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Assessment of testcross performance and genetic diversity of yellow endosperm maize lines derived from adapted × exotic backcrosses

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Abstract Introduction of exotic maize (*Zea mays* L.) into adapted tropical germplasm may enhance genetic variability and lead to greater progress from selection. The first objective of this study was to determine if yellow endosperm lines derived from adapted × exotic backcrosses contain exotic alleles that are superior to the recurrent adapted parental line for yield and other agronomic traits in tropical environments. Thirteen exotic yellow maize inbred lines were crossed to an adapted orange line (KUSR) and the F_1s were backcrossed to KUSR to generate the first backcrosses. Fifty BC_1F_4 lines derived from these backcrosses and the recurrent parent were crossed to a common inbred tester (L4001) to form testcrosses, which were evaluated at eight environments in Nigeria. Testcrosses of the BC-derived lines differed significantly for grain yield and other agronomic traits. Only two testcrosses yielded significantly less than L4001 × KUSR, with the best 15 testcrosses producing between 289 and 1,056 kg/ha more grain yield than L4001 × KUSR. The best testcrosses were similar to or better than L4001 × KUSR for other agronomic traits. The second objective of this study was to assess the extent of genetic diversity present among the BC-derived lines. We genotyped 46 BC-derived lines including KUSR and L4001 with 10 AFLP primer pairs and found 491 polymorphic fragments. The average allelic diversity of the lines was 0.30 ± 0.01. The genetic distance of each BC-derived line from KUSR ranged between 0.49 and 0.91. The average genetic distance for all pairs of the BC-derived lines was 0.68 ± 0.004, varying from 0.34 to 0.92. The increased grain yield and genetic diversity observed in these studies provide evidence that exotic germplasm can contribute new alleles to expand the genetic base of tropical maize and develop high-yielding hybrids.

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Introduction

Maize (*Zea mays* L.) is one of the major staple food crops in West and Central Africa and is grown in diverse environments characterized by high incidence of biotic and abiotic stresses (Fajemisin et al. 1985). The development of maize varieties and hybrids with high yield potential and improved adaptation to the major stresses is important to increasing productivity in the diverse production environments. Useful maize inbred lines, varieties, and hybrids have been commonly selected from adapted tropical germplasm for the different growing environments in West and Central Africa (Efron 1985; Fajemisin et al. 1985; IITA 1992). However, broadening and diversifying the genetic base of adapted maize germplasm through the introduction of new genetic variation can further enhance progress from selection for both grain yield and stability of performance. Exotic germplasm has been suggested as potential source of new beneficial alleles for introgression into adapted germplasm to increase the variability in qualitatively and quantitatively inherited traits (Wellhausen 1965; Goodman 1985; Echandi and Hallauer 1996). Hallauer and Miranda Fo (1988) defined exotics as germplasm having limited direct use without improvement for adaptation. In tropical areas of West and Central Africa, local landraces, temperate and introduced tropical germplasm can be potential sources of unique alleles useful for breeding programs (Efron 1985).

The potential usefulness of exotic germplasm for maize improvement in the temperate areas was review by Goodman (1999). Several studies reported that some

testcrosses of lines derived from tropical hybrids and temperate × tropical crosses were competitive in yielding ability with commercial hybrids in the USA (Godshalk and Kauffmann 1995; Uhr and Goodman 1995b; Holland and Goodman 1995; Tallury and Goodman 1999; Goodman et al. 2000). Exotic maize germplasm has also been a useful source of alleles for resistance to diseases (Kramer and Ullstup 1959) and insect pests (Sullivan et al. 1974) and for broadening the genetic base of temperate germplasm (Goodman 1999). Efron (1985) reviewed the potential use of exotic germplasm for improving grain yield and other agronomic traits in tropical Africa and concluded that its use had been poorly documented because of the limited efforts to incorporate exotic germplasm into breeding populations and the lack of adaptation of exotic germplasm to tropical climate and its susceptibility to diseases and insects. He suggested that exotic germplasm could be used as potential heterotic partner to adapted tropical germplasm in Africa for maximizing heterosis in hybrids.

Eberhart et al. (1995) proposed the use of elite exotic germplasm with high yield potential and resistance to diseases and insects as a good strategy for integrating genetic diversity into maize breeding populations. Inbred lines have been regarded as the most promising sources of exotic germplasm because they should be free from deleterious recessive alleles and possess desirable traits fixed through several generations of inbreeding and selection (Goodman 1999). Also the improved plant type and agronomic traits of exotic maize inbred lines could be integrated into adapted tropical inbred lines fairly quickly to improve performance of hybrids and synthetic varieties. Thirteen yellow endosperm maize inbred lines from Hawaii (Brewbaker et al. 1989) were thus used as sources of exotic germplasm for developing new inbred lines, which were included in this study. The first objective of this study was to determine if the yellow endosperm lines derived from adapted × exotic backcrosses contain exotic alleles that are superior to a recurrent adapted parental line for yield and other agronomic traits in tropical environments. The second objective of this study was to use AFLP markers to determine the level of genetic diversity retained in the yellow endosperm backcross-derived lines after three generations of inbreeding with selection. Combining estimates of genetic distance (GD) with data on agronomic performance of testcrosses may facilitate the successful use of the backcross-derived lines to increase genetic diversity and improve performance of broadly adapted cultivars.

