Breeding Dual-Purpose Maize: Grain Production and Biofuel Conversion of the Stover

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Abstract: The improvement of maize double cropping has become increasingly important in recent years. In order to establish breeding programs for sustainable maize production, the goals of the research were (i) to understand the correlations between grain and stover yield and saccharification efficiency, and (ii) to identify QTL and metabolic pathways to design of breeding programs in a double exploitation approach. We carried out a genome-wide association study (GWAS) and a metabolic pathway analysis using a panel of highly diverse maize inbreds. As results, we have obtained that the regulation of energetic and developmental pathways have been pointed out as key pathways related to stover quantity and utilization, while no specific pathways could be identified in relation to grain yield. However, due to the moderate high heritability of yields and their positive correlation a phenotypic selection approach would be adequate for the improvement of both yields, while for saccharification efficiency improvement upcoming genomic selection models are more advisable. Overall, breeding strategies that manage the dual use of maize are viable and will contribute to a more sustainable maize crop in the near future.

Keywords: maize; yield; saccharification; double exploitation; GWAS; metabolic pathway

1. Introduction

One of the most important and widely used cereals for food, feed, industrial and energy purposes is maize. Improving the double exploitation of maize has become increasingly important in the recent years due to population increases and growing food demand [1,2]. Breeding programs have been focused on traits associated with grain yield and its related traits. Grain yield is a quantitative trait with low heritability, which may indicate several assumptions: (i) yield is determined by a large number of genes; (ii) the largest proportion of the variance is due to environment or experimental error; and/or (iii) the difference between genotypic values depends on environment (G × E effect) [3–5].

Although a large number of quantitative trait loci (QTL) for maize grain yield have been described over the past three decades, the phenotypic variance that they explained has been very low. For example, in a mapping population of 266 $F_{2:3}$ family lines, six genomic regions associated with grain yield were mapped. One of them, located in the region between bnlg1556 and umc128 on chromosome 1 was identified as the major QTL, explaining between 7.1% and 16.8% of the total phenotypic variation [6]. Similarly, in a set of 253 recombinant inbred lines (RILs), Yang et al. [7] identified a single QTL for grain yield that explicated from 8.42% to 8.44% of the phenotypic variance. For grain yield per

plant, 11 genomic regions were detected in eight RIL populations explaining 2.2% to 6.7% of phenotypic variance [8].

So far, breeding efforts have been focused predominantly on improving grain yield, or silage for animal feed, with few of them specializing in exploiting stover for energy production. The production and distribution networks in maize cultivation are well-established and can supply large quantities of lignocellulosic residue with excellent logistics for exploitation [9]. Dependence on fossil biofuels has led to environmental and economic consequences of global concern; thus, lignocellulose derived from agricultural residues can represent a sustainable alternative for the production of biofuels [10].

Mapping QTL associated with lignocellulosic biomass quantity and quality is essential to choose the best breeding programs to produce biofuel [2]. While some studies described three QTL in a RIL progeny derived from the cross between WM13 and Rio for stover yield [11], others have focused on improving the quality of the lignocellulosic residue, based on three major characteristics associated with the cell wall composition: high and low concentration of cellulose and lignin, respectively, and high proportion of glucose released by enzymatic degradation [12]. Truntzler et al. [13], investigated the genetic relationship between cell wall components of the stover and saccharification efficiency (the conversion into fermentable sugars). This QTL meta-analysis included 14 studies of QTL mapping, using 11 independent populations for and cell-wall components and degradability and found 27 QTL related to saccharification. In B73 $\times$ Mo17 recombinant inbred maize population, 10 QTL were identified for sugar release after high-temperature dilute acid treatment [12] and four QTL for glucose release after a steam explosion pretreatment, what is a measure of saccharification [14]. More recently, a study performed in a MAGIC population found two significant Single Nucleotide Polymorphisms (SNPs) on chromosome 6 associated with saccharification efficiency and 16 SNPs associated with stover yield [2].

