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Changes in key volatile components associated with leaf quality of *Pandanus amaryllifolius* Roxb. alongside growth duration

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ABSTRACT

Pandan (*Pandanus amaryllifolius* Roxb.) are one of the traditional food materials in Southeast Asian countries. However, there has long been a lack of understanding of the differences in volatile organic compounds (VOCs) of leaves at different growth periods. Gas chromatography-mass spectrometry (GC–MS) was used to identify and analyze VOCs in different leaf positions of Pandan in this study. The content of 2-Acetyl-1-pyrroline (2AP) was higher in L1-L3 and decreased with leaf growth, while squalene showed the opposite trend. The content of neophytadiene first increased and then decreased, reaching the highest in L15. L8 is the critical point at which the content of each major VOCs is balanced. Combining agronomic traits and VOCs content, leaves in L4-L25 position are suitable for harvesting. This study provides data support for scientific judgment of the harvesting site and time of Pandan, and provides theoretical basis for further utilization of VOCs of Pandan.

1. Introduction

Spice crops play an important role in advancing human historical progress and still play a significant role in the spice and food industries at the present time (Abeywickrema et al., 2022; Chen et al., 2023; Łyczko et al., 2023), Among numerous spice plants, a perennial tropical herbaceous spice plant with a unique aroma of fragrant rice which named Pandan (*Pandanus amaryllifolius* Roxb.) was known as the "Oriental Vanilla". As a traditional food, Pandan's fresh leaves have been consumed by people in Southeast Asian countries for a long time (Arshimny & Syamsu, 2020). The leaves are the main utilization site of Pandan. It has extremely high economic value and development prospects. Currently, the harvesting standard for Pandan primarily requires leaves to exceed 40 cm in length, with harvested leaves subsequently mixed for further processing. However, leaves at different positions on the same plant are at various growth stages and exhibit significant differences in physical properties, nutrient accumulation, and the types and contents of volatile organic compounds. The single harvesting standard and prolonged mixed collection have resulted in inconsistent quality of Pandan.

Agronomic traits are important indicators reflecting crop growth rate and productivity. They can also significantly affect yield and quality. For example, the water content and chlorophyll content of leaves represent the actual growth and development status of plants (Song et al., 2021), and are important indicators reflecting the dynamics of ecological systems such as plant photosynthesis, nutritional status, and growth trends. Chlorophyll and leaf water content can affect photosynthetic rates (Wang et al., 2022), thereby influencing the growth and development of leaves and the accumulation of dry matter, ultimately leading to differences in the quality of Pandan. Therefore, monitoring the agronomic traits at different growth stages can master the growth status of Pandan, which is conducive to clarify the harvest time of Pandan.

The volatile organic compounds (VOCs) of Pandan are closely tied to its physiological state, playing a vital role in its development, utilization, and product quality. Major VOCs include furans, pyrroles, terpenes, alcohols, alkenes, and esters (Jiang, 1999), with 2-Acetyl-1-pyrroline (2AP) being the key characteristic compound (Wakte et al., 2010). Known for its "glutinous rice" aroma, 2AP enhances appetite and

Abbreviations: 2AP, 2-Acetyl-1-pyrroline.

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metabolism and is also found in perfume coconut (Saensuk et al., 2016), pumpkin (Junxing et al., 2022), and fragrant rice (Dutta et al., 2022). Preliminary studies reveal that Pandan contains over ten times more 2AP than fragrant rice(Routray & Rayaguru, 2010), making it a promising raw material for 2AP extraction. Pandan leaves are also rich in bioactive compounds like squalene and phytol (Lomthong et al., 2022). Squalene exhibits antioxidant, bactericidal, and oxygen-transport properties, enhancing cellular vitality, immunity, and metabolism while reducing fatigue (Du et al., 2024; Feng et al., 2022). Neophytadiene, the source of Pandan's "fresh aroma," also imparts characteristic flavors to tobacco and cigars (Banožić et al., 2021; Wen et al., 2023a, 2023b) and has anti-anxiety and sedative effects (Gonzalez-Rivera et al., 2023). Additionally, phytol, with its bactericidal and vitality-enhancing properties, supports tocopherol (vitamin E) synthesis (Duraisamy et al., 2024; Valentin et al., 2006). These active compounds highlight Pandan's potential in medical (Widyaningsih et al., 2022) and cosmetic (Ambarwati et al., 2021) applications.

In summary, this study aims to clarify the quality differences of Pandan leaves at different growth stages. The differences in the types and contents of VOCs and agronomic traits in different leaf positions of the whole plant were analyzed through field experiments. The findings provide data support for scientifically judging the harvesting positions and timing of Pandan, and provide a theoretical basis for the cultivation management, quality control and development and utilization of VOCs.

2. Materials and methods

2.1. Experimental sites

The field experiment was established in the Planting Resource Nursery of the Institute of Spices and Beverages, Chinese Academy of Tropical Agricultural Sciences, Wanning, Hainan Province (100°13′E, 18°15′N). The experimental region belonged to the tropical monsoon climate. The annual mean temperature, precipitation and sunshine hours were 22 °C, 2100–2200 mm and 1750–2650 h, respectively. The soil was tidal sand–mud (US Soil Taxonomy classification). The soil pH and organic matter content were 6.00 and 20.04 $g\cdot kg^{-1}$, respectively.

