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Association analysis of intragenic molecular markers related to fiber quality and tensile strength of abaca (*Musa textilis* Nee)

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Key words: Genome association and prediction integrated tool (GAPIT), Hierfstat, intragenic molecular markers, *Musa textilis* Nee, population structure.

Abstract: Abaca are leaf-fiber plants found predominantly in the Philippines. Our country holds most of the Manila hemp market, but the unknown genetic architecture of the fiber hinders the crop's improvement. We developed intragenic molecular markers from genes related to fiber development and linked them to abaca fiber quality, with the goal of increasing precision of breeding. Pearson's correlation package of the R programming software revealed a high positive relationship between the pseudostem's top and middle girth (r=0.91), while a low negative correlation between the percent fiber percent fiber strain and the number of suckers (r= -0.42). The analysis also showed that the ultimate tensile strength was highly correlated with percent fiber percent fiber strain (r=0.33) and dry weight (r=0.34). Three subpopulations were determined using the STRUCTURE software, while Hierfstat computed an average 0.0648 F_{st} value, indicating moderate genetic diversity. Eight significant marker-trait associations (p-value <0.005) were identified with positive effects and >0.6% phenotypic variance explained (PVE). Eight markers from the COBRA-like protein, expansin, cellulose synthase, and auxin gene families were identified as linked to fiber quality and tensile strength. Our study identified nine abaca accessions with the trait of interest and the candidate genes. The significant molecular markers will be used to identify the hybrids with good fiber quality.

1. Introduction

There are eight widely known plant fibers, sisal, coir, cotton, flax,

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hemp, jute, ramie, and lastly, abaca (*M. textilis* Nee) (FAO, 2009). The latter is an herbaceous perennial *Musa* highly praised for its mechanical strength and industrial applications. In contrast to its close relative banana, this *Musa* species has thinner, glossier pseudostems, and instead of fruits, the succulent stems are the main commodity from abaca. These stems are harvested and processed into fibers internationally known as Manila hemp. There are multiple applications for plant fibers, but the boom of synthetic fiber industries slowly changed the demand for natural fibers (Bemiller, 2007). Nevertheless, natural fibers are sought after because of their sustainability and inexpensive production cost compared to their synthetic counterparts (Radhakrishnan, 2014). Abaca belongs to the leaf or cordage fibers. Their pseudostems are composed of multicelled fibers. These cells provide strength and mechanical resistance to the abaca fibers. The strands have weak bonds and can be easily separated through scraping. Cellulose and hemicellulose content are critical to the tensile strength and quality of fiber crops. The increased cellulose content indicates higher tensile strength. Meanwhile, increased hemicellulose, tends to decrease the mechanical potential of the fibers by lowering their tensile strength due to unstable monomers. Therefore, those plant fibers with high mechanical properties are chosen for structural builds (Djafari Petroudy, 2017). Still, at the molecular level, other factors can affect the cell wall biosynthesis and the strength of the fiber cells, especially when exposed to different environmental conditions and stress.

Since its discovery in 1989 by Litt and Luty, SSR markers have been extensively used on many species. The availability of new software and whole genome sequences eased SSR mining, making new SSR studies robust and relevant. This co-dominant marker type is versatile and reliable for evolutionary analysis, genetic diversity, and marker-assisted breeding. It has helped hasten the classic plant breeding works (Victoria *et al*., 2011) and fingerprinted closely related species (Vinarao *et al.*, 2019). These markers can be developed from genic portions, miRNA regions, chloroplast sequences, expressed sequence tags, and whole genome assembly (Victoria *et al.*, 2011; Sagwal *et al.,* 2022). In addition, SSR markers were also used to uphold the breeder's right over the marketed seeds and prevent variety mislabeling in the field (Palumbo and Barcaccia, 2018). There are several published works on the use of SSRs on abaca (Boguero *et al.,* 2016; Yllano *et al.,* 2020; Mendoza *et al.,* 2024). However, these studies are focused on the genetic diversity of the crop rather than the markers' relationship with specific traits. The genomic markers used by Yllano *et al*. (2020) and Boguero *et al*. (2016) are even designed from other *Musa* species that lessens their specificity for association analysis. This study identified the intragenic SSR markers related to tensile strength and fiber quality. We also analyzed phenotype-genotype trait association using candidate gene-based SSR markers and selected abaca accessions that show promising traits. The information can be used in the genomic selection of elite accessions abaca hybrid production.

