Effect of the few-branched-1 (*Fbr1*) tassel mutation on performance of maize inbred lines and hybrids evaluated under stress and optimum environments

Shorai Dari[1]*, John MacRobert[2], André Minnaar-Ontong[3], Maryke T Labuschagne[3]

[1]Department of Crop Science, University of Zimbabwe, Harare, Zimbabwe
[2]International Maize and Wheat Improvement Centre (CIMMYT), Harare, Zimbabwe
[3]Department of Plant Sciences, University of the Free State, Bloemfontein, South Africa
*Corresponding author: E-mail: shoraidari@yahoo.co.uk

Abstract

Yield can be increased under stress conditions by manipulating the traits that limit yield under these conditions. Tassel size is one such trait. A few branched-1 mutation (*Fbr1*) was introduced into the maize breeding programme of CIMMYT as a strategy to improve drought tolerance. The aim of this study was to evaluate yield performance of Fbr1 maize lines and hybrids under optimum and stress environments, and to determine associations between tassel size and grain yield under stress and optimum environments. Genotype effects were highly significant for all traits and genotype by environment (GxE) interaction effects were significant for all traits except for kernel row number and anthesis silking interval. Generally *Fbr1* x *Fbr1* hybrids had lower grain and pollen yields, and were less adapted to abiotic stress conditions. Positive relationships between grain yield components and pollen yield components were found except for association of prolificacy with tassel branch number and total tassel length under drought stress and optimum conditions. We were expecting that the *Fbr1* genotypes could potentially increase grain yield under drought stress. It seems that grain yield improvement and stress tolerance is determined by multiple factors, which, when put together can additively contribute to increased yield performance. Although reduction in tassel size could be one of these many factors that contribute to improved grain yield under stress conditions, the factor cannot bring significant improvement on its own. Our results show that the *Fbr1* trait reduce pollen production in genotypes with few tassel branches resulting in reduced kernel set and the problem worsens under drought and low N stress. We recommend selection for shorter and lighter tassels to improve grain yield without compromising on tassel size to ensure sufficient pollen availability, especially under stress environments.

Introduction

Maize (*Zea mays* L) breeding programmes are focusing on selection of crop features that can improve grain yield with high regulation of energy conservation. Among such phenomena are tassel characteristics, which influence plant performance and productivity significantly, particularly under stress environments. There are very few studies on the maize tassel and on variation in pollen production (Vidal-Martínez et al, 2004) as research is usually focussed on grain yield (Vidal-Martínez et al, 2001a), and grain yield is based upon the female structures and not the male flowers. Tassel branch number, tassel length and tassel weight are important tassel characters while ear height, ear length and ear diameter are important ear characters that affect maize plant yielding efficiency (Sofi, 2007). The tassel traits affect grain yield either physiologically by competing for photosynthates or physically by shading effect (Guei and Wasson, 1996).

A plant's efficiency can be measured by its ability to allocate most of the photosynthate produced toward the formation of grain (Guei and Wassom, 1996). Traits such as plant height, ear height, leaf area, and leaf number can affect photosynthetic efficiency of maize plants (Moss and Musgrave, 1971). Tassel size and leaf senescence of a plant are other important traits related to productiveness of maize, especially during grain filling. Tassel size affects grain yield, either physiologically by competition for assimilates, as available photosynthates are diverted away from the grain or physiologically by interception of radiation to the leaf canopy (Ribaut et al, 2004). Studies have shown that low yielding plants partition more photosynthates towards the formation of big and heavy tassels, than producing big ears (Hunter et al, 1969). Large tassel size has been found to be negatively correlated with grain yield. In maize hybrid breeding programmes an ideal male parent is supposed to have large tassels that can produce large amounts of pollen whereas an ideal female should partition more assimilates towards big ears and hence should possess small tassels (Upadyayula et al, 2006). Selecting for smaller tassel size should result in increased yield

(Guei and Wassom, 1996). The negative effect of the tassels on yield was demonstrated when de-tasselled plants yielded 19% more grain than plants that had not been de-tasselled or had tassels removed and then rejoined. This yield increase was attributed to interception of radiation by the tassels. Other studies have shown a correlation between detasselling and reducing the number of tassel branches with a positive effect on yield (Lambert and Johnson, 1977; Geraldi et al, 1985). In tropical maize, unlike in temperate maize, the indirect pressure of selection for reduced tassel size by selecting for increased grain production has had relatively modest effects on tassel size.

Although many authors have referred to a negative association between tassel size and yield potential (Hunter et al, 1973; Fakorede and Mock, 1978; Geraldi et al, 1985), positive association of these traits have also been reported (Neto and Miranda Filho, 2001). There is evidence that the correlation between yield and tassel size tends to be higher and negative under stress environments (Neto and Miranda Filho, 2001). Geraldi et al (1978, 1985), Vidal-Martínez et al (2001b), Gyenesne Hegyi et al (2001), and Hegyi (2003) reported that tassel branch number was negatively related to grain yield and the results indicated that selection targeted on decreased tassel branch number and tassel size may have an indirect influence on increased grain yield. Selection for smaller tassels decreases the energy of plant consumed for tassel development and the shading of flag and upper leaves by the tassel (Lambert and Johnson, 1977). It is expected since under stress, as resources become limited, tassel size influences the development of ears and silks, thus limiting grain yield by three different mechanisms: shading of the upper leaves, acting as a competitive sink and modifying the supply of growth regulators. The improvement of stress tolerance relies on manipulation of the traits that limit yield under the particular stress environment.

