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Genetic Diversity for Dual Use Maize: Grain and Second-Generation Biofuel

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Abstract: Maize biomass from agricultural residues can be a substrate for biofuel production. However, commercial breeding programs have focused on grain yield for food and feed, and whole plant yield and nutritive value for silage, with little attention paid directly to stover yield or composition. Enhancing the energy content of crop residues with higher quality cellulosic biomass for ethanol conversion should provide a complementary use to grain use. We also question whether there is maize germplasm predisposed to dual use as second-generation biofuel. Twenty genotypes, including landraces from Spain, Atlantic, and Mediterranean Europe and genotypes derived from Iowa stiff stalk synthetic, Lancaster, and commercial hybrids were studied in a randomized complete block design across environments in Galicia (Spain) in 2010 and 2011. Germplasm was evaluated for agronomic characteristics and fiber parameters. Results show high heritability for all characteristics and parameters, ranging from 0.81 to 0.98. Principal components analysis revealed clear differences among origin of the varieties studied. Hybrids had the highest grain yield values and B73xMo17 and PR34G13 had the highest grain yield overall, at 10133 and 9349 kg/ha, respectively. European landrace varieties had lower harvest indexes (HI) than the hybrid origin, with Faro and BSL having HI of 0.43–0.47, compared to hybrid PR34613 at 0.56. Fiber concentrations were significantly correlated with yield performance, with values ranging from 0.38 to 0.61 for cob fibers and between −0.14 to −0.57 for stover fibers. Fiber concentrations were significantly different, based on the origins, in cobs but not in stover, with the Atlantic European group showing a favorable trend for cob exploitation with low acid detergent lignin and high acid detergent fiber and neutral detergent fiber values. In summary, population origin showed a reservoir of genetic diversity for breeding to improve residue quality, suggesting that adaptation played a role for stover yield and quality. European landraces could be used in prebreeding programs with stover yield and fiber quality as target traits for dual-purpose maize.

Keywords: maize; dual-purpose; biofuels; ADF; ADL; NDF; landraces; PCA; stover

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1. Introduction

Fossil fuels are not renewable and contribute to the increment of CO_2 concentration in the atmosphere. Biomass from agricultural residues could contribute to a sustainable or circular bioeconomy as a feedstock for bioenergy (biofeedstock) or high value bio-based products in integrated biorefineries [1,2]. In Europe, a large amount of residual biomass is generated in the cultivation of cereals, maize, and oilseed crops [3]. The most efficient use of crop residues depends on the energetic goal, the characteristics of the environment, the crop varieties, and the interaction among these [4]. The exploitation of residues could stimulate the economy in rural areas, which are being depopulated, by increasing the value of crops and by generating new jobs in biorefineries [5]. Generally, local crop varieties have large genetic variability, which reduces the vulnerability to new stresses and could provide alleles to improve crop adaptation [6]. These local varieties can be a source of genes to increase adaptation to environmental change of elite germplasm [7] and contribute to a more sustainable agriculture. In low-input or organic farming, there is also an interest in local varieties, whose seeds can be saved by farmers for the next growing season, something that cannot be done with elite varieties, as for example maize hybrids, that must be purchased each growing season [8,9].

Enhancing the energy content of crops by increasing cellulosic biomass, while more efficient biochemical technologies [10–12] to convert biomass to ethanol are being developed, should be the focus to reduce unit cost of biofuels. In maize, exploring alternative traits for high-quality crop varieties with improved fiber digestibility would provide a complementary value for otherwise unused cellulosic energy of residues, converting the plant to a dual-purpose crop, and may reduce risk for growers. For instance, maize stover is a residue and a potential source of for cellulosic ethanol production [13–15]. In the stover, plant cell wall components include lignin, hemicellulose, cellulose, and other organic components, with the least digestible plant components being cellulose and lignin [16]. These cell wall components can be measured as three fiber quality parameters [17]—neutral detergent fiber (NDF), which is composed of cellulose, hemicellulose, and lignin; acid detergent fiber (ADF), which is composed of cellulose and lignin; and acid detergent lignin (ADL). Lignin is an undigestible component that has no energy value as animal feed [18,19], nor value for energy conversion to methane or ethanol, and restricts digestibility of other fiber elements. Lorenz et al. [20] suggested that fodder fiber quality and composition may be used to predict cellulosic ethanol yield due to positive correlations with NDF. However, qualitative changes in fiber quality should not penalize agronomic traits to be considered a high-quality bioenergy crop. Albrecht et al. [21], in research with three cycles of recurrent selection of maize (Iowa synthetic#1), reported that traits associated with stalk lodging resistance did not have a negative impact on digestibility or substantially alter fiber composition or concentration of the maize stalks. Smith et al. [22] showed that qualitative changes of biosynthesis substrates (e.g., feruloyl-CoA) of the cell wall seems to increase the digestibility of the cell wall and may provide higher stover yields and superior biofuels substrates without changes in agronomic traits. Although, greater biomass may be inclined to lodging or may be antagonistic to grain yield and moisture traits [23]. Simultaneous improvement of whole plant fiber characteristics and corn grain yield has the potential for second generation biofuel production.

The ideal moisture concentration of crop residue depends on storage facilities and energy conversion goals. Low moisture concentration is necessary if the residue is used for combustion, but not when residue is fermented for bioethanol or biogas production. For biogas production in Europe, whole-plant maize is usually ensiled at 65–72% according to Grieder et al. [24]. Shinners et al. [25] analyzed different systems of storage and concluded that crop residues can be stored for long periods when dried to below 20% moisture. Or, after wilting in the field to 40% to 55% moisture, stover can be stored, without chopping, as silage in wrapped square or round bales. Depending on storage structure, e.g., bags, bunkers, or piles, chopped forms of residue ranging in moisture from 35% to 67% can be stored as silage.

Maize varieties have shown genetic diversity for stover yield [15,26,27]. A few maize varieties from a common pedigree, Corn Belt dent, including Lancaster, Minnesota 13, and Reid, are the predominant contributors to maize hybrids cultivated in temperate areas. Within Reid, a synthetic comprised of 18 inbreds (Stiff Stalk Synthetic, BSSS), and an early strain, Iodent Reid, are ancestors of many elite inbred lines [28]. One of the most common heterotic patterns in temperate maize is BSSS x no BSSS. Commercial breeding was based on recycling closely related inbred lines within heterotic groups [29,30], resulting in many elite lines being derived from only a few ancestors, i.e., B73 and B14 from BSSS, Mo17 from Lancaster, etc. [31]. Maize was introduced to Europe soon after discovery of America and there are three groups of germplasm in Europe: central-northern European Flints related to eastern US Flints, Mediterranean European varieties close to Caribbean or northern South American varieties, and Atlantic European varieties (Northern Spain and France), which are not close to any known American variety [32,33]. Landraces in Spain were extensively studied at the National Research Institutes in Spain for agronomic and morphological traits [34–36]. Similar to what has been seen in US, only a fraction of the variability of the European maize landraces is present in the European elite lines [37].

In this research, we compared a set of maize landraces from Spain to a representation of flint and dent varieties of other origins, Corn Belt and commercial hybrids, to evaluate the potential bioenergy suitability and agronomic trait performance. We studied fiber parameters to assess whether adaptation predisposes certain materials for dual use or not.

2. Materials and Methods

2.1. Plant Materials and Data Collection

Twenty maize genotypes were grown in two locations in Pontevedra, Spain, to account for genotype (G), environment (E), and G × E interaction effects. The locations used were Misión Biológica de Galicia (42°24′ N, 8°38′ W, and 20 m above sea level) and Ponte Caldelas (42°23′ N, 8°30′ W, and 360 m above sea level), in 2009 and 2010. Trials were conducted with 20 diverse genotypes that included open pollinated varieties from Atlantic Europe, Mediterranean Europe, and the US Corn Belt and commercial hybrids (Table 1). Each year–location combination was considered as an individual environment, resulting in a total of four environments. The experimental design at each environment was a randomized complete block design (RCBD) with three replicates. Each plot had two 5-m rows and each row had 25 plants. Rows were spaced with 0.8 m separation, while within rows plants were separated by 0.21 cm, resulting in a density of 60,000 plants/ha. Standard management practices for the area were used to prepare the soil and fertilize the crop.