Overall, as previously mentioned, increase in both yields, grain and stover, would be a requirement to meet future food and fuel demands. Genome-wide association studies (GWAS) using panels of inbred lines presenting large diversity are an advantageous tool to identify genomic regions related to both grain and stover yield. These studies also assist the design of breeding programs for double crop exploitation [15]. Moreover, due to their being quantitative characters with a small effect for each gene, metabolic pathway analysis focusing on the action of a certain number of genes that are grouped based on their biological function could be a promising approach to complement GWAS [16]. The combination of GWAS with metabolic pathway analysis considers all genetic sequences positively associated with the trait of interest [17]. In recent studies, this approach has been utilized to investigate the genetic basis of complex traits in maize such as aflatoxin accumulation [18,19], corn earworm resistance [20], or lipid biosynthesis [21].

Aiming to advance in the identification of markers that allow to establish breeding programs for sustainable maize production, the objectives were to (i) understand the correlations between grain, stover yield and saccharification efficiency in a double exploitation approach, and (ii) identify QTL and metabolic pathways associated with these traits.

2. Material and Methods

2.1. Plant Material, Experimental Design and Yield Calculations

A subset of 836-inbred lines from the Ames association panel (North Central Regional Plant Introduction Station, Ames, Iowa, USA) [15], along with six inbred controls (A619, A632, A662, A665, PH207, EP42) were grown at Misión Biológica de Galicia in Pontevedra (42°24′ N, 8°38′ W, 20 m above sea level) following an augmented 17-block design in 2018 and 2019 [22]. Three hundred inbred lines well adapted to the environmental conditions in Pontevedra were selected for evaluation, in order to have a quality phenotyping. The inbred lines were divided into five precocity groups based on female flowering (date at which 50% of the plants showed visible silks) and each group was harvested at approximately 70 days post-flowering. Grain and stover yield and saccharification efficiency were recorded on

each experimental plot, each plot consisting of a single row, 2.4 m long, of 13 plants, spaced between consecutive hills in a row by 0.21 m and 0.8 m between rows.

2.1.1. Grain Yield

Grain yield was calculated as the weight of grain per plant and adjusted to 14% grain moisture. It was determined by the following Equation (1):

$$\text{Grain yield} \left(\frac{\text{g}}{\text{plant}} \right) = \frac{Ear\ weight\ per\ plant\ (g) \times (100 - grain\ mosture) \times Grain\ weight\ of\ 5\ ears\ (g)}{Number\ of\ plants\ per\ plot \times 86 \times Total\ weight\ of\ 5\ ears\ (g)} \tag{1}$$

where grain moisture, expressed as a percentage, was recorded using a Kett moisture meter (model PM-400) in a sample of 240 cm^3. The value 86 in the equation corresponds to a constant to adjust the yield to 14% humidity.

2.1.2. Stover Yield

In each plot, the stover (plants without ears) of plants was harvested. The weight of fresh stover was recorded and collected to estimate the percentage of dry matter in the stover. The fresh stover was weighed, dried at 60 °C in an oven and again weighed after seven days (stover dry weight). Stover yield was calculated by the following Equation (2):

$$\text{Stover yield} \left(\frac{\text{g}}{\text{plant}} \right) = \frac{weight\ of\ harvested\ stover\ (g) \times stover\ dry\ weight\ (g)}{Number\ of\ plants \times stover\ fresh\ weight\ (g)} \tag{2}$$

2.1.3. Saccharification Efficiency

The stover samples, once dried (60 °C, 7 days), were ground in a Wiley mill with a 0.75 mm mesh. Saccharification efficiency determinations were carried out following the method described by Gomez et al. [23].

Ground material was weighed into 96-well plates. Each well contained 4 mg of each sample and processed using a high-throughput automated system. Samples were pretreated with 0.5 M NaOH at 90 °C for 30 min, washed four times with 500 μL sodium acetate buffer and finally subjected to enzymatic digestion (Cellic CTEC2, 7FPU/g) at 50 °C for 8 h. The amount of released sugars was assessed against a glucose standard curve using the 3-methyl-2-benzothiazolinone hydrozone method [24].

2.2. Statistical Analysis

2.2.1. Analysis of Variance and Correlation Coefficients

A combined analysis of variance was performed for the traits studied using the PROC MIXED procedure of the SAS software [25]. Best Linear Unbiased Estimators (BLUEs) for each line were calculated based on the pooled data for the 2-year analysis. Genotypes were considered as fixed effect while years and blocks were considered as random. Means comparison were carried out using the minimum significant difference (LSD) method. Traits' heritabilities were estimated following Holland et al. [26].

