UNIVERSIDAD AUTÓNOMA CHAPINGO

DEPARTAMENTO DE FITOTECNIA
COORDINACIÓN DE ESTUDIOS DE POSGRADO

DOCTORADO EN CIENCIAS EN HORTICULTURA

PERFIL METABOLÓMICO DE TRES CULTIVARES DE VERDOLAGA (*Portulaca oleracea* L.) CON FERTILIZACIÓN NITROGENADA EN HIDROPONÍA, MEDIANTE RMN

Como requisito parcial para obtener el grado de:

DOCTOR EN CIENCIAS EN HORTICULTURA

Presenta:

César Omar Montoya García

Bajo la supervisión de: Dra. María del Rosario García Mateos

APROBADA

Chapingo, Estado de México, marzo de 2023

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Tesis realizada por **CÉSAR OMAR MONTOYA GARCÍA** bajo la supervisión del comité asesor indicado, aprobada por el mismo y aceptada como requisito parcial para obtener el grado de

DOCTOR EN CIENCIAS EN HORTICULTURA

DIRECTORA

Dra. Ma. Rosario García Mateos

CODIRECTORA

Dra. Elvia Becerra Martínez

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ASESOR

Dr. Víctor Hugo Volke Haller

LECTOR EXTERNO

Dr. Ramón Marcos Soto Hernández

RESUMEN

La verdolaga (*Portulaca oleracea* L.) es una planta herbácea consumida en diversas partes del mundo y México desde tiempos prehispánicos; proporciona aportes sustanciales de minerales, polisacáridos, ácidos grasos, proteínas, alcaloides, terpenoides, esteroles, fenoles, flavonoides y vitaminas. Las técnicas metabolómicas basadas en NMR han ampliado el panorama del potencial alimenticio y medicinal de esta planta. La presente investigación se divide en tres secciones con los siguientes objetivos: **(i))** mostrar el estado del arte del cultivo de la verdolaga, con énfasis en el efecto del estrés abiótico, causado por el manejo del cultivo en condiciones hidropónicas y a campo abierto, sobre el contenido de compuestos bioactivos con técnicas metabolómicas; **(ii)** caracterizar el rendimiento y altura, así como, elucidar el metaboloma de verdolaga de tres cultivares nativos, en función del momento de cosecha (32, 39 y 46 días después de la emergencia) en invernadero; y **(iii)** elucidar el metaboloma de verdolaga de tres cultivares nativos, por la aplicación de nitrógeno (0.5, 4.0, 8.0, 12.0 y 16.0 mM N L^{-1}) en condiciones controladas de hidroponía. El presente estudio se llevó a cabo en el Estado de México, México (19.491260, -98.872207, 2215 msnm), en condiciones de invernadero e hidroponía en agosto y septiembre de 2021. Los cultivares de verdolaga evaluados fueron provenientes de México, de los estados de Morelos, Estado de México y Ciudad de México, denominados por su zona de producción: Cuautla, Mixquic y Xochimilco, respectivamente. Se empleó un sistema hidropónico de balsas flotantes. Como resultado del segundo objetivo se identificaron 5 azucares, 15 aminoácidos, 7 ácidos orgánicos y 3 isómeros del ácido canfeoilquínico, 2 alcoholes, 3 nucleósidos, entre otros compuestos; 37 compuestos en los cultivares de verdolaga nativos de Xochimilco y Cuautla, y 39 en la verdolaga de Mixquic. El análisis de componentes principales (PCA) y el análisis discriminante de mínimos cuadrados parciales (OPLS-DA) separaron los cultivares en tres clusters; siendo Mixquic el cultivar con mayor número de compuestos diferenciales (aminoácidos y carbohidratos) seguido de Xochimilco y Cuautla. Para el momento de cosecha, se observaron incrementos del metaboloma en las cosechas tardías, los compuestos diferenciales fueron glucosa, fructosa, galactosa, piruvato, colina y 2-hidroxisobutirato. Como resultado del tercer objetivo se observaron 41 metabolitos, incluidos 6 carbohidratos, 15 aminoácidos, 11 ácidos orgánicos, 2 alcoholes, 4 nucleótidos, colina, O-fosfocolina y

trigonelina. El análisis de componentes principales (PCA) y el análisis discriminante de mínimos cuadrados parciales (OPLS-DA) separaron los cultivares en tres clusters de acuerdo a las dosis de nitrogeno: (i) 8 y 16 mM N; (ii) 0.5 y 4 mM N; y (iii) 12 mM N. Los modelos de regresión individuales para cada metabolito indicaron efectos crecientes en concentración en función de la dosis de nitrógeno con excepción del ácido cafeoilquínico; las dosis óptimas metabolómicas se pudieron encontrar entre 11 y 13 mM N, que a su vez produjo el mayor rendimiento y altura de la planta. En conclusión **(i)** la información recabada del estado del arte de la verdolaga indicó que las técnicas metabolómicas basadas en NMR han contribuido a dilucidar el metaboloma en condiciones específicas de crecimiento, con hallazgos de nuevos compuestos bioactivos; **(ii)**, los datos presentados pueden contribuir para seleccionar el cultivar y el momento cosecha con la finalidad de potencializar el contenido nutricional de la verdolaga; **(iii)**, con la información generada mediante regresión se logró identificar la concentración optima metabolómica en función del nitrógeno para producir la mayor concentración de los metabolitos de interés económico y nutrimental en beneficio de los productores, consumidores e investigadores.

Palabras clave: compuestos bioactivos; estrés abiótico; principal component analysis (PCA); análisis de regresión; concentración optima metabolómica.

ABSTRACT

Purslane (Portulaca oleracea L.) is an herbaceous plant consumed in various parts of the world and Mexico since pre-Hispanic times; it provides substantial contributions of minerals, polysaccharides, fatty acids, proteins, alkaloids, terpenoids, sterols, phenols, flavonoids, and vitamins. NMR-based metabolomic techniques have broadened the picture of the dietary and medicinal potential of this plant. The present research is divided into three sections with the following objectives: **(i)** to show the state of the art of purslane cultivation, with emphasis on the effect of abiotic stress, caused by crop management under hydroponic and open field conditions, on the content of bioactive compounds with metabolomic techniques; **(ii)** characterize yield and height, as well as elucidate the purslane metabolome of three native cultivars, as a function of harvest time (32, 39 and 46 days after emergence) in a greenhouse; and, **(iii)** elucidate the purslane metabolome of three native cultivars, by nitrogen application (0. 5, 4.0, 8.0, 12.0 and 16.0 mM N L-1) under controlled hydroponic conditions. The present study was carried out in the State of Mexico, Mexico (19.491260, -98.872207, 2215 masl), under greenhouse and hydroponic conditions in August and September 2021. The purslane cultivars evaluated were from Mexico, from the states of Morelos, State of Mexico, and Mexico City, named for their production zone: Cuautla, Mixquic and Xochimilco, respectively. A floating raft hydroponic system was used. As a result of the second objective, 5 sugars, 15 amino acids, 7 organic acids and 3 isomers of canfeoilquinic acid, 2 alcohols, 3 nucleosides, among other compounds were identified: 37 compounds in the purslane cultivars native to Xochimilco and Cuautla, and 39 in the Mixquic purslane. Principal component analysis (PCA) and partial least squares discriminant analysis (OPLS-DA) separated the cultivars into three clusters; Mixquic being the cultivar with the highest number of differential compounds (amino acids and carbohydrates) followed by Xochimilco and Cuautla. By harvest time, increases in the metabolome were observed in the late harvests, the differential compounds were glucose, fructose, galactose, pyruvate, choline and 2-hydroxybutyrate. As a result of the third objective, 41 metabolites were observed, including 6 carbohydrates, 15 amino acids, 11 organic acids, 2 alcohols, 4 nucleotides, choline, O-phosphocholine and trigonelline. Principal component analysis (PCA) and partial least squares discriminant analysis (OPLS-DA) separated the cultivars into three clusters according to the nitrogen doses: (i) 8 and 16 mM N; (ii) 0.5 and 4 mM N;

and (iii) 12 mM N. Individual regression models for each metabolite indicated increasing effects in concentration as a function of nitrogen dose except for caffeoylquinic acid; the optimal metabolomic doses could be found between 11 and 13 mM N, which in turn produced the highest yield and plant height. In conclusion **(i)** the information gathered on the state of the art of purslane indicated that metabolomic techniques based on NMR have contributed to elucidating the metabolome under specific growth conditions, with findings of new bioactive compounds; **(ii)** the data presented can contribute to select the cultivar and harvest time to enhance the nutritional content of purslane; and, **(iii)** with the information generated by regression, it was possible to identify the optimal metabolomic concentration as a function of nitrogen to produce the highest concentration of metabolites of economic and nutritional interest for the benefit of producers, consumers and researchers.

Key words: bioactive compounds; abiotic stress; principal component analysis (PCA); regression analysis; metabolomic optimum concentration.

AGRADECIMIENTOS

A los **productores del sector agropecuario**, en especial a los horticultores de la Ciudad de México, por su dedicación y esfuerzo diario por conservar su agricultura ancestral.

A las **mexicanas y mexicanos** que, a través de sus impuestos, el **Consejo Nacional de Ciencia y Tecnología (CONACyT)** y la **Universidad Autónoma Chapingo**, financiaron parte de mis estudios doctorales.

A los integrantes de mi consejo particular:

A la Dra. **Ma. Rosario García-Mateos**, por su dedicación, apoyo y confianza a esta investigación y a un servidor; fue un gran orgullo conocerla.

A la Dra. **Elvia Becerra-Martínez**, por compartirme sus conocimientos de metabolómica con RMN, su apoyo en la escritura, análisis estadístico, entrega en esta investigación; y su valiosa amistad.

Al Dr. **Víctor Hugo Volke-Haller** por su orientación, y brindarme sus conocimientos en regresión; máximo respeto para usted

Al Dr. **J. Jesús Magdaleno-Villar** por su recomendaciones y apoyo en campo.

A mis padres, **Casimiro y Guadalupe**, por su dedicación, enseñanzas, consejos, orientación, sabiduría, su infinito amor, por enseñarme que todo es posible, a ustedes mi mayor reconocimiento.

A mis **hermanos y sobrinos** su apoyo fue incondicional y esencial.

A mis amigos que me acompañaron en este gran proceso, en los momentos divertidos y difíciles, con sus conocimientos, consejos y cariño, tanto en campo y laboratorio: **Esmeralda Hernández; Fabian Guzmán; Ricardo Miranda; Guillermo Enciso; Dinora Manzano; Luz Riviello; Antonio Barrón; Gabriela Rodríguez; Nahum Luna**; y **Emmanuel Reyes**, por disipar el silencio con su música.

DEDICATORIA

A mí padre, **Casimiro Montoya Ruíz**, te agradezco por tus enseñanzas, consejos e infinito amor, ***eres y siempre serás un chingón***. Te recuerdo todo el tiempo y te extraño demasiado. Siempre estarás con nosotros.

Solo adelantaste el viaje con **Alex** para viajar por el universo, algún día nos encontraremos.

Te amo.

Con amor a mi madre, **Guadalupe García Solano**, por tu cariño, apoyo incondicional, fortaleza y gran corazón lleno de amor, gracias por estar para nosotros siempre.

A mis hermanos y sobrinos: **Karina, Jessica, Emiliano, Yael, Ángel, Iker** y **Camila**, ustedes son geniales.

DATOS BIBLIOGRAFICOS

César Omar Montoya García (MOGC890919HDFNRS03) nació el 19 de septiembre de 1989 en el Distrito Federal, CDMX. Sus estudios nivel licenciatura fueron cursados en la Universidad Autónoma Metropolitana (2007-2012), egresó como Ingeniero Agrónomo.

En 2016 obtuvo el grado de Maestro en Ciencias en Edafología en el Colegio de Postgraduados con especialidad en fertilidad de suelos y nutrición vegetal.

Se desempeñó como extensionista en Zacatecas (2016 y 2017); y fue Investigador en el área de fertilidad de suelos en el Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) en Iguala Guerrero (2018).

Ha impartido clases con diversos cursos enfocados en fertilidad de suelos y nutrición vegetal en: la Universidad Autónoma Metropolitana en el área de Educación Continua y a Distancia (2016 a 2023); en la Carrera de Ingeniería Agronómica de la Facultad de Ciencias Agrícolas en la Universidad Central del Ecuador (2019–2020); en la Unidad Pedagógica Hohenau Universidad Católica Nuestra Señora de la Asunción, en Paraguay (2020); Centro de Desarrollo e Innovación Tecnológica, en Paraguay (2020). Fue ponente en diferentes congresos científicos nacionales e internacionales.

Se desempeñó como codirector en una tesis de licenciatura; fue asesor en una tesis nivel licenciatura, y en una tesis de nivel maestría en la Universidad Autónoma de Guerrero.

Ha publicado seis artículos científicos como primer autor y a la vez como autor de referencia; además de participar en seis artículos científicos como coautor, enfocado en análisis estadísticos; todos estos documentos se han publicado en revistas científicas de alto impacto, indexadas en el Journal Citation Report.

CONTENIDO

CAPÍTULO I

INTRODUCCIÓN GENERAL

INTRODUCCIÓN GENERAL

La verdolaga (*Portulaca oleracea* L.) es uno de los cultivos que ha tenido mayor atención desde el descubrimiento de su contenido de ácido linoléico (18:2ω6), α-linolénico (18:3ω3), además de contener compuestos esenciales para la nutrición humana como minerales, polisacáridos, ácidos grasos, proteínas, alcaloides, terpenoides, esteroles, vitaminas, lignanos, compuestos fenólicos, flavonoides (Palaniswamy et al., 2000; Fontana et al., 2006; Siriamornpun & Suttajit, 2010; Szalai et al., 2010; Montoya-García et al., 2018a, 2018b; Montoya-García et al., 2019); además en investigaciones recientes se han reportado nuevos compuestos como lignanos, flavonoides, homoisoflavonoides, homoisoflavones, glucosidos de amida, alcaloides, alcaloides de amida e isoindol, y alcaloides fenólicos como las oleraceínas (Balabanova et al., 2020; Camalle et al., 2020; Farag & Shakour, 2019; Fernández-Poyatos et al., 2021; Nemzer et al., 2020; Zaman et al., 2019; Zaman et al., 2020); al consumir la verdolaga se han demostrado propiedades farmacológicas como propiedades analgésicas, antiinflamatorias, broncodilatadoras, neuroprotectoras, antivirales, antiproliferativas, antidiabéticas e hipocolesterolémicas (Cai-Xia et al., 2010; El-Sayed, 2011; Sharma et al., 2010; Shen et al., 2013; Zhao et al., 2013; Zidan et al., 2014).; esta información ha contribuido a ampliar el panorama potencial alimentario y medicinal de esta planta.

La verdolaga se consume en Italia, Reino Unido, España, Grecia, Turquía, Irán, Malasia, Filipinas, China, Norte de África, Australia, Estados Unidos, Brasil y México (desde épocas prehispánicas) (Mera-Ovando et al., 2014; Alu'datt et al., 2019; Nemzer et al., 2020). La verdolaga comenzó a producirse en Xochimilco, México desde 1960 en campo abierto con cultivares nativos, actualmente se reportan diversos cultivares nativos pertenecientes al sitio de producción, pues se han adaptado a las diversas condiciones edafoclimáticas; actualmente se produce de forma comercial en la Ciudad de México, Morelos, Estado de México y Baja California (Montoya-García et al., 2017).

Se han determinado diversos compuestos bioactivos de verdolaga a través de diferentes análisis químicos, como cromatografía líquida y de gases acopladas a espectrometría de masas (EM), electroforesis capilar (EC), espectrometría infrarroja (IR), y resonancia magnética nuclear (RMN) (Cevallos-Cevallos et al., 2009; Koda et al., 2012; Veenstra, 2012). Las técnicas basadas

en RMN identifican y cuantifican de forma precisa y con alta selectividad los metabolitos disueltos en extractos polares y no polares, no es necesaria la purificación de los extractos (Markley et al., 2017; Villa-Ruano et al., 2019).

Los estudios metabolómicos tienen en común que se colectan muestras de verdolaga de diversas regiones del mundo, pero no se toman en consideración la influencia que puede tener el proceso de cultivo y el estrés abiótico o biótico.

Con la aplicación de GC–MS, Camalle et al. (2020) determinaron efectos de la relación amonio/nitrato en dos cultivares de verdolaga, observaron clorofila, carotenoides, proteínas y ácidos grasos totales, y seis azucares, doce aminoácidos y tres ácidos orgánicos.

Por GC–MS, se reportaron 132 metabolitos en dos accesiones de verdolaga cultivadas en América y China, de los cuales fueron 35 acidos organics, 26 aminoacidos, 20 carbohidratos, 20 aminas y 13 lípidos (Zaman et al., 2020).

En verdolaga proveniente de México, la concentración de compuestos fenólicos y flavonoides se incrementaron a bajas y altas dosis de N en campo (0 y 300 kg N ha^{-1}), mientras que los ácidos grasos poliinsaturados incrementaron en proporción a la dosis de N aplicada (Montoya-García et al., 2018a).