Table 1. Plant material evaluated in a randomized complete block design in Galicia, Spain, in 2010 and 2011.

Number	Genotype	Origin	Type	Mating System
1	Lazcano [x]	Europe Atlantic	Flint	OP
2	Aranga [x]	Europe Atlantic	Flint	OP
3	Ribadumia [x]	Europe Atlantic	Flint	OP
4	Tui [x]	Europe Atlantic	Flint	OP
5	Norteño Largo [x]	Europe Atlantic	Flint	OP
6	Posada de Llaneras [x]	Europe Atlantic	Flint	OP
7	Faro [x]	Europe Mediterranean	Flint	OP
8	Rastrojero [x]	Europe Mediterranean	Semi-Dent	OP
9	Vejer [x]	Europe Mediterranean	Semi-Dent	OP
10	Hembrilla × Queixalet [x]	Europe Mediterranean	Semi-Dent	OP
11	Basto × Blanco [x]	Europe Mediterranean	Semi-Dent	OP
12	BS17 "BSSS" [y]	US Corn Belt	Dent	OP
13	BSL "Lancaster" [z]	US Corn Belt	Dent	OP
14	Minnesota 13	US Corn Belt	Dent	OP
15	BSTE "Prolifica"	US Corn Belt	Dent	OP
16	B73 × Mo17	Hybrid	Dent	Hybrid
17	A619 × A632	Hybrid	Dent	Hybrid
18	NKThermo	Hybrid	Dent	Hybrid
19	PR34G13	Hybrid	Dent	Hybrid
20	PR36B08	Hybrid	Dent	Hybrid

[x] Described by Sanchez-Monge (1962) [35]; [y] derived from Iowa Stiff Stalk Synthetic; [z] derived from Lancaster; OP = open pollinated.

Field data collected included: days to silking (DTS), indicating the number of days from planting to 50% of plants showing silks; plant height (average value of 10 plants in cm); lodging at harvest (percentage of plants broken below the main ear and plants leaning more than 45° from the vertical); grain and cob moisture at harvest (%); and dry grain and cob yield (kg/ha) at harvest. Proportion cobs consisted of the central fibrous rachis of the female inflorescence to the whole ear. Plots were harvested at physiological maturity of the grain. Ten representative plants of each plot were chosen after removing the ears, and the vegetative fraction was chopped with a yard waste chipper and weighed to estimate the stover biomass yield (kg/ha). Stover samples consisted of stalks, leaf blades, leaf sheaths, husk leaves, and ear shanks. Two stover subsamples of approximately 400 g were obtained from each plot. The two subsamples were dried for 5 days at 60 °C. One subsample was used to estimate the stover dry weight and stover moisture, while the other subsample was ground in a knife mill to pass a 1-mm screen and used for fiber analysis. Similarly, a sample of 10 cobs of each plot was dried at 60 °C and ground for subsequent analysis.

2.2. Fiber Analysis

The acid detergent fiber (ADF), acid detergent lignin (ADL), and neutral detergent fiber (NDF) of stover and cobs were analyzed according to Van Soest et al. [17] using the Fibercap system (Foss Electric, Hillerød, Denmark). These methods are based on different solubilities of the cell wall fractions in neutral and acid detergent solutions and acid. Neutral detergent fiber is an estimate of total cell wall, including cellulose, hemicellulose, and lignin; ADF is composed primarily of cellulose and lignin; and ADL estimates lignin insoluble in acid [38]. For biological conversion to bioethanol, high carbohydrate concentration and low recalcitrance would be ideal, [39] that is, high values of NDF and ADF and a low value of ADL are desirable. However, for thermochemical conversion the recalcitrance levels are not relevant [39], and high values of ADL would not be detrimental.

2.3. Statistical Analysis

Analysis of variance (ANOVA), mixed models, and heritability were conducted using R Studio (Version 1.1.463, Boston, MA, USA) [40]. Each year–location combination was considered as an individual environment. Technical replicates of each sample from each plot were averaged for downstream analysis. The experimental model design for the ANOVA broke treatments down into three components: G, E, and G × E effects:

$$Y_{ijk} = \mu + \alpha_i + \gamma_j + \alpha\gamma_{ij} + \beta_{ik} + \varepsilon_{ijk} \quad (1)$$

where Y_{ijk} is the average value of the dependent variable of genotype i in environment j in the kth, block, μ is a mean value for all data points, α_i is the effect of the ith genotype, γ_j is the effect of the jth environment, $\alpha\gamma_{ij}$ is the effect of the ith genotype by the jth environment, β_{jk} is the block effect at the jth environment in the kth block, and ε_{ijk} is the residual error term. Mean differences were compared using multiple comparisons of treatments with genotypes as fixed effects through a Tukey test with a significance threshold of $p < 0.05$. Best linear unbiased estimates were calculated using restricted maximum likelihood method estimates (RMEL) according to the experimental design with factors as random. Variance components were estimated using the *lmer* package in R [40] and broad sense heritabilities were calculated using the Fehr equation [41].

$$H^2 = \sigma_g^2 / (\sigma_g^2 + \left(\sigma_{gl}^2 / t\right) + (\sigma_e^2 / rt)) \quad (2)$$

where ${\sigma^2}_g$ is the genetic variance, ${\sigma^2}_{gl}$ is the genotype by environment interaction variance, ${\sigma^2}_e$ is the residual variance, t is the number of environments, and r is number of replicates. Genetic correlations were calculated using the *Meta-R* package [42].

2.4. Principal Component Analysis (PCA)

Principal component analysis (PCA) reduces high-dimensional data into a lower-dimensional space, utilizing a few new variables, PCs, to summarize the attributes of original variables to the extent possible [43,44]. Here, we used sixteen variables, including agronomic, yield, and fiber phenotypes. Principal component analysis was performed in R [40] using the *factoextra* package and the *prcomp* function. Each dimension is a linear transformation of the original variables, arranged in descending order of percentage of explained variance. To visualize the results of the PCA, we used functions *fviz_pca_ind* and *fviz_pca_var*.

3. Results

3.1. Variation in Yield and Agronomic Traits

Genotypes showed a wide range of phenotypic values for yield and agronomic traits across environments (Supplementary Tables S1–S3). Mean values of the agronomic and yield traits showed 1.17–2.70-fold differences across genotypes, with the exception of proportion of lodging, which differed 7.5-fold (Table 2). For yield traits, on average, BLUPS showed that the hybrid B73xMo17 had the highest grain yield at 10,133 kg/ha followed by the other hybrids PR34G13, PR36B08, and NKThermo at 9349, 8233, and 7771 kg/ha, respectively (Supplementary Table S1). Hembrilla Queixalet had the lowest grain yield (3799 kg/ha) followed by BastoxBlanco (4228 kg/ha), Norteño Largo (4276 kg/ha), and RastrojeroC3 (4456 kg/ha), all genotypes with EU Mediterranean origin, with the exception of Norteño Largo from the EU Atlantic region. For cob yield, hybrid B73xMo17 was also the highest at 1723 kg/ha, followed by hybrid A619xA632 at 1410 kg/ha and Posada Llanera from the EU Atlantic (1405 kg/ha) (Supplementary Table S1). Interestingly, for stover yield, the highest biomass was measured in the US Corn Belt accession BSL at 6870 kg/ha, followed by Faro (EU Mediterranean) at 6507 kg/ha and hybrid B73xMo17 at 6506 kg/ha (Figure 1). The lowest value was Hembrilla Queixalet at 3404 kg/ha, followed by Minnesota13 at 3638 kg/ha from EU Mediterranean and US corn belt, respectively (Supplementary Table S1).