CIMMYT initiated hybrid maize breeding programmes in the mid-1980s and breeding programmes have invested resources in tropical maize germplasm development and improvement in Africa (Dhliwayo et al, 2009). The drought tolerant maize for Africa (DTMA) initiative is one such project that is focusing on developing maize germplasm tolerant to drought. Yield can be increased under drought stress by manipulating «adaptive traits» that limit yield under such stress. Tassel size is one such trait, since tassels act as competitive sinks under stress (Ribaut et al, 2004), and the bigger tassel exerts high apical dominance at the expense of developing ears, thus reducing grain yield (Sangoi and Salvador, 1996). Working with the hypothesis of tassel size effect on yield under stress, CIMMYT breeders have successfully introduced an ethyl-methane sulfonate (EMS)-induced, few-branched-1602: designated as *Fbr1* by Neuffer (1989), tassel mutation from a Mexican donor line of tropical adaptation into elite CIMMYT maize

lines by backcrossing and it seems that this mutation has great potential to reduce resources channelled by the plants to the tassels, and rather using this to fill the seeds, without any detrimental effect on pollen production and pollination. The effect of the *Fbr1* tassel mutation on grain yield and likely response of genotypes to drought and low N stress has not been investigated. This study aims to (i) evaluate *Fbr1* inbred lines and hybrids for yield performance under normal, drought and low N stress conditions and (ii) determine association between grain yield and tassel size in *Fbr1* maize genotypes evaluated under optimal, low N and drought stress conditions; in order to determine effects of the tassel mutation on maize stress tolerance.

Materials and Methods

Germplasm and mating design
Six CIMMYT maize near isogenic inbred lines adapted to the mid-altitude zones of southern Africa (altitudinal range of 850-1,520 masl) were selected for this study i.e. three with the *Fbr1* tassel morphology and three with normal tassels. These lines were selected to represent the few-branched mutants and the normal-tasselled elite CIMMYT maize lines. These inbred lines were crossed in a half diallel mating design (Griffing, 1956) during the off-season of 2009 under irrigation at Muzarabani in Zimbabwe to make 15 F_1 hybrids constituted of (*Fbr1* x *Fbr1*) and (*Fbr1* x normal) and (normal x normal) F_1 hybrids. Seed for the six parents was also produced during the same season for evaluation in replicated experiments.

Agronomic management, environments and stress management of trials
Two sets of trials, the six inbred parents and the 15 hybrids plus five normal-tasselled hybrid checks, were grown adjacent to each other in three environments in Zimbabwe during 2010 and again in 2011. The three trial environments were CIMMYT-Harare Maize Research Station (17.80S; 31.05E, 1468 masl) (optimum conditions), CIMMYT-Harare Maize Research Station under low N during the summer wet season, and Chiredzi Research Station (21.03S; 31.57E, 392 masl) during the winter dry season (under managed drought). Trials under optimal and low N conditions were rain-fed, but a light irrigation was applied immediately after planting to facilitate seed germination and seedling emergence. Irrigation was also applied in the case of a long dry spell. Irrigation of 7 mm hr^{-1} for six hours was applied just after planting to facilitate germination. Total water application per irrigation was 42 mm. Thereafter, the irrigation interval varied from 9 to 15 days depending on temperature and crop development stage. Average rainfall was 700-800 mm and 650-700 mm potential evapotranspiration was experienced during the growing seasons for Harare in 2010 and 2011. For trials under managed drought stress in Chiredzi, three to four irrigations totalling 250 mm of water were applied at the begin-

ning of the season and irrigation stopped at 43 to 57 days after planting (about 50 days before anthesis). The crop completed its life cycle without any further irrigation or rain.

Under optimum growing conditions in all sites, a basal application of 400 kg ha^{-1} of compound Z fertilizer (8% N, 14% P$_2$O$_5$, 7% K$_2$O, 0.8% Zn) was broadcast and disc-incorporated by a tractor. Ammonium nitrate (33% N) was split applied at 200 kg ha^{-1}. The first application of 100 kg ha^{-1} was done at four weeks after crop emergence and the second split was given at six weeks after emergence.

The experiments under low N were also conducted at Harare using, except for N management, the same crop management practices as under recommended agronomic management. Low N experiments were grown in fields that were depleted of N by continuously cropping maize (main season) or irrigated wheat (winter dry season), removing all stover biomass after harvest and not applying any N fertilizer. No chemical N fertilizer was applied to the low N experiments. For trials under managed drought stress in Chiredzi, three to four irrigations totalling 250 mm of water were applied at the beginning of the season and irrigation stopped at 43 to 57 days after planting (about 50 days before anthesis). The crop completed its life cycle without any further irrigation or rain.

For all trials, seedbeds were kept weed-free throughout the season. A mixture of atrazine (Atrazine WP), dual (Metolachlor) and gramoxone (Paraquat), at 4.5, 1.8 and 1.0 l ha-1, respectively was applied as a post-planting pre-emergence spray for weed control. At three weeks after crop emergence, Basagram was applied at 3 l ha^{-1} to control nutsedge (Cyperus spp) and broad leaf weeds. At three to four weeks after crop emergence, Bentazon (Basagram) was applied to control all weeds. From seven weeks on, the field was kept weed-free by hand weeding.

