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A note on potential candidate genomic regions with implications for maize stover fodder quality

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ABSTRACT

A panel of 276 inbred lines from CIMMYT's Drought tolerant maize for Africa program was test crossed to maize line CML312 and the single crosses were evaluated for grain and stover yields, plant height (PH), days to 50% anthesis (DtA_{50}) and silking, stover nitrogen (N), neutral (NDF) and acid detergent fiber (ADF), acid detergent lignin (ADL), *in vitro* organic matter digestibility (IVOMD) and metabolizable energy (ME) content. Most stover fodder quality traits were highly significantly different among the lines except ADF. These differences were substantial among best and worst lines for the traits, with stover N varying threefold and NDF, ADF and IVOMD by more than 10 percentage units. Among the agronomic traits, significant positive associations were observed among grain and stover yield. Grain yield was significantly negatively associated with DtA_{50} and Anthesis to silking interval (AtS) and positively with PH. Stover yield was significantly negatively associated with DtA_{50} and positively with PH. Desirable stover quality traits N, IVOMD and ME were significantly negatively associated with grain yield ($R^2 = 0.25–0.28$) while undesirable quality traits NDF, ADF and ADL were significantly positively associated with grain yield ($R^2 = 0.04–0.23$). Stover yields were largely unrelated fodder quality traits except for a significant negative association with NDF and ADF ($R^2 = 0.04$ to 0.08). GWAS analysis carried out using GBS (genotyping by sequencing) and a 55K SNPs genotypic dataset revealed several regions of significant association for N, ADF and IVOMD, each explaining from 3 to 9% of phenotypic variance for these fodder quality traits. SYN7725 from the 55K chip on chromosome 4 explained the largest proportion of phenotypic variance (~9%) for ADF and had a robust minor allele frequency (MAF) of 0.35. A specific genomic region on chromosome 3 (132.7–149.2 Mb) was found to be significantly associated with all the three forage quality traits, with the largest effect on IVOMD. This region merits attention for further validation and marker-assisted introgressions. A cellulose-related candidate gene, *Xyloglucan endotransglucosylase/hydrolase* (*xth1*, GRMZM2G119783) was also identified closer to the peak on chr.10 (~76.9 Mb) for ADF, which has been previously demonstrated to have a significant role in fiber elongation in cotton.

1. Introduction

Higher stover fodder quantity and quality is emerging as an important trait in maize improvement programs (Berhanu et al., 2012). Genome-wide association study (GWAS) or association mapping provides a powerful tool for crop improvement and has resulted in identification of several important genomic regions for plant height, flowering time and a range of disease resistance traits in maize (Ducrocq et al., 2008, 2009; McMullen et al., 2009; Kump et al., 2011; Yan et al., 2011). With the discovery of several rapid, cost effective and efficient molecular marker systems in maize, identification of candidate genomic regions with significant effects on fodder quality and their subsequent use in quality improvement program is feasible and practical. The objectives of this investigation were to evaluate a diverse collection of tropical and sub-tropical maize lines for a range of pertinent agronomic and stover fodder quality traits – IVOMD, ADF and N; and to identify tentative candidate genomic regions that are associated with important stover fodder quality parameters for further validation in appropriate populations.

2. Materials and methods

2.1. Plant material and field trials

A collection of 276 inbred lines from CIMMYT's tropical and sub-tropical programs (DTMA) were test-crossed to CML312 (a widely used sub-tropical tester with a very good general combining ability across germplasm and maturity groups) were evaluated for agronomic and stover fodder quality traits at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) during February–June 2011. These crosses were planted in two row 4 m plots with 2 replications following an α-lattice design. The soil type of the experimental plot was black clay loam with a pH of 8.5. The average daily minimum and maximum temperature during the cropping season was 25 °C and 36 °C, respectively, with an average relative humidity of 32%. Before planting 60 kg nitrogen (N) ha^{-1} in the form of urea, 60 kg phosphorous ha^{-1} as single super phosphate, 40 kg potassium ha^{-1} as muriate of potash and 10 kg zinc as zinc sulfate were applied as a basal dose. Second and third doses of N (each 30 kg N ha^{-1}) were side-dressed when plants were about knee-high and at tasseling, respectively. Pre-emergence application of pendimethalin and atrazine (both at 0.75 kg/ha a.i., tank mixed) were used for weed control.