2. Materials and Methods

Phenotyping

The abaca gene bank composed of 73 accessions collected from 11 administrative regions of the Philippines, was used in the study (Suppl. Materials Table 1S). A total of 56% of this collection was planted in 2020, while the remaining 44% was established in 2016. The pseudostem were harvested when the flag leaf emerged. The number of suckers (S) were counted from each hill while and the leaf sheaths (LS) were counted from the harvested pseudostems. The circumference of the base of the crown leaf crown (GT), the middle girth (GM), and the base (GB) of the pseudostem was measured in centimeters. The stem length (SL) was measured from end to end of the pseudostem. The fresh weight (FW) was measured in kilograms, while the fiber dry weight (DW) was obtained in grams using an analytical balance after air drying. The tensile strength and percent fiber strain were obtained using the Shimadzu AGX series tensile strength machine. The ultimate tensile strength (*UTS*) was computed using the formula:

$$UTS = F/A$$

Where: *F* is the force applied and *A* the fiber cross sectional area.

DNA extraction and polymerase chain reaction

A total of 34 genes related to fiber development and quality were obtained from the list of transcripts identified by Reamillo (2018) and other related studies (Table 1). The identified sequences were

Table 1 - Profile of the intragenic molecular markers that were used for association mapping of fiber traits in Abaca (*M. textilis* Nee)

Marker	Motif	Annealing temperature	Product size	Functional identity
51	AGAGAGAG	59.9	309	Cellulose Synthase-like protein D2
52	TATATATA	59.95	307	Cellulose Synthase-like protein D2
53	TCTTCTTCT	60.15	306	Cellulose Synthase-like protein D2
54	TCTCTC	60.35	301	Cellulose Synthase-like protein D2
55	GAGAGA	60.25	302	Cellulose Synthase-like protein D5
56	AGGAGGAGGAGGAGGAGG	59.45	201	Cellulose Synthase-like protein E6
57	TCTCTC	59.55	301	Cellulose Synthase-like protein D2
58	TCTCTC	59.45	405	cobra-like protein 4
59	CGTCGTCGT	59.8	203	Expansin A2
60	CAACAACAA	60.9	205	Expansin A10
61	TTCTTCTTCTTCTTCTTCTTCTTC	58.9	306	Expansin A10
62	TCTCTCTC	59.95	336	Expansin A10
63	GCTGCTGCTGCT	59.6	403	Expansin A4
64	AGAGAG	59.2	268	Auxin response factor 9
65	TCTCTCTC	59.7	407	Auxin-responsive protein IAA6-like
66	CTCTCT	59.5	309	Auxin responsive IAA30 like protein
67	GTCGTCGTC	59.85	316	Auxin-induced protein 22D
68	GAGAGA	59.7	302	putative protein auxin response 4
69	CCTCCTCCTCCTCCT	59.8	201	AP2_like_ethylene_responsive_transcription_factor_AIL5
70	CTCCTCCTC	60.4	207	AP2-like ethylene-responsive transcription factor AIL5
71	AGAGAGAG	58.9	323	Ethylene insensitive 3-like protein
72	AGAGAG	59.7	313	probable_ethylene_response_sensor_1
73	AAGAAGAAGAAGAAGAAGAAG	59.9	224	glycolipid_transfer_protein_3
74	AGAGAGAG	60.15	331	Cellulose Synthase-like protein D2_5'UTR
75	TATATATA	60	322	Cellulose Synthase-like protein D2_5'UTR
76	TCTTCTTCT	60.1	246	Auxin_response_factor_9_5'UTR
77	TCTCTC	60.6	323	auxin-responsive protein IAA6-like_5'UTR
78	GAGAGA	59.3	244	Ethylene insensitive 3-like protein_5'UTR
79	AGGAGGAGGAGGAGGAGG	59.1	301	Ethylene insensitive 3-like protein_5'UTR
80	TCTCTC	58.9	306	Expansin A10
81	TCTCTC	58.7	201	auxin-responsive protein IAA30-like
82	CGTCGTCGT	58.5	215	putative protein auxin response 4
83	CAACAACAA	59.9	224	AP2-like ethylene-responsive transcription factor AIL5
84	TTCTTCTTCTTCTTCTTCTTC	58.7	112	glycolipid_transfer_protein_3

scanned from the NCBI website (https://www.ncbi.nlm.nih.gov/). The BLASTN command line version was used to determine the percent identity of the sequences and high bit score with the whole genome sequence of *Musa textilis* Nee cv. Abuab (Galvez *et al.,* 2021). Only those that passed the modified BLASTN threshold (e-value = 0.005, high identity and bit score) were scanned for tandem repeats using the microsatellite finder (http://insilico.ehu.es/mini_tools/microsatellites/). The molecular markers were generated from the 250 bp upstream and downstream of the tandem repeats using the primer3 website (https://primer3.ut.ee/). The parameters used in the development of the primers were: 18-27 primer size, 57°C to 63°C primer Tm, -10.0 to 110 primers bound %, and 20%-80% percent GC content.

The Doyle and Doyle (1990) CTAB DNA extraction protocol, modified by Sandoval (2011), was used to isolate the DNA. The 10 µL PCR reaction comprised of 1x PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs and primers (forward and reverse), and a 0.05 U/µL *Taq* polymerase cocktail. The running conditions for each reaction were 94°C for 4 minutes, then 35 cycles at

94°C for 30 s, 58-60°C for 30 s, 72°C for 45 s, and a final extension at 72°C for 10 min. The amplified bands were visualized using 6% polyacrylamide gel stained using GelRed and photodocumented in the GenoSens photo-documentation system. The amplified polymorphic bands were scored using the GelAnalyzer v.23.1 software (Lazar and Lazar, 2023).