Correlation coefficients between grain and stover yield and saccharification efficiency were calculated using REML estimates according to a published SAS mixed model procedure [27].

2.2.2. Association Mapping

Inbred lines were genotyped using a genotyping-by-sequencing method (GBS) at the Institute of Biotechnology of Cornell University using version 4 of the B73 genome [22]. The genotype matrix was filtered, i.e., SNPs with more than 50% missing data and a minor allele frequency of less than 5% were omitted, as well as monomeric and multi-allelic SNPs and insertion/deletion polymorphisms (INDELs). Heterozygous genotypes were considered missing data. After filtering, 147.428 SNPs distributed across the maize genome were retained.

A genome-wide association study (GWAS) of the BLUEs across years was carried out with BLINK based on a mixed linear model using R [28]. Genotypic (GD) and phenotypic (Y) data

are included in the model. BLINK eliminates the requirement of even distribution of markers in the genome and the bin method was replaced by linkage disequilibrium information [28].

An association between a trait and a SNP was considered significant when the observed $-\log10$ (p) values showed a clear derivation from the distribution of the expected $-\log10$ (p) that holds under the null hypothesis (i.e., no significant association) in quantile–quantile (QQ) plots [22,29].

2.2.3. Pathway Association

Significant metabolic pathways associated with grain and stover yield and saccharification efficiency were assigned using PAST tool, implemented through MaizeGDB [16,30,31]. PAST processes data in four main steps. First, GWAS output data are loaded into PAST. These data come in the form of statistics that reflect the effects of specific loci (e.g., SNPs) on a trait(s) of interest and LD data between loci. Second, the SNPs are distributed in linkage groups and each group is associated with a gene based on genomic distance between SNPs and the gene. Once SNPs are assigned to genes, attributes of SNPs, allelic effects and p-values, are transferred to genes according to the method described in Tang et al. [19]. The genes and their effects are used to find significant pathways and calculate a running enrichment score, which is plotted in a rug plot for each pathway in the fourth step [17]. In the current study, linkage groups were stablished based on all SNP pairs having LD exceeding an R^2 of 0.8. The search window for the causative gene was set at ±1 Kb, which was based on the distances between linked SNPs and only metabolic pathways with five or more genes were considered to reduce the bias of the small sample size.

3. Results

3.1. Analysis of Variance and Correlation Coefficients

The combined analyses of variance across years showed that differences between inbreds were significant for grain and stover yield (F = 2.75, F = 2.47, $p < 0.0001$, respectively) and for saccharification efficiency (F = 1.26, $p = 0.0342$) (Table S1). Significant genotype-by-environment interactions were observed for stover yield (Z = 2.93, $p = 0.0017$) and saccharification efficiency (Z = 2.07, $p = 0.0194$). The estimated genetic heritability for grain and stover yield was moderately high (Table 1). The genetic heritability for the estimated saccharification efficiency was not significantly different from zero (Table 1). The genotypic and phenotypic correlation coefficient between stover yield and saccharification was not significant (-0.599 ± 0.317, -0.096 ± 0.046, respectively). The genotypic and phenotypic correlation coefficients between stover and grain yield were positive, moderate and significant (0.614 ± 0.079, 0.547 ± 0.035, respectively).

Table 1. Means, ranks, least significant difference (LSD) and h^2 for stover yield, grain yield and saccharification efficiency (SaccEff) of the Ames association panel lines evaluated in 2018 and 2019. The h^2 is considered significantly different from zero when it is two times greater than the standard deviation.

	Grain Yield (g/Plant)	Stover Yield (g/Plant)	SaccEff (nmol Glucose/mg/h)
Mean	63.21	68.04	158.43
Rank	8.83–142.98	14.14–142.52	99.05–198.04
LSD	26.13	38.28	19.04
h^2	0.61 ± 0.05	0.59 ± 0.05	0.18 ± 0.11

3.2. Association Mapping

Following Yi et al. [22], a marker was considered significantly associated ($\alpha = 0.05$) with a trait at p values less than 1.00×10^{-4}. We considered a region of $+/-3$ Kb around the SNP as the SNP confidence interval following the distance at which the LD decay ($R^2 < 0.1$). Two SNPs were assigned to the same QTL when their confidence intervals overlapped. We found a total of 22 significant SNPs that corresponded to 17 QTL. We

identified nine independent SNPs associated with grain yield, eight SNPs significantly associated with stover yield that were grouped into five QTL, and five SNPs grouped into three QTL, associated with saccharification efficiency (Table 2, Figure S1).

