

Leaf margin phenotype and the genetic diversity of collected pineapple germplasm: a validation of the P locus on Spiny/Pipe leaf phenotype

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Short Report

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Abstract

Pineapple (*Ananas comosus* (L.) Merr.) is a commercially important tropical fruit, with global production exceeding 28 million metric tons. Breeding programs require the assessment of genetic diversity and trait-specific markers across diverse germplasms. This study evaluated 60 pineapple accessions, including cultivars, mutants, and breeding lines, using 20 SSR markers and investigated the relationship between leaf spine traits and the presence of the *P* locus. The SSR analysis revealed moderate genetic diversity, with an average of 2.65 alleles per locus and a mean polymorphism information content (PIC) of 0.70. Clustering analysis revealed distinct groupings based on genetic background. A strong negative correlation ($\rho = -0.848$, p value $< 2.2e-16$) was observed between the presence of the *P* locus and the leaf spiny phenotype, supported by a significant Wilcoxon rank sum test (p value = $7.667e-11$), which suggested an inverse relationship. These results validate the *P* locus control of the spine/pipe leaf phenotype and demonstrate the utility of SSR markers and the *P* locus in marker-assisted selection, providing insights into the genetic control of spineless leaf traits in pineapple.

Introduction

Pineapple (*Ananas comosus* (L.) Merr.) is one of the most important tropical fruits, which plays a key role in agricultural production worldwide. Pineapple production exceeds 28 million metric tons and expected to reach 32.2 million metric tons by 2026, significantly contributing to the income of countries such as Indonesia, the Philippines, Costa Rica, Thailand, Brazil, and China (Shahbandeh 2025). The economic value of pineapple is not only in fresh use but also in processed products (Li et al. 2022). Under the global climate change and high demand for pineapple quality, the evaluation of genetic diversity and trait exploitation on collected pineapple germplasms, including wild types, cultivars, and breeding lines, is crucial for breeding programs that improve quality and climate adaptation (Wang et al. 2017; Li et al. 2022; Yow et al. 2022; Abike Chalotte Adjé et al. 2024). SSR molecular markers are powerful tools for discriminating the diversity and clustering individuals, which can be used as potential parents in breeding programs (Abdalah et al. 2018; Ismail et al. 2020; Nashima et al. 2020).

One of the major traits of the pineapple is the spine/pipe on leaf margins. The spiny leaves cause problems in growing plants, harvesting, and transporting fruits on big farms. The pineapples with pipe leaves are preferred for cultivation and production. However, spiny/pipe leaf traits are widely diverse in pineapples. This trait varies from leaves of pipe (no spines) to spines on the leaf tip, spines on one marginal side, to spines fully on both sides. Spiny leaves, common in wild varieties, make handling difficult and increase labor costs, while pipe varieties, a result of domestication, are favored for their ease of harvesting (Sanewski 2020; de Lira Júnior et al. 2021).

Recent studies of genes controlling spiny/pipe leaf trait on *P* locus have shown that *AcWOX3* on the chromosome 8 may play a key role in the formation of leaf margin spine beside other modifier genes (Sanewski 2020; Nashima et al. 2022). This study focused on assessing genetic diversity using SSR

markers and exploring the relationship between leaf spine traits and *P* locus, aiming to provide a scientific basis for marker-assisted breeding programs.

Materials and Methods

A collection of pineapples at Southern Horticultural Research Institute (Vietnam) used in this study including 28 cultivars, 9 mutants, 25 breeding line from different parents (Queen x MD2, MD2 x Queen, DL8 x MD2). Leaves from these pineapple plants were collected for leaf margin phenotyping and DNA extraction for SSR and target gene analysis. Leaf spiny/pipe phenotyping by placing a section of the leaf (15 cm from the leaf tip) on a Canon LiDE300 scanner at 300dpi resolution. The score applied based on the presence of leaf spines were 0 = no spines, 1 = leaf tip spines, 2 = spines on one margin side, 3 = full spines on both margin sides (Fig. 1B).

PCR-based SSR markers were performed in a total reaction volume of 15 μ L containing 7.5 μ L Magic PCR Mix 2X with Hot Start Polymerase (Phu Sa Genomics), 10 pmol each forward and reverse primer (Table 1) (Shoda et al. 2013; Ismail et al. 2020), and 10 ng of genomic DNA. Primers for *P* locus were followed by description in Nashima et al. (Nashima et al. 2022). PCR conditions were set at 95⁰C for 5 min, 30 cycles of 95⁰C for 10 sec, 60⁰C for 40s, and final extension at 72⁰C for 2 min. Pig-tail primers were used as Pig-tail primers were used as described by Shoda et al. (Shoda et al. 2013). Genomic DNA was extracted following La et al. (Thang et al. 2020), 20 mg of young leaves ground in 200 μ L NaOH 0,5 M with stainless beads using Bioblock Scientific at 60 Hz 1 min, then adding 350 μ L Tris 1M pH 8.0 and centrifuge at 13000 rpm in 3 min. DNA extracts can be stored at -20⁰C and used for PCR reaction. SSR marker genotyping data analysis was performed using GenAlEx 6.51b2 (Peakall and Smouse 2012). Data visualization, correlation, and Mann–Whitney test on spiny trait and target gene were performed on R software with packages '*ComplexHeatmap*', '*circlize*', '*grid*'.