Data analysis

Pearson's correlation between the observed traits was computed in the R software. STRUCTURE software v.2.3.4 (Pritchard *et al.,* 2000) generated the population structure and the q matrix used for association mapping. Each K was run over in ten replications in a burn-in period of 20,000 with 50,000 Monte Carlo Markov Chain (MCMC) replicates. The best grouping was determined using the R package Pophelper v.2.3.1 (Francis, 2017) (https://github.com/royfrancis/pophelper). The Hierfstat package was used to determine the F_{st} value between the populations. The q matrix generated by the STRUCTURE software was used to analyze the association between markers and traits using the FarmCPU (Fixed and Random Model Circulating Probability Unification) of the GAPIT (genomic association and prediction tool) package (Lipka *et al*., 2012).

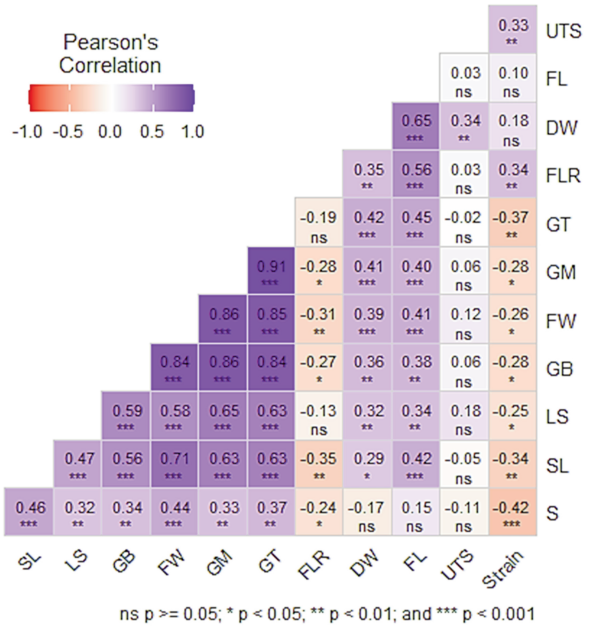

Fig. 1 - Pearson's correlation shows the correlation between the morphological traits of the 73 abaca (*Musa textilis* Nee) accessions. The highest positive correlations were found among the girth measures while the percent fiber strain and fiber length recovery (FLR) exhibited the lowest negative correlations among the traits.

3. Results

Figure 1 shows the results from Pearson's correlation analysis. The highest positive correlation was obtained from the girth measurements of the pseudostem's middle and top sections (r= 0.91). In contrast, the lowest negative correlation (r= -0.42) was obtained between the percent fiber strain and the number of suckers (S). The number of suckers also has a positive correlation with seven out of ten morphological traits. Pseudostem length (SL) correlation with fresh weight (r= 0.71) was its strongest correlation among the other characters. The leaf sheath (LS) shows an average positive correlation of r= 0.61 with girth and fresh weight characters. A strong mean correlation (r= 0.85) was observed among the base girths and fresh weight. Percent fiber strain and fiber length recovery (FLR) are the traits with the most negative correlations with other traits. The percent fiber strain and dry weight (dry weight) are the only traits that have a positive correlation with the ultimate tensile strength at r= 0.33 and r= 0.34, respectively. The correlation shows the trait values that increase together and characters with negative relationships.

Population structure

The F_{st} value indicates the allele frequency distribution between the subpopulations or clusters of abaca. The F_{st} value of >0.25 indicates high genetic differentiation and suggests that the accessions came from different genera or completely different species. The mean F_{st} value obtained from the abaca subpopulation in this study was F_{st} = 0.0648, suggesting moderate genetic variation between the three populations (Li *et al.,* 2014; Luo *et al.,* 2019). The lowest F_{st} value at 0.0229 was obtained between cluster 1 and cluster 2 followed by F_{st}=0.0728 between cluster 1 and 3. The highest F_{st} value of 0.0988 was obtained between cluster 2 and 3. All the computed F_{st} values indicate that the subpopulation are moderately diverse. Most members of cluster 2 are abaca accessions from Mindanao, while members of subpopulation 1 and 3 are from Luzon, particularly