For pest management in all trials, scouting for cutworm (Agrotisipsilon) damage started immediately after crop emergence. Where cutworms were found to be a problem, a mixture of 60 g dipterex (Trichlorfon WP), 20 kg of maize meal and 20 l of water was applied as bait along crop rows in the evening. At five weeks after crop emergence, scouting for the maize stalk borer (Busseola fusca) started and endosulfan 1% granules (thionex) at 2 kg ha^{-1} in a mixture of two parts sand and one part chemical, was applied by hand every 10 days alternating with dipterex at 2 kg ha^{-1}. Maize streak virus disease was controlled by applying carbofuran (curater) mixed with sand in a ratio of three parts chemical to four parts sand in the planting hole to kill the vectors of the disease, Cicadulina leafhoppers.

Experimental design and data collection

The experimental design was an alpha lattice (0.1) (Patterson et al, 1978) with two replications for hybrid and inbred line trials in each environment. The 15 hybrids and five hybrid checks were laid out as a 4 x 5

incomplete lattice design in all three environments in 2010 and 2011, for evaluation of tassel and ear traits, where pollen yield, pollen yield components, grain yield and grain yield components were recorded. A separate trial of six inbred parents, laid out as a 2x3 incomplete lattice design, with two replications was planted adjacent to the hybrid trial in the same field. Plot size at all locations was a single 4 m row with 0.75 m between rows and 0.25 m between plants within rows, giving final plant populations of ≈ 53,000 plants per hectare at all sites.

Pollen production/pollen yield was estimated by the bagging method, which is similar to the technique followed in maize controlled pollinations, where the tassels of four selected plants were sampled for pollen production (Vidal-Martínez et al, 2001b). Pollen samples were collected daily from the same plant throughout the entire shedding period (6 to 12 d). Anthers and insects were removed by sieving the collected pollen through a #35 US standard testing sieve (500 μm opening) (Vidal-Martínez et al, 2004). The collected pollen was dried under normal day temperatures and favourable atmospheric shedding conditions, to a moisture content of less than 10% (Goss, 1968), prior to weighing with a precision balance to obtain the daily and total production of pollen during anthesis.

Four tassels, visually selected by comparing anthesis development in unbagged plant-tassels per plot were used for estimating tassel characteristics (pollen production components) – tassel branch number and total tassel length (Upadyayula et al, 2005). Total tassel length in centimetres was measured as the distance from the non-branching node present below the lowermost primary branch to the tip of the central spike. Average tassel branch number was the number of primary branches per plant-tassel. The amount of pollen produced by a maize plant depended on the number of staminate flowers per plant and the amount of pollen per anther (Goss, 1968). Therefore, plants had different amounts of collected pollen throughout the entire shedding period. Pollen yield in grams was measured on a sample of four plants per plot during the pollen shedding period.

Ear weight, kernels per ear, ear-row number, kernel-row number and ear length were measured and considered as grain yield components. Twelve ears per plot were used for estimating these grain yield components (Vidal-Martínez et al, 2001a). Grain yield (adjusted to 12.5% moisture content) was obtained considering harvested plot area and counting number of plants and harvested ears per plot. Days to anthesis (number of days from planting to 50% pollen shed), days to silking (number of days from planting to 50% silk emergence), and ASI were measured on plot basis for both inbred parent and hybrid trials.

Statistical analysis

All trials were first analysed individually (including checks) according to an alpha lattice (0.1) design

Table 1 - Analysis of variance of grain yield, grain yield components, pollen yield and pollen yield components for the six maize inbred lines and 15 maize hybrids plus five hybrid checks for experiments conducted in 2010-2011 across the three environments (low N, drought stress and optimum conditions).

Source	df	GYD	1000-kw	Ear weight	KRN	KPE	TTL	TBN	PYD	ASI	DTA
Inbreds											
Entry	5	0.56**	13913.01***	2368.37***	12.98***	4343.46**	123.87***	199.04***	0.54***	0.13*	227.64***
Site	2	5.42***	50562.92***	8946.84***	0.44	5784.22*	153.53***	19.69*	0.095**	0.40**	2779.83***
Year	1	0.006	12679.00***	20.9	0.74	139093.18***	706.50***	0.47	0.027	0.45**	9320.69***
Entry x site	10	0.13	2823.48**	601.45**	0.44	905.38	17.14	9.39*	0.11***	0.051	14.23**
Entry x year	5	0.076	1321.21	238.9	0.27	3419.21*	17.22	2.46	0.0022	0.045	10.95*
Entry(site x year)	6	0.055	6435.43***	401.89*	0.14	33355.47***	94.66***	13.37**	0.015	0.069	1073.24***
Fbr1 vs Norm	2	0.098	877.24	167.95	0.39	1069.39	78.45	438.96***	0.68*	0.3	189.87
Error	39	0.098	877.24	167.95	0.39	1069.39	13.24	4.44	0.016	0.049	3.99
Hybrids and checks											
Entry	19	1.72***	12151.02***	5880.56***	2.20***	15504.93***	226.01***	417.90***	4.30***	0.11***	176.97***
Site	2	103.75***	341826.9***	26134.39**	11.20***	397327.94***	1882.27***	1131.6***	21.68***	2.82***	15575.6***
Year	1	1.93***	1813.92	606.13	14.47***	5652.68	3059.88***	0.34	0.094	0.29***	31088.8***
Entry x site	37	0.56***	2207.24***	2328.19***	0.58	10402.77***	39.08**	16.97***	0.62***	0.038	14.78**
Entry x year	19	0.27	917.53	527.71	0.65	2858.71	23.64	14.25*	0.28	0.042	5.04
Entry(site x year)	19	0.59***	4523.23***	2386.66***	0.67	13981.83***	122.77***	32.59***	0.20	0.064**	1236.18***
Contrast (hybrids)	2	0.049	13.38	89.57	0.0069	939.93	0.43	480.03***	9.84***	0.18*	145.83
Error	139	0.18	805.30	591.61	0.61	3451.82	19.22	7.73	0.23	0.028	4.57

