BIODIVERSITAS
Volume 22, Number 8, August 2021
Pages: 3127-3135

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d220806

Metabolite profile of two *Allium cepa* L. aggregatum group cultivars by Nuclear Magnetic Resonance

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Manuscript received: 26 March 2021. Revision accepted: 8 July 2021.

Abstract. *Basundari FRA, Sulistyaningsih E, Murti RH, Nuringtyas TR. 2021. Metabolite profile of two* Allium cepa *L. aggregatum group cultivars by Nuclear Magnetic Resonance. Biodiversitas 22: 3127-3135.* Shallot, an *Allium* species with high economic value, is widely cultivated in Indonesia. The species includes numerous cultivars with unique characteristics, such as harvesting time, yield, taste, odor, and bulb color. Tuk Tuk and Trisula, the cultivars used in this experiment, have different bulb colors. Tuk Tuk's bulb color is light red, while that of Trisula is dark red. This color difference may be attributed to variations in the metabolite contents of the shallot bulbs. The present experiment aimed to reveal differences in the metabolite contents of the two shallot cultivars by using Nuclear Magnetic Resonance (NMR) and then determine the specific metabolites contributing to these differences. Bulbs of Tuk Tuk and Trisula were planted in the field under the same conditions and then collected. The bulb samples were powdered, freeze-dried, and subjected to a two-phase extraction method with $CDCl_3$ and D_2O as solvents to separate the nonpolar metabolites from polar ones. The extracts were analyzed by ^{1}H-NMR, and the spectra collected were analyzed using MNOVA software and Metaboanalyst.ca. A total of 23 metabolites were successfully identified and characterized in this experiment. The contents of eight of these metabolites, namely, sucrose, glutamine, citric acid, choline, methiin, propiin, threonine, and formic acid, were significantly higher in Trisula than in Tuk Tuk. These differences may be correlated with variations in the color intensity, pungency, and other traits of the cultivars. The results demonstrate that NMR metabolite profiling could effectively differentiate metabolite profile variations among shallot cultivars.

Keywords: *Allium*, metabolomics, ^{1}H-NMR, shallot

INTRODUCTION

Allium, one of the most important horticultural crops in the world, is cultivated under various climatic conditions. Shallot is a high-value *Allium* species that is widely grown in Indonesia. The species includes many varieties that have been developed by both the government and private sector on account of their specific characteristics, such as yield, disease resistance, taste, odor, and color. Sulistyaningsih et al. (2020) reported that superior accessions with high bulb yield could be developed from the true seeds of shallot. Roldan et al. (2014) and Dong et al. (2015) revealed that plant phenotypes are affected by the synthesis and accumulation of metabolites in a specific organ, growth stage, or environmental condition. Thus, cultivars may feature various plant metabolites or tissue characteristics in specific organs.

Tuk Tuk and Trisula are two shallot cultivars frequently grown by farmers in Indonesia. These cultivars are well adapted to lowlands and usually planted in the dry season. The cultivars show similarities in several characteristics, such as seed and flower color and number of leaves in each bulb. However, these cultivars can also be easily differentiated according to their morphological characteristics, especially bulb color. In addition, Tuk Tuk has a round-shaped bulb, while Trisula has a cone-shaped one. Hong et al. (2016) suggested that variations in metabolite compounds may cause differences between the cultivars. Thus, a comparison of the metabolic profiles of these two cultivars would be of great interest.

Nuclear Magnetic Resonance (NMR) metabolomics is extensively applied in plant metabolomics (Jahangir et al. 2018). This approach has proven to be effective in differentiating the characteristics of cultivars of certain species, such as the flower color of *Catharanthus roseus* (Pan et al. 2014) and resistance to root-knot nematode of tomato (Afifah et al. 2019). NMR metabolomics involves a wide range of metabolites, including primary metabolites, such as sugar, organic acids, and amino acids, and secondary metabolites, such as flavonoids, alkaloids, and terpenoids. Holmes et al. (2006) and Jahangir et al. (2018) reported that NMR metabolic profiling can characterize and identify different metabolite profiles at the tissue and even cell level within the same plant. NMR metabolite profiling of *Allium* species has been conducted for garlic and onion (Jo et al. 2020; Saviano et al. 2019) but not shallot bulb. Thus, the present study was designed to collect the metabolite information of two shallot cultivars possessing different morphological characteristics.

MATERIALS AND METHODS

Plant materials

Two cultivars, Tuk Tuk and Trisula, were used in this experiment. Seven bulbs of each cultivar were grown in the field under similar conditions and then collected in September 2019. Each fresh bulb was cut into small pieces and pulverized with a mortar and pestle in liquid nitrogen. The samples were freeze-dried for 72 h to remove their water content and then prepared for ^{1}H NMR analysis.

Bulb color characterization

Tuk Tuk and Trisula bulbs were harvested from the field, dried to remove approximately 20% of their water content, and then kept in a net bag for 2 months. The Royal Horticultural Society (RHS) color chart was used to identify the skin and flesh color of the shallot bulbs.

Sample preparation for ^{1}H-NMR

^{1}H NMR sample preparation was conducted according to the biphase extraction method described by Schripsema and Dagnino (2018) with a slight modification. Two internal standard solutions (ISSs) were used in this experiment. The first ISS (ISS1) consisted of 2.5 mg of trimethylsilyl-3-propionic acid (TMSP) in 5 ml of D_2O, and the second one (ISS2) consisted of 2 mg of TMS in 4 ml of $CDCl_3$. Approximately 100 mg of the dried samples was carefully weighed and placed in a 2 ml Eppendorf tube. Exactly 0.80 ml of D_2O was added to the tube to wet the sample thoroughly. Next, 0.80 ml of $CDCl_3$ was added to the tube. The mixture was vortexed and placed in an ultrasonicator for 10 min. After incubation, the mixture was centrifuged at 12000× *g* for 10 min. Exactly 0.10 ml of ISS1 was added to an NMR tube, and 0.5 ml of the D_2O phase (upper phase) was extracted from the Eppendorf tube and transferred to the same NMR tube. The NMR tube was sealed and placed in a vortex mixer to combine the extract and ISS1 completely. Another NMR tube was prepared, and added with 0.10 ml of ISS2. The $CDCl_3$ phase (lower phase) was pipetted out from the Eppendorf tube and then loaded into the NMR tube containing ISS2. The tube was sealed and vortexed to mix the extract and ISS2. Thus, two NMR tubes, one containing D_2O extracts and another containing $CDCl_3$ extract, were prepared for NMR analysis.

