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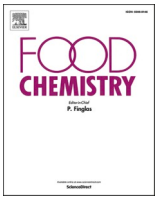

Amino acids, UPLC-MS phenolic metabolites and multivariate approach for elucidating the effect of two growing conditions on growth and yield attributes in okra pods and leaves

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ABSTRACT

Okra is one of the highly nutritious indigenised food crops in Southern Africa. The study intended to evaluate plant growth, yield, amino acids, and untargeted semi-targeted phenolic metabolites grown concurrently at greenhouse and micro-plot under open-field conditions. Yield and growth attributes: stem diameter, plant height, number of branches per plant, number of pods per plant, pod weight, pod length and pod diameter were higher in the micro-plot under open-field conditions. Sixteen amino acids were quantified and arginine, serine, glycine, aspartate, glutamate, alanine, proline, histidine, threonine, methionine, lysine, tyrosine, leucine, valine, and isoleucine were higher in leaf samples grown in micro-plots under open-field than greenhouse samples. UPLC-MS phenolic metabolites associated with the two growth conditions were quercetin 3-galactoside, succinyl adenosine, quercetin 3-lathyroside, isotan b and icariside F2b which were either highly upregulated or downregulated. Growth conditions can be used to manipulate the accumulation of free amino acids and phenolic metabolites.

1. Introduction

Okra (*Abelmoschus esculentus* (L.) Moench) is a subtropical food crop that belongs to the Malvaceae botanical family. It is now one of the indigenised vegetable crops in African countries due to its nutrition and human health medicinal properties. The African Union have set a priority to improve Africa's food nutrition security through regenerating resilience in crop adaptation (Africa United Nations, 2021). Given the estimated population growth that will be reaching 9.8 billion in 2050 (Africa United Nations, 2021), there is more pressure against food production for maximised growth and yield attributes. The problem of food security further contributes to the awareness of use-efficiently in the production of land and water (Africa United Nations, 2021). Many households in Africa face challenges related to food insecurity (Mudzielwana et al., 2022). This issue continues to be a major factor contributing to malnutrition, especially in low and middle-income countries (Siddiqui et al., 2020).

Okra is a drought-tolerant crop that performs best in open field conditions where the optimum climate reaches the subtropical to tropical degree in the summer period (Mkhabela et al., 2022). Due to this reason, okra growth is seasonal and its production becomes inadequate during the winter season. Farming in a greenhouse condition has gained popularity in South Africa due to the fact related to crop seasonality which affects the off-season fruit and vegetable availability (Yano & Cossu, 2019). Greenhouse growth condition is often temperature regulated to match growing crops optimum requirements (Yano & Cossu, 2019). In okra production, greenhouse-modified macro-climate induced growth and quality two-fold higher than in an open-field condition during the winter season (Mishra et al., 2011). However, such a growing environment could influence a holistic profile of primary metabolites which further influences the secondary metabolites and plant adaptation. The introduction of production systems that will result in the enhancement of phytochemicals in plant production while improving the crop adaptation, growth and yield requires extensive metabolite control because background levels of other metabolites are largely unknown. Plant metabolomics is a new research discipline, which aims to develop a comprehensive approach to metabolite detection, identification and quantification (Mishra et al., 2017). They allow the identification of the most important compounds underlying differences between genotypes or phenotypes and they can improve crop

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adaptations (Mishra et al., 2017). Plant metabolomics is the large-scale study of metabolites within plant tissues, which helps to understand plant physiology and biology because metabolites represent the endpoint of biological activities and they assist in measuring crop response to environmental cues (Barbehenn & Constabel, 2011).

Okra pod and leaf are rich sources of primary metabolites including protein, amino acids and some nutritional elements (Mokgalabone et al., 2023; Salameh, 2014). Okra may be considered a medicinal plant due to its nutritional enhancement in dipping the blood glucose level in hyperglycemia induced by diabetes (Kumar et al., 2010). It is also an important component of preventive therapy in the management of diabetes and its related complications. Moreover, the pods are also used to reduce diarrhoea and acute inflammation dysentery (Middleton, 2000). The plant is also used to reduce kidney catarrhal infections, ardor urine, dysuria irritation of the stomach and gonorrhea. Okra has found medical applications as a plasma replacement or blood volume expander (Kumar et al., 2010; Middleton, 2000). Mokgalabone et al. (2023) showed how different amino acids, proteins and nutritional elements can be interrelated with growth conditions. On the other hand, okra is a powerhouse for secondary metabolites such as isoleucine, fatty acids, γ-aminobutyrate, glutamine, asparagine, unsaturated lipids, choline, phosphocholine and cinnamic acid which were associated with quality changes in okra during postharvest senescence (Liu et al., 2017). According to a Liquid Chromatography-Mass Spectrometry (LC-MS) report by D'Urso et al. (2020), okra owns specialised phenolic acid and flavonoid metabolites associated with antioxidant activity. Furthermore, phenolic acids including those belonging to the hydroxybenzoic and hydroxycinnamic acids such as 4-hydroxybenzoic acid, benzoic acid β-D-glucopyranosyl ester, tecomin, 3-methoxy benzoic acid, 3,4-dimethoxy benzoic acid, ferulic acid, 4-O-β-D-glucopyranoside, coumaric acid, sinapic acid, and 4-hydroxycinnamic acid were predominated I n okra pods (D'Urso et al., 2020). In addition, the pods exhibited some flavonoid components from the quercetin and myricetin glycosides (D'Urso et al., 2020). However, no information is available on the effect of growing conditions on primary and secondary metabolites in the case of okra despite their direct or indirect contribution to growth and yield attributes. This study is the first to document the primary and secondary metabolites in okra cultivated under two different growing conditions to enhance its growth and yield attributes. Therefore, the current study objectives envisaged comparing growth, yield and some metabolites of an okra pod and leaf tissues grown in a greenhouse and micro-plot under open-field conditions.

2. Material and methods

2.1. Growing conditions, plant material and okra seedling establishment

The experiments were conducted concurrently in a greenhouse and under open field in micro-plot conditions at the Green Biotechnologies Research Centre of Excellence (GBRCE), University of Limpopo, South Africa (23°53"10′S, 29°44″15′E) from October to December 2021 after some preliminary optimisation trials (Mokgalabone et al., 2023). The greenhouse structure had ambient day/night temperatures averaging 22–28 °C, with maximum temperatures controlled using thermostatically activated fans and a wet wall on the other end to regulate relative humidity averaging 45 %. Open field micro-plots were characterised by hot-dry weather with day/night temperatures ranging between 17 and 31 °C and precipitation mean average of less than 560 mm (Mokgalabone et al., 2023).