Table 2. Significant QTL associated with stover and grain yield and saccharification efficiency (SaccEff) in a subset of the Ames association panel lines evaluated in two years.

Trait	QTL [a]	Marker	Chr	Pos [b]	p Value	MAF [c]	Effect [d]
Grain yield	QTL_1_1	S1_27627320	1	27818428	5.064×10^{-5}	0.352	8.067
	QTL_1_2	S1_29190896	1	29445156	1.9397×10^{-5}	0.261	−9.508
	QTL_2_1	S2_4514070	2	4481454	4.3931×10^{-5}	0.187	10.264
	QTL_2_2	S2_11756881	2	12049766	1.4789×10^{-5}	0.250	−10.191
	QTL_3_1	S3_179251623	3	182111795	9.3472×10^{-5}	0.109	12.663
	QTL_3_2	S3_212022847	3	215694276	9.3124×10^{-5}	0.476	7.294
	QTL_4_1	S4_196824058	4	201140915	9.7931×10^{-5}	0.367	−7.751
	QTL_7_1	S7_5108781	7	5367270	4.0454×10^{-5}	0.428	7.755
	QTL_10_1	S10_148116222	10	148928081	8.8683×10^{-5}	0.172	−11.301
Stover yield	QTL_2_1	S2_38324920	2	39888908	4.5751×10^{-5}	0.336	−8.883
	QTL_2_1	S2_38324925	2	39888913	4.5751×10^{-5}	0.336	8.883
	QTL_2_1	S2_38324933	2	39888921	1.8528×10^{-5}	0.300	9.500
	QTL_2_1	S2_38324945	2	39888933	4.657×10^{-5}	0.330	−8.757
	QTL_4_1	S4_240526011	4	246038631	6.7154×10^{-5}	0.269	−9.633
	QTL_7_1	S7_40413622	7	41898418	8.6845×10^{-5}	0.090	−14.817
	QTL_8_1	S8_28160359	8	29092941	8.0953×10^{-5}	0.292	8.624
	QTL_10_1	S10_148116222	10	148928081	1.552×10^{-5}	0.174	−12.996
SaccEff	QTL_3_1	S3_201309933	3	204497949	3.7353×10^{-5}	0.146	5.019
	QTL_5_1	S5_178352276	5	182432287	8.4243×10^{-5}	0.249	−3.903
	QTL_5_1	S5_178352277	5	182432288	8.5921×10^{-5}	0.249	−3.898
	QTL_6_1	S6_150466053	6	154564447	6.4079×10^{-5}	0.177	−4.102
	QTL_6_1	S6_150466085	6	154564479	5.8653×10^{-5}	0.181	4.114

[a] The number before the underscores indicates the chromosome and the number after the underscores indicates the QTL within the chromosome. [b] Physical position of marker within the chromosome. [c] MAF: Minority allele frequency. [d] Additive effect calculated as half the difference between the mean of homozygotes of the second allele in alphabetical order and the mean of homozygotes of the first allele in alphabetical order.

3.3. Pathway Association

None of the 363 pathways found with at least five genes was associated with grain yield, while aerobic, pyridine and long-day-regulated expression of florigens pathways were significantly (FDR < 0.15) enriched among genes with an effect on stover yield. On the other hand, genes of the indole-3-acetyl-amide were overrepresented (FDR < 0.15) among genes with an effect on saccharification efficiency (Table 3).

Table 3. Pathways affecting stover yield and saccharification efficiency (SaccEff) with enrichment scores better than $q < 0.15$.