Results and Discussion

The genetic diversity of 60 pineapple accessions was assessed using 20 polymorphic SSR markers, revealing a mean number of alleles (*N_a*) of 2.65 per locus, with a range from 1.00 (TsuAC035_Pigtail) to 4.00 (TsuAC023_Pigtail) (Table 1). Observed heterozygosity (*H_o*) averaged 0.31 (0.00–0.83), while expected heterozygosity (*H_e*) was 0.74 (0.45–0.91), indicating moderate genetic diversity. The polymorphism information content (PIC) averaged 0.70 (0.26–0.91), with Acom9.9 and TsuAC004_Pigtail showing the highest values (0.89 and 0.91, respectively). Shannon's information index (*I*) averaged 1.22 (range: 0.64–1.63), reflecting variability across loci. These genetic parameters indicate that pineapple accessions have moderate to high diversity. A cluster dendrogram based on SSR data (Fig. 1) illustrated the genetic relationships among accessions, with clear clusters influenced by genetic background. Pineapple accessions revealed the presence of a *P* locus that causes the pipe leaf trait, except in Thom_Trung Quoc, which needs to be further validated. Moreover, the *P* locus presence in the breeding lines and the leaf spine/pipe phenotype were both closely linked in a cause – effect manner. The parents carrying the *P* locus and pipe leaf phenotype produced a hybrid that also carried the *P* locus

and leaf spineless (DL08 x MD2) (Fig. 1A). Similarly, the hybrids from the Queen x MD2 or MD2 x Queen crosses all expressed the genetic motif of leaf spine/pipe trait and *P* locus following their parental leaf phenotype (Fig. 1A).

Statistical analysis showed a strong negative Spearman's rank correlation ($\rho = -0.848$, $p < 2.2e-16$) between the spiny leaf trait and *P* locus, suggesting an inverse relationship. The Wilcoxon rank sum test further confirmed a significant difference in spiny leaf trait distribution between groups with and without the *P* locus ($W = 790$, $p = 7.667e-11$), indicating distinct phenotypic patterns.

The SSR analysis demonstrated moderate genetic diversity in the pineapple germplasm, consistent with previous studies (Chen et al. 2019; Ismail et al. 2020; Nashima et al. 2020), with high PIC values (> 0.7) at loci like Acom9.9 and TsuAC004_Pigtail indicating their utility for marker-assisted selection. The dendrogram (Fig. 1) likely reflects clustering by genetic type, such as Cultivars, Mutants, and Breeding lines, supporting the role of SSRs in assessing population structure (Ming et al. 2015). The low H_o compared to H_e suggests strong selection of hybrid lines for pineapple cultivation. The strong negative correlation ($\rho = -0.848$) between spiny leaf trait and *P* locus, coupled with the significant Wilcoxon test result ($p = 7.667e-11$), indicates that more leaf spines are strongly associated with the presence of *P* locus, and *vice versa*. This evidence was confirmed on MD2_WT (wild type) vs MD2_Mutant (mutated MD2), where MD2_Mutant lost its *P* locus, causing spiny leaves compared to the pipe leaf of MD2 wild type. This inverse relationship may reflect pleiotropic effects or tightly linkage between loci controlling spine density and presence *P* locus, possibly involving AcWOX3 or QTLs on chromosome 23 (Sanewski 2020; Nashima et al. 2022). However, the unexpected distribution suggests environmental or epigenetic influences, as noted in prior studies (Sanewski et al., 2019). These findings highlight the need for genome-wide association studies to figure out causal variants, enhancing breeding strategies for spineless cultivars of pineapples.

Conclusion

In summary, twenty SSR markers were used to assess genetic diversity of 60 accessions of pineapples showed high diversity in the germplasm. The expected heterozygosity (H_e) is higher than the observed heterozygosity (H_o) suggesting that the pineapple population is highly hybrid. The *P* locus was tightly associated with spine/pipe leaf phenotype and the high genetic diversity of the pineapple population may imply the use of the *P* locus as an effective molecular marker for selection pineapple varieties with or without leaf spine in breeding programs.

Declarations

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Author contributions

Bui Thanh Liem conceived and designed the project, analyzed the data, and wrote the original draft. Nguyen Nhat Truong and Luong Bao Duy performed the experiments. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

Data Availability

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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Tables

Table 1
Diversity information parameters on the 20 polymorphic SSR markers

SSR locus	Na	Ho	He	I	PIC
Acom9.9	3.00	0.13	0.89	1.28	0.89
Acom22.22	3.00	0.58	0.69	1.44	0.65
Acom39.5	3.00	0.60	0.61	1.43	0.53
Acom68.3	2.00	0.25	0.74	0.96	0.74
Acom82.8	2.00	0.00	0.85	0.85	0.85
APCT136B	2.00	0.27	0.83	0.97	0.82
TsuAC004	3.00	0.50	0.60	1.49	0.51
TsuAC008	3.00	0.83	0.64	1.32	0.59
TsuAC010	3.00	0.28	0.81	1.38	0.80
TsuAC013	2.00	0.13	0.76	0.77	0.76
TsuAC018	3.00	0.08	0.45	1.56	0.26
TsuAC023	2.00	0.20	0.86	0.86	0.86
TsuAC030	3.00	0.23	0.63	1.55	0.55
TsuAC035	3.00	0.38	0.76	1.33	0.74
TsuAC004_Pigtail	3.00	0.02	0.91	1.22	0.91
TsuAC008_Pigtail	2.00	0.05	0.83	0.90	0.83
TsuAC010_PigTail	3.00	0.47	0.81	1.43	0.79
TsuAC018_PigTail	3.00	0.42	0.78	1.28	0.77
TsuAC023_PigTail	4.00	0.80	0.54	1.63	0.43
TsuAC035_PigTail	1.00	0.00	0.77	0.64	0.77
Mean	2.65	0.31	0.74	1.22	0.70
Notes: Na = Number of alleles; Ho = observed heterozygosity; He = expected					

Heterozygosity, I = Shannon's information index, and PIC = polymorphic information content.

Figures

of *P* locus leads to the reappearance of leaf spines in the MD2 mutant, Queen is the most common cultivar with fully spiny leaves used as reference.