Materials and methods

Genetic materials

A total of 101 inbred lines received from the University of Hawaii under the Maize Inbred Resistance program (Brewbaker et al. 1989) were grown at Ibadan (3°58′E, 7°22′N, altitude of 150 m) in Nigeria in 1996. Among these, 13 yellow endosperm inbred lines with good standability, short plant type, low ear placement, and well-filled ears were selected as exotic parents and crossed to an adapted orange endosperm inbred line (KUSR). KUSR is an inbred line converted for resistance to the maize streak virus through four generations of backcrossing to a line introduced from Thailand (KU1414). The donor parent for resistance to the maize streak virus during conversion was a yellow endosperm maize inbred line, L4001. KUSR is also resistant to southern corn leaf blight (*Bipolaris maydis* (Nisikado & Miyake) Shoemaker) and southern corn rust (*Puccinia polysora* Underw) and Curvularia leaf spot (*Curvularia lunata* (Wakk)). As shown in Table 1, the selected exotic inbred lines originated from nine breeding programs with diverse genetic backgrounds. Each F_1 was backcrossed once to KUSR at Ibadan in 1996/1997 to generate the first backcross (BC_1). From 1997 dry season to 1999 rainy season, ear-to-row selection was made to develop inbred lines from each BC_1 population. At each generation of inbreeding, visual selection within and among lines was made on the basis of synchrony between pollen shed and silking, low ear placement, well-filled ears and resistance to lodging and diseases, including southern corn rust, southern corn leaf blight, and Curvularia leaf spot, under naturally occurring disease pressure at Ibadan. A total of 50 uniform lines (BC_1F_4) derived from the 13 adapted × exotic backcross populations, which are hereafter referred to as BC-derived lines, were selected for this study (Table 1).

Field trials

Each backcross-derived yellow endosperm line was crossed to an elite inbred tester, L4001, which is a heterotic partner to the adapted recurrent parent (KUSR), to generate 50 testcrosses during the 2000 dry season. L4001 was derived from a cross between a maize population from CIMMYT, Sete Lagoas 7728, and an IITA maize streak virus resistant population, TZSR. L4001 is late maturing with flint endosperm texture and good general combining ability. It has been extensively used as a tester to characterize the heterotic patterns of yellow endosperm maize inbred lines emanating from the IITA maize breeding program. A trial composed of the 50 testcrosses and a hybrid between KUSR and L4001, a commercial hybrid marketed in Nigeria as Oba Super II, was evaluated at Bagauda (8°19′E, 12°2′N, altitude 520 m), Ikenne (3°42′E, 6°54′N, altitude 30 m), Saminaka (8°39′E, 10°34′N, altitude 760 m), and Zaria (7°21′E, 11°7′N, altitude 640 m) in Nigeria, in 2000 and 2001. The hybrids were arranged in a randomized complete block design with two replications and were planted in single row plots, 5 m long with 0.75 m between rows and 0.50 m between plants within a row. Within a row, three seeds were planted in a hill and thinned to two plants after emergence to attain a

Table 1 List of exotic inbred lines and an adapted recurrent parent used as parents of the BC-derived lines as well as an inbred tester used to generate testcrosses

Inbred lines	Parentage[a]	Origin	Number of BC-derived lines	BC-derived lines designation
Exotic parent				
B37(Hi)[b]	Iowa stiff stalk synthetic	Iowa	6	B37-1–B37-6
CIMA 21	Poza Rica 698-149#	CIMMYT	6	CIM21-1–CIM21-6
CM 116	Puerto Rico Gr.1	India	3	CM116-1–CM116-3
CM 117	Cuba 11J-A46	India	5	CM117-1–CM117-5
CMA 103(Hi)[b]	Colombia 1 × 38-11	India	2	CM103-1–CM103-2
Fla 2AT 116	–	Florida	3	FLA116-1–FLA116-3
Fla 2AT 98	–	Florida	1	FLA98-1
Hi 28[c]	Peru333##329c#	Hawaii	7	HI28-1–HI28-7
Hi 29[c]	Cuba342-2-2-#	Hawaii	3	HI29-1–HI29-3
ICAL 36	–	Colombia	6	CAL36-1–CAL36-6
MIT2-S6	Mi2 (Phil.)	Thailand	2	MI2S6-1–MI2S6-2
SC 43	SC246C × Pioneer 3009	South Carolina	4	SC42-1–SC43-4
VA 35(Hi)[b]	(C103 × T8) × T8	Virginia	2	VA35-1–VA35-2
Adapted parent				
KU1414-SR	$(KU1414)^5$/4001BC	IITA		KUSR
Tester line				
4001	(TZSR × 7,728)BC	IITA		L4001

[a]Source of parentage (Brewbaker et al. 1989)
[b]Inbred lines converted to the Mv gene for resistance to maize mosaic virus in Hawaii
[c]Inbred lines developed in Hawaii

population density of 53,000 plants/ha in each trial. A compound fertilizer was applied at the rates of 60 kg N, 60 kg P, and 60 kg K/ha at the time of sowing. An additional 60 kg N/ha was applied as top dressing four weeks later. In the testcross trial, Atrazine and Gramoxone were applied as pre- and post-emergence herbicides at 5 l/ha each of Primextra and Paraquat, respectively. Subsequent manual weeding was done to keep the trials weed-free.

Days from planting to anthesis and silking were recorded in each plot as the number of days from planting to when 50% of the plants were shedding pollen and displaying visible silks, respectively. Plant and ear heights were measured in centimeter as the distance from the base of the plant to the height of the first tassel branch and the node bearing the upper ear, respectively. Plant aspect was rated on a scale of 1–5, where 1 = excellent overall phenotypic appeal and 5 = poor overall phenotypic appeal. Ear aspect was scored on a 1–5 scale, where 1 = clean, uniform, large, and well-filled ears and 5 = rotten, variable, small and partially filled ears. Southern corn rust, southern corn leaf blight, and Curvularia leaf spot were scored at Ikenne for a season on a scale of 1–5, where 1 = slight leaf infection and 5 = severe leaf infection. All ears harvested from each plot were weighed and representative samples of ears were shelled to determine percent moisture. Grain yield adjusted to 15% moisture was computed from ear weight assuming a shelling percentage of 80%.

