Selection efficiency of tunnel length and stalk breakage to obtain maize inbred lines resistant to stem borer attack

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Abstract

There is not any conclusive result about the most suitable trait for performing pedigree selection for improving maize performance against corn borer attack: tunnel length or stalk breakage. We have used simultaneously both selection traits in the same genetic backgrounds and the objective of the present work has been to compare the suitability of pedigree selection for tunnel length and stalk breakage in order to obtain inbred lines that *per se* and in hybrid combination show lower stem damage and good agronomic performance. Tunnel length is better as selection criteria for increasing resistance to corn borers, but, in some genetic backgrounds, genetic linkage between yield and stalk tunneling by stem borers could make that selection for reduced tunnel length resulted in significant yield reductions.

Introduction

Pedigree selection has been and remains the backbone of hybrid maize breeding (Duvick 2005). It was performed not only for increasing yield but for broadening the genetic base of cultivated maize and improving other agronomical traits such as adaptation, and resistance to drought stress, diseases and pests (Mayfield et al. 2012; Ordás et al. 1994; Panouille et al. 1998; Tarter et al. 2003; Carena et al. 2009; Reid et al. 2009).

Efforts to develop selection inbred lines resistant to the first or second generation of corn borers by pedigree or backcross selection have been successful (Russell et al. 1975; Abel et al. 2000a; Abel et al. 2000b; Willmot et al. 2005; Butron et al. 2006). However, transfer of second-generation corn borer resistance from an American inbred line to adapted early European inbred lines has failed probably due to linkage between resistance to stem tunneling by borers and unfavorable characters which have been systematically contra selected (Panouille et al. 1998). Consequently, pedigree selection in Central Europe has been made for tolerance rather than for resistance using a discontinuous scale that takes into account stalk breakage (1 = little damage, 2 =broken tassel, 3 =broken tassel and upper leaves, 4.5 = broken upper stalk and 9 = broken stalk under the ear)(Panouille et al. 1998). Therefore, there is not any conclusive result about the most suitable trait for performing pedigree selection for improving maize performance against corn borer attack: tunnel length or stalk breakage. We have used simultaneously both selection traits in the same genetic backgrounds and the objective of the present work has been to compare the suitability of pedigree selection for tunnel length and stalk breakage in order to obtain inbred lines that per se and in hybrid combination show low stem damage by the Mediterranean corn borer (MCB, *Sesamia nonagrioides* Lef.) and good agronomic performance.

Material and methods

Genetic materials and breeding methodology

Ten second-cycle inbred lines were obtained from each cross: A671 x A295, B93 x Oh43 and B98 x W572. A671, A295, B93 and Oh43 are classified as Lancaster inbred lines, W572 is Reid and B98 is mixed because it cannot be classified as a typical Lancaster inbred because it was developed from a diverse composite, BS11 FR C5, but it behaves better in crosses to the Iowa Stiff Stalk Synthetic (BSSS) than in crosses to Lancaster (Hallauer et al. 1994).Inbred lines involved in these crosses had shown resistance to the Mediterranean stem borer (Butrón et al. 1999a; Butrón et al. 2006). The parental lines were chosen to obtain second cycle inbreds that combine resistance and good agronomic performance. In 2004, the F_1 were selfed to obtain the corresponding F_2 populations; in 2005, 300 plants from each F_2 population were planted, selfed and infested with approximately 40 MCB eggs per plant. At harvest, two traits were used for selection were applied: plants with the least tunnel length (stems were dissected and the lengths of tunnels made by borers were measured, TL method) and plants that did not show stalk breakage conditions (SB method). Twenty-five F_3 families (ears) were selected based on tunnel length and 25 based on stalk lodging. In 2006, each selected F_3 family was planted in a row with 15 two-kernel/hills, selfed, infestations were performed, and 15 F_4 families were selected attending to each selection method. In 2007 and 2008, 10 F_5 and 5 F_6 families, respectively, were selected. In 2009, the 30 experimental inbred lines (5 inbred line × 2 selection criteria × 3 crosses) were multiplied and, in 2010, they were crossed to two inbred testers. The tester was Reid when experimental inbreds were derived from Lancaster materials (A671 x A295 and B93 x Oh43) and Lancaster when inbreds were derived from the B98 x W57 cross. A scheme of the method used to obtain inbred lines from A671 x A295 cross is showed in figure 1. Similar methods were used in the other two crosses