2.2. Experimental materials

The leaves of Pandan are generally harvested after 10 months of growth. In this study, healthy, disease-free, and pest-free plants were selected as test samples. These plants had been transplanted and grown in the field for one year. Leaf samples were cut at the base of the leaves and immediately transferred to the laboratory in March 2023 for determination.

2.3. Experiment design

Randomly select three Pandan plants with the same growth period and vigor. Single factor experiment was set up to investigate the different levels of agronomic traits and VOCs in different leaf positions of Pandan under the same treatment. All plants had more than 25 leaves in Pandan, so based on the botanical and morphological characteristics of Pandan, the leaves of the plant were arranged in the order of leaf position from the tip to the base of the plant, labeled in order as L1-L25 in this study (Fig. 1). The management (e.g., fertilization, irrigation, shading, etc.) of all the Pandan plants prior to sample collection was consistent.

2.4. Sample collection

The Pandan samples were pre-treated immediately after being brought back to the laboratory. Firstly, all the leaves of each Pandan plant were carefully removed at the base and labeled according to their respective leaf positions. The surface of the leaves were then cleaned of dirt and dust using gauze and distilled water. After cleaning, The leaves were left to stand for 5 min at an ambient room temperature (22 ± 3 °C).

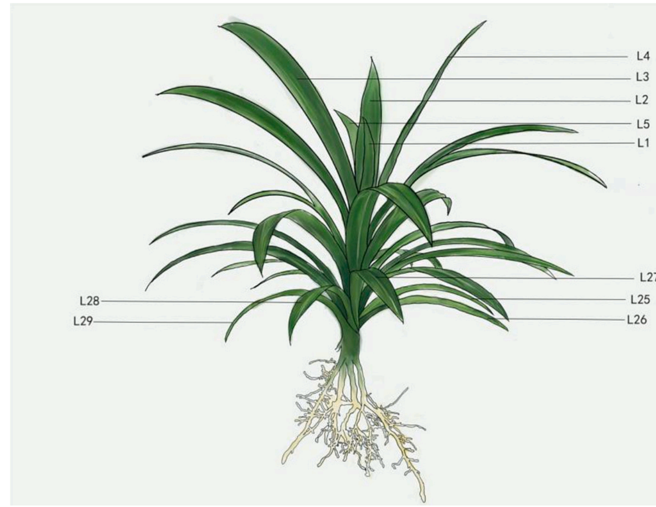

Fig. 1. Structural pattern diagram of Pandan.

2.5. Determination of agronomic and photosynthetic traits in leaves of Pandan

Leaf fresh weight was immediately determined using a one-thousandth electronic balance (Mettler-Toledo Instruments Shanghai Co., Ltd.), after sample were pretreatment.

Leaf length, leaf width, and leaf area were measured using a leaf area measuring instrument (YMJ-B, Zhejiang Top Yunnong Technology Co., Ltd.). Repeat three times for each leaf position.

The chlorophyll content was measured by chlorophyll measuring instrument (Zhejiang Top Yunnong Technology Co., Ltd.). This study used the measured SPAD (Soil and Plant Analyzer Development) values as a quantitative indicator of chlorophyll content. The SPAD values of the upper 1/3, the middle and the lower 1/3 of the leaves of each leaf were determined respectively, and the average value was taken as the SPAD value of each blade.

After the determination of the above indexes was completed, placed in the oven and subjected to fixation at 105 °C for 30 min. Then the oven temperature was lowered to 75 °C to dry for 24 h to a constant weight, and the dry weight of the leaves was measured by using one-thousandth of the electronic balance (Mettler Toledo Instruments Shanghai Co., Ltd.). Repeat three times for each leaf position.

The photosynthetic traits of the leaves were measured using a Li-6400XT portable photosynthesis system, including the net photosynthesis (P_n), transpiration (T_r), stomatal conductance (G_s).

The measurement started at 9:00 am on a sunny day, and the upper surface of the top 1/2 to 1/3 of the leaf was measured. Each leaf position was measured three times in a row and the average value was taken. And water use efficiency (WUE) was calculated using the formula:

$$WUE = P_n / T_r \quad (1)$$

2.6. Metabolomics profiling

2.6.1. Sample preparation

The extraction method was described by Zhang et al. (2023). Then the VOCs of the extracts were determined using gas chromatography-mass (GC–MS) spectrometry (Aglient-7890B/5977B GC–MS, Agilent, USA).

2.6.2. Chromatographic conditions

DB-WAX (30 m × 0.25 mm × 0.25 μm) flexible quartz capillary column was used. The inlet temperature was set to 250 °C, with an initial temperature of 50 °C held for 2 min. The temperature was then increased to 100 °C at 5 °C·min^{-1}, followed by a further increase to 240 °C at 6 °C·min^{-1}, and held for 5 min. The carrier gas used was high-purity helium (99.999 %), with a flow rate of 1 mL·min^{-1}, and the injection was performed without split.

2.6.3. Mass spectrometry conditions

The ion source was operated in EI mode with an ionization energy of 70 eV. The ion source temperature was set to 230 °C, the quadrupole temperature to 150 °C, and the inlet temperature to 250 °C. The scanning mass range was 30–450 amu.