Seeds of okra (Starke Ayres Pty Ltd., South Africa), cultivar Clemson of moderately ribbed and medium green straight spineless pods, were sown in a disinfected 200 polystyrene seedling trays filled with Hygromix® growing medium up to 4 weeks of post-emergence as described by Mokgalabone et al. (2023). The growth medium enclosed consisted Hygromix® growing medium, pasteurized (300 °C for 45 min) loam soil and fine sand at a 2:1:1 (*v*/v/v) ratio. Before transplanting, 30 cm pots were filled with the growth medium and in each pot, one okra seedling was planted. The micro-plot condition was prepared in the field by demarcating an area of 10 × 5 m, where the pots (with growth medium) were set underneath at 30 cm height down the surface, to enable plants growth in a homogenous growth medium as described by Mokgalabone et al. (2023). Then some pots were kept on the benches of the greenhouse using a spacing of 50 × 40 cm. Irrigation was carried out using 250 mL of non-chlorinated tap water whenever the moisture probes (T10 Bodentester, South Africa) indicated a status of dryness as described by Mpai et al. (2022).

2.2. Determination of growth and yield okra attributes

After initiating the experiment, growth parameters including stem diameter and plant height were measured once per week for up to eight weeks of vegetative growth as described by Mokgalabone et al. (2023). Yield parameters including the number of branches per plant, number of pods per plant, fresh pod weight (g), pod length and diameter (mm), were measured and recorded as described by Mokgalabone et al. (2023). The leaf and pod okra samples from greenhouse and micro-plot condition were then oven dried at 40 °C until a constant dried mass was achieved. All the samples were then ground to pass through a 250 μm diameter mesh screen and they were stored in a cold room (4 °C) condition until analysis the analysis of metabolites.

2.3. Determination of amino acids contents

Amino acid sample preparation was performed following the manufacturer's instructions for the Ultra Derivitization Kit as described in Mpai et al. (2018). The analysis of individual amino acids including Arginine (Arg), Serine (Ser), Glycine (Gly), Aspartate (Asp), Glutamate (Glu), Alanine (Ala), Proline (Pro), Histidine (His), Threonine (Thr), Methionine (Met), Lysine (Lys), Tyrosine (Tyr), Leucine (Leu), Phenylanine (Phe), Asparagine (Asn) and Glutamine (Gln) was performed using the ultra-performance liquid chromatography analysis following the methods described by Mpai et al. (2018) without modification.

2.4. Determination of UHPLC-QTOF-MS bioactive metabolites of okra grown in different growing conditions

The sample preparation for analysis of Ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS) phenolic metabolites was based on the methods described by Mpai and Sivakumar (2020), whereby 0.2 g of the okra sample grown in different growing conditions was homogenised with 15 mL of 50 % methanol acidified by 1 % formic acid. Thereafter the samples were vortexed for 1 min, followed by extraction in an ultrasonic bath for 1 h, centrifuged at 14,000 rpm for 5 min and the supernatant was transferred into 1.5 mL vials for analysis. The untargeted and targeted metabolites analysis was performed using a high-resolution Waters Synapt G2 Quadrupole time-of-flight (QTOF) mass spectrometer (MS) connected to a Waters Acquity ultra-performance liquid chromatograph (UPLC) (Waters, Milford, MA, USA). This was further connected to a column eluate that first passed through a Photodiode Array (PDA) detector before going to the mass spectrometer, allowing simultaneous collection of UV and MS spectra. Electrospray ionization was used in negative mode with a cone voltage of 15 V, desolvation temperature of 275 °C, desolvation gas at 650 L/h, and the rest of the MS settings optimized for best resolution and sensitivity. Data were acquired by scanning from *m/z* 150 to 1500 *m/z* in resolution mode as well as in MSE mode. In MSE mode two channels of MS data were acquired, one at a low collision energy (4 V) and the second using a collision energy ramp (40–100 V) to obtain fragmentation data as well. Leucine enkaphalin was used as lock mass (reference mass) for accurate mass determination and the instrument was calibrated with sodium formate. Separation was achieved on a Waters HSS T3, 2.1 × 100 mm, 1.7 μm column. An injection volume of 2

μL was used and the mobile phase consisted of 0.1 % formic acid (solvent A) and acetonitrile containing 0.1 % formic acid as solvent B. The gradient started at 100 % solvent A for 1 min and changed to 28 % B over 22 min in a linear way. It then went to 40 % B over 50 s and a wash step of 1.5 min at 100 % B, followed by re-equilibration to initial conditions for 4 min. The flow rate was 0.3 mL/min and the column temperature was maintained at 55 °C. Compounds were quantified relatively against a calibration curve established by injecting a range of quercetin standards from 0.5 to 100 mg/L quercetin.

2.5. Statistical analysis

Generated data between the two-growing condition (greenhouse and micro-plot under open-field) were subjected to statistical analysis using GenStat 18th version statistical package (VSN International, Hempstead, UK), to separate for significance among treatments, *t*-test was employed at the significance level of 5 %. Chemometric data analysis were performed using SIMCA ver 13.0 (Umetrics, Malmo, Swedan) software to create an explorative Principle Component Analysis (PCA) and supervised Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) models similar to the description of Mpai et al. (2022).

3. Results and discussion

3.1. Effect of different growing conditions of growth and yield parameters in okra

In this study, growing okra in a micro-plot under open-field and greenhouse conditions affected growth parameters: stem diameter and plant height. Fig. 1A and B demonstrated the effectiveness of the micro-plot under open-field growing conditions in improving the stem diameter and plant height over the greenhouse condition. Changes in the stem diameter initiated in week two when samples grown under the micro-plot under open-field were 50, 53, 56, 70, 71, 75 and 80 % higher than their counterparts' samples grown in a greenhouse condition during their respective week period interval. As shown in Fig. 1B, plant height variation between samples grown in the two compared growth conditions initiated in week four when samples grown in a micro-plot under an open field were higher than samples grown in a greenhouse condition. On the other hand, yield components including the number of branches per plant, number of pods per plant, fresh pod weight, pod length and pod diameter were significantly ($p \leq 0.05$) higher in samples grown in micro-plots under the open-field condition in comparison to those in a greenhouse setting as shown in Table 1. The number of branches and number of pods per plant in micro-plots under open-field condition were three times higher than in greenhouse samples.

The results herein were in contrast with those concluded by Mishra et al., 2011 which demonstrated a greenhouse to promote growth and yield in okra pod. Potential attribution of the obtained results could be related to temperature regulation, light emission and humidity of the greenhouse condition (Katsoulas & Kittas, 2008). Given the trend of the results, it is obvious that the micro plots under open-field conditions induced the drought and light adaptation mechanisms which have synergistic relationships with primary metabolites and further to secondary metabolites (Batista-Silva et al., 2019). According to Romaisa et al. (2015), stem diameter and plant height share a common goal to strive for acquiring optimum sunlight used during the photosynthesis process. Furthermore, stem diameter and plant height are important agronomic traits for increasing okra yield, therefore thin stems and short plant height are undesirable due to their potential of inducing water lodging (Eshiet & Brisibe, 2014; Yadav et al., 2010).

