

Linear relationships between grain yield and tassel traits in maize

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Abstract

This study aimed to verify the linear relationships between maize grain yield and tassel traits, identifying traits for selection through direct and indirect effects over three crop years. Three experiments were performed with 20 maize genotypes in a randomized complete block design with three replicates. Individual variance analysis showed significant genotype effects for all traits in the three crop years. However, a joint analysis demonstrated a significant genotype effect at 5% error probability for all traits, but branching space length. Furthermore, only the number of secondary branches and tassel branch number did not show significant genotype × crop year interaction. These results revealed a genotype differentiated behavior over the evaluated years, emphasizing the need to evaluate genotypes in more than one crop year. Based on phenotypic and genotypic correlation matrices and path analyses, the number of secondary tassel branches showed a positive linear relationship with grain yield; therefore, it can be used for the indirect selection of maize plants in breeding programs.

Keywords: *Zea mays* L., multicollinearity, path analysis, indirect selection.

Abbreviations: CV_Coefficient of variation; Fc_F calculated for genotype; SA_Selective accuracy; PL_Peduncle length (considering the distance between the collar of the flag leaf and the first branch), in cm; BSL_Branching space length, in cm; CSL_Central spike length, in cm; TL_Tassel length, in cm; NPB_Number of primary branches; NSB_Number of secondary branches; TBN_Tassel branch number; PDM_Peduncle dry matter (considering the region between the flag leaf collar and the first branch), in g; BSDM_Branching space dry matter, in g; CSDM_Central spike dry matter, in g; TDM_Tassel dry matter, in g; GY_Grain yield, in the plot and expressed as Mg ha⁻¹ at 13% moisture; rp_Correlation coefficients phenotypic; rg_Correlation coefficients genotypic; g_Gran; Mg_Megagram; cm_Centimeters; m_Meter; °C_Degrees Celsius; kg ha⁻¹_Kilogram per hectare; N_Nitrogen; K₂O_Potassium monoxide; P₂O₅_Pentaóxido de difósforo; Oct_October; Nov_November; FEPAGRO_State Foundation for Agricultural Research of the state of Rio Grande do Sul; CN_Condition number; VIF_Variance inflation factor

Introduction

Maize (*Zea mays* L.) is a cereal grain used in several sectors of the production chain, from the production of staple foods such as flour, hominy, and oils to its use in the formulation of glucose syrup, maltodextrins, and dyes. Due to its socioeconomic importance, breeding studies have been carried out on grain yield as one of the main components for cultivar selection (Porto et al., 2011).

Maize breeding programs have sought to develop inbred lines and high-yielding hybrids with smaller tassel sizes (length) and fewer branches, but maintaining enough pollen production to favor fertilization (Duvick 2005; Fischer and Edmeades 2010). According to Edwards (2011) and Brewbaker (2015), plants with larger tassels inhibit solar radiation passage through the plant canopy, reducing photosynthetic activity and acting as a drain of photoassimilates, which may reduce grain yield.

In a study of a long-term commercial plant-breeding program, Duvick et al. (2004) reported negative linear regression coefficients for tassel-branch number, tassel weight, and tassel size score on released high-yielding U.S. maize hybrids with respective coefficients of determination (R²) of 0.66, 0.70, and 0.90. Moreover, a reduction in tassel size was sorted as a promoter of

grain production efficiency intrinsic to maize plants exposed to intense selection for higher and more stable yields. However, Brewbaker (2015) concluded that there is little evidence that reductions in tassel size would result in higher grain yield, besides that tassels cannot become much smaller. Meanwhile, modern maize breeders are aware of increased tassel sizes in male inbred lines for greater pollen production and dispersal to assure the economic viability of hybrid seed production (Gage et al., 2018). Thus, studies of linear associations based on phenotypic and genotypic correlation coefficients can be used to measure the degree of association among traits. Linear correlations are valuable for selecting low-heritability and difficult-to-measure traits of agronomic interest.

A linear association among traits is used to quantify the magnitude and direction of influences but it does not allow assessing direct and indirect effects. Meantime, the correlation coefficients are unfolded into direct and indirect effects in path analysis, allowing to measure the influence of one variable on another, independently of the others (Cruz et al., 2014). Studies on correlations and path analysis involving maize tassel traits were

developed (Upadyayula et al., 2006; Parvez, 2007; Ci et al., 2012; Nardino et al., 2016a; Öner, 2018).

However, to the best of our knowledge, there are no studies of linear relationships with a higher number of tassel traits in the literature, i.e., with the better technical detail of linear relations between tassel components and grain yield. We assume that such linear relationships exist and can be used for the indirect selection of higher grain yield genotypes. Therefore, this study aimed to verify the linear relationships between grain yield and maize tassel traits and identify traits for selection through direct and indirect effects over three crop years.

Results and discussion

Analysis of variance and experimental precision

Individual analyses of variance exhibited significant genotype effects for all traits in the three crop years (Table 1). Meantime, the joint analysis of variance showed a significant genotype effect at a 5% probability of error for all traits but BSL. Furthermore, only the traits NSB and TBN did not show significant genotype \times crop year interaction (Table 2). These results evidenced differentiated behavior of genotypes in the evaluated years, emphasizing the need to evaluate them in more than one crop year.

Regarding the selective accuracy (SA) among the 36 cases (12 traits \times three crop years), 35 presented very high experimental accuracy ($SA \geq 0.90$), and only one case, in experiment 1, displayed high accuracy ($0.70 \leq SA < 0.90$), according to the classification established by Resende and Duarte (2007) (Table 1). When the three experiments were jointly examined, all traits exhibited very high experimental accuracy ($SA \geq 0.90$) (Table 2). Therefore, given the large dataset (12 traits, 20 genotypes, and three crop years) and the high experimental precision and variability among genotypes, we can infer that the dataset is suitable for studying the linear relationships between tassel traits and grain yield through correlation and path analysis.