2.6.4. Process of GC–MS data

Qualitative analysis: Combined with mass spectrometry and retention index (RI) to qualify the aroma components of Pandan, in which the results of mass spectrometry were searched in the NIST 2017 spectral library, compared with the qualitative results; using the C7-C40 n-alkanes mixing standard (Shanghai Anspectrum Experimental Science and Technology Co. Ltd.), with the same conditions of the GC–MS analysis, the use of its retention time in accordance with the linear equation to calculate the retention index of VOCs of the retention index of the RI, the retention index calculated by the actual measured with the literature to carry out the qualitative analysis.

Quantitative analysis: The squalene standard (GC ≥ 98 %, Shanghai Yuanye Biotechnology Co.) and Methanol (chromatographically pure) were precisely aspirated and configured in accordance with the concentration gradient of 50, 100, 150, 300 and 500 μg·mL^{-1}. According to the chromatographic conditions of the samples, the samples were injected in the order of the gradient concentration of the standard from low to high, and the samples were injected three times for each concentration, and the gradient concentration of each component was taken as the horizontal coordinate, and the average of the peak areas of the three measurements was taken as the vertical coordinate to get the quantitative linear relationship of the components. The quantitative linear relationship was obtained. The content of squalene was calculated according to the linear equation using the peak area. Other substances were semi-quantified according to the squalene standard curve.

$$\text{Calculation formula}: X_i = (A_i/A_s) \times C_s \tag{2}$$

(X_i is the content of the substance to be measured; A_i is the peak area of the substance to be measured; A_s is the peak area of squalene; C_s is the squalene content in the sample).

2.7. Data analysis

One-way ANOVA was used to analyze the differences in content of co-contained VOCs and agronomic traits among different leaf positions by SAS V8. All data were tested for homogeneity of variances and conformed to normal distribution before analysis. Data analysis was performed by using the Duncan test, the difference between mean values was determined by using the least significant difference (LSD, $P < 0.05$) as indicated by different letters. The correlations between the main VOCs content and agronomic traits were determined by Spearman's correlation analysis (SPSS 23.0, SPSS Inc., Chicago, USA). Correlation analysis plots, histograms, and cluster analyses were plotted using Origin 2021. The co-occurrence network interaction between VOCs and agronomic and photosynthetic traits was analyzed and painted by Cytoscape V3.8.2.

3. Results

3.1. Differences in the types and contents of VOCs at different leaf positions of Pandan

A total of twenty-nine VOCs were detected in twenty-five leaf positions of Pandan, which belonged to nine categories of pyrroles, hydrocarbons, furans, furanone, alcohols, acids, ketones, esters, and phenols, respectively. There are significant differences in both the quantity and composition of VOCs among leaves from different positions of Pandan (Fig. 2a). The leaf VOCs of L1-L25 consisted of 16–25 organic compounds, of which the VOCs of L1 consisted of 8 types of compounds, and the VOCs of all other leaf positions consisted of nine types of compounds.

The content of VOCs varied greatly among different leaf positions, and the total VOCs of L1-L25 showed a gradual increase, which could be visualized as a significant change in the content of pyrroles, furanones and hydrocarbons. The content of pyrroles and furanones showed a gradual decrease, compared with L1, the content of pyrroles in L25 decreased by 51.26 %, and the content of furanones decreased by 60.27 %; the content of hydrocarbons showed a significant increase, and the content of hydrocarbons in L1 was only 4.75 % of that in L25(Fig. 2b).

Cluster analysis of the VOCs of the different leaf positions of Pandan was carried out, and the differences in VOCs between the different leaf positions could be visualized through the colors. Based on the cluster analysis of VOCs, the 25 leaf positions were divided into two categories, L1 and L2 as one category, and L3-L25 as the other category. The L3-L25 category was divided into two subcategories (L3-L8 and L9-L25), which showed that the leaves clustered into one category were all from similar parts of the Pandan plant (Fig. 2c).

3.2. Differences in the contents of characteristic VOCs in different leaf positions of Pandan

The 2AP content in the leaves of Pandan showed a decreasing trend with plant growth and development, and the 2AP content of L1-L6 was significantly higher than that of L9-L25 (Fig. 3a, $p < 0.05$), and the 2AP content of the leaves of L9-L25 were below the mean and remained at a low level. The squalene content of L11-L25 was higher than the average and significantly higher than that of L1-L7 ($p < 0.05$), and the squalene content of L25 at the base of the plant was as high as 5427.33 ± 987.27 μg·g^{-1} with the growth of leaves, which was 33.92-fold increase compared with that of L1(Fig. 3b); The content of neophytadiene showed a trend of first increasing and then decreasing (Fig. 3c). The content of neophytadiene in the leaves from the top to the middle of the plant increased significantly, reaching a peak at L15, with a content of 613.53 ± 125.08 μg·g^{-1}, and then began to decrease significantly. The neophytadiene contents in the leaves of L7-L20 were all higher than the average and significantly higher than those of L1-L2 at the top of the plant and L24-L25 at the base of the plant ($p < 0.05$, Appendix Table A2). The phytol content of leaves from L11 to the base of the plant was stable above the average value (Fig. 3d). There was no significant difference in phytol content between leaves in different leaf positions of Pandan (Appendix Table A3).

Note: The dashed markers in the graph are the mean values of 2AP content (a) Squalene content (b) Neophytadiene content (c) Phytol content (d) at 25 leaf positions.