Con base a esta información, se plantearon los siguientes objetivos

(i) Elaborar una revisión exhaustiva con la finalidad de mostrar el estado del arte de la verdolaga, con énfasis en el efecto del estrés abiótico, causado por el manejo del cultivo en condiciones hidropónicas y de campo abierto, sobre el contenido de compuestos nutracéuticos, calidad nutricional, propiedades medicinales, y compuestos bioactivos recientemente identificados a través de diferentes técnicas de análisis como la metabolómica.

(ii) fue caracterizar el rendimiento y altura, y elucidar el metaboloma de verdolaga de tres cultivares nativos, en función del momento de cosecha (etapa fenológica) bajo condiciones de invernadero e hidroponía.

(iii) Fue elucidar el metaboloma de verdolaga de tres cultivares nativos y la influencia por la aplicación de nitrógeno en condiciones controladas de hidroponía, con la finalidad de obtener las concentraciones optimas metabolómicas en función del nitrógeno aplicado

REFERENCIAS

Alu'datt, M. H., Rababah, T., Alhamad, M. N., Al-Tawaha, A., Al-Tawaha, A. R., Gammoh, S., Ereifej, K.I., Al-Karaki, G., Hamasha, H. R., Tranchant, C. C., & Kubow S. (2019). Herbal yield, nutritive composition, phenolic contents and antioxidant activity of purslane (*Portulaca oleracea* L.) grown in different soilless media in a closed system. *Industrial Crops and Products*, *141*(1), 111746. https://doi.org/10.1016j.indcrop.2019.111746

Balabanova, V., Hristov, I., Zheleva-Dimitrova, D., Sugareva, P., Lozanov, V., & Gevrenova, R. (2020). Bioinformatic insight into *Portulaca oleracea* L. (Purslane) of bulgarian and Greek origin. *Acta Biologica Cracoviensia Series. Botanica*, *62*(1), 7-21. https://doi.org/: 10.24425/abcsb.2020.131662

Cai-Xia, D., Kyoko, H., Jung-Bum, L., Toshimitsu, H., 2010. Characterization of structures and antiviral effects of polysaccharides from *Portulaca oleracea* L. *Chemical and Pharmaceutical Bulletin*, 58 (4), 507–510. http://dx.doi.org/10.1248/cpb.58.507.

Camalle, M., Standing, D., Jitan, M., Muhaisen, R., Bader, N., Bsoul, M., et al. (2020). Effect of Salinity and Nitrogen Sources on Leaf Quality, Biomass, and Metabolic Responses of Two Ecotypes of *Portulaca oleracea*. https://www.preprints.org/manuscript/202004.0044/v1

Cevallos-Cevallos, J. M., Reyes-De-Corcuera, J. I., Etxeberria, E., Danyluk, M. D., & Rodrick, G. E. (2009). Metabolomic analysis in food science: a review. *Trends in Food Science & Technology*, 20(11-12), 557-566. https://doi.org/10.1016/j.tifs.2009.07.002

El-Sayed, M. I. K. (2011). Effects of *Portulaca oleracea* L. seeds in treatment of type-2 diabetes mellitus patients as adjunctive and alternative therapy. *Journal of ethnopharmacology*, 137(1), 643-651. https://doi.org/10.1016/j.jep.2011.06.020

Farag, M.A., & Shakour, Z.T.A. (2019). Metabolomics driven analysis of 11 Portulaca leaf taxa as analysed via UPLC-ESI-MS/MS and chemometrics. *Phychemestry*, *161*, 117-129. https://doi.org/10.1016/j.phytochem.2019.02.009

Fernández-Poyatos, M.D.P., Llorent-Martínez, E.J., Ruiz-Medina, A., 2021. Phytochemical composition and antioxidant activity of *Portulaca oleracea*: influence of the steaming cooking process. *Foods* 10 (1), 94. https://doi.org/10.3390/foods10010094.

Fontana, E., Hoeberechts, J., Nicola, S., Cros, V., Battista-Palmegiano, G., & Giorgio Peiretti, P. (2006). Nitrogen concentration and nitrate/ammonium ratio affect yield and change the oxalic

acid concentration and fatty acid profile of purslane (*Portulaca oleracea* L.) grown in a soilless culture system. *Journal of the Science of Food and Agriculture*, 86(14), 2417-2424. https://doi.org/10.1002/jsfa.2633

Koda, M., Furihata, K., Wei, F., Miyakawa, T., & Tanokura, M. (2012). Metabolic discrimination of mango juice from various cultivars by band-selective NMR spectroscopy. *Journal of agricultural and food chemistry*, 60(5), 1158-1166. https://pubs.acs.org/doi/abs/10.1021/jf2041438

Markley, J. L., Brüschweiler, R., Edison, A. S., Eghbalnia, H. R., Powers, R., Raftery, D., & Wishart, D. S. (2017). The future of NMR-based metabolomics. *Current Opinion in Biotechnology*, *43*, 34-40. https://doi.org/10.1016/j.copbio.2016.08.001

Mera-Ovando, L.M., Bye, B.R., & Solano, L.M., (2014). La verdolaga (*Portulaca oleracea* L.) fuente vegetal de omega 3 y omega 6. *Agro-Productividad*, 7, 3–7. http://www.colpos.mx/wb_pdf/Agroproductividad/2014/AGROPRODUCTIVIDAD%20I_2014.pdf.

Montoya-García, C. O., Volke-Haller, V., Santillán-Ángeles, A., López-Escobar, N.F., & Trinidad-Santos, A. (2019). Relación NH_4^+/NO_3^- en la producción de biomasa y el contenido nutrimental de *Portulaca oleracea* L. *Agrociencia*, 53: 521-533. https://agrociencia-colpos.mx/index.php/agrociencia/article/view/1825

Montoya-García, C. O., Volke-Haller, V., Trinidad-Santos, A., & Villanueva-Verduzco, C. (2018a). Change in the contents of fatty acids and antioxidant capacity of purslane in relation to fertilization. *Scientia Horticulturae*, 234: 152-159. https://doi.org/10.1016/j.scienta.2018.02.043

Montoya-García, C. O., Volke-Haller, V., Trinidad-Santos, A., & Villanueva-Verduzco, C. (2018b). Concentración nutrimental de la verdolaga (*Portulaca oleracea* L.) en respuesta a la fertilización con NPK. *Agrociencia*, 52: 241-254. http://www.scielo.org.mx/scielo.php?pid=S1405-31952018000200241&script=sci_abstract

Montoya-García, C. O., Volke-Haller, V., Trinidad-Santos, A., Villanueva-Verduzco, C. & Sánchez-Escudero, J. (2017). Respuesta de la verdolaga (*Portulaca oleracea* L.) a la fertilización con NPK. *Revista Fitotecnia Mexicana*, 40: 325-332. https://doi.org/10.35196/rfm.2017.3.325-332

Nemzer, B., Al-Taher, F., & Abshiru, N. (2020). Phytochemical composition and nutritional value of different plant parts in two cultivated and wild purslane (*Portulaca oleracea* L.) genotypes. *Food Chemistry*, *320*, 126621. https://doi.org/10.1016/j.foodchem.2020.126621

Palaniswamy, U., R. Mcavoy, and B. Bible. (2000). Omega-3-fatty acid concentration in *Portulaca oleracea* is altered by nitrogen source in hydroponic solution. *Journal of the American Society for Horticultural Science*, 125: 190-194.

Sharma, A., Kaithwas, G., Vijayakumar, M., Unnikrishnan, M. K., & Rao, C. V. (2012). Antihyperglycemic and antioxidant potential of polysaccharide fraction from P*ortulaca oleracea* seeds against streptozotocin-induced diabetes in rats. *Journal of Food Biochemistry*, 36(3), 378-382. https://doi.org/10.1111/j.1745-4514.2011.00547.x

Shen, H., Tang, G., Zeng, G., Yang, Y., Cai, X., Li, D., Liu, H., & Zhou, N. (2013). Purification and characterization of an antitumor polysaccharide from *Portulaca oleracea* L. *Carbohydrate polymers*, 93(2), 395-400. https://doi.org/10.1016/j.carbpol.2012.11.107

Siriamornpun, S., & Suttajit, M. (2010). Microchemical components and antioxidant activity of different morphological parts of Thai wild purslane (*Portulaca oleracea*). *Weed Science*, 58(3), 182-188. https://doi.org/10.1614/WS-D-09-00073.1

Szalai, G., Dai, N., Danin, A., Dudai, N., & Barazani, O. (2010). Effect of nitrogen source in the fertilizing solution on nutritional quality of three members of the *Portulaca oleracea* aggregate. *Journal of the Science of Food and Agriculture*, 90(12), 2039-2045. https://doi.org/10.1002/jsfa.4049

Veenstra, T. D. (2012). Metabolomics: the final frontier?. *Genome Medicine*, *4*(4), 1-3. https://link.springer.com/article/10.1186/gm339

Villa-Ruano, N., Pérez-Hernández, N., Zepeda-Vallejo, L.G., Quiroz-Acosta, T., Mendieta-Moctezuma, A., Montoya-García, C., García-Nava, M.L., & Becerra-Martínez, E. (2019). ^{1}H-NMR based metabolomics profiling of citrus juices produced in Veracruz, México. *Chemistry & Biodiversity*, *16*(5), e1800479. https://doi.org/10.1002/cbdv.201800479

Zaman, S., Bilal, M., Du, H., & Che, S. (2020). Morphophysiological and comparative metabolic profiling of purslane genotypes (*Portulaca oleracea* L.) under salt stress. *Biomedical Research International*, *2020*, 1-17 https://doi.org/10.1155/2020/4827045

Zaman, S., Hu, S., Alam, M. A., Du, H., & Che, S. (2019). The accumulation of fatty acids in different organs of purslane under salt stress. *Scientia Horticulturae*, *250*, 236-242. https://doi.org/10.1016/j.scienta.2019.02.051

Zhao, R., Gao, X., Cai, Y., Shao, X., Jia, G., Huang, Y., Qin, X., Wang, J., & Zheng, X. (2013). Antitumor activity of *Portulaca oleracea* L. polysaccharides against cervical carcinoma in vitro and in vivo. *Carbohydrate polymers*, 96(2), 376-383. https://doi.org/10.1016/j.carbpol.2013.04.023

Zidan, Y., Bouderbala, S., Djellouli, F., Lacaille-Dubois, M. A., Bouchenak, M. (2014). *Portulaca oleracea* reduces triglyceridemia, cholesterolemia, and improves lecithin: cholesterol acyltransferase activity in rats fed enriched-cholesterol diet. *Phytomedicine*, 21(12), 1504-1508. https://doi.org/10.1016/j.phymed.2014.07.010

CAPÍTULO II

BIOACTIVE COMPOUNDS OF PURSLANE (P*ortulaca oleracea* L.) ACCORDING TO THE PRODUCTION SYSTEM: A REVIEW

PUBLICADO EN:

Montoya-García, C. O., García-Mateos, R., Becerra-Martínez, E., Toledo-Aguilar, R., Volke-Haller, V. H., & Magdaleno-Villar, J. J. (2023). Bioactive compounds of purslane (Portulaca oleracea L.) according to the production system: A review. *Scientia Horticulturae*, *308*, 111584.

BIOACTIVE COMPOUNDS OF PURSLANE (*Portulaca oleracea* L.) ACCORDING TO THE PRODUCTION SYSTEM: A REVIEW

Montoya-García, César Omar[1 *]; García-Mateos, Rosario[1]; Becerra-Martínez, Elvia [2]; Volke-Haller, Víctor Hugo [3]; Magdaleno-Villar, J. Jesús[1]

[1]Universidad Autónoma Chapingo - Departamento de Fitotecnia. Km. 38.5, Carretera México-Texcoco, 56230, Chapingo, Estado de México, México.

[2]Centro de Nanociencias y Micro y Nanotecnologías, Instituto Politécnico Nacional, Av. Luis Enrique Erro S/N, Unidad Profesional Adolfo López Mateos, Zacatenco, Delegación Gustavo A. Madero, Ciudad de México, 07738, México.

[3]Colegio de Postgraduados - Campus Montecillo. Km. 36.5, Carretera México-Texcoco, Montecillo, Texcoco, 56230, Estado de México, México.

*** Corresponding author:**
Montoya–García César Omar, montoya-garcia@outlook.com

ABSTRACT

Purslane (*Portulaca oleracea* L.) is an herbaceous plant consumed in Italy, the United Kingdom, Spain, Greece, Turkey, Iran, Malaysia, the Philippines, China, North Africa, Australia, the United States, Brazil, and Mexico. Purslane contains nutraceutical compounds that can be potentiated based on biotic and abiotic stress practices. It provides substantial contributions of minerals, polysaccharides, fatty acids, proteins, alkaloids, terpenoids, sterols, phenols, flavonoids, and vitamins, which together or individually provide neuroprotective, antimicrobial, antidiabetic, antioxidant, anti-inflammatory, antiulcerogenic, anticancer, and anticholinesterase properties. The preference for foods with high nutraceutical value, which provide benefits to human health and contribute to the prevention of diseases, mainly chronic-degenerative diseases, is increasing. In this review, the information regarding the effects of crop management on the chemical composition, medicinal effects, and new functional compounds with techniques based on metabolomics is updated. Under adverse edaphoclimatic conditions, an increase in nutraceutical compounds has been observed, which represents a production alternative given its nutritional and commercial value. Metabolomics techniques based on ^{1}H-NMR have helped elucidate the metabolome of purslane under specific growth conditions (stress, hydroponics, open field, and controlled), with reports of new compounds from the groups of lignans, flavonoids, homoisoflavonoids, homoisoflavones, amide glycosides, alkaloids, amide and isoindole alkaloids, and phenolic alkaloids such as oleraceins; this has broadened the potential food and medicinal panorama of this plant.

Keywords: metabolomics; nutraceuticals; abiotic stress; linoleic acid; α-linolenic; oleraceins.

I. INTRODUCTION

It is estimated that the world population will reach 10 billion inhabitants in the year 2050; this population increase will result in a greater demand for food. Unsustainable farming practices and the use of conventional crops will not address these challenges; coupled with the effects of abiotic factors such as temperature variations, precipitation, CO_2 levels, increased fertilizer costs, loss of soil fertility, salinity, etc., which further potentiate the problem (Jacobsen *et al*., 2013; FAO, 2019; Raza *et al*., 2020).

Throughout human history, 7,000 plants have been used for food, forage, medicine, clothing, and shelter; currently only about 200 plant species are used (Meyer *et al*., 2012). This has caused the loss of traditional foodstuffs which are often part of the culture and diet of the people who grow them (Gruber, 2017). Given this scenario, it is necessary to revalue plant species for their nutritional and nutraceutical characteristics, mainly those that are underutilized, which can contribute to food security, specifically in some rural regions and areas of extreme poverty.

The nutraceutical quality of various foods has been the subject of study, as well as their contribution to maintaining good health and preventing chronic-degenerative diseases in consumers, mainly due to the content of antioxidant components (Daliu *et al*., 2018; Santini *et al*., 2018; Sharif & Khalid, 2018).

Purslane (*Portulaca oleracea* L.) is one of the crops that has received the most attention since the discovery of its content of linoleic acid (18:2ω6), α-linolenic acid (18:3ω3), and various antioxidant compounds. Its cultivation under adverse conditions causes stress in the plant, which generates a higher concentration of nutraceutical compounds, which have been identified through several chemical techniques. Metabolomics is a tool that contributes to elucidating the biochemical changes caused by biotic and abiotic stress (Velásquez-Valle *et al*., 2020; Matamoros *et al*., 2021).

The objective of this review is to the state of the art of purslane is shown, with emphasis on the effect of abiotic stress, caused by the management of the crop under hydroponic and open field conditions, on the content of nutraceutical compounds, nutritional quality, medicinal properties, and recently identified bioactive compounds through different analysis techniques such as metabolomics. Research articles from the Scopus, Pubmed, ScienceDirect, and Google Scholar databases were consulted to consolidate the effect of the various factors involved in purslane

production and how they influence its nutraceutical composition and elucidation of potentially active molecules.

II. PURSLANE CULTIVATION

2.1 Plant description

Portulaca oleracea L. belongs to the Portulacaceae family and is commonly known as purslane, pigweed, little hogweed, and/or pusley in various countries. It is an herbaceous, succulent, cosmopolitan plant that includes more than 15 microspecies. Purslane is described in the Florentine Codex as a plant with a thick stem, small round leaves, and a creeping habit (Dibble & Anderson, 1963). The leaves are grouped at the joints and ends of the stem; they are fleshy, adapted to store water, flat and oval, with an obtuse apex and a wedge-shaped base. The stems are smooth, reddish and swollen at the nodes. The flowers, up to 6 mm wide, are yellow, without petioles, axillary, with five petals almost as long as the sepals and incisions at the tip, and they open individually. The seeds form in a small pod that splits open when ripe (Banerjee & Mukherjee, 2003; Akbar, 2020).