Table 1
Effect of growing conditions on some yield components in okra.

	Number of branches per plant	Number of pods per plant	Fresh pod weight (g)	Pod length (mm)	Pod diameter (mm)
Greenhouse	3.00 ± 0.17	5.00 ± 0.29	7.73 ± 0.88	139.45 ± 6.3	24.27 ± 0.62
Micro-plot	8.00 ± 0.24**	16.00 ± 0.4**	11.64 ± 2.02**	172.43 ± 6.2**	27.26 ± 0.8[a]**

Values are expressed as means ± standard error ($n = 15$). For all the values within a column, different letters superscripts are significantly different at ns = not significant, **$p \leq 0.05$.

3.2. Effect of growing condition on the amino acid profile in okra leaves and pods

Amino acids are directly associated with the growth and development of plants. Okra leaf samples grown on the micro-plots under open-field conditions outperformed the samples grown in a greenhouse condition such that: all amino acids including Arg (0.97 mg/kg), Ser (0.81 mg/kg), Gly (0.96 mg/kg), Asp (2.07 mg/kg), Glu (2.74 mg/kg), Ala (0.95 mg/kg), Pro (0.90 mg/kg), His (0.46 mg/kg), Thr (0.84 mg/kg), Met (0.52 mg/kg), Lys (1.32 mg/kg), Tyr (0.66 mg/kg), Leu (0.66 mg/kg), ILe (0.81 mg/kg) and Val (0.81 mg/kg) were significantly ($p > 0.05$) higher than those in a greenhouse condition in exception of Phe (0.61 mg/kg) which showed a non-significant variation as shown in Table 2. On the other hand, some amino acid compounds such as Ser (0.51 mg/kg), Gly (0.52 mg/kg), Ala (0.48 mg/kg), His (0.27 mg/kg), Thr (0.41 mg/kg), Met (0.22 mg/kg), Lys (0.70 mg/kg) Tyr (0.22 mg/kg), Val (0.52 mg/kg) and ILe (0.38 mg/kg) were significantly ($p > 0.05$) maintained by the micro-plots under open-field growing condition in comparison to those grown in a greenhouse condition in okra pod samples (Table 2). Yet, the growing conditions did not affect the contents of Arg, Asp, Glu, Pro, Leu and Phe in pod okra samples as shown in Table 2.

The results herein can be attributed to the fact that amino acids are precursors of photosynthesis which were reported to be higher in the old leaves than in the young leaves and pods (Gupta, 2019). The rate of

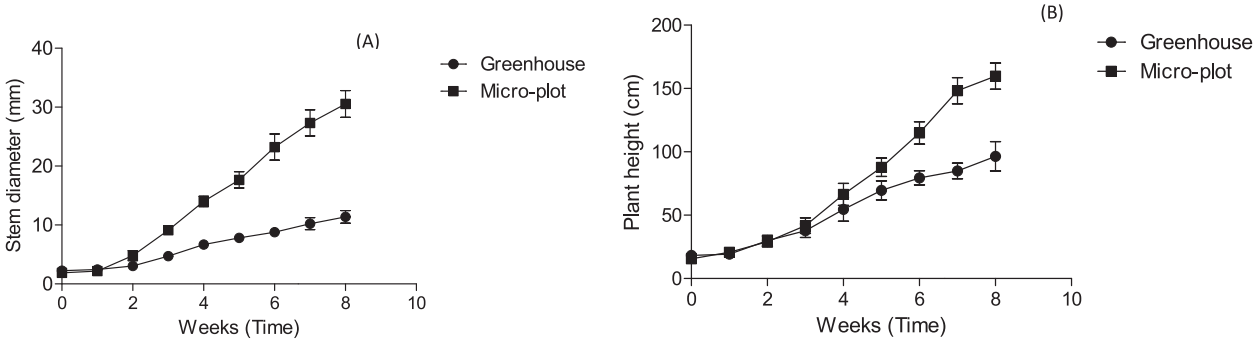

Fig. 1. Plant growth in okra seedlings grown in open-field and greenhouse conditions. A = Stem diameter; B = Plant height. $n = 15$.

Table 2
Amino acid concentrations in okra leaf and pod grown in different growing conditions (mg/kg).

	Okra leaf		Okra pod	
Amino acids (mg/kg)	Greenhouse	Microplot	Greenhouse	Microplot
Arginine (Arg)	0.51 ± 0.21	0.97 ± 0.44**	0.94 ± 0.34 ns	1.06 ± 0.76 ns
Serine (Ser)	0.57 ± 0.67	0.81 ± 0.55**	0.28 ± 0.56	0.51 ± 0.86**
Glycine (Gly)	0.56 ± 0.12	0.96 ± 0.24**	0.28 ± 0.67	0.52 ± 0.45**
Aspartate (Asp)	1.50 ± 0.77	2.07 ± 0.76*	1.11 ± 0.33 ns	1.11 ± 0.64 ns
Glutamate (Glu)	2.22 ± 0.52	2.74 ± 0.89**	3.66 ± 0.12 ns	3.64 ± 0.23a
Alanine (Ala)	0.51 ± 0.66	0.95 ± 0.07**	0.23 ± 0.66	0.48 ± 0.55**
Proline (Pro)	0.58 ± 0.84	0.90 ± 0.95**	0.38 ± 0.71 ns	0.40 ± 0.67 ns
Histidine (His)	0.30 ± 0.76	0.46 ± 0.43**	0.17 ± 0.23	0.27 ± 0.99**
Threonine (Thr)	0.53 ± 0.71	0.84 ± 0.39**	0.24 ± 0.43	0.41 ± 0.43**
Methionine (Met)	0.23 ± 0.54	0.52 ± 0.83**	0.14 ± 0.07	0.22 ± 0.19**
Lysine (Lys)	0.75 ± 0.33	1.32 ± 0.41**	0.36 ± 0.45	0.70 ± 0.12**
Tyrosine (Tyr)	0.33 ± 0.21	0.66 ± 0.28**	0.13 ± 0.98	0.22 ± 0.54**
Leucine (Leu)	0.81 ± 0.00	0.91 ± 0.78**	1.4 ± 30.22 ns	1.36 ± 0.13 ns
Valine (Val)	0.65 ± 0.11	1.08 ± 0.43**	0.27 ± 0.13	0.52 ± 0.67**
Isoleucine (ILe)	0.49 ± 0.42	0.80 ± 0.36**	0.19 ± 0.56	0.38 ± 0.33**
Phenylalanine (Phe)	0.65 ± 0.98	0.61 ± 0.48 ns	0.32 ± 0.34 ns	0.29 ± 0.03 ns

Values are expressed as means ± standard error (n = 15). For all the values within row, different letters superscripts are significantly different at ns = not significant, * $p \leq 0.01$, ** $p \leq 0.05$.

photosynthesis is directly affected by the temperature, light and carbon dioxide rate which were unregulated in the open field condition (Tanda et al., 2022).