3.3. The correlation between VOCs content and agronomic and photosynthetic traits in different leaf positions of Pandan

In the co-occurrence network of the absolute content of different categories of VOCs and agronomic and photosynthetic traits, the values of average connectivity (avgK), average clustering coefficient (avgCC), average path length (APL) and graph density were 4.63, 0.37, 2.72 and 0.16 (Table 1). The absolute contents of different categories of VOCs in the co-occurrence network diagram showed more positive co-occurrence relationships with the agronomic and photosynthetic traits. It is worth noting that furanones and pyrroles showed more negative co-occurrence relationships with the agronomic and photosynthetic traits. But there are more negative co-occurrence relationships between water content and the absolute contents of different categories of VOCs

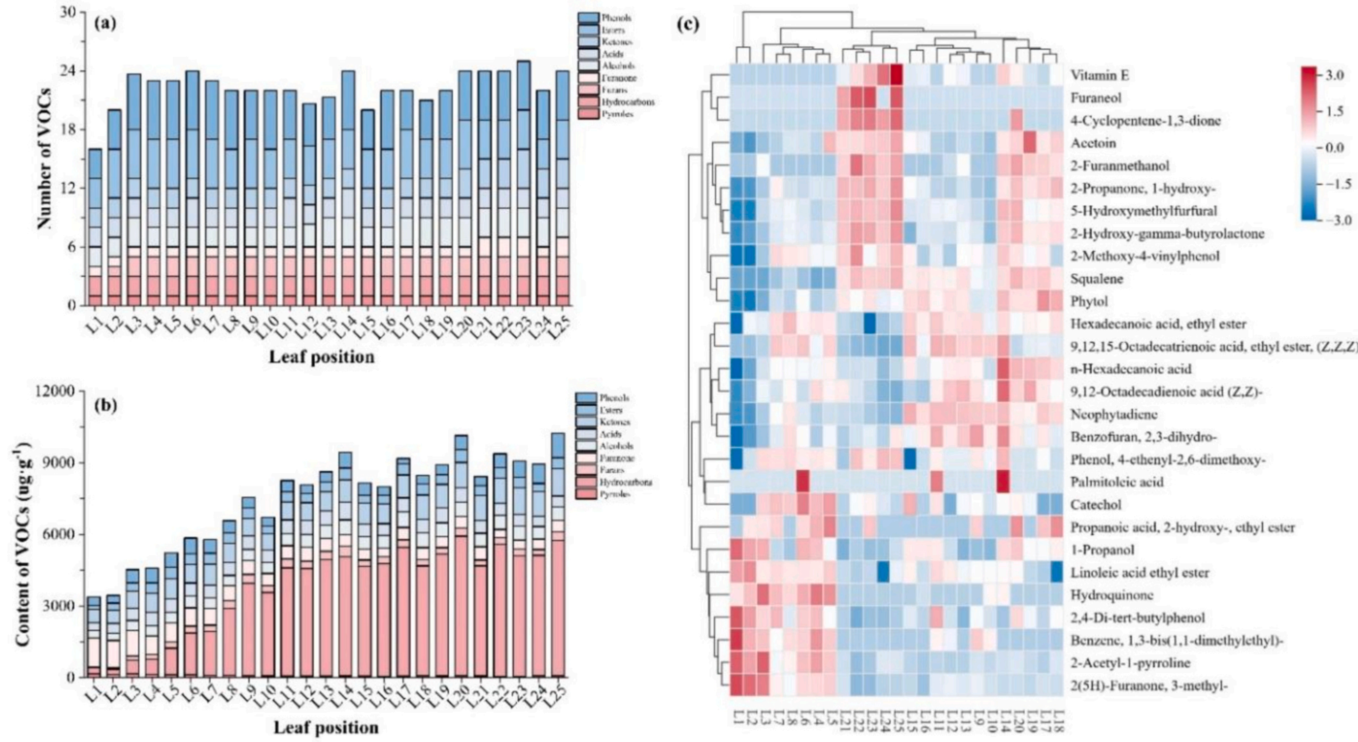

Fig. 2. Comparison of the number of categories (a), absolute content (b) and clustering heat map analysis (c) of VOCs in different leaf positions of Pandan.

