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Impact of rice agro-system on aroma accumulation of Phka Rumdoul rice variety in Cambodia

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Abstract: *Phka Rumdoul is a premium aromatic rice variety highly valued in Cambodia for its fragrance, primarily attributed to 2-Acetyl-1-pyrroline (2-AP). The intensity of this aroma varies across regions, suggesting strong environmental and agronomic influences. This study investigated the impact of different agro-systems on 2-AP accumulation in Phka Rumdoul by examining the relationship between soil's physicochemical properties and grain 2-AP content. Samples were collected from 33 fields across nine provinces, representing five cultivation practices: conventional tillage (CT), conservation agriculture (CA), sustainable rice platform (SRP), organic rice farming (ORF), and green manure (GM). The soil samples were analyzed for pH, soil moisture, total carbon, organic matter, total nitrogen, total phosphorus, cation exchange capacity, exchangeable Ca, Mg, K, and Na, and C/N ratio. Rice grains were analyzed for 2-AP concentration (µg/g) using gas chromatography mass spectrometry (GC-MS) based on the internal standard and ion fragments. Principal component analysis (PCA) was employed to see the correlation of soil properties contributing across different agro-systems and 2-AP levels. The results showed that CA and SRP practices produced significantly higher 2-AP levels compared to CT. In Kampong Thom, CA improved soil properties such as organic carbon and exchangeable nutrients, which correlated with increased 2-AP content. However, variation in 2-AP levels under the same practice (e.g., ORF) across provinces suggests that environmental factors and possible genetic differences within the variety also play roles. In conclusion, CA improves both soil quality and aroma accumulation in Phka Rumdoul rice. Future studies using genetically uniform seeds and standardized post-harvest handling are recommended to better understand the effects of agroecological factors on rice aroma.*

Keywords: Phka Rumdoul, Aroma, 2-Acetyl-1-pyrroline, Agro-system, Gas Chromatography-Mass spectrometry

1. INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food for almost half the world's population, particularly 95% of production is located in Southeast Asia [1]. In Cambodia, rice is the most important crop. It covers 75% of the country's cultivated area [2]. Aroma rice is a type of rice that produces a pleasant floral odour during and after cooking. Among rice varieties, aroma is one of the most important characteristics of rice, especially when taking consumer acceptance as a criterion. Today, consumers are increasingly conscious of the quality of the rice they consume. Hence, demand for aromatic rice is increasing in both domestic and international markets [3]. Consequently, rice aroma has gained a leading importance as a quality characteristic [4]. Jasmine and Basmati rice are the best-known aromatic rice varieties, with distinct flavors and textures. Cambodian Phka Rumdoul rice, renowned for its jasmine-like aroma, has received global recognition, winning the "World Best Rice of the Year" award six times. More than 300 volatile compounds have been identified in aromatic rice across various studies [5]. Among them, 2-Acetyl-1-pyrroline (2-AP) is recognized as the key aroma compound and is considered one of the most flavorful and important natural chemical compounds in aromatic rice. The