Trait	Pathway ID	Pathway Name	No. of Genes	p Value	q Value
Stover yield	PWY-4302	aerobic	41	9.16×10^{-4}	0.133
	PWY-5381	pyridine	20	2.33×10^{-4}	0.084
	R-ZMA-8934036.1	Long-day-regulated expression of florigens	23	1.10×10^{-3}	0.133
SaccEff	PWY-6219	indole-3-acetyl-amide	7	3.80×10^{-4}	0.138

The plots of the pathways for stover yield and saccharification efficiency illustrate how the running enrichment score values change with genetic rank (Figure 1). The pyridine pathway (PWY-5381, Figure 1a) for stover yield had a high enrichment score (0.58) due to genes in the pathway having the highest and lowest effects on the trait and thus, increased the enrichment score value. The long-day-regulated expression of florigens pathway (R-ZMA-8934036.1, Figure 1b) for stover yield had moderate enrichment scores (0.49). This was in contrast to the aerobic pathway (PWY-4302, Figure 1c), which had a lower enrichment score (0.41) as few genes in this pathway had high effects (positive or negative) on the trait but were outnumbered by genes having low effects. For saccharification efficiency, the indole-3-acetyl-amide pathway (PWY-6219, Figure 1d) had a very high enrichment score (0.81), partly because, with the exception of one gene, the few genes assigned to this pathway were among those with the highest or lowest ranks.
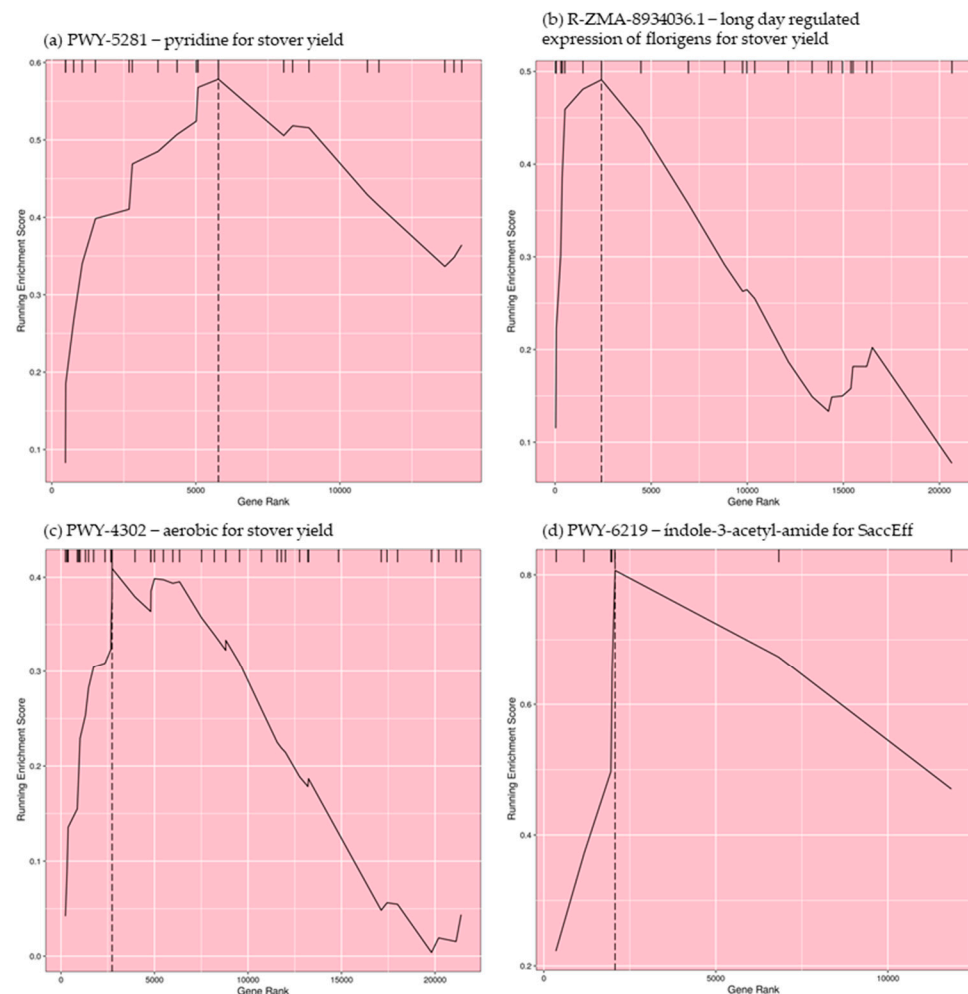
Figure 1. Graphs of the running enrichment score calculation for (**a**) pyridine pathway (PWY-5381), (**b**) long-day-regulated expression of florigens pathway (R-ZMA-8934036.1), (**c**) aerobic pathway (PWY-4302) for stover yield and (**d**) indole-3-acetyl-amide pathway (PWY-6219) for saccharification efficiency. The *X*-axis shows the rank of each gene effect value; the *Y*-axis shows the value of the enrichment score (ES) running sum statistic as each consecutive gene effect value is processed. The x-intercept line indicates the highest point of the ES. Small hatch marks at the top of the image indicate the rank position of the effect of all genes in the pathway.