2.2 Purslane consumption

Purslane is considered a weed in some regions of the world; however, it is consumed in much of Italy, the United Kingdom, Spain, Greece, Turkey, Iran, Malaysia, the Philippines, China, North Africa, Australia, the United States, the Caribbean, Brazil, and Mexico (Mera-Ovando *et al*., 2014; Alu'datt *et al*., 2019; Nemzer *et al*., 2020). It can be consumed in various ways: in the Middle East, Greece, and India, it is eaten in salads or soups; in the Netherlands, as "*sop selam korkot*"; in France, "bonne femme"; in Indonesia, steamed with various vegetables and legumes called "*pecel*" and fried "*oseng-osen*"; in China, fried with garlic, sesame oil, and soy sauce; in Sri Lanka, with Maldivian fish, garlic, leek, chili powder, and lemon juice; in the United States, in pickles, pancakes, and salad; in Turkey, stewed with lamb and beans (Tarkergari *et al*., 2013; Popescu *et al*., 2018); and in Mexico, it is traditionally stewed with pork and tomato and chili sauce (Vibrans, 2016; Linares *et al*., 2017).

Poeydomenge & Savage (2007) observed that cooking and pickling purslane reduced the oxalate content of the tissue, while adding yogurt to fresh purslane leaves decreased the concentration of oxalates from 53 to 10% (Moreau & Savage, 2009).

2.3 Management and nutrition

Purslane is grown in various edaphoclimatic systems, hydroponics, and floating systems; with modifications in plantation density, nutritional management, production time from sowing to harvest, and aerial biomass yield (Table 1). Figure 1 shows the commercial production, and two purslane cultivars in Mexico.

In hydroponics, purslane can adapt to high levels of ammonium (NH_4^+) and salinity (NaCl), and low levels of oxygen in the nutrient solution. Montoya-García *et al*. (2019) reported that the 25/75 ratio of NH_4^+/NO_3^- in the nutrient solution increased the physical characteristics of two purslane cultivars, in terms of fresh and dry aerial biomass production.

Zaman *et al*. (2019) observed that purslane from Pakistan and China is tolerant to the application of NaCl (100 mM Na L^{-1}), since it did not reduce its productive potential of leaves, stems, and roots. In purslane from Spain, Cros *et al*. (2007) and Franco *et al*. (2011) reported tolerances between 7.5 and 15.0 dS m^{-1}, with low decreases in germination, height, number of leaves, leaf area, yield, and root development (fresh and dry weight, length and diameter).

In the field, a low demand for fertilizers has been reported. Santos *et al*. (2016), Montoya-García *et al*. (2017), and Hosseinzadeh *et al*. (2020) observed high responses in fresh biomass production to nitrogen fertilizers with 60 and 65 kg N ha^{-1}.

The maximum increases in purslane yield under field conditions have been established with 100 kg ha^{-1} N, and optimal economic doses of 65 kg N ha^{-1} and 2,500 plants/m^2, at three harvest dates (27, 34, and 42 days after emergence), with yields of 58, 95, and 130 t ha^{-1}, and net income of 30,338; 67,828; and 102,440 Mexican pesos per hectare (Montoya-García *et al*., 2017).

2.4 Chemical composition of purslane in function of harvest time

Purslane represents an excellent nutritional source for the consumer due to the quantity and concentration of elements necessary for human nutrition, since it offers nitrogen (N), phosphorus (P), sulfur (S), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), boron (B), manganese (Mn), and copper (Cu), as well as sodium (Na) and chlorine (Cl) in relatively low concentrations (Egea-Gilabert et al., 2014; Viana *et al*., 2015; Montoya-García *et al*., 2018b; Santiago-Saenz *et al*., 2018; Montoya-García *et al*., 2019).

Estimates of the nutritional content indicate that 5 g of dehydrated purslane, or between 87 and 119 g fresh (which depends on the moisture content, related to the date of harvest and fertilization with N, P, and K) can provide 0.8 to 1.6 g protein, 19 to 23 mg P, 36 to 77 mg K, 41 to 49 mg Ca, 66 to 81 mg Mg, 10 to 20 mg Na, 0.3 to 0.4 mg Mn, 3.1 to 7.4 mg Fe, 0.1 mg Cu, 0.2 to 0.3 mg Zn, and 0.1 to 0.2 mg B (Montoya-García *et al*., 2018b).

The makeup of proteins, lipids, carbohydrates, fiber, ashes, phenols (Uddin *et al*., 2012), and saturated and monounsaturated fatty acids (Stroescu *et al*., 2013) showed variations in purslane harvested in different seasons of the year. In late harvests, total fatty acids, α-linolenic and linoleic acid (Mortley *et al*., 2012) increase, as do concentrations of Ca, Mg, Zn (Uddin *et al*., 2012), N, K, Na, Mn, Fe, and Cu, flavonoids, chlorophyll *a* and *b*, *β*-carotene, antioxidant activity, palmitic acid, as well as stearic acid in the aerial biomass of purslane (Montoya-García *et al*., 2018a; Montoya-García *et al*. 2018b). Moreover, Petropoulos *et al*. (2019) point out that the early harvest contains high levels of ω3 fatty acids, phenolic compounds, and oleraceins, and a lower content of oxalic acid.

III. MEDICINAL USE

In traditional medicine, purslane has been reported to promote ethnopharmacological effects such as a hypoglycemic (Andrade-Cetto & Heinrich, 2005), for the treatment of hypertension (Castillo-España *et al*., 2009), wounds, intestinal infections, headache, stomach and intestinal inflammation, constipation, diabetes, anxiety (Hernández *et al*., 2003; Juárez-Vázquez *et al*., 2013; Guzmán-Gutiérrez *et al*., 2014; Alonso-Castro *et al*., 2017; Delgado-Altamirano *et al*.,

2017), blood circulation, and cardiovascular disorders (Camou-Guerrero *et al*., 2008; Lara *et al*., 2019).

It has been shown that the consumption of foods with omega 3 (ω-3 α-linolenic acid) and omega 6 (ω-6 linoleic acid) polyunsaturated fatty acids, precursors of eicosapentaenoic and docosahexaenoic acid, can reduce the risk of cardiovascular and cerebral diseases (Carrero *et al*., 2005). ω-3 fatty acids inhibit carcinogenesis through anti-inflammatory activity (Larsson *et al*., 2007); ω-6 fatty acids reduce carcinogenesis through oxidative and pro-inflammatory damage mechanisms, as well as endoplasmic reticulum stress, and apoptosis in human hepatoma cells (Gerber, 2009).

The consumption of purslane has medicinal effects and possible benefits for human health, these findings were observed in experiments with rodents fed with purslane (Table 2); the effects include anti-inflammatory, analgesic (Chan *et al*., 2000), improvement in scar formation (Rashed *et al*., 2003), bronchodilators (Malek *et al*., 2004), antimutagenic (Alam *et al*., 2014a; Caballero-Salazar *et al*., 2002), and contraceptive (Londonkar & Nayaka, 2013).

Purslane consumption is useful in the prevention of cardiovascular and neurodegenerative diseases (Dkhil *et al*., 2010; Moneim *et al*., 2013), improves learning capacity, memory and environmental stimuli, and decreases anxiety, (Hongxing *et al*., 2007). It also increases enzyme activity, such as glutathione (GSH), glutathione reductase (GR), glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD) (Wang & Yang, 2010), and glutathione-S-transferase (GST) and increases defenses against neurodegeneration caused by environmental neurotoxins and hypoxia (Al-Quraishy *et al*., 2012; Wanyin *et al*., 2012).

The consumption of leaves, stems, and seeds of *P. oleracea* by mice, caused increases in body weight, white blood cells, splenocytes, serum cytokines (interleukin-2, interleukin-4). Furthermore, it controlled blood glucose and lipid levels, and reduced the reactive substances of thiobarbituric acid, in addition to contributing to the increase in the enzymatic activity of GPx, CAT, and SOD. Finally, it enhanced cytochrome-C and malondialdehyde activity in heart tissues (Li *et al*., 2009; El-Sayed, 2011; Sharma *et al*., 2012; Li *et al*., 2014; Ariyanfar *et al*., 2020).

The pectic polysaccharides extracted from the purslane stem showed antiviral activity (HSV-2) (Dong *et al*., 2010), increased T lymphocytes, leukocytes, $CD4_+/CD8_+$ ratio in blood, and decreased levels of aspartate transaminase, alanine transaminase, urea nitrogen, and creatinine (Shen *et al*., 2013). Other results include inhibition of hemolysis of red blood cells, increase of T and B lymphocytes of thymocytes (YouGuo *et al*., 2009), inhibited the cell growth of cervical cancer *in vitro* and *in vivo* (Zhao *et al*., 2013), and immunoactivity, antispasmodic activity, and immunosuppressive action in mice (Catap *et al*., 2018).

Taha & Osman (2015) and Zidan *et al*. (2015) observed that feeding *P. oleracea* L. to rats restores the level of enzymatic and non-enzymatic antioxidants in the liver, decreases total serum cholesterol (LDL-HDL1, HDL2, and HDL3), triacylglycerols, and increases lecithin: cholesterol acyltransferase activity, due to the high antioxidant potential *in-vitro* and *in-vivo*.

IV. NUTRIENT AND NUTRACEUTICAL CHARACTERISTICS DEPENDING ON CROP MANAGEMENT

The classification of nutraceutical foods can be based on the action mechanism and chemical nature, such as dietary fiber, probiotics, prebiotics, polyunsaturated fatty acids, antioxidant vitamins, and polyphenols (Kokate *et al*. 2002; Kalia, 2005). These compounds, such as vitamins and antioxidants (vitamins C and E, carotenoids: β-carotene, lutein, and zeaxanthin) and polyphenols (flavonoids, flavones, flavan-3-ols, flavanones, and anthocyanins), commonly known as phytochemicals, are functional metabolites biosynthesized by plants as a response to biotic and abiotic factors, which provide defense against predators, some are attractive to the same or another species, or as coloring agents to attract or warn other species (Harborne, 2001).

In the nutraceutical aspect, some natural antioxidant substances are of great interest, since they can inhibit the oxidation caused by reactive oxygen and/or nitrogen species in humans (Al-Gubory *et al*., 2010; Piccolella *et al*., 2019). These substances arise in living cells as a by-product of the aerobic respiration process and metabolism (Al-Guboryet al., 2010). Their uncontrolled production implies the generation of liver, cardiovascular, and degenerative diseases associated with aging, as well as various types of cancer (Alzheimer's, arthritis, Parkinson's and atherosclerosis) (Ali *et al*., 2008; Moon & Shibamoto, 2009).

Purslane is considered one of the richest plant sources of fatty acids, particularly linoleic and α-linolenic acids (Erkan, 2012; Montoya-García *et al*., 2018a). The fatty acids with the greatest presence are α-linolenic, palmitoleic, palmitic, linoleic, oleic, and stearic acids (Dubois *et al*., 2007), and trace amounts of eicosapentaenoic and docosahexaenoic acids (Harris *et al*., 2013).

In terms of phytochemical compounds in purslane, concentrations of α-tocopherol, ascorbic acid, *β*-carotene, lutein, and zeaxanthin have been determined (Dias *et al*., 2009; Oliveira *et al*., 2013; Viana *et al*., 2015; Egea-Gilabert et al., 2014; Montoya-García *et al*., 2018a; Santiago-Saenz *et al*., 2018), B complex vitamins, such as thiamin, riboflavin, niacin, pantothenic acid, pyridoxine, and folate (Uddin et al., 2014), and various phenolic compounds (gallic, protocatechuic, *p*-hydroxybenzoic, chlorogenic, vanillic, caffeic, syringic, and *p*-coumaric, cinnamic umbelliferone), and flavonoids (rutin, myricetin, quercetin, apigenin and kameferol, isoquercetin, kaempferol, luteolin, genistein, ferulic) (Siriamornpun & Suttajit , 2010; Egea-Gilabert et al., 2014; Gatea *et al*., 2017; Santiago-Saenz *et al*., 2018).

Likewise, the nutritional and nutraceutical content in purslane can vary from the nutritional source provided to the plant (ammonium/nitrate ratio, salinity (NaCl), fertilization with N, P, and K, ZnO nanoparticles) and culture system (hydroponics and open field) (Table 3). The following subheadings provide details on the effects of crop management on purslane properties.

4.1 Use of substrates

Cross *et al*. (2007) observed that purslane develops better with the use of peat than with vermiculite and coconut fiber in a floating hydroponic system, since it promoted the growth and yield of aerial and root biomass. Furthermore, it improved the concentration of α-linolenic, linoleic, palmitic, stearic, myristic, and arachidic acids.

For their part, Alu'datt *et al*. (2019) found that wild purslane has a favorable response depending on the type of substrate used in hydroponics. A combination of tuff:peatmoss (2:1) increased fresh and dry aerial biomass production, and plant height; and with tuff:peatmoss:perlite (2:1:1) increased proteins and lipids. Meanwhile, the plants produced in soil had a higher concentration of phenolic compounds, flavonoids, and anthocyanins. The highest antioxidant

capacity measured in IC_{50} was obtained with plants grown on peatmoss, followed by tuff, and tuff:peatmoss (1:1).

4.2 Ammonium/nitrate ratio

The application of an ammonium/nitrate ratio (NH_4^+/NO_3^-) in the nutrient solution could contribute to improving certain nutritional characteristics of purslane. Results of review are presented in Table 3. Fontana *et al*. (2006) found no differences with the 40/60 and 60/40 ratios in size and weight of leaves and stems. The opposite was observed for the concentration of fatty acids, mainly α-linolenic and linoleic, and they obtained lower concentrations of protein and oxalic acid with 60/40. Similar results were obtained by Szalai *et al*. (2010) in purslane subspecies.

When producing purslane in a hydroponic system with a 25/75 NH_4^+/NO_3^- ratio in the nutrient solution, the result was an increase in plant height, number of leaves, production of fresh and dry biomass, and concentrations of nitrogen, phosphorus and potassium; the opposite is observed for calcium and magnesium (Montoya-García *et al*., 2019).

Camalle *et al*. (2020) identified changes in the phytochemical composition of purslane with 66/33 and 25/75 NH_4^+/NO_3^- in the nutrient solution in the concentration of (i) chlorophylls, corotenoids, proteins, total fatty acids, α-linolenic, alanine, aspartic acid, leucine, and (ii) fructose, myo-inositol, raffinose, glutamate, glycine, isoleucine, ornithine, phenylalanine, proline, serine, tyramine, and urea.

4.3 Saline stress

Salinity is an abiotic stress factor that limits crop production. In the field, production land can be affected by salinity, with potentially serious implications for crop yields. High salinity in soils disrupts the ability of roots to extract water, resulting in the inhibition of physiological and biochemical processes. In contrast, the dosage of NaCl in the nutrient solution is one of the most studied abiotic factors in purslane and its influence on the content of nutritional compounds and phytochemicals. This crop shows great tolerance to salts and increases the concentration of primary and secondary metabolites; however, the effects vary depending on the cultivar and its geographic distribution. Impact of saline stress is summarized in Table 3.

In this context, the application of 70 and 140 mM NaCl L^{-1} increased the concentration of malondialdehyde and proline. This implies a better tolerance to oxidative stress and the reduction of superoxide radicals and decreases the enzymatic activity of superoxide dismutase, peroxidase, catalase, and ascorbate peroxidase after 30 days of exposure to salinity (Yazici *et al*., 2007). Likewise, α-linolenic, linoleic, palmitic, palmitoleic, stearic, and arachidic acids increased in function of the NaCl concentration, between 120 and 240 mM NaCl L^{-1} (AnastáCio & Carvalho, 2013).

The origin of the purslane influences the response to salinity. In this context, Alam *et al*. (2014b) evaluated 25 accessions from Malaysia and classified the tolerant individuals into five salinity levels (0 to 40 dS m^{-1}) in relation to the observed morphological characteristics. Based on this, they identified two individuals tolerant to 40 dS m^{-1} and six others moderately tolerant at 30 dS m^{-1}; however, the concentrations of phosphorus, potassium, calcium, magnesium, iron, and zinc decreased when the concentration of sodium in aerial biomass increased (Alam *et al*., 2016). Meanwhile, in two purslane cultivars from Pakistan and China, with 200 mM NaCl L^{-1}, the levels of monounsaturated fatty acids in leaves, and α-linolenic and linolenic acid in leaves, stems, and roots increased (Zaman *et al*., 2019).

With 50 mM NaCl L^{-1} in solution, there was an increase in the concentration of chlorophylls, carotenoids, proteins, total fatty acids, α-linolenic acid, fructose, myo-inositol, galactinol, ononitol, raffinose, pinitol, alanine, aspartic acid, leucine, glutamate, glycine, isoleucine, serine, and citric acid; and the concentration of leucine, and oxalic and ascorbic acids decreased (Camalle *et al*., 2020).

On the other hand, the concentration of soil salinity measured in EC (dS m^{-1}), did not limit the growth of purslane in terms of fresh and dry biomass, unlike production in the hydroponic system, and the concentration of nutrients such as N, P, Ca, Mg, S, Fe, Al, Ba, Sr, Zn, and Cu in leaves decreased; while K, Na, and Cl increased. The authors state that purslane cultivation could extract between 400 and 500 kg NaCl ha^{-1} from the soil (Bekmirzaev *et al*., 2021). This information is important to rehabilitate or cultivate in highly saline areas and carry out bio-extraction of sodium and chlorine.