Table 2. Summary of agronomic traits with units plus minimum, mean, and maximum BLUP values; fold difference between minimum; and broad sense heritability (H^2) for ten agronomic traits—yield grain, yield cob, yield stover, silking, height, lodging, proportion of cob (Prop cob), moisture of the grain (Mgrain), cob moisture (Mcob), and stover moisture (Mstover) estimated from 20 maize genotypes grown at four environments in Pontevedra, Spain, in 2010 and 2011.

Trait (Units)	Minimun	Mean	Maximun	Fold	H^2
Yield grain (kg/ha)	3799	6168	10,133	2.67	93.3
Yield cob (kg/ha)	637	1144	1723	2.70	93.4
Yield stover (kg/ha)	3404	5101	6870	2.02	91.3
Silking (DTS *)	72	80	88	1.22	98.1
Height (cm)	191	224	270	1.41	96.0
Lodging (%)	3.7	16.1	28.1	7.55	81.3
Prop_cob	0.17	0.22	0.29	1.74	92.3
Mgrain (g/kg)	210	260	330	1.58	85.0
Mcob (g/kg)	330	480	620	1.86	91.9
Mstover (g/kg)	450	590	660	1.47	86.6

* DTS, is days to silking.

For agronomic traits, on average, Minnesota13 was the first line to reach silking stage at 72 DAP, and BSL was the last line at 87.5 DAP (Supplementary Table S2). In terms of plant height, on average, B73xMo17 was the tallest line at 270 cm, whereas Hembrilla Queixalet was the shortest line at 191 cm from hybrid and EU Mediterranean origin, respectively (Supplementary Table S2). For lodging, the most affected genotype on average was Norteño Largo at 28% with an EU Atlantic origin, followed by Faro and RibadumiaC2 at 27%, from EU Atlantic and EU Mediterranean origin, respectively. The most tolerant to lodging was the hybrid A619xA632 at 3.5% (Supplementary Table S3). In terms of cob proportion, hybrid origin has the smallest proportion with NKThermo at 0.166, followed by PR36B08 and PR34G13, at 0.176, and 0.179, respectively; only the hybrid A619xA632 had a slightly higher ratio at 0.208. The greatest proportion of cob was for the European genotypes: Posada de Llanera had the largest value, on average, at 0.289, followed by Faro at 0.283, and Aranga and Lazcano at 0.259 and 0.246, respectively (Supplementary Table S3). For moisture traits, the Vejer genotype had the greatest grain moisture at 327 g/kg followed by Faro at 315 g/kg, both from EU Mediterranean, whereas Minnesota13, from the US Corn Belt, had the lowest grain moisture at 210 g/kg, followed by the hybrid NKThermo at 220 g/kg. Similarly, Vejer had the greatest cob moisture at 620 g/kg followed by Faro at 600 g/kg, whereas Minnesota13 and Norteño Largo had the lowest cob moisture on average at 330 g/kg and 380 g/kg, respectively. For cob moisture, the EU Atlantic origin genotypes Vejer and Faro had the largest values at 620 g/kg and 600 g/kg, respectively. However, Minnesota13 had the lowest cob moisture value at 330 g/kg, on average, followed by Norteño Largo and NKThermo at 380 g/kg and 410 g/kg, respectively (Supplementary Table S3). For the stover moisture, six out of eight of the genotypes with the lowest moisture were European landraces, although the lowest stover moisture value was for Minnesota13 at 450 g/kg and the greatest in the BSL genotype at 663 g/kg, both with a US origin.

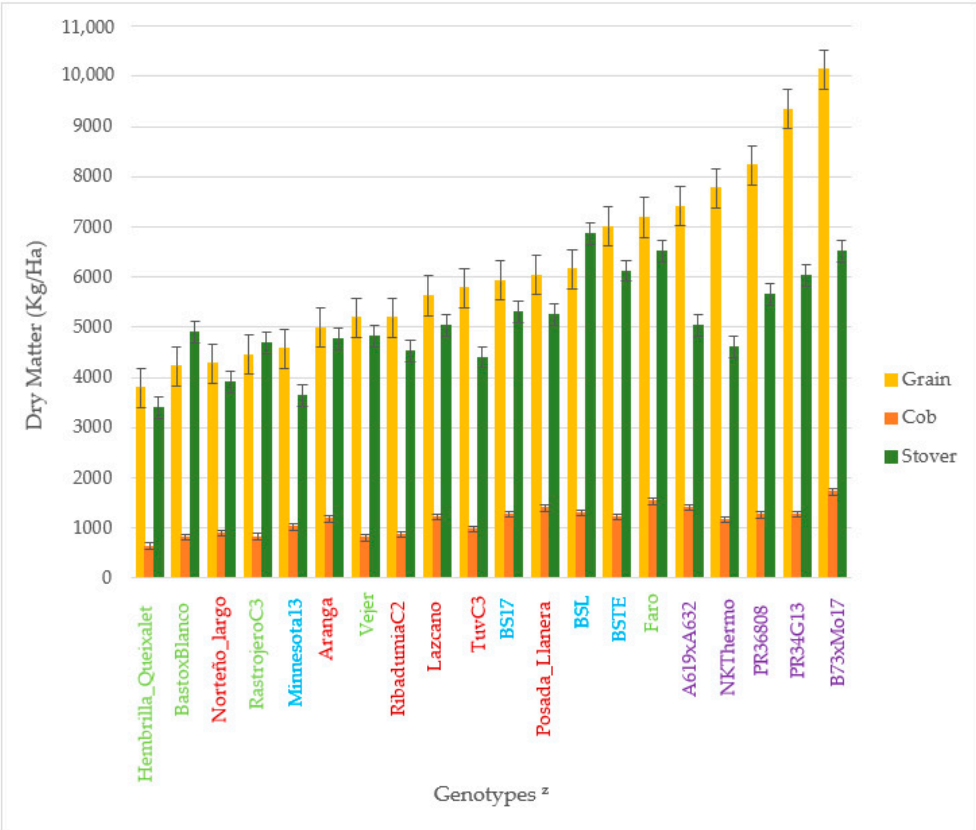

Figure 1. Yield in dry matter amounts for grain, cobs, and stover of 20 maize genotypes grown at four environments in Galicia, Spain, in 2010 and 2011. Ordered in ascending order for grain yield. [z] Genotypes are colored by origin: EU Atlantic (maroon), EU Mediterranean (green), hybrid (purple), and US Corn Belt (blue).

Mean heritability estimates ranged from 0.81 for plot lodging to 0.98 for silking (Table 2). The environment effect was significant ($p < 0.001$) for each trait and explained between 2.85% for stover yield and 50.28% of the phenotypic variance for proportion of lodging. The genotype effect was significant ($p < 0.001$) for each trait and explained between 18.09%, for M_stover, and 64.55%, for silking, of the phenotypic variance (Figure 2). The genotype term explained the largest proportion of the phenotypic variation for grain yield, cob yield, stover yield, silking, height, prop_cob, lodging, Mgrain, and Mcob. Lodging and Mstover were largely explained by the environment term. The G × E interaction term was significant ($p < 0.05$) for all traits except stover yield ($p = 0.199$), Mstover ($p = 0.216$), and height ($p = 0.467$). This term explained between 0.09% for height and 10.18% for grain yield, of phenotypic variance. The factor block nested within environment explained the smallest proportion of the phenotypic variance among all the sources of variation, between

0.12 for silking and 7.61% for stover yield. Residuals represented ranged from 6.96%, for silking, to 48.69%, for Mgrain, of the phenotypic variance. The residual error represented the largest portion of the phenotypic variance for Mgrain (Figure 2).