NMR experiments

^{1}H-NMR was conducted at 30 °C by using a 500 MHz spectrometer (JEOL, USA). A total of 128 scans were conducted over 10 min under the following parameters: 0.16 Hz/point, pulse width of 30 (11.3 ms), and 26 s acquisition time with relaxation delay of 1.5 s. Pre-saturation mode was applied to reduce the excess water signal. The internal lock was set to the deuterated solvent. The spectral width was recorded from 0 to 10 ppm.

Data analysis

The ^{1}H-NMR spectra were analyzed using MNOVA software. The spectra were processed by manual phasing, baseline correction, and calibration to internal standard solution signals (TMSP) placement at the chemical shift of 0.0 ppm. Metabolites were identified by comparison with the data in a metabolite database as described in previous research (Kim et al. 2010; Jo et al. 2020; Ritota et al. 2012; Saviano et al. 2019). Semi-quantitatively signal analysis was performed by comparing the area of a signal to that of the corresponding internal standard. Multivariate data analysis via principal component analysis (PCA) and partial least-squares discriminant analysis (PLS-DA) was performed using Metaboanalyst software (Metaboanalys.ca). The spectra were centered and scaled using the autoscaling method. The predictive ability of the model (Q^2) was calculated via cross-validation, and a permutation test was performed to determine the statistical significance of the model. The *t*-test and PatternHunter analysis were used to check significant differences and the correlations of metabolites between the two cultivars. Important compounds in the model were assessed by variable importance of projection (VIP) scores. A VIP score of >1 indicates that the variable contributes to the cultivar's variance. Finally, the identified metabolites were statistically analyzed by ANOVA with α = 0.05; if a significant result was obtained, post-hoc analysis using Tukey's test was conducted to see the difference.

RESULTS AND DISCUSSION

Bulb characteristics

Tuk Tuk and Trisula have round and cone-shaped bulbs, respectively. According to the RHS color chart, Tuk Tuk skin is gray-red in color (178-A), while Trisula skin is red-purple in color (61-A) as it can be seen in Figure 1-A1 and 1-B1. The flesh color of Tuk Tuk bulbs is purple (77-B), while that of Trisula bulbs is red-purple (71-A). The flesh color photos (Figure 1-A2 and 1-B2) were taken with the RHS color chart card as the background, which has the same color as the bulb from each cultivar.

Figure 1. The skin (1) and flesh of bulb (2) color of Tuk Tuk (A) and Trisula (B) according to the Royal Horticultural Society color chart. Tuk Tuk skin is gray-red (178-A) in color, while that of Trisula is red-purple (61-A). Tuk Tuk flesh is purple (77-B) in color, while that of Trisula is red-purple (71-A)