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biosynthesis of 2-AP occurs through two distinct pathways: the non-enzymatic and enzymatic pathways [6]. The non-enzymatic pathway involves the conversion of proline, glutamate, and ornithine to 1-pyrroline-5-carboxylate (P5C) by the action of the Δ1-pyrroline-5-carboxylate synthetase (P5CS), proline dehydrogenase (PDH), and ornithine aminotransferase (OAT) enzymes. Subsequently, 1-pyrroline is further converted to 2-AP through a non-enzymatic reaction involving methylglyoxal [7]. However, the activity of these pathways is dependent on Betaine Aldehyde Dehydrogenase (*BADH*), which catalyzes the conversion of gamma-aminobutyraldehyde (GABald) into GABA [8]. *BADH2* gene expression inhibits 2-AP production in non-aromatic rice; however, the *BADH2* gene is inactive in aroma rice. The inactive *BADH2* gene in fragrant rice converts GABald to 1-pyrroline, which leads to the biosynthesis of 2-AP [9]. 2-AP imparts a characteristic "popcorn" or "toasted" aroma. Traditionally, aroma evaluation in breeding programs relies on sensory assessment [10]. However, without a trained sensory panel, such evaluations are subjective and not quantitative. 2-AP was first identified as an important aroma component of cooked rice [11], using a time and sample-intensive atmospheric steam distillation followed by Gas chromatography-mass spectrometry (GC-MS). Khao Dawk Mali 105 rice (Jasmine), Basmati, and some Italian rice varieties contain relatively high 2-AP accumulation. Since 2-AP is a highly volatile compound, it can easily evaporate and dissipate into the atmosphere during processing steps such as drying, milling, or cooking [12], leading to aroma loss. The 2-AP content in the aromatic rice is influenced by biotic (living organisms) and abiotic factors (genotypes, climates, crop management, and the physical and chemical properties of soil) [13]. Khush and Jukiano (1985) studied the 2-AP content and found that it also varies with temperature, rainfall, day length, light intensity, humidity, soil type, and fertility [14]. Similarly, Yoshihashi et al. (2004) confirmed that the ecological conditions of an area, such as the weather and soil conditions, are highly correlated with the quality of the fragrant rice [15]. Additionally, several studied have reported the variability of aromatic quality of grain when rice has been grown in traditional fragrant rice area, enotype, certain characteristics of the growing environment [16], such as soil salinity [17], soil nutrients [18], storage time and temperature [19], and harvest time [20], have a significant effect on the strength and quality of the aroma. Therefore, agro-systems play a crucial role in the biosynthesis and accumulation of the 2-AP compound.

In the present study, Phka Rumdoul rice grains and soil samples were collected from 33 rice fields representing different agro-systems across nine provinces in Cambodia. Various agro-systems factors such as farming practices, fertilizer application, seed generation, sowing dates, and harvest times etc were considered. However, this study focused more specifically on agricultural practices. Five main farming practices were identified. Conventional tillage (CT) is a traditional practice involving intensive tillage and the use of chemical fertilizers, which can negatively affect both the environment and soil quality [21]. In contrast, Conservation agriculture (CA) promotes soil health through practices such as no tillage, cover crops, and crop rotation. The Sustainable Rice Platform (SRP) represents a more integrated system, incorporating sustainable methods like integrated pest management (IPM), though it can be challenging for farmers to implement. Green manure (GM) emphasizes minimal tillage and relies on natural cover crops to enhance soil nitrogen [22]. Meanwhile, organic rice farming (ORF) adheres to organic principles, emphasizing practices that preserve and improve soil vitality while avoiding synthetic inputs [23]. Soil samples were analyzed for their physico-chemical properties, and the aromatic rice was identified based on 2-AP content in rice grain using advanced analytical techniques by using gas chromatography-mass spectrometry (GC-MS), which has improved the accuracy of 2-AP quantification. Subsequently, the correlation between soil physico-chemical properties and 2-AP content under different agricultural practices was investigated. Understanding the impact of agro-systems on 2-AP accumulation is essential for improving the quality of aromatic rice varieties in Cambodia and enhancing their internationally recognized value. Therefore, this study aimed to examine the impact of different agricultural practices on 2-AP accumulation in Phka Rumdoul rice grain by sampling 33 fields across nine provinces in Cambodia.

2. METHODOLOGY

2.1 Sample Collection

Phka Rumdoul rice grains (approximately 1 kg per site) were collected from the farmers participating in the HealthyRice Consortium (FEF project). After being dried to 14% moisture content, the grains were sent to the HealthyRice laboratory at ITC to be stored at 4ºC until analysis. Simultaneously, soil samples were also collected from each site where the Phka Rumdoul rice variety was cultivated and stored in a laboratory at room temperature. Both rice grains and soil samples were collected during the harvest season in November 2024 from nine provinces across Cambodia, including Phnom Penh, Kampong Speu, Kampong Chhnang, Kampong Thom, Battambang, Siem Reap, Preah Vihear, Stung Treng, and Mondulkiri (**Table 1**). These rice fields were managed using different farming practices, fertilizer application, generation of rice seeds, sowing date, harvest times, etc.