The mean values obtained in our study were in agreement with those observed by Upadyayula et al. (2006), who reported similar values for the traits TL, NPB, BSL, CSL, and TDM. Moreover, Andrade and Miranda Filho (2008) observed consonant values for TL, TDM, and TBN. They also agreed with the results described by Nardino et al. (2016b) for TL, NPB, and GY, as well as those observed by Yi et al. (2018) and Öner (2018) for TL and TBN (Table 2). Hence, the mean values of TL, NPB, TBN, BSL, CSL, TDM, and GY display an adequate crop development over the three experiments.

Phenotypic and genotypic correlation coefficients

Phenotypic and genotypic correlation matrices were similar in the three crop years in the joint analysis (Table 3). Linear associations of PL with the traits BSL, CSL, NPB, NSB, TBN, BSDM, CSDM, TDM, and GY showed a low magnitude correlation, i.e., between -0.398 and 0.375 , while PL correlations with TL and PDM were significant and of high magnitude, with values ranging from 0.602 to 0.901 . The phenotypic and genotypic correlations of BSL were positive with the traits NPB, NSB, TBN, PDM, BSDM, TDM, and GY. Regarding CSL, the correlations were negative and significant with the traits BSL, NPB, NSB, TBN, and BSDM. The trait TBN correlated positively and significantly with BSL, NPB, NSB, PDM, BSDM, TDM, and GY. For TDM, the phenotypic and genotypic correlations were positive and significant with BSL, NPB, NSB, TBN, PDM, BSDM, and GY. When studying tassel and ear traits, Upadyayula et al. (2006) observed negative and significant correlations between CSL and the traits BSL and TBN. These authors also found a positive and significant association among the traits TBN, BSL and TDM, corroborating the results obtained in our research. Andrade and

Miranda Filho (2008) observed low magnitude correlations between TL and the traits TDM and TBN, whereas those between TDM and TBN were positive and of greater magnitude ($r_g = 0.626$ and $r_p = 0.603$), also supporting our results. Lastly, Öner (2018) observed low magnitude correlations of TL with TBN and TDM, which are similar to those in our investigation.

The GY exhibited positive and significant phenotypic and genotypic correlations with the traits NSB, BSDM, and TDM in the first experiment. In the second, it showed a positive and significant correlation with BSL, NPB, NSB, TBN, BSDM, and TDM. And, in the third experiment, the correlation was positive and significant with BSL, TL, NPB, NSB, TBN, BSDM, and TDM. Between CSL and GY, the phenotypic and genotypic correlations were negative and of low magnitude (Table 3). Ci et al. (2012) observed that the association between GY and TL was positive and of low magnitude, corroborating our results as well. However, it is impossible to infer which of the traits has a direct effect on GY only using correlation coefficients. Thus, the unfolding of the coefficients through path analysis is important, as it unfolds direct and indirect effects and reveals the real cause and effect associations among the traits (Wright, 1921).

Diagnosis of multicollinearity

The diagnosis of the phenotypic and genotypic correlation coefficient matrices among explanatory traits (PL, BSL, CSL, TL, NPB, NSB, TBN, PDM, BSDM, CSDM, and TDM), evaluated in each experiment and jointly, showed a high degree of multicollinearity. Thus, after eliminating the traits PL, CSL, TL, TBN, PDM, CSDM, and BSDM in individual and joint analyses, the correlation matrices exhibited a condition number between 18.19 (phenotypic correlation matrix in experiment 3) and 26.80 (genotype correlation matrix in experiment 1), i.e., weak multicollinearity according to the criteria of Montgomery, Peck, Vinning (2012). Variance inflation factors (VIF) were lower than 10 when the traits PL, CSL, TL, TBN, PDM, CSDM, and BSDM were eliminated, indicating weak multicollinearity (Table 4). Thus, we can infer that the path analyses of the main trait (GY) as a function of the explanatory traits (BSL, NPB, NSB, and TDM) were performed under appropriate conditions (Figure 2).

Path analyzes

The direct effects of NSB on GY obtained from the phenotypic and genotypic correlation matrices were positive and of high magnitude ($0.501 \leq \text{direct effect} \leq 0.855$), which demonstrates a cause and effect association between these traits. Meantime for TDM on GY, the direct effects were positive but with a lower magnitude ($0.099 \leq \text{direct effects} \leq 0.693$). However, the associations can be explained by the greater indirect effects via NSB. Direct effects were of low magnitude ($-0.482 \leq \text{direct effect} \leq 0.001$) for NPB on GY, which can be explained by the high positive indirect effect of NSB on GY ($0.413 \leq \text{indirect effects} \leq 0.698$), featuring the absence of cause-and-effect association between NPB and GY. For BSL on GY, the direct effects were of low magnitude ($-0.402 \leq \text{direct effect} \leq 0.412$). However, the association can be explained by the greater indirect effects via NSB ($0.366 \leq \text{indirect effects} \leq 0.698$) (Table 4).

Based on the phenotypic and genotypic correlation matrices and path analyses, we can infer that the number of secondary tassel branches had a positive linear association with grain yield. Therefore, it can be used in indirect plant selection for grain yield. In practice, a direct non-destructive selection in the field is feasible after anthesis, by counting the secondary branches without removing tassels from maize plants.

Table 1. Summary of analysis of variance with degrees of freedom (DF) and mean square for the sources of variation block, genotype and residue, mean, coefficient of variation (CV%), F-test value for genotype (Fc), and selective accuracy (AS) for 12 traits in 20 maize genotypes in the 2015-2016 (experiment 1), 2016-2017 (experiment 2) and 2017-2018 (experiment 3) crop years.