Mean squares

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, GYD - grain yield in kilogram per m², 1,000-kw - thousand kernel weight, KRN - mean kernel row number per cob, KPE - mean number of kernels per ear, TTL - total tassel length in centimeter, TBN - average tassel branch number, PYD - pollen yield in gram, DTA - mean number of days to anthesis, DTS - mean days to silking.

(Patterson et al, 1978) using Proc Mixed in SAS (SAS Institute, 2003), to determine either individual or combined significant response of traits among genotypes. The combined analysis of variance of a response included the factors genotype (G), location (L) and incomplete block (replication) within location (B) and was done using the general linear model (GLM) procedure in SAS System for Windows, Version 9.1 (SAS Institute, 2003). Analysis of variance (ANOVA) for grain yield, grain yield components, pollen yield and pollen yield components was done for each location and a combined ANOVA was computed across all locations using a GLM procedure, by considering locations as fixed, and genotypes (inbred lines or hybrids), replication and incomplete blocks as random factors. ASI data was normalised using the transformation of LN (ASI +10) (Bolaños and Edmeades, 1996), before ANOVA. Linear contrasts were performed using SAS to test linearity of performance in *Fbr1* x *Fbr1*, *Fbr1* x normal tasselled, and normal tasselled x normal tasselled hybrids. Multiple comparisons of means (Tukey, 0.05) were carried out using SAS (SAS Institute, 2003).

Pearson's phenotypic correlation coefficients were computed to estimate associations among traits – pollen yield components, grain yield components, pollen yield and grain yield, using the least squares means for parameters measured in hybrid trials. Pollen yield components and grain yield components data were analysed using the multivariate analysis (SAS Institute, 2003) i.e. the principal component analysis. The relationship between pollen yield components and grain yield components were displayed by means of Gabriel's Biplot (Rawling, 1988). A biplot illustrates relationships among the independent variables, the relative similarities of the individual data points, and the relative values of the observations for each independent variable.

Results and Discussion

Evaluation of maize lines and hybrids for grain and pollen yield

Results of the combined ANOVA across sites (environments) and years for the inbred lines revealed significant differences among lines for all traits measured (Table 1). For the maize hybrids, combined ANOVA across sites and years also showed highly significant differences for all traits measured. Environmental effects for grain yield, 1,000-kernel weight, ear weight, kernels per ear, total tassel length, tassel branch number, pollen yield, anthesis silking interval, days to anthesis, and days to silking were all significant, for both inbred lines and hybrid progenies, indicating that these traits are influenced by environmental conditions. Variation due to environment was, however, not significant for kernel row number for inbred lines, indicating that the trait is not affected by environment.

GxE interaction effects were significant for pollen yield, 1,000-kernel weight, ear weight, days to anthesis, tassel branch number, and days to silking. This means inbred lines did not respond to the environment similarly for these traits. For the hybrid progenies, GxE interaction was significant for all traits except for kernel row number and anthesis silking interval.

Non-significant GxE interaction for kernel row number and anthesis silking interval suggests the genotypes maintained their rank for these traits across environments and selection for the traits in one environment might be effective when selecting for a broad range of environments.

For maize inbred lines, orthogonal contrasts were highly significant ($P \leq 0.001$) for tassel branch number and were significant ($P \leq 0.05$) for pollen yield and days to silking (Table 1). For the maize hybrids, the contrasts were highly significant ($P \leq 0.001$) for tassel

Table 2 - Mean grain yield, total tassel length, tassel branch number, and pollen yield for the inbred lines and hybrids, measured in 2010 and 2011 under optimum, low N, and drought stress conditions.

