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Genetic resources of maize (*Zea mays* L.): double purpose hybrids to generate grain and lignocellulosic biomass.

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Abstract

Maize stover (*Zea mays* L.) can be converted into fermentable sugars to produce ethanol by fermentation processes,similar to those occurring to forage in the rumen. The efficiency of these processes depends on the stover cell wall structure. Some authors suggested that selection for grain yield and resistance to stalk lodging could reduce forage quality and, as a result, its potential ability to produce ethanol. Therefore, finding sources of maize genetic variability appears to be a good alternative for lignocellulosic bioethanol production without compromising grain yield. During three years, 144 maize genotypes were evaluated to found favorable alleles to generate double purpose hybrids for grain and lignocellulosic biomass. They included native Argentinean populations (landraces), commercial hybrids, maize composites, and experimental silage hybrids, differing in improvement level, cycle length, grain type, and presence of BMR genes. Selection indexes were constructed using a nonparametric rank-sum index to select dual-purpose genotypes. Thus, indexes allowed to identify the superior genotypes for bio-energetic Potential and stability. Two native populations were selected for their good performance to produce grain and lignocellulosic biomass (ARZM03003, ARZM18022).

Introduction

Stover is the residue of grain harvest and is an abundant, cheap and promising source of lignocellulosic biomass for ethanol production (Lorenz et al., 2009). This is an unexploited resource in many countries such as Argentina, where maize production area is around 8.5 million hectares, of which 7 million hectares are used to produce grain (Minagri, 2018), and the remaining area is used for fodder reserves.

The rumen microorganisms cross several barriers to access the carbohydrates of the cell wall to produce lactic acid, a similar process occurs in the simultaneous saccharification and fermentation (SSF) to obtain ethanol (Dowe and Mcmillan, 2001). Therefore, lignocellulosic biomass quality would be estimated with forage quality methods, such as *in vitro* ruminal digestibility of dry matter, neutral detergent fiber digestibility (NDFD) and lignin content (ADL) (Lorenz et al., 2009), which are easy to calibrate and perform using NIRS technology (Grabber, 2005; Lorenz et al., 2009a).

Comparisons between maize genotypes suggest that genetic variability could be exploited to increase ethanol yield per unit area, like changing lignin content (LORENZ et al., 2009a) as in "brown midrib" (bmr) mutants (Barrière et al., 2017). However, lignin content reduction is generally accompanied by a reduction in biomass production and an increase in susceptibility to stem diseases (Pedersen et al., 2005).

Another way to increase ethanol yield could be using germplasm with high cell wall digestibility as proposed by Barrière et al. (2018). Selection towards grain yield and stalk lodging resistance could have reduced forage quality and, consequently, the stover potential to produce bioethanol (Barrière et al. 2005, 2017). Lorenz et al. (2010a) proposed increasing plant height, stem diameter and density, leaf area and delaying senescence, to achieve higher yield and lignocellulosic biomass production.

One solution to increase the stover quality to produce lignocellulosic bioethanol without compromising the grain yield could be to explore local genetic resources.

Examples of this are the Latin American Maize Project (LAMP) and the Maize Germplasm Improvement Project (GEM) whose objective is to introduce new germplasm into breeding programs (Pollak, 2001). Other authors when evaluating Argentinean maize landraces found sources of favorable alleles for forage yields and quality (Bertoia et al., 2006, Incognito et al., 2013, 2016, Bertoia and Aulicino, 2014). In this sense, we propose that Argentine Landrace maize could be used as sources of favorable alleles to obtain dual purpose genotypes (grain and lignocellulosic energy) due to its low level of improvement for grain yield and high biomass yields. The objectives of this work were: i. Characterize local and exotic maize genetic resources variability based on grain yield and stover biomass for bioethanol. ii. Use selection indices to identify local genotypes with superior aptitudes for grain and lignocellulosic bioethanol production.

Materials and Methods

This study included 144 different maize accessions representing a wide range of racial origins, cycle, and grain type: 100 Argentinean Native maize populations (Landraces) were ceded by Germplasm Bank of INTA Pergamino (from 1 to 100); 12 commercial hybrids used for grain and forage production in Argentina (from 101 to 112); 5 early hybrids ceded by Limagrain S.A. (from 116 to 120); 10 Maize Composites (MC) conformed by groups of genotypes selected by grain and silage (from 121 to 130) ceded by Dow Agrosciences S.A. (DAS), as also 17 silage experimental hybrids (SEH) (from 113 to 115 and 131 to 144).