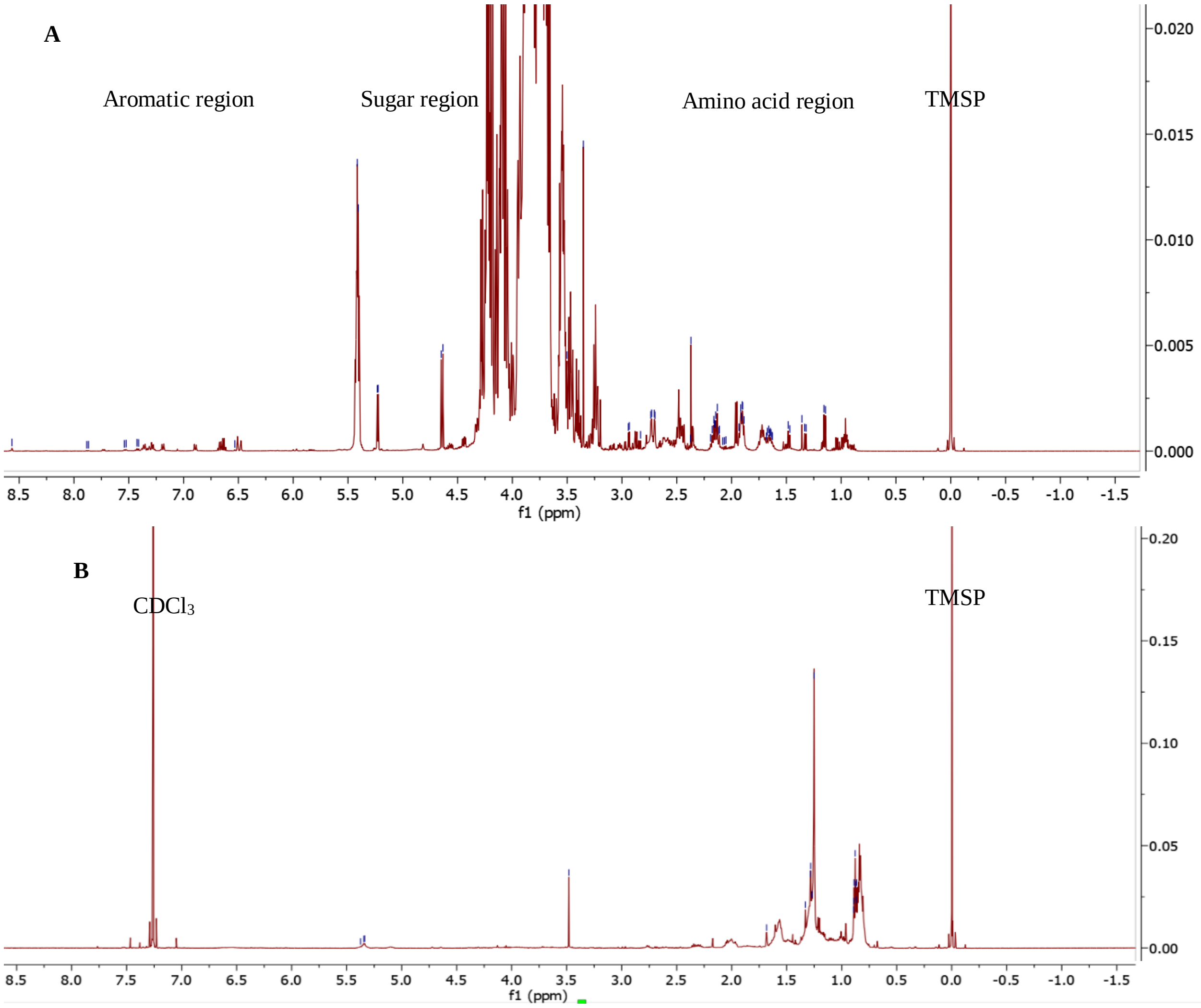

Figure 2. Representative of 1H-NMR spectra of shallot bulb extracted using D2O (A) and CDCl3 (B) solvent

Assignment of [1]H NMR signals

The [1]H-NMR spectra of the shallot bulbs were divided into three regions, i.e., amino acids, sugars, and aromatics ranging from 0.0 ppm to 3.0 ppm, from 3.0 ppm to 5.5 ppm, and from 5.5 ppm to 8.6 ppm, respectively. The polar extract was dominated by the high-intensity peaks of sugars. Minor signals from amino acids and aromatics were also observed (Figure 2A). The spectra collected from the $CDCl_3$ extract were not analyzed in this study because the chemical compounds detected could not be identified on account of the limited data available in the database. However, the nonpolar extract yielded less signals compared with those obtained from the polar extract because the collection of crowded signals at 0.5–2.0 ppm, which belong to long hydrocarbon chain, were eliminated by the $CDCl_3$ extract (Figure 2B). Thus, the spectra collected from the D_2O extract were further analyzed.

A total of 23 metabolites were identified in this research, as detailed in Table 1. The amino acids identified included isoleucine, threonine, cycloalliin, alanine, arginine, GABA, glutamate, glutamine, methiin, propiin, glycine, phenylalanine, tryptophan, and histidine. The sugars identified were α-glucose, β-glucose, and sucrose. Finally, the organic compounds identified included acetic acid, citric acid, fumaric acid, formic acid, and succinic acid. Choline was also identified in this study.

Multivariate data analysis

As the first step of multivariate data analysis, the metabolites identified in both cultivars were analyzed by PCA. The five-component model explained 92% of the total variance observed. Among the five components, the first two components explained 71.5% of the variance. However, the model did not result in good separation between the two cultivars (Figure 3A). Hence, PLS-DA was conducted. The five components assessed in the PLS-DA were found to explain 88.9% of the total variance. PC1and PC2 explained 45.5% and 10.7%, respectively of the variance observed. The score plot obtained showed that Tuk Tuk and Trisula could be well separated from each other. Tuk Tuk was located in the positive quadrant whereas Trisula was located in the negative quadrant of PC1 (Figure 3B).