Inbred lines

	Grain yield (kg m^{-2})			Total tassel length (cm)			Tassel branch number			Pollen yield (g plant^{-1})		
	Optimum	Low N	Drought	Optimum	Low N	Drought	Optimum	Low N	Drought	Optimum	Low N	Drought
[CML443/TAS]	0.93	0.31	1.51	43.78	39.74	34.63	3.25	2.82	2.69	0.09	0.11	0.04
[CML444/TAS]	1.48	0.69	2.02	35.04	36.57	27.57	4.19	2.83	3.25	0.14	0.10	0.11
[CML488/TAS]	0.76	0.23	1.13	33.05	33.84	33.06	2.07	4.38	5.65	0.14	0.24	0.10
CML443	1.09	0.24	0.85	42.64	41.11	38.49	10.25	8.25	14.69	0.17	0.31	0.14
CML444	1.65	0.28	1.66	35.77	36.15	34.51	10.88	11.25	13.07	0.25	0.27	0.88
CML488	0.88	0.30	1.52	37.08	38.79	35.22	10.69	9.00	9.82	0.69	0.44	0.94
Mean	1.13	0.34	1.45	37.89	37.70	33.91	6.89	6.42	8.19	0.25	0.25	0.37
LSD$_{0.05}$	0.45	0.32	1.19	10.85	5.52	5.46	6.12	3.12	1.58	0.28	0.10	0.17
Hybrid progeny and checks												
Fbr1 x Fbr1	2.73	0.73	1.18	50.25	46.67	42.42	3.40	3.02	6.34	0.62	0.35	0.78
Fbr1 x Normal	2.74	0.70	1.39	50.29	44.80	41.25	7.76	6.77	12.74	0.98	0.51	1.39
Normal x Normal	3.28	0.72	1.57	55.01	47.53	44.37	16.63	12.98	24.15	1.94	1.13	3.49
Check 1	4.24	0.71	0.75	61.82	49.90	45.58	20.82	18.38	27.69	1.91	1.60	3.13
Check 2	1.24	.	0.17	50.88	34.80	37.33	11.19	9.69	17.07	0.47	0.30	0.64
Mean	2.92	0.72	1.38	51.85	46.33	42.68	9.26	7.59	14.41	1.18	0.66	1.89
LSD$_{0.05}$	1.06	0.57	0.10	9.98	9.69	6.37	5.00	5.40	6.14	1.59	0.58	0.90

check1: best performing hybrid check and check 2: poorest performing hybrid check. Only two out of five checks were selected for comparison with the hybrid

branch number, pollen yield and were significant at P ≤ 0.05 for anthesis silking interval. This indicates that there was a significant linear trend for tassel branch number in *Fbr1* versus normal tasselled inbred lines. Inbred lines with the *Fbr1* mutation had lower mean tassel branch numbers compared to normal tasselled lines (Table 2). Pollen yield in both inbred lines and hybrids also showed a decreasing trend from normal x normal, *Fbr1* x normal, and *Fbr1* x *Fbr1* tasselled hybrids, which is indicative of additive gene action governing pollen yield.

For the hybrids, there was a linear trend in tassel branch number of *Fbr1* x *Fbr1*, *Fbr1* x normal and normal x normal hybrids. *Fbr1* x *Fbr1* hybrids had the lowest tassel branch number, followed by *Fbr1* x normal, then normal x normal tasselled hybrids which had the highest number of tassel branches (Table 2). Indirectly this is indicative of the predominance of additive gene action governing the tassel branch number trait. Mean grain yields for hybrids ranged from 0.72 kg m^{-2} under low N stress to 2.92 kg m^{-2} under optimum conditions. For the parental inbred lines mean grain yield ranged from 0.32 kg m^{-2} under low N to 1.19 kg m^{-2} under drought stress. Hybrid vigour was expressed for total tassel length as maize hybrids had higher mean tassel lengths compared to parental inbred lines (Table 2).

Inbred lines and hybrids that had many tassel branches produced more pollen than those with few branches (Table 2). This implies that breeding for few branches could pose a challenge in increasing grain yield under stress since number of tassel branches is considered as a vital pollen yield component (Vidal-Martínez et al, 2001b). Although Chinwuba et al (1961) indicated that reduction in tassel size decreases apical dominance and consequently improves grain yield under stress environments, particularly at high plant populations, our results conform to questions previously raised on the viability of breeding programmes targeting tassel reduction as a way of increasing grain yield, since pollen production is heavily compromised and the effects are significant under stress conditions and when the lines are used as males in hybrid seed production (Monneveux et al, 2006).

Pollen and grain yield components variation

Variation in pollen yield components and grain yield components of genotypes was found (Table 3). Similarly, Vidal-Martínez (2001a) reported significant genetic variation in Mexican and Corn Belt genotypes of maize for similar quantitative traits. Differences due to environment and GxE interaction were significant for all pollen yield components. For grain yield components, environmental variation was found for most traits except for ear length, and GxE interaction was significant for most components except for kernel row number and cob circumference. Thus, for all pollen yield components and most grain yield components, there was differential genotypic response to the different environmental conditions (optimum, low N, and drought stress conditions). This GxE interaction is normally associated with changes in genotypic rankings and limits the identification of superior, stable hybrids for yield performance (Epinat-Le Signor et al, 2001). Although a substantial amount of variation among entries for pollen yield components and grain yield components was genotypic, environmental variation explained the larger part of differences among entries for pollen yield components and grain yield components.

Association among pollen yield, pollen yield components, grain yield, and grain yield components

A positive relationship between pollen yield and grain yield was found for analysis done across the three sites (r = 0.37) and for data collected under optimum conditions (r = 0.48) and in both cases the relationship was significant at P ≤ 0.01 (Table 4). Vidal-Martínez (2001b) also found a moderate and

Table 3 - Mean squares for pollen and grain yield components for genotypes and environments.