SV	DF	Mean Square											
		PL	BSL	CSL	TL	NPB	NSB	TBN	PDM	BSDM	CSDM	TDM	GY
Block	2	0.265	0.870	0.337	3.450	3.637	0.172	5.335	0.000	0.020	0.002	0.031	0.771
Genotype	19	10.393*	19.027*	40.976*	24.426*	52.709*	5.009*	85.015*	0.022*	1.986*	0.060*	2.378*	5.754*
Residue	38	0.891	0.833	0.626	1.484	1.411	0.318	2.628	0.001	0.080	0.003	0.102	2.224
Mean		8.780	12.110	26.600	47.500	11.410	2.590	14.000	0.260	2.170	0.670	3.110	9.970
CV(%)		10.750	7.530	2.970	2.560	10.410	21.730	11.580	12.060	13.060	7.410	10.270	14.970
Fc		11.665	22.851	65.461	16.465	37.344	15.766	32.349	22.442	24.701	23.836	23.397	2.587
SA ⁽¹⁾		0.956	0.978	0.992	0.969	0.987	0.968	0.984	0.977	0.980	0.979	0.978	0.783
Precision		VH	VH	VH	VH	VH	VH	VH	VH	VH	VH	VH	H
SV	DF	Mean Square											
		PL	BSL	CSL	TL	NPB	NSB	TBN	PDM	BSDM	CSDM	TDM	GY
Block	2	1.063	0.463	3.465	3.193	1.451	0.293	2.949	0.003	0.011	0.007	0.053	1.849
Genotype	19	12.447*	17.291*	22.092*	35.002*	33.669*	3.783*	55.212*	0.022*	0.993*	0.034*	1.275*	8.455*
Residue	38	0.414	0.611	2.032	2.402	1.317	0.199	1.857	0.000	0.022	0.002	0.034	0.959
Mean		9.130	12.200	27.050	48.380	9.910	2.480	12.390	0.230	1.640	0.530	2.400	9.260
CV(%)		7.040	6.410	5.270	3.200	11.580	17.980	11.000	9.180	8.960	8.080	7.700	10.570
Fc		30.058	28.285	10.870	14.572	25.574	19.022	29.725	49.662	46.008	18.316	37.355	8.819
SA ⁽¹⁾		0.983	0.982	0.953	0.965	0.980	0.973	0.983	0.990	0.989	0.972	0.987	0.942
Precision		VH	VH	VH	VH	VH	VH	VH	VH	VH	VH	VH	VH
SV	DF	Mean Square											
		PL	BSL	CSL	TL	NPB	NSB	TBN	PDM	BSDM	CSDM	TDM	GY
Block	2	0.637	0.083	5.547	8.299	0.158	0.332	0.429	0.002	0.079	0.001	0.115	2.539
Genotype	19	14.207*	22.506*	33.761*	35.013*	32.862*	3.149*	52.775*	0.019*	1.308*	0.056*	1.611*	4.318*
Residue	38	0.440	0.499	2.698	2.936	0.614	0.104	0.957	0.001	0.052	0.005	0.081	0.759
Mean		7.800	12.080	24.860	44.740	9.160	2.080	11.240	0.210	1.800	0.590	2.600	8.320
CV(%)		8.510	5.850	6.610	3.830	8.550	15.480	8.700	11.180	12.720	11.500	10.970	10.470
Fc		32.297	45.062	12.513	11.925	53.530	30.384	55.171	32.960	25.074	12.273	19.884	5.688
SA ⁽¹⁾		0.984	0.989	0.959	0.957	0.991	0.983	0.991	0.985	0.980	0.958	0.975	0.908
Precision		VH	VH	VH	VH	VH	VH	VH	VH	VH	VH	VH	VH

Traits: PL: peduncle length (considering the distance between the collar of the flag leaf and the first branch), in cm; BSL: branching space length, in cm; CSL: central spike length, in cm; TL: tassel length, in cm; NPB: number of primary branches; NSB: number of secondary branches; TBN: tassel branch number; PDM: peduncle dry matter (considering the region between the flag leaf collar and the first branch); BSDM: branching space dry matter, in g; CSDM: central spike dry matter, in g; TDM: tassel dry matter, in g; and GY: grain yield in Mg ha⁻¹ at 13% moisture.

*Significant effect by F-test at 5% probability of error.

(1) Classification limits for selective accuracy (SA) established by Resende and Duarte (2007): VH: very high (SA ≥ 0.90), H: high (0.70 ≤ SA < 0.90) and M: moderate (0.50 ≤ SA < 0.70).

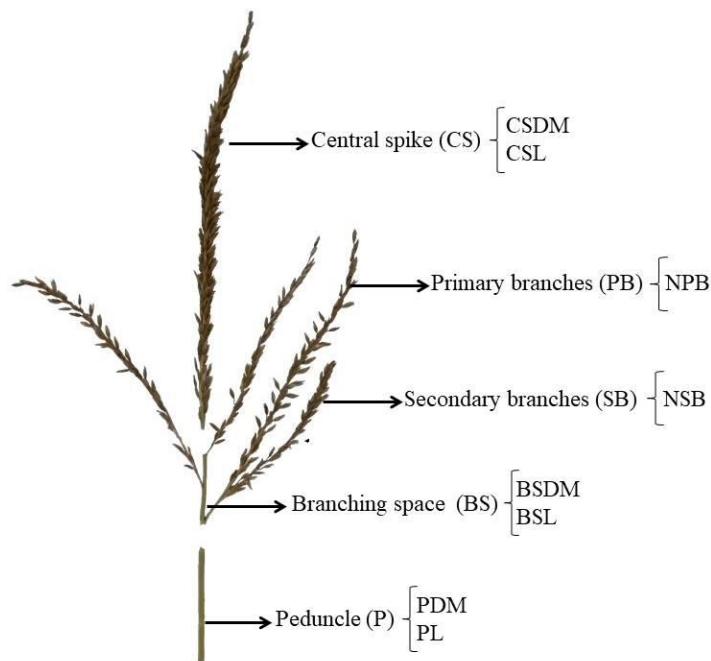


Figure 1. Representation of the traits evaluated in maize tassels, PL: peduncle length (considering the distance between the collar of the flag leaf and the first branch), in cm; BSL: branching space length, in cm; CSL: central spike length, in cm; TL: tassel length, in cm; NPB: number of primary branches; NSB: number of secondary branches; PDM: peduncle dry matter (considering the region between the flag leaf collar and the first branch), in g; BSDM: branching space dry matter, in g; CSDM: central spike dry matter, in g. Adapted from Wartha et al. (2016).