4. Discussion

4.1. Combined Analysis of Variance, Heritabilities and Correlation Coefficients

In the current study, the high heritability observed for grain and stover yield indicates that additive variance was more important than variances due to G x E interaction and

experimental error for these traits. These results agree with previous studies, where the broad-sense heritability for grain yield was very high, with values ranging from 0.77 to 0.91 [3,4,32]. However, there are no studies to contrast the heritability found here for stover yield, although there are studies on the quality and yield of corn silage around the world [33]. Unlike other crops, stover yield and grain yield were positively correlated in maize [34,35]. Based on the observed positive high genotypic and phenotypic correlation between grain yield and stover yield, these traits could be managed together. This is supported by SNP S10_14811622, which is associated to both traits with the same sign (Table 2). This put forward that increasing stover biomass does not negatively compromise grain yield. In addition, the moderately high heritability suggests that both traits can be successfully improved using phenotypic selection, although genomic selection could also be an alternative.

Increases in biomass or stover yield have been a target for biofuel production and forage digestibility [2,12,36]. The genotypic correlation found between saccharification and stover yield was high and negative ($r_g = -0.60$), although it was not significantly different from zero, probably due to the low heritability for saccharification efficiency. In this context, optimization of maize stover as a biofuel feedstock can be achieved by plant breeding to increase yield but the quality of the stover should also be taken into account. Considering the genotypic variability for saccharification efficiency and that the heritability for that trait did not differ from zero, there is hardly any variability for improving residue quality. Herein, our study represents a novel contribution for dual exploitation of the crop, since ethanolic production from lignocellulosic material does not compete with grain production.

4.2. Association Mapping

For grain yield, many QTL have been previously described in the last decades. Our results show co-localization with previously detected QTL. For example, marker S1_29190896 co-localized in bin 1.03 with previously described QTL in a RIL population [4] and in an F$_{2:3}$ population [6]. The QTL significantly associated with grain yield located in chromosomes 2 and 3 in this study co-localized with those found by Lima et al. [5] using a set of 256 F$_{2:3}$ families derived from tropical maize germplasm. The S3_212022847 marker also co-localized at bin 3.08 with QTL identified by Wang et al. [37]. However, few consistent results have been obtained, probably due to the use of different parental materials, segregating population sizes, marker densities or environments, which could influence QTL detection.

In the current study, QTL for stover yield co-localize with QTL found in a multiparent advanced generation intercross (MAGIC) population by López-Malvar et al. [2]. As the decay linkage disequilibrium occurred at a distance greater than 100.000 bp in that MAGIC population [38,39], SNPs located far apart less than 100 kpb from those found by López-Malvar et al. [2] could be associated to the same QTL. They previously found 13 QTL significantly associated with stover yield, three of them less than 100 kpb apart from markers S2_38324933, S7_40413622 and S10_148116222 associated to stover yield in the present study.

None of the markers significantly associated with saccharification efficiency in this study coincide in the same bin with those previously described for glucose yield or saccharification [12,14]. In the multiparent advanced generation intercross (MAGIC) population, López-Malvar et al. [2] identified two QTL for saccharification efficiency less than 100 kpb apart from markers associated to saccharification efficiency in the current GWAS, S6_150466085 and S6_150466053.