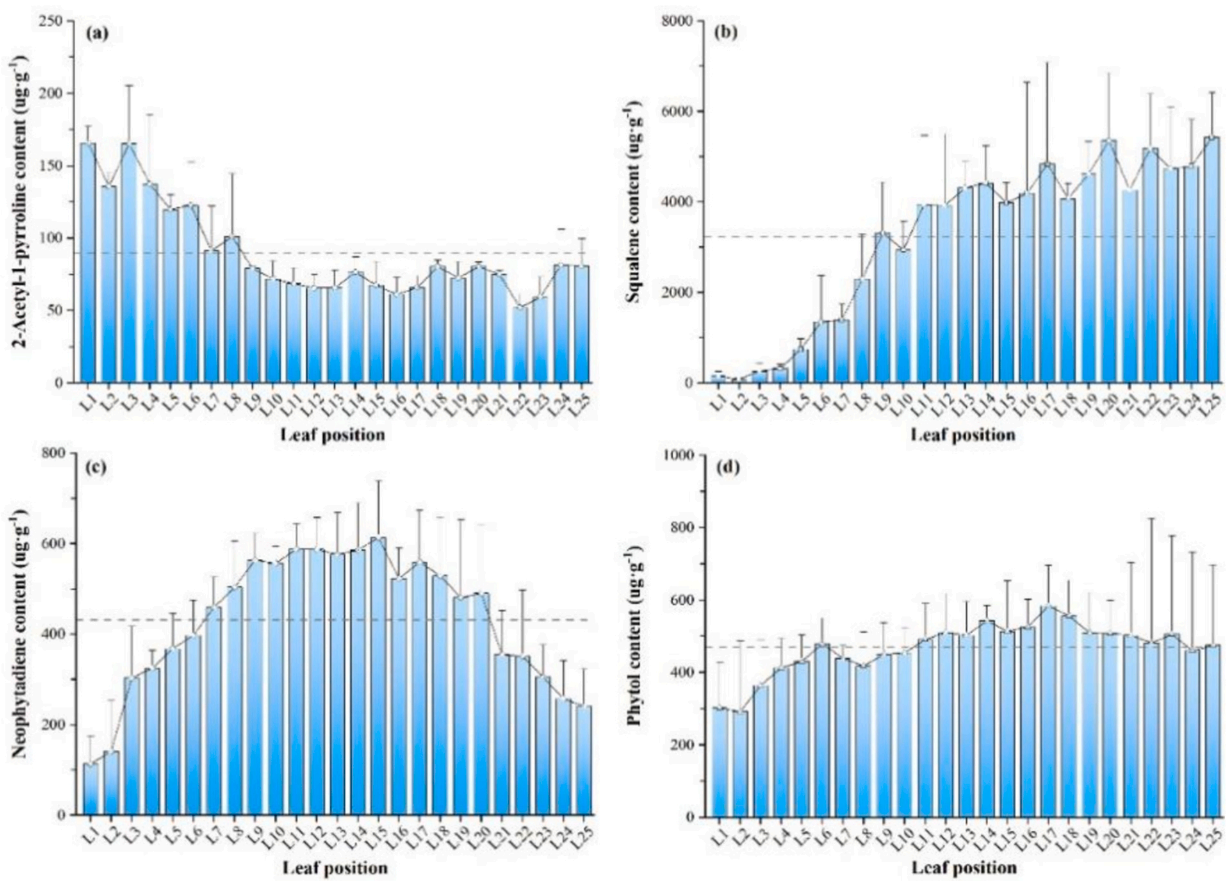

Fig. 3. Comparison of characteristic VOCs in leaves of different leaf positions of Pandan.

Table 1
Topological properties of the co-occurrence network of contents of different categories of VOCs and agronomic, photosynthetic traits.

Network metrics	The co-occurrence network relationship between the content of different categories of VOCs and agronomic, photosynthetic traits
Number of Nodes	17
Number of Edges	106
Number of Positive Correlations	61
Number of Negative Correlations	45
Percentage of the Positive Link (P%)	57.55 %
P% from agronomic and photosynthetic traits to VOCs of Pandan	56.60 %
P% among VOCs of Pandan	66.67 %
P% among agronomic and photosynthetic traits of Pandan	52.00 %
Average Connectivity (avgK)	4.63
Average Clustering Coefficient (avgCC)	0.37
Average Path Length (APL)	2.72
Graph Density	0.16

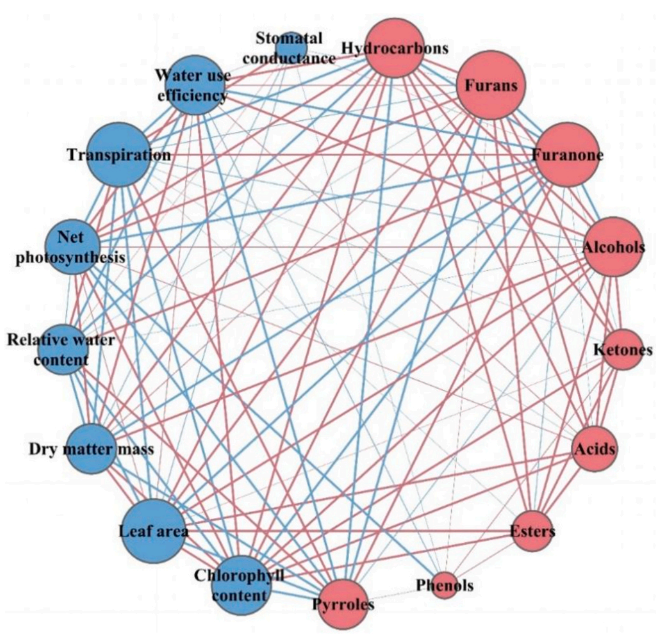

Fig. 4. Analysis of co-occurrence network relationships between absolute content of different categories of VOCs and agronomic and photosynthetic traits. (Red lines indicate positive correlations, while blue lines indicate negative correlations. The thickness of the lines represents the correlation size. The size of the points represents the magnitude of absolute contents of different categories of VOCs.) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 4).

3.4. The correlation between agronomic and photosynthetic traits and characteristic VOCs of Pandan

Correlation analysis between the four characteristic VOCs of Pandan and agronomic traits showed that 2AP content was highly significantly positively correlated with relative moisture content ($r = 0.88$, $P < 0.01$), significantly positively correlated with T_r, and an extremely significant negative correlation with dry matter mass, chlorophyll content, leaf area, P_n and WUE ($P < 0.01$), the correlation levels reached −0.79, −0.93, −0.61, −0.71 and − 0.63 respectively; Contrary to the correlation shown by 2AP, squalene has a highly significant positive correlation with dry matter mass ($r = 0.64$, P < 0.01), chlorophyll content ($r = 0.87$, P < 0.01), P_n ($r = 0.81$, P < 0.01) and a significant positive correlation with leaf area ($r = 0.42$, $P < 0.05$), it highly significantly negatively correlated with relative water content ($r = -0.93$, P < 0.01) and significantly negatively correlated with T_r ($r = -0.49$, P < 0.05). Phytol, neophytadiene and squalene showed almost the same correlation with agronomic and photosynthetic traits (Fig. 5).