Laboratory analyses

We included 46 of the 50 BC-derived yellow endosperm inbred lines, the adapted recurrent parent and the inbred tester in the diversity study (Table 1). For each inbred line, bulk samples of 15–20 seedlings grown in a screen house and harvested 10 days after planting were frozen and ground into fine powder (Table 1). The DNA was extracted using the CTAB procedure (Saghai Maroof et al. 1994). The AFLP analysis was performed following the standard procedure described by Vos et al. (1995). Genomic DNA (0.3 μg) of each line was digested with a pair of restriction enzymes (*Eco*RI and *Mse*I) and ligated to double stranded adapters. The ligate was preamplified with nonselective primers and selective amplification was carried out using pairs of selective primers with *Eco*RI/*Mse*I extensions (AAC/CAC, AGC/CAA, ACC/CTC, ACT/CTA, ACA/CAC, ACG/CAT, ACA/CAG, ACT/CTA, AGG/CTA, AGG/CTT). The products were separated on polyacrylamide gel and were silver stained using the instructions from Promega (Madison, WI). Only polymorphic bands with strong intensity were scored manually in binary form as 1 or 0 for their presence or absence in each line.

Statistical analysis

Analyses of variance combined over environments were computed for each trait with PROC GLM in SAS (SAS Institute 2000) using a RANDOM statement with the TEST option. In the combined analysis of variance, testcrosses were considered as fixed effects for each trait, while replications and location–year combinations, hereafter referred to as environments, were considered as random effects. Correlation coefficients were calculated between pairs of agronomic traits using PROC CORR in SAS (SAS Institute 2000).

Frequencies of polymorphic fragments detected with AFLP primer pairs were calculated for the 48 yellow endosperm maize lines. As AFLPs are dominant markers, only two states (present and absent) can be distinguished at each band position assuming that each band position corresponds to a locus with two alleles. Allele diversity was estimated as AFLP marker

diversity based on Nei's (1987) gene diversity as: $h = n(1 - \sum x_i^2)/(n-1) = (2pqn)/(n-1)$, where x_i was the allele frequency at the ith locus, p was the frequency of presence, and q was the frequency of absence of AFLP fragments among n inbred lines for the ith AFLP marker. Genetic similarities (GS) between pairs of yellow endosperm inbred lines were calculated from the 491 AFLP fragments with a SAS macro (Mumm and Dudley 1995) based on the formula developed by Jaccard (1908). GD estimates were computed from GS as GD = 1 − GS. Standard deviation (SD) of GD estimates between each line and KUSR was calculated using the formula of Bar-Hen and Charcosset (1994).

$SD = \sqrt{GD(1-GD)/N}$,where N is the number of polymorphic bands and GD is the genetic distance between each line and KUSR. The 95% confidence interval for GD estimates between each line and KUSR was then calculated as $Z_{\alpha/2} \times SD$, where $Z_{\alpha/2}$ is the value of standardized normal with $\alpha/2$ probability.

Dendrograms were constructed from the GD matrix by UPGMA and neighbor jointing clustering methods with NTSYS-pc package (Rohlf 1998) to visualize the patterns of diversity among the 48 yellow endosperm lines. Bootstrap analysis was performed using the software package "WinBoot" developed at IRRI with the number of iterations set at 1,000 (Yap and Nelson 1996). The cophenetic correlation coefficient (Rohlf and Fisher 1968) was calculated, and Mantel's test (Mantel 1967) was performed to check the goodness of fit of the UPGMA clustering to the GD matrix on which it was based using the appropriate routines of NTSYS-pc package (Rohlf 1998).

Results

Field performance of testcrosses of BC-derived yellow endosperm maize lines

Performance evaluation of testcross of the BC-derived yellow endosperm maize lines was carried out at test locations representing a broad range of climatic conditions (Menkir et al. 2000). Mean grain yields of the test environments averaged over testcrosses varied from 3,593 to 7,491 kg/ha. As shown in Table 2, the combined analyses of variance found significant environmental effects on all agronomic traits measured except plant aspect. Environmental effects were large on days to anthesis, days to silking, plant height, ear height, and grain yield and were small on other traits. Despite the wide range of environments in which the trial was grown, the testcrosses × environment interaction mean square was significant only for days to anthesis and days to silking (Table 2). Testcrosses of the BC-derived lines exhibited significant differences for all agronomic traits including resistance to foliar diseases. The testcross sum of squares accounted for 3–15% of the total variation for agronomic traits and 66–72% of the total variation for disease scores (Table 2).

One method of evaluating the potential of the BC-derived lines to enhance productivity in hybrids is to compare the performance of their testcrosses with the performance of the recurrent parent testcross to the same inbred tester (L4001 × KUSR). This hybrid is currently marketed as a commercial hybrid (Oba Super-II) in Nigeria. Mean grain yields of individual testcrosses of the BC-derived yellow endosperm lines averaged over eight environments varied from 5,040 to 6,753 kg/ha (Table 3). Sixty-two percent of all the testcrosses of the BC-derived lines yielded as high as or higher than L4001 × KUSR (data not shown). Only two of the remaining testcrosses yielded significantly less than the L4001 × KUSR testcross (Table 3). Fifteen testcrosses of BC-derived lines involving seven exotic donor parents yielded between 289 and 1,056 kg/ha more than L4001 × KUSR (Table 3). About two-third of these testcrosses did not differ significantly from L4001 × KUSR for days to anthesis and silking as well as plant and ear heights. Most of them were similar to or significantly better than L4001 × KUSR in plant aspect, ear aspect, and disease scores (Table 3). Five of the highest yielding testcrosses produced significantly higher grain yields than L4001 × KUSR but had agronomic traits that were similar to or better than L4001 × KUSR (Table 3).

