Volatile organic compound profiling of *Capsicum annuum* var. *longum* grown under different concentrations of nitrogen

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Summary Emission of volatile organic compounds (VOCs) in plants is triggered by several biotic and abiotic factors, such as nutrient deficiency, environmental stress, and pathogenic attacks. For instance, plants suffering from limited or excessive nitrogen (N) supply may experience internal stress which can ultimately lower their stability and immunity making them susceptible to infection and infestation. In this study, VOCs from *Capsicum annuum* var. *longum* (Solanaceae) exposed to nitrogen (1.8 g/L, 4.5 g/L, and 9 g/L urea) were extracted using a 100 µm Solid Phase Microextraction (SPME) fiber coated with polydimethylsiloxane (PDMS). Using Gas Chromatography-Mass Spectrometry (GC-MS), extracted VOCs from N-treated plants were identified as Butanoic acid, 3-hexenyl ester, (E)-; Butanoic acid, hexyl ester; Hexanoic acid, 3-hexenyl ester, (Z)-; Hexanoic acid, 4-hexen-1-yl ester; cis-3-Hexenyl cis-3-hexenoate and 4-Pentenoic acid 2-methyl-, hexyl ester. Among these volatiles, butanoic acid, 3-hexenyl ester showed the most distinctive peak from the N-treated plants in comparison with the untreated. In addition, the Green Leaf Volatiles (GLV) 3-Hexenal; 2-Hexenal; 3-Hexen-1-ol, (Z)-; 2-Hexen-1-ol, (E) and 1-Hexanol were also detected from the N-treated plants. The identification of plant volatiles provides useful information that can be used in agricultural practices and plant phenotyping.

Additional keywords: Butanoic acid, GC-MS, Green Leaf Volatiles, Polydimethylsiloxane, Solid Phase Microextraction Fiber

Introduction

The *Capsicum* genus (Solanaceae) represents a diverse plant group where varieties have been produced from different climatic conditions and cultivation periods (Gaytan *et al.*, 2017). Among the 20-27 identified species (Walsh and Hoot, 2001), *Capsicum chinense*, *Capsicum frutescens*, *Capsicum annuum*, *Capsicum baccatum*, and *Capsicum pubescens* are globally cultivated (Heiser and Pickersgill, 1969). Among these five reared capsicums, *C. annuum* is the most extensively cultivated across the globe. Varieties of *C. annuum* are further categorized

based on its fruit shape, namely, *C. annuum* var. *glabriusculum*, *C. annuum* var. *abbreviatum*, *C. annuum* var. *groszum*, *C. annuum* var. *anuum* and *C. annuum* var. *accuminatum* (Zhigila *et al.*, 2014). *C. annuum* generally grows in areas with subtropical to tropical climate where there is a temperature and an annual rainfall range of 20-25°C and 120-850 mm, respectively. *C. annuum* var. *longum*, commonly known as Green long chili or “siling haba”, originated from tropical America and has been widely cultivated in the Philippines for food purposes, predominantly used as a spice and for seasoning, and medicinal uses (McBride, 2016). *Capsicum* usually requires four to five months before it reaches complete growth (Bargavi and Elumalai, 2010) while yield is also significantly affected by nutrient availability (Anitha and Geethakumari, 2006). Some of the key factors affecting the growth and productivity of capsicums are pests, diseases, temperature, soil characteristics, and water availability (Bhutia *et al.*, 2018). The tillage, irriga-

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tion, spacing, and sowing strategies can also have an impact on the development of capsicums (Sharma and Kumar, 2017; Islam *et al.*, 2011).

Nitrogen, one of the essential nutrients in plant productivity, is usually supplied as fertilizer given directly to the soil or blended with the irrigation water. Upon contact with soil, N-fertilizers, such as urea, begin to breakdown and hydrolyze into ammonium and carbon dioxide. Although the addition of urea can increase dry weight and enhance drought tolerance of plants (Gou *et al.*, 2017), their response to the availability of nitrogen varies in plant species, cultivars, plant organs, and tissues (Effan *et al.*, 2019; Moreau *et al.*, 2015). This variation can be attributed to the plants' efficiency to absorb nitrogen and carbon, which can be measured as radiation use efficiency, root weight ratio, nitrogen uptake efficiency, and morphogenic efficiency (Moreau *et al.*, 2015).