Table 2. Summary of joint analysis of variance with degrees of freedom (DF) and mean square for the sources of variation block nested in the crop year, genotype, crop year, genotype × crop year interaction and residue, mean, coefficient of variation (CV%), F-test value for genotype (Fc), and selective accuracy (AS) for 12 traits in 20 maize genotypes in the 2015-2016 (experiment 1), 2016-2017 (experiment 2) and 2017-2018 (experiment 3) crop years.

SV	DF	Mean Square					
		PL	BSL	CSL	TL	NPB	NSB
Block/Agricultural year	6	0.655	0.472	3.116	4.981	1.748	0.266
Genotype	19	32.970*	56.053*	86.068*	80.715*	115.452*	11.517*
Agricultural year	2	28.849*	0.220ns	80.303*	216.841*	78.501*	4.365*
Genotype × Agricultural year	38	2.039*	1.386*	5.380*	6.862*	1.894*	0.212ns
Residue	114	0.582	0.648	1.785	2.274	1.114	0.207
Mean		8.570	12.131	26.172	46.874	10.161	2.385
CV(%)		8.899	6.635	5.105	3.217	10.388	19.068
Fc		16.173	40.450	15.997	11.762	60.951	54.357
SA ⁽¹⁾		0.969	0.988	0.968	0.957	0.992	0.991
Precision		MA	MA	MA	MA	MA	MA
SV	DF	Mean Square					
		TBN	PDM	BSDM	CSDM	TDM	GY
Block/Agricultural year	6	2.904	0.002	0.036	0.003	0.066	1.720
Genotype	19	187.588*	0.057*	3.979*	0.140*	4.854*	13.936*
Agricultural year	2	115.286*	0.032*	4.475*	0.317*	7.936*	40.765*
Genotype × Agricultural year	38	2.707ns	0.003*	0.154*	0.005*	0.205*	2.296*
Residue	114	1.814	0.001	0.051	0.003	0.072	1.314
Mean		12.545	0.234	1.869	0.597	2.701	9.184
CV(%)		10.736	10.991	12.126	9.112	9.953	12.482
Fc		69.306	20.199	25.822	31.141	23.697	6.070
SA ⁽¹⁾		0.993	0.975	0.980	0.984	0.979	0.914
Precision		MA	MA	MA	MA	MA	MA

Traits: PL: peduncle length (considering the distance between the collar of the flag leaf and the first branch), in cm; BSL: branching space length, in cm; CSL: central spike length, in cm; TL: tassel length, in cm; NPB: number of primary branches; NSB: number of secondary branches; TBN: tassel branch number; PDM: peduncle dry matter (considering the region between the flag leaf collar and the first branch); BSDM: branching space dry matter, in g; CSDM: central spike dry matter, in g; TDM: tassel dry matter, in g; and GY: grain yield in Mg ha⁻¹ at 13% moisture.

*Significant effect by F-test at 5% probability of error. ns Non-significant.

(1) Classification limits for selective accuracy (SA) established by Resende and Duarte (2007): VH: very high (SA ≥ 0.90), H: high (0.70 ≤ SA < 0.90) and M: moderate (0.50 ≤ SA < 0.70).

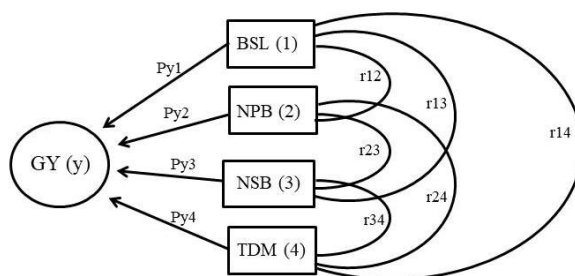


Figure 2. Causal diagram illustrating the direct and indirect effects of explanatory variables: PL: BSL: branching space length, in cm; NPB: number of primary branches; NSB: number of secondary branches; PDM: peduncle dry matter (considering the region between the flag leaf collar and the first branch) on the dependent variable GY: grain yield in Mg ha⁻¹ at 13% moisture. Pyi: Direct effect of each of the four explanatory variables on the dependent variable. Rij: correlation coefficient between explanatory variables.

Table 3. Estimates of phenotypic (above diagonal) and genotypic (below diagonal) correlation coefficients among 12 measured traits of 20 maize genotypes evaluated over three crop years.