Table 1. List of Phka Rumdoul rice fields across nine provinces in Cambodia

Provinces	GPS coordoner	Agriculture Practices*
Phnom Penh	11°28'42.3"N 104°48'25.0"E	CA (n=1)
Kampong Speu	11°46'04.2"N 104°18'42.6"E	CT (n=1)
Kampong Chnang	12°12'48.2"N 104°33'05.3"E	CT (n=1)
Battambang	12°57'34.0"N 103°15'52.7"E	CA (n=1)
Siem Reap	13°13'42.8"N 104°08'58.0"E	SRP (n=1)
Preah Vihear	13°58'27.3"N 104°53'33.4"E	ORF (n=1)

Preah Vihear	13°24'32"N 105°08'56"E	GM (n=2)
Preah Vihear	13°24'55"N 105°08'40"E	CT (n=2)
Stung Treng	13°23'17.7"N 105°45'01.4"E	ORF (n=2)
Mondulkiri	13°09'40.7"N 106°54'01.8"E	ORF (n=1)
Kampong Thom	12°32'58.4"N 105°08'51.1"E	CA (n=10)
Kampong Thom	12°32'58.4"N 105°08'51.1"E	CT (n=10)

*n: number of field samples

2.2 Soil's physicochemical properties analysis

Soil samples were collected from 33 fields across nine provinces. In each field, five plots were sampled (150 g per plot) at a depth of 10 to 15 cm [24]. The composite samples (300 g each) were then air-dried at room temperature on an Agro-food wrapping paper to prevent chemical changes from sunlight, then ground with a pestle to break clumps and sieved through a 2mm mesh. The prepared samples were analyzed for physical and chemical properties, including Organic Carbon and Organic matter, by using the Black & Walkey method. [25], Total Nitrogen (Kjeldahl digestion method) [26], Soil Moisture content % (Oven 105°C, 48 h method), available phosphorus (Bray II method), Cation Exchange Capacity (Ammonium acetate pH7.0 method), Exchangeable Ca and Mg (EDTA titration), Exchangeable K and Na (Flame photometer), pH (H_2O, KCl), C/N ratio (unit), total exchangeable bases, basic saturates [27]. The analysis was conducted at the Soil Science Laboratory in the Royal University of Agriculture.

2.3 Chemical reagent

The authentic standard of 2-Acetyl-1-pyrroline (2-AP) (solution in Toluene 1000 µg/mL) was purchased from A Chemtek Inc., Wobum, USA.

2.4 Extraction of 2-AP

All Phka Rumdoul rice samples collected from each province were extracted using the liquid extraction method. First, 100 grams of rice seeds were decorticated using a rice hulling machine, resulting in brown rice. Then, the outer layer of rice seeds was removed using a polishing machine, resulting in white rice. The white rice was ground into rice powder using the CT 293 Cycletec machine with a particle size of rice powder 0.5mm. Next, 2 g of rice powder was weighed into a 15 ml Falcon tube, and 2 ml of ethanol was added. The sample was mixed well using a vortexing machine and then placed in an ultrasonic bath for 30 minutes at 80 °C. During the ultrasonic bath, the liquid sample was mixed every 2 minutes to prevent it from sticking to the tip of the Falcon tube. After the ultrasonic treatment, the liquid sample was placed in a freezer at -20°c for 5 minutes. It was then mixed again by inversion and centrifuged at 1800g for 5 mins. Next, 2 ml of the liquid sample was pumped with a Nylon syringe and filtered through a 0.45µm filter into a 2 ml vial tube. Finally, the extract was ready for quantification of the amount of 2-AP compound using GC-MS. Additionally, 2-AP was detected based on the internal standard and mass spectral fragment ions (m/z 83). So, an internal standard solution of 2-AP was prepared.

2.5 Identification of 2-AP by gas chromatography-mass spectrometry (GC-MS)

The GC-MS analysis was performed using a Shimadzu GC-MS-TQ8040, which was coupled with a GC-2010 Plus. This instrument was equipped with an AOC-20S autoinjector and -20i autosampler, manufactured by Shimadzu (Japan). The injection mode was splitless, with an injection volume of 1 µL. The capillary column was DB-5ms (30 m x 0.25 mm inner diameter, 0.25 µm film thickness, Agilent, USA). The oven temperature programmed was initially set at 40 °C with a holding time of 1 min, and then increased to 310 °C (8 $°C.min^{-1}$) with a holding time of 4 mins. The injector and interface temperatures were 250 °C and 270 °C, respectively. Helium (purity ≥ 99.999%) was used as carrier gas with a flow rate of 50 $mL.min^{-1}$. For MS, the electron impact energy (EI) was 70 eV. The ion source and interface temperatures were 200 °C and 300 °C, respectively. The sample was analyzed in full-sim mode within the range of m/z 45-600 to confirm in spectral library search of the presence of 2-AP compounds. The 2-AP (µg/g) was identified by a data treatment system and computer that calculated the monoisotopic mass and predicted the structural formula of the compounds, and compared them using the MS database.