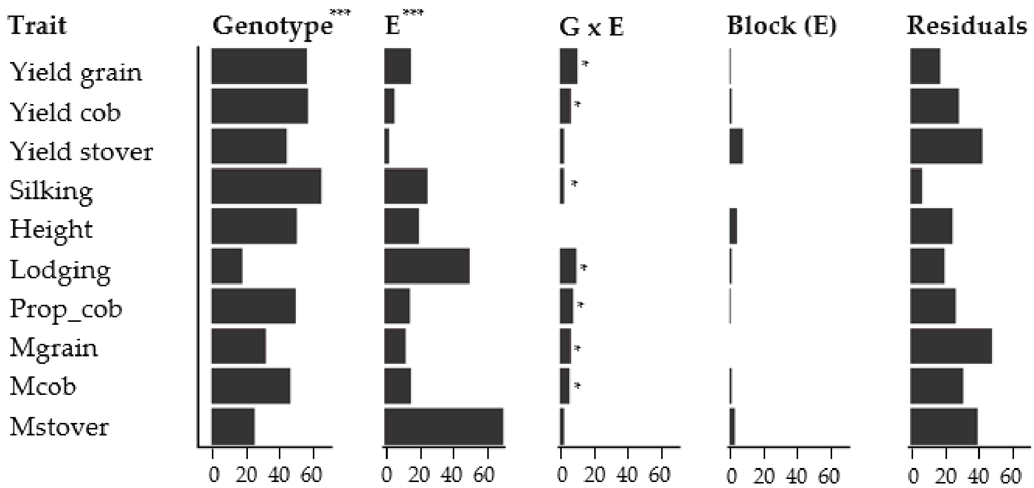

Figure 2. Percentage of phenotypic variance explained by each analysis of variance with the model fit as random effects for 10 quantitative traits—yield of grain, cob, and stover; flowering time (silking); plant height; proportion of cob (prop_cob); lodging; moisture of the grain (Mgrain); cob (Mcob); and stover (Mstover)—measured on twenty lines in four environments in Pontevedra (Spain), 2010 and 2011. Genotype (G), environment (E). * and *** denote significance at p = 0.05 and p = 0.001, respectively.

3.2. *The Energy Content of Crop Residue*

The energy content of corn residue is defined by the amount and digestibility of fiber. Plant cell wall components include lignin, hemicellulose, cellulose, and other organic components, with the least digestible plant components being lignin and cellulose. The results of the analysis of variance (Table 3) revealed that the mean squares for genotype were significant for all energy phenotypes studied. Large variability was observed for cob ADF, ADL, and NDF, and for stalk and leaf NDF, ADL, and ADF, in descending order. Based on the origin (Mediterranean, Atlantic, Corn Belt, or hybrid) of the material, the energy content showed significant differences (Table 4). These differences were greater in cobs than in stover, suggesting that selection may have acted more strongly on cobs than vegetative tissues. Overall, the mean values for the three fiber components were greater in maize cobs than in stalks and leaves (Table 5).

3.2.1. NDF

Cobs

For NDF, the environment term contributed the most to the total variance, at 59.8%. The genotype term represented 19.1%, whereas the G × E factor represented 8.1% of the total variance (Table 3). Genotypes were significantly different (p < 0.001), with genotype Lazcano, with EU Atlantic origin, having the greatest NDF concentration at 87.6%. The genotypes RastrojeroC3 and Faro, both with EU Mediterranean origin, had lowest concentrations at 78.7% and 79.2%, respectively (Table 5). There were significant differences in cob NDF concentrations based on origin (p = 0.002), with EU Atlantic the having the greatest value for NDF at 84.1%, followed by US Corn Belt and the Hybrids at 83.3% (Table 4). The lowest group for NDF was the EU Mediterranean origin. Heritability for cob NDF was 59.19% (Table 3).

Stover

Environment contributed the most to the total variance in stover NDF, at 74.7%. The genotype term represented 7.7%, whereas the G × E factor represented 12.0% of the total variance (Table 3). Genotypes were significantly different ($p < 0.001$), with genotype Minnesota13 having the greatest NDF concentration at 73.1% from the US Corn Belt, followed by B73xMo17 at 72.5, and Norteño Largo at 71.9%, with hybrid and EU Atlantic origins, respectively. The genotypes Vejer, with EU Mediterranean origin, and BSTE, with US Corn Belt, origin had the lowest stover NDF concentrations, with 67.1 and 67.6%, respectively (Table 5). No significant difference was seen based on origin ($p = 0.0756$), but the US Corn Belt was numerically higher (70.1%), followed by hybrid, EU Atlantic, and EU Mediterranean origins at 70.0, 69.4, and 68.4% (Table 4). Heritability for stover NDF was 63.3% (Table 3).

Table 3. Analysis of variance, source of variation, and broad sense heritability (H^2) for acid detergent fiber (ADF), acid detergent lignin (ADL), and neutral detergent fiber (NDF) from 20 maize genotypes grown at four environments in Galicia, Spain, in 2010 and 2011.

Source [y]	Cob						Stover					
	Mean Squares [z]						Mean Squares [z]					
	ADF	%	ADL	%	NDF	%	ADF	%	ADL	%	NDF	%
Genotype	34.59 ***	25.0	19.75 ***	24.5	59.16 **	19.1	14.63 **	14.6	22.72 *	7.8	36.54 ***	7.7
Environment	75.78 ***	54.8	45.75 ***	56.8	185.04 ***	59.8	32.94 **	32.9	195.69 ***	67.1	353.43 ***	74.7
Block(E)	16.89 **	12.2	8.42	10.5	15.88	5.1	34.65 ***	34.7	46.77 ***	16.0	56.77 ***	12.0
G × E	5.08	3.7	2.38	2.9	25.20	8.1	10.64 *	10.6	13.54	4.6	13.4	2.8
Residuals	5.85	4.2	4.28	5.3	23.93	7.7	7.13	7.1	12.75	4.4	12.79	2.7
H^2	82.51		82.56		59.19		27.37		40.39		63.34	

[z] Denotes the proportion of means square to the total for each phenotype, respectively. [y] Source of variations denoted environment as E, genotype as G, and G × E is genotype by environment interaction. *, **, and *** denotes significance at $p = 0.05$, $p = 0.01$, and $p = 0.001$, respectively.

Table 4. Origin means follow by standard error (SE) of fiber components: acid detergent fiber (ADF), acid detergent lignin (ADL), and neutral detergent fiber (NDF) described in Table 2 grown at four environments in Galicia, Spain, in 2010 and 2011. Values ordered based on cob ADF means.

Origins	Cobs [z]									Stover [z]								
	ADF	SE		ADL	SE		NDF	SE		ADF	SE		ADL	SE		NDF	SE	
EU Mediterranean	42.6	0.8	a	8.9	0.6	ab	80.5	1.2	a	38.8	0.7	a	7.0	1.0	a	68.4	1.3	a
US Corn Belt	44.3	0.9	ab	9.3	0.6	ab	83.3	1.3	ab	39.5	0.7	a	5.9	1.0	a	70.1	1.3	a
EU Atlantic	44.6	0.9	b	8.7	0.6	b	84.1	1.2	b	39.3	0.7	a	7.3	1.0	a	69.4	1.3	a
Hybrid	45.3	0.8	b	10.0	0.6	a	83.3	1.2	ab	39.1	0.7	a	6.3	1.0	a	70.0	1.9	a

[z] Means with the same letters within a column are not significantly different at $p = 0.05$ based on a Tukey's test.

Table 5. Means of fiber components: acid detergent fiber (ADF), acid detergent lignin (ADL), and neutral detergent fiber (NDF) from 20 maize genotypes grown at four environments in Galicia, Spain, in 2010 and 2011. Values ordered based on the cob ADF means.