In *C. annuum*, the introduction of nitrogen is reported to have led to an increased pod number (83.47%), seed number (19.27%), plant height (42.7%), branch number (37.7%) and leaf number (63.5%) (Molla *et al.*, 2019). High accumulation of nitrogen can trigger the release of growth hormone factors such as cytokines and gibberellins resulting in an increased plant height. The phenological properties, such as flowering, maturity, and pod settings of hot pepper plants are also affected by nitrogen (Molla *et al.*, 2019). The addition of appropriate N concentration could improve production yield in *C. annuum*, however, excessive nitrogen may damage its reproductive structures (Aliyah, 2000). Besides, differences in available nitrogen levels significantly affect plant VOC production (Holopainen and Gershenzon, 2010) while deficiency alters VOC emission (Lou and Baldwin, 2004). For instance, tomato plants exposed at high nitrogen concentration were able to produce various terpenes, such as β -caryophyllene, α -terpinene, β -pinene, (+)-4-carene, α -copaene, p-cymene, β -phellandrene, and α -humulene (Islam *et al.*, 2017).

Approximately 1,700 of the 100,000 phy-

tochemicals are classified as volatile organic compounds (VOCs) (Holopainen and Gershenzon, 2010; Spinelli *et al.*, 2011). As plants remain the top VOC emitter, its annual emission reaches around 760 Tg ($^{\circ}\text{C}$) across the globe (Samburova *et al.*, 2019). Its crucial role in plants can be attributed to the plant-plant and plant-biota interaction to act as a defense mechanism against invasive insects, alleviation of environmental stress, and signal between and within plants (Spinelli *et al.*, 2011). These volatile emissions could be traced from the bursting of plant organs with stored VOCs due to herbivory or de novo synthesis as a response to damaged organs (Holopainen and Gershenzon, 2010). In some cases, VOCs are released by carnivorous plants to attract their prey (Kreuzwieser *et al.*, 2014). Some of the most common VOCs identified from plants include isoprene, monoterpenes, and sesquiterpenes (Samburova *et al.*, 2019). Its emission can greatly vary depending on the plant species, type of organ, stage of development, and ecological conditions (Jassbi *et al.*, 2010).

The main classes of VOCs previously identified from different species of capsicums belong to aliphatic branched-chain hydrocarbons, terpenes, aldehydes, and alcohols (Ziino *et al.*, 2009). Previous studies also identified the different VOCs that are naturally produced in chilli pepper plants. For instance, a total of 64 plant volatiles identified as terpenes and its derivatives, pyrazines, alcohols, aldehydes, ketones, esters, hydrocarbons, and carotenoid derivatives were detected from powdered chilli pepper fruits using HS-SPME/GC-MS method (Cirlini *et al.*, 2019). These VOCs, in combination with non-VOCs are often associated with the fruit's flavor and aroma giving it its unique taste and smell (Eggink *et al.*, 2012a, 2012b; Cirlini *et al.*, 2019). Similarly, a variety of VOCs were also detected from chili pepper seeds (Silva *et al.*, 2013). From these studies, only few reported the influence of elevated nitrogen concentrations on the profile of VOCs in capsicums. Therefore, this study aims to determine the effects of nitrogen nutrition on the VOCs profile of *C. annuum* var. *longum*.

Materials and Methods

Plant cultivation, nitrogen treatment, and sample collection

Forty-five-day old seedlings of *C. annuum* var. *longum* were acquired from the Bureau of Plants Industry, Manila, Philippines. Plants were allowed to grow for 4 weeks in a nursery at De La Salle University, Manila for the experimental process. Plants received similar treatments such as watering and occasional rotation to attain uniform treatments. For nitrogen (N) treatments, urea was used as N source. Three concentrations were prepared by dissolving 1.8 g (1x), 4.8 g (2.5x) and 9 g (5x) of urea in 1L distilled water. The N-treatment started when 500 mL of N solutions were introduced to the soil. Untreated plants were given the same amount of water (0x). Control and treated *C. annuum* var. *longum* leaves were collected after 4 weeks. All treatments were conducted in triplicates.