Three commercial hybrids widely used for grain and forage production in Argentina were used as controls: DK72-10VT3P (Monsanto) with high grain performance and good adaptability (122 days to maturity), BMR126HX (DAS) a BMR hybrid (115 days to maturity) and DK390VT3P (Monsanto) a tropical hybrid with high grain performance (150 days to maturity). This information was provided by seed companies.

Experimental design

Experimentals were conducted in the Facultad de Ciencias Agrarias, Universidad Nacional de Lomas de Zamora (FCA-UNLZ) (34°49'59.45''S, 58°43'17.98''W) during 3 years. Sowing dates were: 12/10/2012, 10/30/2013 and 10/29/2014, respectively over typical Argiudoll soils. Due to the high number of genotypes and low seeds numbers, an augmented design was used (Federer, 1961). Plots consisted of two 5 m rows spaced 0.5 m apart. We arbitrary used a final density of 80,000 plants ha-1, obtained by thinning when the plants reached the V_3 stage (Ritchie et al., 1993). Three controls were randomized repeated every 4 units to estimate the experimental error and to test the differences between the genotypes (144 accessions), using adjusted means (Federer et al., 2001; Federer, 1961).

Stover quality analysis

Plants of each genotype were hand-harvested once reaching physiological maturity and with less than 20% moisture content in the grains. Ten plants were harvested and weighed at random per plot, their ears were removed previously. Stover sample was taken of the complete phytomer that carried the ear. After drying in an oven at 60 °C until constant weight, the percentage of dry matter was calculated to determine the dry matter yield of stover (SY, t ha-1) and grain yield (GY, t ha-1) per plot corrected to 14.5 % humidity. Determinations of root lodging (RL) as a percentage of plants leans from the vertical at an angle of 45° or more, and of stalk lodging (SL) as a percentage of plants with stalks broken below the ear, were both made at the time of harvesting.

Dried stover samples (minus cob) were ground with a hammer mill to pass a 1 mm screen and subsequently scanned with a NIR Systems 6500 near infrared reflectance spectrophotometer. We used our NIRS calibration curve developed at Cereals and forage Laboratory of the FCA-UNLZ to obtain a detailed compositional profile of all stover samples (Bertoia and Aulicino, 2014). For the determination of NDFD, the NDF and IVD techniques were applied sequentially on the same sample (Van Soest et al., 1991). Stover samples were incubated with enzyme solution together with the buffer solution in the Daisy II incubator (Ankom Technology, Macedon, NY, USA). Subsequently, the NDF content of the residue was determined on an ANKOM-200 fiber analyzer. The IVD and NDF values were used to calculate NDFD according to the Goering and Van Soest, (1970) method. Cellulose was determined as the percentage of tissue lost between ADF and ADL and Hemicellulose was determined as difference between NDF and ADF.

We used the equation cited by Zhao et al. (2009) to estimate ethanol yield (CEY, l ha^{-1}) based on cellulose and hemicellulose content:

CEY (l ha^{-1}) = [cellulose (%) + hemicellulose (%)] * SY (T ha^{-1}) * 1.11 (conversion factor of sugar from cellulose and hemicellulose) * 0.85 (process efficiency of sugar from cellulose and hemicellulose) * 0.51 (conversion factor of ethanol from sugar) * 0.85 (process efficiency of ethanol from sugar) * 1000 * 0.79-1 (specific gravity of ethanol, g ml^{-1}). It is a standardized protocol that can be used to compare different biomass sources.

Statistical analysis

Data for each year were reported separately, using an analysis of variance (ANOVA) with an augmented design of incomplete blocks. ANOVA of all the variables was performed using the Adjusted Values (AjV) (Federer, 1961). The CF was estimated for each block with the data of the checks by applying the equation:

$CF = (1\ g^{-1})$ (total of each block - total of all blocks/r), where: "g" is the number of checks and "r" number of blocks.