Therefore, these results postulate a higher photosynthesis rate in the open-field condition which could have contributed to the deprivation of abundant proteins including the subunits of photosystems and ribosomes and further resulted in the accumulation of free amino acids (Batista-Silva et al., 2019). Furthermore, the low amino acid in samples grown in the greenhouse indicates the use-up as an alternative substrate for respiration resulting from a low photosynthesis rate (Batista-Silva et al., 2019).

3.3. Identification and characterisation of UHPLC-QTOF-MS bioactive metabolites in okra grown in different growing conditions

Phenolic compounds including phenolic acids and flavonoids contribute to plant adaptation in different growing conditions. These metabolites were identified based on the chromatographic molecular mass, fragmentation pattern, absorption wavelength and retention time as shown in Table 3. Furthermore, the generated molecule was authenticated by literature for tentative identification. n-O-caffeoyl glucaric acid was detected at retention time: 6.735; *m/z*: 6.735; MSE fragments: 209, 191, 147, 85 (Neugart et al., 2017). The results showed sinapoyl-glucaric acid detected at rt.: 12.863; *m/z*: 415.087 and MSE fragments: 191,85 (Jamali et al., 2017). Based on the ESI MS fragments: 353 of peak eluting at rt.: 13.51 with *m/z*: 351.01869, tentatively the peak was identified as chlorogenic acid (Fig. 5). Results further corroborate the presence of ferulic acid with two detected isomers (Table 3). The first ferulic acid isomer was detected at rt.: 9.890, *m/z*:385.0814, MSE: 209, 191,147, 85 and this were tentatively identified as feruloyl galactaric acid in the MS detector (Fig. 5). The second isomer was detected at rt.: 11:213, *m/z*:385.078 with MSE: 209. 191, 147, 129 and 85 which demonstrate a sugar moiety gain (Jamali et al., 2017). The other ferulic acid were detected at rt.:13.102; *m/z:* 457.13428 and MSE fragments: 406, 163, 119, 85 tentatively identified as 2′-(*E*)-Feruloyl-3-(arabinosylxylose). Whilst the compound eluting at rt.:18.528, *m/z*:547.10437, MSE:385, 223, 191, 129, 85 were identified as feruloyl glucarate hexoside as shown in Table 3 and igure 5. Such results are in agreement with a compressive metabolites profile in okra pods reported by D'Urso et al. (2020) about the presence of sinapic acid, and ferulic acid derivatives in okra pods. The predominated phenolic acid of the hydroxycinnamic acid was also detected in pumpkin-blanched leaves (Mashitoa et al., 2021).

However, for the first time, three ferulic acid isomers were detected in okra pods and leaves. Other compounds that have contributed to the total phenolic compounds included the O-glycosyl derivatives of Icariside F2; Benzyl beta-D-Apiofuranosyl-O-beta-D-glucopyranoside (rt: 13.663; *m/z*: 401.144 and MSE fragment: 269, 161, 101) and isotan B (rt: 14.22, *m/z*: 308.079; and MSE fragment: 209, 193, 134, 85) (Fig. 5). The phenethyl rutinoside: a member of the O-glycosyl compound was detected at rt.: 19.429, *m/z*: 429.176 and MSE fragment: 267, 223.96, 59 (Table 3).

The members of flavonoid-O-glycosides which included the quercetin moieties tentatively identified as quercetin dihexoside (rt: 16.108, *m/z*: 625.14024 and MSE: 300, 271. 151), quercetin hexoside-xyloside (rt: 16.494 and *m/z*: 595.13226. MSE: 300, 271, 151), and quercetin 3-hexoside (rt: 18.188, *m/z*: 463.08798, and MSE: 300, 271, 255, 151) as shown in Table 3 and Fig. 5 (Shen et al., 2019). The quercetin derivatives differed based on the sugar compound losses or gains. Other derivatives of the flavonoid-O-glycosides were the tentatively identified kaempferol O-xylosyl-hexoside eluting at rt.: 18.091 and *m/z:* 579.13428 and MSE: 284, 044, 227. Such results are in agreement with the report published by D'Urso et al. (2020), about the predominant presence of quercetin molecules in okra pods.

The remaining were constituted by metabolites of different classes such as terpene glycoside, tetrapyrroles, glucotonics and phenylalanine derivatives. The terpene glycoside eluted at rt.: 14.546 and *m/z*: 431.19186 was tentatively identified as citroside A. Whilst, *L*-phenylalanine tentatively identified at rt.: 7.266 and *m/z*: 164.0717 were recorded. Of interest was the three analysts detected at rt.: 19.681, 20.212 and 21.098 with *m/z* of 839.33777, 839.33289 and 837.31866 which were unknown metabolites from the tetrapyrroles and glucoronic acid derivatives (Table 3). These metabolites shared almost similar *m/z* but their fragments and mass error values showed their degree of variation (Table 3). These metabolites were all present in all the studied samples, however, their regulations varied based on the treatments. Therefore, these results validate abundant metabolites reported by D'Urso et al. (2020) about the presence of specialised phenolic metabolites in okra pod. Yet this study generated the first report about the metabolites found in okra pods and leaves harvested from different growing conditions.

3.4. Chemometric approach for understanding metabolites profile in different growing environments

To summarize the results of metabolite identification profile distinctions or similarities, and to highlight metabolome fingerprint change induced by the growing conditions, multivariate analyses were performed. Fig. 2A shows the explorative principal component analysis (PCA) that demonstrate two main sample clusters based on plant tissue and growing condition treatments (greenhouse and micro-plot under field condition). The cluster (horizontal PC 1 and PC 2) showed major (86.7 %) metabolites heterogeneity between okra samples grown under the greenhouse and micro-plot under field conditions aligned within the

Table 3
Tentative peak assignment and quantification of the UHPLC-QTOF-MS metabolites detected from okra leaf and pods grown in different growing conditions.