2.6 Statistical analysis

Microsoft Excel was used to collect and arrange the data. R version 4.4.3 was used for the statistical analyses of phenotypic data, using the packages rstatix, ggplot2, and multcompView. The confidence interval at 95% was selected. As all of our data did not follow a normal distribution ($p<5\%$ with the Shapiro-Wilk test), non-parametric Kruskal-Wallis tests ($\alpha=5\%$) followed by pairwise Wilcoxon tests (p.adjust.method = "Bonferroni") were used to assess the significance of differences between means. Principal component analysis (PCA) was performed to evaluate the relationships between soil physicochemical properties and 2-AP concentrations from 33 fields across nine provinces in Cambodia.

3. RESULTS AND DISCUSSION

Phka Rumdoul is regarded as one of the most aromatic rice varieties due to its distinctive fragrance. This study was based on the hypothesis that agricultural practices and soil properties influence the accumulation of 2-AP in rice grains. To test this, the effects of different farming practices on soil properties and their correlation with 2-AP accumulation were investigated using samples from 33 fields across nine provinces in Cambodia.

3.1 Variability of soil physicochemical properties

A principal component analysis (PCA) based on these soil physicochemical properties from 33 fields in nine provinces across Cambodia was presented in **Fig 1.** The first principal component (Dim1) explained 32.3% of the total variance, while the second component (Dim2) explained 18.3%. Soil organic carbon (SOC), soil organic matter (SOM), C/N ratio, and clay content and total N are clustered together, indicating a strong positive correlation among these variables. In contrast, pH (both H_2O and KCl) and Na are closely aligned, suggesting a related pattern or influence by similar soil chemical properties. Sand appears in the opposite direction to cation exchange capacity (CEC) and clay, SOM, SOC, and total N, suggesting that soils with high sand content tend to have lower CEC and clay, SOM, SOC, and total N. In addition, electrical conductivity (EC), available phosphorus (P) also show some correlation. The black dots represented individual soil samples coded by field. Their distribution in PCA space indicates the diversity of soil properties among the sampled fields.

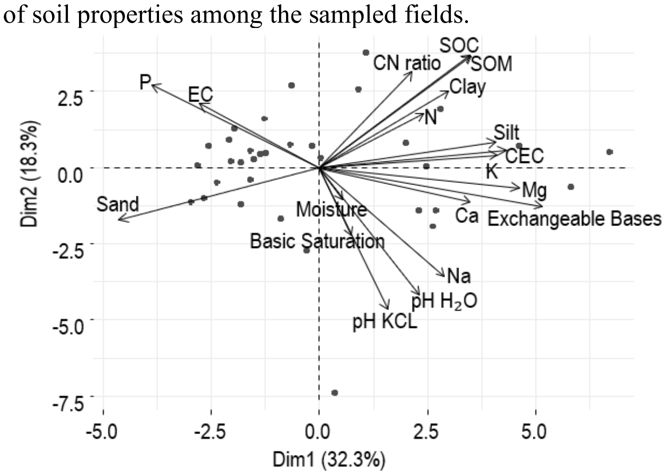

Fig. 1. Principal component analysis (PCA) of soil physico-chemical properties in 33 fields. Each black dot represented a soil sample. EC µS/cm, CEC: Total Cationic Exchange Capacity; C/N Ratio Carbon/Nitrogen, Calcium (Ca), Magnesium (Mg), Potassium (K), Sodium (Na), Exchangeable Bases meq/100g, Basic Saturation, SOM: Soil organic matter, SOC: soil organic carbon, N: total nitrogen, P: Available Phosphorus, Clay, Silt, Sand, and Moisture Content.