ANOVA was calculated with the SAS® macro program, augment.sas (Iasri, 2013) using SAS Stat Sofware (SAS INSTITUTE, 2009).

Tukey Non Additivity test was applied to evaluate Genotype × Environment (year) interaction (G × E). This method was calculated by a randomized complete blocks design, considering the years as blocks. The total sum of squares was partitioned into sum of squares for non-additivity (with 1 degree of freedom) and in addition of residual squares (Steel and Torrie, 1960). When the F of the non-additivity is significant, it would indicate presence of multiplicative effects.

The genetic parameters were: Phenotypic ($VP=MSg+MSe$), Genotypic ($VG=MSg$) and Environmental ($VE=MSe$) Variances, Broad Heritability ($He^2\ (\%) = VG/VP$), Coefficient of Genetic Variation in percentage ($CVg\%=(100\sqrt{VG})/Mg$), Coefficient of Environmental Variation in percentage($CVe\%=(100\sqrt{VE})/Mg$), and the CVg/CVe ratio, where: Mg is genotype means, and MS is Mean Squares. We calculated using GENES program (Cruz, 2006).

Selection indices

We created a rank-sum index (SI) (Kang, 1993) to select superior genotypes for grain and lignocellulosic biomass production. The selection indexes (SI) were established using CVg/CVe ratio as weights for the grain and stover yield variables, as well as for the quality variables related to convertibility (IVD and NDFD) and to the content of structural carbohydrates (NDF, ADF and ADL). This ratio identified which genetic or non-genetic variation was the greatest (Falconer and Mackay, 1996). Negative signs were applied only for ADL, RL and SL. An arbitrary value of "-1" was assigned to RL and SL, excluding genotypes that presented this undesired behavior.

Selection of the genotypes was done using the total sum of indices by genotypes (TSI), which was calculated by adding the SI through the variables corresponding to each year. The TSI of each genotype was added over the years, constituting the General Selection Index (GSI). The genotypes were ranked in decreasing order by their GSI. Then, genotypes with AjV greater than the mean of the controls + 1 LSD (least significant difference) were selected as superior (Cotes and Ñústez, 2001; Kang, 1993). We arbitrary decided to select the top 20% of the genotypes with the highest index. Wricke Ecovalence method (Wricke, 1962) was applied to evaluate agronomic stability of the indices through the years.

Weather data

Weather data (air temperature and precipitation) were recorded continuously at the meteorological station located at a distance of about 100 m from the field experiment. Temperature values (mean, minimum and maximum) and rainfall were calculated for the planting to harvest period of each trial (Table 1).

Table 1 - Climatic conditions for each experimental period. Air Temperature (mean, minimum and maximum, in °C) for sowing-silking period, and rainfall (in mm) for sowing-harvest period.

	Air Temperature			Rainfall
	Mean	Minimum	Maximum	
Years	°C	°C	°C	mm
2012	23.4	7.9	37.1	531
2013	23.3	6.4	38.5	930
2014	22.3	5.6	35.0	567

Results and discussion

Grain, stover and calculated ethanol yield

ANOVAS showed significant differences ($p<0.01$) between Treatments, Genotypes and Controls for all yield variables. The Controls means were significantly higher than Genotypes ($p <0.01$), except for SY in 2012 (Table 2). Likewise Bertoia et al. (2006) found similar results for GY, but not for SY, where the Controls were lower than the Genotypes. On the contrary, CEY showed the most dissimilar results, with significant differences at 1% in 2014, at 5% in 2013 and not significant in 2012. According to Maddonni (2012) and Mercau et al. (2014), late sowing in the Argentina's Maize Belt is used as a strategy for decreasing the deficit hydric risk during silking period. This ensures more stable harvests but not guarantee high yields. Highest levels of SY (10.61 t ha-1) and CEY (3.70 1000 l ha-1) were also found in 2012, which would be also related to the sowing date of the experiment. Cirilo and Andrade (1994) found that early sowings favored reproductive growth, with highest grain yield, whereas late sowings favored

vegetative growth. Although the highest levels of GY were also found in 2012, these could be due to a good distribution of effective rainfall (> 10 mm day^{-1}) and air temperature under 36°C during the silking period (data not shown), despite being a late planting date. Tukey Non Additivity test was applied for all variables. The test was significant for SY and CEY ($p<0.01$) indicating G × E interaction effects. Other authors (Bertoia and Aulicino, 2014; Sah et al., 2016) found similar results for genotypes evaluated in the silage harvest moment and double-purposed genotypes (grain and stover production).