Trait	PL	BSL	CSL	TL	NPB	NSB	TBN	PDM	BSDM	CSDM	TDM	GY
Experiment 1 (2015-2016)												
PL	-	0.359	-0.284	0.602*	0.300	0.302	0.310	0.901*	0.340	-0.068	0.387	0.166
BSL	0.397	-	-0.794*	0.088	0.794*	0.839*	0.828*	0.579*	0.764*	-0.396	0.691*	0.354
CSL	-0.303	-0.806*	-	0.409	-0.812*	-0.789*	-0.830*	-0.501*	-0.660*	0.526*	-0.568*	-0.121
TL	0.595*	0.077	0.414	-	-0.155	-0.085	-0.142	0.450*	0.041	0.288	0.126	0.264
NPB	0.327	0.796*	-0.818*	-0.166	-	0.840*	0.991*	0.515*	0.839*	-0.243	0.778*	0.372
NSB	0.338	0.850*	-0.815*	-0.106	0.851*	-	0.904*	0.451*	0.852*	-0.315	0.773*	0.540*
TBN	0.339	0.831*	-0.840*	-0.156	0.992*	0.910*	-	0.515*	0.868*	-0.268	0.800*	0.424
PDM	0.908*	0.605*	-0.519*	0.435	0.535*	0.476*	0.536*	-	0.566*	-0.033	0.608*	0.230
BSDM	0.369	0.766*	-0.671*	0.029	0.843*	0.868*	0.872*	0.583*	-	0.111	0.986*	0.531*
CSDM	-0.079	-0.412	0.535*	0.292	-0.252	-0.331	-0.278	-0.046	0.095	-	0.257	0.247
TDM	0.412	0.694*	-0.579*	0.115	0.782*	0.787*	0.805*	0.622*	0.986*	0.241	-	0.546*
GY	0.265	0.393	-0.162	0.305	0.438	0.614*	0.492*	0.324	0.656*	0.324	0.683*	-
Experiment 2 (2016-2017)												
PL	-	0.300	0.019	0.822*	0.202	0.038	0.168	0.837*	0.229	0.000	0.313	-0.053

BSL	0.319	-	-0.672*	0.348	0.723*	0.686*	0.744*	0.526*	0.758*	-0.382	0.676*	0.474*
CSL	0.021	-0.707*	-	0.334	-0.696*	-0.646*	-0.713*	-0.134	-0.539*	0.596*	-0.396	-0.333
TL	0.853*	0.355	0.291	-	0.075	-0.009	0.057	0.762*	0.242	0.204	0.347	0.038
NPB	0.222	0.725*	-0.722*	0.087	-	0.787*	0.987*	0.455*	0.845*	-0.083	0.792*	0.614*
NSB	0.049	0.706*	-0.700*	-0.014	0.809*	-	0.876*	0.156	0.788*	-0.221	0.680*	0.757*
TBN	0.185	0.748*	-0.743*	0.064	0.988*	0.889*	-	0.396	0.866*	-0.123	0.797*	0.677*
PDM	0.845*	0.537*	-0.151	0.779*	0.470*	0.165	0.409	-	0.561*	0.144	0.650*	0.124
BSDM	0.243	0.767*	-0.585*	0.237	0.861*	0.803*	0.878*	0.565*	-	0.141	0.980*	0.641*
CSDM	0.008	-0.401	0.603*	0.191	-0.088	-0.232	-0.128	0.138	0.126	-	0.306	-0.014
TDM	0.328	0.685*	-0.441	0.344	0.810*	0.695*	0.811*	0.654*	0.980*	0.290	-	0.580*
GY	-0.051	0.497*	-0.350	0.050	0.647*	0.804*	0.713*	0.130	0.681*	-0.006	0.619*	-
Experiment 3 (2017-2018)												
PL	-	0.375	-0.333	0.611*	0.240	0.107	0.215	0.868*	-0.009	-0.398	0.012	0.219
BSL	0.387	-	-0.622*	0.430	0.749*	0.731*	0.769*	0.595*	0.682*	-0.353	0.613*	0.641*
CSL	-0.345	-0.649*	-	0.271	-0.615*	-0.545*	-0.619*	-0.304	-0.223	0.794*	-0.086	-0.200
TL	0.636*	0.443	0.220	-	0.149	0.119	0.147	0.732*	0.323	0.244	0.415	0.457*
NPB	0.248	0.752*	-0.629*	0.166	-	0.824*	0.990*	0.422	0.786*	-0.195	0.718*	0.538*
NSB	0.116	0.741*	-0.547*	0.152	0.834*	-	0.895*	0.248	0.816*	-0.211	0.723*	0.665*
TBN	0.224	0.773*	-0.629*	0.168	0.991*	0.900*	-	0.394	0.820*	-0.205	0.743*	0.587*
PDM	0.877*	0.608*	-0.336	0.747*	0.437	0.264	0.409	-	0.333	-0.176	0.375	0.379
BSDM	0.001	0.692*	-0.270	0.308	0.807*	0.850*	0.843*	0.331	-	0.251	0.984*	0.573*
CSDM	-0.402	-0.364	0.793*	0.216	-0.189	-0.203	-0.198	-0.195	0.230	-	0.393	-0.094
TDM	0.023	0.627*	-0.136	0.400	0.744*	0.762*	0.772*	0.373	0.984*	0.370	-	0.539*
GY	0.257	0.690*	-0.244	0.500*	0.589*	0.717*	0.639*	0.424	0.620*	-0.119	0.587*	-
General												
PL	-	0.352	-0.197	0.729*	0.251	0.143	0.233	0.871*	0.181	-0.178	0.228	0.104
BSL	0.355	-	-0.746*	0.288	0.762*	0.768*	0.788*	0.575*	0.735*	-0.396	0.660*	0.559*
CSL	-0.187	-0.775*	-	0.285	-0.752*	-0.712*	-0.766*	-0.344	-0.541*	0.643*	-0.418	-0.294
TL	0.757*	0.280	0.258	-	0.020	-0.004	0.014	0.681*	0.169	0.220	0.264	0.228
NPB	0.255	0.768*	-0.771*	0.019	-	0.831*	0.991*	0.478*	0.839*	-0.186	0.780*	0.593*
NSB	0.139	0.777*	-0.735*	-0.010	0.838*	-	0.900*	0.290	0.842*	-0.259	0.750*	0.749*
TBN	0.234	0.794*	-0.786*	0.013	0.991*	0.904*	-	0.446*	0.867*	-0.210	0.797*	0.651*
PDM	0.875*	0.581*	-0.352	0.697*	0.484*	0.288	0.450*	-	0.483*	-0.046	0.539*	0.279
BSDM	0.179	0.739*	-0.572*	0.154	0.847*	0.854*	0.875*	0.481*	-	0.151	0.983*	0.685*
CSDM	-0.181	-0.408	0.646*	0.207	-0.187	-0.261	-0.211	-0.054	0.143	-	0.301	0.041
TDM	0.226	0.664*	-0.446*	0.251	0.789*	0.761*	0.806*	0.535*	0.983*	0.294	-	0.657*
GY	0.111	0.617*	-0.355	0.231	0.662*	0.820*	0.722*	0.316	0.771*	0.033	0.739*	-

*Significant at 5% probability of error by Student's t-test with 18 degrees of freedom.