Table 2 Sums of squares of sources of variation, expressed as percentages of the corrected total sums of squares, from the combined analyses of variance for BC-derived yellow endosperm lines evaluated in crosses with a testers at four locations in Nigeria in 2000 and 2001

Source	*df*	Days to anthesis (days)	Days to silking (days)	Plant height (cm)	Ear height (cm)	Plant aspect (1–5)[a]	Ear aspect (1–5)[b]	Southern corn rust (1–5)[c]	Southern corn leaf blight (1–5)[d]	Curvularia leaf spot (1–5)[e]	Grain yield (kg/ha)
Environment (ENV)	7	82*	82*	60*	35*	5	3	–	–	–	58*
Rep (environment)	8	2*	1*	2*	2*	2**	2	5*	4**	3**	1
Testcross (TC)	49	3*	3*	7*	12*	10*	15*	66*	72*	67*	4*
TC**ENV	343	7*	7*	15	23	42	39	–	–	–	19

Mean squares significantly different from zero at $*P < 0.01$ and $**P < 0.05$ levels, respectively
[a]Plant aspect (1–5): where 1 = excellent overall phenotypic appeal and 5 = poor overall phenotypic appeal
[b]Ear aspect (1–5): where 1 = clean, uniform, large, and well-filled ears and 5 = rotten, variable, small and partially filled ears
[c]Southern corn rust (1–5): 1 = slight leaf infection and 5 = severe leaf infection
[d]Southern corn leaf blight (1–5): 1 = slight leaf infection and 5 = severe leaf infection
[e]Curvularia leaf spot (1–5): 1 = slight leaf infection and 5 = severe leaf infection

Table 3 Mean performance of the highest yielding 15 and lowest yielding five testcrosses of BC-derived lines crossed to an inbred tester and check hybrids evaluated at eight test environments in Nigeria in 2000 and 2001

Testcrosses	Dates to anthesis (days)	Days to silking (days)	Plant height (cm)	Ear height (cm)	Plant aspect (1–5)[a]	Ear aspect (1–5)[b]	Southern corn rust (1–5)[c]	Southern corn blight (1–5)[d]	Curvularia leaf spot (1–5)[e]	Grain yield (kg/ha)
L4001 × B37-2	58	60*	212	108*	2.6	2.4	2.0	2.0	2.0	6,743*
L4001 × CAL36-5	57*	59*	211	100	2.4	2.2*	2.0	2.0	2.3	6,700*
L4001 × B37-4	59	61	210	101	2.5	2.4	1.8*	1.8*	2.3	6,499*
L4001 × CIM21-1	59	61	215*	102	2.6	2.8*	2.0	2.0	2.0	6,212*
L4001 × CM103-2	60*	62	206	96*	2.5	2.2*	2.0	2.0	2.3	6,201*
L4001 × CAL36-4	60*	61	217*	111	2.8*	2.6	2.0	2.3	2.5	6,188
L4001 × CIM21-4	59	61	209	102	2.5	2.3*	2.0	2.0	2.5	6,177
L4001 × SC43-2	59	61	209	101	2.6	2.3*	2.3	2.3	2.5	6,159
L4001 × HI29-2	58	61	210	104	2.3*	2.3*	2.0	1.8*	1.8*	6,126
L4001 × CM103-1	59	61	202*	93*	2.6	2.5	1.8*	1.8*	1.8*	6,028
L4001 × SC43-1	59	61	206	99	2.5	2.2*	2.0	2.0	2.0	6,023
L4001 × SC43-1	58	60*	199*	94*	2.5	2.4	1.5*	1.5	2.0	6,017
L4001 × B37-3	57*	59*	206	100	2.7	2.3*	2.5	2.8*	2.8*	6,015
L4001 × B37-5	58	60*	200*	102	2.3*	2.4	2.0	2.0	2.0	5,996
L4001 × CAL36-3	59	62	214*	103	2.6	2.4	2.0	2.0	2.0	5,976
L4001 × SC43-3	58	61	203	97*	2.6	2.6	2.0	2.0	2.0	5,310
L4001 × CIM21-2	60*	62	215*	109*	2.4	2.3*	1.8*	1.8*	1.8*	5,293
L4001 × CIM21-3	59	61	206	102	2.7	3.0*	2.0	2.0	2.5	5,221
L4001 × HI28-7	58	60*	206	104	2.5	2.6	2.0	2.0	2.3	5,108*
L4001 × HI28-1	57*	59*	191*	92*	2.5	2.6	2.5	3.0*	3.0	5,043*
Check hybrid										
L4001 × KUSR	59	61	208	102	2.6	2.6	2.2	2.3	2.3	5,687
Mean	59	61	208	101	3	2	2.0	2.1	2.2	5,787
LSD (0.05)	0.7	0.8	5.6	4.2	0.2	0.2	0.3	0.3	0.4	504
CV	2	3	6	10	15	17	11	12	12	19

Significantly different from the recurrent parent at $*P < 0.05$ level
[a]Plant aspect (1–5): where 1 = excellent overall phenotypic appeal and 5 = poor overall phenotypic appeal
[b]Ear aspect (1–5): where 1 = clean, uniform, large, and well-filled ears and 5 = rotten, variable, small and partially filled ears
[c]Southern corn rust (1–5): 1 = slight leaf infection and 5 = severe leaf infection
[d]Southern corn blight (1–5): 1 = slight leaf infection and 5 = severe leaf infection
[e]Curvularia leaf spot (1–5): 1 = slight leaf infection and 5 = severe leaf infection

Grain yield of testcrosses, the primary trait of interest, was significantly correlated with plant height but not with all other agronomic traits measured (Table 4). The correlation of days to anthesis with days to silking, plant height, and ear height was significant and positive, while its correlation with southern corn leaf blight and southern corn rust was significant and negative (Table 4). Days to silking was positively and significantly correlated with plant height and, negatively and significantly correlated with southern corn leaf blight, southern corn rust and Curvularia leaf spot. Plant height was positively correlated with ear height and negatively

Table 4 Simple correlation coefficients between pairs of traits recorded in testcrosses of the BC-derived yellow lines evaluated at four locations in Nigeria in 2000 and 2001