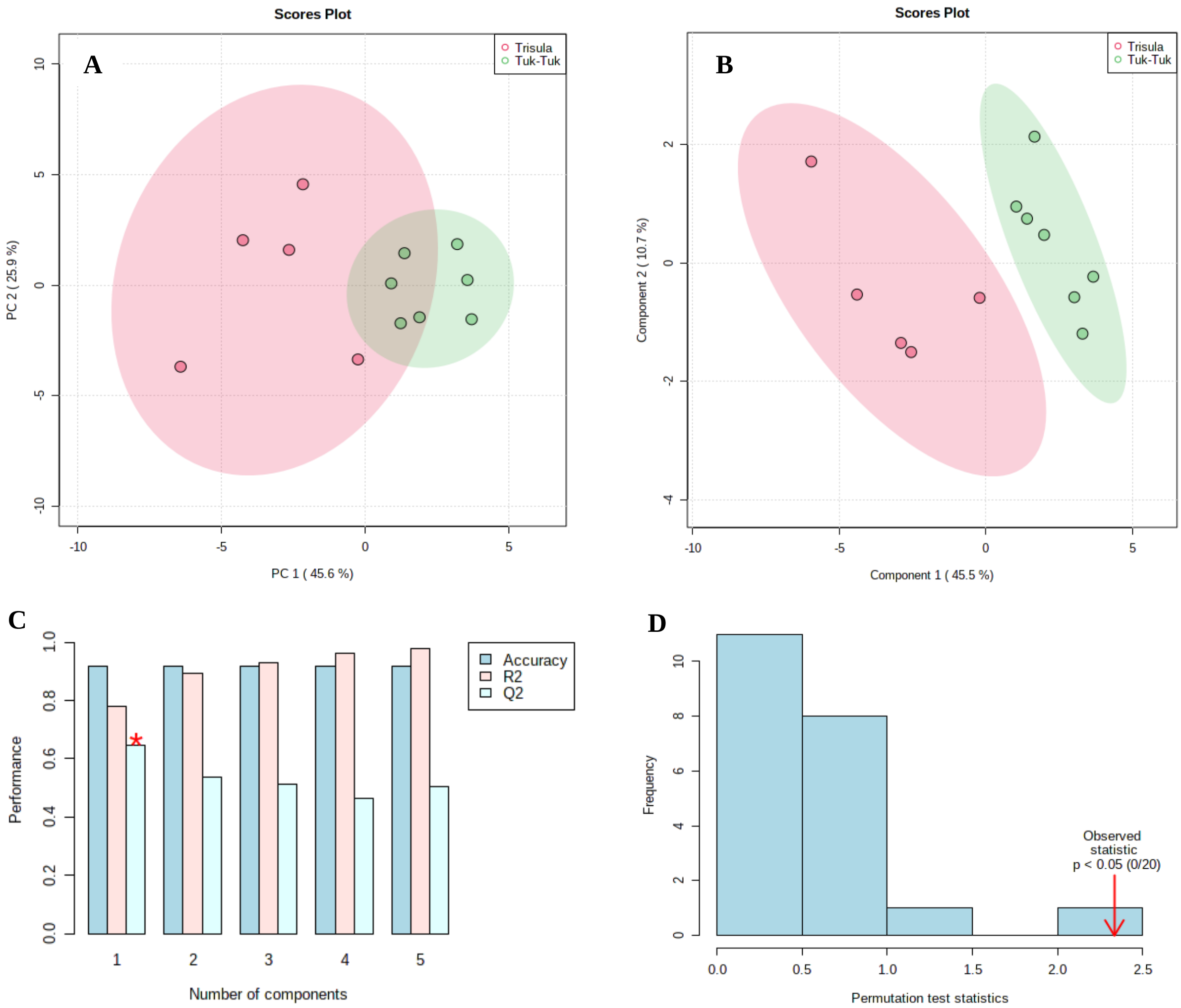

Figure 3. Score plots obtained from the (A) principal component analysis and (B) partial least-squares discriminant analysis of the metabolite profiles of Trisula (red ellipse) and Tuk Tuk (green ellipse). (C) Cross-validation and (D) permutation test statistics confirming the ability of the PLS-DA model to discriminate between the metabolite profiles of the two cultivars. The asterisk symbol (*) shows the optimum number of components included to make the reliable model

Q^2 was calculated through cross-validation to estimate the predictive ability of the PLS-DA model. When $R^2 = 1$ and $Q^2 = 1$, the model could perfectly describe and predict the data, respectively (Triba et al. 2015). In this work, the PLS-DA model yielded a Q^2 of 0.64 and R^2 of 0.7 (Figure 3C). This result indicates that the model is reliable for multivariate analysis. This finding is supported by Leiss et al. (2009), who indicated that Q^2 is a good value if it is greater than 0.5 ($Q^2 > 0.5$). A permutation test was conducted to determine whether the null hypothesis would be rejected on the basis of the *p*-value. The result of this test showed that the observed statistics have a *p*-value of >0.05 (Figure 3D). This finding reveals that the mean difference in metabolites between the two cultivars is statistically significant. Overall, these results indicate that the PLS-DA model for metabolite profiling developed in this study is trustworthy.

The *t*-test and PatternHunter analysis were employed to evaluate significant differences and the correlations of metabolites of two cultivars. The *t*-test result showed that there were eight metabolites with a level of significance (*p*-value) less than 0.05 ($p < 0.05$). Those metabolites were methiin, citric acid, sucrose, propiin, glutamine, choline, threonine, and formic acid. To investigate the correlation coefficient among metabolites found in shallot, a further analysis using PatternHunter based on feature of interest was performed. By using a pattern of interest of citric acid, the result showed that all of the metabolites had a positive correlation with citric acid. However, only eight metabolites had correlation coefficient (*r*) value greater than 0.5, and it was linear with the result of *t*-test analysis (Figure 4).

The advantage of using PLS-DA is the ability to calculate VIP scores, which reflect the importance of a variable in the model. VIP scores are well known to be useful in selecting important variables contributing the most to the observed variance. A VIP score of >1 is generally used as a criterion for variable selection (Farrés et al. 2015). According to the VIP score graph in Figure 5, eight metabolites have VIP scores >1.

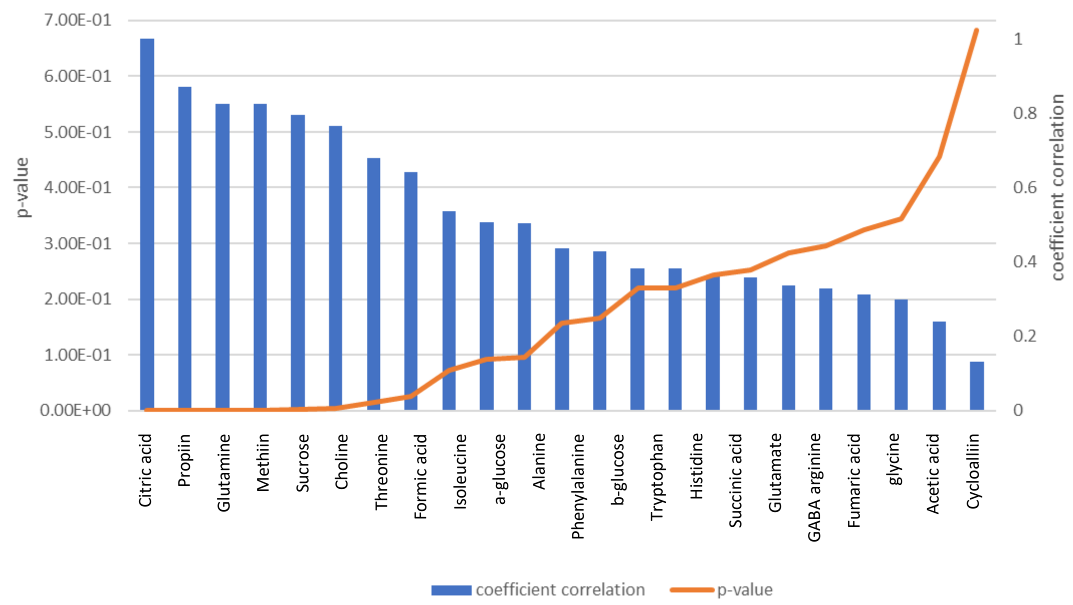

Figure 4. Coefficient correlations and *p*-values of the metabolite profiles of Tuk Tuk and Trisula. The *p*-values on the left side of the graph indicate the level of significance of differences in metabolites between the two cultivars. The correlation coefficients indicated on the right side of the graph indicate the strength of the correlation between metabolites and citric acid as a metabolite of interest

The metabolites identified in this study were primary metabolites and were dominated by sucrose, glutamine, citric acid, choline, methiin, threonine, propiin, and formic acid. Significant differences in metabolites between the two cultivars are illustrated in Figure 6.