2.2. Agronomic observations and stover fodder quality traits

Plot-wise five agronomic traits were recorded. Days to 50% anthesis and silking were determined, when 50% of the plants had shed pollen and silk had emerged, respectively. The anthesis silking interval was calculated as the difference between days to 50% silking and 50% anthesis. At physiological maturity, plant height was measured on five representative plants per plot. Grain and stover yields were recorded plot wise and grain weights were adjusted to 12.5% moisture content. Ten representative stover samples from each of the plots harvested were analyzed for stover fodder quality traits [nitrogen (N), neutral (NDF) and acid detergent fiber (ADF), acid detergent lignin (ADL), in vitro organic matter digestibility (IVOMD) and metabolizable energy (ME) content] using near infrared spectroscopy (NIRS) (Ramana Reddy et al., 2013).

2.3. Genotyping and association mapping

DNA was extracted from greenhouse grown seedlings at the 3–4 leaves stage. Normalized DNA was used for genotyping with 55K infinium illumina SNP chip and genotyping by sequencing (GBS) platform (Elshire et al., 2011) at Cornell University. SNPs with less than 0.02 Minor Allele Frequency (MAF) and 0.8 call rate (CR) in the 55K genotype dataset were excluded from the analysis. For GBS SNP markers with less than 0.01 MAF and 0.5 CR were eliminated. For the GWAS (genome wide association study) analysis, ~443 K SNPs that remained after filtering for the above-said criterion from both the platforms were combined.

2.4. Statistical analysis

Association test results on three major stover quality traits (N, ADF and IVOMD) were corrected for population structure using general linear model (GLM) algorithm as well as population structure + kinship (Q + K) using Mixed Linear Model (MLM) algorithm (Flint-Garcia et al., 2005; Yu and Buckler, 2006). The GLM and MLM analyses were performed using TASSEL (Bradbury et al., 2007) and SVS (http://www.goldenhelix.com/SNP_Variation/).

The mixed model used for estimating the best linear unbiased estimates (BLUE) for the phenotypes is as: $y = XB + Z\mu + e$ where all fixed effects are modeled in the "XB" term, including the population structure (Q). Random effects are modeled in "$Z\mu$" term including

Table 1
Descriptive statistics for agronomic traits in 276 lines from CIMMYT's tropical and subtropical program for drought tolerant maize for Africa (DTMA).

Trait	Mean	Range	P > F
Grain yield (kg/ha)	1486	150–3356	0.0001
Stover yield (kg/ha)	2816	1453–4757	0.08
Days to 50% Anthesis (d)	58.5	52–65	0.0001
Anthesis to Silking Interval (d)	6.2	0.5–18	0.54
Plant height (cm)	129	94–170	0.96

Table 2
Descriptive statistics for forstover quality traits among 276 lines from CIMMYT's tropical and subtropical program for drought tolerant maize for Africa (DTMA).

Trait	Mean	Range	P > F
Nitrogen (%)	1.1	0.5–1.6	0.0001
Neutral detergent fiber (%)	72.5	62.4–77.9	0.0001
Acid detergent fiber (%)	38.5	31.2–45.3	0.0001
Acid detergent lignin (%) n	4.1	3.1–5.4	0.31
In vitro digestibility (%)	52.6	47.9–60.6	0.0001
Metabolisable energy (MJ/kg)	7.6	7.0–8.9	0.0001

Kinship coefficients (K) and "e" is the vector of residual effects. Analysis of variance and trait relationships were estimated using SAS 9.2 (SAS Institute, 2009). Principal component analysis was performed using TASSEL (Bradbury et al., 2007) and the first 10 principal components (PCs) estimated based on the SNP dataset were used to correct the association tests in the GLM analysis.

3. Results

3.1. Variations in agronomic and stover fodder quality traits

Mean and ranges in grain and stover yields, days to 50% anthesis (DtA$_{50}$), anthesis to silking (AtS) interval and plant height (PH) in 276 lines from the DTMA association mapping panel are reported in Table 1. Highly significant differences between lines were found for grain yield and DtA$_{50}$ ($P = 0.08$). Stover yield tended to be different among the lines while differences in AtS and PH were insignificant.

Except for ADL, stover fodder quality traits were highly significantly different among the lines (Table 2). These differences were substantial with stover N varying threefold and NDF, ADF and IVOMD by more than 10 percentage units among the best and the worst lines.