4. Discussion

4.1. Differences in agronomic traits and photosynthetic physiology at different leaf positions in Pandan

At the outset of this study, variations in agronomic traits and photosynthetic physiological indicators of Pandan at different leaf positions were first observed. Chlorophyll content increased initially and peaked at L15-L20 (Appendix Fig. A1a), indicating optimal physiological function. Photosynthetic variation followed a similar trend (Appendix Fig. A1a, Appendix Table A2). Then decreased, reflecting its role in photosynthesis. During senescence, chloroplast loss and membrane disruption lead to pigment degradation (Gao et al., 2024; Gorfer et al., 2022). However, reduced photosynthesis at the leaf base suggests inhibited physiological activity during senescence, despite stable chlorophyll levels, which is supported by decreased dry matter content (Appendix Fig. A1b).

As Pandan plants grow, leaf dry matter mass increases rapidly in the L1-L3 stages due to vigorous photosynthesis and then peaks at L7, reflecting a balance in physiological activities and stabilized substance synthesis and migration (Appendix Fig. A1b). Leaf water content decreases from top to base, with tender leaves containing more free water for metabolism and older leaves retaining bound water for structural stability (Appendix Fig. A1c). Leaf transpiration decreases with senescence, consistent with this trend (Appendix Fig. A1f). During the L1-L3 stage, leaves are smaller (Appendix Fig. A1d) with lower chlorophyll content and dry matter mass, not meeting traditional harvest standards. In contrast, L15-L20 leaves exhibit optimal water use efficiency and photosynthetic productivity (Appendix Fig. A1g, Appendix Table A2). Therefore, if only the biomass of Pandan is considered, harvesting leaves below the L7 stage is recommended.

4.2. Differences in the categories and content of VOCs of Pandan among different leaf positions

The categories and content of VOCs are important indicators for measuring the quality of pandan. This study used GC–MS technology to detect VOCs in the leaves of Pandan at different leaf positions. The VOCs of Pandan in this study are composed of nine categories of compounds: pyrroles, furanones, hydrocarbons, furans, ketones, alcohols, esters, phenols and acids (Fig. 2a), which is basically consistent with the results of previous studies (Zhang et al., 2023). However, the quantity and type composition of VOCs in various leaf positions of Pandan are quite different may be related to the different growth duration of the leaves. As the leaves grow and mature, the content of VOCs also changes significantly, which is consistent with the findings in tobacco leaves (Wen et al., 2023a, 2023b).

The leaf area, chlorophyll content, and transpiration of Pandan are the main influencing factors regulating VOCs synthesis in this study. Chlorophyll content and leaf area may stimulate the synthesis and accumulation of VOCs, while increased transpiration adversely affects VOC accumulation, likely due to the significant loss of partially water-soluble VOCs. Moreover, the positive effects of photosynthesis and water use efficiency on VOCs accumulation cannot be ignored. A similar study found that the recovery of photosynthesis in northern evergreen tree leaves during spring aligns with the rapid synthesis of volatile substances such as monoterpenes. This indicates that the synthesis of volatile substances plays a protective functional role for the foliage