Stover quality traits

ANOVA showed significant differences ($p<0.01$) between Genotypes for IVD in all years. However, the rest of quality traits produced significant differential responses depending on the year considered. We found differences at 1% in 2012 for NDFD and for ADF in 2014, differences at 5% for NDF in 2013; for ADF, and ADL in 2012 (Table 2). The year 2014 presented higher values of IVD (49.05%), NDFD (39.04%), NDF (73.58%); moderate ADF (36.73%) and low ADL (5.72%) (Table 3). This year presented the lowest temperature (Table

Table 2 - Augment design's ANOVA by environment. Tukey Non-Addivity Test. Estimated mean squares for the variables Grain Yield (GY, t ha-1), Stover Yield (SY, t ha-1), Acid Detergent Fiber (ADF, %), Neutral Detergent Fiber (NDF, %), Lignin Detergent Acid (ADL, %), in vitro Digestibility (IVD, %), Neutral Detergent Fiber Digestibility (NDFD, %), Calculated Ethanol Yield (CEY, 1000 l ha-1).

FV	DF	GY		SY		ADF		NDF		ADL		IVD		NDFD		CEY	
2012																	
Block	15	1.6	NS	1.6	NS	13.4	**	44.9	**	0.7	NS	9.4	**	18.2	*	0.2	NS
Treatments	146	12.3	**	9.2	**	8.4	*	23	NS	0.7	*	17.6	**	30.9	**	1.3	**
Genotypes	143	7.5	**	8.5	**	7.6	*	22.4	NS	0.7	*	10.7	**	19	**	1.2	**
Controls	2	65.4	**	62.2	**	69.1	**	77.4	*	4.6	**	497	**	849.2	**	9.9	**
Genotypes vs. Controls	1	651	**	0.8	NS	10.3	NS	15.8	NS	5.8	**	66.2	**	208.2	**	0.5	NS
Error	30	2		2		4.4		14.6		0.4		2.8		8.4		0.2	
Corrected Total	191	.		.		.		.		.				.		.	
2013																	
Block	9	0.7	NS	0.7	NS	5.1	NS	21.5	NS	1.6	**	3.9	NS	7.6	NS	0.1	NS
Treatments	146	10.4	**	7.3	**	8.5	NS	25.2	*	0.9	*	18.5	**	25.2	*	0.8	**
Genotypes	143	6.5	**	7	**	6.6	NS	20.6	*	0.7	NS	10.9	**	16.1	NS	0.7	**
Controls	2	108	**	26.4	**	144	**	340	**	11	**	573	**	613.5	**	5.7	**
Genotypes vs. Controls	1	363	**	11.4	**	5.1	NS	48.8	*	7.9	**	0.8	NS	155	**	0.6	*
Error	18	1.9		1		5		9.9		0.4		3.9		11.2		0.1	
Corrected Total	173	.		.		.		.		.		.		.		.	
2014																	
Block	9	1.5	NS	0.9	NS	5.1	NS	11	NS	1.6	*	8.5	*	4.3	NS	0.1	NS
Treatments	146	10.5	**	6.9	**	9.9	**	19	**	1.1	NS	22.2	**	39.3	**	0.8	**
Genotypes	143	7	**	5.3	**	8	**	17.1	**	0.9	NS	14	**	22.7	**	0.6	**
Controls	2	30.6	**	34	**	136	**	166	**	10	**	615	**	1225	**	6.1	**
Genotypes vs. Controls	1	469	**	179	**	25.1	**	0.1	NS	8.9	**	5.8	NS	45.4	**	22	**
Error	18	0.8		0.7		2.5		4.5		0.6		2.6		3.7		0.1	
Corrected Total	173							.		.						.	
Non Additivity	1	0	NS	54.5	**	2.7	NS	0.1	NS	2.6	*	4.1	NS	29.4	NS	11	**
Remainder	285	3		5.2		7.3		21.3		0.6		10.5		12.6		0.6	