Traits	Days to silking (days)	Plant height (cm)	Ear height (cm)	Plant aspect (1–5)[a]	Ear aspect (1–5)[b]	Southern corn rust (1–5)[c]	Southern corn leaf blight (1–5)[d]	Curvularia leaf spot (1–5)[e]	Grain yield (kg/ha)
Days to anthesis (days)	0.91*	0.46*	0.36**	0.09	0.12	−0.34**	−0.42*	−0.27	0.05
Days to silking (days)		0.46*	0.26	−0.01	0.09	−0.33**	−0.52*	−0.42*	0.08
Plant height (cm)			0.69*	0.10	−0.11	−0.28	−0.41*	−0.22	0.32**
Ear height (cm)				0.05	−0.02	−0.34**	−0.38*	−0.23	0.20
Plant aspect (1–5)					0.38*	0.22	0.30**	0.25	−0.03
Ear aspect (1–5)						0.02	0.18	0.13	−0.28
Lowland rust (1–5)							0.80*	0.66*	−0.08
Lowland blight (1–5)								0.83*	−0.25
Curvularia leaf spot (1–5)									−0.21

Significantly different from zero at $*P < 0.01$ and $**P < 0.05$ levels, respectively
[a]Plant aspect (1–5): where 1 = excellent overall phenotypic appeal and 5 = poor overall phenotypic appeal
[b]Ear aspect (1–5): where 1 = clean, uniform, large, and well-filled ears and 5 = rotten, variable, small and partially filled ears
[c]Southern corn rust (1–5): 1 = slight leaf infection and 5 = severe leaf infection
[d]Southern corn blight (1–5): 1 = slight leaf infection and 5 = severe leaf infection
[e]Curvularia leaf spot (1–5): 1 = slight leaf infection and 5 = severe leaf infection

correlated with southern corn leaf blight. The relationship of ear height with southern corn rust and southern corn leaf blight was also negative and significant. Plant aspect was positively correlated with ear aspect and southern corn leaf blight. The relationships among the three disease scores were positive and highly significant.

AFLP-based genetic diversity of the BC-derived yellow endosperm lines

AFLP genotyping of 43 white inbred lines with 10 *Eco*RI/*Mse*I primer combinations revealed 491 distinguishable polymorphic fragments. The number of polymorphic fragments detected across the yellow endosperm lines varied from 35 for ACA/CAG to 69 for ACT/CTA, with an average of 49 per primer pair. Among the 491 polymorphic AFLP fragments, 279 were present in the BC-derived lines and absent in the recurrent parent, KUSR. These fragments occurred at frequencies varying from 0.02 to 0.79, with nearly 69% of them having low frequencies (< 0.25). The remaining 212 AFLP fragments were present in both the BC-derived lines and the recurrent parent at frequencies ranging from 0.02 to 0.94, with 72% of them having frequencies equal to or greater than 0.25. As shown in Table 5, only 7–46% of the 491 AFLP fragments were present in each BC-derived line, the recurrent parent, and the inbred tester. The proportion of AFLP fragments present in each BC-derived line and absent in the recurrent parent, KUSR, can be one measure of diversity between a BC-derived line and its recurrent parent. The BC-derived lines shared 21–146 AFLP fragments with KUSR (Table 5), representing 51–68% of the total fragments detected in each line. The proportion of fragments shared with the recurrent parent exceeded 60% for 31 BC-derived lines. The number of AFLP fragments scored in the BC-derived lines, which was not shared with KUSR, varied from 15 to 79. Allele diversity was calculated to assess the extent of polymorphism detected in the 48 yellow endosperm lines. Average allele diversity (PIC) values for the 491 AFLP markers was 0.30 ± 0.01, with a range of 0.04–0.50. Close to 57% of the 491 markers had allele diversity values exceeding 0.30, reflecting the higher level of genetic diversity embodied in the BC-derived lines.

The genetic distance of each BC-derived yellow endosperm line from its recurrent parent, KUSR, was also used as another measure of genetic diversity in the BC-derived lines. The GD estimates of each BC-derived line from KUSR varied from 0.49 to 0.91, with a mean of 0.66 ± 0.02 (Table 5). More than 50% of the BC-derived lines had GD estimates exceeding 0.60, suggesting that they shared less that 40% of the AFLP fragments with the recurrent parent, KUSR. These GD estimates fell within the bounds of the 95% confidence interval. The GD estimates between all pairs of the BC-derived lines varied from 0.34 to 0.92, with an average of

Table 5 Total number of bands detected in BC-derived lines and the number of bands shared with the recurrent parent, KUSR, and estimates of genetic distance of the BC-derived lines from KUSR and L4001