Amino acids

Threonine, methiin, and propiin were significantly higher in Trisula than in Tuk Tuk (Figure 6). Methiin and propiin are the chemical compounds of S-alk(en)ylcysteine S-oxides (ACSOs), which are considered health-beneficial compounds in *Allium* vegetables (Ramirez et al. 2017). The ACSO composition of *Allium* is affected by various factors. Previous studies indicated that the species regulates the type of ACSOs obtained, while intra-specific genetic variations (e.g., different cultivars) determine the concentration of ACSOs. Environmental factors, such as growing conditions and sulfur fertilization, and post-harvest treatment might also influence the contents of ACSOs in plants (Ramirez et al. 2017). In the present study, the contents of methiin and propiin significantly differed between Trisula and Tuk Tuk. A previous study revealed that differences in the relative concentration of these compounds between Trisula and Tuk Tuk may be related to genetic variations. Trisula's bulb is dark red in color, while that of Tuk Tuk is light red. Thus, significant differences in the contents of methiin and propiin between the cultivars may be related to differences in their bulb color. This assumption is supported by Montaño et al. (2011), who found that purple-type garlic cultivars have the highest contents of alliin and methiin and the strongest pungency among different samples of *Allium* species, and Pardo et al. (2007), who demonstrated that purple-type cultivars are the most pungent cultivars of garlic. An earlier study of shallot indicated that a shallot cultivar with high contents of ACSOs, especially methiin and isoalliin, would have high levels of pungency (Abdelrahman et al. 2020; Ariyanti et al. 2018). Furthermore, Abdelrahman et al. (2020) also suggested that high ACSO contents in shallots may be attributed to a metabolic adaptation that enables the latter to thrive in a hot and humid tropical climate and act as an antimicrobial agent against a variety of microorganisms as well as a repellent for insects and herbivores. Given these findings, the significant difference in methiin contents between the two cultivars may also be related to their pungency traits and resistance to insects. Trisula may have a higher level of pungency and show increased adaptation to various insects than Tuk Tuk. However, because information on these traits in both cultivars is lacking, further studies on this topic are recommended.

The content of threonine was also significantly higher in Trisula than in Tuk Tuk. Dhillon and Kumar (2017) reported that threonine concentrations are higher in *Sorghum bicolor* seedlings that are resistant to stem borer (*Chillo partellus*) than in the susceptible variety. Threonine and other amino acid compounds (e.g., alanine and valine) were previously found to affect the resistance of gladiol to thrips (Wahyuni 2021). Thus, Trisula may be more resistant to abiotic and biotic stress in Tuk Tuk. This finding is supported by Abdelrahman et al. (2015), who described the threonine and methionine metabolic pathway in doubled haploid shallots (*A. cepa* L. Aggregatum group). This report highlighted the correlation between the metabolic and genomic datasets and indicated the increased adaptability of doubled haploid shallot to abiotic stress compared with doubled haploid onion (*A. cepa* L. Onion group).

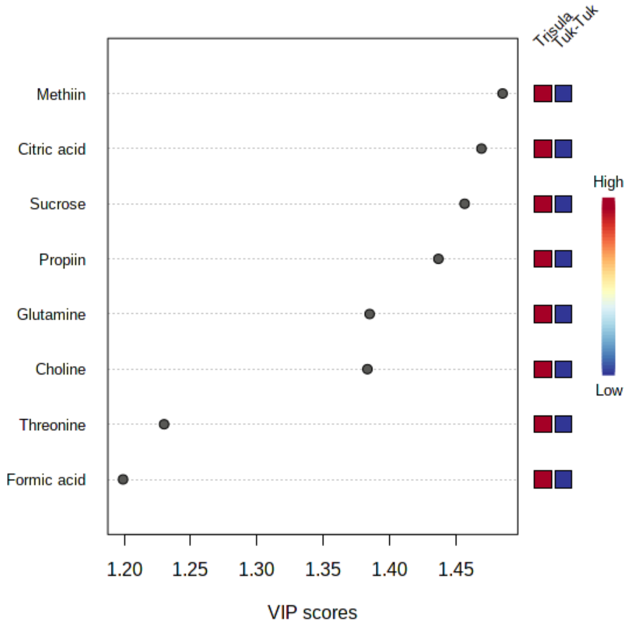

Figure 5. Ranking of eight metabolites identified by variable importance in projection (VIP) scores based on the PLS-DA of Tuk Tuk and Trisula. The relative concentration of the metabolite in each group is indicated by the color boxes on the right side of the figure

Compared with threonine, methiin, and propiin, glutamine was relatively higher than those in shallot. This finding agrees with the result of Fredotović et al. (2020), who reported that considerable amounts of glutamine are obtained in garlic. Glutamine regulates plant growth, development, and responses to environmental stress (Qiu et al. 2020). It is also known to induce the expression of key transcription factor genes involved in the nitrogen and stress responses of rice roots (Kan et al. 2015). Under drought conditions, drought-tolerant onion genotypes show increased expression levels of the vacuolar amino acid transporter and amino acid transporter gene (Abdelrahman et al. 2020; Khan et al. 2019). Thus, glutamine may help shallot cope with drought stress. As no information related to the resistance of the cultivars used in this study to biotic and abiotic stresses is available, further research on this topic is necessary.

Carbohydrates

The sugars of shallot consist of β-glucose, α-glucose, and sucrose. Metabolomics analysis revealed that sucrose makes up the greatest proportion of sugar and other chemical compounds of shallot (Figure 6). This result agrees with the findings of Saviano et al. (2019), who revealed that 80% of the chemical compounds accumulated in onion bulbs are composed of carbohydrates, such as glucose, sucrose, and fructans. Sucrose is also the most abundant soluble carbohydrate in most onion cultivars (Pöhnl et al. 2018). In recent research, carbohydrates were reported to be a factor influencing the bulb initiation and growth of *Allium* species (Atif et al. 2020). A previous study also reported that abiotic stress tolerance in plants, especially drought tolerance, is correlated with carbohydrate metabolism. Sugars could modify the transcription of various stress-related genes (Abdelrahman et al. 2015).