RT (min)	Generated *M/z*	Tentative identification	Formula	Fragments	UV	Mass error (ppm)	Greenhouse	Micro-plot	Greenhouse	Micro-plot
							Leaves (mg/kg)		Pods (mg/kg)	
6.735	371.06009	n-O-caffeoylglucaric acid	$C_{15}H_{16}O_{11}$	209, 191, 147, 85	324	−2.2	226.13 ± 9.89	286.13 ± 12.32**	2.20 ± 0.23**	0.07 ± 0.01
9.890	385.08149	Feruloyl -galactaric acid	$C_{16}H_{18}O_{11}$	209,191, 147, 85			57.13 ± 5.21**	52.83 ± 7.23	11.31 ± 1.43**	4.00 ± 0.02
11.421	355.06287	Coumaroyl glucaric acid	$C_{15}H_{16}O_{10}$	209, 191, 147, 129, 85	312	−2.8	53.01 ± 4.45*	30.15 ± 3.45	2.04 ± 0.93**	0.99 ± 0.03
12.520	385.07825	Feruloyl glucaric acid 4	$C_{16}H_{18}O_{11}$	209, 191, 147, 129, 85	327	−3.9	100.28 ± 7.88	111.69 ± 16.23**	2.94 ± 0.45*	2.62 ± 0.43
12.863	415.08722	Sinapoyl-glucaric acid	$C_{17}H_{20}O_{12}$	191, 85	311	−1.2	14.23 ± 4.09***	6.42 ± 1.23	0.24 ± 0.02**	0.14 ± 0.00
13.102	457.13428	Feruloyl-(arabinosylxylose)	$C_{20}H_{26}O_{12}$	406, 163, 119, 85	288	−3.1	55.76 ± 6.12	153.73 ± 10.23***	1.25 ± 0.22	3.25 ± 0.43**
19.791	415.19815	Ethyl 7-epi-12-hydroxyjasmonate glucoside	$C_{20}H_{32}O_9$	209, 179, 89	309, 415	2.2	133.14 ± 8.76***	68.49 ± 6.67	0.37 ± 0.04	1.17 ± 0.05**
18.528	547.10437	Feruloyl glucarate hexoside	$C_{16}H_{18}O_{11}$	209,191, 147, 85			7.17 ± 0.42	14.69 ± 4.23***	1.21 ± 0.05**	0.77 ± 0.00
19.429	429.17688	Phenethyl rutinoside	$C_{20}H_{30}O_{10}$	267, 223.96, 59	318	0.7	57.32 ± 2.56***	4.03 ± 0.23	0.14 ± 0.01	0.24 ± 0.23**
13.663	401.14426	Icariside F2;Benzyl beta-D-Apiofuranosyl-(1- > 6)-O-beta-D-glucopyranoside	$C_{18}H_{26}O_{10}$	269, 161, 101	300	−2.0	194.89 ± 17.39***	99.20 ± 9.32	23.47 ± 2.32 ns	22.56 ± 2.12
16.108	625.14024	Quercetin dihexoside	$C_{27}H_{30}O_{17}$	300, 271, 151	255, 354	0.2	0.33 ± 0.01	2.02 ± 0.32**	128.41 ± 15.33***	62.80 ± 4.33
16.494	595.13226	Quercetin hexoside-xyloside	$C_{26}H_{28}O_{16}$	300, 271, 151	255, 354	−3	88.17 ± 3.87	393.87 ± 12.43**	26.07 ± 4.21	39.67 ± 4.22**
18.091	579.13428	Kaempferol O-xylosyl-hexoside	$C_{26}H_{28}O_{15}$	284, 44, 227	263, 318	0.5	30.00 ± 2.78	112.21 ± 5.21**	0.31 ± 0.01	0.43 ± 0.33*
18.188	463.08798	Quercetin 3-hexoside	$C_{21}H_{20}O_{12}$	300, 271, 255, 151	254, 350	−2.6	11.69 ± 4.88	201.17 ± 10.23**	63.55 ± 5.54**	50.32 ± 2.32
14.220	308.07919	Isotan B	$C_{14}H_{15}NO_7$	209, 193, 134, 85	321	2.9	47.33 ± 5.78**	36.54 ± 2.34	32.54 ± 3.45**	3.05 ± 0.21
13.510	191.01869	Chlorogenic acid	$C_{16}H_{18}O_{09}$	353	324	−4.5	18.78 ± 4.32**	11.57 ± 1.23	2.07 ± 0.12**	0.49 ± 0.01
14.546	431.19186	Citroside A	$C_{19}H_{30}O_8$	223, 153, 89	301	0	412.04 ± 8.97**	247.61 ± 22.56	10.64 ± 0.18 ns	10.13 ± 0.09 ns

Values are expressed as means ± standard error ($n = 3$). For all the values within a column, different letters superscripts are significantly different at ns = not significant, * $p \leq 0.05$, ** 556 $p \leq 0.01$, *** $p \leq 0.001$.

horizontal PC1 and PC2. Whilst the observed sub-cluster demonstrated metabolic variation (9.5 %) within vertical PC1 and PC2 between okra leaf and pods samples. The results verify the correct sampling on leaves and pods and the effects of growing condition on the metabolome profile. These trends were observed on the orthogonal partial least squares discriminant analysis (OPLS-DA) plot in Fig. 2B which demonstrated a holistic metabolome variation between okra samples (leaf and pod) grown under the studied growing condition (greenhouse and micro-plot) within the vertical PC1 and PC2. Furthermore, a clear metabolome distinction between the sample type (leaf or pod) showed along the horizontal PC1 and PC2. Both the unsupervised and supervised plots were of great accuracy due to the obtained higher predictive statistics model of 96.2 and 93.3 % respectively.

Fig. 3A highlighted the metabolite's contribution to the overall variation. Metabolites including quercetin 3-galactoside, succinyl adenosine, quercetin 3-lathyroside, quercetin 3-galactoside-7-glucoside, neocarlinoside, and 2′-(*E*)-Feruloyl-3-(arabinosylxylose) were loaded positively in a vertical PC2, whilst, isotan B and UNPD130810 (unnamed) were loaded negatively in the vertical PC1. Therefore, these biomarker compounds have attributed to metabolome profile variation. The plots in Fig. 3B suggested that the larger the distance between a point and its original point, the more contribution of that compound to the total variation.

The hierarchical cluster analysis was applied to observe similarities or distinctions in metabolome profiles among and within the studied samples. Fig. 4A, defined and verified the two main clusters based on growing conditions which further introduced some two sub-clusters that can be associated with the plant tissue type (pod and leaf). Okra leaf samples showed moderate to high intensity for most of the detected metabolites irrespective of the growing conditions. Similarly, the okra pod samples showed a downregulated intensity for most of the detected metabolites. However, Fig. 4B shows variable importance in the projection (VIP) plot with summarised intensity and biomarker identification. Quercetin 3-galactoside, quercetin 3-lathyroside, succinyl adenosine and Isotan B were the main analysts contributing to variation among and within the treatments. Samples grown under a greenhouse condition show-cased higher expression of isotan B and a down-regulation of quercetin 3-galactoside, succinyl adenosine, and quercetin 3-lathyroside. This trend was found to be in the opposite venture in okra samples grown in micro-plot under open-field condition. The use of chemometric plots has been used as a tool in the crop sciences to demonstrate the effect of agronomical treatment of metabolites variation (Mashitoa et al., 2021; Mpai et al., 2022).