Volatile organic compound analysis

From each plant, a total of three leaves were collected from the top, middle, and bottom parts of the plant. Then, samples were acclimatized for 1 hour before the analysis. Collected leaf samples were placed in a 500 mL Pyrex® Erlenmeyer flask sealed with parafilm and covered with aluminum foil. The flask served as a headspace chamber for VOC analysis (Tavera *et al.*, 2018). Volatile organic compounds were extracted using a Supelco® 100 µm Polydimethylsiloxane Solid Phase Microextraction fiber (SPME). PDMS fiber was selected in this study to include both low and high molecular mass volatile compounds in addition to the short desorption time and thermal stability of the fiber as reported by the group of Perera *et al.* (2020). Initially, the fibers were conditioned at 250°C for 30 min to remove chemical residues. The flasks containing the samples and the fiber were heated at 30°C-40°C for 15 min to initiate the release of VOCs (Silva *et al.* 2017). While the samples were heating, the fiber was exposed inside the flask to collect VOCs from the headspace. For the control,

the SPME fibers were exposed to flask without the leaf samples (Tavera *et al.*, 2018).

Gas Chromatography-Mass Spectrometry

The analysis of VOCs was performed using Gas Chromatography-Mass Spectrometry (Shimadzu GC-MS QP2020) equipped with SH-Rxi-5Sil MS capillary column (30m x 0.25 mm x 0.25 µm). The samples were passed through a pre-installed SPME Inlet liner and Merlin Microseal Septa General Purpose Kit. The injection temperature was set to 250°C with an initial temperature of 50°C for 5 minutes and a ramping temperature of 1°C per minute until it reached 200°C with a constant helium gas flow rate of 1 mL/min. The mass spectra of the N treated samples were compared with the control and the distinct VOC mass spectra found were identified using the NIST 2017 Mass Spectral Library and Wiley Registry 11th edition (Tavera *et al.*, 2018).

Results

The effect of high N-supply on the VOC profile of *C. annuum* var. *longum* is presented in Figure 1. After the four-day treatment, the plants which received urea higher than 1.8 g/L concentration started to exhibit curling and wrinkling of leaves. The plants treated with 5x N dried up within 48 h and were not included for further analysis. This type of response is typical in plants when urea is suddenly introduced. N-treated plants also developed black spots on the leaves which may be caused by a weakened innate defense system or N toxicity. Furthermore, aphids and whiteflies appeared on the N-treated plants four days after treatment. On the other hand, the untreated control plants showed normal growth and absence of insects, suggesting that the N supply may have caused the observed responses and the attraction of insects to N-treated plants. The sensitivity of *C. annuum* var. *longum* to N-treatment further suggests that this species is a very good candidate for N-stress studies.

Figure 1. Representatives of *C. annuum* var. *longum* treated with 0 g/L, 1.8g/L, 4.5 g/L and 9 g/L Urea after 48 h supply.

The VOCs collected from the leaves of N-treated setups (1x and 2.5x) and compared to those of the control (0x) are shown in Figure 2A-F. In general, the VOCs common in N-treated and untreated *C. annuum* var. *longum* belonged to the group of alkanes, sesquiterpenes, carboxylic acids, aldehydes,

ketones, alcohols, and alkenes. Among these plant volatiles, Butanoic acid, 3-hexenyl ester [peak #6], (E)-, Butanoic acid, hexenyl ester [peak #7], Hexanoic acid, 3-hexenyl ester, (Z)- [peak #8], Hexanoic acid, 4-hexen-1-yl ester [peak #9], cis-3-Hexenyl cis-3-hexenoate [peak #10], and 4-Pentenoic acid

Figure 2. (A) GC-MS analysis of the volatile organic compounds identified from *C. annuum* var. *longum*. Magnified version of the chromatograms with retention times are shown in (B) 3.40 – 3.60 (C) 5.05 – 5.80 (D) 12.60 – 12.80 (E) 15.45 – 15.70 (F) 19.02 – 19.12. The detected compounds were 3-Hexenal [1, 3.527], 2-Hexenal [2, 5.120], 3-Hexen-1-ol, (Z)- [3, 5.207] and 2-Hexen-1-ol, (E)- [4, 5.581], 1-Hexanol [5, 5.692], Butanoic acid, 3-hexenyl ester [6, 12.776], (E)-, Butanoic acid, hexyl ester [7, 12.674], Hexanoic acid, 3-hexenyl ester, (Z)- [8, 15.557], Hexanoic acid, 4-hexen-1-yl ester [9, 15.561], cis-3-Hexenyl cis-3-hexenoate [10, 15.61315], and 4-Pentenoic acid 2-methyl-, hexyl ester [11, 15.666] and Octane, 1,1'-oxybis- [12, 19.096]. Samples for 1x, 2.5x and control are shown as black, purple and blue lines, respectively.

2-methyl-, hexyl ester [peak #11] were the ones detected from N-treated plants.