Field evaluations

Inbreds and hybrids were evaluated in adjacent split-plot trials with two replications at two locations in 2011. Locations are 10 km far, they have different orientation and only one was irrigated. Crosses were the main plot while inbreds derived from the same cross were randomly allocated in subplots. In the hybrid trial, subplots consisted of two rows spaced 0.80 m apart with 17 plants within each row spaced 0.18 m apart; while, in the inbred trial, subplots consisted of one row with 15 plants. Plots were overplanted and thinned to obtain a final population density of about 70,000 plants ha^{-1}. At flowering, 10 adjacent and competitive (equally spaced apart from adjacent plants) plants per subplot were infested by placing egg masses of about 40-50 MCB eggs between the upper ear and the stem. The MCB rearing method used has been described by Eizaguirre & Albajes (1992).

Observations were recorded for days to pollen shed (days from planting when 50% of plants had shed pollen), days to silking (days from planting to when 50% of plants had silks emerged), plant height (recorded on ten competitive plants as the distance from the ground to the top of the plant), stalk lodging(percentage of plants in the plot with the stem broken below the main ear), root lodging (percentage of plants in the plot leaning more than 45º to the vertical), kernel moisture (g of water in 100g of kernels), yield (Mg ha^{-1} of kernels at 140 g H_2O kg^{-1}), tunnel length (total length in cm per plant of stem tunnels made by borers), and visual ratings for kernel, and shank damages (on a 9 point subjective scale determined as follows: 1 > 90% damage, 2 = 81 to 90% damage, 3 = 71 to 80% damage, 4 = 61 to 70% damage, 5 = 41 to 60% damage, 6 = 31 to 40% damage, 7 = 21 to 30% damage, 8 = 1 to 20% damage, and 9 = no damage).

Combined analyses of variance were performed with the GLM procedure of SAS. Location and replication were considered random effects and method and genetic background fixed effects. Each combination method-genetic background consisted in a random sample of five and ten (five inbreds crossed to two testers) genotypes for the inbred and hybrid trials, respectively. Comparisons of means were computed using the Fisher's Least Significant Difference. Finally, mean comparisons among experimental hybrids and two hybrid checks (PR36B08 and PR34G13) were performed.

Results

In the inbred experiments, there were significant differences between selection efficiency for stalk lodging, but the method x background interaction was significant for important traits such as days to pollen shedding, shank damage and tunnel length (Table 1). Similarly, in the hybrid experiments, the method x background interaction was significant for relevant traits like days to pollen shedding, kernel moisture and yield (Table 2), while differences between methods were only significant for tunnel length. Therefore mean comparison between methods was separately made for each genetic background, except for stalk lodging and tunnel length in the inbred and hybrid trials, respectively.

The average stalk lodging across genetic backgrounds of inbreds selected for reduced tunnel length was less than the average of inbreds selected for stalk lodging (Table 3). Selection for tunnel length was also more efficient than selection for stalk lodging in achieving less damage by borers among Lancaster materials, while the opposite was observed for inbreds derived from B98 x W572 cross although in the latter germplasm group the differences for shank damage and tunnel length were not significant (Table 3 and Figure 2). Parental lines means are also showed in Table 3.

The hybrids of inbreds selected for reduced tunnel length presented reduced tunnel length compared to hybrids of inbreds obtained by selection for stalk lodging (Table 4 and Figure 2). However, differences for hybrid yield between selection criteria greatly depended on the genetic background; selection for tunnel length was beneficial for yield compared to selection for stalk lodging when performed in materials derived from A671 x A295, detrimental in those derived from B93 x Oh43, and there were not significant differences for yield between selection methods when used in lines derived from B98 x W572 (Table 4 and Figure 3).