**, *: Significant at the 0.01 and 0.05 probability level, respectively. NS: Not significant.

Table 3 - Genetics parameters: General Mean, Controls Mean, Genotype Mean, CV%, VP, VE, VG, He^2(%), CVg%, CVg/CVe, for the next variables: Grain Yield (GY, t ha^{-1}), Stover Yield (SY, t ha^{-1}), Acid Detergent Fiber (ADF, %), Neutral Detergent Fiber (NDF, %), Acid Detergent Lignin (ADL, %), in vitro Digestibility (IVD, %), Neutral Detergent Fiber Digestibility (NDFD, %), Calculated Ethanol Yield (CEY, 1000l ha^{-1}).

Trait	General Mean	Controls Mean	Genotype Mean	CV%	VP	VE	VG	He^2	CVg%	CVg/CVe
					2012					
GY	9.38	12.93	8.20	14.96	12.42	1.97	10.45	84.14	39.43	2.30
SY	10.61	10.53	10.64	13.35	10.59	2.01	8.58	81.02	27.52	2.07
ADF	38.61	39.54	38.29	5.42	9.60	4.38	5.22	54.34	5.97	1.09
NDF	73.39	73.54	73.34	5.20	28.86	14.57	14.30	49.53	5.16	0.99
ADL	5.95	6.39	5.81	10.20	0.79	0.37	0.42	53.26	11.16	1.07
IVD	44.38	42.97	44.85	3.74	13.72	2.76	10.96	79.89	7.38	1.99
NDFD	29.60	27.07	30.45	9.79	26.31	8.40	17.92	68.08	13.90	1.46
CEY	3.70	3.66	3.72	12.53	1.45	0.22	1.23	85.13	29.86	2.39
					2013					
GY	7.90	11.07	7.24	17.32	11.83	1.87	9.96	84.17	43.57	2.31
SY	8.55	9.10	8.43	11.87	10.03	1.03	9.00	89.73	35.57	2.96
ADF	35.38	35.76	35.31	6.32	7.56	5.00	2.56	33.82	4.53	0.71
NDF	68.78	67.62	69.02	4.57	24.60	9.89	14.72	59.81	5.56	1.22
ADL	6.20	6.67	6.10	10.55	0.93	0.43	0.50	54.08	11.63	1.09
IVD	48.03	47.88	48.06	4.14	11.61	3.95	7.66	65.97	5.76	1.39
NDFD	27.69	25.24	27.75	12.22	18.66	11.14	7.51	40.28	9.88	0.82
CEY	2.76	2.89	2.74	10.44	0.98	0.08	0.89	91.47	34.53	3.27
					2014					
GY	8.62	12.23	7.87	10.65	10.94	0.84	10.10	92.30	40.38	3.46
SY	7.84	10.06	7.37	10.43	5.88	0.67	5.21	88.63	30.95	2.79
ADF	36.73	37.55	36.56	4.33	8.94	2.53	6.41	71.70	6.93	1.59
NDF	73.58	73.52	73.59	2.89	19.31	4.53	14.79	76.55	5.23	1.81
ADL	5.72	6.21	5.62	13.08	1.10	0.56	0.54	49.16	13.09	0.98
IVD	49.05	48.66	49.14	3.31	15.50	2.63	12.87	83.00	7.30	2.21
NDFD	39.04	37.71	39.09	4.93	27.31	3.67	23.64	86.57	12.44	2.54
CEY	2.74	3.52	2.58	12.20	0.69	0.11	0.58	83.83	29.51	2.28

**, *: Significant at the 0.01 and 0.05 probability level, respectively. NS: Not significant

1), which is associated with a better stover quality, increasing its potential capacity to produce ethanol (Lorenz et al., 2009a). Likewise, Fairey (1983), and Darby and Lauer (2002) showed that high temperature increased the synthesis of stover components and reduced the translocation of photosynthates during grain filling. Therefore, the high temperature could be considered an environmental factor which contributes to decrease in digestibility (Buxton, 1996). Furthermore, Hansey et al. (2010) demonstrated genetic differences in stover quality.

Controls differed significantly for most of the traits in almost all years. Genotypes vs. controls differed significantly ($p < 0.01$) in the 3 years for ADL and NDF. However, the contrast was only significant for IVD in 2012, and for ADF in 2014. NDF only showed differences at 5% in 2013.