3.2. 2-AP content in Phka Rumdoul rice grain from different regions

Phka Rumdoul rice grains were collected from 33 fields across Cambodia. However, each sample of Phka rumdoul rice collected had a different story. Based on the survey, variation in Phka Rumdoul arises due to differences in generation seeding, water management, day of sowing and harvesting, farming practices, weather conditions, soil physical and chemical properties, and field location and topography, etc. All these factors play a significant role in determining 2-AP production [15]. As shown in **Fig. 2**, the comparison of 2-AP concentration in Phka Rumdoul rice grains across different regions is presented. Based on the data shown in Figure 2, 2-AP concentrations in Phka Rumdoul rice grains vary significantly across provinces, ranging from 0.2 µg/g in Mondulkiri to 2.0 µg/g in Siem Reap. Samples from Mondulkiri province exhibited the lowest 2-AP levels. If compared to other regions, there were no significant differences except for Phka Rumdoul rice grain from Kompong Thom and Siem Reap provinces, which showed statistically significant differences with p-values of 0.017 and 0.0083, respectively. In the Mondulkiri survey fields, which practice organic rice farming, the result obtained from triplicate analyses showed lower 2-AP levels. This may be due to the short duration of organic practices or slower nitrogen mineralization, which limits the availability of substrates necessary for 2-AP biosynthesis [28]. A combination of high nitrogen imbalance, lack of moderate water stress, high soil organic matter buffering, and yield aroma trade-offs can contribute to reduced 2-AP content under certain agro-systems [29]. In fact, Mondulkiri had less raw data than the practices ORF. Thus, we cannot conclude that ORF practices are responsible for the low 2-AP content. Moreover, as a term of agro-system factors such as rice seed generation, climate change (temperature, weather, humidity), and edaphic factors (e.g., soil properties, soil microbiota) [30] should be further studied to understand which factors affect the 2-AP accumulation. Therefore, these results support the idea that different agro-systems can lead to varying levels of 2-AP accumulation within the same rice variety [15].

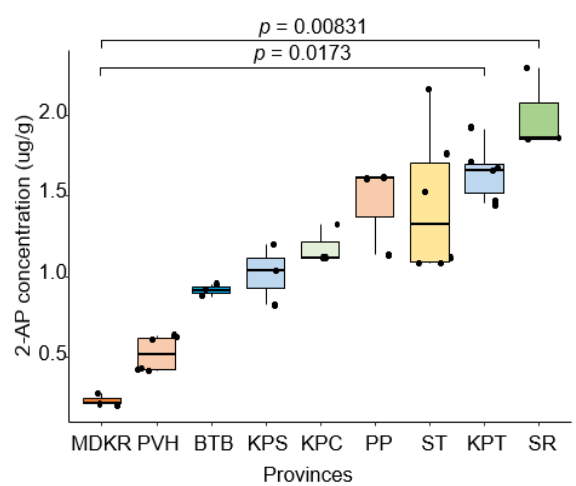

Fig. 2. 2-AP concentration (µg/g) in Phka Rumoul rice grain with different regions. MDKR: Mondulkri, PVH: Preah Vihear, BTB: Battambang, KPC: Kampong Chhnang, KPS: Kampong Speu, PP: Phnom Penh, ST: Stung Treng, KPT: Kampong Thom, SR: Siem Reap.

3.3 2-AP content in Phka Rumdoul rice grain under different farming practices

In **Fig. 3,** 2-AP concentration (µg/g) in Phka Rumdoul rice grain was compared across five different farming practices, including conventional tillage (CT), conservation agriculture (CA), sustainable rice platform (SRP), organic rice farming (ORF), and green manure (GM). The high median of 2-AP concentration was observed under CA and SRP, with median of 1.8 µg/g and 2 µg/g, respectively. In contrast, GM and CT recorded the lowest 2-AP levels, both with median concentrations of 0.7 µg/g and 0.8 µg/g, respectively. Statistical analysis showed that rice grown under CA and SRP had significantly higher 2-AP levels compared to GM, with p-values of 0.002 and 0.004, respectively. The lowest 2-AP concentration was observed under GM practices, particularly in fields located in Preah Vihear province, where no fertilizer inputs were applied. Preah Vihear is located in northern Cambodia and has a distinct agro-ecological zone with upland areas with lower rainfall compared to the southern regions [31]. This environmental stress may affect 2-AP production in Phka Rumdoul rice. Results from this study suggest that farming practices such as CA and SRP are associated with higher 2-AP concentrations in aromatic rice, particularly Phka Rumdoul. For instance, in the boxplot presented ORF practice, each black dot indicated the raw data of 2-AP content. The ORF practices were collected from three different fields, such as Mondulkiri, Stung Treng, and Preah Vihear. As a result, the variation in 2-AP concentration under ORF may reflect regional differences in environmental factors and field management, even within the same farming practices. Due to unequal sample sizes and limitations in the available raw data, the next result shown in **Fig. 4** focuses on a more localized comparison of 2-AP content between CA and CT practices within Kampong Thom province, where the agro-systems are more directly comparable and the sample distribution is more consistent.