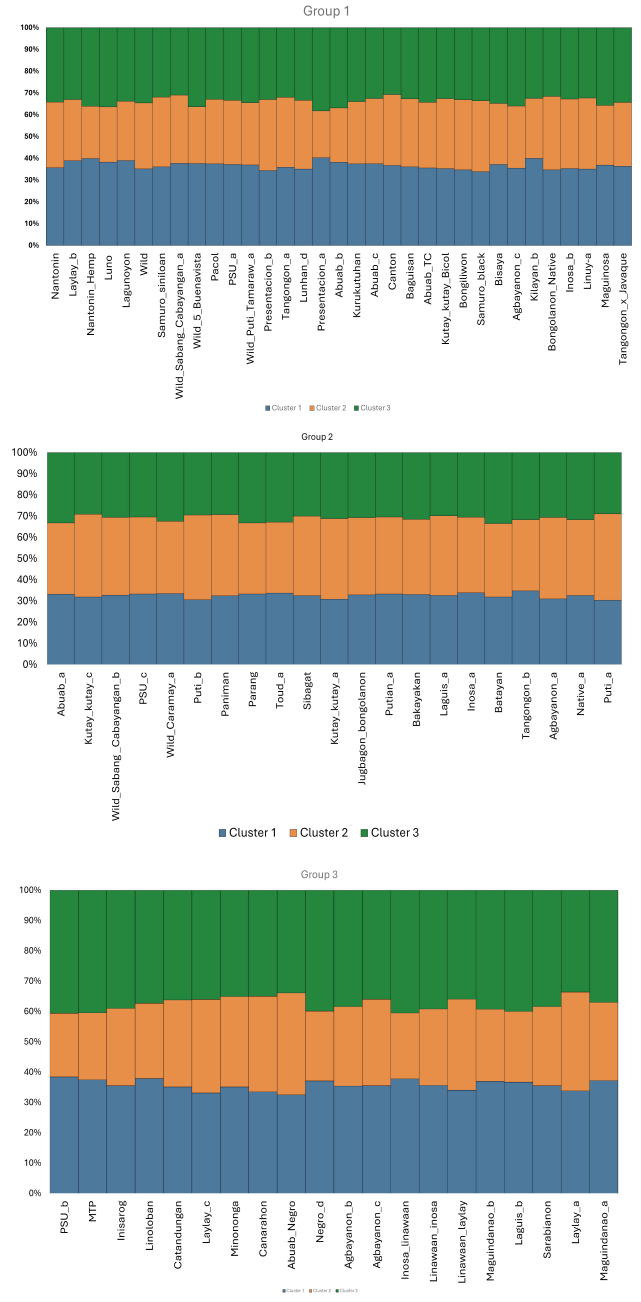

Fig. 2 - The inferred subpopulation assignment of the 73 abaca accessions showing three groupings. Each bar and percent color represent the allelic distribution possessed by each individual in each cluster.

from Region 5 (Fig. 2). The best K clustering was three clusters, the markers used are gene-specific markers hence, a few clusters are expected (Fig. 3).

Association mapping

All the marker-trait associations (MTAs) that are less than the threshold (p-value ≤0.005) were

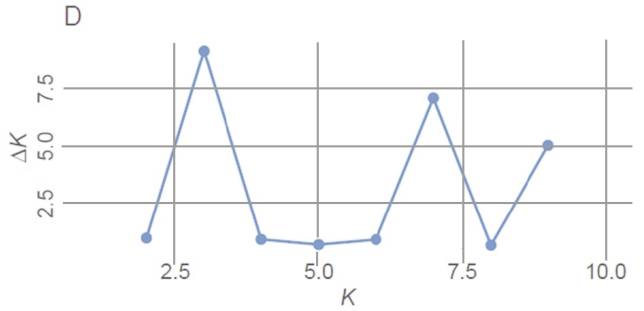

Fig. 3 - Three groupings identified by pophelper package of Rstudio as best k clustering for the 73 abaca accessions. The results for K=3 was then used as Q matrix or kinship in the GAPIT analysis.

considered significant in this study (Fig. 4). Table 2 shows the significant MTAs identified using the FarmCPU model implemented in the GAPIT package of the R software. This model was able to control the false positives effectively by iterative running of the fixed and random effects. The Q-Q plot showed the relationship of the expected and observed p-values for each trait (Fig. 4). The data points that lie beyond the gray area or the 95% confidence interval of the qqplot indicate rejection of the null hypothesis of no association. Eight significant MTAs for five traits were considered for this study. The number of suckers exhibited the highest number of associated markers with no negative effects (MK59, MK58, and MK69). The percent phenotypic variance explained (%PVE) by the markers on the number of suckers ranged from 0.6-57% while its minor allele frequency or MAF value ranges from 0.01-0.06. MK55 and MK63 were significantly associated with the ultimate tensile strength exhibited the highest positive effect among the MTAs. Their explained phenotypic variance was 35.91 and 7.16, respectively. Only one marker (MK55) was strongly associated with the girth size (middle) which explained 42.99% of the observed phenotypic variation. The markers associated with dry weight and percent fiber strain showed the lowest positive effect among the MTA at 0.01 and 0.4, respectively. Overall, by combining the results from phenotyping and the significant marker trait association, accessions that possess genes of interest and exhibits high agronomic trait values are PSU a and b for the number of suckers, Tangongon and Laylay for girth, Native and Parang for dry weight, Kutay-kutay and Native for ultimate tensile strength, and Laylay for percent fiber strain. The team had