Tukey Non Additivity test was separately applied for all the variables. The test was significant at 5% for ADL, revealing the existence of G × E interaction effects. ANOVA of RL (Root Lodging) and SL (Stalk Lodging) was not made because they are categorical traits.

The Genotypes showed significant differences over the controls for the quality variables. Similarly results were found by Bertoia et al. (2006) when selecting Landraces as sources of favorable alleles to generate silage maize hybrids, and Controls showed good performance for grain and forage yield in all years. Barrière et al. (2005)

Table 4 - Rank of 30 Genotypes selected through the rank sum index, where TSI1, TSI2 and TSI3 sum of ranges for year 2012, 2013 and 2014 respectively. General Selection Index (GSI): Sum of total selection index (TSI).

Rank	Genotype	Characteristics / Company	TSI1	TSI2	TSI3	GSI
1	SEH 5	Silage Experimental Hybrid / DAS	1434	923	1128	3485
2	SEH 4	Silage Experimental Hybrid / DAS	843	1001	1393	3236
3	Tropical SC 3	Tropical Maize Composites / DAS	1130	1089	979	3198
4	ARZM03003	CAMELIA / INTA	1064	893	1148	3104
5	ARZM14063	Cristalino colorado y dentado / INTA	1073	1073	958	3104
6	BMR SC	BMR Maize Composites /DAS	921	735	1416	3071
7	SEH 6	Silage Experimental Hybrid / DAS	948	958	1152	3058
8	Silage SC 2	Silage Maize Composites / DAS	999	870	1176	3045
9	SEH 14	Silage Experimental Hybrid / DAS	982	937	1095	3014
10	Silage SC 3	Silage Maize Composites / DAS	895	833	1242	2971
11	Silage SC 4	Silage Maize Composites / DAS	1000	775	1192	2966
12	Tropical SC 2	Tropical Maize Composites / DAS	922	1068	950	2940
13	SY 900 VIPTERA3	Grain Commercial Hybrid / Syngenta	1032	797	1072	2901
14	Tropical SC 4	Tropical Maize Composites / DAS	1088	941	872	2901
15	ARZM17008	Cristalino Colorado / INTA	1063	759	1075	2898
16	Tropical SC 1	Tropical Maize Composites / DAS	904	856	1130	2889
17	ARZM04011	No Clasificable / INTA	896	735	1213	2843
18	DK 747 VT3P	Grain Commercial Hybrid / Monsanto	806	951	1059	2817
19	SEH 7	Silage Experimental Hybrid / DAS	937	831	1033	2802
20	ARZM17035	Cristalino Colorado / INTA	1162	703	915	2780
21	ARZM06051	Cristalino Colorado /INTA	926	929	917	2772
22	Grain SC	Grain Maize Composites / DAS	798	891	1047	2736
23	ARZM18022	No Clasificable / INTA	936	735	1062	2733
24	ARZM03026	Calchaqui /INTA	1083	469	1175	2727
25	Silage SC 1	Silage Maize Composites / DAS	875	805	1043	2724
26	ARZM17029	Cristalino Colorado / INTA	1101	824	796	2721
27	PAN 5E-203	Silage Commercial Hybrid / PANNAR	900	733	1078	2711
28	SEH 1	Silage Experimental Hybrid / DAS	817	1054	824	2694
29	KM 4020 G	Silage Commercial Hybrid / KWS	855	972	867	2694
30	SRM553 MG	Grain Commercial Hybrid / SURSEM	1005	662	1003	2670

proposed that the future development of forage maize hybrids would be enhanced by the introgression of germplasm that has never been used in maize breeding programs.

We did not found G × E interaction for quality variables, except to ADL. The ADL variability was high in comparison with its mean value, and the ratio between CVg and CVe reached values near to 1, indicating a similar contribution of genetic variance versus environmental to the phenotypic variance. Similarly, BERTOIA AND AULICINO (2014) pointed that the quality variables evaluated at the silage moment were principally determined by the additive components: genotype and environmental. On the contrary, Ertiro et al. (2013) found significant G×E interaction for stover quality traits across locations. They noted that those traits which had shown significant G×E interaction had an erratic performance across locations or years.