Pollen yield components

Variable	PYLD†	TBN‡	TTL	ASI	AD
Genotype	4.30***	417.90***	226.010***	15.79***	136.61***
Environment	21.68***	1131.60***	1882.27***	321.48***	11640.66***
Genotype x E	0.62***	16.97***	39.081**	5.63***	8.89***

Grain yield components

Variable	GYLD§	1,000-KW	KPE	EW	KRN	EL	CC	CW	SD
Genotype	1.72***	10466.027****	15504.93***	5880.56***	2.20***	16.20***	7.098***	83782.77***	176.97***
Environment	103.75***	329160.85***	397327.94***	261349.39***	11.20***	602.15	213.56***	3092845.29***	155.60***
Genotype x E	0.56***	2215.19***	10402.77***	2328.19***	0.58ns	3.29**	1.19	29560.51***	3.24***

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

†PYLD - Pollen yield (g plant⁻1) Grain yield in kilograms, measured on plot basis, adjusted to 12.5% grain moisture content and to number of plants per plot. ‡TBN - Tassel branch number, TTL - Total tassel length (cm), ASI - Anthesis silking interval, and DTA - mean number of days to anthesis. §GYLD = Grain yield (kg m⁻²); 1,000-KW - 1,000- kernel weight, KPE - Kernels per ear, EW - Ear weight, KRN - Kernel row number, EL - Ear length

positive relationship between pollen yield and grain yield. Under low N and drought stress conditions, there was no significant association between pollen yield and grain yield, and pollen yield explained only 0.41% of the variation in grain yield: r = 0.0041. Under drought conditions pollen yield explained only 5.3% of variation in grain yield. Under stress conditions, pollen yield is reduced, but the major contributor to yield reduction are effects imposed by stress on the plants' metabolic and physiological processes that in turn affect grain yield (Bänziger et al, 2000). Highly significant (P ≤ 0.001) and positive relationships between pollen yield and tassel branch number were obtained across sites, under optimum, low N, and under drought stress conditions. These ranged from r = 0.64 under optimum conditions to r = 0.79 under drought stress. Under stress conditions, tassel branch number explained a considerable amount of variation in pollen yield (r = 0.45 under low N and r = 0.62 under drought stress). This is true because the bigger the tassel: with many primary branches, the more the pollen yield, especially because of extended pollen availability, as the many branches shed pollen at different times. That is one reason why breeders have conflicting interests in selection of tassel traits. From the standpoint of yielding efficiency and shading effect, a smaller tassel is ideal but in case of certain situations such as stress environments, larger tassels are selected for to ensure sufficient and extended pollen availability (Sofi, 2007). This is especially crucial for stress environments where pollen production is drastically reduced. Across sites and under optimum conditions, significant and positive relationships of pollen yield with grain yield components were found. Their correlation values ranged from 0.19 (P ≤ 0.05) to 0.42 (P ≤ 0.001) across site and 0.34 (P ≤ 0.05) to 0.55 (P ≤ 0.001) under optimum conditions, suggesting a moderate relationship between these traits. Significant and small association was found for pollen yield with grain yield components under low N and drought stress conditions with pollen yield explaining not more than 3.6% and 7.3% of variation in kernel row number under low N and drought stress respec-

tively. Although under stress pollen yield is drastically reduced, the effects on grain yield could be a result of different genotypes responding and adjusting for yield differently in reaction to stress.

The association of grain yield with pollen yield components: total tassel length, was positive, moderate and significant and ranged from 0.24 under optimum to 0.65 under drought stress conditions. The relationship was also significant (P ≤ 0.01) and positive between tassel branch number and grain yield across sites and under optimum conditions, and a significant and very small association was found between tassel branch number and grain yield under low N and drought stress conditions. Tassel branch number explained not more than 0.6% and 8.4% of variation in grain yield under low N and drought stress respectively. Afzal et al (1997) also found positive correlations between grain yield and tassel branch number. However, Vidal Martínez (2001b) found negative associations between pollen yield components and grain yield. Many authors have referred to a negative association between tassel size and yield potential (Hunter et al, 1973; Fakorede and Mock, 1978; Geraldi et al, 1985). There is evidence that the correlation between yield and tassel size tends to be higher and negative under stress caused by unfavourable environments (Neto and Miranda Filho, 2001). They emphasized that environmental factors such as photoperiod, solar radiation and rainfall affect the yield potential of maize and consequently the association between traits may change if there is differentiated variety response to the environmental factors.

Relationships between tassel and ear traits have been previously reported (Sharma and Dhawan, 1968; Vidal Martínez et al, 2001a). Vidal Martínez et al (2001a) found negative phenotypic correlations between pollen yield components and grain yield components. Negative associations between pollen yield components and grain yield components were expected in this study, especially under stress environments, as a consequence of either a trade-off phenomenon between male and female functions which are in competition for resources; or apical dominance

Table 4 - Phenotypic correlations between pollen yield (PYLD) and grain yield (GYLD), PYLD with pollen yield components (PYC) and grain yield components (GYC), and association between PYC and GYC with GYLD in maize hybrids under optimum conditions.