3.2. Relationships between agronomic traits and stover fodder quality traits

Among the agronomic traits, grain and stover yield were highly correlated (Table 3) but the overall relation was weak ($R^2 = 0.08$). Grain yield was significantly negatively associated with DtA$_{50}$ and AtS and positively with PH. Stover yield was significantly negatively associated with DtA$_{50}$ and positively with PH.

Desirable stover quality traits N, IVOMD and ME were significantly negatively associated with grain yield ($R^2 = 0.25$–0.28) while undesirable stover quality traits NDF, ADF and ADL were significantly positively associated with grain yield ($R^2 = 0.04$–0.23). Stover fodder quality traits were largely unrelated to stover yield except for a significant negative association with NDF and ADF ($R^2 = 0.04$–0.08).

3.3. Genome wide association study

Based on GLM and MLM P values, significant candidate associations could be identified and their values along with chromosome and physical positions of the top 10 significant associations are reported in Tables 4–6 for three selected stover fodder quality traits N, ADF and IVOMD.

Table 3

Correlations among agronomic measurements and fodder quality traits determined in 276 lines from CIMMYT's tropical and subtropical programs for DTMA program in post rainy season of 2012.

[a]Traits	GY	SY	DtA$_{50}$	AtS	PH	N	NDF	ADF	ADL	IVOMD	ME
GY	1.0										
SY	0.27[***]	1.0									
DtA$_{50}$	−0.14[*]	−0.28[***]	1.0								
AtS	−0.45[***]	−0.1	−0.22[***]	1.0							
PH	0.49[***]	0.47[***]	−0.36[***]	−0.18[**]	1.0						
N	−0.53[***]	0.06	−0.11	0.42[***]	−0.24[***]	1.0					
NDF	0.36[***]	−0.29[***]	0.03	−0.25[***]	0.02	−0.5[***]	1.0				
ADF	0.48[***]	−0.21[***]	0.1	−0.34[***]	0.25[***]	−0.61[***]	0.75[***]	1.0			
ADL	0.2[***]	−0.04	−0.15[*]	−0.06	0.33[***]	−0.06	0.15[*]	0.43[***]	1.0		
IVOMD	−0.52[***]	0.08	−0.04	0.35[***]	−0.18[*]	0.58[***]	−0.70[***]	−0.67[***]	−0.38[***]	1.0	
ME	−0.50[***]	0.1	−0.04	0.33[***]	−0.17[**]	0.52[***]	−0.69[***]	−0.68[***]	−0.42[***]	0.99[***]	1.0

[a] GY: grain yield; SY: stover yield; DtA$_{50}$: days to 50% anthesis; AtS: anthesis to silking interval; PH: plant height; N: stover nitrogen; NDF: neutral detergent fibre; ADF: acid detergent fiber; ADL: acid detergent lignin; IVOMD: *in vitro* organic matter digestibility; ME: metabolizable energy content.

[*] significance at $P = 0.05$.
[**] significance at $P = 0.01$.
[***] significance at $P = 0.001$.

Table 4

Ten most significant genomic regions identified for stover nitrogen (N) based on genotyping by Sequencing and 55K SNPs (GBS/55k).

SNP	[a]C	P$_{bp}$	P$_{MLM}$	P$_{GLM}$	MAF	AE	R^2 (%)
S8_152142322	8	152142322	3.12E-06	4.01E-06	0.02	0.165	5.51
S3_141296855	3	141296855	3.87E-06	3.75E-06	0.05	0.123	6.05
S7_152778541	7	152778541	6.11E-06	5.69E-07	0.10	−0.068	6.02
S9_148622192	9	148622192	6.70E-06	1.49E-05	0.10	−0.097	4.27
S5_147624511	5	147624511	1.82E-05	1.59E-06	0.19	−0.068	9.11
S1_260621489	1	260621489	2.93E-05	3.19E-05	0.12	0.082	7.63
S3_37360006	3	37360006	3.18E-05	6.42E-05	0.04	0.120	4.69
S8_127258377	8	127258377	3.42E-05	7.17E-06	0.01	−0.172	2.92
S5_13303317	5	13303317	3.67E-05	2.82E-06	0.13	−0.075	5.18
S1_62230813	1	62230813	4.71E-05	1.99E-06	0.50	0.038	4.99

[a] C = chromosome, P$_{bp}$ = position in base pair, P$_{MLM}$ = probability of significant association using Mixed Linear Models, P$_{GLM}$ = probability of significant association using General Linear Model, MAF = minor allele frequency, AE = allele effect.