The sucrose concentration of Trisula was significantly higher than that of Tuk Tuk. Genetic variations have been suggested to account for this difference. This result agrees with findings in leek (Soininen et al. 2014); specifically, minor variability in mitochondrial genome levels may be observed among leek cultivars. This variability could also be altered by environmental factors, such as sulfur supply, nitrogen supply, and temperature (Böttcher et al. 2018). Lee et al. (2009) demonstrated a correlation between the sugar content and pungency level of onion. According to the researchers, no relationship exists between either the individual or total sugar content and pungency level of onion. The flavor compounds that make up the pungency of onion could mask the sweetness of sugars. In other words, the pungency level of onion could reduce its perceived sweetness. Further research should be conducted to investigate the role of carbohydrates in *Allium* species in general and shallot in particular.

Organic acids

The organic acid compounds identified in this study were citric acid and formic acid, and the contents of these compounds were significantly higher in Trisula than in Tuk Tuk (Figure 6). Citric acid is a plant metabolite that is essential for photosynthesis and cellular respiration (Tusei 2019). It also plays a key role in plants' mechanisms for coping with nutrient shortages, metal exposure, rhizosphere plant-microbe interactions, and environmental stress (Trejo-Téllez et al. 2012). The relative concentration of citric acid in Trisula was higher than that in Tuk Tuk. Thus, Trisula may have higher tolerance to environmental stress than Tuk Tuk.

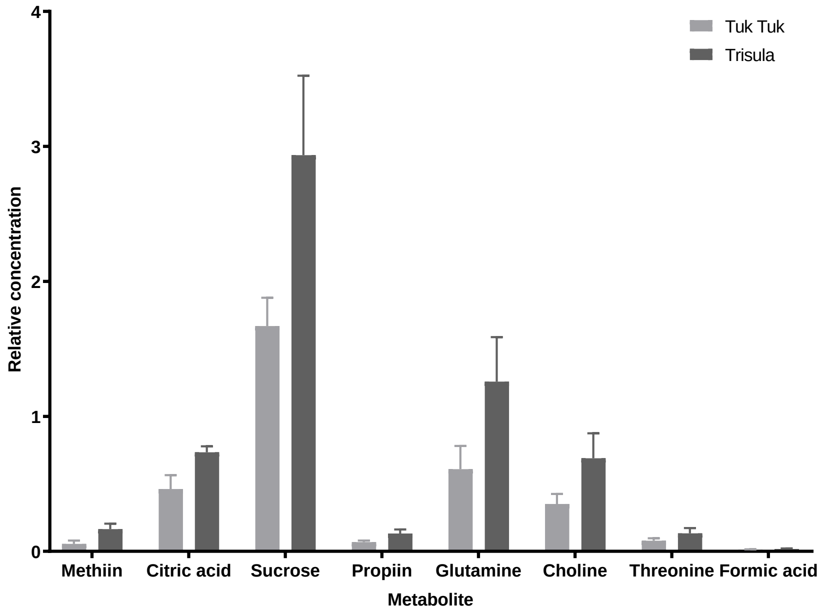

Figure 6. Metabolites identified as important contributors to the difference between Tuk Tuk and Trisula. Results are presented as the mean and standard deviation of the relative concentration.

Formic acid contents also significantly differed between Tuk Tuk and Trisula. The effect of formic acid in shallot is not well known, and the information related to the role of formic acid in plants is limited. Formic acid is useful in agricultural applications as an animal feed and silage additive (Ricke et al. 2020). Berregi et al. (2007) suggested that the presence of formic acid in nature can provide plants and fruits with an additional protection mechanism against diseases caused by bacteria and fungi. For example, certain varieties of apples are naturally resistant to such diseases. Given the higher concentration of formic acid in Trisula compared with that in Tuk Tuk, the former may be more disease resistant than the latter.

Another metabolite demonstrating a significant difference in relative concentration between the two cultivars is choline (Figure 6). In this study, choline in Trisula was significantly higher than that in Tuk Tuk. The role of choline in *Allium* species is yet known. However, choline and glycine betaine are known to play important roles in osmotic adjustment and responses to environmental stress, including drought stress in maize (Gou et al. 2015). A study in wheat revealed that choline priming improves wheat salt tolerance. Choline promotes glycine betaine accumulation, retains beneficial elements (e.g., K^+, Ca^{2+}), minimizes toxic elements (e.g., Na^+, Cl^-), and reduces oxidative stress. These findings may explain why choline priming improves wheat salt tolerance (manifested as reduced lipid peroxidation) in terms of increased shoot and root fresh mass, dry mass, and relative growth rate under NaCl salinity (Salama et al. 2011). Thus, choline may affect the ability of Trisula and Tuk Tuk to cope with environmental stress. Given its relatively higher choline content, Trisula may be more resistant to environmental stress, such as drought or salinity, than Tuk Tuk.

Other studies have revealed that choline is an essential nutrient for human health. Choline or its metabolites are required for cell membrane structural integrity, methyl synthesis, transmembrane signaling, lipid and cholesterol transport, metabolism, and cholinergic neurotransmission; thus, this substance is essential during the critical period of brain growth (Biswas and Giri, 2015). Several vegetables, such as soybean and potato, are the major dietary sources of choline (Arias et al. 2020). Considering the role of choline in transmembrane signaling, the substance may help in nutrient absorption. Thus, it could be biosynthesized into specific compounds influencing the performance of a specific trait. Because Trisula has a higher relative concentration of choline and a darker bulb color than Tuk Tuk, higher relative concentrations of choline may lead to a darker bulb color. In addition, the higher relative concentration of choline in Trisula compared with that in Tuk Tuk may indicate that the former is a better source of nutrition for humans than the latter. The findings described above should be investigated in future research.