	Across	Optimum	Low-N	Drought
PYLD – GYLD	0.37**	0.48**	0.064ns	0.23ns
PYLD – PYC				
Pollen yield - Total tassel length	0.013ns	0.018ns	0.13ns	0.32**
Pollen yield - Tassel branch number	0.78***	0.64***	0.67***	0.79***
Pollen yield - Anthesis silking interval	-0.37***	-	-0.20ns	0.093ns
Pollen yield - Anthesis date	-0.22**	-	0.0052ns	0.32**
Pollen yield - Silking date	-0.26**	-	-0.018ns	0.37**
PYLD – GYC				
Pollen yield - 1000-kw	0.30***	0.50**	0.084ns	0.21ns
Pollen yield - Ear weight	0.30***	0.53***	0.088ns	0.042ns
Pollen yield - Kernel row number	0.29***	0.34*	0.19ns	0.27ns
Pollen yield - Ear length	0.29***	0.35*	0.014ns	0.082
Pollen yield - Kernels per ear	0.19*	0.42**	0.0036ns	0.014ns
Pollen yield - Cob circumference	0.32***	0.55***	0.16ns	0.12ns
Pollen yield - cob weight	0.42***	0.51***	0.15ns	0.073ns
PYC – GYLD				
Total tassel length - Grain yield	0.37***	0.24*	0.27*	0.65**
Tassel branch number - Grain yield	0.22**	0.30**	0.077ns	0.29ns
Anthesis silking interval - Grain yield	-0.62***	-0.33**	-0.46***	-0.42ns
Anthesis date - Grain yield	-0.28**	-0.037ns	-	-0.36
Silking date - Grain yield	-0.33***	-0.20ns	-0.19ns	-0.48*
GYC – GYLD				
1000-kernel weight - Grain yield	0.86***	0.77***	0.46***	0.49***
Ears per plant - Grain yield	-0.50***	-0.63***	-	-0.70***
Ear weight - Grain yield	0.92***	0.81***	0.86***	0.86***
Kernel row number - Grain yield	0.37***	0.21*	0.29*	-0.20*
Ear length - Grain yield	0.88***	0.73***	0.76***	-0.77***
Cob circumference - Grain yield	0.82***	0.69***	0.59***	0.72***
Cob weight - Grain yield	0.95***	0.89**	0.87**	0.97***

* P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001

which provides a negative effect of the tassel on the development of the female ear (Devlin, 1989; Garnier et al, 1993). These results would indicate physiological agreement with those models when more than one trait is involved in expressing pollen yield components-grain yield components relationships (Vidal Martínez, 2001b). The general negative association of prolificacy with tassel branch number and total tassel length (tassel size) was also found by Souza Junior et al (1985) who reported on a negative correlation between tassel size and prolificacy which was explained by a large amount of indol-acetic-acid (IAA) produced by larger tassels and causing inhibition of prolificacy, or vice versa (Anderson, 1967).

Correlation matrix biplots of pollen production and grain yield components

Maize hybrids evaluated under drought stress conditions accounted for 64.83% of phenotypic variability according to the first two principal components (PC) (Figure 1). Under low N stress, hybrids accounted for 65.98% of phenotypic variation, where PC1 accounted for 45.53% and PC2 accounted for 20.44% of the phenotypic variation in the hybrids (Figure 2).

The contribution of traits to the eigenvectors for the principal components can be represented as a biplot, where X and Y-axes represent the proportion of variation attributable by PC1 and PC2 (Figure 1 and Figure 2). The lengths of these vectors reflect the magnitude of variation for each trait, whereas the

angle between vectors reflects the degree of association between the traits (Gabriel, 1971). Thus, Figure 1 shows kernel row number and days to silking/female flowering (FF) having larger variation than the other traits under drought stress conditions, while anthesis silking interval shows greater variation under low N stress conditions (Figure 2).

Tassel branch number is closely associated but inversely correlated with prolificacy (EPP) under drought stress conditions. Under stress, larger tassels produce large amounts of indol-acetic-acid (IAA), which inhibits prolificacy, or vice versa (Anderson, 1967). Under drought stress, tassel branch number was not closely correlated with grain yield and most of the grain yield components (Figure 1). However, phenotypic variation for tassel branch number, anthesis silking interval and pollen yield under drought stress was larger than that of grain yield components. Under low N stress conditions, grain yield and cob weight, ear weight and ear length, tassel branch number and cob weight were positively and closely associated but were all inversely associated with anthesis silking interval (Figure 2). This showed that delayed silking has a large effect on grain yield under low N stress. All vectors showed equally large variation under low N stress. Tassel branch number had positive association with most grain yield components under low N stress unlike under drought stress conditions.

The lack of consistency of PC scores and the dif-

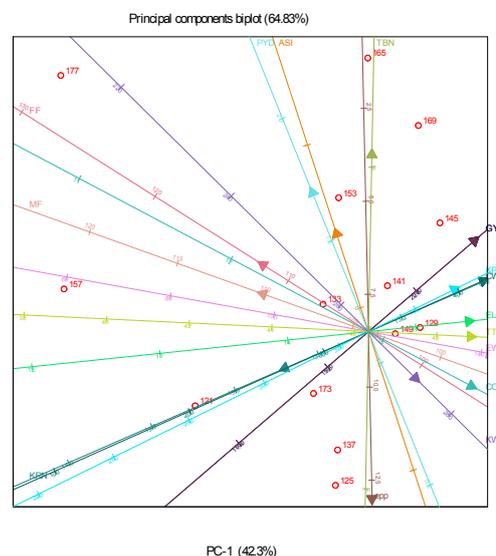Principal components biplot (64.83%)

PC-1 (42.3%)