For stover N, significant associations were observed on chromosomes 1, 3, 5, 8 and 9. The highest percentage of phenotypic variance of 9.1% was explained by the GBS SNP, S5_147624511, located on chromosome 5. The SNP S8_127258377 on chromosome 8 had the highest effect size (average phenotypic difference between two genotype classes of the given marker), but had low minor allele frequency (MAF) of 0.01 (Table 4).

Significant SNP associations were detected for acid digestible fiber (ADF) on chromosomes 3, 4, 5, 6, 7, 8 and 10 (Table 5). Phenotypic variance explained by any individual SNP varied from 4 to 9% and the allele effects ranged from 0.65 to 1.7% for the top 10 significant genomic regions. The 55K SNP SYN7725, on chromosome 4 at 224 Mb explained the largest proportion of phenotypic variance (∼9%) and also had a robust MAF of 0.35.

Significant genomic regions were identified on chromosomes 1,3,4,5, 6 and 9 for IVOMD (Table 6). The phenotypic variance explained by the significant SNPs varied from 3 to 7% and the effects ranged from 0.5 to 1.7%. The genomic regions on chromosomes 4, 5 and 6, had positive effects on IVOMD. The highest magnitude of allelic effect was observed for the SNP on chromosome 3 (S3_149240557, −1.46). Incidentally, this region was also detected for stover N percent (S3_141296855) and ADF (S3_13273100) and may possibly either contain a cluster of genes governing forage quality traits or may be pleiotropic.

4. Discussion

4.1. Stover fodder quality traits and grain-stover relationships

Stover, or more generally fodder quality is ultimately only determined by livestock response in the form of meat, milk production and draught power. Laboratory analysis of fodder quality traits both desirable as well as undesirable ones, are shortcuts to actual quality assessment, as actual livestock productivity trials are often unsuitable and cumbersome for routine evaluations. Further, laboratory fodder quality traits are also helpful in identifying components and plant constituents contributing to overall fodder quality. In fodder rich in structural carbohydrates, such as cereal straws and stover, total structural carbohydrates or plant cell wall (NDF), cellulose content of plant cell wall (ADF) and lignin content (ADL) are key constituents determining overall fodder quality (Van Soest, 1994). Nitrogen is another important essential nutrient for rumen microbes, and low N content is a major constraint in cereal straw and stover feeding (Sundstøl and Owen, 1984). Several studies have indicated the importance of IVOMD in animal productivity with Kristjanson and Zerbini (1999) suggesting that an increase of 1 percentage unit in IVOMD can lead to 6–8% increase in animal productivity. The variations observed in the current study in stover N, NDF, ADF, IVOMD and ME among the lines in the germplasm were statistically and nutritionally significant for livestock (Ravi et al., 2012; Anandan et al., 2012). The germplasm lines in this study showed a wide diversity in stover IVOMD ranging from 47.9 to 60.6% (Table 2), suggesting a diverse pool of lines for use in breeding programs and genetic studies. The ranges in stover fodder quality traits were larger than observed in other maize stover breeding work (Ertiro et al., 2013; Zaidi et al., 2012). Further, in contrast to their findings, this study revealed a significant inverse association between stover fodder quality traits and grain yield (Table 2). These negative associations reported might be the result of very large ranges in grain yields (150–3356 kg/ha) which could have resulted in some outlier-type relationships or may be due to the pleiotropic effects. However, further investigations needs to be done to decipher these findings.

Table 5
Ten most significant genomic regions identified for acid detergent fiber (ADF), based on Genotyping by Sequencing and 55K SNPs (GBS/55k).