Other plant volatiles, such as 3-Hexenal [peak #1], 2-Hexenal [peak #2], 3-Hexen-1-ol, (Z)- [peak #3], 2-Hexen-1-ol, (E)- [peak #4] and 1-Hexanol [peak #5], which are part of the green leaf volatile synthesis were detected as shown in peak areas on the GC-MS analysis (Fig. 2A-F). The matching factor and structure of the plant volatiles are shown in Table 1. Among these volatiles, only 2-hexenal, 3-Hexen-1-ol, (Z)- and 1-Hexanol were identified from the control samples. The 3-Hexen-1-ol, (Z)- and 1-Hexanol identified from the treated samples have increased peak areas, whereas 2-hexenal only increased in plants treated with 2.5x urea. On the other hand, 3-hexenal was identified only from plants treated with 1x and 2.5x urea, whereas 2-Hexen-1-ol, (E)- was only identified in plants treated with 2.5x urea. Also, the peak area of Octane, 1,1'-oxybis- [peak #12] increased as N concentration increased. These results suggest that the VOCs detected were in response to the increased concentration of N. The characteristics of each VOCs, including the retention time, matching factor, and structure can be found in Table 1.

Discussion

In general, plants respond to changes in the concentration of exogenous nitrogen (N) present in soil from molecular up to the physiological level. These changes elicit an appropriate response that may benefit or harm the plants (Redillas *et al.*, 2011; Wahocho *et al.*, 2016; Jansson *et al.*, 1986; Jauset *et al.*, 1998; Lu *et al.*, 2007; Veromann *et al.*, 2013; Islam *et al.*, 2017). The application of nitrogen in chili plants in an appropriate amount can affect the production of carbohydrates, protein, ash contents, fibers, fats, and nutrients. An increased nitrogen level (up to 250 Kg/ha Urea) in the cultivation of capsicum can improve the plant's height, number of branches, number of days before flowering, and length of fruits (Wahocho *et al.*, 2016).

However, improper application of nitrogen may trigger negative effects on the plants. For instance, increased N in plants could increase the population of insects which often leads to insect infestation and herbivory (Jansson *et al.*, 1986; Jauset *et al.*, 1998; Lu *et al.*, 2007; Veromann *et al.*, 2013; Islam *et al.*, 2017). In our study, we observed that whiteflies and aphids became attracted to chili plants when treated with N. The silverleaf whitefly (*Bemisia tabaci*) is one of the most common insect pests in chili plants. It serves as a vector to transmit *Begomovirus* which can hamper the growth and development of the plant (Firdaus *et al.*, 2011; Salas *et al.*, 2015; Saad *et al.*, 2015). Similarly, the green peach aphid (*Myzus persicae*) and the cotton aphid (*Aphis gossypii*) have been reported as pests and viral vectors in Chili plants (Talaga-Taquinas *et al.*, 2020). This attraction of insects to plants with higher nitrogen content has also been documented in other plants, such as potato and tomato plants (Jansson *et al.*, 1986; Jauset *et al.*, 1998; Islam *et al.*, 2017; Veromann *et al.*, 2013). In tomato plants, the greenhouse whitefly (*Trialeurodes vaporariorum*) has shown a high preference for plants with higher N content for egg-laying and oviposition compared to plants with lower N content (Jauset *et al.*, 1998). Green peach aphids infestation has also been detected in potato plants with high N content (Jansson *et al.*, 1986). Similarly, pollen beetles and cabbage seed weevils became more attracted to oilseed rape plants when high N concentration was introduced to the plant (Veromann *et al.*, 2013).

The emergence of black spots on chili plants treated with 2.5x N has also been reported in case of oilseed rape plants exposed to high nitrogen concentration (Veromann *et al.*, 2013). The appearance of black spots may have been due to either a weakened innate defense system or N toxicity. However, further studies are required to confirm the cause of the emergence of these black spots on N-treated chili plants.

It is widely known that plants release VOCs as a response to abiotic stresses, to reduce their negative impact, and trigger

Table 1. Plant volatile organic compounds identified from *C. annuum* var. *longum* treated with 1x and 2.5x urea. The retention time, matching factor and structure of the VOCs are shown in the figure.