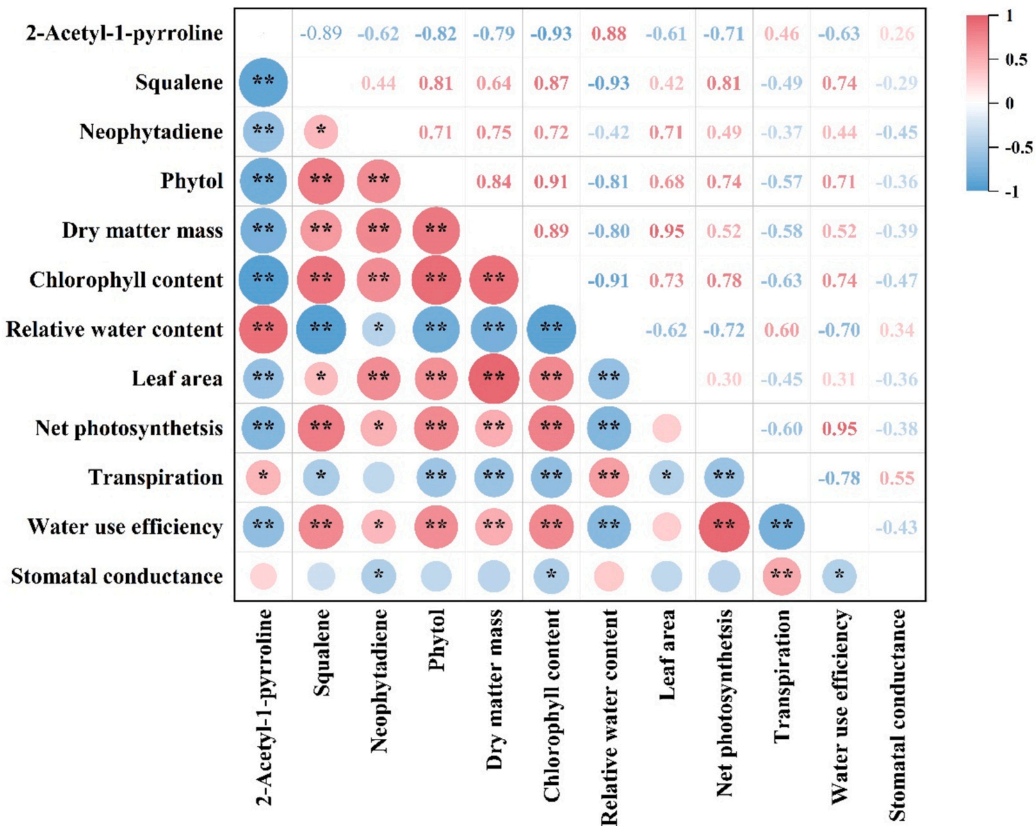

Fig. 5. Correlation analysis between characteristic VOCs and agronomic and photosynthetic traits. (Red indicates a positive correlation between the two parameters. Blue indicates a negative correlation between the two parameters. The numbers in each cell represent correlation coefficients. *, * * indicates differ significantly at the P < 0.05 and P < 0.01 levels, respectively.) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

during a critical transitional state (Aalto et al., 2015). A higher proportion of positive correlation was found between the agronomic traits and photosynthetic indicators of Pandan and the VOCs components in this study, indicating that the improvement of physiological indicators during Pandan's growth process contributes to the synthesis and accumulation of VOCs. Similarly, the higher proportion of positive correlation between different categories of VOCs in Pandan leaves indicates that the synergistic effect between the synthesis and accumulation of VOCs in Pandan leaves during aging is significantly higher than the antagonistic effect.

Specifically, the content of pyrroles and furanones gradually decreased, while the content of hydrocarbons increased significantly (Fig. 2b), in current study may owing to various factors such as leaf physiological functions, metabolic pathways, and environmental adaptability (Liaotrakoon et al., 2021). It is also possible that as leaves grow, the content of hydrocarbons such as squalene with antioxidant activity increases to regulate the aging process of plants (Feng et al., 2022). The furanones and pyrroles exhibit negative co-occurrence relationships with other categories of VOCs and agronomic traits, while showing positive co-occurrence relationships with each other and with leaf water content. This may indicate that agronomic traits have similar effects on the absolute content of furanones and pyrroles. Leaf water content shows negative co-occurrence relationships with the absolute content of furanones, alcohols, acids, ketones, hydrocarbons, phenols, and esters, suggesting that the content of these VOCs increases with decreasing leaf water content.

4.3. Effects of different leaf positions on the characteristic VOCs synthesis of Pandan

The organic compounds in Pandan play a crucial role in the quality evaluation of Pandan because they have a significant impact on the aroma of Pandan and its functions in health care and other aspects. Therefore, the tendency of characteristic VOCs such as 2AP, squalene, neophytadiene and phytol in different positions of Pandan leaves and their correlation with agronomic traits were analyzed in this study to clarify the dynamic changes in key VOCs during its growth and development process (Fig. 3).

As one of the main VOCs of Pandan's distinctive aroma, the 2AP content could serve as one of the main quality indicators of Pandan. A gradually decreasing trend of the 2AP during the growth and development of Pandan plants indicates that the synthesis or accumulation of 2AP may be inhibited during the growth of plants, or reduced due to competition from other metabolic pathways (Zhao et al., 2022). Studies in fragrant rice have shown that water and temperature regulation can significantly affect the biosynthesis of 2AP, low temperature increased 2AP content and high temperature decreased 2AP content (Luo et al., 2024). Alternate wetting and drying can affect the accumulation of precursors for the synthesis of 2AP, the activity of related enzymes and the expression of related genes by regulating moisture, thereby affecting the biosynthesis of 2AP rather than the yield of Pandan (Bao et al., 2018). 2AP content is positively correlated with transpiration and relative water content, while negatively correlated with photosynthesis, dry matter mass, chlorophyll content and leaf area in current study confirm that the synthesis or accumulation of 2AP is closely related to the water status, growth stage of the plant and the degradation of chlorophyll. The increase of leaf water content may significantly promote leaf physiological activity and accelerate the synthesis rate of 2AP in Pandan, thus, increasing irrigation volume may promote the biosynthesis rate of 2AP, but the specific molecular mechanism needs further research. However, it seems that Pandan needs to maintain a balance between the synthesis of 2AP and photosynthetic products, and the accumulation of photosynthetic products may have a negative impact on the synthesis of 2AP during the growth process of Pandan in current study.