SNP	[a]C	P_{bp}	P_{MLM}	P_{GLM}	MAF	AE	R^2 (%)
S3_10514106	3	10514106	2.63E-06	4.20E-06	0.09	−1.69	5.13
S10_76950155	10	76950155	8.33E-06	5.80E-07	0.29	0.78	6.94
S8_173321133	8	173321133	1.61E-05	3.84E-07	0.08	−1.24	5.84
S3_211534263	3	211534263	1.64E-05	1.52E-06	0.35	0.76	7.23
SYN7725	4	224955107	3.14E-05	2.89E-06	0.35	0.72	8.95
S7_10554620	7	10554620	4.09E-05	7.82E-06	0.37	0.69	4.67
S5_23723532	5	23723532	5.54E-05	7.77E-06	0.08	−1.25	5.58
S10_27541681	10	27541681	6.72E-05	5.84E-06	0.04	−1.51	5.31
S6_105845244	6	105845244	7.03E-05	6.82E-06	0.13	1.18	4.03
S3_13273100	3	13273100	1.14E-04	3.72E-06	0.29	0.65	4.62

[a] C = chromosome, P_{bp} = position in base pair, P_{MLM} = probability of significant association using Mixed Linear Models, P_{GLM} = probability of significant association using General Linear Model, MAF = minor allele frequency, AE = allele effect.

Table 6
Ten most significant genomic regions identified for IVOMD, based on genotyping by sequencing and 55K SNPs (GBS/55k).

SNP	[a]C	P_{bp}	P_{MLM}	P_{GLM}	MAF	AE	R^2 (%)
S9_152460495	9	152460495	5.16E-06	1.51E-06	0.19	−0.85	4.86
S5_170821008	5	170821008	8.29E-06	8.11E-06	0.18	1.00	2.47
PZE-104000566	4	766291	1.15E-05	2.22E-06	0.23	0.70	4.09
PZE-109054815	9	95130774	1.18E-05	1.73E-05	0.15	−1.14	4.66
PZE-106084436	6	141591405	1.55E-05	3.31E-06	0.16	0.78	5.00
S3_149240557	3	149240557	2.69E-05	2.56E-06	0.10	−1.46	2.47
S1_164645015	1	164645015	2.87E-05	8.88E-06	0.23	−0.64	6.79
S5_23574731	5	23574731	2.99E-05	6.77E-05	0.08	−1.45	3.01
PZE-104036129	4	47369264	5.18E-05	2.57E-05	0.20	−0.67	3.90
S1_21387831	1	21387831	6.82E-05	5.70E-05	0.45	−0.52	3.71

[a] C = chromosome, P_{bp} = position in base pair, P_{MLM} = probability of significant association using Mixed Linear Models, P_{GLM} = probability of significant association using General Linear Model, MAF = minor allele frequency, AE = allele effect.

The structural carbohydrate associated traits (NDF, ADF, ADL) are often undesirable, while N content is a desirable fodder quality trait. IVOMD and ME content are two favorable fodder characteristics that, in a way, reflect the cumulative actions and interactions of these (and more) plant constituents when incubated in an artificial (i.e. *in vitro*) rumen environment. Some of the traits, such as NDF, ADF and ADL, will be interrelated particularly within a crop or forage species (Van Soest, 1994). In fact, in the present work, stover NDF and ADF were highly inter-correlated and both were negatively correlated with IVOMD and ME (R^2 = 0.48–0.56 Table 3). These strong negative associations between desirable and undesirable components suggest proximity of genomic regions governing these traits. If validated, this association could be extremely beneficial in breeding perspective, as close proximity between these regions would indicate high linkage disequilibrium and would result in co-segregation of positive and negative alleles of desirable and undesirable traits respectively. A similar association trend was also observed between stover N, and NDF and ADF, however the magnitude of association was less strong (R^2 = 0.25–0.37) (Table 3). Different genomic regions should, therefore, be identifiable, for traits such as N and ADF.

4.2. Genome association studies and fodder quality traits

Genome-wide association studies (GWAS) are fast becoming standard tools for rapidly uncovering of marker-trait associations in many crop species, where generation of high density marker information is feasible and affordable. The compilation of a reasonably diverse and large panel of lines evaluated in the same environment without major adaptation differences is a key requirement for the success of GWAS analysis. We evaluated a large panel of 276, developed at CIMMYT that have wide adaptation in tropical and sub-tropical regions for a range of forage and agronomic traits. GWAS analyses were carried out for the forage quality traits. Linkage disequilibrium (LD) is a measure of non-random association of alleles at two or more loci. Crude estimations of genome-wide LD in a given association panel helps in the determination of possible resolution of causal locus or loci. Higher the LD decay, better is the mapping resolution. In high density marker data, genome-wide LD estimates are generally obtained by studying the recombination rates between adjacent markers. The average adjacent pair-wise LD estimate was 0.24 (R^2, calculated by Expectation Maximization method) in the panel and the average adjacent pair-wise physical distance was 4.5 kb, based on 443 K SNPs. The low LD estimate (0.24) in this panel indicates the diverse nature of tropical germplasm, which potentially can lead to higher mapping resolution in GWAS studies. The extent of population structure in an association panel could be inferred using principal component analysis (PCA). The first 10 principal components (PCs) in the PCA analysis based on all the SNPs, explained close to 35% of the genotypic variation and accordingly the association tests were corrected using the first 10 PCs in the GLM analysis.