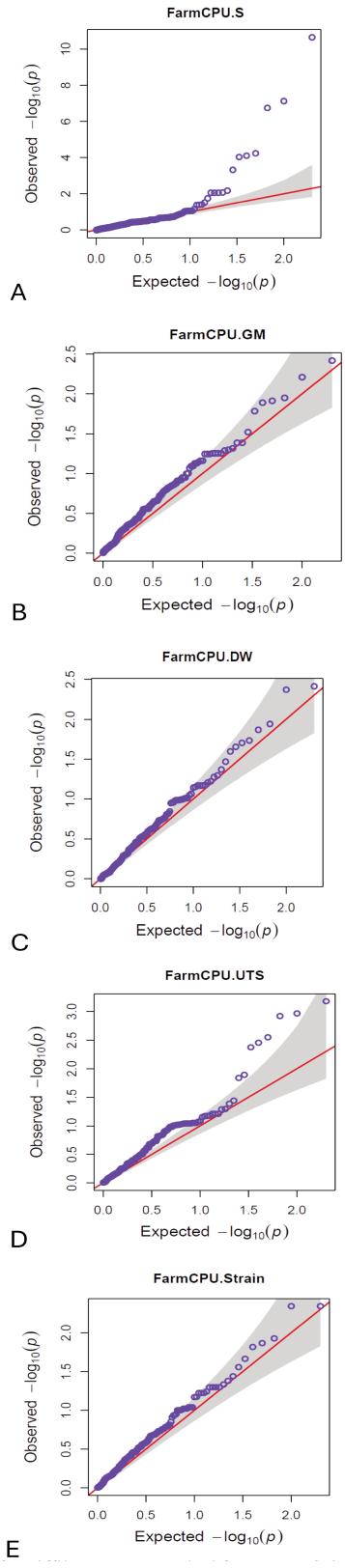

Fig. 4 - The QQ plot generated for each trait using the FarmCPU model. A. suckers, B. Girth (middle), C. Dry weight, D. Ultimate tensile strength, E. Percent fiber strain. The markers that are above the regression line (red line) and beyond the 95% confidence interval (gray area) are significantly related to the traits- of-interest.

Table 2- The significant marker-trait-associations (MTAs) related to the important agromorphological traits of abaca (Musa textilis Nee) determined using the association mapping tool GAPIT

Traits	Marker	Product size (bp)	P. value	Minor allele frequency (MAF)	Effect	%Phenotypic variance explained	Functional identification
Number of suckers	MK59	400	7,81E-05	0.013699	8.735656	0.603816	Expansin A2
Number of suckers	MK58	550	7,46E-08	0.027397	10.50474	7.864861	cobra-like protein 4
Number of suckers	MK69	250	2,25E-11	0.068493	7.862466	57.61	AP2_like_ethylene_responsive_transcription_factor_AIL5
Girth (Middle)	MK55	315	0.003813	0.054795	4.982266	42.99	Cellulose Synthase-like protein D5
Dry weight	MK63	400	0.003858s	0.082192	0.013531	51.2039	Expansin A4
Tensile strength	MK55	390	0.000658	0.082192	263.4273	35.91	Cellulose Synthase-like protein D5
Tensile strength	MK63	400	0.00281	0.08219	222.399	7.16	Auxin response factor 9
Strain	MK66	160	0.004529	0.013699	0.46045	9.89	Auxin responsive IAA30 like protein

produced 49 crosses out of the selected parentals, however only 15 hybrids survived. Applying the markers with high correlation with the traits of interest can hasten the breeding work on abaca. The hybrids that show amplicons for these markers will be considered for advanced trial. The marker aided selection increases the accuracy of crop improvement for abaca and remains a crucial step in breeding.

4. Discussion and Conclusions

Abaca fiber composition

The cell wall thickness was not only the determining factor for tensile strength. Sisal (*Agave sisalana*), a fiber plant species, with 73% cellulose showed a higher tensile strength of 484MPa than jute (*Corchorus capsularis*) with 249MPa and has 65% cellulose content (Alves Fidelis *et al*., 2013). The study of Saragih *et al.* (2020) focused on the thermal analysis of the fibers and their chemical composition. Natural fibers are composed of 66.43% cellulose, 24.7% hemicellulose, and 13.6% lignin. It can be deduced that the amount of cellulose in the fibers is higher than the other two fiber components and contributes much to the strength of the fibers. With their results, they also found the potential of abaca as bioplastic fillers. Different industrial applications require various chemical components for abaca fiber. For instance, the pulping industry would require abaca fibers with low ignin and ash content while having high α-cellulose concentration (Moreno and Protacio, 2012). An amount of >99% cellulose is considered pure. This is achievable through processing but difficult for certain fiber crops like abaca. Only cotton which is composed of 93% cellulose and 0% lignin would require just hot alkali treatment to produce high grade pulps (Bemiller, 2007). The input cost for locally producing cotton is higher than importing them causing a decline in the cotton industry of the Philippines (Asis, 2017). Looking for other pulp sources such as abaca and optimizing the protocol by adding or removing a few steps during production of good pulp can be a solution.