Peak Number	Retention time (min)	Match Factor	Compound	Structure
1	3.527	95	3-Hexenal	
2	5.120	97	2-Hexenal	
3	5.207	97	3-Hexen-1-ol, (Z)-	
4	5.581	98	2-Hexen-1-ol, (E)-	
5	5.692	97	1-Hexanol	
6	12.674	97	Butanoic acid, hexyl ester	
7	12.776	96	Butanoic acid, 3-hexenyl ester, (E)-	
8	15.557	93	Hexanoic acid, 3-hexenyl ester, (Z)-	
9	15.561	93	Hexanoic acid, 4-hexen-1-yl ester	
10	15.613	97	cis-3-Hexenyl cis-3-hexenoate	
11	15.666	83	4-Pentenoic acid 2-methyl-, hexyl ester	
12	19.096	95	Octane, 1,1'-oxybis-	

plant-to-plant interactions i.e. water stress (Salerno *et al.*, 2017), drought stress (Hansen and Seufert, 1999), salt stress (Teuber *et al.*, 2008), oxidative stress (Vuorinen *et al.*, 2004), increased temperature (Maleknia *et al.*, 2009), light intensity (Hansen and Seufert, 2003), mechanical damage (Kirstine and Galbally, 1998; 2004), and nutrient deficiency (Gouinguene and Turlings, 2002). A range of plant volatiles has been identified in this study that may function in defense against a wide array of stresses i.e. Butanoic acid, 3-hexenyl ester, (E)-; Butanoic acid, hexyl ester; Hexanoic acid, 3-hexenyl ester, (Z)-; Hexanoic acid, 4-hexen-1-yl ester; cis-3-Hexenyl cis-3-hexenoate; and 4-Pentenoic acid 2-methyl-, hexyl ester. The different compounds detected in this study that have hexanoic acid-containing moiety may play an important role in the plant's defense system. Studies report that hexanoic acid is a priming inducer that triggers plants defense against fungi (Aranega-Bou *et al.*, 2014; Finiti *et al.*, 2014). For instance, treatment with hexanoic acid helps the plant to regulate oxidative stress caused by plant-pathogen interaction. This oxidative stress protection was previously reported in tomato plants infected with *Botrytis cinerea* (Finiti *et al.*, 2014). The protective property of hexanoic acid was also reported to be effective against *Pseudomonas syringae* (Vicedo *et al.*, 2009). Similarly, a total of 16 VOCs were detected from tomato plants treated with different concentration of N. These include an alkane (Farnesan), Sesquiterpene (α -Humulene, β -Caryophyllene, α -Cedrene, Longifolene, α -Copaene, d-Elemene), Alkyl aldehyde (Nonanal), Monoterpene (β -Phellandrene, p-Cymene, α -terpiene, (+)-4-carene, Myrcene, β -pinene, α -pinene), and Alkyl aldehyde (Heptanal) (Islam *et al.*, 2017). Among these VOCs, the nonanal and p-cymene were also detected in chili plants, however, both were detected on treated and untreated plants.

The green leaf volatiles (GLV) with an increased peak in the N- treated capsicum plants were 2-Hexenal, 1-Hexanol, and 3-Hexen-1-ol, (Z)-. The detection of the

3-Hexenal, 2-Hexenal, 3-Hexen-1-ol, (Z)- and 2-Hexen-1-ol, (E)- suggest that the GLV synthesis is active in the N-treated plant. The synthesis of GLV begins when galactolipids and phospholipids in the thylakoid membranes are digested with lipase enzymes releasing linoleic acid; the oxidation of a diene catalyzed by the 13-Lipoxygenase produces 13-hydroperoxide, which will then undergo further oxidation to produce (Z)-3-Hexenal or n-Hexenal through isomerization; when treated with aldehyde reductase, aldo/keto reductase or alcohol dehydrogenase 3-Hexen-1-ol, (Z)- and 2-Hexen-1-ol, (E)- can be produced, respectively (Kunishima *et al.*, 2016). The emission of (Z)-3-Hexen-1-ol in plants can have different functions such as the enhancement of defense against insect pests e.g. the (Z)-3-Hexen-1-ol function to elicit a defense system in tea plants, *Camellia sinensis*, against the attack by the tea geometrid *Ectropis oblique* by increasing VOC emission and the activity of polyphenol oxidase. In return, the release of VOCs attracted a parasitoid of the tea geometrid (Xin *et al.*, 2016). The (Z)-3-Hexen-1-ol is also known to attract other beneficial insects, such as *Stethorus p. picipes*, *Orius tristicolor*, *Anagrus daanei*, Syrphidae, Braconidae, and Micro-Hymenoptera (James, 2005). Similarly, the detected 4-Pentenoic acid, 2-methyl-, hexylester, in plant treated with nitrogen, initiates plants' mechanism for insect control (Janzantti *et al.*, 2012; Tewari *et al.*, 2014). On the other hand, the detected hexan-1-ol was previously observed to have a repellent effect on *Ceutorhynchus assimilis* (Smart and Blight, 1997). Nevertheless, the GLVs detected in this study suggest the presence of compounds that establish an insect-plant relationship (Smart and Blight, 1997; James, 2005; Xin *et al.*, 2016; Kunishima *et al.*, 2016).