In general, the effects of the top 10 associations varied from 0.04 to 0.17% for stover N, while the R^2 (phenotypic variance explained by the SNP) varied from 2.9 to 9.1%. The highest percentage of variation explained by the identified region on Chromosome 5 (147.6 Mb) was in close proximity to a previously identified QTL (at 180 Mb) for crude protein in maize under elevated nitrogen conditions (Xie et al., 2009). Similarly, another QTL on chromosome 8 for crude protein content at ~115 Mb reported, was closer to the region identified in the present GWAS study at 127 Mb. However, these regions had cumulative negative effects on stover N content. Another putative region on chromosome 8 in proximity to the other identified region with desirable positive effect on stover N was also identified in this study at 152 Mb. This warrants further investigation and validation of this region on chromosome 8, on a panel of bi-parental population.

For ADF, SYN7725 from the 55K chip on chromosome 4 at 224 Mb, explained the largest proportion of phenotypic variance (~9%) and also had robust MAF of 0.35. This region co-localizes to a QTL (at bin 4.10, 240 Mb) previously identified with ADF content for maize stalks (Krakowsky et al., 2005). A QTL identified

on chromosome 10 for ADF on leaf sheath tissue (10.03 bin; npi105 at ~13–14 Mb) (Krakowsky et al., 2006) also co-localized to the SNP S10_27541681 detected in the present GWAS analysis explaining 5.3% of phenotypic variance. This region is in proximity to a cellulose-related candidate gene *Xyloglucan endotransglucosylase/hydrolase* (*xth1*, GRMZM2G119783) identified based on the maize genome annotation track (www.maizesequence.org), that has previously been demonstrated to have significant role in fibre elongation in cotton (Shao et al., 2011).

Roussel et al. (2002) reported a number of QTLs for *in vitro* NDF digestibility (IVNDFD) on a set of recombinant inbred line populations derived from (F288 × F271); Two of the regions identified on bin 1.02 (18.6–26.9 Mb) and on 6.06 (146.6–149.8 Mb) coincided with the results of the present investigation (S1_21387831, PZE-106084436). Similarly, (Barriere et al., 2008) detected two QTLs for cell wall digestibility on bin 1.07 (196.2–198 Mb) and 4.05 (39.6–44.1), which overlapped with the genomic regions identified (S1_164645015, PZE-104036129) in this study for IVOMD, thereby lending credence to the GWAS results. Incidentally, the two SNP's on chromosome 4 and 6 had favorable effect on the IVOMD content and explained a large portion (>5%) of the phenotypic variation.

The genomic regions identified for the three fodder quality traits were mostly different with two notable exceptions. A genomic region on chromosome 3, between 132.7 and 147.2 Mb was found to be significantly associated with all the three traits, thereby indicating its potential use in marker assisted introgressions in breeding programs that are aimed at improving maize germplasm specifically for these fodder quality traits. However, the cumulative effect of these traits in the current panel of test cross studied contributed unfavorably. Similarly, another region on chromosome 9, between 148.6 and 152.4 Mb was found to be significantly associated with IVOMD and N, however the effect was negative. Further studies will need to be carried out to gain a better understanding of whether a cluster of genes with related functions or pleiotropy is responsible for such an effect and its utility in a breeding program would need to be further assessed in a bi-parental population.

5. Conclusion

This study revealed several putative regions contributing substantially to fodder quality traits. While GWAS enables rapid identification of putative genomic regions for a given phenotype, it can also generate several spurious associations, especially when sample sizes are too small. The preliminary genomic leads identified in the present study would serve the purpose of an ensemble of regions contributing to fodder quality, and can therefore serve as a starting point for further validation through independent and/or bi-parental populations in future. Upon validation these SNPs will also enable carrying out a large-scale allele mining study for fodder quality associated genomic regions in tropical and sub-tropical maize germplasm.

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