Genotype	Cobs [z]									Stover [z]								
	ADF	Std		ADL	Std		NDF	Std		ADF	Std		ADL	Std		NDF	Std	
Aranga	47.9	2.1	a	10.2	2.6	abc	84.9	3.0	ab	38.4	2.0	ab	6.6	4.5	ab	67.9	3.3	abc
A619 × A632	47.4	2.3	a	12.8	2.6	a	84.5	2.0	ab	37.7	2.4	ab	6.7	3.1	ab	67.7	5.1	bc
BS17	47.2	1.6	ab	11.5	0.6	ab	85.7	2.3	ab	38.9	2.7	ab	6.8	3.5	ab	71.1	3.9	abc
PR36B08	47.0	2.0	ab	9.6	1.3	abc	85.7	3.4	ab	39.2	1.9	ab	4.8	2.0	ab	70.2	3.3	abc
B73 × Mo17	45.7	2.1	ab	10.7	2.0	abc	85.0	2.8	ab	39.6	3.4	ab	9.3	5.5	ab	72.5	2.5	ab
Faro	45.7	9.1	ab	9.4	4.2	bc	79.2	4.2	b	39.7	2.1	ab	6.9	5.3	ab	68.2	3.1	abc
Lazcano	45.3	1.9	ab	9.3	1.6	bc	87.6	10.9	a	38.3	2.2	ab	5.2	2.8	ab	68.8	3.5	abc
RastrojeroC3	45.0	2.4	ab	11.4	2.7	ab	78.7	15.5	b	37.4	4.9	b	7.1	3.7	ab	68.2	4.8	abc
TuyC3	45.0	2.7	ab	9.5	2.1	bc	84.6	2.5	ab	39.1	2.6	ab	7.0	3.1	ab	69.4	3.3	abc
RibadumiaC2	44.6	1.6	abc	8.2	3.2	bc	84.3	1.7	ab	39.5	2.8	ab	7.9	5.2	ab	68.8	4.6	abc
BSL	44.6	2.6	abc	9.3	1.7	bc	82.9	3.2	ab	39.0	1.9	ab	5.2	2.0	ab	68.6	3.4	abc
Minnesota13	44.5	2.8	abc	8.6	1.6	bc	85.0	2.7	ab	41.6	2.9	ab	6.8	4.4	ab	73.1	3.3	a
PR34G13	44.2	1.8	abc	9.6	3.1	bc	81.7	2.2	ab	38.9	2.8	ab	6.2	3.1	ab	68.2	3.4	abc
NKThermo	44.2	2.6	abc	7.9	2.1	bc	82.4	2.1	ab	40.2	3.0	ab	4.7	1.6	b	71.5	4.4	abc
Basto × Blanco	44.1	1.9	abcd	8.8	1.9	bc	85.6	2.1	ab	40.7	2.7	ab	7.6	3.6	ab	69.8	2.6	abc
Posada_Llanera	43.9	2.8	abcd	8.2	2.0	bc	81.4	2.7	ab	38.3	2.4	ab	7.3	2.8	ab	69.8	3.5	abc
BSTE	43.8	2.9	abcd	8.9	1.5	bc	82.7	3.6	ab	38.4	2.7	ab	5.1	2.3	ab	67.6	10.5	bc
Norteño_largo	43.3	2.3	bcd	7.6	1.6	c	82.8	2.8	ab	41.9	9.5	a	10.0	8.9	a	71.9	4.6	abc
Hembrilla_Queixalet	41.1	1.7	cd	8.2	1.5	bc	81.2	2.8	ab	38.8	2.4	ab	6.9	4.7	ab	68.8	4.4	abc
Vejer	40.4	2.0	d	7.6	0.8	c	79.3	2.0	b	37.5	2.9	b	6.4	2.7	ab	67.1	3.5	c
Mean	44.5			9.2			83.1			39.2			6.7			69.4		

[z] Means with the same letters within a column are not significantly different at $p = 0.05$ based on a Tukey's test.

3.2.2. ADF

Cobs

For ADF, the environment term contributed most to total variance at 74.8%. The genotype term represented 25.0%, whereas G × E factor represented 3.7% of the total variance (Table 3). Genotypes were significantly different ($p < 0.001$) with genotypes Aranga at 47.93 and A619xA632 at 47.36 having the highest NDF amounts, and EU Atlantic and Hybrid origins, respectively. The genotype Vejer had the lowest with 40.38 and has EU Mediterranean origin (Table 5). In terms of origin, there was significant variation among origins ($p < 0.001$) with hybrid having the highest average ADF value at 45.30 and EU Mediterranean the lowest value at 42.60, with US Corn Belt at 44.30 and EU Atlantic at 44.60 intermediate (Table 4). Heritability for cob ADF was very high at 82.51% (Table 3).

Stover

Environment contributed the most to the total stover ADF variance, at 32.9%. The genotype term represented 14.6%, whereas G × E factor represented 10.6% of the total variance (Table 3) and the term block nested in environment accounted for the largest proportion, 34.7%. Genotypes were significantly different ($p < 0.01$), with genotype Norteño Largo, with EU Atlantic origin, having the highest ADF_stover amount at 41.9%. The genotypes Vejer and RastrojeroC3, both with EU Mediterranean origin, had the lowest average values at 37.4% (Table 5). In terms of origin, there was not significant variation among origins ($p = 0.751$), but numeric values ranged from 39.5% in the US Corn Belt group to 38.8% in the EU Mediterranean collection (Table 4). Heritability for stover ADF was the lowest at 27.37% (Table 3).

3.2.3. ADL

Cobs

For cob ADL concentration, the environment term contributed the most to the total variance at 56.8%. The genotype term represented 24.5%, whereas the G × E factor represented 2.9% of the total variance, and the term block nested in environment accounted for 10.5% (Table 3). Genotypes were significantly different ($p < 0.001$) with genotype A619xA632, with hybrid origin, having the highest cob ADL amount at 12.8%. The genotypes Vejer and Norteño Largo, both with EU Mediterranean origin, had the lowest cob ADL at 7.6 and 7.6% (Table 5). In terms of origin, there was significant variation among origins ($p = 0.014$), with the hybrid group having greatest ADL at 10.1, and EU Atlantic the lowest value at 8.7%, followed by EU Mediterranean and US Corn Belt at 8.9 and 9.3%, respectively (Table 3). Heritability for cob ADL was the highest at 82.56% (Table 3).

Stover

Environment was the primary contributor to the total variance for stover ADL concenteration, at 67.1%. The genotype term represented 7.8%, whereas the G × E factor represented 4.6% of the total variance and the term block nested in environment accounted for 16.0% (Table 3). Genotypes were significantly different ($p < 0.001$), with genotype Norteño Largo, with EU Atlantic origin, having the highest ADL concentration at 10.1%. The genotype NKThermo, from the hybrid group, had the lowest at 4.7% (Table 5). In terms of origin, there were not significant differences among origins ($p = 0.1889$) but the EU Atlantic contained 7.3, followed by EU Mediterranean at 7.0, and hybrid at 7.0%. The lowest numeric value was US Corn Belt at 5.9% (Table 4). Heritability for stover ADL was 40.39% (Table 3).

3.3. Principal Component Analysis (PCA)

To explore separation based on genotype origin, PCA was performed to extract the intercorrelation information from a multidimensional variable approach. We used sixteen variables that include agronomic, yield, and fiber traits. The principal component (PC) score plot of all the samples is illustrated in Figure 3. The results of PCA analysis showed that the first six components reflected 90% of the information in the original phenotypic data. The percentage of explained variance of the first four components was 78.4%, of which the contribution rate of the first principal component (PC1) was 36.2%, the contribution rate of the second component (PC2) was 21.3%, and the contribution rate of the third component (PC3) was 12.4% and for the fourth component (PC4) was 8.5%. Such percentage of contribution explains the majority of variance.