In conclusion, excessive concentration of nitrogen weakens the overall plant health making it more susceptible to herbivory and infestation (Islam *et al.*, 2017; Veromann *et al.*, 2013). In this study, we have identified a range of volatiles that may function in defense against a wide array of stresses. The majority of the detected volatiles were pro-

duced by plants in response to nitrogen-induced insect infestation and the presence of black spots. Although these volatiles have been reported in previous studies, this is the first study that reports their involvement in *C. annuum* when exposed to increased nitrogen conditions. We believe that the results obtained from this study will contribute in understanding the mechanisms behind volatile emission in *C. annuum* var. *longum* in response to N-nutrition. Also, the profile of plant volatiles is useful especially in the field of agriculture to avoid infestation and disease outbreak.

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Προσδιορισμός πτητικών οργανικών ενώσεων σε φυτά *Capsicum annuum* var. *longum* που αναπτύσσονται κάτω από διαφορετικές συγκεντρώσεις αζώτου

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Περίληψη Η έκλυση πτητικών οργανικών ενώσεων (VOC) στα φυτά ενεργοποιείται από διάφορους βιοτικούς και αβιοτικούς παράγοντες, όπως η ανεπάρκεια σε θρεπτικά στοιχεία, η περιβαλλοντική καταπόνηση και οι προσβολές από παθογόνα και εχθρούς. Για παράδειγμα, η ελλειπής ή υπερβολική παροχή αζώτου (N) στα φυτά είναι δυνατό να προκαλέσει εσωτερική καταπόνηση που θα μειώσει τη σταθερότητα και την ανοσία τους καθιστώντας τα ευπαθή σε προσβολές από παθογόνα και εχθρούς. Στην παρούσα εργασία πραγματοποιήθηκε εκχύλιση των πτητικών οργανικών ενώσεων από φυτά *Capsicum annuum* var. *longum* (Solanaceae) μετά από εφαρμογή αζώτου (1,8 g/L, 4,5 g/L, και 9 g/L ουρία) με τη χρήση 100 μm ίνας Στερεάς Φάσης Μικροεκχύλισης (Solid Phase Microextraction, SPME) επικαλυμμένης με πολυδιμεθυλοσιλοξάνιο (PDMS). Με τη χρήση αέριας χρωματογραφίας-φασματομετρίας μάζας (GC-MS) οι πτητικές ενώσεις, που εκλύθηκαν από τα φυτά στα οποία είχε γίνει εφαρμογή αζώτου, προσδιορίστηκαν ως 3-εξενυλεστέρας του βουτανοϊκού οξέος, (E)-εξυλεστέρας του βουτανοϊκού οξέος, (Z)-3-εξενυλ εστέρας του εξανοϊκού οξέος, 4-εξεν-1-υλεστέρας του εξανοϊκού οξέος, cis-3-εξενυλ-cis-3-εξανοϊκός εστέρας, και εξυλεστέρας του 2-μεθυλ-4-πεντενοϊκού οξέος. Μεταξύ αυτών των πτητικών ενώσεων, ο 3-εξενυλεστέρας του βουτανοϊκού οξέος έδειξε την πιο χαρακτηριστική κορυφή (peak) στα φυτά που είχαν δεχθεί επέμβαση με άζωτο σε σύγκριση με το μάρτυρα. Επιπλέον, στα φυτά αυτά ανιχνεύθηκαν τα πτητικά πράσινων φύλλων (Green Leaf Volatiles, GLV) 3-εξανάλη, 2-εξανάλη, (Z)-3-εξεν-1-όλη, (E)-2-εξεν-1-όλη, και 1-εξανόλη. Ο προσδιορισμός των πτητικών ουσιών στα φυτά παρέχει χρήσιμες πληροφορίες που μπορούν να αξιοποιηθούν σε γεωργικές πρακτικές και στη φαινοτύπιση των φυτών.

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