Genetics parameters

Yield variables (GY, SY, CEY) produced the highest VP (Table 3). Thus, the yield variables showed higher heritability values, in comparison with the quality variables. Although, You et al. (2013) explained that He2 is overestimated in augmented designs. The VG greatly exceeded their VE, which determined maximum

values for the CVg/CVe ratio (>2).

The variables associated with the quality presented a CVg/CVe ratio close to 1 and less than 2 (range 0.71-1.63), except for IVD and NDFD that showed the highest but fluctuating ratios in accordance to the year (range 0.82 - 2.54). As consequence, IVD and NDFD reached higher values of He2 specially in 2012 and 2014, due to a high VG that exceeded the VE in 1.5 to 2.5 times, respectively. In this sense, Lorenz et al. (2009a) also used digestibility traits in direct selection for the production of lignocellulosic biomass. Likewise, Lewis et al. (2010) estimated a higher heritability for glucose, glucose release, and lignin than for grain yield and stalk and root lodging and suggested that maize stover quality would be easier to improve than grain productivity. Genetic parameters showed that the Genotypes have a high genetic variability for the variables associated with convertibility such as IVD and NDFD (Table 3). Despite the CVg/CVe ratio for both did not remain constant throughout the years, which indicates that it is less repeatable; the higher heritability values could assure a rapid genetic advance.

Zhao et al. (2009) proposed to use the combined variable CEY in the indirect selection to increase the quality of the tracker. Although CEY showed high heritability, it was calculated assuming that all carbohydrates are converted to ethanol, in contrast to the findings of Lorenz et al. (2009b) who said that not every component is really converted to that.

Selection indices

We found a positive and significant correlation between TSI and CEY (r: 0.43, $p<0.01$). This showed that TSI indexes can serve as a useful tool to selected genotypes with a greater capacity to produce lignocellulosic ethanol. CEY was a composite variable that comes from combining SY, ADL, ADF and NDF, but does not take into account grain yield variables or other agronomic interest variables (RL and SL). Therefore, TSI would be the best index for identifying genotypes with a dual purpose, for grain and lignocellulosic ethanol yield. Although, Lewis et al. (2010) suggested that stover quality is a more stable breeding target than stover yield and he proposed the use of an index of selection which considered yield + stover quality to select better genotypes for cellulosic ethanol. On the contrary, other authors (Hallauer and Sears, 1972; Holland et al., 1996) pointed out the importance of use in breeding non adaptive or exotic germplasm which usually has lower grain yield and a poor agronomic performance but they present different linkage groups between genes that could determine new combinations of characters.

After applying the selection indices, we selected the 20% out of a total of 144 accessions. The selected genotypes included: 9 landraces, 10 Maize Composites, 6 silage experimental hybrids and 5 double-purpose (grain and forage) commercial hybrids (Table 4). Kang (1993) proposed that a greater emphasis by researchers on the stability component during the selection process would be beneficial to growers. It would decrease the probability of disastrous Type II errors, when an unstable genotype would not be penalized for instability. Ten genotypes showed greater agronomic stability (W = 0.01 to 0.77): 3 silage experimental hybrids (SEH 7, SEH 14, SEH 6), 2 commercial hybrids (SY 900 VIPTERA3, PAN 5E-203), 3 Maize Composites (Silage MC 1, Silage MC 2, Tropical MC 1) and 2 Landraces (ARZM03003, ARZM18022). The 20% of the selected genotypes showed low ecovalence, which would indicate a constant response to environmental changes, maintaining the same relative positions in the rankings. The introduction of exotic genetic resources into breeding programs is a long and laborious task that has often discouraged for maize breeders. It is necessary to point out, that two of the not improved native populations displayed good performance and stability as well. It would ensure a rapid advance in the acquisition of new inbreed lines with good yields, quality and wide adaptability.

Conclusions

We were able to generate selection indexes that allowed us to select genotypes with high CEY, without omitting grain production and agronomic traits, as well as assessing their agronomic stability.

Some of the maize compounds and landraces would be used in future research with the aim of broadening the genetic base in dual-purpose search genotypes, grain and energy uses. This will speed up the implementation of the bioenergy industry for the efficient production of lignocellulosic ethanol at regional and international level.

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