Line designation	Number of bands		Genetic distance			Line designation	Number of bands		Genetic distance		
	Present in each line	Shared with KUSR	With KUSR	95% CI[a]	With L4001		Present in each line	Shared with KUSR	With KUSR	95% CI[a]	With L4001
B37-1	161	109	0.59	(0.51, 0.66)	0.62	HI28-1	108	69	0.72	(0.66, 0.79)	0.73
B37-2	200	127	0.55	(0.48, 0.63)	0.54	HI28-2	165	104	0.62	(0.54, 0.69)	0.64
B37-3	85	52	0.79	(0.73, 0.84)	0.77	HI28-3	196	130	0.53	(0.45, 0.61)	0.52
B37-4	90	53	0.79	(0.73, 0.84)	0.80	HI28-4	215	143	0.49	(0.41, 0.58)	0.45
B37-5	194	126	0.55	(0.47, 0.63)	0.55	HI28-5	177	111	0.60	(0.52, 0.67)	0.59
CIM21-1	82	44	0.82	(0.77, 0.88)	0.81	HI28-6	104	65	0.74	(0.68, 0.80)	0.75
CIM21-2	180	117	0.57	(0.50, 0.65)	0.57	HI29-1	182	118	0.57	(0.49, 0.65)	0.57
CIM21-3	225	146	0.50	(0.41, 0.58)	0.50	HI29-2	161	109	0.59	(0.51, 0.66)	0.58
CIM21-4	211	136	0.52	(0.44, 0.60)	0.52	HI29-3	126	75	0.71	(0.65, 0.78)	0.68
CIM21-5	201	128	0.55	(0.47, 0.63)	0.54	CAL36-1	190	122	0.56	(0.49, 0.64)	0.55
CIM21-6	185	121	0.56	(0.48, 0.64)	0.56	CAL36-2	81	44	0.82	(0.77, 0.88)	0.82
CM103-1	187	121	0.56	(0.49, 0.64)	0.56	CAL36-3	122	67	0.75	(0.69, 0.81)	0.73
CM103-2	213	141	0.50	(0.42, 0.58)	0.50	CAL36-4	136	78	0.71	(0.65, 0.77)	0.70
CM116-1	154	100	0.62	(0.55, 0.70)	0.60	CAL36-5	191	122	0.56	(0.49, 0.64)	0.56
CM116-2	107	57	0.78	(0.72, 0.84)	0.76	CAL36-6	92	47	0.82	(0.76, 0.87)	0.78
CM117-1	195	127	0.54	(0.47, 0.62)	0.54	MI2S6-1	83	46	0.81	(0.76, 0.87)	0.80
CM117-2	86	51	0.79	(0.74, 0.85)	0.76	MI2S6-2	40	23	0.90	(0.86, 0.94)	0.89
CM117-3	205	138	0.50	(0.42, 0.59)	0.49	SC43-1	36	21	0.91	(0.87, 0.95)	0.91
CM117-4	145	86	0.68	(0.61, 0.75)	0.67	SC43-2	55	33	0.86	(0.81, 0.91)	0.87
CM117-5	108	67	0.73	(0.67, 0.80)	0.72	SC43-3	140	95	0.63	(0.55, 0.70)	0.66
FLA116-1	164	111	0.58	(0.50, 0.66)	0.62	VA35-1	213	134	0.54	(0.46, 0.62)	0.51
FLA116-2	189	120	0.57	(0.49, 0.65)	0.56	VA35-2	194	122	0.57	(0.49, 0.65)	0.54
FLA116-3	76	39	0.84	(0.79, 0.89)	0.82	KUSR	211	211	0.00	(0.00, 0.00)	0.43
FLA98-1	150	99	0.62	(0.55, 0.70)	0.63	L4001	223	157	0.43	(0.34, 0.52)	0.00

[a]Lower and upper limits of a 95% confidence interval on AFLP-based GD estimates between each line and KUSR

0.68 ± 0.004. Although the BC-derived lines from different backcross populations had a common recurrent parent, 68% of all pairs of these lines had GD estimates that exceeded 0.60, suggesting that they shared less than 40% of the AFLP fragments with one another. The GD value between the recurrent parent, KUSR, and the inbred tester, L4001, was 0.43. As the inbred tester was a donor parent for resistance to the maize streak virus during conversion of KUSR, the two lines share some common alleles in their genetic background that resulted in small GD estimate between the lines. Each BC-derived line in combination with the inbred tester had a GD estimate varying from 0.45 to 0.91. The GD estimates of the BC-derived lines from the inbred tester, 4001, was not significantly correlated with their testcross mean grain yields ($r = 0.10$, $P = 0.49$). Also testcross grain yields of the BC-derived lines were not significantly ($P > 0.60$) correlated with the total number of AFLP