The osmotic effects induced by NaCl can negatively affect CO_2 accumulation, inhibition of electron transport, inactivation of photosystem II (PSII) reaction centers, and destruction of the oxygen evolving complex (OEC). Proline accumulation lies in its ability to stabilize proteins, membranes, and subcellular structures, and protect them from damage through the elimination of ROS (He *et al*., 2021; Hnilickova *et al*., 2021).

In context, in purslane it was observed that with doses of 300 mM NaCl L^{-1}, the water potential, CO_2 assimilation, stomatal conductance, and PSII yield (ΔFv/Fm) in leaves decreased, while substomatal CO_2 and proline concentration increased. This shows a transition from C4 metabolism to CAM, and tolerance to a lower level of saline stress and the possibility of its use in saline localities (Hnilickova *et al*., 2021).

He *et al*. (2021) observed that purslane subjected to 200 and 300 mM NaCl L^{-1} had lower contents of water content, chlorophyll, carotenoids, electron transport rate, PSII yield (ΔFv/Fm), P_N light response, Cyt b_6f, nitrogen compounds (NO_3^-, total reduced nitrogen and proteins), RUBISCO activity, ascorbic acid, and phenolic compounds. However, with 300 mM NaCl L^{-1}, there was an increase in CAM acidity and proline, while with 100 mM NaCl L^{-1}, greater biomass and root area, number of leaves, and leaf area were obtained.

4.4 Water stress

One of the factors of vital importance in production in closed hydroponic systems and that generally receives little attention is the supply of dissolved oxygen in the nutrient solution. When oxygen is lacking, the plant reduces water and mineral uptake, which can limit crop growth and yield. Details about using water stress are shown in Table 3.

The effect of aeration levels in the nutrient solution in purslane (null, low, and high, that varied between 8 to 1 (i), 8 to 4 (ii), and 8 mg O_2 L^{-1} (iii)) was studied. Lower levels of aeration showed a slight decrease in purslane growth (leaf area, biomass fresh and dry weight, and root area), nitrate and K^+ concentration in leaves, and increased the content of phenols, chlorophyll (SPAD), and antioxidant capacity (Lara *et al*., 2011). This indicates the ability of purslane to adapt to the gradual decrease in oxygen in the nutrient solution and increase phytochemical compounds.

Uddin *et al*. (2017) evaluated the response of purslane to five soil water conditions, continuous field capacity (H_i), continuous saturation (H_{ii}), continuous flooding (H_{iii}), 10 days of flooding followed by saturation condition (H_{iv}), and 10 days saturation followed by field capacity (H_v). They reported greater plant height and stem diameter with H_{ii}, leaf area with H_{iii}, decrease in phenolic compounds from 3.06 to 1.33 mg QAE g^{-1} from treatments H_i to H_v, and higher content of flavonoids 1.2 mg QE g^{-1} in H_{ii} to H_{iv}. The increased intensity and duration of drought stress increases the production of reactive oxygen species (ROS) in the stem, such as H_2O_2 and O_2^-, which can cause damage to protein, lipid, and DNA macromolecules. Acclimatization mechanisms such as cell wall modifications, osmotic adjustment, and activation of the antioxidant defense system such as catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), and ascorbate peroxidase (APX) are induced.

Saheri *et al*. (2020) evaluated three levels of water stress in purslane (90, 60, and 30% field capacity (FC)) and leaf salicylic acid (0, 0.5, and 1.0 mM). They observed decreases, depending on water stress, in plant height, water use, fresh and dry weight of biomass, number of leaves, chlorophyll *a* and *b*, carotenoids, stomatal conductance, net photosynthetic rate, transpiration rate, and intercellular CO_2 concentration. They also observed increases in sugars, proline, and antioxidant defense enzymes such as CAT, SOD, POD, and APX, and antioxidants (phenolic compounds and total flavonoids). Meanwhile, fatty acids such as palmitic and linoleic acid increased with 60% FC; stearic, oleic, and α-linolenic acid decreased at lower FC; and with 30% FC, arachidic and behenic acids increased. Leaf application of salicylic acid contributed to mitigate the negative effects of water stress, due to the increase of proline and soluble sugars, and secondary metabolites and phenolic compounds and flavonoids. Additionally, it improved the activity of antioxidant enzymes and the regulation of the fatty acids profile.

4.5 Fertilization and nutrition

Fertilization is one of the main factors that can limit agricultural production; air quality, groundwater and surface water, as well as the physical, biological, and physicochemical properties of the soil, and with greater emphasis it can affect production costs. Excessive or insufficient applications and doses of fertilizers negatively affect the environment, and the quantity and quality of crops. The tools that contribute to obtaining optimal doses of fertilization to decrease the

environmental impact and production costs, as well as maximize yields, are highly valued today. This contributes to producing more and better-quality food. The effects of fertilization and nutrition in the purslane crop and its influence on the nutritional and metabolic compounds of the leaves are presented below. Data about fertilization of purslane in context the increase in the of chemical composition are presented in Table 3.

In purslane production, influence was reported from the use of nitrogen fertilizer in two years of evaluation. One such fertilizer is ammonium sulfate, which contributed to increasing plant height and root length; whereas, ammonium nitrate had an effect on purslane leaf yield, followed by urea application. The nutritional concentrations of Ca, K, Mg, P, and S were improved with calcium ammonium nitrate. Ammonium sulfate improved Fe and B, and ammonium nitrate improved Zn (Kaymak, 2013). This information coincides with Mohammed & Suliman (2019), who reported that ammonium sulfate increased the nutrient concentration and decreased the nitrate content in purslane leaves.

The concentration of nitrate in purslane leaves and stems is influenced by the addition of nitrogen fertilizers. At higher doses (from 0 to 90 kg N ha^{-1}), it increased by 38.8 and 56.6 mg NO_3^- g^{-1} in stem. Contrastingly, a dose of 60 kg N ha^{-1} resulted in 42.4 and 33.5 mg NO_3^- g^{-1}. The concentration of oxalic acid had similar performance with 0.55 and 1.46 mg g^{-1} in leaves with 60 and 90 kg N ha^{-1} (Santos *et al*., 2016).

Montoya-García *et al*. (2017) reported that the concentration of nitrate in leaves and stems is influenced by the dose of N and P applied. This can be reduced to ranges that are not harmful to human health, with values of 516 mg NO_3^- kg^{-1} of fresh matter, without application of N and P in three commercial harvests, and increases to values of 1329, 2005, and 1700 mg NO_3^- kg^{-1} obtained with 300 kg ha^{-1} N and 90 kg P_2O_5.

When applying organic fertilizers (cow and chicken manure; 120 kg N ha^{-1}), the nitrate concentration in the purslane stem is reduced, between 30 and 60 mg NO_3^- kg^{-1}, compared to inorganic fertilizers where the content was over 100 mg NO_3^- kg^{-1} (Fallah & Omrani *et al*., 2018).

In increasing doses of organic fertilizer enriched with bacteria (0, 67, 134, 200 g kg^{-1}) and their interaction with 6.67 g NPK kg^{-1}, raising increments were observed in purslane plant height,

stem diameter, and leaf area, concentration of nitrates, oxalic acid, and total saturated fatty acids in leaves with the application of organic fertilizer. Meanwhile, the organic and inorganic interaction enhanced the concentrations of soluble sugars, vitamin C, anthocyanins, carotenoids, flavonoids, and, α-linolenic and linoleic acids (Yang *et al*., 2020).

In soil-grown purslane, the doses of nitrogen fertilization modified the concentration of fatty acids in stems. Higher N doses cause an increase of linoleic acid (15–22%) and α-linolenic acid (23–27%), unlike stearic acid (11–13%) and palmitic acid (38–45%). The concentration of phytochemicals of an antioxidant nature showed modifications from fertilization with N, P, and K. At higher doses of fertilizer, ascorbic acid and flavonoids decrease, with more drastic effects at higher doses of P and K. A lower dose of N increases total phenols, chlorophyll *a* and *b*, while the dose of K decreases the phenolic compounds and chlorophylls. The *β*-carotene concentration increases with N and K fertilization, and the antioxidant activity was higher with doses of 300 kg N ha^{-1} and late harvest dates, with similar effects of DPPH reduction (Montoya-García *et al*., 2018a).

Hosseinzadeh *et al*. (2020; 2021) evaluated the concentration of nutraceutical compounds of purslane based on the application of organic fertilizer (8 t ha^{-1} at 0, 25, 50, 75, and 100%) and inorganic fertilizer (120 kg urea ha^{-1} at 0, 25, 50, 75, and 100 %) and the application of arbuscular mycorrhizae (*Rhizophagus irregularis*) under 70% irrigation conditions and drought stress, in two years of evaluation. They reported a greater increase in flavonoids with 25/75 organic/inorganic (O/I) under drought stress conditions and inoculation. They found increased linoleic and *α*-linolenic acids with 0/100 O/I, and, palmitic and stearic acids without the application of fertilizers. The isolated effects were: mycorrhizal inoculation increased purslane biomass, seed production, antioxidant activity (AA), and content of unsaturated fatty acids (UFA). Drought stress reduced the concentrations of P and N, AA, seed production, and biomass, and increased the concentration of malondialdehyde, flavonoids (FT), saturated fatty acids (SFA), and UFA. Nitrogen fertilizer decreased malondialdehyde and increased biomass, P, N, FT, AA, SFA, and UFA.

Leaf application of nanoparticles (NPs) showed changes in the phytochemical composition of purslane in roots, stems, and leaves. Zinc oxide (500 mg ZnO NPs L^{-1}) increased seed germination, growth and biomass production, contents of chlorophyll *a* and *b*, carotenoids, phenols,

flavonoids, antioxidant capacity, and peroxidase and catalase activity, compared to plants that did not receive the Zn treatment (Iziy *et al*., 2019).

In italian purslane microgreens production (23 days after sowing) identified phenolic acids: caffeic, caffeoyl feruloyl, tartaric, caffeoyl quinic, ferulic, feruloyl hexoside, feruloyl quinic, and sinapinic acid hexose; and flavonoids: kaempferol-3-glucoside, luteolin-7-o-glucoside, quercetin rhamnoside and rutin (Corrado et al., 2021). In purslane microgreens (14 days after sowing), biofortified with selenium at increasing doses (0 to 10 mg Se L^{-1}), increased the concentration of chlorophylls, carotenoids, anthocyanins, antioxidant capacity, phenolic compounds and flavonoids (Puccinelli et al., 2021).

Purslane production under heavy metal stress conditions, caused by Cr and Cd, reduced aerial biomass but stimulated chlorophylls and antioxidant enzymes production (CAT, SOD, GP, and APX); moreover, these metals accumulated in plant roots, mainly (Thalassinos et al., 2021 and 2022; Akandi et al., 2022).

In purslane from Iran, foliar application with salicylic acid (100 M), cerium oxide (50 mg L^{-1}) and cerium oxide:salicylic acid nanoparticles, in interaction with salinity (0, 50 and 100 mM NaCl) can increase the concentration of total soluble solid, chlorophyll, oil yield, chlorophylls, total phenolics, flavonoids, anthocyanins, malondialdehyde, proline, H_2O_2, CAT activity and nutrients (N, P, Na, K, Mg, Ca, Fe, Zn and Mn). In addition, increases plant height, aerial biomass yield in fresh and dry by salicylic acid and cerium oxide, but decreases as NaCl concentration increases (Hassanpouraghdam et al., 2022).

In purslane wild and commercial plants from Italy, the biofortification with foliar zinc (0, 1.3, 2.6 and 5.2 mg Zn L^{-1}) in hydroponics can increased concentration of neoxanthin, lutein β-carotene, stearic acid, arachidic acid, behenic acid, myristic acid and oleic acid, with higher contents at 1.3 mg Zn L^{-1} dose; but not for biomass yield, total saturated fatty acids content, n-6/n-3 ratio, palmitic and linoleic acids content (D'Imperio et al., 2022).

V. ANALYTICAL METHODS FOR THE IDENTIFICATION OF NEW ACTIVE COMPOUNDS

The concentrations of nutraceutical compounds vary widely, and precise analyzes are required to determine the metabolites present in crops (Fujimura *et al*., 2011). New phytochemical compounds have been identified in purslane through high-performance liquid chromatographic (HPLC) and its variants, gas chromatography–mass spectrometry (GC–MS), spectroscopic methods, including one-dimensional (1D) and two-dimensional (2D) proton nuclear magnetic resonance (^{1}H-NMR). For example, in purslane the concentrations of dopamine and norepinephrine were determined with techniques of (i) derivatization in a dark ultrasonic, fluorescein isothiocyanate (FITC) separated by capillary zone electrophoresis (CZE) and detected with laser-induced fluorescence; (ii) HPLC coupled with photodiode array (PDA); and, (iii) simple and rapid capillary electrophoretic. Reported concentrations range between 0.004–6.00 µg and 0.011–8.25 µg of norepinephrine and dopamine (Zhang *et al*., 2002; Chen *et al*., 2003; Yue *et al*., 2005).

Below are detailed the compounds identified with various analytical methods that contribute to the elucidation of new molecules in the stem of purslane with potential pharmacological and nutritional use.

5.1 Oleraceins

Among the alkaloids characterized in purslane extracts, there is a class of indoline amide glycosides, called oleraceins or cyclo-dopa amides, which have the 5,6-dihydroxyindoline-2-carboxylic acid N-acylated with cinnamic acid derivatives, and are highly glycosylated (Voynikov *et al*., 2021).

Five oleraceins: named A, B, C, D, and E, were identified with the use of ^{1}H–^{13}C–RMN (500 and 125 MHz, DMSO-d_6). These oleraceins are yellow pigments, highly water-soluble, that have a cyclodopa residue (cyclic 3,4-dihydroxy-phenylalanine, or 5,6-dihydroxy-indoline-2-carboxylic acid), derived from the condensation of cyclodopa with ferulic acid or *p*-coumaric acid. The cyclodopa fraction may possibly be the 2S configuration, similar to those of betalains. The antioxidant potential of these phenolic-alkaloids influences the inhibition of DPPH, measured in inhibition (EC_{50}). Three oleraceins (A, B and E) were evaluated in comparison with butylated hydroxyl anisole (9.08 µM), ascorbic acid (11.70 µM), and α-tocopherol (13.14 µM), with results

of B: 5.56 µM, A: 8.96 µM, and E: 9.87 µM. Additionally, there was a reducing effect on lipid peroxidation in the brains of rats. These data demonstrate the potential of a new class of antioxidant agents in purslane (Xiang *et al*., 2005).

With ^{1}H–^{13}C–RMN (500 and 125 MHz, DMSO-d_6), two new oleraceins, F and G, were reported. These showed an antioxidant potential measured in DPPH inhibition of 21.0 and 37.7 µM, respectively, compared to butylated hydroxyl anisole (5.2 µM) and ascorbic acid (16.4 µM) (Liu *et al*., 2011).

In HPLC-DAD analysis combined with HPLC-ESI-MS/MS, eight new oleraceins (H, I, J, K, L, M, N, and O) and four previously reported (A, B, C, and D) were detected (Jiao *et al*., 2014). These were corroborated with ^{1}H–^{13}C–RMN (500 and 125 MHz, DMSO-d_6) by Jiao *et al*. (2015); each of them has four different functional R groups; and antioxidant activities of 42.8, 40.1, 29.0, 27.6, 15.3, 16.1, 43.5, 40.9, 31.5, 35.4, 35.1, 38.1, 38.2, 34.5, and 30.1 µM to inhibit DPPH (EC_{50}) for oleraceins H, I, N, O, K, L, P, Q, R, S, A, B, C, D, and vitamin C, respectively.

With UHPLC-MS and UPLC techniques, various origin-dependent compounds were observed in purslane from Greece and Bulgaria; 14 phenolic acids and 2 flavonoids were reported, such as: vanillin, ellagic, gallic, gentisic, protocatechuic, 4-hydroxybenzoic, 2-hydroxybenzoic, *p*-coumaric, *m*-coumaric, caffeic, ferulic, neochlorogenic, and chlorogenic acids, rutin, and quercetin-3-O-glucoside. Also detected were oleraceins with different functional R groups, four oleraceins (A – D) with 2 R groups, five (N – Q, and S) with 3 R groups, and four (U, W, C, and D) with 1 R group. All of the above had antioxidant and DPPH-reducing potential (Voynikov *et al*., 2019).

In Spanish purslane were identified 24 compounds with HPLC–DAD/ESI–MS analysis, six were phenolic acids, six flavonoids, hydroxytyrosol hexoside, citric and isocitric acids and eight alkaloids compounds, specifically oleraceins A, B, N, U, J, X and Y; when purslane samples were steamed, concentration of metabolome generally maintained the compounds content initial, and decrease antioxidant activity (Fernández-Poyatos et al., 2021).

Using ^{13}C-NMR and HPLC analysis, 15 alkaloids were identified in Chinase purslane, including one imidazolidine (1), two quinolizinium inner salts (2–3), three 2,5-dioxopyrrolidins

(4–6), one β-carboline derivative (7), three amide glycosides (8–10) and five indoline amide glucosides (11–15) denominated as oleracein (X, Y, Z, ZA and ZB, respectively) (Fu et al., 2021).