Table 4. Path analysis direct and indirect effects of the phenotypic and genotypic correlation matrices of explanatory traits on grain yield (GY) of 20 maize genotypes evaluated in three crop years.

Effects	Phenotypic correlation matrix				Genotypic correlation matrix			
	BSL	NPB	NSB	TDM	BSL	NPB	NSB	TDM
Experiment 1 (2015/2016)								
Direct on GY	-0.286	-0.402	0.748	0.479	-0.402	-0.482	0.821	0.693
Indirect via BSL		-0.227	-0.240	-0.198		-0.320	-0.342	-0.279
Indirect via NPB	-0.319		-0.338	-0.313	-0.384		-0.410	-0.377
Indirect via NSB	0.628	0.628		0.578	0.698	0.698		0.646
Indirect via TDM	0.331	0.373	0.370		0.481	0.542	0.545	
Total	0.354ns	0.372ns	0.540*	0.546*	0.393ns	0.438ns	0.614*	0.683*
Coefficient of determination	0.415				0.608			
Condition number	23.61				26.80			
Maximum variance inflation factor	3.713	4.275	5.118	2.885	3.876	4.395	5.761	2.991
Experiment 2 (2016/2017)								
Direct on GY	-0.158	0.001	0.740	0.183	-0.205	-0.086	0.855	0.234
Indirect via BSL		-0.114	-0.109	-0.107		-0.148	-0.144	-0.140
Indirect via NPB	0.001		0.001	0.001	-0.062		-0.069	-0.069
Indirect via NSB	0.507	0.582		0.503	0.604	0.692		0.594
Indirect via TDM	0.124	0.145	0.125		0.160	0.190	0.163	
Total	0.474*	0.614*	0.757*	0.580*	0.497*	0.647*	0.804*	0.619*
Coefficient of determination	0.592				0.675			
Condition number	18.42				21.28			
Maximum variance inflation factor	2.374	4.123	2.861	2.883	2.426	4.582	3.173	3.097
Experiment 3 (2017/2018)								
Direct on GY	0.391	-0.253	0.501	0.119	0.412	-0.245	0.540	0.099
Indirect via BSL		0.293	0.286	0.240		0.310	0.305	0.258

Indirect via NPB	-0.189		-0.208	-0.181	-0.184		-0.204	-0.182
Indirect via NSB	0.366	0.413		0.362	0.400	0.450		0.411
Indirect via TDM	0.073	0.085	0.086		0.062	0.074	0.075	
Total	0.641*	0.538*	0.665*	0.539*	0.690*	0.589*	0.717*	0.587*
Coefficient of determination	0.512				0.585			
Condition number	18.19				19.58			
Maximum variance inflation factor	2.519	3.919	3.772	2.331	2.555	4.086	4.163	2.629
Effects	Phenotypic correlation matrix				Genotypic correlation matrix			
	BSL	NPB	NSB	TDM	BSL	NPB	NSB	TDM
	General							
Direct on GY	-0.030	-0.239	0.738	0.311	-0.044	-0.254	0.785	0.372
Indirect via BSL		-0.023	-0.023	-0.020		-0.034	-0.034	-0.029
Indirect via NPB	-0.182		-0.199	-0.186	-0.195		-0.213	-0.200
Indirect via NSB	0.566	0.613		0.553	0.609	0.657		0.597
Indirect via TDM	0.205	0.242	0.233		0.247	0.293	0.283	
Total	0.559*	0.593*	0.749*	0.657*	0.617*	0.662*	0.820*	0.739*
Coefficient of determination	0.598				0.723			
Condition number	19.82				20.71			
Maximum variance inflation factor	2.780	4.331	4.013	2.799	2.855	4.495	4.216	2.909

* Significant at 5% probability of error by Student's t-test with 18 degrees of freedom.

Table 5. Version, technology, company, type, cycle, use, kernel, color and investment of 20 maize genotypes.

Hybrid	Version	Technology ⁽¹⁾	Company	Type ⁽²⁾	Cycle	Use ⁽³⁾	Kernel	Color ⁽⁴⁾	Investment
20A55	PW	PowerCore	Morgan Seeds	TH	E	G/S	Semi-flint	LO	Medium
30F53	YH	Optimum Intrasect	Pioneer	SH	E	G/S	Semi-dent	O	High
AG8780	PRO 3	VT PRO 3	Agrocere seeds	SH	E	G	Semi-dent	LO	High
BM3066	PRO2	VT PRO 2	Biomatrix	SH	E	G/S	Semi-dent	O	High
DKB 290	PRO 3	VT PRO 3	Dekalb	SH	E	G	Semi-dent	LO	High
MS 2010	-	Conventional	Melhoramento Agropastoril	SH	E	G	Semi-dent	Y/LO	High
MS 2013	-	Conventional	Melhoramento Agropastoril	SH	E	G	Semi-flint	LO	High
MS 3022	-	Conventional	Melhoramento Agropastoril	TH	E	G	Flint	O	Medium
Status	VIP	Agrisure Viptera	Syngenta Seeds	SH	E	G	Flint	LO	High
SX7331	VIP	Agrisure Viptera	Syngenta Seeds	SH	E	G	Flint	O	High
30A68	PW	PowerCore	Morgan Seeds	SH	SE	G	Semi-flint	LO	High
AG9025	PRO 3	VT PRO 3	Agrocere seeds	SH	SE	G	Semi-dent	LO	High
AM9724	-	Conventional	Melhoramento Agropastoril	SH	SE	G	Dent	Y/LO	High
AS1666	PRO 3	VT PRO 3	Agroeste	SH	SE	G	Semi-dent	Y/LO	High
AS1677	PRO 3	VT PRO 3	Agroeste	SH	SE	G	Semi-dent	LO	High
Celeron	TL	Agrisure TL	Syngenta Seeds	SH	SE	G	Flint	LO	High
DKB 230	PRO 3	VT PRO 3	Dekalb	SH	SE	G	Semi-dent	Y	High
P1630	H	Herculex I	Pioneer	SH	SE	G	Semi-dent	LO	High
P2530	-	Conventional	Pioneer	SH	SE	G	Semi-flint	O	High
SHS 7915	PRO	YieldGard VT PRO	Santa Helena Seeds	SH	SE	G/S	Semi-flint	LO	High