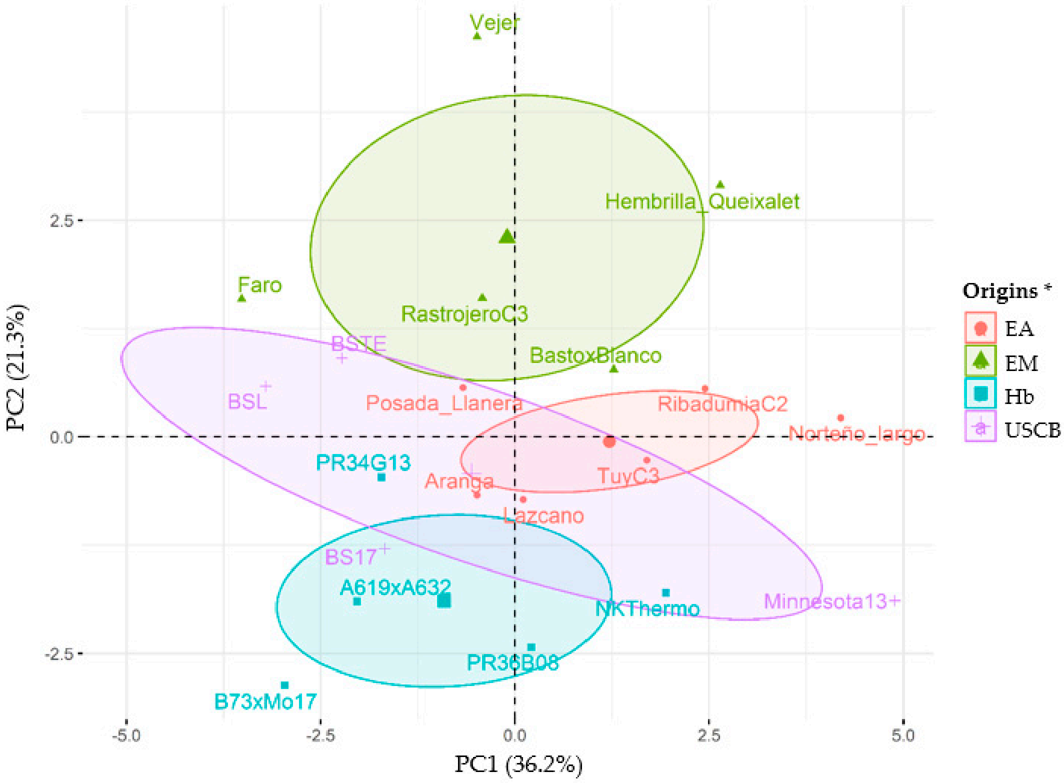

Figure 3. Plot of principal components analysis (PCA) of component 1 (PC1) versus 2 (PC2) across origins described in Table 2, with the genotypes grown across four environments in 2010 and 2011 in Pontevedra, Spain. * Europe Atlantic = EA, Europe Mediterranean = EM, US Corn Belt = USCB, and hybrid = Hb.

A graph of individuals with a similar profile are grouped together in Figure 3, showing four distinctive clusters according to genotype origin. EU Mediterranean and the Atlantic origin tend to align separately of all others, whereas hybrids and US Corn Belt tend to overlap, suggesting that they may have a common origin and/or evolved similarly due to the similar latitudes. Consistent results were depicted using PC1 (36.2%) versus PC2 (21.3%) (Figure 3), and when we plotted the PC2 (21.3%) versus PC3 (12.4%) (Supplementary Figure S1).

A graph of variables (Figure 4) indicated positively correlated variables pointing to the same side of the plot and negatively correlated variables to opposite sides of the graph. On one side, ADF of stover, NDF of stover, NDF of cob, and the proportion of grain were positively correlated traits as opposed to the negatively correlated Mcob, Mgrain, silking, and prop_cob. On the other hand, the traits stover yield, cob yield, grain yield, height, and cob ADL were highly correlated, as opposed to lodging and stover ADL (Figure 4). In addition, the length of the arrow signifies the importance of the contribution of each variable to the analysis. The traits with the largest contribution to the analysis were stover yield, cob yield, and Mcob (Figure 4), followed by grain yield and cob ADF. In terms of source of energy, the largest contributor was cob ADF (Figure 4), followed by cob ADL (Figure 4), then cob NDF and stover NDF (Figure 4), and stover ADF (Figure 4).

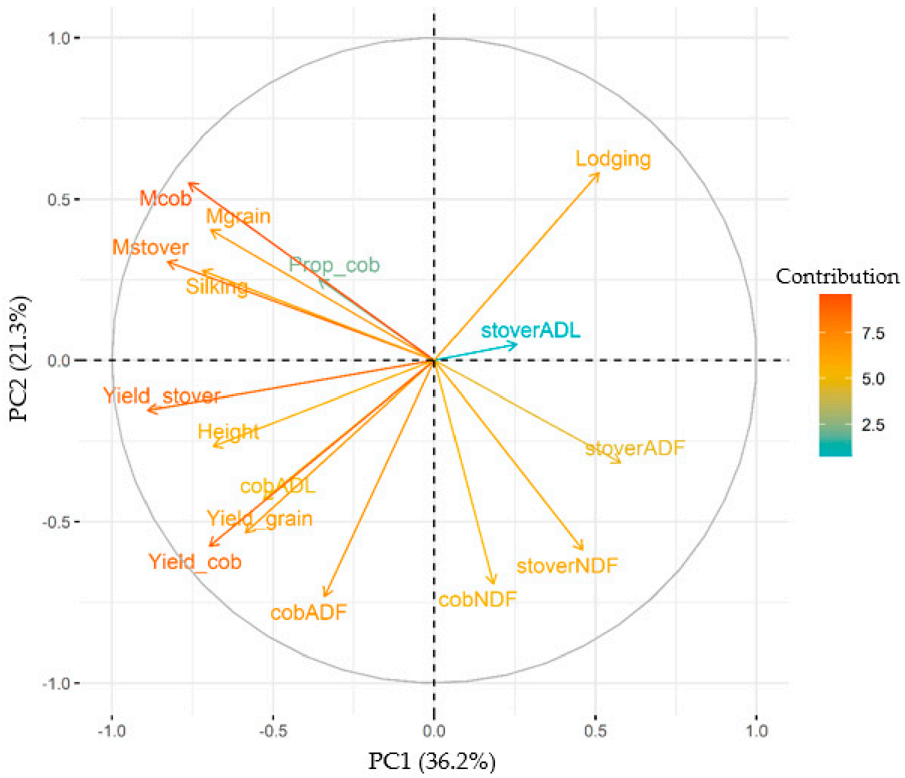

Figure 4. Plot of variable contributions of the principal components analysis (PCA) of components 1 (PC1) and 2 (PC2) across four environments in 2010 and 2011 in Pontevedra, Spain. Each arrow represents one trait: grain yield (yield_grain), cob yield (yield_cobs), and stover yield (yield_stover); NDF, ADF, and ADL (cobs and stover); agronomic traits, defined as silking, height, proportion of cob (prop_cob), and lodging; grain moisture (Mgrain), cob moisture (Mcob), and stover moisture (Mstover). The length of each arrow indicates the contribution of the trait, and the orientation shows the correlation between variables.