Hybrids of experimental inbreds developed by both methods were compared with hybrid checks for agronomical traits. There was an inbred, EP105, that in both hybrid combinations showed values for agronomic performance comparable to those presented by hybrid checks (Table 5).

Discussion

As expected, selection for stalk strength rendered similar results for stem tunneling to selection for tunnel length when performed in segregating materials from the cross B98xW572. The inbred W572 is derived from the Stiff Stalk Synthetic and B98 is partially related to this population and previous studies had shown a good relationship between stalk strength and resistance to corn borers among Stiff Stalk Synthetic materials (Butron et al. 2002; Martin et al. 2004). However, among segregating Lancaster materials, tunnel length was more suitable as selection trait for reducing damage by borers than stem breakage agreeing with the idea that resistance mechanisms other than stalk strength are involved in the stem resistance to corn borers (Butron et al. 2002). Among inbreds developed from the same inbred cross, differences between selection methods for shank damage were similar to differences for tunnel length, suggesting the same mechanisms could be involved in shank and stem resistance.

Inbreds selected for tunnel length rendered less stalk lodging than inbreds selected for stalk strength probably because stem tunneling by borers is the main cause of stalk lodging under the high borer pressure obtained by performing artificial infestation. However, under low borer pressure other factors besides borer damage should account for stem breakage making direct selection for stalk strength more efficient.

Hybrids of inbreds obtained by selecting for reduced tunnel length showed less stem damage by borers than the hybrids of inbreds obtained by selecting for increased stalk strength, independently of the background. Since differences for selection criteria were significant when hybrids were analyzed regardless genetic background (Table 5). Therefore, the improvement achieved for resistance to stem tunneling through inbred selection is transmitted, in general, to hybrids confirming that the inheritance of stem tunneling is basically under additive control (Butron et al. 1999b; Cartea et al. 1999). However, differences for hybrid yield between selection criteria greatly depended on the genetic background, making impossible to choose the best selection criterion for yield across genetic backgrounds.

In general, tunnel length is better as selection criteria for increasing resistance to corn borers, but, in some genetic backgrounds, genetic linkage between yield and stalk tunneling by stem borers could make that selection for reduced tunnel length resulted in significant yield reductions (Schulz et al. 1997; Kreps et al. 1998; Butrón et al. 2012). Pedigree selection for reduced tunnel length was successful for developing inbreds, such as EP105, that in hybrid combinations were comparable to hybrid checks for agronomic performance.

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Table 1. Mean squares of the analysis of variance of maize inbreds obtained using two different selection criteria from three backgrounds evaluated in two locations in 2011.

Source of variation	df	Stalk lodging	Root lodging	Days to Pollen shedding	Days to silking	Plant height	Kernel moisture	Yield	Kernel damage	Shank damage	Tunnel length
Background (B)	2	1005	359*	7	98	8357*	65	6.24	0.14	1.94	1101
B x Location (L)	2	974*	98	10	51	257	105	5.15	3.17	2.07	246
Replication x B (L)	4	121	34	31	32	159	8	3.39	1.32	1.10	301
Criteria (C)	1	704*	3	4	39	2761[1]	23	0.06	0.07	3.03	475
L x C	1	421	55	5	1	115	18	4.15	0.11	3.43	30
B x C	2	162	88	54**	42	1189	26	1.95	0.54	9.68*	401*
B x L x C	2	99	91	2	4	178	18	1.94	0.92	1.05	47
Error	100	149	137	10	15	709	18	1.94	1.26	2.28	121

*, ** Significant at 0.05 and 0.01 probability level, respectively.

[1] Significant at 0.0511 probability level.

Table 2. Mean squares of the analysis of variance of maize hybrids derived from inbred lines obtained using two different selection criteria methods from three backgrounds evaluated in two locations in 2011.