⁽¹⁾The genetic composition of maize plants is modified, being able to confer resistance or tolerance inherent in the development of corn hybrids, that is, to develop materials with specific traits, such as: resistance to attack by insect pests and tolerance to different herbicides. ⁽²⁾SH: single-cross hybrid; HT: three-way cross hybrid; E: early; SE: super early. ⁽³⁾G: grain; S: silage. ⁽⁴⁾LO: light orange; O: orange; Y: yellow. Information provided by the State Foundation for Agricultural Research (FEPAGRO).

Materials and methods

Conduction of study and experimental design

Three experiments were conducted with maize in the 2015/2016 (experiment 1), 2016/2017 (experiment 2), and 2017/2018 (experiment 3) crop years. According to the Köppen climate classification, the climate in the region is defined as *Cfa*, which stands for humid subtropical, with hot summers and no dry season defined (Alvares et al., 2013). The soil of the area is classified as sandy-loam typic Paleudalf (Santos et al., 2018). The experiments were constituted of 20 genotypes from a network of evaluation trials of maize cultivars in the state of Rio Grande do Sul, which is coordinated by the State Foundation for Agricultural Research (FEPAGRO) (Table 5).

The experimental design used was randomized complete blocks with three replicates. Plots consisted of two 5-m-long rows spaced 0.80 m apart and 0.20 m between plants within rows, totaling a useful area of 8 m² per plot. The experiments were conducted for three years, using the same hybrids, which are grown commercially on a large scale. In this way, consistent data and results were obtained.

Cultural management

Sowings were carried out on Oct/21/2015, Nov/19/2016, and Oct/31/2017 respectively for experiments 1, 2, and 3. Basal dressing was performed along with sowing in the three experiments. In the 2015/2016 crop year (experiment 1), an NPK 05-20-20 commercial formulation was used to provide 37.5 kg ha⁻¹ N, 150 kg ha⁻¹ P₂O₅, and 150 kg ha⁻¹ K₂O, and topdressing fertilization with 121.5 kg ha⁻¹ N in the form of urea into three splits. In the 2016/2017 crop year (experiment 2), an NPK 05-20-20 commercial formulation was used to provide 20 kg ha⁻¹ N, 80 kg ha⁻¹ P₂O₅, and 80 kg ha⁻¹ K₂O, and topdressing with 180 kg ha⁻¹ N in the form of urea into two splits. In the 2017/2018 crop year (experiment 3), an NPK 05-20-20 commercial formulation was used to provide 15 kg ha⁻¹ N, 60 kg ha⁻¹ P₂O₅, and 60 kg ha⁻¹ K₂O, and topdressing with 157.5 kg ha⁻¹ N in the form of urea into two splits. Plant density was adjusted by manual thinning to five plants per meter in each row, totaling 62,500 plants per hectare. The cultural practices were carried out according to the recommendations for maize cropping, maintaining the

experimental area in a competition-free condition of weeds, pests, and diseases (Fancelli and Dourado Neto, 2009).

Data collection

When plants were at the reproductive stage, we randomly collected 20, 11, and 20 tassels per plot respectively in the 2015/2016, 2016/2017, and 2017/2018 crop years. As reported by Wartha et al. (2016), sample sizes containing 11, 20, 43, and 169 tassels are enough to estimate averages of tassel traits at precision levels of 40, 30, 20, and 10%, respectively (for a 95% confidence level). After being collected in the field, tassels were identified, stored in paper packaging, and dried in a forced-air ventilation oven (60°C) until a constant weight was reached. The following traits were measured for each tassel: peduncle length (PL, considering the distance between the collar of the flag leaf and the first branch), in cm; branching space length (BSL), in cm; central spike length (CSL), in cm; tassel length (TL=PL+BSL+CSL), in cm; number of primary branches (NPB); number of secondary branches (NSB); tassel branch number (TBN=NPB+NSB); peduncle dry matter (PDM, considering the region between the flag leaf collar and the first branch), in g; branching space dry matter (BSDM), in g; central spike dry matter (CSDM), in g; and tassel dry matter (TDM=PDM+BSDM+CSDM), in g (Figure 1). All plant in the plot were assessed for grain yield (GY), which was expressed as Mg ha⁻¹ at 13% moisture.