Squalene has several biological activities, including enhancing the immunity, resisting skin senility, and exerting hypolipidemic, antioxidant, antitumor, antibacterial, and detoxification activities (Cheng et al., 2024). Traditional squalene is extracted from olive oil and seeds of pseudograin *Amaranthus* sp. (Popa et al., 2015). However, as a tropical herbaceous plant which is rich in squalene, the faster growth rate of Pandan provides the possibility of obtaining squalene at a lower cost. As an important intermediate of cholesterol/phytosterol biosynthesis in animal and plant organisms, the synthesis of squalene is similar in all organisms, although the enzymes implicated in its formation can have different properties (Popa et al., 2015). Therefore, the content of squalene is positively correlated with dry matter mass and chlorophyll content, and negatively correlated with relative water content, which is contrary to 2AP (Fig. 5). This may be attributed to differences in enzyme activity in Pandan leaves at different growth stages rather than differences in synthesis mechanisms. The content of squalene shows an increasing trend during the growth and development of Pandan in current study. The content of squalene in L11-L25 is significantly higher than that in L1-L7, which means that squalene may play an important role in the growth process of the plant, especially in the mature stage of leaves, participating in the metabolism and stress resistance of leaf cells. Therefore, it is feasible to increase the amount of irrigation or moderate drought stress to change the physiological and biochemical status of Pandan, thereby changing the water content of leaves and promoting the synthesis of squalene (Amnan et al., 2022a; Amnan et al., 2023). This result also provides data support for regulating the synthesis of characteristic VOCs by adjusting agricultural management measures (Amnan et al., 2022b; Gui et al., 2022; Li et al., 2016).

Previous studies have shown that neophytadiene is a diterpenoid compound with antibacterial, anti-inflammatory, analgesic, antipyretic, antioxidant activities, anti-anxiety, and anti-convulsant properties (Gonzalez-Rivera et al., 2023), and provides a refreshing flavor to Pandan. It was observed that tobacco leaves exhibit a high level of accumulation throughout leaf senescence (Wen et al., 2023a, 2023b), because the increased accumulation of these substances might protect plants against herbivores and pathogen attacks at late developmental stages of tobacco (Ovali, 2024). However, the content of neophytadiene in Pandan showed a trend of first increasing and then decreasing, despite this trend may reflect the synthesis and degradation process of neophytadiene during the growth and development of Pandan. The scientific hypothesis that causes this phenomenon may be divided into two parts: On the one hand, the significant increase in structural carbon, such as lignin and cellulose, can strengthen the support for plant structure and resist external environmental pressure and pathogen invasion during the senescence process of Pandan leaves, thus reduce the synthesis of neophytadiene; On the other hand, studies have shown that neophytadiene will undergo thermal decomposition under high-temperature aerobic conditions (Ovali, 2024), while Pandan generally grows in tropical lowland rainforest areas, and long-term high temperatures may accelerate the decomposition of neophytadiene. Nevertheless, the physiological mechanism of neophytadiene synthesis and degradation during the growth and development of Pandan requires further research.

Phytol is widely present in plant leaves, which has the function of insecticidal, bactericidal, and antioxidant. As a component of chlorophyll, its content may also be related to the anabolism of chlorophyll (Durrett & Welti, 2021). The significant positive correlation between phytol and chlorophyll content in this study confirms the above results. Although there was no difference in phytol content between different leaf positions (Fig. 3d), this may indicate that the synthesis and utilization of phytol in Pandan leaves remain stable and are not affected by growth stage or leaf position.

4.4. Harvesting recommendations for the different leaf positions of Pandan

2AP and squalene are the main organic compounds influencing the aroma and nutritional value of Pandan. The content of 2AP gradually decreases from the top to the base of the plant, decreasing below the average from L7-L9; meanwhile, the content of squalene increases rapidly, rising above the average from L7-L9. Therefore, this study preliminarily suggests the balance point of these two compounds is at L8. If the target quality trait is 2AP, it is recommended to harvest the tender leaves from L1-L8. If squalene is the target, it is suggested to harvest the older leaves from L9-L25.

The relative water content, dry matter mass, chlorophyll content, leaf area and the VOCs have all undergone changes during the growth and development of Pandan that reflect the physiological state and growth patterns of the leaves. Meanwhile, it was found that there is a close correlation between the characteristic VOCs and agronomic traits in different leaf positions of Pandan, which provides us with clues to explore the synthesis, accumulation and possible functions of these compounds in Pandan. The above research results not only help to deeply understand the physiological and ecological characteristics of Pandan leaves and provide theoretical basis for targeted harvesting, but also provide important scientific basis for the cultivation management, quality control and aroma resource development and utilization of Pandan. In the future, more experiments and research will be conducted to further explore the specific relationship between VOCs and leaf physiological processes, as well as how to optimize the aroma quality of Pandan by regulating environmental factors.

5. Conclusion

As new leaves at the top of Pandan, The VOCs in the leaves at positions of L1-L3 are significant different from those at mature positions (L4-L25). Although the content of pyrrole and furan is high in new leaves, their insufficient growth time results in lower photosynthesis, water use efficiency, chlorophyll content, dry matter content, and total VOCs content, making them unsuitable for harvesting. The accumulation of dry matter reaches a stable state in mature leaves, and the chlorophyll content and the total VOCs content begins to reach a relatively high level. Among them, the content of squalene, neophytadiene, and phytol gradually increases with the growth of the leaves, except for the decrease of neophytadiene in aging leaves, although the 2AP content begins to slowly decrease and is significantly lower than that of new leaves. In summary, this study suggests that L8 is the critical point at which the content of each major VOCs is balanced, and harvesting the L4-L8 position leaves of Pandan for obtaining 2AP, leaves below L11 position for obtaining squalene, and leaves at L7-L20 position for obtaining neophytadiene.

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CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Zhang, Ang (2024), "Changes in key volatile organic components associated with leaf quality of *Pandanus amaryllifolius* alongside the growth duration", Mendeley Data, V1, doi: 10.17632/fyp9g8j467.1

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.102126.

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