Voynikov et al. (2021) identified 51 compounds as oleraceins in Bulgarian purslane with UHPLC-Orbitrap-HRMS analysis, conducted by negative ionization; of these, 26 already known: A, B, C, D (2 isoformas), H, I (2 isoformas), J, K, L, M (2 isoformas), N/S (4 isoformas), O (3 isoformas), P, Q, W, X (3 isoformas) are N-acylated with either coumaric, caffeic or ferulic acid; in addition, 25 previously undescribed oleraceins were identified, linked with several moieties or alone, that include hydroxybenzoyl, coumaroyl, caffeoyl, feruloyl, sinapoyl and glucopyranosyl.

5.2 Metabolomic profile

The metabolic profile comprises qualitative and quantitative measurements of small molecules (50 - 1500 MW) (Villa-Ruano *et al*., 2019). Metabolic profiling techniques are used to assess the nutraceutical content of a cultivar, individuals, or between different environmental conditions (Scalbert *et al*., 2009). Changes in metabolism caused by external factors can be analyzed with analytical techniques, such as liquid and gas chromatography coupled to mass spectrometry (MS), capillary electrophoresis (CE), infrared spectrometry (IR), and nuclear magnetic resonance (NMR) (Cevallos-Cevallos *et al*., 2009; Koda *et al*., 2012; Veenstra, 2012). NMR-based techniques accurately identify and quantify dissolved metabolites in polar and non-polar extracts with high selectivity (Markley *et al*., 2017; Villa-Ruano *et al*., 2019).

Farag & Shakour (2019) performed the first metabolomic study in three taxa of *P. oleracea* L. (*P. oleracea*, *P. rausii*, and *P. granulatostellulata*) from Egypt using UPLC–ESI–MS/MS. They identified 85 metabolites, including 6 amino acids, 22 phenolic compounds, 16 alkaloids, and 11 fatty acids. They also observed seven oleraceins, named A, B, C, D, K, N, and O, and discovered four new ones, not previously reported: T, U, V, and W.

Meanwhile, Nemzer *et al*. (2020) detected differences between wild and commercial cultivars and identified 17 amino acids with HPLC in wild purslane. The ones with the greatest amount were glutamic acid, aspartic acid, leucine, and alanine, with smaller quantities of serine, proline, glycine, tyrosine, arginine, cysteine, threonine, valine, isoleucine, phenylalanine, lysine, histidine, and methionine.

Using LC-HRAMS, 38 accessions of purslane originating from Greece and Bulgaria were analyzed; 50 functional metabolites were identified, dependent on geographical origin. Of these, 29 were reported for the first time. In the metabolomic profile, the outstanding compounds were phenolic compounds, flavonoids, terpenoids, hydroxybenzoic acids, hydroxycinnamic acids, acylquinic acids, cinnamic acids, amines, coumarins, hydroxy and methoxy-coumarins, lignins, quinones, iridoids, cyclo-dopa amines (oleraceins A, B, C, and D), amino acids, vitamins, indole derivatives, tetrahydroisoquinoline, and three acids (citric, isoscitric, and quinic) (Balabanova *et al*., 2020).

Farag et al. (2021) characterized Egyptian purslane seed with ultra-performance liquid chromatography (UPLC) metabolomics, and identified 48 fatty acyl/lipids, seven carbohydrates, two glycosylated hydroxy-cinnamic acid derivatives, terpenoids, steroids, lignans, purine nucleosides, flavonoids subclasses, such as flavone, biflavonoid, polyflavonoid, flavan, chalcone and flavonoid glycosides. Seed extract, when was consumed by rodents, had beneficial effects in reducing brain damage; in addition, it increased enzymes responsible for protecting the brain from oxidative stress.

Free phenolic compounds were reported in Tunisian purslane by RP-HPLC system, 18 compounds were identified (catechol, catechin hydrate, epigallocatechin, chlorogenic acid, epicatechin-3-gallate, caffeic acid, p-coumaric acid, sinapic acid, ferulic acid, myricitrin, luteolin-7-O-glucoside, rosmarinic acid, myricetin, isorhamnetin-3-O-glucoside, apigenin, lutolin, quercetin and kaempferol). Phytochemical content varied with cooking treatment; raw and microwaved plant material showed higher levels of epigallocatechin, p-coumaric acid, isorhamnetin-3-O-glucoside, luteolin-7-O-glucoside and total phenolic content compared with pressure-cooked and microwaved samples (Farhat et al., 2022). Data about the metabolomic profile of purslane in context of genotype or cultivar are presented in Table 4.

Recently, more than 50 new compounds were identified in purslane by ^{1}H and ^{13}C analysis (500~600 and 150 MHz, DMSO-d_6) (Table 5): These compounds possess high antioxidant capacity, anticholinesterase activity, and reduced cytotoxic effects.

5.3 Influence of abiotic factors in the metabolomic profile

The metabolomic studies presented above have in common that purslane samples are collected from various regions, but the influence that the cultivation process may have is not taken into consideration, as shown in Chapter IV. There is currently little evidence of the influence of biotic or abiotic factors on the metabolomic profile of purslane.

For example, with the use of UPLC/MS, the presence of various compounds in purslane in dry weight was detected. These compounds increase through harvest time at 29, 43, and 52 days after planting (dap), mainly olerazin A at 1.03, 0.082, and 0.349 mg g in leaves, and in stems at 0, 7.5, 2.8 µg g; and oleracein C at 1.43, 0.212, 1.02 mg g in leaves, and in stems at 152, 67, 33 µg g. Four tocopherols, sugars, organic acids, and 20 fatty acids were also reported (Petropoulos *et al*., 2019).

Camalle *et al*. (2020) determined, with GC–MS, effects of the ammonium/nitrate ratio in interaction with salinity (NaCl) in two purslane cultivars. They observed higher contents of chlorophyll, carotenoids, proteins, and total fatty acids, as well as six sugars, twelve amino acids, and three organic acids from the effect of higher levels of NaCl.

By GC–MS, 132 metabolites were reported in two purslane accessions grown in America and China, of which 35 were organic acids, 26 amino acids, 20 sugars, 14 sugar alcohols, 20 amines, 13 lipids and sterols, and 4 other acids. The influence of the NaCl dose of 200 mM caused an increase in sugars in roots and sugar alcohols in leaves and roots in both accessions. In the American variety, organic acids, amines in leaves, and amino acids in leaves and roots increased (Zaman *et al*., 2020). The potential perspectives of influence of cultivar and abiotic factors in the metabolomic profile are presented in Table 4.

Most of the reported metabolites are involved in biochemical pathways, such as the TCA cycle, GS/GOGAT cycle, GABA, glycolysis, proline synthesis, shikimic acid, aromatics, and the phenylpropanoid, flavonoid and alkaloid pathway through tyrosine. The latter serves as a precursor for cinnamic acid and cyclodopaamides, where L-dopa is the precursor for the synthesis of cyclodopa amides and dopamine. Figure 2 shows a general diagram of the possible routes of primary and secondary metabolism of purslane.

VI. CONCLUSIONS

Purslane cultivation is a production alternative as it presents many expectations given its nutritional and nutraceutical properties, low production costs, and high productivity. Moreover, it is a short cycle crop from planting to harvest, easy to manage, with low nutritional demand, and high tolerance to abiotic factors; characteristics that can increase its nutritional and nutraceutical quality. The consumption of purslane in human diets should be revalued, since it has substantial contributions of (i) minerals, a wide variety of metabolites (ii) primaries such as sugars, amino acids, organic acids, and nucleosides, and (iii) secondaries with various chemical structures such as lignans, flavonoids, homoisoflavonoids, homoisoflavones, amide glycosides, alkaloids, amide and isoindole alkaloids, and phenolic alkaloids such as olerazines. These provide food and medicinal properties as a whole or by themselves and are part of the response mechanisms to abiotic stress. The recently published information on the various analytical methods and the report of new molecules suggests that biochemical compounds of great importance for human health can be identified in the future. The metabolomic profiles contribute to elucidate the effects of crop management on their production, showing a wealth of active principles or bioactive compounds to be used for medicinal and nutraceutical ends. In the future, well-designed clinical trials are hoped for to provide substantial evidence to demonstrate the beneficial effects of the bioactive compounds present in purslane and their possible use for medicinal and nutraceutical purposes. The dissemination of this information can contribute to increasing the preference for the scientific research, consumption, and production of purslane.

Acknowledgments

This research received financial support from Universidad Autonoma Chapingo and CONACyT in México. In memory of Casimiro Montoya Ruíz, father of the first autor, thank you dad for invaluable support and love.

REFERENCES

Akandi, Z. N., Makarian, H., Pirdashti, H., Amerian, M. R., Firozabadi, M. B., & Ghanbary, M. A. T. (2022). Iron Nanoparticles-induced Improvement of Antioxidant Enzymes and Photosynthetic Pigments of Purslane (*Portulaca oleracea* L.) Under Cadmium Stress. *Gesunde Pflanzen*, 1-10. https://doi.org/10.1007/s10343-022-00680-9

Akbar, S. (2020). *Portulaca oleracea* L. (Portulacaceae). In *Handbook of 200 Medicinal Plants* (pp. 1491-1504). Springer Nature Switzerland AG. https://doi.org/10.1007/978-3-030-16807-0_154

Alam, M. A., Juraimi, A. S., Rafii, M. Y., Hamid, A. A., Aslani, F., Hasan, M. M., Zainudin M. A. M., & Uddin M. K. (2014a). Evaluation of antioxidant compounds, antioxidant activities, and mineral composition of 13 collected purslane (*Portulac aoleracea* L.) accessions. *BioMed Research International*, 296063. https://doi.org/10.1155/2014/296063

Alam, M. A., Juraimi, A. S., Yusop, R. M., Hamid, A. A., & Hakim, A. (2014b). Morphophysiological and mineral nutrient characterization of 45 collected purslane (*Portulaca oleracea* L.) accessions. *Bragantia*, *73*(4), 426-437. https://doi.org/10.1590/1678-4499.253

Alam, M., Juraimi, A. S., Rafii, M. Y., Hamid, A. A., Aslani, F., & Hakim, M. A. (2016). Salinity-induced changes in the morphology and major mineral nutrient composition of purslane (*Portulaca oleracea* L.) accessions. *Biological research*, 49, 1-19. https://doi.org/10.1016/j.tifs.2016.06.010

Al-Gubory, K. H., Fowler, P. A., & Garrel, C. (2010). The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. *The international journal of biochemistry & cell biology*, *42*(10), 1634-1650. https://doi.org/10.1016/j.biocel.2010.06.001

Ali, S. S., Kasoju, N., Luthra, A., Singh, A., Sharanabasava, H., Sahu, A., & Bora, U., (2008). Indian medicinal herbs as sources of antioxidants. *Food Research International*, *41*(1), 1-15. https://doi.org/10.1016/j.foodres.2007.10.001

Alonso-Castro, A. J., Domínguez, F., Maldonado-Miranda, J. J., Castillo-Pérez, L. J., Carranza-Álvarez, C., Solano, E., Isiordia-Espinoza, M. A., Juárez-Vázquez, M. C., Zapata-Morales, J. R., Argueta-Fuertes, M. A., Ruíz-Padilla, A.J., Solorio-Alvarado, C. R., Rangel-Velázquez, J. E., Ortiz-Andrade, R., González-Sánchez, I., Cruz-Jiménez, G., & Orozco-Castellanos, L. M. (2017). Use of medicinal plants by health professionals in Mexico. *Journal of Ethnopharmacology*, *198*, 81-86. https://doi.org/10.1016/j.jep.2016.12.038

Al-Quraishy, S., Dkhil, M. A., & Moneim, A. E. A. (2012). Protective effects of *Portulaca oleracea* against rotenone mediated depletion of glutathione in the striatum of rats as an animal model of Parkinson's disease. *Pesticide biochemistry and physiology*, *103*(2), 108-114. https://doi.org/10.1016/j.pestbp.2012.04.005

Alu'datt, M. H., Rababah, T., Alhamad, M. N., Al-Tawaha, A., Al-Tawaha, A. R., Gammoh, S., Ereifej, K.I., Al-Karaki, G., Hamasha, H. R., Tranchant, C. C., & Kubow S. (2019). Herbal yield, nutritive composition, phenolic contents and antioxidant activity of purslane (*Portulaca oleracea* L.) grown in different soilless media in a closed system. *Industrial Crops and Products*, *141*(1), 111746. https://doi.org/10.1016j.indcrop.2019.111746

AnastáCio, A., & Carvalho, I. S. (2013). Accumulation of fatty acids in purslane grown in hydroponic salt stress conditions. *International journal of food sciences and nutrition*, 64(2), 235-242. https://doi.org/10.3109/09637486.2012.713915

Andrade-Cetto, A., & Heinrich, M. (2005). Mexican plants with hypoglycaemic effect used in the treatment of diabetes. *Journal of ethnopharmacology*, *99*(3), 325-348. https://doi.org/10.1016/j.jep.2005.04.019

Arias-Rico, J., Macías-León, F. J., Alanís-García, E., Cruz-Cansino, N. D. S., Jaramillo-Morales, O. A., Barrera-Gálvez, R., & Ramírez-Moreno, E. (2020). Study of edible plants: Effects of boiling on nutritional, antioxidant, and physicochemical properties. *Foods*, *9*(5), 599. https://doi.org/10.3390/foods9050599

Ariyanfar, H., Matinhomaee, H., Hosseini, & Ghazalian, F. S. A. (2020). The effect of endurance training and purslane (*Portulaca oleracea*) seed consumption on Cytochrome-C and Malondialdehyde in the heart tissue of rats poisoned with H2O2. *Journal of Nutrition, Fasting and Health*, *8*(2), 80-86. https://doi.org/10.22038/jnfh.2020. 44729.1237

Balabanova, V., Hristov, I., Zheleva-Dimitrova, D., Sugareva, P., Lozanov, V., & Gevrenova, R. (2020). Bioinformatic insight into *Portulaca oleracea* L. (Purslane) of bulgarian and Greek origin. *Acta Biologica Cracoviensia s. Botanica*, 62(1). DOI: 10.24425/abcsb.2020.131662

Banerjee, G., & Mukherjee, A. (2003). Antibacterial activity of a common weed, *Portulaca oleracea* L. *Geobios* *30*, 143-144. https://www.koreascience.or.kr/article/JAKO200430710403274.pdf

Bekmirzaev, G., Ouddane, B., Beltrao, J., Khamidov, M., Fujii, Y., & Sugiyama, A. (2021). Effects of Salinity on the Macro-and Micronutrient Contents of a Halophytic Plant Species (*Portulaca oleracea* L.). *Land*, 10(5), 481. https://doi.org/10.3390/land10050481

Caballero-Salazar, S., Riveron-Negrete, L., Ordaz-Tellez, M. G., Abdullaev, F., & Espinosa-Aguirre, J. J. (2002). Evaluation of the antimutagenic activity of different vegetable extracts using an in vitro screening test. In Proceedings of the Western Pharmacology Society (Vol. 45, pp. 101-103). Seattle, Wash.: The Society. https://fikrat0.tripod.com/sitebuildercontent/sitebuilderpictures/procwest.pdf

Camalle, M., Standing, D., Jitan, M., Muhaisen, R., Bader, N., Bsoul, M., et al. (2020). Effect of Salinity and Nitrogen Sources on Leaf Quality, Biomass, and Metabolic Responses of Two Ecotypes of *Portulaca oleracea*. https://www.preprints.org/manuscript/202004.0044/v1

Camou-Guerrero, A., Reyes-García, V., Martínez-Ramos, M., & Casas, A. (2008). Knowledge and use value of plant species in a Rarámuri community: a gender perspective for conservation. *Human ecology*, *36*(2), 259-272. https://link.springer.com/article/10.1007/s10745-007-9152-3

Carrero, J. J., Martín-Bautista, E., Baró, L., Fonollá, J., Jiménez, J., Boza, J. J., & López-Huertas, E. (2005). Efectos cardiovasculares de los ácidos grasos omega-3 y alternativas para incrementar su ingesta. *Nutrición Hospitalaria*, *20*(1), 63-69. https://scielo.isciii.es/scielo.php?script=sci_abstract&pid=S0212-16112005000100010

Castillo-España, P., Cisneros-Estrada, A., Garduño-Ramírez, M. L., Hernández-Abreu, O., Ramírez, R., & Estrada-Soto, S. (2009). Preliminary ethnopharmacological survey of plants used in Mexico for the treatment of hypertension. *Pharmacognosy Reviews*, *3*(5), 48. https://www.phcogrev.com/article/2009/3/5-4

Castro-Lara D, Basurto-Peña F, Mera-Ovando L, & Bye R. (2011). Los quelites, tradición milenaria. Universidad Autónoma de Chapingo, Texcoco, Estado de México. 36 p.