Statistical analyses

The Kolmogorov-Smirnov and Levene tests were used to verify the normality of errors and homogeneity of residual variances for all 12 traits measured, respectively. Subsequently, individual and joint analyses of variance were performed at a 5% level of significance. For the joint analysis, the effects of genotypes were considered fixed, and crop years deemed as random. Estimates of the means, coefficient of variation (CV), F-calculated for genotype (F_c), and selective accuracy (SA) were recorded for each trait (PL, BSL, CSL, TL, NPB, NSB, TBN, PDM, BSDM, CSDM, TDM, and GY), as described by Resende and Duarte (2007). Linear relationships were investigated using estimated matrices of phenotypic (rp) and genotypic (rg) correlation coefficients among the traits with significant differences by individual variance analysis (F-test) and by joint variance analysis. Besides, the significance of phenotypic and genotypic correlation coefficients was tested as per the Student's t-test at a 5% probability of error. The multicollinearity of the phenotypic and genotypic correlation matrices was diagnosed by a variance inflation factor (VIF) and condition number (CN), as established by Montgomery, Peck and Vinning (2012). The criteria used were VIF <10 and CN <100 as indicative of weak multicollinearity. In the presence of a high degree of multicollinearity (detrimental to path analysis), one or more traits highly correlated were excluded. A path analysis was performed for each experiment (crop year), considering grain yield as the main trait and tassel traits as explanatory. Additionally, a general path analysis (all experiments) was also performed. The path analyses enabled identifying explanatory traits with direct and indirect effects on the main trait. Statistical analyses were performed using the Genes software (Cruz, 2016) and Microsoft Office Excel® application.

Conclusions

The number of secondary tassel branches had a positive linear relationship with grain yield and can be used for the indirect selection of maize plants in breeding programs.

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References

- Alvares CA, Stape JL, Sentelhas PC, Gonçalves JLM, Sparovek G (2013) Köppen's climate classification map for Brazil. *Meteorol Z.* 22 (6): 711-728.
- Andrade JAC, Miranda Filho JB (2008) Quantitative variation in the tropical maize population, ESALQ-PB1.. *Sci Agric.* 65 (2): 174-182.
- Brewbaker JL (2015) Diversity and genetics of tassel branch numbers in maize. *Crop Sci.* 55: 65-78.
- Ci X, Li M, Xu J, Lu Z, Bai P, Ru G, Liang X, Zhang D, Li X, Bai L, Xie C, Hao Z, Zhang S, Dong, S (2012) Trends of grain yield and plant traits in Chinese maize cultivars from the 1950s to the 2000. *Euphytica.* 185: 395-406.
- Cruz CD, Carneiro PCS, Regazzi AJ (2014) Modelos biométricos aplicados ao melhoramento genético. 5 rd edn Viçosa: UFV.
- Cruz CD (2016) Genes Software - extended and integrated with the R, Matlab and Selegen. *Acta Sci-Agron.* 38 (4): 547-552.
- Duvick DN, Smith JSC, Cooper M (2004) Long-term selection in a commercial hybrid maize breeding program. In: *Plant Breeding Reviews. Part 2: Long-term selection: crops, animals, and bacteria.* 1rd edn. John Wiley & Sons, New Jersey. 109-152.
- Duvick D (2005) Genetic progress in yield of United States maize (*Zea mays* L.). *Maydica.* 50 (3): 193-202.
- Edwards J (2011) Changes in plant morphology in response to recurrent selection in the Iowa stiff stalk synthetic maize population. *Crop Sci.* 51 (6): 2352-2361.
- Fischer RA, Edmeades GO (2010) Breeding and cereal yield progress. *Crop Sci.* 50: 85-98.
- Fancelli AL, Dourado Neto D (2009) Milho: manejo e produtividade. 1rd edn. ESALQ/USP, Piracicaba. 181.
- Gage JL, White MR, Edwards JW, Kaeppler S, De Leon N (2018) Selection signatures underlying dramatic male inflorescence transformation during modern hybrid maize breeding. *Genetics.* 210 (3): 1125-1138.
- Montgomery DC, Peck EA, Vinning GG (2012) Introduction to linear regression analysis. 5rd edn. Wiley, New York. 672.
- Nardino M, De Souza VQ, Baretta D, Konflanz VA, Carvalho IC, Follmann DN, Caron BO (2016a) Association of secondary traits with yield in maize F 1 's. *Cienc Rural.* 46 (5): 776-782.
- Nardino M, De Souza VQ, Baretta D, Konflanz VA, Follmann DN, Carvalho IC, Ferrari M, Caron BO, Schmidt, D (2016b) Partial diallel analysis among maize lines for characteristics related to the tassel and the productivity. *Afr J Agric Res.* 11 (11): 974-982.
- Öner F (2018) Assessment of genetic variation in turkish local maize genotypes using multivariate discriminant analysis. *Appl Ecol Env Res.* 16 (2): 1369-1380.
- Parvez AS (2007) Genetic Analysis of Tassel and Ear Characters in Maize (*Zea mays* L.) Using Triple Test Cross. *Asian J Plant Sci.* 6 (5): 881-883.
- Porto APF, Vasconcelos RC, Viana AES, Almeida MRS (2011) Variedades de milho a diferentes espaçamentos no Planalto de Vitória da Conquista - BA. *Rev. Bras. Cienc. Agrar.* 6 (2): 208-214.
- Resende MDV, Duarte JB (2007) Precisão e controle de qualidade em experimentos de avaliação de cultivares. *Pesq. Agropec. Trop.* 37 (3): 182-194.
- Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Lumberas JF, Coelho MR, Almeida JA, Cunha, TJF, Oliveira JB (2013) Sistema brasileiro de classificação de solos. 5rd edn. Brasília: Embrapa.

Wartha CA, Cargnelutti Filho A, Lúcio AD, Follmann DN, Kleinpaul JA, Simões FM (2016) Sample sizes to estimate mean values for tassel traits in maize genotypes. *Genet Mol Res.* 15 (4): 1-13.

Wright S (1921) Correlation and causation. *J Agric Res.* 20, 557-585.

Upadyayula N, Da Silva HS, Bohn MO, Rocheford TR (2006) Genetic and QTL analysis of maize tassel and ear inflorescence architecture. *Theor Appl Genet.* 112: 592-606.

Yi Q, Liu Y, Zhang X, Hou X, Zhang J, Liu H, Hu Y, Yu G, Huang, Y (2018) Comparative mapping of quantitative trait loci for tassel-related traits of maize in F2:3 and RIL populations. *J Genet.* 97: 253-266.