SSR Markers for association mapping

The candidate gene mapping using gene-derived SSR markers have been used in maize (Dubey *et al*., 2009), barley (Matthies *et al.,* 2012), sorghum (Wang *et al*., 2011), bread wheat (Mehta *et al*., 2021), cotton (Buyyarapu *et al*., 2011; Baytar *et al.,* 2022), durum wheat (Alsaleh, 2022), rice (Pritesh *et al*., 2023; Pradhan *et al*., 2023), potato (Schumacher *et al*., 2021), wheat (Singh *et al.*, 2018), flax (Nag *et al.*, 2020) and sugarcane (Divakar *et al*., 2023). These studies used varying sample sizes and different numbers of molecular markers; Divakar *et al*. (2023) even used a set of 70 highly related sugarcane that exhibited moderate genetic diversity. Their number is similar to this study where we used 73 abaca germplasm collection for determining the association of fiber traits with newly developed molecular markers. Nag *et al*. (2020) used only 28 molecular markers on flax to determine significant MTAs with different fiber quality traits. Matthies *et al*. (2012) also used 22 SSR markers to determine important association with barley's kernel quality. The polymorphic nature of SSR markers makes them effective in distinguishing genotypes from the same genome (Lin *et al*., 2012). These show that even with limited sample sizes and molecular markers significant associations can be determined.

The intragenic molecular markers were also found related to different plant architecture traits and grain yield components of rice. Seventeen cgSSR markers with varying percent explained PVE (4% to 17%) were observed to be linked with panicle length and weight, number of primary branches, and grain yield. The associated markers increase the accuracy of rice breeding (Sah *et al*., 2023). Bareto *et al*. (2019) also utilized SSR markers to improve the breeding work and elite genotype selection in sugarcane. A total of 23 significant MTAs were highly associated with not only sugar content but also the crop's plant architecture, including stalk height, weight, and number, and can yield. Further investigation on the MTAs role in traits should be done in future studies. Divakar *et al*. (2023) also looked into the association of 30 EST-SSR markers with yield trait components in 70 sugarcane accessions. The marker SEM407 was found to be highly associated with %Brix, sucrose content, and percent sugar recovery. In fiber crops, a total of 28 SSR markers were used to characterize the genetic structure of the fiber genes of flax. Mixed linear model analysis revealed that Lu10-600 and LU15-300 were observed associated with fiber length while LU7-150 was the lone allele correlated with fiber strength. Four markers (LU5-50, LU7-150, LU10-600, and LU15-300), on the other hand, were observed highly related to fiber yield. These markers

can be utilized to screen flax genotypes with good fiber quality potential for breeding (Nag *et al*., 2020). Qin *et al*. (2015) evaluated the relationship of genic SSR markers with 3 fiber quality traits in cotton. They found that 26 molecular markers were positively correlated with quality and fiber strength, exhibiting most of the association (12 markers). The genome-wide SSR markers were also used in association mapping in cotton. A total of 106 gene markers were determined to be significantly associated with six fiber quality traits, including fiber strength, fiber upper half length, short fiber, micronaire value, and fiber uniformity. The fiber strength had the greatest number of correlated markers (61 markers) in the study of Nie *et al*. (2016). Marker-assisted breeding (MAB) programs are especially challenging when few available markers cover the upland cotton's complex genetic background (Qin *et al*., 2015). With the increase in the identified correlated molecular markers, hybrids were developed from upland cotton, and 52 significant loci were found related to six fiber quality traits from the parents and hybrids (Huang *et al*., 2018) showing the success of gene specific markers in marker assisted breeding.