Catap, E. S., Kho, M. J. L., & Jimenez, M. R. R. 2018. In vivo nonspecific immunomodulatory and antispasmodic effects of common purslane (*Portulaca oleracea* Linn.) leaf extracts in ICR mice. *Journal of ethnopharmacology*, 215, 191-198. https://doi.org/10.1016/j.jep.2018.01.009

Cevallos-Cevallos, J. M., Reyes-De-Corcuera, J. I., Etxeberria, E., Danyluk, M. D., & Rodrick, G. E. (2009). Metabolomic analysis in food science: a review. *Trends in Food Science & Technology*, 20(11-12), 557-566. https://doi.org/10.1016/j.tifs.2009.07.002

Chan, K., Islam, M. W., Kamil, M., Radhakrishnan, R., Zakaria, M. N. M., Habibullah, M., & Attas, A. (2000). The analgesic and anti-inflammatory effects of *Portulaca oleracea* L. subsp. sativa (Haw.) Celak. *Journal of ethnopharmacology*, 73(3), 445-451. https://doi.org/10.1016/S0378-8741(00)00318-4

Chen, J, Shi, Y. P. & Liu J. Y. (2003). Determination of noradrenaline and dopamine in Chinese herbal extracts from *Portulaca oleracea* L. by high-performance liquid chromatography. *Journal of Chromatography A*, 10. https://doi.org/10.1016/S0021-9673(03)00786-6

Corrado, G.; El-Nakhel, C.; Graziani, G.; Pannico, A.; Zarrelli, A.; Giannini, P.; Ritieni, A.; De Pascale, S.; Kyriacou, M.C.; Rouphael, Y. (2021). Productive and Morphometric Traits, Mineral Composition and Secondary Metabolome Components of Borage and Purslane as Underutilized Species for Microgreens Production. *Horticulturae*, 7, 211. https://doi.org/10.3390/horticulturae7080211

Cros, V., Martínez-Sánchez, J. J., & Franco, J. A. (2007). Good yields of common purslane with a high fatty acid content can be obtained in a peat-based floating system. *HortTechnology*, 17(1), 14-20. https://doi.org/10.21273/HORTTECH.17.1.14

Cui, X., Ying, Z., Ying, X., Jia, L., & Yang, G. (2021). Three new alkaloids from Portulaca oleracea L. and their bioactivities. *Fitoterapia*, 154, 105020. https://doi.org/10.1016/j.fitote.2021.105020

Daliu, P., Santini, A., & Novellino, E. (2018). A decade of nutraceutical patents: where are we now in 2018?. *Expert opinion on therapeutic patents*, 28(12), 875-882. https://doi.org/10.1080/13543776.2018.1552260

Delgado-Altamirano, R., Monzote, L., Piñón-Tápanes, A., Vibrans, H., Rivero-Cruz, J. F., Ibarra-Alvarado, C., & Rojas-Molina, A. (2017). In vitro antileishmanial activity of Mexican medicinal plants. *Heliyon*, 3(9), e00394. https://doi.org/10.1016/j.heliyon.2017.e00394

Dias, M. G., Camões, M. F. G. F. C., & Oliveira, L. (2009). Carotenoids in traditional Portuguese fruits and vegetables. *Food Chemistry*, 113, 808e815. https://doi.org/10.1016/j.foodchem.2008.08.002

Dibble, C. E., & Anderson, A. J. O. (1963). Florentine Codex: General History of the Things of New Spain. Book 11 — Earthly Things. Fray Bernardino de Sahagun. Translated by Dibble Charles E. & Anderson Arthur J. O. School of American Research and University of Utah.

Monographs of the School of American Research and the Museum of New Mexico, No. 14, Pt. 12, University of Utah Press, Salt Lake City. pp:297.

D'Imperio, M., Durante, M., Gonnella, M., Renna, M., Montesano, F. F., Parente, A., Mita, G., & Serio, F. (2022). Enhancing the nutritional value of Portulaca oleracea L. by using soilless agronomic biofortification with zinc. *Food Research International*, 155, 111057. https://doi.org/10.1016/j.foodres.2022.111057

Dkhil, M. A., Moniem, A. A., Al-Quraishy, S., & Saleh, R. A. (2011). Antioxidant effect of purslane (*Portulaca oleracea*) and its mechanism of action. *J Med Plants Res*, 5(9), 1589-1563. https://doi.org/10.5897/JMPR.9000222

Dong, C. X., Hayashi, K., Lee, J. B., & Hayashi, T. (2010). Characterization of structures and antiviral effects of polysaccharides from *Portulaca oleracea* L. *Chemical and Pharmaceutical Bulletin*, 58(4), 507-510. https://doi.org/10.1248/cpb.58.507

Duan, Y., Ying, Z., He, F., Ying, X., Jia, L., & Yang, G. (2021). A new skeleton flavonoid and a new lignan from *Portulaca oleracea* L. and their activities. *Fitoterapia*, 153, 104993. https://doi.org/10.1016/j.fitote.2021.104993

Duan, Y., Ying, Z., Zhang M., Ying, X., & Yang, G. (2020) Two new homoisoflavones from *Portulaca oleracea* L. and their activities. *Natural Product Research* 36.7: 1765-1773. https://doi.org/10.1080/14786419.2020.1815742

Dubois, V., Breton, S., Linder, M., Fanni, J., & Parmentier, M. (2007). Fatty acid profiles of 80 vegetable oils with regard to their nutritional potential. *European Journal of Lipid Science and Technology*, 109, 710e732. https://doi.org/10.1002/ejlt.200700040

Egea-Gilabert, C., Ruiz-Hernández, V., & Angeles, P. (2014). Characterization of purslane (*Portulaca oleracea* L.) accessions: Suitability as ready-to-eat product. *Scientia Horticulturae*. 172: 73-81. https://doi.org/10.1016/j.scienta.2014.03.051

El-Sayed, M. I. K. (2011). Effects of *Portulaca oleracea* L. seeds in treatment of type-2 diabetes mellitus patients as adjunctive and alternative therapy. *Journal of ethnopharmacology*, 137(1), 643-651. https://doi.org/10.1016/j.jep.2011.06.020

Erkan, N. (2012). Antioxidant activity and phenolic compounds of fractions from *Portulaca oleracea* L. *Food Chemistry*, 133, 775e781. https://doi.org/10.1016/j.foodchem.2012.01.091

Fallah, S., & Omrani, B. (2018). Substitution of inorganic fertilizers with organic manure reduces nitrate accumulation and improves quality of purslane. *Plant Physiology*, 9(1), 2651-2660. DOI: 10.22034/IJPP.2018.545667

FAO IFAD UNICEF WFP WHO. (2019). The State of Food Security and Nutrition in the World 2019. Safeguarding against economic slowdowns and downturns. FAO. http://www.fao.org/3/ca5162en/ca5162en.pdf

Farag, M.A., Shakour, Z.T.A. 2019. Metabolomics driven analysis of 11 *Portulaca* leaf taxa as analysed via UPLC-ESI-MS/MS and chemometrics. *Phychemestry* 161:117-129. https://doi.org/10.1016/j.phytochem.2019.02.009

Farag, O. M., Abd-Elsalam, R. M., Ogaly, H. A., Ali, S. E., El Badawy, S. A., Alsherbiny, M. A., Li, C. G., & Ahmed, K. A. (2021). Metabolomic profiling and neuroprotective effects of purslane seeds extract against acrylamide toxicity in rat's brain. *Neurochemical Research*, 46(4), 819-842. https://link.springer.com/article/10.1007/s11064-020-03209-6

Farhat, M. B., Beji-Serairi, R., Selmi, S., Saidani-Tounsi, M., & Abdelly, C. (2022). *Salicornia fruticosa* L. and *Portulaca oleracea* L. antioxidants as affected by domestic cooking processes. *International Journal of Gastronomy and Food Science*, 27, 100462. https://doi.org/10.1016/j.ijgfs.2021.100462

Fernández-Poyatos, M. D. P., Llorent-Martínez, E. J., & Ruiz-Medina, A. (2021). Phytochemical composition and antioxidant activity of *Portulaca oleracea*: influence of the steaming cooking process. *Foods*, 10(1), 94. https://doi.org/10.3390/foods10010094

Fontana, E., Hoeberechts, J., Nicola, S., Cros, V., Battista-Palmegiano, G., & Giorgio Peiretti, P. (2006). Nitrogen concentration and nitrate/ammonium ratio affect yield and change the oxalic acid concentration and fatty acid profile of purslane (*Portulaca oleracea* L.) grown in a soilless culture system. *Journal of the Science of Food and Agriculture*, 86(14), 2417-2424. https://doi.org/10.1002/jsfa.2633

Franco, J. A., Cros, V., Vicente, M. J., & Martínez-Sánchez, J. J. (2011). Effects of salinity on the germination, growth, and nitrate contents of purslane (*Portulaca oleracea* L.) cultivated under different climatic conditions. *The Journal of Horticultural Science and Biotechnology*, 86(1), 1-6. https://doi.org/10.1080/14620316.2011.11512716

Fu, J., Wang, H., Dong, C., Xi, C., Xie, J., Lai, S., Che, R., & Kang, J. (2021). Water-soluble alkaloids isolated from *Portulaca oleracea* L. *Bioorganic Chemistry*, 113, 105023. https://doi.org/10.1016/j.bioorg.2021.105023

Fujimura, Y., Kurihara, K., Ida, M., Kosaka, R., Miura, D., Wariishi, H., ... & Tachibana, H. (2011). Metabolomics-driven nutraceutical evaluation of diverse green tea cultivars. *PloS one*, 6(8), e23426. https://doi.org/10.1371/journal.pone.0023426

Gatea, F., Teodor, E. D., Seciu, A. M., Nagodă, E., Radu, G. L. 2017. Chemical constituents and bioactive potential of *Portulaca pilosa* L vs. *Portulaca oleracea* L. *Medicinal Chemistry Research*, 26(7), 1516-1527. https://link.springer.com/article/10.1007/s00044-017-1862-5

Gerber, M. (2009). Background review paper on total fat, fatty acid intake and cancers. *Annals of Nutrition Metabolism*. 55: 140–161. https://pubmed.ncbi.nlm.nih.gov/19752540/

Gruber, K. 2017. Agrobiodiversity: The living library. *Nature*, 544(7651), S8-S10. https://www.nature.com/articles/544S8a.pdf

Gu, Y., Leng, A., Zhang, W., Ying, X., & Stien, D. (2022). A novel alkaloid from *Portulaca oleracea* L. and its anti-inflammatory activity. *Natural Product Research*, 36(2), 595-600. https://doi.org/10.1080/14786419.2020.1795855

Gu, Y., Ying, Z., Lan, X., Ying, X., & Yang, G. (2021). Two new esters from the aerial parts of *Portulaca oleracea* L. and their bioactivities. *Phytochemistry Letters*, 44, 98-101. https://doi.org/10.1016/j.phytol.2021.06.009

Guzmán-Gutiérrez, S. L., Reyes Chilpa, R., & Bonilla Jaime, H. (2014). Medicinal plants for the treatment of "nervios", anxiety, and depression in Mexican Traditional Medicine. *Revista Brasileira de Farmacognosia*, 24(5), 591-608. https://doi.org/10.1016/j.bjp.2014.10.007

Harborne, J. B. (2001). Twenty-five years of chemical ecology. *Natural product reports*, 18(4), 361-379. https://pubs.rsc.org/en/content/articlelanding/2001/np/b005311m/unauth

Harris, M., Pellegrino, N., Giacomino, M. S., Chiesa, A., & Frezza, D. (2013). Comportamiento agronomico-productivo y contenido de acidos grasos poliinsaturados (omega 3) de *Portulaca oleracea* y *Montia perfoliata*. *Chilean Journal of Agricultural & Animal Science, ex Agro-Ciencia*. 29(2): 139-150. https://www.researchgate.net/profile/Nestor-Pellegrino/publication/269403152_Comportamiento_agronomico_-_productivo_y_contenido_de_acidos_grasos_poliinsaturados_Omega_3_de_Portulaca_oleracea_y_Montia_perfoliata/links/55d3317808ae0b8f3ef925d5/Comportamiento-agronomico-

productivo-y-contenido-de-acidos-grasos-poliinsaturados-Omega-3-de-Portulaca-oleracea-y-Montia-perfoliata.pdf

Hassanpouraghdam, M. B., Vojodi Mehrabani, L., Bonabian, Z., Aazami, M. A., Rasouli, F., Feldo, M., Strzemski, M., & Dresler, S. (2022). Foliar Application of Cerium Oxide-Salicylic Acid Nanoparticles (CeO2: SA Nanoparticles) Influences the Growth and Physiological Responses of *Portulaca oleracea* L. under Salinity. *International Journal of Molecular Sciences*, 23(9), 5093. https://doi.org/10.3390/ijms23095093

He, J., You, X., & Qin, L. (2021). High salinity reduces plant growth and photosynthetic performance but enhances certain nutritional quality of C4 Halophyte *Portulaca oleracea* L. grown hydroponically under LED lighting. *Frontiers in Plant Science*, 12, 651341. https://doi.org/10.3389/fpls.2021.651341

Hernández, T., Canales, M., Avila, J. G., Duran, A., Caballero, J., De Vivar, A. R., & Lira, R. (2003). Ethnobotany and antibacterial activity of some plants used in traditional medicine of Zapotitlán de las Salinas, Puebla (México). *Journal of ethnopharmacology*, 88(2-3), 181-188. https://doi.org/10.1016/S0378-8741(03)00213-7

Hnilickova, H., Kraus, K., Vachova, P., & Hnilicka, F. (2021). Salinity stress affects photosynthesis, malondialdehyde formation, and proline content in *Portulaca oleracea* L. *Plants*, 10(5), 845. https://doi.org/10.3390/plants10050845

Hongxing, Z., Nancai, Y., Guofu, H., Jianbo, S., Yanxia, W., Hanju, H., Quian, L., Yandong, Y., & hao, H. (2007). Neuroprotective effects of purslane herb aquenous extracts against D-galactose induced neurotoxicity. *Chemico-biological interactions*, 170(3), 145-152. https://doi.org/10.1016/j.cbi.2007.07.009

Hosseinzadeh, M. H., Ghalavand, A., Boojar, M. M. A., Modarres-Sanavy, S. A. M., & Mokhtassi-Bidgoli, A. (2021). Application of manure and biofertilizer to improve soil properties and increase grain yield, essential oil and ω3 of purslane (*Portulaca oleracea* L.) under drought stress. *Soil and Tillage Research*, 205, 104633. https://doi.org/10.1016/j.still.2020.104633

Hosseinzadeh, M. H., Ghalavand, A., Mashhadi-Akbar-Boojar, M., Modarres-Sanavy, S. A. M., Mokhtassi-Bidgoli, A. 2020. Increased Medicinal Contents of Purslane by Nitrogen and Arbuscular Mycorrhiza under Drought Stress. *Communications in Soil Science and Plant Analysis*, 51(1), 118-135. https://doi.org/10.1080/00103624.2019.1695828

Iziy E., Majd A., Vaezi-Kakhki, M.R., Nejadsattari T., & Kazemi-Noureini S. (2019). Effects of zinc oxidenano particles on enzymatic and nonenzymatic antioxidant content, germination, and biochemical and ultrastructural cell characteristics of *Portulaca oleracea* L. *Acta Societatis Botanicorum Poloniae*, 88 (4):3639. DOI: 10.5586/asbp.3639

Jacobsen, S.-E., Sørensen, M., Pedersen, S. M., & Weiner, J. (2013). Feeding the world: genetically modified crops versus agricultural biodiversity. *Agronomy for sustainable development*, 33(4), 651-662. https://link.springer.com/article/10.1007/s13593-013-0138-9

Jiang, M., Zhang, W., Yang, X., Xiu, F., Xu, H., Ying, X., & Stien, D. (2018). An isoindole alkaloid from *Portulaca oleracea* L. *Natural product research*, 32(20), 2431-2436. https://doi.org/10.1080/14786419.2017.1419226

Jiao, Z. Z., Yue, S., Sun, H. X., Jin, T. Y., Wang, H. N., Zhu, R. X., & Xiang, L. (2015). Indoline amide glucosides from Portulaca oleracea: isolation, structure, and DPPH radical scavenging activity. *Journal of natural products*, 78(11), 2588-2597. https://doi.org/10.1021/acs.jnatprod.5b00524

Jiao, Z., Wang, H., Wang, P., Sun, H., Yue, S., & Xiang, L. (2014). Detection and quantification of cyclo-dopa amides in *Portulaca oleracea* L. by HPLC-DAD and HPLC-ESI-MS/MS. *Journal of Chinese Pharmaceutical Sciences*, 23(8). DOI: 10.5246/jcps.2014.08.069

Juárez-Vázquez, M., Carranza-Álvarez, C., Alonso-Castro, A. J., González-Alcaraz, V. F., Bravo-Acevedo, E., Chamarro-Tinajero, F. J., Solano, E. (2013). Ethnobotany of medicinal plants used in Xalpatlahuac, Guerrero, Mexico. *Journal of Ethnopharmacology*, 148(2), 521-527. https://doi.org/10.1016/j.jep.2013.04.048

Kalia, A.N. (2005) Textbook of Industrial Pharmacognocy, CBS publisher and distributor, New Delhi, pp 204–208

Kaşkar, C., Fernández, J. A., Ochoa, J., Niñirola, D., Conesa, E., & Tüzel, Y. (2008). Agronomic behaviour and oxalate and nitrate content of different purslane cultivars (*Portulaca oleracea*) grown in a hydroponic floating system. In International Symposium on Strategies Towards Sustainability of Protected Cultivation in Mild Winter Climate 807 (pp. 521-526). https://www.actahort.org/books/807/807_76.htm

Kaymak, H. C. (2013). Effect of nitrogen forms on growth, yield and nitrate accumulation of cultivated purslane (*Portulaca oleracea* L.). *Bulgarian Journal of Agricultural Science*, 19, 444-449. https://www.agrojournal.org/19/03-08.pdf

Kiliç, C. C., Kukul, Y. S., & Anaç, D. (2008). Performance of purslane (*Portulaca oleracea* L.) as a salt-removing crop. *Agricultural water management*, 95(7), 854-858. https://doi.org/10.1016/j.agwat.2008.01.019

Koda, M., Furihata, K., Wei, F., Miyakawa, T., & Tanokura, M. (2012). Metabolic discrimination of mango juice from various cultivars by band-selective NMR spectroscopy. *Journal of agricultural and food chemistry*, 60(5), 1158-1166. https://pubs.acs.org/doi/abs/10.1021/jf2041438

Kokate, C. K., Purohit, A. P., & Gokhale, S. B. (2002). Nutraceutical and cosmaceutical. Pharmacognosy, 21st edition, Pune, India: Nirali Prakashan, 542, 549.