3.4. Genetic Correlations and Trait Contributions

The genetic correlation matrix among the 16 traits reveals statistically significant correlations at the 0.05 level (Table 6). Fiber traits were highly correlated with yield performance—for instance, cob ADF was positively associated with grain yield, cob yield, and stover yield at 0.414, 0.614, and 0.412, respectively. Cob ADL also had a positive relationship with grain yield, cob yield, and stover yield at 0.380, 0.466, and 0.440, respectively. However, there was a negative association between lodging and cob ADF and cob ADL of −0.758 and −0.777, respectively. Contrarily, fiber traits studied in the stover were negatively correlated with yield performance—for instance, stover ADL was −0.356, −0.231, and −0.539 with grain yield, cob yield, and stover yield, respectively. Stover ADF was associated at −0.200, −0.144, and −0.576 with grain yield, cob yield, and stover yield, respectively. Positive relationships between stover ADF and lodging (0.732) and stover ADL and lodging (0.624) were observed. Neutral detergent fiber of stover was negatively correlated with stover at −0.307. In addition, other traits showed interesting correlation trends, such as stover ADF and cob ADL with the highest genetic correlation among all at −0.824, and stover ADF and stover ADL with 0.852 genetic correlation (Table 6).

Table 6. Genetic correlation matrix of yield traits (grain, cobs, and stover); fiber traits—NDF, ADF, and ADL (cobs and stover); agronomic traits (silking, height, proportion of cob, and lodging); and moisture (Mgrain, Mcob and Mstover) across environments in North of Spain in 2010 and 2011.

Traits	ADF_cob	ADL_cob	NDF_cob	ADF_stover	ADL_stover	NDF_stover	Silking	Height	Prop_cob	Mgrain	Mcob	Mstover	Grain Yield	Cob Yield	Stover Yield
ADL_cob	0.764 [z]														
NDF_cob	0.586	0.261 [z]													
ADF_stover	−0.020	−0.824 [z]	0.884 [z]												
ADL_stover	−0.074	0.049	−0.176 [z]	0.852 [z]											
NDF_stover	0.075 [z]	−0.265 [z]	0.358 [z]	1.000 [z]	0.918 [z]										
Silking	−0.223 [z]	0.140	−0.480 [z]	−0.323 [z]	−0.047	−0.284 [z]									
Height	0.291 [z]	0.316 [z]	−0.172 [z]	−0.179 [z]	−0.104	0.056	0.484 [z]								
Prop_cob	0.002	−0.104	−0.376 [z]	−0.236 [z]	0.112	−0.239 [z]	0.337 [z]	0.350 [z]							
Mgrain	−0.189 [z]	0.227 [z]	−0.414 [z]	−0.678 [z]	−0.032	−0.488 [z]	0.831 [z]	0.449 [z]	0.205 [z]						
Mcob	−0.226 [z]	0.088	−0.591 [z]	−1.000 [z]	−0.240 [z]	−0.789 [z]	0.712 [z]	0.371 [z]	0.508 [z]	0.810 [z]					
Mstover	0.180 [z]	0.537 [z]	−0.729 [z]	−1.000 [z]	−0.080	−0.833 [z]	0.651 [z]	0.419 [z]	0.297 [z]	0.679 [z]	0.764 [z]				
Grain yield	0.414 [z]	0.380 [z]	0.000	−0.200 [z]	−0.356 [z]	0.111	0.250 [z]	0.600 [z]	−0.232 [z]	0.243 [z]	0.255 [z]	0.330 [z]			
Cob yield	0.614 [z]	0.466 [z]	0.093	−0.144	−0.231 [z]	0.169 [z]	0.361 [z]	0.850 [z]	0.355 [z]	0.249 [z]	0.307 [z]	0.370 [z]	0.796 [z]		
Stover yield	0.412 [z]	0.440 [z]	−0.084	−0.576 [z]	−0.539 [z]	−0.307 [z]	0.745 [z]	0.777 [z]	0.265 [z]	0.654 [z]	0.666 [z]	0.767 [z]	0.732 [z]	0.797 [z]	
Lodging	−0.758 [z]	−0.777 [z]	−0.306 [z]	0.732 [z]	0.624 [z]	−0.006	−0.186 [z]	−0.296 [z]	0.190 [z]	0.009	−0.094	−0.325 [z]	−0.716 [z]	−0.616 [z]	−0.492 [z]

[z] Significant pairwise genetic correlations at 0.05 level.

Genetic correlations of agronomic traits were high. Height was positively correlated with grain yield, cob yield, and stover yield, at 0.600, 0.850, and 0.777; this trend was also shown in the variable contribution graph (Figure 4) with cob ADL, height, and yields having the same proportions and arrow direction. Likewise, silking date was significantly positively correlated with stover yield at 0.745, and with moisture of the grain, cob, and stover at 0.831, 0.712, and 0.651, which was also shown by the variable contributions in Figure 4, suggesting that maturity time and moisture (Table 6) do play a role in stover yield potential [5]. This aspect will allow selecting for fiber contents, such as low ADL, and favor stover yield at the same time.

4. Discussion

Elite maize varieties selected for grain yield may not be ideal for dual exploitation, as selection for grain may negatively affect some relevant characteristics for a dual purpose biofuel resource [23,39]. To reduce the cost per unit of biofuels, biomass yield, residue use, and the efficiency in plant fiber conversion digestibility are key factors. We evaluated several agronomic and compositional characteristics relevant to the dual exploitation in open pollinated varieties that did not undergo intense selection and could have favorable characteristics for dual exploitation not present in the elite varieties. We used open pollinated varieties from three important heterotic groups—Atlantic Europe, Mediterranean Europe, and the US Corn Belt. Three of the four US Corn Belt varieties—Lancaster, Minnesota 13, and BSSS—have historical relevance in maize breeding that are not in the background of the European varieties.

Results showed that population origin presents a reservoir of genetic diversity for breeding to improve biomass quality to efficiently convert maize stover into fermentable sugars for bioenergy [15,26,27]. Multivariate analysis clearly separated the Atlantic Europe, the Mediterranean Europe, and the commercial hybrid group, indicating that the clusters differed for the variables under study. Landraces from Mediterranean Europe cluster independently and had, on average, lower NDF, ADF, and ADL values (Table 4). This difference from the other origins agrees with Mir et al. [45], who suggested they were introduced from northern South America to Europe via Spain. Likewise, the EU Atlantic populations may share common US Corn Belt origin, based on the PCA cluster (Supplementary Figure S1) and the similarity of latitudes from the northern US flint landrace.

The varieties of the US Corn Belt origin, especially Minnesota 13, grouped separately from the others in the first axis of the PC analysis (Figure 3). Late-flowering positively correlated with stover yield and grain yield [46], and Minnesota13 differed from the others because it had the earliest flowering and the lowest grain and stover moisture, although considering that earliness, it still had a relatively high grain yield. This variety was very important to adapt maize for environments with shorter growing seasons, such as the northern Corn Belt and Central Europe, and contributed to the development of one of the most important Iodent lines, PH207 [47]. Furthermore, BSSS was close to the group of commercial hybrids, which was also expected given that BSSS has contributed to elite germplasm, with such outstanding lines as B37, B14, and B73. Specifically, one of the parents of the hybrids with open formula is B73 and one of the parents of the other open formula hybrid is A632, which is derived from B14. The other hybrids probably also have a parent of BSSS origin. In the PCA, the loading contributions of fiber composition variables (i.e., NDF, ADF, ADL) were similar from the stover and the cob, only cob ADL seems to contribute more than stover ADL, which was the lowest loading contribution of the analysis (Figure 4). Agronomic variables, on average, contributed slightly more to the loadings of fiber components (Figure 4). This may be because agronomic traits have shown larger phenotypic variability than the compositional traits.