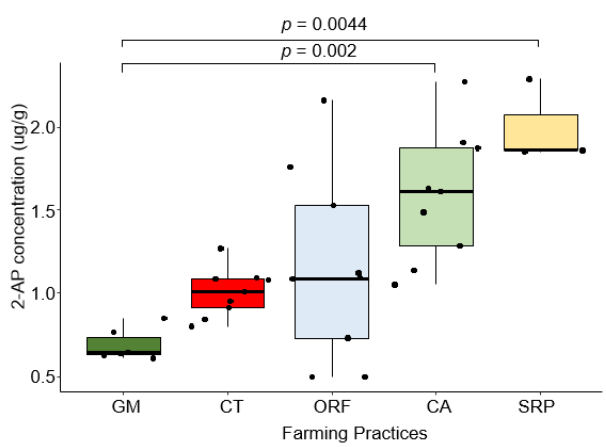

Fig. 3. 2-AP concentration (µg/g) in Phka Rumoul rice grain under different rice farming practices.

3.3 2-AP content under CA and CT practices in Kampong Thom province

Fig. 4 presents a comparison of 2-AP concentrations (µg/g) between Conservation agriculture (CA) and Conventional tillage (CT) in Kampong Thom province, where the environment is relatively consistent. This comparison is based on data from 10 fields under practices (CA=10 fields, CT=10 fields), with triplicated measurements, resulting in a total of 60 samples (n = 60). The result showed that the median 2-AP concentration under CA was significantly higher than CT ($p = 0.027$). Furthermore, the distribution of data points shows that CA not only had a higher median but also a greater proportion of samples exceeding 2 µg/g. These findings are consistent with those shown in **Fig. 3** and suggest that CA practice had greater 2-AP accumulation. This is true even within a single agroecological region. The principle of CA practice emphasizes minimal soil disturbance, permanent organic soil, permanent residue cover, and planned crop rotations, which are considered essential to its effectiveness [32]. Moreover, these practices promote beneficial microbial activity, improve soil organic matter content, and maintain optimal soil moisture levels, all of which contribute to increased 2-AP production in rice [19, 20].