3.5. Effect of growing condition on the concentration level of some detected phenolic metabolites in okra leaves and pods

Okra leaves grown in a micro-plot under open-field condition had the highest contents of n-O-caffeoylglucaric acid (286.13 mg/kg), feruloyl glucaric acid (111.69 mg/kg), feruloyl-(arabinosylxylose) (153.73 mg/kg), feruloyl glucarate hexoside (14.69 mg/kg), quercetin dihexoside (2.02 mg/kg), quercetin hexoside-xyloside (393.87 mg/kg) over those grown in a greenhouse condition (Table 3). Whilst okra leaf samples grown in a greenhouse condition exhibited the highest contents of

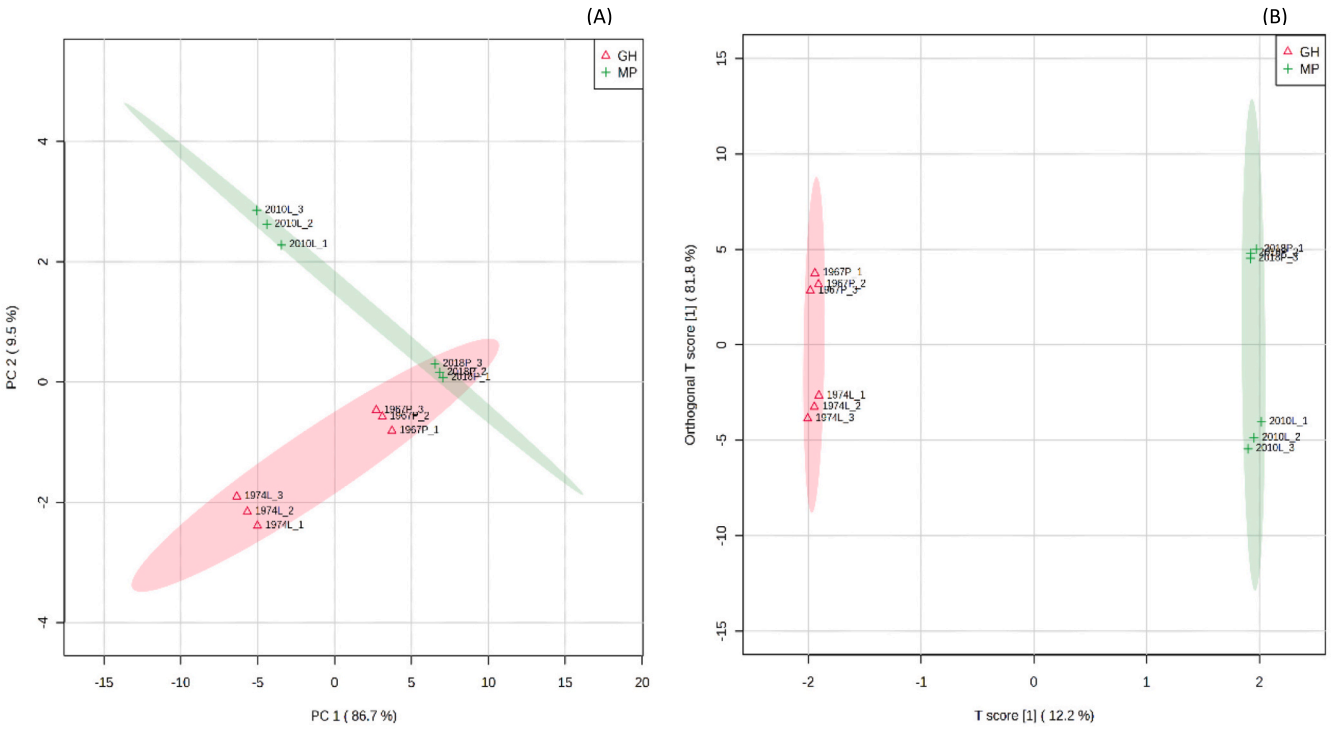

Fig. 2. A) Explorative PCA and B). supervised OPLS-DA plot for metabolite profile of okra leaves grown on in a greenhouse and micro-plot field condition.

feruloyl glucaric acid (57.13 mg/kg), coumaroyl glucaric acid (53.01 mg/kg), sinapoyl-glucaric acid (14.23 mg/kg), ethyl 7-epi-12-hydroxy-jasmonate glucoside (133.14 mg/kg), phenethyl rutinoside (57.32 mg/kg), Icariside F2;Benzyl beta-D-Apiofuranosyl-(1- > 6)-O-beta (194.89 mg/kg), isotan b (47.33 mg/kg), terpene glycoside (18.78 mg/kg), citroside A (412.04 mg/kg), Phyllanthostatin (4607 mg/kg) and L-Phenylalanine (131.94 mg/kg).

On the other hand, metabolites including: feruloyl-(arabinosylxylose) (3.25 mg/kg), Ethyl 7-epi-12-hydroxyjasmonate glucoside (1.17 mg/kg), phenethyl rutinoside (0.24 mg/kg), quercetin hexoside-xyloside (39.67 mg/kg), kaempferol O-xylosyl-hexoside (0.43 mg/kg), and L-Phenylalanine (13.59 mg/kg) were predominated in okra pod samples grown on a micro-plot under open-field conditions. Whilst, metabolites including: n-O-caffeoylglucaric acid (2.20 mg/kg), feruloyl glucaric acid (11.31 mg/kg), coumaroyl glucaric acid (2.04 mg/kg), feruloyl glucaric acid (2.94 mg/kg), sinapoyl-glucaric acid (0.24 mg/kg), feruloyl glucarate hexoside (1.21 mg/kg), quercetin dihexoside (128.41 mg/kg), quercetin 3-hexoside (63.55 mg/kg), isotan B (32.54 mg/kg), terpene glycoside (2.07 mg/kg), and phyllanthostatin (100 mg/kg) were predominated the okra pods grown in a greenhouse (Table 3). These results concede with some published reports about predominant metabolites in okra and the effect of growing conditions on secondary metabolites in 'Taşköprü garlic', lettuce, tomato, and strawberry grown concurrently in different growing conditions including tunnel, greenhouse and open-field (Angmo et al., 2021; Gecer et al., 2022; Oh et al., 2011). Metabolic quantities are directly affected by the growing conditions, in this case, phenolic metabolites showed metabolic profile alterations as a result of greenhouse and open field growth condition. From the crop establishment and transplanting procedures, the seedling transplanted in a micro-plot under field condition were exposed to 'transplanting shock' from the greenhouse to the open field. Although the growth medium was the same in both conditions, the light intensity from the greenhouse was trapped by the polyethylene film and temperature was regulated within a range of 22–28 °C, whilst these conditions were unregulated in the micro-plot under an open field. Therefore, the results postulated a semi-drought condition caused by the extreme weather temperatures which could have resulted to the accumulation of some free phenolic metabolites. Yet those metabolites could be advocating for the antioxidants against hydrogen peroxide molecules produced by drought stress. However, due to the nature of okra, growing optimally in open-field conditions, the results indicated that the greenhouse condition strained the plants which could have led to some type of chilling injury. Therefore, the most phenolic compounds accumulating in the greenhouse condition from the flavonoids and hydroxycinnamic phenolic acid indicate the induction of antioxidant activity against lipids peroxidation (Fattahi et al., 2023). Similar polyphenols including caffeic acid derivatives, quercetin and kaempferol glycosides were affected by growing conditions such that open-air-grown lettuce samples outperformed those in a greenhouse condition (Romani et al., 2002).