Auxin

Significant MTAs from the CDS of auxin response factor 9 and auxin-responsive IAA30-like protein were positively correlated with tensile strength and percent fiber strain. Four abaca accessions, namely, Tangongon, Laylay, Kutay-kutay, and Native have showed amplification in these markers and exhibited high girth and tensile strength. Auxin promotes fiber elongation in cotton (Zhu *et al*., 2022). Its accumulation in fiber cells during growth is also responsible for the development of cotton fiber cells (Zhang *et al*., 2017). In a gene expression analysis, auxin was observed to control the activity of reactive oxygen species and secondary wall deposition in fiber cells of cotton (Zhang *et al*., 2020). Buyyarapu *et al.* (2011) used cgSSR markers from auxin to determine its relationship with fiber development in cotton and these markers were found polymorphic for the 26 species of cotton, suggesting their varying number and effect in the cotton genome. The SAUR gene family, also known as Small Auxin-up RNA genes are responsible for the growth of plant tissues at different stages in flax (*L. usitatissimum* L.) The genome-wide analysis of the SAUR genes revealed 86 *Lus*SAUR genes. It was also found that these genes are highly influenced by the application of exogenous IAA. Some of the identified *Lus*SAUR genes were also found to be regulated in the cell wall thickening of the phloem bundle and the salt response of flax (Bao *et al*., 2024). The response of the 15 randomly selected SAUR genes of ramie was also observed under drought and high-temperature conditions. Eight of the *Bn*SAUR genes were regulated by both abiotic stress but mostly downregulated during high temperatures. Most notably, BnSAUR43 showed enhanced expression when subjected to drought conditions. These identified SAUR genes can be used for early detection of auxin-influenced traits in ramie (Huang *et al*., 2016). Various *SAUR* genes are differentially expressed in the leaves of Agave indicating their role in the leaf development of the crop (Deng *et al*., 2019). The studies mentioned revealed not only the possible role of auxin during stress conditions for fiber crops but also auxin's accumulation in the fiber cells meant a better fiber strength. The markers identified in this study can be used later to determine the relationship of abiotic stress with the fiber quality of abaca. The preliminary nature of this study also calls for further studies on expression analysis of auxin.

Cellulose synthase genes

In this study, the molecular marker generated from cellulose synthase-like protein D5 explains 35% of the phenotypic variation in the tensile strength of the abaca accessions. Cellulose is an essential carbohydrate that makes up the plant cell wall. It is a chain typically composed of 36 β-1,4-glucose units synthesized from the *CesA* proteins produced by the cellulose synthase genes. The functions of *CesA* genes were first studied in *Arabidopsis* using mutation. From the study of Beeckman *et al*. (2002), it appears that out of the 10 *CesA* genes, only four - *CESA1, CESA2, CESA3*, and *CESA9* - function in synthesizing primary cell walls in the embryo of *A. thaliana* while *AtCesA 4,7, and* 8 are responsible for secondary cell wall synthesis. The latter set of genes was confirmed to be upregulated in the secondary cell walls of cotton fibers (Betancur *et al*., 2010). Based on differential gene expression analysis, four cellulose synthase genes have been identified in flax, namely LusCESA1, LusCESA3-B, LusCESA4, and LusCESA8-A while ramie has CESA3 and CESA8 (Xie *et al*., 2020). These genes were observed to be upregulated during cell wall biosynthesis in these fiber crops.

Elucidating the history of gene families can result

to better functional analysis for *Musa* species, given the transcriptomic analysis for the crop is limited (Cenci *et al*., 2014). In abaca, only *CesA 7-5* and *CesA 9-7* were upregulated in Abuab while expansin and sucrose synthase genes were upregulated in Pacol. Based on the generated gene expression data, BC_1 resembles Abuab while BC_2 and BC_3 are more like the Pacol parent (Reamillo, 2018). The mutation in *CesA7* was found to show reduced fiber cell wall thickness and cellulose content as well as the absence of secondary cell walls (Maleki *et al*., 2016). This shows the importance of cellulose synthase genes in the strength and thickness of the fibers.

Tinawagang Puti is an abaca variety from the Bicol region and is one of the recommended varieties of PhilFIDA. Increasing its alpha cellulose and hemicellulose content was seen possible through addition of phosphorus sources (Bondad *et al*., 1979); however, the reason behind this physiological response was not fully identified Elucidating the underlying molecular mechanism would provide more valuable information and a more systematic approach to improving such abaca accessions. Because there are few studies on *M. textilis* Nee transcriptomics, focusing on the gene sequences of prominent fiber gene families and their probable phylogenetic relationship with model species will provide a better understanding of the expression control of the plant. In our study, the markers designed from the cellulose synthase genes studied by Reamillo (2018) exhibited an association with the abaca accessions' tensile strength and pseudostem girth size. With these molecular markers, simpler molecular approaches (i.e. PCR amplification) can be applied to identify accessions with the cellulose synthase gene and good fiber quality.