Figure 1 - Biplot of first (PC1) and second (PC2) principal components expressing the proportion of variation derived from grain yield components and pollen production components (vectors) in the maize hybrids under drought stress conditions. MF - days to anthesis, FF - days to silking, PYD - pollen yield, ASI - anthesis silking interval, TBN - tassel branch number, GYG - grain yield, KPE - kernels per ear, CW - cob weight, EL - ear length, TTL - total tassel length, EW - ear weight, CC - cob circumference, KW - kernel weight, and EPP - ears per plant. PC1 and PC2 cumulatively explained 64.83% of total variation in yield components.
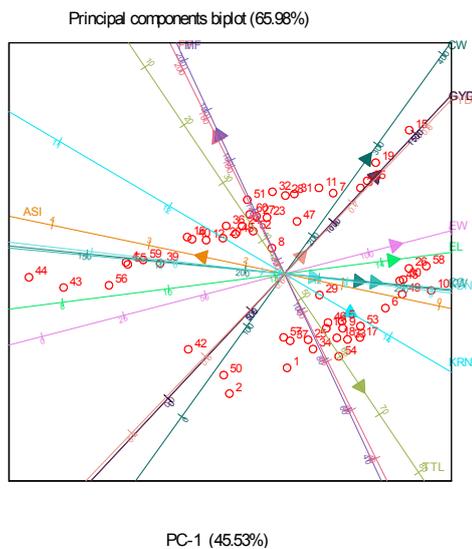
Principal components biplot (65.98%)

PC-1 (45.53%)

Figure 2 - Biplot of first (PC1) and second (PC2) principal components expressing the proportion of variation derived from grain yield components and pollen production components (vectors) in the maize hybrids under low N stress conditions. MF - days to anthesis, FF - days to silking, PYD - pollen yield, ASI - anthesis silking interval, TBN - tassel branch number, GYG - grain yield, KPE - kernels per ear, CW - cob weight, EL - ear length, TTL - total tassel length, EW - ear weight, CC - cob circumference, KW - kernel weight, and EPP - ears per plant. PC1 and PC2 cumulatively explained 65.98% of total variation in yield components.

ferent magnitude of variation, as well as the different degrees of associations among traits measured under drought and low N stress, may be due to the presence of phenotypic plasticity, which is the amount of change in the expression of traits in different environments (Bradshaw, 1965; Vidal-Martínez et al, 2004). This plastic response of genotypes to different environments is also shown in an analysis of variance in Table 3 where environmental and GxE interaction mean squares were significant for most traits. The presence of genotypic variation for all the measured traits suggests genetic differences between genotypes and indicates that phenotypic plasticity could itself be under genetic control and would, therefore, be subject to selection pressures (Bradshaw, 1965; Vidal-Martínez et al, 2001b).

One way of improving performance of maize under stress that is centered on mechanisms that improve partitioning of assimilates to the ear at flowering, at the expense of tassel and stem growth, is reducing tassel weight. Reports showed that reduction of tassel weight was much greater than the reduction in primary branch number as reported by Bolaños et al (1993), who found reduction of tassel weight by 2.6% per cycle of recurrent selection. Chapman and Edmeades (1999) also reported a reduction in tassel weight of 5.9% per cycle of recurrent selection, suggesting that selection for reduced tassel weight may be performed without decreasing tassel branch

number and pollen production at the same time (Monneveux et al, 2006). Thus, selecting for reduced tassel weight rather than few tassel branch number can increase maize grain yield thus reducing complications of reduced pollen amount in few branched genotypes. Sofi (2007) also concluded that selecting for upright tassel branches may compensate for yield reduction without compromising tassel size to ensure sufficient pollen availability.

Conclusions

Fbr1 x *Fbr1* hybrids were generally low yielding under drought and low N stress environments. Although literature has shown that breeding for small tassels could improve grain yield under stress conditions, it remains elusive considering these results. Grain yield improvement and stress tolerance can be determined by multiple factors, which, when put together can additively contribute to increased yield performance. Reduction in tassel size could be one of these many factors that contribute to improved grain yield under stress conditions, but the factor cannot bring significant improvement on its own. Secondary traits like stay green, synchrony between male and female flowering, factors associated with premature senescence, and decreased barrenness can affect yield and should be selected for, together with small tassel size, as they are mechanisms associated with tolerance in water and low N limiting environments.

Although reduced tassel size appears to be a relevant breeding objective under stress, especially in tropical germplasm and also considering the fact that this trait can be easily altered by selection, and is highly heritable, our results were contrary. In this study, reduction in tassel size caused a reduction in pollen and grain yield and the effects of this reduction were pronounced under drought and low N stress. Biplots of PC1 and PC2 showed negative association between ASI and grain yield components and prolificacy with tassel branch number under drought stress. This could be a consequence of apical dominance exerted by a larger tassel on the ear, under stress environments. We were anticipating that the *Fbr1* genotypes could potentially increase grain yield under drought stress. However, reduced pollen production in genotypes with few tassel branches, can result in reduced kernel set. The desiccating drought conditions can exacerbate the problem as pollen and silk viability is reduced. Selecting for shorter and lighter tassels may result in higher yields without compromising on tassel size to ensure sufficient pollen availability, especially under stress environments and in hybrid seed production.

Genotypic variation and environmental differences on tassel morphological traits and grain yield components indicated that pollen production and grain yield components rely on phenotypic plasticity and genetic variation. Thus, selection of genotypes with plastic response to different environments effectively improves breeding progress and grain yield.

Acknowledgements

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