Source of variation	df	Stalk lodging	Root lodging	Days to Pollen shedding	Days to silking	Plant height	Kernel moisture	Yield	Tunnel length
Background (B)	2	116	4.18	6.61	5.36*	2335	129**	8.16	4029
B x Location (L)	2	46	14.12	-	-	412	1	10.46	3237**
Replication x B (L)	4	21	7.24	0.53	0.11	1012	5	1.84	164
Criteria (C)	1	1	0.03	7.01	12.68	26	3	0.65	1290*
L x C	1	129	9.14	-	-	103	4	1.63	565
B x C	2	45	24.28	13.86*	8.13	527	13*	74.78**	8
B x L x C	2	16	40.65	-	-	142	9	0.20	8
Error	222	37	14.12	3.62	4.28	338	4	4.70	183

*, ** Significant at 0.05 and 0.01 probability level, respectively.

Table 3. Means of the inbred lines obtained by selecting for tunnel length (TL) or stalk breakage (SB) in three different backgrounds and evaluated in two locations in 2011. Means of the parental lines are correspondingly included.

Experimental inbred background	Method	Stalk lodging (%)	Root lodging (%)	Days to pollen shedding (days)	Days to silking (days)	Plant height (cm)	Kernel moisture (%)	Yield (Mg ha^{-1})	Kernel damage (1-9)[1]	Shank damage (1-9)[1]	Tunnel length (cm)
A671xA295	TL	3.4 a	3.5 a	77 a	80 a	157 a	27.0 a	2.48 a	7.9 a	5.9 a	23 b
	SB	6.8 a	1.7 a	77 a	81 a	159 a	30.1 a	2.06 a	7.7 a	5.3 a	30 a
	A671	7.1 A	0.0 A	75 A	76 A	175 A	22.0 A	3.2 A	8.4 A	7.1 A	9 A
	A295	0.0 A	0.0 A	79 B	82 B	140 B	33.7 B	0.9 B	6.4 B	4.2 B	14 B
B93xOh43	TL	8.7 a	0.0 a	75 b	75 b	184 a	30.3 a	2.94 a	7.9 a	5.9 a	21 b
	SB	17.8 a	3.3 a	78 a	79 a	165 b	31.8 a	3.35 a	7.8 a	4.8 b	27 a
	B93	31.7 A	0.0 A	75 A	75 A	140 A	30.5 A	3.86 A	8.1 A	5.7 A	25 A
	Oh43	0.0 A	12.5 A	77 A	78 A	152 A	31.1 A	3.26 A	8.3 A	5.8 A	20 A
B98xW572	TL	3.0 a	8.4 a	77 a	79 a	194 a	28.9 a	2.89 a	7.8 a	5.4 a	35 a
	SB	4.5 a	6.4 a	76 a	78 a	180 a	28.3 a	2.63 a	8.0 a	6.2 a	32 a
	B98	0.0 A	16.7 A	81 A	83 B	214 A	31.9 B	3.49 A	8.6 A	6.9 A	31 A
	W572	4.2 A	11.9 A	77 A	78 A	166 B	25.2 A	3.21 A	8.2 A	6.1 A	27 A

Across	TL	5.0 b	4.0 a	76 a	78 a	178 a	28.8 a	2.78 a	7.9 a	5.7 a	26 a
	SB	9.7 a	3.8 a	77 a	79 a	168 a	30.1 a	2.69 a	7.8 a	5.4 a	30 a
	Mean	7.4	3.9	77	78	173	29.5	2.7	7.8	5.6	28

Within each genetic background and trait, means followed by the same letter did not differ significantly at 0.05 probability level. Lowercase letters compare selection criteria. Capital letters compare parental lines.

[1] visual ratings for kernel, and shank damages on a 9 point subjective scale determined as follows: 1 > 90% damage, 2 = 81 to 90% damage, 3 = 71 to 80% damage, 4 = 61 to 70% damage, 5 = 41 to 60% damage, 6 = 31 to 40% damage, 7 = 21 to 30% damage, 8 = 1 to 20% damage, and 9 = no damage.