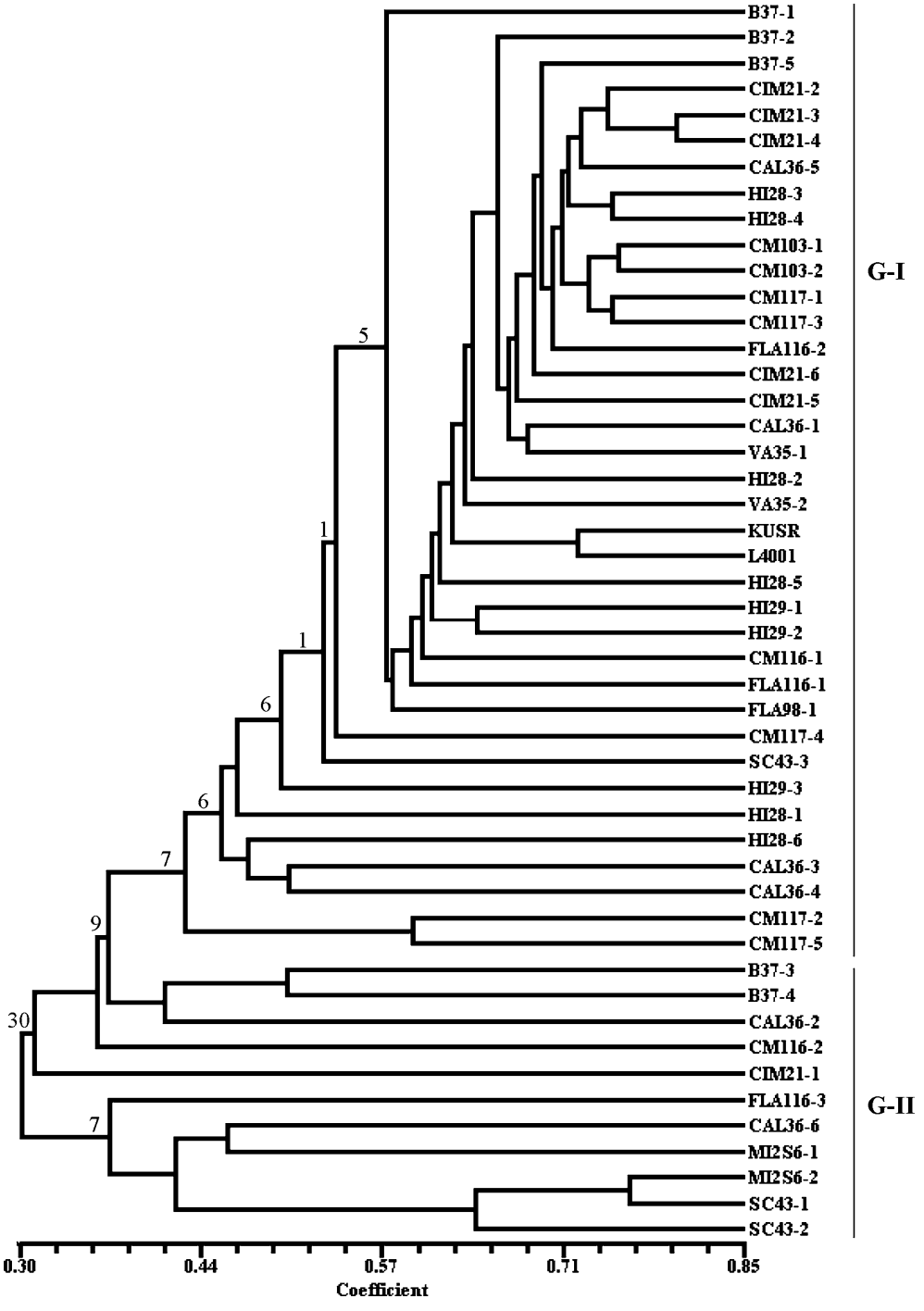

Fig. 1 Dendrogram of 48 BC-derived yellow endosperm lines obtained using 491 AFLP fragments

fragments present in each line and the number of fragments shared with the recurrent parent ($r = -0.08$ and $r = -0.06$, respectively).

Two groups, denoted by G-I and G-II, were formed by cluster analysis of AFLP-based GD estimates (Fig. 1). The first group (G-I) included 35 BC-derived lines, the adapted parent (KUSR) and the inbred tester (L4001). The BC-derived lines included in G-I involved all exotic lines, except MI2S6, as non-recurrent parents. When examining the specific subgroups found within G-I, the BC-derived lines with the same exotic parent tended to cluster together. The second group contained 11 inbred lines derived from backcrosses with six exotic non-recurrent parents (Fig. 1). Again, the BC-derived lines with a common exotic parent tended to cluster together within G-II. Cluster analysis using the neighbor jointing method also separated the BC-derived lines into the same two groups consistent with clustering using UPGMA (data not shown). The BC-derived lines with a common exotic parent were not separated into distinct subgroups but were intermixed in the various subgroups within each main group. In general, the bootstrap probability values for nodes formed with AFLPs were low. Such low bootstrap values for subgroups in the two main groups further indicated no evidence of higher-order groups within the BC-derived lines. The cophenetic correlation coefficient between the dendrogram constructed using UPGMA and the original GD matrix ($r = 0.96$) showed that the clusters accurately represented the estimates of GD.

In the principal component analysis of AFLP-based GD estimates, the first (PC1) and the second (PC2) axes accounted for 48 and 5% of the total variation, respectively, in the GD matrix. The BC-derived lines included in G-I of the cluster analysis had PC1 scores varying from −5.7 to 4.6, while those included in G-II had PC1 scores ranging from 5.3 to 11.0. As a result of these non-overlapping PC1 scores, this axis separated the inbred lines into two distinct groups consistent with the results of cluster analysis (Fig. 2). In contrast, the BC-derived lines in G-I had scores ranging from −3.3 to 3.5 for PC2 that overlapped with the PC2 scores of −3.6 and 3.2 in G-II.

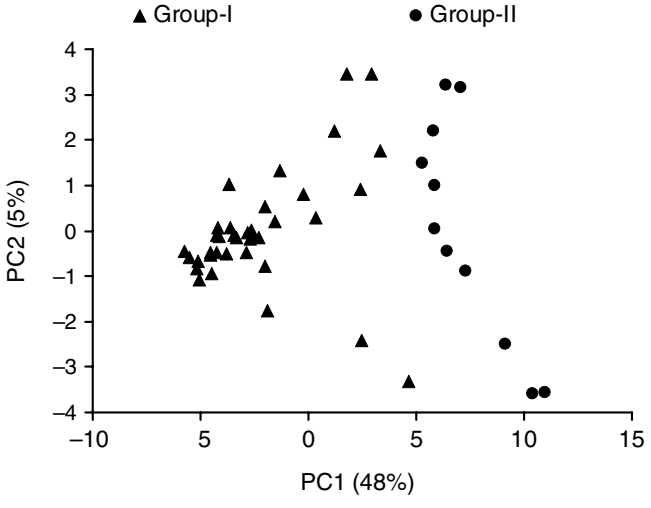

Fig. 2 Scatter plot of 48 BC-derived yellow endosperm lines determined on the basis of principal component analysis of AFLP-based GD estimates, with the two groups shown in the scatter plot being predefined based on UPGMA

Discussion

The BC-derived yellow endosperm lines examined in this study displayed significant variation in grain yield and other agronomic traits possibly representing a diverse array of alleles extracted from exotic lines of different origin and introgressed into the recurrent parent. Mean grain yield and other agronomic traits of testcrosses of the BC-derived lines were consistent across test environments. Similarly, Grauffret et al. (2000) reported that temperate × tropical highland hybrids were more stable across diverse growing environments than the temperate × temperate or the tropical highland × tropical highland hybrids. The yield instability of the hybrids in their study was related to the major adaptation factors of resistance to diseases, temperature and photoperiod insensitivity. These results suggest that the BC-derived yellow endosperm lines could serve as potential donor parents to introduce desirable alleles for broad adaptation in diverse production environments.