Expansin genes

The expansin markers explained the phenotypic variation of the number of suckers and dry weight by 0.6% and 57%, respectively. Cell walls must be sturdy enough to avoid damage brought by cell enlargement or external force and should also be flexible enough to permit some impact and induce wall relaxation. This ability is known as cell wall loosening. Expansin proteins are released to mediate the cell wall loosening (Sampedro and Cosgrove, 2005). These proteins are also used by grass pollens to soften the maternal cell walls of stigmas (Cosgrove, 2016). They also affect the germination and the fruiting stage of plants. Expansin, like *CesA* and sucrose synthase, is a large gene family composed of four subfamilies, namely, α-expansin, β-expansin, expansin-like A, and expansin-like B (Ding *et al*., 2016). The expansin genes that belonged to the α and β evolved from a common ancestor and shared similar introns. Based on the analysis of rice expansin genes, the promoter possessed responsive elements to hormones (Lee *et al.*, 2001). The relationship of expansin proteins with hormones was also observed in poplar trees (*Populus nigra* L.) and aspen (*Populus tremula* L.). The increase in salt stress caused the decrease of *PtrEXPA3* and *PnEXPA3* activities resulting in decreased size of petioles, leaves, and internodes but increased size of epidermal cells (Kuluev *et al.*, 2017). This shows that expansin proteins are responsible for stress-related events. Phylogenetics provided vital information and association analysis of expansin genes to distantly related species. This also directs researchers to a more systematic approach when manipulating *EXP* genes. Screening genomic libraries and PCR amplification revealed that out of the six expansin genes of cotton (*GhExp1-GhExp6*), *GhExp1* and *GhExp2* are involved in the elongation of the cotton cells during fiber development (Harmer *et al.*, 2002). In bast fibers like flax, expansin was observed to be upregulated during cell wall thickening, indicating its role in fiber cell rearrangement during maturation (Mokshina *et al*., 2020). Expansin genes are upregulated during oxidative and osmotic stresses such as drought and high salt conditions (Gao *et al*., 2018). The genes identified in the abaca accessions were Expansin A2 and A4 and were highly correlated with the number of suckers and dry weight. This indicates that using simple laboratories and computation techniques can link genes to their functions and accessions with the trait of interest.

COBRA-like genes

This protein family is glycosylphosphatidylinositol-chained proteins that modulate the orientation of cell expansion and cellulose crystallinity. The tetraploid *G. hirsutum* has 14-15 COBL genes more than the *G. raimondii* and *G. arboretum*. *GhCOBL9* and *GhCOBL13* were upregulated with cellulose synthase genes during secondary cell wall biosynthesis. Based on QTL analysis, *GhCOBL9* is correlated with fiber quality (Niu *et al.*, 2015). Expression analysis also revealed that *GhCOBL9* was constantly upregulated during secondary cell wall thickening in cotton (He *et al*., 2025). Mechanical resistance to lodging in sorghum (*Sorghum bicolor*)

was observed reduced by only one mutation in *SbBC1* genes that encode COBRA-like proteins. Not only did it affect mechanical strength but also the cellulose content and lignin content. These components decreased and increased, respectively. This suggests that *SbBC1* genes are involved in cell wall biosynthesis in sorghum (Li *et al*., 2019). This protein was found along the sides of *Arabidopsis* roots where there is an increased elongation activity (Schindelman *et al*., 2001). COBRA acts as a "polysaccharide chaperone" that binds to individual β-1,4-linked glucan units resulting in cellulose crystallization (Sorek *et al*., 2014). *In-silico* analysis revealed thirteen glycophosphatidylinositol anchors encoding genes from nine *Hevea* genomes that share similar functions with their *Arabidopsis* counterparts. Out of these thirteen genes, nine clustered in the COBRA gene subfamily-1 while the rest clustered with COBRA gene subfamily-II (Putranto *et al*., 2017). The mutated *SlCOBRA*-like proteins of tomato caused cell wall degradation by upregulating wall-degrading genes while overexpressed *SlCOBRA*-like proteins resulted in the thicker cell wall of fruits. This indicates that COBRA-like proteins participate in cell wall biosynthesis (Cao *et al*., 2012). In flax, their role is vague, but are upregulated during the primary, secondary, and tertiary cell wall formation (Mokshina *et al*., 2020). Based on expression analysis, a similar role was also observed in hemp, where the CBL 4 gene is active during cell wall formation (Koziel, 2010). In poplar trees, the cobra-like genes are involved in cellulose microfibril orientation (Gritsch *et al*., 2015). In the study of Reamillo (2018), COBRA-like proteins were upregulated in Abuab and downregulated in the backcrosses. In our work, only the cobra-like protein 4 was highly correlated with the number of suckers, explaining 7.8% of the phenotypic variation in the abaca collection. This trait showed a negative relationship with tensile strength and can be used to determine the fiber quality even at the juvenile stage of abaca.

This study explores the use of intragenic molecular markers to identify significant MTAs (p-value <0.005) related to fiber quality and tensile strength of abaca. Eight MTAs related to five traits were found to have a positive significant effect of up to 263 and percent phenotypic variance explained ranging from 0.6% to 57%. The molecular markers from the auxin, cellulose synthase, expansin, and COBRA-like gene families related to fiber development were identified in this study. However, it is suggested that these genes should undergo subsequent experiments to solidify their role in the abaca fiber quality and tensile strength. The markers identified in this study can be used to fast-track the crop improvement of abaca, for only the hybrids that possess the gene of interest shall be tested in advanced yield trials. The marker-assisted selection increases the precision of breeding in abaca and lowers agricultural input waste during field testing. With this research, we determined nine abaca accessions that possess both traits and markers of interest and were used to generate 49 seeds. These hybrids will be evaluated using morphological and molecular markers in a future study.

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