Table 4. Means of the crosses between maize tester and experimental inbred lines obtained by selecting for tunnel length (TL) or stalk breakage (SB) in three different backgrounds and evaluated in two locations in 2011.

Experimental inbred background	Method	Stalk lodging (%)	Root lodging (%)	Days to pollen shedding (days)	Days to silking (days)	Plant height (cm)	Kernel moisture (%)	Yield (Mg ha^{-1})	Tunnel Length (cm)
A671xA295	TL	4.3 a	2 a	68 a	69 a	282 a	27 b	12.2 a	17 a
	SB	2.5 a	2 a	69 a	70 a	281 a	28 a	10.5 b	23 a
B93xOh43	TL	5.7 a	2 a	68 b	69 b	273 a	29 a	10.4 b	14 a
	SB	6.0 a	1 a	70 a	71 a	277 a	29 a	12.4 a	19 a
B98xW572	TL	4.2 a	1 a	69 a	69 a	274 a	26 a	11.1 a	28 a
	SB	5.3 a	2 a	68 a	69 a	268 a	26 a	10.5 a	32 a
Across	TL	4.7 a	2 a	68 a	69 a	276 a	27 a	11.2 a	20 b
	SB	4.6 a	2 a	69 a	70 a	276 a	27 a	11.1 a	25 a
	Mean	4.7	2	69	69	276	27	11.2	22

Within each genetic background and trait, means followed by the same letter did not differ significantly at 0.05 probability level.

1 Table 5. Means of maize hybrid checks and the outstanding experimental inbred in hybrid combination for

2 agronomical traits evaluated at two locations in 2011. Hybrid checks PR36B08 and PR34G13.

Experimental inbred	Selection method	Background	Hybrid	Days to silking (days)	Plant height (cm)	Tunnel length (cm)	Yield (Mg ha^{-1})	Kernel moisture (%)
EP2008-30	TL	A671xA295	EP105 x Tester 1	70	312	15.7	15.0	25.8
EP2008-30	TL	A671xA295	EP105 x Tester 2	71	293	20.2	13.3	25.9
			PR36B08	67	247	13.2	12.8	25.9
			PR34G13	70	275	18.6	14.9	25.3
LSD (P≤0.05)				3	19	-	2.3	2.2

Figure 1. Method for obtaining inbred lines from A671 x A295 using two selection criteria: tunnel length (TL) and stalk breaking (SB). MCB: Mediterranean Corn Borer.

Year	Genotype		Task
2004	(A671 × A295)F_1		Selfing
2005	(A671 × A295)F_2 300 plants		Selfing Infestation with MCB eggs
2006	(A671 × A295)F_3 25 families TL method	(A671 × A295)F_3 25 families SB method	Selfing Infestation with MCB eggs
2007	(A671 × A295)F_4 15 families TL method	(A671 × A295)F_4 15 families SB method	Selfing Infestation with MCB eggs
2008	(A671 × A295)F_5 10 families TL method	(A671 × A295)F_5 15 families SB method	Selfing Infestation with MCB eggs
2009	(A671 × A295)F_6 5 inbred lines TL method	(A671 × A295)F_6 5 inbred lines SB method	Selfing for multiplication
2010	5 TL inbred lines × 2 testers	5 SB inbred lines × 2 tester	Crossing inbreds by tester

Figure 2. Means for tunnel length (cm) of inbred lines *per se* and crossed to two testers (hybrid 1 and hybrid 2) in three different backgrounds and evaluated in two locations in 2011. Inbreds were obtained by selecting for tunnel length (TL) or stalk breakage (SB). LSD for inbreds= 13 cm; LSD for hybrids= 2 cm.

