borer resistance and grain yield in maize (Beyene et al., 2011; Udaykumar et al., 2013).

Heterotic orientations of maize inbred lines under *C. partellus* infestation

The evaluation of testcrosses showed relative responses of the parent lines. Using two genetic testers, different probable heterotic orientations were identified for inbred lines used in the current study. Relative heterosis data and the magnitude of the SCA effects for the testcrosses were used in the clustering of heterotic orientations and the identification of response patterns for *C. partellus* in the various locations. Most testcrosses showed positive heterosis for grain yield across locations, indicating the presence of heterosis in the hybrids. Relative heterosis was highest for grain yield, which is in tandem with other reports on maize (Sanghera et al., 2012; Liberatore et al., 2013). These may imply that the various lines and groups identified may be useful in *C. partellus* borer's specific breeding programmes for the formation of hybrids. In addition, the results show that for lines that have good general combining ability, probably, all new testers with new genetic structures may be able to distinguish them for *C. partellus* resistance (Morais and Pinheiro, 2012).

The high genetic variability detected for *C. partellus* resistance in the testcross hybrid's clustering with the two testers is a desirable characteristic for a good maize tester. However, some lines that did not show any heterotic orientation with both testers. Tentatively, these lines may be useful in breeding; however, in practice they would be discarded. The heterotic orientations identified based on specific combining effects suggest need for new testers with new genetic structures since continuous introduction of new and diverse germplasm into breeding programs may render some testers insensitive to discriminating materials. Similar observations have been reported in previous studies (Fato et al., 2012; Guimaraes et al., 2012).

In addition, the grain yield observed under *C. partellus* infection was 1.2 t ha⁻¹, which is even lower than the Kenya's national average which is 1.5 t ha⁻¹. This is a reflection of the impact *C. partellus* can have on grain yield during an epidemic.

Conclusions

Genetic combining ability effects were significant for *C. partellus,* stem borers resistance. The results suggest that additive gene effects were most important in the control of resistance for *C. partellus,* stem borers. It is possible to identify specific lines that may be useful for hybrid breeding for specific ecologies where these borers occur exclusively or in league. For *C. partellus* resistance traits and grain yield the specific combining ability effects were significant demonstrating that non-additive gene effects were also essential in explaining variations in the borer resistance traits and grain yield.

The testers CML312/CML442 and CML395/CML444 were able to discriminate these materials based on the general and specific combining ability for stem borer resistance, agronomic characters and grain yield. This implies that the single cross testers from CIMMYT can be used for line evaluations in breeding programmes for the identification of heterotic orientations in a stem borer resistance hybrid breeding programme. However, in this study there was a high number of lines which could not be classified based on testers CML312/CML442 and

CML395/CML444. Consequently, there is a need for new testers with new genetic structures since continuous introduction of new and diverse germplasm into breeding programs may render some testers insensitive to discriminating materials.

Using specific combining ability effects various heterotic orientations identified lines that showed positive significant SCA effects for C. partellus resistance and grain yield. The genotypes indicating high desirable GCA and SCA effects and with heterotic orientations that are favourable for grain yield may be deployed in breeding programmes across Kenya with emphasis on stem borers where these borers occur exclusively or in league. The corollary is that these superior lines may be used in hybrid pedigree breeding programmes that focus on C. partellus stem borer resistance in the tropics. The products from the line x tester evaluations were three way cross hybrids that can be nominated directly into the national performance trials for further evaluation and deployment into maize growing areas where these borers occur exclusively or in league with other stem borers.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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