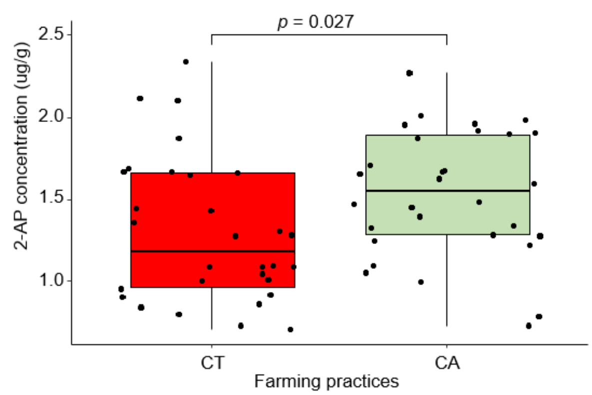

Fig. 4. 2-AP content under CA and CT practices within Kampong Thom province

3.4 Correlation between soil physicochemical properties with 2-AP content under CA and CT in Kompong Thom province

The Principal Component Analysis (PCA) biplot displays the distribution of samples under two different farming practices: Conservation Agriculture (CA) and Conventional Tillage (CT) in Kampong Thom province in relation to key soil properties and the concentration of 2-AP as presented in **Fig. 5**. The first two components explained 49.4% of total variability: First principal component (Dim1) explained 29.8% of the total variance, while the second component (Dim2) explained 19.6%. The PCA plot reveals a distinct separation between the CA (green circle) and CT (red circle) groups. The CA samples are predominantly located to the right of Dim1, associated with higher 2-AP content, better soil quality (SOM, SOC, K, Ca), and less acidity. In contrast, CT samples are more concentrated on the left, while soil quality is poor, with more sand content, and has lower 2-AP content. Importantly, 2-AP showed a bit positive correlation with Ca, under CA, and a negative correlation with sand content CT. This indicates that farming practices influence both soil conditions and 2-AP production. Soil richer in these components, particularly under CA, may support higher 2-AP accumulation. Ca deserves further study to examine its specific role in 2-AP biosynthesis. Overall, the results suggest that farming practices significantly affect soil properties and 2-AP levels. CA practices, such as reduced tillage and maintaining organic matter in the soil, may help boost both soil fertility and 2-AP production.

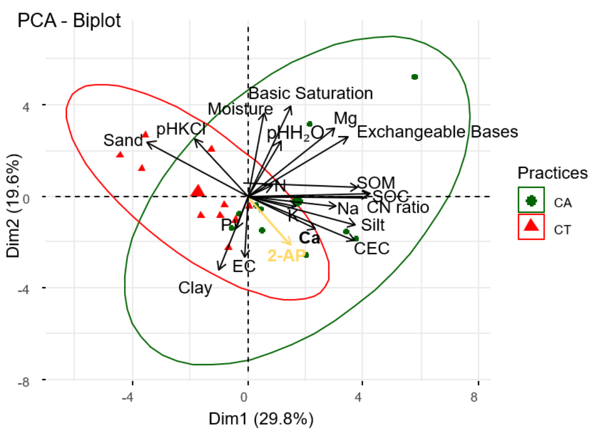

Fig. 5. Principal component analysis (PCA) for farming practices, soil physicochemical properties, and 2-acetyl-1-pyrroline (2-AP) content.

4. CONCLUSIONS

2-Acetyl-1-pyrroline (2-AP) content of Phka Rumdoul rice grains from 33 fields across nine provinces in Cambodia was analyzed. Based on the results, different agro-systems showed varying levels of 2-AP concentration. Among the five agricultural practices, conventional tillage (CT), conservation agriculture (CA), sustainable rice platform (SRP), organic rice farming (ORF), and green manure (GM), we observed that CA and SRP are likely to contribute to higher 2-AP levels in aromatic rice, particularly Phka Rumdoul variety. ORF practice in different fields across Mondulkiri, Stung Treng, and Preah Vihear showed considerable variation in 2-AP concentration, even though the same practice was applied. This suggests that 2-AP levels may be influenced not only by farming practices but also by other environmental factors. Therefore, variations in rice genetics, climate conditions (e.g., temperature, weather, humidity), and edaphic factors (e.g., soil properties, soil microbiota) should be further studied to better understand their influences on 2-AP accumulation. Moreover, although all analyses were conducted using the Phka Rumdoul variety, the seeds used were not sourced from the same origin. Moreover, potential genetic variation within the variety may have contributed to the differences observed. Repeating the experiment with genetically homogeneous or certified seed stock would help distinguish the effects of environmental and agronomic factors from genetic influences. Furthermore, in Kampong Thom, where agro-systems are more directly comparable and the sample distribution is more consistent, we observed that Phka Rumdoul rice grown under CA had significantly higher 2-AP content than rice grown under CT. Soil properties under CA are also associated with higher levels of soil organic carbon, silt, and exchangeable nutrients (Na, K, Ca), which Ca cluster closely with 2-AP content. These results indicate that the improved soil quality under CA has a strong positive impact on 2-AP content in Phka Rumdoul rice grains. Our findings highlight the key role of CA practices in improving soil quality and 2-AP accumulation in rice grain. CA practice is highly recommended in the Kampong Thom region for the improvement of soil properties and rice quality, particularly in terms of 2-AP production. Similarly, studies with larger sample sizes should be conducted for all agricultural practices (GM, SRP, ORF) before drawing broader conclusions. These experiments should be replicated across all provinces using the same certified Phka Rumdoul variety and standardized post-harvest processing, as both genetic uniformity and post-harvest conditions are known to influence 2-AP accumulation significantly.

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