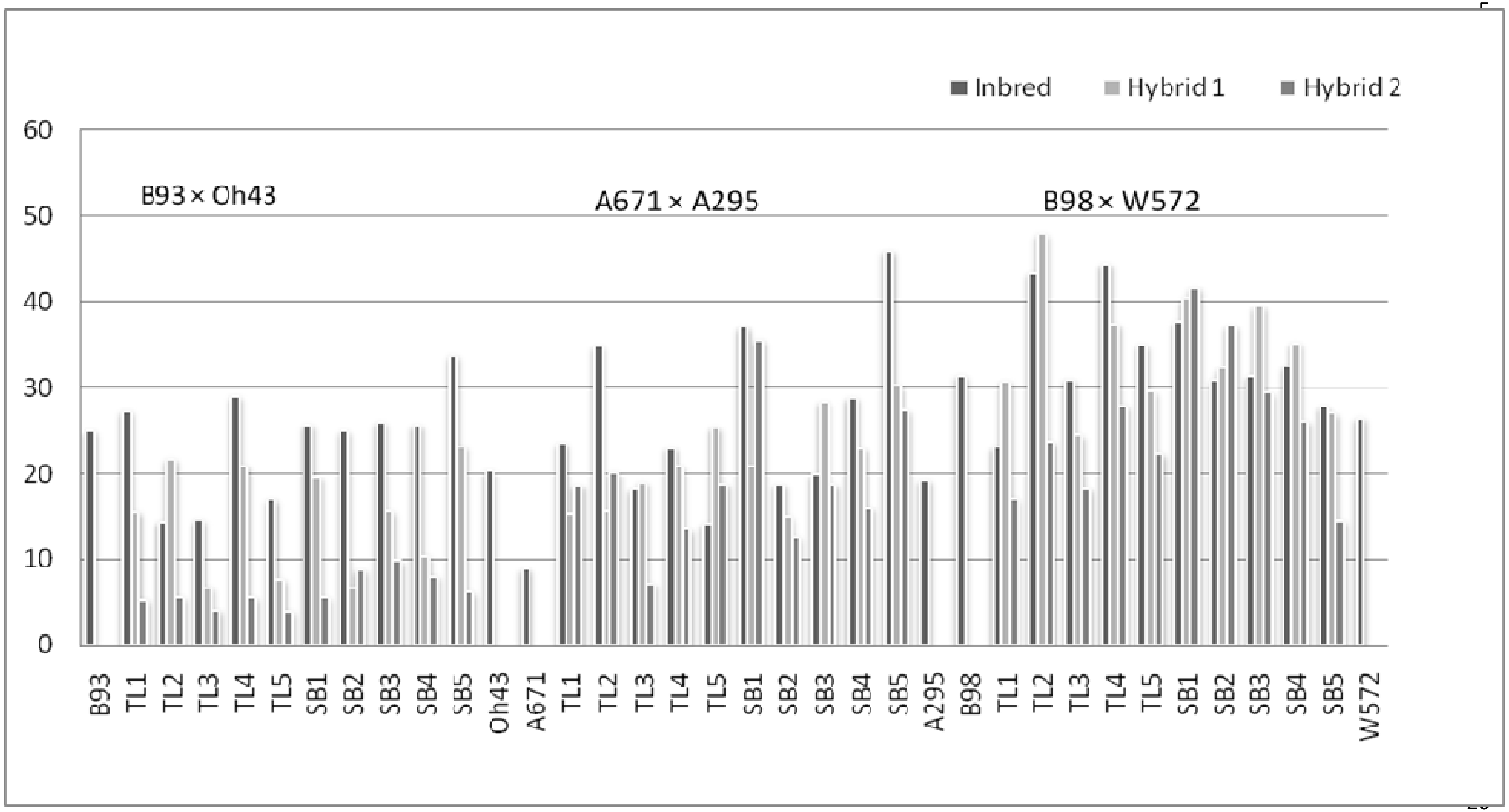

Figure 3. Yield (t/ha) of crosses between inbred lines obtained by selecting for tunnel length (TL) or stalk breakage (SB) and two testers (hybrid 1 and hybrid 2) in three different backgrounds and evaluated in two locations in 2011. Hybrid checks PR36B08 and PR34G13. LSD=2.3 t/ha.