As expected, our results showed that successful commercial maize breeding focused on grain yield [48], because the hybrids had higher grain yield than landraces, even though the later had higher grain moisture on average (Supplementary Table S2). There was a substantial difference in grain yield between the most productive modern hybrid, PR34G13, and the most productive landrace, Faro, of 2000 kg/ha (Supplementary Figure S2). However, Faro is a late genotype and its productivity was at the cost of higher stover moisture. Within the varieties with lower grain moisture, Posada de Llanera had the highest grain yield, although 3000 kg/ha lower than PR34G13. This large difference in grain yield could be a limitation to introducing favorable features of the landraces to elite material, and prebreeding using genomic multistage or index selection is needed [7]. In the case of stover yield there is not a gap between local varieties and commercial hybrids. For example, Faro had higher stover yield than PR34G13 and Posada de Llanera had only 800 kg/ha difference in spite of its lower grain moisture compared to the hybrid PR34G13 (Supplementary Tables S1 and S2). We observed that the gap between selected and unselected material was greater for grain yield, which was the direct target of selection, than for stover yield, which was only indirectly selected (Supplementary Figure S2). As a consequence of this, the harvest index (HI) of the local varieties was lower (0.42–0.52) than the HI of the hybrids (0.53–0.57). In fact, two of EU Mediterranean landraces (BastoxBlanco and RastrojoC3) showed greater amounts of stover than grain yield. In contrast, Lorenz et al. [49], in a literature survey, concluded that the HI did not change over time in US Corn Belt germplasm. Within the landraces, Faro from the Mediterranean area and Lancaster from the Corn Belt had high stover yield in addition to acceptable grain yield, which suggests potential for dual-purpose use. However, these varieties have late flowering, which contribute to high grain moisture at harvest. A breeding goal would be to reduce grain moisture without affecting the grain and stover yield for a dual purpose of grain and residue production. For instance, Posada de Llanera has better agronomic characteristics for dual exploitation because it was among the five best landraces for stover, cob, and grain production and had the second lowest grain moisture after Minnesota 13. This variety seems to have characteristics that are valuable for dual exploitation, such as a relatively late flowering line that allows large accumulation of vegetative biomass and rapid kernel growth and dry down, which explains the relatively high grain yield and low grain moisture in spite of the short grain filling and dry down period [50]. The moisture of the residues of all varieties would be satisfactory for storage in silos [25,51], although, data in more environments, for example in Atlantic Europe, would be needed to confirm this hypothesis.Regarding the composition of the residues, fiber amounts studied in cobs were significantly different based on the origin, whereas the fiber studied in stover did not differ among origins (Table 4). We found higher values of ADF, ADL, and NDF in the cobs than in the stover. The cellulose content can be approximated as ADF-ADL, while the hemicellulose as NDF-ADF. The average quotient of hemicellulose in stover divided by hemicellulose cob was 0.78 in landrace varieties, while the quotient of stover cellulose dived by cob cellulose was 0.92. These values are similar to the values (0.76 and 0.98) reported by Lorenz et al. [52], in spite of the different origin and characteristics of the germplasm, suggesting the potential for bioethanol production is greater in cob than in stover [20].

Fiber composition differences for stover, based on origins, were not detected. This may be due to it not being a breeding target, or due to a greater environmental effect on stover measurements. At the variety level, there were significant differences in fiber composition. Only a few varieties were different from the rest, for example, Norteño Largo had high ADL, which would be detrimental for conversion to ethanol or biogas, and Minnesota 13 had a high concentration of NDF, which could mean greater concentration of hemicellulose. This lack of variation among such a diverse sample of germplasm, including local varieties from Mediterranean Spain that are the most variable in Europe [53], suggests that the selection for stover fiber could be not very effective in temperate maize [52]. We found more variation in fiber composition of cobs than in fiber composition of the stover, suggesting that may be a better breeding target for biofuel production. The Atlantic European group had a favorable composition of cobs for bioenergy with more NDF and ADF and less ADL, and, within this group, Aranga stood out due to its high concentration of ADF and Lazcano for high concentration of NDF. The general trend of our results suggest that EU Atlantic landraces have evolved towards a lower value of ADL, which is an indicator of suitability in the degradation process of energy production for biofuels. The lower the ADL the more suitable for processing residue to biofuel.

Previous studies reported that lodging reduces grain and stover yield [23], although, fiber improvement may not affect lodging [21]. Despite the high values of heritability for each trait, correlations between agronomic traits and stover composition were less relevant; this may be due to the low variability detected for stover fibers. Only the correlation between stover ADF and lodging was greater than 0.7 and positive, suggesting that increased ADF in the stover, favorable for bioenergy production, may lead to an undesirable lodging effect (Table 6). Interestingly, as stover ADL and ADF increase, the stalks tend to lodge more, which is the opposite of what one would expect, but is consistent with results reported by Albrecht et al. [21]. For cob composition, there were also high correlations with lodging, however, the negative relationship of ADF and lodging may be beneficial if negative selection occurred for ADF, which would drive positive agronomic effect due to lodging reduction [21]. However, there were negative correlations between cob ADL and lodging, indicating that decreasing ADL in the cob in order to favor its digestibility may have an undesirable effect on lodging. How a characteristic of the cob influences lodging, which depends on the roots and stalks, deserves more research. In general, genotypic correlations between fiber composition and agronomic trait were not of such magnitude that they may hamper the simultaneous selection for these two traits, in agreement with Lorenz et al. [54] and Lewis [23].

According to these trends, silage maize maybe more appropriate for dual exploitation when the final use is bioethanol or biogas because the digestibility of the whole plant is an important target in silage breeding. However, Barrière et al. [55] observed than the degradability of the modern silage hybrids in Europe is worse than the old ones and pointed out that new investigation of genetic resources is needed. Furthermore, harvest of silage hybrids is performed before the complete maturity of the grain, while in dual purpose hybrids harvest is performed at grain maturity. The ideal composition, yield, and moisture of both types of hybrids could be different, for example, in dual purpose hybrids the moisture of the grain should be as low as possible at harvest. The ideal biomass structure and composition is system dependent and in the case of combustion the degradability is not an issue [39].

5. Conclusions

In summary, our study revealed general trends of evolutionary adaptation based on origin. We showed that commercial breeding focused on ear performance rather than other traits such as, stover yield. As biomass production and fiber parameters are important for second-generation biofuels, the genetic diversity observed in this study suggests than European landraces have breeding potential for lignocellulosic biomass production for use in bioethanol conversion, and could be used in future prebreeding programs for a dual-purpose variety development. Further research should advance breeding material discussed here, coupled with marker-assisted selection increasing biomass, or focus on altering morphology or anatomy of the maize plant. An alternative strategy would be to engineer bioenergy feedstocks targeting genes responsible for the amount and composition of fiber.

Supplementary Materials: The following are available online at https://www.mdpi.com/2073-4395/11/2/230/s1, Figure S1: Plot of principal components analysis (PCA) of components 2 (PC2) and 3 (PC3), across origins described in Table 2, with the genotypes grown across four environments in 2010 and 2011 in Pontevedra, Spain. Figure S2: Yield performance comparison plot of stover yield (left) and grain yield versus grain moisture evaluated on twenty lines from divergent origins—European Atlantic (EA), European Mediterranean (EM), US Corn Belt (USCB), and commercial hybrids (Hb)—in four environments across 2010 and 2011 in Galicia, Spain. Vertical axis is grain moisture (g/kg) and horizontal axis are stover yield (left) and grain yield (right) in kg/ha. Table S1: Means of yields (kg/Ha) of grain, cob, and stover and standard error (SE) from 20 maize genotypes grown at four year_loc environments in Galicia, Spain, in 2010 and 2011. Grain yield (yield_grain), cob yield (yield_cobs), stover yield (yield_stover). Table S2: Means of agronomic descriptive traits and standard error (SE) from 20 maize genotypes grown at four year_loc environments in Galicia, Spain, in 2010 and 2011. Table S3: Means of agronomic morphological traits and standard error (SE) from 20 maize genotypes grown at four year_loc environments in Galicia, Spain, in 2010 and 2011. Traits defined as proportion of cob (prop_cob) and lodging, and grain moisture.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

Europe Atlantic = EA, Europe Mediterranean = EM, US Corn Belt = USCB, hybrid = Hb, Prop_cob = proportion of cob, Mgrain = grain moisture, Mcob = cob moisture, Mstover = stover moisture.

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