Jointly, mapping QTL associated with stover yield is an important step in optimizing selection programs to improve biofuel production. However, the correlation between stover yield and saccharification efficiency was not significant enough to support indirect selection programs to improve saccharification based on stover biomass. These results support a direct breeding strategy for saccharification, preferentially based on genomic selection. Variation in saccharification efficiency was not well explained by the significant SNPs found to recommend marker-assisted selection and the heritability is too low to recommend phenotypic selection. Genomic selection is based on trait values predicted as

the sum of the individual's genetic value at all markers distributed across the genome, and this selection strategy could lead to high correlations between the actual and predicted genetic values [40].

4.3. Pathway Association

The pyridine nucleotides NAD^+ and $NADP^+$ are ubiquitous coenzymes that can have an impact on virtually all metabolic pathways in the cell [41]. Mutant studies provide evidence that changes in NAD status can alter photosynthesis and stress responses in plants, suggesting that NAD content may be a potent modulator of metabolic integration [42,43]. In this sense, the pyridine nucleotide cycle was significantly associated with maize stover yield in the current study. The regulation of dehydrogenases in mitochondria by the level of reduced and oxidized pyridine nucleotides could be a critical point of fine regulation. The plant metabolic system has features associated with remarkable metabolic plasticity of mitochondria that allow the use of energy accumulated during photosynthesis so that all anabolic and catabolic pathways are optimized and coordinated impacting yield [44].

The regulation of respiration in light also represents a phenomenon that coordinates all major pathways of metabolism during photosynthesis, including reactions of secondary metabolism. Oxidative phosphorylation produces ATP, but it is also a central sink for high-capacity electrons required by many metabolic pathways that must be coordinated and integrated in a flexible way [45]. In this context, the aerobic respiration III pathway is enriched among genes with high effect on stover yield. This alternative oxidase (AOX) pathway branches from the cytochrome pathway (aerobic respiration I) at the internal mitochondrial membrane in the ubiquinone pool and passes electrons to a single terminal oxidase. The production of reactive oxygen species (ROS) is an inevitable consequence of the mitochondrial electron transport chain (ETC), and excessive ROS production is critical for plant development. In this respect, ROS would act as a signaling compound that initiates feedback mechanisms to keep photosynthetic cell function under control [46].

Finally, the third pathway significantly impacting stover yield, long-day-regulated expression of florigens, denotes the importance of flowering time in the final biomass production. Flowering time is a very complex trait that controls the adaptation of plants to their local environment, but differences in flowering time in maize are not caused by a few large effect genes, but by the cumulative effects of numerous quantitative trait loci, each of which has only a small impact on the trait [47]. The maize genome encodes several florigen genes, i.e., floral genes expressed in the leaf vasculature that promote flowering at the shoot apex. Because flowering time is highly dependent on temperature, it is shorter for a given non-photoperiodic genotype at lower latitudes than at higher latitudes in the temperate zone. Florigens play an essential role in the genetic variability for flowering time in those lines where breeding suppressed photoperiodism [48–50].

Lastly, the only pathway significantly associated with saccharification efficiency was indole-3-acetyl-amide. Indole-3-acetic acid (IAA), a predominant form of auxin, is an important phytohormone that affects many aspects of plant development throughout the plant life cycle [51]. Stamatiou et al. [52] have observed a link between auxin transport and increased sugar release in maize. Application of a specific auxin transport inhibitor, N-1-naphthylphthalamic acid (NPA), to two different maize cultivars resulted in a significant increase in saccharification. Lines showing enhanced sugar release displayed an incompletely penetrant bishop-shaped inflorescence phenotype, which may mean that altered cell wall integrity contributes to aberrant inflorescence development. We can highlight that auxin transport may influence cytosolic sugar or starch accumulation in vegetative tissues and lead to enhanced saccharification.

5. Conclusions

In brief, a phenotypic selection approach using appropriate inbreds from the Ames panel will be a good starting point concerning the improvement of both yields, whereas forthcoming genomic selection models are advisable for saccharification enhancement. Overall, breeding

strategies managing dual purpose uses of maize are feasible, and will contribute in the near future to a more sustainable maize cultivation. Regulation of energy and development pathways have pointed out as key routes related to stover quantity and exploitation, whereas no specific pathways could be identified in relation to grain production.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13051352/s1, Table S1: BLUEs for saccharification efficiency, stover yield and grain yield; Figure S1: Manhattan and Q-Q plot for grain and stover yield and saccharification efficiency.

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