4. Conclusion

This study aimed to evaluate plant growth, yield, amino acids, and untargeted phenolic metabolites in okra cultivated under two growth environments: a greenhouse and a micro-plot in open field conditions. Results indicated that growth and yield attributes, including stem diameter, plant height, number of branches per plant, number of pods per plant, pod weight, pod length, and pod diameter, were significantly higher ($p \leq 0.05$) in the open field setting. A total of sixteen amino acids were quantified, all amino acids including Arg (0.97 mg/kg), Ser (0.81 mg/kg), Gly (0.96 mg/kg), Asp (2.07 mg/kg), Glu (2.74 mg/kg), Ala (0.95 mg/kg), Pro (0.90 mg/kg), His (0.46 mg/kg), Thr (0.84 mg/kg), Met (0.52 mg/kg), Lys (1.32 mg/kg), Tyr (0.66 mg/kg), Leu (0.66 mg/kg), ILe (0.81 mg/kg) and Val (0.81 mg/kg) were significantly ($p > 0.05$) higher in leaf samples from microplots under open-field conditions compared to those grown in the greenhouse. UPLC-MS analysis identified phenolic metabolites associated with growth condition markers, including quercetin 3-galactoside, succinyl adenosine, quercetin 3-lathyroside, isotan b, and Icariside F2b, which showed significant upregulation or downregulation depending on the growing conditions. The varying levels of phenolic acids and flavonoids suggest that these metabolites respond differently to environmental factors, highlighting the

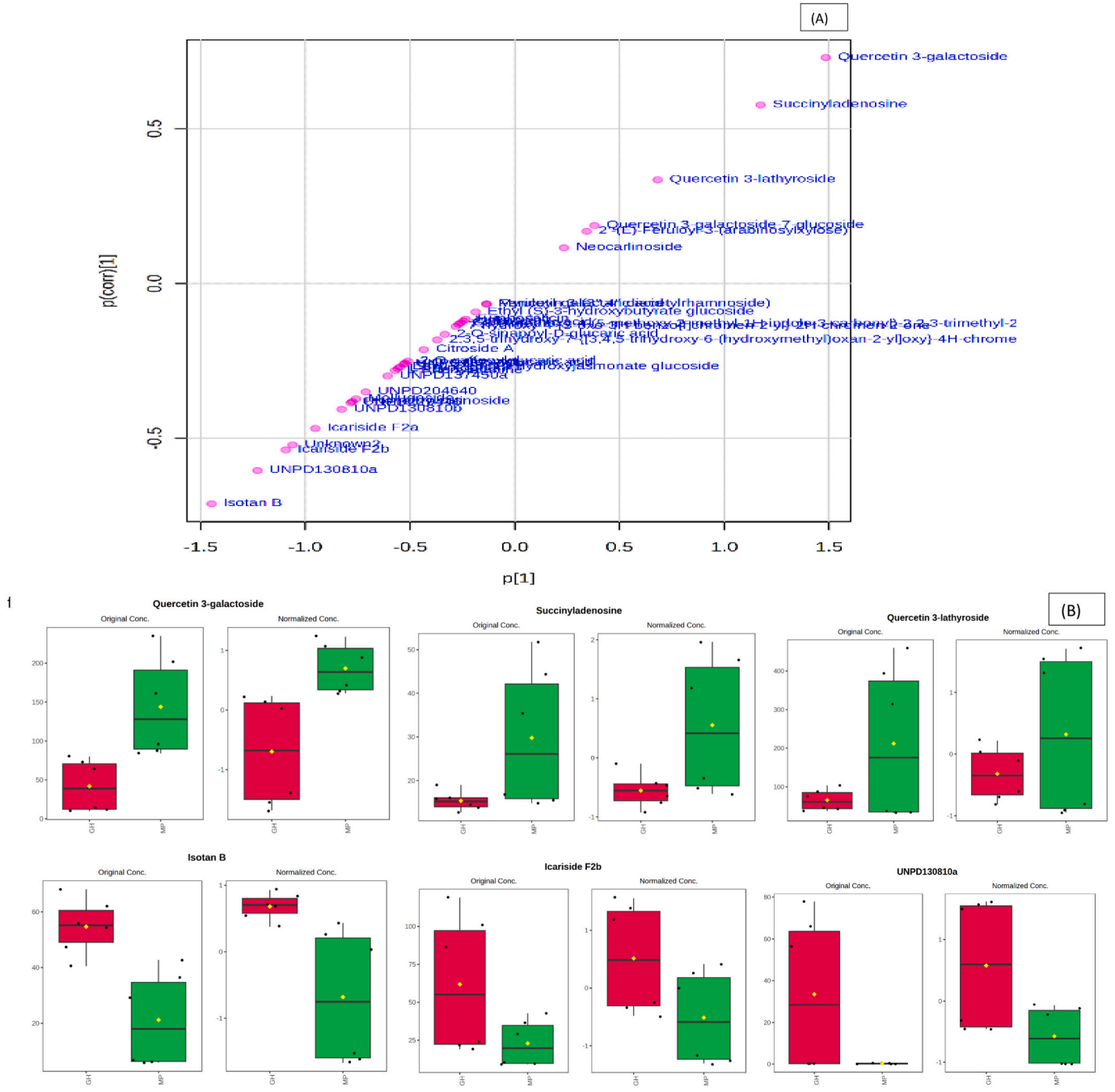

Fig. 3. A). Metabolite's contribution to total variation and B). Overall response of compound's marker to different growing conditions.

need for further research into the accumulation of specific metabolites during semi-drought and semi-chilling abiotic stress. This insight could enhance our understanding of plant resilience and metabolic adaptation, paving the way for strategies to improve crop yields under challenging environmental conditions.