In conclusion, the NMR metabolomics approach could successfully differentiate between the metabolite profiles of two shallot cultivars. Specifically, eight compounds mainly contribute to the differences between Tuk Tuk and Trisula. These metabolites include four amino acids and their derivatives, namely, threonine, methiin, propiin, and glutamine. Sucrose and two organic acids, including citric acid and formic acid, and another metabolite, choline, were also found to be significantly different between the two cultivars. Further study is needed to investigate the effect of these metabolites on the characteristics of the two cultivars besides differences in their bulb color.

ACKNOWLEDGEMENTS

We thank Universitas Gadjah Mada, Yogyakarta, Indonesia for providing the financial support necessary to accomplish this research through the program of "*2020-Rekognisi Tugas Akhir*".

REFERENCES

Abdelrahman M, Ariyanti NA, Sawada Y, Tsuji F, Hirata S, Hang TTM, Okamoto M, Yamada Y, Tsugawa H, Hirai MY, Shigyo M. 2020. Metabolome-based discrimination analysis of shallot landraces and bulb onion cultivars associated with differences in the amino acid and flavonoid profiles. Molecules 25 (22): 5300. DOI: 10.3390/molecules25225300.

Abdelrahman M, Sawada Y, Nakabayashi R, Sato S, Hirakawa H, El-Sayed M, Hirai MY, Saito K, Yamauchi N, Shigyo M. 2015. Integrating transcriptome and target metabolome variability in doubled haploids of *Allium cepa* for abiotic stress protection. Mol Breeding 35: 195. DOI: 10.1007/s11032-015-0378-2.

Afifah EN, Murti RH, Nuringtyas TR. 2019. Metabolomics approach for the analysis of resistance of four tomato genotypes (*Solanum lycopersicum* L.) to root-knot nematodes (*Meloidogyne incognita*). Life Sci 14 (1): 141-149. DOI: 10.1515/biol-2019-0016.

Arias N, Arboleya S, Allison J, Kaliszewska A, Higarza SG, Gueimonde M, Arias JL. 2020. The relationship between choline bioavailability from diet, intestinal microbiota composition, and its modulation of human diseases. Nutrients 12 (8): 2340. DOI: 10.3390/nu12082340.

Ariyanti NA, Torikai K, Kirana RP, Hirata S, Sulistyaningsih E, Ito SI, Yamauchi N, Kobayashi N, Shigyo M. 2018. Comparative study on phytochemical variations in Japanese F1 varieties of bulb onions and South-East Asian shallot landraces. Hortic J 87 (1): 63-72. DOI: 10.2503/hortj.OKD-066.

Atif MJ, Ahanger MA, Amin B, Ghani MI, Ali M, Cheng Z. 2020. Mechanism of *Allium* crops bulb enlargement in response to photoperiod: A review. Intl J Mol Sci 21 (4): 1325. DOI: 10.3390/ijms21041325.

Berregi I, del Campo G, Caracena R, Miranda JI. 2007. Quantitative determination of formic acid in apple juices by 1H NMR spectrometry. Talanta 72 (3): 1049-1053. DOI: 10.1016/j.talanta.2006.12.031.

Biswas S, Giri S. 2015. Importance of choline as essential nutrient and its role in prevention of various toxicities. Prague Medical Report 1: 5-15.

Böttcher C, Krähmer A, Stürtz M, Widder S, Schulz H. 2018. Effect of cultivar and cultivation year on the metabolite profile of onion bulbs (*Allium cepa* L.). J Agric Food Chem 66 (12): 3229-3238. DOI: 10.1021/acs.jafc.8b00254.

Dhillon MK, Kumar S. 2017. Amino acid profiling of *Sorghum bicolo*r vis-à-vis *Chilo partellus* (Swinhoe) for biochemical interactions and plant resistance. Arthropod-Plant Interact 11 (4): 537-550. DOI: 10.1007/s11829-016-9491-3.

Dong X, Gao Y, Chen W, Wang W, Gong L, Liu X, Luo J. 2015. Spatiotemporal distribution of phenolamides and the genetics of natural variation of hydroxycinnamoyl spermidine in rice. Mol Plant 8 (1): 111-121. DOI: 10.1016/j.molp.2014.11.003.

Farrés M, Platikanov S, Tsakovski S, Tauler R. 2015. Comparison of the variable importance in projection (VIP) and of the selectivity ratio (SR) methods for variable selection and interpretation. J Chemometric 29 (10): 528-536. DOI: 10.1002/cem.2736.

Fredotović Ž, Soldo B, Šprung M, Marijanović Z, Jerković I, Puizina J. 2020. Comparison of organosulfur and amino acid composition between triploid onion Allium cornutum clementi ex visiani, 1842, and common onion *Allium cepa* L., and evidence for antiproliferative activity of their extracts. Plants 9 (1): 98. DOI: 10.3390/plants9010098.

Gou WEI, Tian LI, Ruan ZHI, Zheng P, Chen F, Zhang L, Cui Z, Zheng P, Li Z, Gao MEI, Shi WEI, Zhang L, Liu J, Hu J. 2015. Accumulation of choline and glycinebetaine and drought stress tolerance induced in maize (*Zea mays*) by three plant growth-promoting rhizobacteria (PGPR) strains. Pak J Bot 47 (2): 581-586.

Holmes E, Tang H, Wang Y, Seger C. 2006. The assessment of plant metabolite profiles by NMR-based methodologies. Planta Medica 72 (9): 771-785. DOI: 10.1055/s-2006-946682.

Hong J, Yang L, Zhang D, Shi J. 2016. Plant metabolomics: An indispensable system biology tool for plant science. Intl J Mol Sci 17 (6): 767. DOI: 10.3390/ijms17060767.

Jahangir M, Nuringtyas TR, Ali K, Wilson EG, Choi YH, Verpoorte R. 2018. NMR-based metabolomics: understanding plant chemistry and identification of biologically active compounds. In: Keun HC (ed.). NMR-based Metabolomics. Royal Society of Chemistry, London. DOI: 10.1039/9781849736435-00246.

Jo S, Song Y, Jeong JH, Hwang J, Kim Y. 2020. Geographical discrimination of *Allium* species (garlic and onion) using 1H NMR spectroscopy with multivariate analysis. Intl J Food Properties 23 (1): 241-254. DOI: 10.1080/10942912.2020.1722160.