Lan, X., Ying, Z., Guo, S., Duan, Y., Cui, X., Leng, A., & Ying, X. (2021). Two novel amide alkaloids from *Portulaca oleracea* L. and their anti-inflammatory activities. *Natural Product Research*, 1-8. https://doi.org/10.1080/14786419.2021.2021519

Lara, L. J., Egea-Gilabert, C., Niñirola, D., Conesa, E., & Fernández, J. A. (2011). Effect of aeration of the nutrient solution on the growth and quality of purslane (*Portulaca oleracea*). *The Journal of Horticultural Science and Biotechnology*, 86(6), 603-610. https://doi.org/10.1080/14620316.2011.11512810

Larsson, S.C., Kumlin, M., Ingelman-Sundberg, M., & Wolk, A. (2004). Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. *The Americal Journal of Clinical Nutrition*, 79: 935–945. https://doi.org/10.1093/ajcn/79.6.935

Li, F., Li, Q., Gao, D., Peng, Y., & Feng, C. (2009). Preparation and antidiabetic activity of polysaccharide from *Portulaca oleracea* L. *African Journal of Biotechnology*, 8(4). https://www.ajol.info/index.php/ajb/article/view/59871

Li, Y., Hu, Y., Shi, S., & Jiang, L. (2014). Evaluation of antioxidant and immuno-enhancing activities of Purslane polysaccharides in gastric cancer rats. *International journal of biological macromolecules*, 68, 113-116. https://doi.org/10.1016/j.ijbiomac.2014.04.038

Linares, E., Bye, R., Ortega, N., & Arce, A.E. (2017). Quelites: sabores y saberes del sureste del Estado de México. México, CDMX: Universidad Nacional Autónoma de México. 90 p.

Liu, F., Cui, X., Duan, Y., Guo, S., Liu, J., & Ying, X. (2021). A new alkaloid from *Portulaca oleracea* L. and its anti-inflammatory activity. *Natural Product Research*, 1-5. https://doi.org/10.1080/14786419.2021.2000984

Liu, P., Wang, L., Li, H., Tan, L., Ying, X., & Ju, B. (2021). Two new organic acids from *Portulaca oleracea* L. and their anti-inflammatory and anticholinesterase activities. *Natural Product Research*, 1-9. https://doi.org/10.1080/14786419.2021.1999945

Liu, X., Wu, H., Tao, X., Ying, X., & Stien, D. (2019). Two amide glycosides from *Portulaca oleracea* L. and its bioactivities. *Natural Product Research*, 1-5. https://doi.org/10.1080/14786419.2019.1660333

Londonkar, R. L., & Nayaka, H. B. (2013). Effect of ethanol extract of *Portulaca oleracea* L on ovulation and estrous cycle in female albino rats. *Journal of pharmacy research*, 6(4), 431-436. https://doi.org/10.1016/j.jopr.2013.02.030

Ma, Y., Bao, Y., Zhang, W., Ying, X., & Stien, D. (2018). Four lignans from *Portulaca oleracea* L. and its antioxidant activities. *Natural product research*, 1-7. https://doi.org/10.1080/14786419.2018.1534852

Ma, Y., Li, X., Zhang, W., Ying, X., & Stien, D. (2019). A trace alkaloid, oleraisoindole A from *Portulaca oleracea* L. and its anticholinesterase effect. *Natural product research*, 1-4. https://doi.org/10.1080/14786419.2019.1627356

Malek, F., Boskabady, M. H., Borushaki, M. T., & Tohidi, M. (2004). Bronchodilatory effect of *Portulaca oleracea* in airways of asthmatic patients. *Journal of ethnopharmacology*, 93(1), 57-62. https://doi.org/10.1016/j.jep.2004.03.015

Markley, J. L., Brüschweiler, R., Edison, A. S., Eghbalnia, H. R., Powers, R., Raftery, D., & Wishart, D. S. (2017). The future of NMR-based metabolomics. *Current opinion in biotechnology*, 43, 34-40. https://doi.org/10.1016/j.copbio.2016.08.001

Matamoros, V., Rendón-Mera, A. M., Piña, B., Tadić, Đ., Cañameras, N., Carazo, N., & Bayona, J. M. (2021). Metabolomic and phenotypic implications of the application of fertilization products containing microcontaminants in lettuce (*Lactuca sativa*). *Scientific reports*, 11(1), 1-13. https://doi.org/10.1038/s41598-021-89058-x

Mera-Ovando, L.M., Bye, B.R., & Solano, L.M., (2014). La verdolaga (*Portulaca oleracea* L.) fuente vegetal de omega 3 y omega 6. *Agro-Productividad*, 7, 3–7. http://www.colpos.mx/wb_pdf/Agroproductividad/2014/AGROPRODUCTIVIDAD%20I_2014.pdf.

Meyer, R. S., DuVal, A. E., & Jensen, H. R. (2012). Patterns and processes in crop domestication: an historical review and quantitative analysis of 203 global food crops. *New Phytologist*, 196(1), 29-48. https://nph.onlinelibrary.wiley.com/doi/full/10.1111/j.1469-8137.2012.04253.x

Mohammed, T. M. S., & Suliman, F. S. O. (2019). The Response of *Portulaca oleracea* L to Different Concentration of Nitrogen Fertilizer. *American Journal of Bio-Science and Bio-Technology*, 7(1), 19-21. https://ijbsbt.com/index.php/ijbsbt/article/view/9384

Moneim, A. E. A. (2013b). The neuroprotective effects of purslane (*Portulaca oleracea*) on rotenone-induced biochemical changes and apoptosis in brain of rat. *CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders)*, 12(6), 830-841. https://www.ingentaconnect.com/content/ben/cnsnddt/2013/00000012/00000006/art00015

Moneim, A. E. A., Dkhil, M. A., Al-Quraishy, S. 2013a. The potential role of *Portulaca oleracea* as a neuroprotective agent in rotenone-induced neurotoxicity and apoptosis in the brain of rats. *Pesticide Biochemistry and Physiology*, 105(3), 203-212. https://doi.org/10.1016/j.pestbp.2013.02.004

Montoya-García, C. O., Volke-Haller, V., Santillán-Ángeles, A., López-Escobar, N.F., & Trinidad-Santos, A. (2019). Relación NH_4^+/NO_3^- en la producción de biomasa y el contenido nutrimental de *Portulaca oleracea* L. *Agrociencia*, 53: 521-533. https://agrociencia-colpos.mx/index.php/agrociencia/article/view/1825

Montoya-García, C. O., Volke-Haller, V., Trinidad-Santos, A., & Villanueva-Verduzco, C. (2018a). Change in the contents of fatty acids and antioxidant capacity of purslane in relation to fertilization. *Scientia Horticulturae*, 234: 152-159. https://doi.org/10.1016/j.scienta.2018.02.043

Montoya-García, C. O., Volke-Haller, V., Trinidad-Santos, A., & Villanueva-Verduzco, C. (2018b). Concentración nutrimental de la verdolaga (*Portulaca oleracea* L.) en respuesta a la fertilización con NPK. *Agrociencia*, 52: 241-254. http://www.scielo.org.mx/scielo.php?pid=S1405-31952018000200241&script=sci_abstract

Montoya-García, C. O., Volke-Haller, V., Trinidad-Santos, A., Villanueva-Verduzco, C. & Sánchez-Escudero, J. (2017). Respuesta de la verdolaga (*Portulaca oleracea* L.) a la fertilización con NPK. *Revista Fitotecnia Mexicana*, 40: 325-332. https://doi.org/10.35196/rfm.2017.3.325-332

Moon, J. K. & Shibamoto, T. (2009). Antioxidant Assays for Plant and Food Components. *Journal of Agricultural and Food Chemistry*, 57: 1655–1666. https://doi.org/10.1021/jf803537k

Moreau, A. G., & Savage, G. P. (2009). Oxalate content of purslane leaves and the effect of combining them with yoghurt or coconut products. *Journal of Food Composition and Analysis*, 22(4), 303-306. https://doi.org/10.1016/j.jfca.2009.01.013

Mortley, D. G., Oh, J. H., Johnson, D. S., Bonsi, C. K., & Hill, W. A. (2012). Influence of harvest intervals on growth responses and fatty acid content of purslane (*Portulaca oleracea*). *Hortscience*, 47(3), 437-439. https://doi.org/10.21273/HORTSCI.47.3.437

Nemzer, B., Al-Taher, F., & Abshiru, N. 2020. Phytochemical composition and nutritional value of different plant parts in two cultivated and wild purslane (*Portulaca oleracea* L.) genotypes. *Food Chemistry*, 320, 126621. https://doi.org/10.1016/j.foodchem.2020.126621

Oliveira, D. C. S., Wobeto, C., Zanuzo, M. R., & Severgnini, C. (2013). Mineral composition and ascorbic acid content in four non- conventional leafy vegetables species. *Horticulturae Brasileira*, 31, 472e475. https://doi.org/10.1590/S0102-05362013000300021

Petropoulos, S. A., Fernandes, Â., Dias, M. I., Vasilakoglou, I. B., Petrotos, K., Barros, L., & Ferreira, I. C. (2019). Nutritional value, chemical composition and cytotoxic properties of common purslane (*Portulaca oleracea* L.) in relation to harvesting stage and plant part. *Antioxidants*, 8(8), 293. 10.3390/antiox8080293

Petropoulos, S. A., Karkanis, A., Fernandes, Â., Barros, L., Ferreira, I. C., Ntatsi, G., Petrotos, K., Lykas, C., & Khah, E. (2015). Chemical composition and yield of six genotypes of common purslane (*Portulaca oleracea* L.): an alternative source of omega-3 fatty acids. *Plant foods for human nutrition*, 70(4), 420-426. https://link.springer.com/article/10.1007/s11130-015-0511-8

Piccolella, S., Crescente, G., Candela, L., & Pacifico, S. 2019. Nutraceutical polyphenols: New analytical challenges and opportunities. *Journal of pharmaceutical and biomedical analysis*. 175, 112774. https://doi.org/10.1016/j.jpba.2019.07.022

Poeydomenge, G. Y., & Savage, G. P. (2007). Oxalate content of raw and cooked purslane. *Journal of food agriculture and environment*, 5(1), 124. https://hero.epa.gov/hero/index.cfm/reference/details/reference_id/511303

Popescu, C., Vlăduţ, V., Voicea, I., Covaliu, C., Abbas, H., Dune, A., & Lupuleasa, D. (2018). The influence of extraction solvent on the active principles content of *Portulaca oleracea* native species. *Revista de Chimie*, (10), 2682-2692. https://doi.org/10.37358/RC.18.10.6604

Puccinelli, M., Pezzarossa, B., Pintimalli, L., & Malorgio, F. (2021). Selenium biofortification of three wild species, *Rumex acetosa* L., *Plantago coronopus* L., and *Portulaca oleracea* L., grown as microgreens. *Agronomy*, 11(6), 1155. https://doi.org/10.3390/agronomy11061155

Rashed, A. N., Afifi, F. U., & Disi, A. M. (2003). Simple evaluation of the wound healing activity of a crude extract of *Portulaca oleracea* L. (growing in Jordan) in Mus musculus JVI-1. *Journal of ethnopharmacology*, 88(2-3), 131-136. https://doi.org/10.1016/S0378-8741(03)00194-6

Raza, A., Zahra, N., Hafeez, M. B., Ahmad, M., Iqbal, S., Shaukat, K., & Ahmad, G. (2020). Nitrogen fixation of legumes: Biology and Physiology. *In The Plant Family Fabaceae* (pp. 43-74). Springer, Singapore. https://link.springer.com/chapter/10.1007/978-981-15-4752-2_3

Saheri, F., Barzin, G., Pishkar, L., Boojar, M. M. A., & Babaeekhou, L. (2020). Foliar spray of salicylic acid induces physiological and biochemical changes in purslane (*Portulaca oleracea* L.) under drought stress. *Biologia*, 75(12), 2189-2200. https://link.springer.com/article/10.2478/s11756-020-00571-2

Santiago-Saenz, Y. O., Hernández-Fuentes, A. D., Monroy-Torres, R., Cariño-Cortés, R., & Jiménez-Alvarado, R. (2018). Physicochemical, nutritional and antioxidant characterization of three vegetables (*Amaranthus hybridus* L., *Chenopodium berlandieri* L., *Portulaca oleracea* L.) as potential sources of phytochemicals and bioactive compounds. *Journal of Food Measurement and Characterization*, 12(4), 2855-2864. https://link.springer.com/article/10.1007/s11694-018-9900-7

Santini, A., & Novellino, E. (2018). Nutraceuticals-shedding light on the grey area between pharmaceuticals and food. *Expert Review of Clinical Pharmacology*, 11(6), 545-547. https://doi.org/10.1080/17512433.2018.1464911

Santos, R. V., Machado, R. M. A., Alves-Pereira, I., & Ferreira, R. M. A. (2016). The influence of nitrogen fertilization on growth, yield, nitrate and oxalic acid concentration in purslane (*Portulaca oleracea*). *In VI Balkan Symposium on Vegetables and Potatoes*, 1142 (pp. 299-304). 10.17660/ActaHortic.2016.1142.45

Scalbert, A., Brennan, L., Fiehn, O., Hankemeier, T., Kristal, B. S., van Ommen, B., ... & Wopereis, S. (2009). Mass-spectrometry-based metabolomics: limitations and recommendations for future progress with particular focus on nutrition research. *Metabolomics*, 5(4), 435-458. https://link.springer.com/article/10.1007/s11306-009-0168-0

Sharif, M. K., & Khalid, R. (2018). Nutraceuticals: myths versus realities. *In Therapeutic foods* (pp. 3-21). Academic Press. https://doi.org/10.1016/B978-0-12-811517-6.00001-5

Sharma, A., Kaithwas, G., Vijayakumar, M., Unnikrishnan, M. K., & Rao, C. V. (2012). Antihyperglycemic and antioxidant potential of polysaccharide fraction from P*ortulaca oleracea* seeds against streptozotocin-induced diabetes in rats. *Journal of Food Biochemistry*, 36(3), 378-382. https://doi.org/10.1111/j.1745-4514.2011.00547.x

Shen, H., Tang, G., Zeng, G., Yang, Y., Cai, X., Li, D., Liu, H., & Zhou, N. (2013). Purification and characterization of an antitumor polysaccharide from *Portulaca oleracea* L. *Carbohydrate polymers*, 93(2), 395-400. https://doi.org/10.1016/j.carbpol.2012.11.107

Siriamornpun, S., & Suttajit, M. (2010). Microchemical components and antioxidant activity of different morphological parts of Thai wild purslane (*Portulaca oleracea*). *Weed Science*, 58(3), 182-188. https://doi.org/10.1614/WS-D-09-00073.1

Song, M., Ying, Z., Ying, X., Jia, L., & Yang, G. (2022). Three novel alkaloids from *Portulaca oleracea* L. and their anti-inflammatory bioactivities. *Fitoterapia*, 156, 105087. https://doi.org/10.1016/j.fitote.2021.105087

Stroescu, M., Stoica-Guzun, A., Ghergu, S., Chira, N., & Jipa, I. (2013). Optimization offatty acids extraction from *Portulaca oleracea* seed using response surface methodology. *Industrial Crops and Products*, 43, 405e411. https://doi.org/10.1016/j.indcrop.2012.07.051

Szalai, G., Dai, N., Danin, A., Dudai, N., & Barazani, O. (2010). Effect of nitrogen source in the fertilizing solution on nutritional quality of three members of the *Portulaca oleracea* aggregate. *Journal of the Science of Food and Agriculture*, 90(12), 2039-2045. https://doi.org/10.1002/jsfa.4049

Taha, H., & Osman, A. (2015). Assessment of antioxidant capacity of ethanolic extract of *Portulaca oleracea* leaves in vitro and in vivo. *Journal of Medicinal Plants Research*, 9(10), 335-342. https://doi.org/10.5897/JMPR2014.5757