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CRediT authorship contribution statement

Tyson T. Mokgalabone: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Semakaleng Mpai:** Supervision, Resources, Project administration, Methodology, Formal analysis, Data curation, Conceptualization, Writing, Review and editing manuscript. **Trevor T. Nyakudya:** Writing – review & editing, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Ashwell R. Ndhlala:** Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

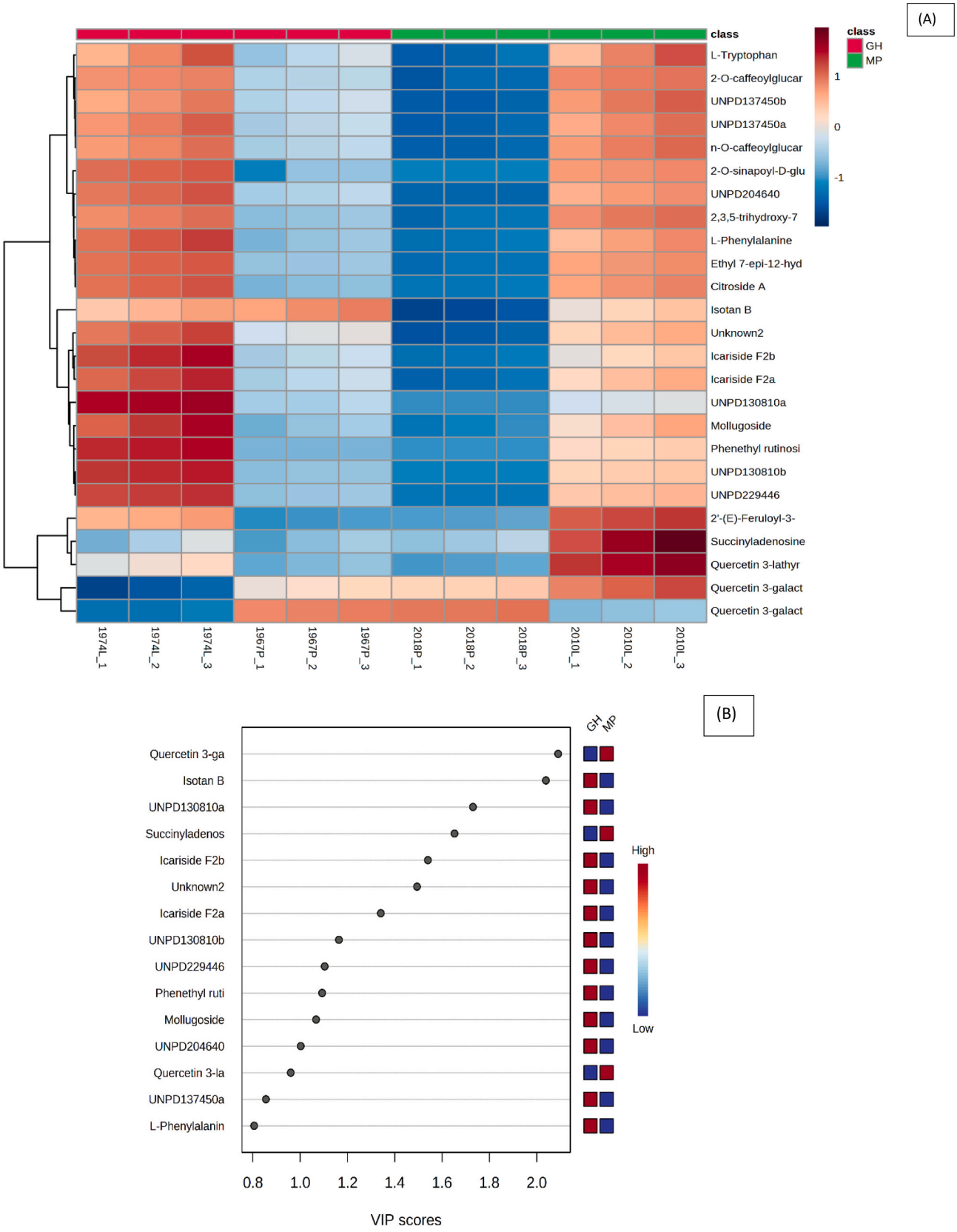

Fig. 4. A). Hierarchical cluster and B). VIP plot for distinguishing metabolites between greenhouse and micro-plot field condition.

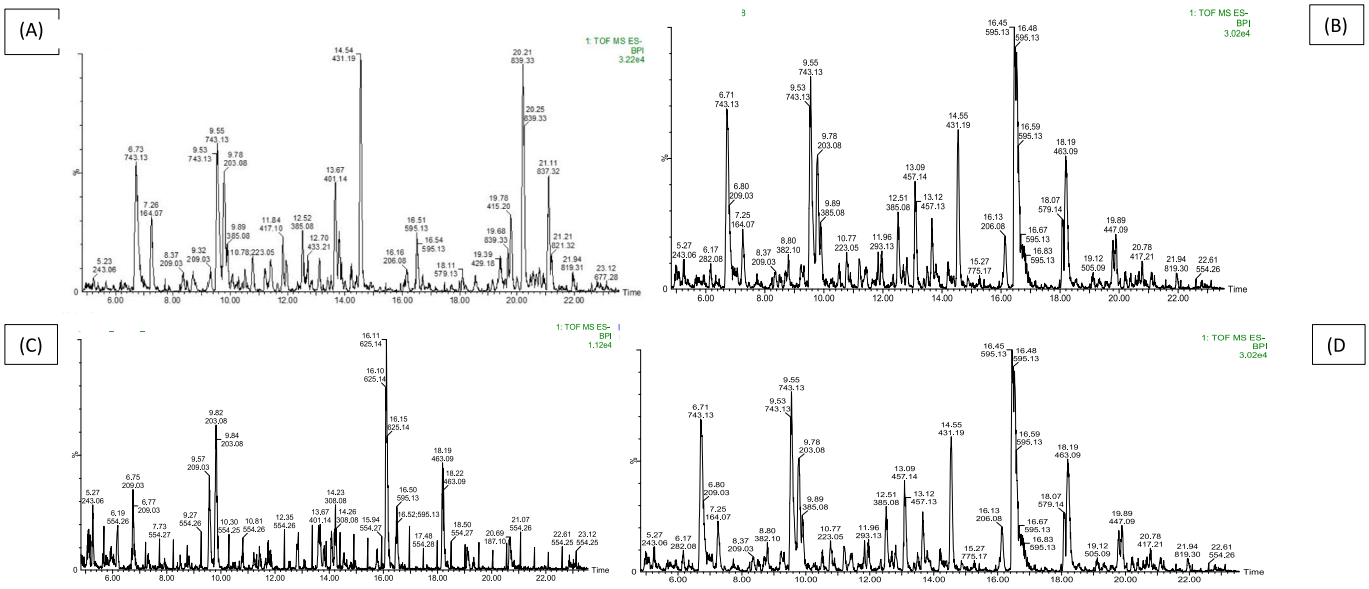

Fig. 5. Base peak chromatograph of okra leaves (A and B) and okra pods (C and D) grown under greenhouse and micro-plot field condition respectively.

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

Data will be made available on request.

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