Kan CC, Chung TY, Juo YA, Hsieh MH. 2015. Glutamine rapidly induces the expression of key transcription factor genes involved in nitrogen and stress responses in rice roots. BMC Genomics 16: 731. DOI: 10.1186/s12864-015-1892-7.

Khan S, Yu H, Li Q, Gao Y, Sallam BN, Wang H, Liu P, Jiang W. 2019. Exogenous application of amino acids improves the growth and yield of lettuce by enhancing photosynthetic assimilation and nutrient availability. Agronomy 9 (5): 266. DOI: 10.3390/agronomy9050266.

Kim HK, Choi YH, Verpoorte R. 2010. NMR-based metabolomic analysis of plants. Nature Protocols 5 (3): 536-549. DOI: 10.1038/nprot.2009.237.

Lee EJ, Yoo KS, Jifon J, Patil BS. 2009. Characterization of short-day onion cultivars of 3 pungency levels with flavor precursor, free amino acid, sulfur, and sugar contents. J Food Sci 74 (6): C475-C480. DOI: 10.1111/j.1750-3841.2009.01243.x.

Montaño A, Beato VM, Mansilla F, Orgaz F. 2011. Effect of genetic characteristics and environmental factors on organosulfur compounds in garlic (*Allium sativum* L.) grown in Andalusia, Spain. J Agric Food Chem 59 (4): 1301-1307. DOI: 10.1021/jf104494j.

Pan Q, Dai Y, Nuringtyas TR, Mustafa NR, Schulte AE, Verpoorte R, Choi YH. 2014. Investigation of the chemomarkers correlated with flower colour in different organs of Catharanthus roseus using nmr-based metabolomics. Phytochem Analys 25 (1): 66-74. DOI: 10.1002/pca.2464.

Pardo JE, Escribano J, Gomez R, Alvarruiz A. 2007. Physical chemical and sensory quality evaluation of potato varieties. J Food Quality 30 (5): 609-622. DOI: 10.1111/j.1745-4557.2007.00146.x.

Pöhnl T, Schweiggert RM, Carle R. 2018. Impact of cultivation method and cultivar selection on soluble carbohydrates and pungent principles in onions (*Allium cepa* L.). J Agric Food Chem 66 (48): 12827-12835. DOI: 10.1021/acs.jafc.8b05018.

Qiu XM, Sun YY, Ye XY, Li ZG. 2020. Signaling role of glutamate in plants. Front Plant Sci 10: 1743 DOI: 10.3389/fpls.2019.01743.

Ramirez DA, Locatelli DA, González RE, Cavagnaro PF, Camargo AB. 2017. Analytical methods for bioactive sulfur compounds in *Allium*: an integrated review and future directions. J Food Compos Analys 61: 4-19. DOI: 10.1016/j.jfca.2016.09.012.

Ricke SC, Dittoe DK, Richardson KE. 2020. Formic acid as an antimicrobial for poultry production: A review. Front Vet Sci 7: 563. DOI: 10.3389/fvets.2020.00563.

Ritota M, Casciani L, Han BZ, Cozzolino S, Leita L, Sequi P, Valentini M. 2012. Traceability of Italian garlic (*Allium sativum* L.) by means of HRMAS-NMR spectroscopy and multivariate data analysis. Food Chem 135 (2): 684-693. DOI: 10.1016/j.foodchem.2012.05.032

Roldan M, Engel B, de Vos RCH, Vereijken P, Astola L, Groenenboom M, van de Geest H, Bovy A, Molenaar J, van Eeuwijk F, Hall R. 2014. Metabolomics reveals organ-specific metabolic rearrangements during early tomato seedling development. Metabolomics 10 (5): 958-974. DOI: 10.1007/s11306-014-0625-2.

Salama KHA, Mansour MMF, Hassan NS. 2011. Choline priming improves salt tolerance in wheat (*Triticum aestivum* L). Aust J Basic Appl Sci 5 (11): 126-132.

Saviano G, Paris D, Melck D, Fantasma F, Motta A, Iorizzi M. 2019. Metabolite variation in three edible Italian *Allium cepa* L. by NMR-based metabolomics: A comparative study in fresh and stored bulbs. Metabolomics 15 (8): 105. DOI: 10.1007/s11306-019-1566-6.

Schripsema J, Dagnino D. 2018. Two-phase extraction for comprehensive analysis of the plant metabolome by NMR. Methods Mol Biol 1738: 195-202. DOI: 10.1007/978-1-4939-7643-0.

Soininen TH, Jukarainen N, Auriola SOK, Julkunen-Tiitto R, Karjalainen R, Vepsäläinen JJ. 2014. Quantitative metabolite profiling of edible onion species by NMR and HPLC-MS. Food Chem 165: 499-505. DOI: 10.1016/j.foodchem.2014.05.132

Sulistyaningsih E, Pangestuti R, Rosliani R. 2020. Growth and yield of five prospective shallots selected accessions. Ilmu Pertanian 5 (2): 92-97. DOI: 10.22146/ipas.52457

Trejo-Téllez LI, Gómez-Merino FC, Schmitt JM. 2012. Citric acid: Biosynthesis, properties and applications on higher plants. In D Vargas, J Medina (Eds.), Citric Acid: Synthesis, Properties and Applications. Nova Science Publishers, Inc.

Triba MN, Le Moyec L, Amathieu R, Goossens C, Bouchemal N, Nahon P, Rutledge DN, Savarin P. 2015. PLS/OPLS models in metabolomics: The impact of permutation of dataset rows on the K-fold cross-validation quality parameters. Mol BioSystems 11 (1): 13-19. DOI: 10.1039/c4mb00414k

Tusei C. 2019. The effects of citric acid on pH and nutrient uptake in wheatgrass (*Triticum aestivum*). IdeaFest: Interdisciplinary J Creative Works Res 3 (7): 31-35.

Wahyuni D. 2021. Thrips resistance in gladiolus. An eco-metabolomic approach. Universiteit Leiden, Nederland.