Five BC-derived lines formed testcrosses that were significantly superior to the testcross of their recurrent parent (L4001 × KUSR) for yield potential and other agronomic traits. These results demonstrated that the BC-derived lines contained alleles derived from exotic lines that contributed to enhanced testcross yields. The lack of significant correlation between grain yield and most other agronomic traits in this study provides an indication of the possibility to incorporate limited amounts of exotic germplasm into adapted tropical maize to increase yielding ability without affecting agronomic performance. Also, the significant and positive correlations among the three disease scores indicate that a testcross that was susceptible to one disease was so week that it was also severely infected by other diseases. Inbred lines containing limited amount of exotic germplasm with good combining ability and multiple resistance to the three major diseases can thus be derived from backcrosses with exotic lines as donor parents. Work reported by Tallury and Goodman (1999) also showed that hybrids containing 10–60% tropical germplasm produced higher yields and displayed better overall performance than hybrids with more than 60% tropical germplasm in temperate environments. When an adapted parent possesses more desirable alleles than an exotic parent, Dudley (1982) emphasizes the importance of at least one backcross to an adapted parent to increase the probability of extracting useful lines. Selig et al. (1999) also recommend introgression of a relatively small amount of exotic genome into adapted germplasm

for the improvement of quantitative traits and development of productive lines.

The BC-derived lines evaluated in this study are at the BC_1F_4 stage and would require additional generations of selfing to develop highly homozygous lines. The lines had an average GD estimate of 0.66 ± 0.02 from KUSR instead of the expected 0.25. Although most of the BC-derived yellow endosperm lines clustered with their recurrent parent, consistent with the results reported by Selig et al. (1999), the GD estimates showed that the BC-derived lines shared only a small fraction ($\leq$ 40%) of the AFLP fragments with the recurrent parent. This was contrary to the findings of Tarter et al. (2004) that reported a 31% average occurrence of exotic alleles in their semi-exotic lines. This suggests that a large fraction of the AFLP genetic diversity captured in the BC-derived yellow endosperm lines was not derived from the recurrent parent. Tarter et al. (2004) argue that further inbreeding and selection could result in the loss of exotic alleles in highly homozygous lines due to selection against alleles contributing to or linked to poor adaptation to a target environment. However, the potential loss of exotic alleles during additional generations of selfing would be minimal because the early generations of inbreeding allowed for selection against deleterious recessive alleles from exotic lines (Tarter et al. 2003). Selig et al. (1999) demonstrated that the proportions of RFLP fragments from the exotic donor parents detected in backcross-derived maize inbred lines varied widely, but they did not differ significantly from the expected values of the backcross method in most of the lines. Lewis and Goodman (2003) also showed that tropical maize germplasm can be incorporated at high rates into a temperate line using a pedigree method. It is, therefore, possible to develop highly homozygous yellow endosperm lines with significant amount of exotic germplasm and good combining ability from the BC-derived lines evaluated in this study.

The inbred tester (L4001) was a donor parent for resistance to the maize streak virus during the conversion of KUSR. Although KUSR was expected to have less than 5% of the genome of L4001 in its genetic background, the two inbred lines showed great genetic similarity between them (GD = 0.43). One potential reason for this discrepancy between the observed result and theoretical expectation could arise from fixation of high proportion of the donor alleles through deliberate selection for L4001 type of resistance to the maize streak virus and other foliar diseases during conversion of KUSR. Selig et al. (1999) demonstrated that the proportion of the genome from the donor parent retained in some backcross-derived lines could be significantly higher than the expected values. In spite of the great genetic similarity between KUSR and L4001, however, the single cross between these lines is a productive commercial hybrid marketed in Nigeria. The effect of selection during the conversion process and the different origin of KUSR and L4001 may be adequate to cause differences in allele frequencies, resulting in heterosis in the single cross between the two inbred lines. This is consistent with the results of Benchimol et al. (2000) that demonstrated the potential to develop a commercial hybrid from inbred lines derived from the same heterotic population. Nonetheless, use of inbred lines derived from unrelated heterotic groups offers better opportunities for developing high-yielding hybrids than using related lines (Melchinger 1999).

Kraja et al. (2000) pointed out that the difference in the genetic background of adapted germplasm could significantly affect the performance of exotic × adapted crosses. Although the temperate lines used as testers in their study were susceptible to gray leaf spot, the tropical × Mo17 crosses had consistently lower scores for gray leaf spot and other diseases than the same exotic germplasm crossed to B73. A significant exotic × adapted line interaction also occurs for yield in testcross of lines containing exotic germplasm (Godshalk and Kauffmann 1995). Thus a potential exists to uncover hybrids that are more productive than the ones tested in this study if the BC-derived lines are evaluated in crosses with multiple testers.

The variation among backcrosses involving diverse exotic lines as donor parents can significantly affect grain yield and other traits in testcrosses of the BC-derived lines. Work reported by Uhr and Goodman (1995a) demonstrated that the variation among sources of lines was more important in determining testcross grain yields than the variation among lines within a source. Also, the choice of exotic parents had a major effect on the development of maize inbred lines derived from adapted × exotic crosses (Uhr and Goodman 1995b). These results emphasize the importance of using diverse exotic lines as donor parents in backcross populations to enhance the probability of extracting productive inbred lines with good combining ability. As suggested by Kraja et al. (2000), the breeding value of a large number of exotic lines with diverse genetic background could be assessed using a common line as a tester.

In conclusion, the identification of BC-derived lines whose testcrosses produced 5–19% higher yields than the testcross of the recurrent parent with the common inbred tester suggest that a proportion of the genome of the exotic donor lines retained in these lines enhanced testcross yields. The observed substantial amount of genetic diversity among the BC-derived lines in our study also represents the retention of different alleles derived mainly from the exotic maize inbred lines. The BC-derived yellow endosperm lines can thus be used as potential sources of new alleles to broaden and diversify the genetic base of adapted tropical germplasm and to further increase yield and stability of production in tropical maize growing environments.

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