Tarkergari, S., Waghray, K., & Gulla, S. (2013). Acceptability studies of value added products with purslane (*Portulaca oleracea*). *Pakistan Journal of Nutrition*, 12(1), 93-96. 10.3923/pjn.2013.93.96

Thalassinos, G., Nastou, E., Petropoulos, S. A., & Antoniadis, V. (2021). Nitrogen Effect on Growth-Related Parameters and Evaluation of *Portulaca oleracea* as a Phytoremediation

Species in a Cr (VI)-Spiked Soil. *Horticulturae*, 7(7), 192. https://doi.org/10.3390/horticulturae7070192

Thalassinos, G., Nastou, E., Petropoulos, S. A., & Antoniadis, V. (2022). Soil dynamics of Cr (VI) and responses of *Portulaca oleracea* L. grown in a Cr (VI)-spiked soil under different nitrogen fertilization regimes. *Environmental Science and Pollution Research*, 29(10), 14469-14478. https://link.springer.com/article/10.1007/s11356-021-16413-w

Tian, J., Ying, Z., Lan, X., Li, Y., Leng, A., & Ying, X. (2022). Two new metabolites from *Portulaca oleracea* and their anti-inflammatory activities. *Phytochemistry Letters*, 48, 114-119. https://doi.org/10.1016/j.phytol.2022.02.007

Uddin, M. K., Juraimi, A. S., Hossain, M. S., Nahar, M. A. U., Ali, M. E., & Rahman, M. M. (2014). Purslane weed (*Portulaca oleracea*): A prospective plant source of nutrition, omega-3 fatty acid, and antioxidant attributes. *The Scientific WorldJournal*. Article ID 951019, 6 pp https://doi.org/10.1155/2014/951019

Uddin, M. K., Sam, S. G., Awang, A., Juraimi, A. S., Jalloh, M. B., Madon, M. S., & Shamsuzzaman, S. M. (2017). Effect of water regimes on growth, total flavonoid and phenolic content of purslane (*Portulaca oleracea* L.). *Bangladesh Journal of Botany*, 46(1), 255-262. https://www.cabdirect.org/cabdirect/abstract/20173360972

Uddin, M., Juraimi, A. S., Ali, M., & Ismail, M. R. (2012). Evaluation of antioxidant properties and mineral composition of purslane (*Portulaca oleracea* L.) at different growth stages. *International journal of molecular sciences*, 13(8), 10257-10267. https://doi.org/10.3390/ijms130810257

Veenstra, T. D. (2012). Metabolomics: the final frontier?. *Genome medicine*, 4(4), 1-3. https://link.springer.com/article/10.1186/gm339

Velásquez-Valle, R., Villa-Ruano, N., Hidalgo-Martínez, D., Zepeda-Vallejo, L. G., Pérez-Hernández, N., Reyes-López, C. A., Reyes-Cervantes, E., Medina-Melchor, D. L., & Becerra-Martínez, E. (2020). Revealing the ^{1}H NMR metabolome of mirasol chili peppers (*Capsicum annuum*) infected by Candidatus Phytoplasma trifolii. *Food Research International*, 131, 108863. https://doi.org/10.1016/j.foodres.2019.108863

Viana, M. M. S., Carlos, L. A., Silva, E. C., Pereira, S. M. F., Oliveira, D. B., & Assis, M. L. V. (2015). Phytochemical composition and antioxidant potential of unconventional vegetables. *Horticultura Brasileira*, 33, 504e509. https://doi.org/10.1590/S0102-053620150000400016

Vibrans, H. (2016). Ethnobotany of Mexican weeds. *In Ethnobotany of Mexico* (pp. 287-317). Springer, New York, NY. https://link.springer.com/chapter/10.1007/978-1-4614-6669-7_12

Villa-Ruano, N., Pérez-Hernández, N., Zepeda-Vallejo, L.G., Quiroz-Acosta, T., Mendieta-Moctezuma, A., Montoya-García, C., García-Nava, M.L., & Becerra-Martínez, E. (2019). ^{1}H-NMR based metabolomics profiling of citrus juices produced in Veracruz, México. *Chemistry & Biodiversity*, 16 (5), e1800479. https://doi.org/10.1002/cbdv.201800479

Voynikov, Y., Gevrenova, R., Balabanova, V., Doytchinova, I., Nedialkov, P., & Zheleva-Dimitrova, D. (2019). LC-MS analysis of phenolic compounds and oleraceins in aerial parts of *Portulaca oleracea* L. *Journal of Applied Botany and Food Quality*, 92, 298-312. DOI:10.5073/JABFQ.2019.092.041

Voynikov, Y., Nedialkov, P., Gevrenova, R., Zheleva-Dimitrova, D., Balabanova, V., & Dimitrov, I. (2021). UHPLC-Orbitrap-HRMS Identification of 51 Oleraceins (Cyclo-Dopa Amides) in *Portulaca oleracea* L. Cluster Analysis and MS2 Filtering by Mass Difference. https://www.preprints.org/manuscript/202109.0048/v1

Wang, C. Q., & Yang, G. Q. (2010). Betacyanins from *Portulaca oleracea* L. ameliorate cognition deficits and attenuate oxidative damage induced by D-galactose in the brains of senescent mice. *Phytomedicine*, 17(7), 527-532. https://doi.org/10.1016/j.phymed.2009.09.006

Wang, C., Guo, S., Tian, J., Liu, P., Song, M., Zhang, W., & Ying, X. (2022). Two new lignans with their biological activities in *Portulaca oleracea* L. *Phytochemistry Letters*, 50, 95-99. https://doi.org/10.1016/j.phytol.2022.06.003

Wanyin, W., Liwei, D., Lin, J., Hailiang, X., Changquan, L., & Min, L. (2012). Ethanol extract of *Portulaca oleracea* L. protects against hypoxia-induced neuro damage through modulating endogenous erythropoietin expression. *The Journal of nutritional biochemistry*, 23(4), 385-391. https://doi.org/10.1016/j.jnutbio.2010.12.015

Xiang, L., Xing, D., Wang, W., Wang, R., Ding, Y., & Du, L. (2005). Alkaloids from *Portulaca oleracea* L. *Phytochemistry*, 66(21), 2595-2601. https://doi.org/10.1016/j.phytochem.2005.08.011

Xiu, F., Li, X., Zhang, W., He, F., Ying, X., & Stien, D. (2019). A new alkaloid from *Portulaca oleracea* L. and its antiacetylcholinesterase activity. *Natural product research*, 33(18), 2583-2590. https://doi.org/10.1080/14786419.2018.1460833

Xu, W., Wang, J., Ju, B., Lan, X., Ying, X., & Stien, D. (2022). Seven compounds from *Portulaca oleracea* L. and their anticholinesterase activities. *Natural Product Research*, 36(10), 2547-2553. https://doi.org/10.1080/14786419.2021.1916928

Xu, W., Ying, Z., Tao, X., Ying, X., & Yang, G. (2021). Two new amide alkaloids from *Portulaca oleracea* L. and their anticholinesterase activities. *Natural Product Research*, 35(21), 3794-3800. https://doi.org/10.1080/14786419.2020.1739040

Yan, J., Sun, L. R., Zhou, Z. Y., Chen, Y. C., Zhang, W. M., Dai, H. F., & Tan, J. W. (2012). Homoisoflavonoids from the medicinal plant *Portulaca oleracea*. *Phytochemistry*, 80, 37-41. https://doi.org/10.1016/j.phytochem.2012.05.014

Yang, X., Zhang, W., Ying, X., & Stien, D. 2018. New flavonoids from *Portulaca oleracea* L. and their activities. *Fitoterapia*, 127, 257-262. https://doi.org/10.1016/j.fitote.2018.02.032

Yang, Y., Syed, S., Mao, S., Li, Q., Ge, F., Lian, B., & Lu, C. (2020). Bioorganic–Mineral Fertilizer Can Remediate Chemical Fertilizer-Oversupplied Soil: Purslane Planting as an Example. *Journal of Soil Science and Plant Nutrition*, 1-9. https://link.springer.com/article/10.1007/s42729-020-00175-4

Yang, Z., Liu, C., Xiang, L., & Zheng, Y. (2009). Phenolic alkaloids as a new class of antioxidants in *Portulaca oleracea*. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 23(7), 1032-1035. https://doi.org/10.1002/ptr.2742

Yazici, I., Türkan, I., Sekmen, A. H., & Demiral, T. (2007). Salinity tolerance of purslane (Portulaca oleracea L.) is achieved by enhanced antioxidative system, lower level of lipid peroxidation and proline accumulation. *Environmental and Experimental Botany*, 61(1), 49-57. https://doi.org/10.1016/j.envexpbot.2007.02.010

YouGuo, C., ZongJi, S., & XiaoPing, C. (2009). Evaluation of free radicals scavenging and immunity-modulatory activities of Purslane polysaccharides. *International journal of biological macromolecules*, 45(5), 448-452. https://doi.org/10.1016/j.ijbiomac.2009.07.009

Yue, M.E., Jiang, T.F., & Shi, Y.P. (2005). Simultaneous determination of noradrenaline and dopamine in *Portulaca oleracea* L. by capillary zone electrophoresis. *Journal of Separation Science*, 4, 360–364. https://doi.org/10.1002/jssc.200400045

Zaman, S., Bilal, M., Du, H., & Che, S. (2020). Morphophysiological and comparative metabolic profiling of purslane genotypes (*Portulaca oleracea* L.) under salt stress. *BioMed research international*, 2020. https://doi.org/10.1155/2020/4827045

Zaman, S., Hu, S., Alam, M. A., Du, H., & Che, S. (2019). The accumulation of fatty acids in different organs of purslane under salt stress. *Scientia Horticulturae*, 250, 236-242. https://doi.org/10.1016/j.scienta.2019.02.051

Zhang, J., Chen, X., Hu, Z., & Ma, X. (2002). Quantification of noradrenaline and dopamine in Portulaca oleracea L. by capillary electrophoresis with laser-induced fluorescence detection. *Analytica Chimica Acta*, 471, 203–209. https://doi.org/10.1016/S0003-2670(02)00775-4

Zhao, R., Gao, X., Cai, Y., Shao, X., Jia, G., Huang, Y., Qin, X., Wang, J., & Zheng, X. (2013). Antitumor activity of *Portulaca oleracea* L. polysaccharides against cervical carcinoma in vitro and in vivo. *Carbohydrate polymers*, 96(2), 376-383. https://doi.org/10.1016/j.carbpol.2013.04.023

Zidan, Y., Bouderbala, S., Djellouli, F., Lacaille-Dubois, M. A., Bouchenak, M. (2014). *Portulaca oleracea* reduces triglyceridemia, cholesterolemia, and improves lecithin: cholesterol acyltransferase activity in rats fed enriched-cholesterol diet. *Phytomedicine*, 21(12), 1504-1508. https://doi.org/10.1016/j.phymed.2014.07.010

TABLES AND FIGURES

Table 1. *Portulaca oleracea* L. yield given diverse cultivation conditions.

Cultivation conditions	Conditions to promote biomass production.	DAS	SD m^2	Y kg m^{-2}	Country	Reference
Average air temperature 25.9 and 26.3 °C, minimum relative humidity (RH) 19 %, maximum 91%. The system was on floating beds. Electrical conductivity (EC) at 2.2–2.45 dS m^{-1}; and pH at 5.5–6.5.	H + Substrate (peatmoss)	18	2200	1.27	Spain	(Cros et al., 2007)
The glasshouse provided with automatic environmental control for temperature and shading (35/15 °C day/night).	H + 7.2 mM NH_4^+ y 4.8 mM NO_3^- L^{-1}	20	3105	2.44	Spain	(Fontana et al., 2006)
The average air temperature was 22°C, with a minimum and maximum of 13.6 and 38.1 °C, respectively, and a minimum and maximum RH of 10 and 85%, respectively. The greenhouse received only natural light, with an	H + 2.5 dS m^{-1} CE	50	–	3.84	Spain	(Franco et al., 2011)

average level of photosynthetically active radiation (PAR) of 530 μmol m^{-2} s^{-1} at 12.00 h. The initial EC of the solution was 1.6 dS m^{-1} whithout NaCl.						
Irrigation and fertilization were practiced by diluted seawater and half-strength Hoagland solution.	H + 3.50 dS m^{-1} CE	38	–	9.25	Turkey	(Kiliç et al., 2008)
Average air temperature 29.1 ºC, with minimum and maximum of 18.6 and 48.9 ºC; minimum and maximum RH of 14.1 and 82.8%. With natural light and an average midday PAR of 353 μmol m^{-2} s^{-1}. EC of the solution was between 2.56 to 3.30 dS·m^{-1} and the pH was between 5.82 to 6.10.	H + Several cultivars	13	3200	1.68	Turkey	(Kaskar et al., 2009)
The soil of the experimental area was sandy loam texture (clay 22.88%, silt 33.16%, sand 43.96%) Ustorthent great soil group with neutral pH (7.23) and EC 403 μmhos cm^{-1}. It had 1.16 % organic matter.	H + Fertilizer: NO_3^- NH_4^+	35	–	1.52	Turkey	(Kaymak, 2013)
Hydroponically system used nutrient film tecnique	H + DAS	77	25	1.56	Argentina	(Harris et al., 2013)

The soil was loam (38 % sand, 36 % silt, and 26 % clay), the pH was 7.4 and the percent organic matter was 1.3 %. Average air temperature 26 – 28 ºC, and solar radiation 200 – 280 Wm^{-2} throughout the growing season. The precipitation during the growing season was 78 mm of rain.	C + Several cultivars	65	–	3.31	Iran	(Petropoulos et al., 2015)
The rainfall was 411.8 mm. The mean maximum daily temperatures varied between 19 and 28 °C, while the minimum varied between 6 and 11 °C. The soil of the experimental area was clay loam texture, the pH was 8.2; the EC 0.36 dS m^{-1}; 2.29–3.0 % organic matter.	C + Nitrogen dose at 65 kg N ha^{-1}.	27	2500	5.88	México	(Montoya-García et al., 2017)
Temperature not exceeded 28 °C. Clark's nutrient solution was pumped from independent tanks. Electrical conductivity at 2.0–2.5 dS m^{-1}; and pH at 5.5–6.5.	H+ Substrate (peatmoss:peat)	–	–	3.88	Jordan	(Alu´datt et al., 2019)

DAS: days after sowing; SD: sowing density; Y: fresh aerial biomass yield; H: hydroponics; C: open field.

Table 2. Medicinal effects reported for *Portulaca. oleracea* L.

Medicinal effect	Plant part	Country of origin	Reference
Anti-inflammatory and analgesic	Whole	Arabia	(Chan et al., 2000)
Cicatrization	Aerial	Jordan	(Rashed et al., 2003)
Bronchodilator	Aerial	Iran	(Malek et al., 2004)
Neuroprotector	Aerial	China	(Hongxing et al., 2007; Wang y Yang, 2010; Wanyin et al., 2012)
Neuroprotector	Whole	Egypt	(Al-Quraishy et al., 2012; Moneim et al., 2013a and 2013b)
Antidiabetic	Aerial	China	(Li et al., 2009)
Antidiabetic	Seeds	Yemen	(El-Sayed, 2011)
Antidiabetic	Seeds	India	(Sharma et al., 2012)
Antiovulatory	Aerial	India	(Londonkar and Nayaka, 2013)
Immunomodulators	Whole	China	(YouGuo et al., 2009; Li et al., 2014)
Immunomodulators and antispasmodic	Leaves	Philippines	(Catap et al., 2018)
Antitumoral	Aerial	China	(Shen et al., 2013; Zhao et al., 2013)
Antiviral	Whole	Japan	(Dong et al., 2010)
Antioxidant *in vivo*	Leaves	Egypt	(Taha y Osman, 2015)

Anticholesterolemic and Anti-triglycerides	Leaves	Sahara	(Zidan et al., 2014)
Antimutagenic	Whole	Malaysia	(Alam et al., 2014)
Antimutagenic	Whole	México	(Caballero-Salazar et al., 2002)

Table 3. Increase in the chemical composition of *Portulaca oleracea* L as a function of crop management.

Cultivation conditions	Compounds[1]	Reference
Substrate used		
Peatmoss	C18:3ω3, C18:2ω6, palmitic, stearic, myristic, arachidic	(Cros et al., 2007)
	AAox	(Alu´datt et al., 2019)
Ammonia/nitrate ratio		
60/40	C18:3ω3, C18:2ω6	(Fontana et al., 2006)
66/33	Chl, TC, TP, PUFA, C18:3ω3, aspartic acid, AA	(Camalle et al., 2020)
Salinity		
240 mM NaCl	Palmitic, palmitoleic, stearic, arachidic acids	(AnastáCio y Carvalho, 2013)
120 mM NaCl	C18:3ω3, C18:2ω6	
200 mM NaCl	C18:3ω3, C18:2ω6	(Zaman et al., 2019)
50 mM NaCl	Chl, TC, TP, PUFA, C18:3ω3, AA	(Camalle et al., 2020)
Water stress		
8 a 1 mg O_2 L^{-1}	TPh, Chl and AAox	(Lara et al., 2011)
Saturation and continuous flooding condition	TPh and TFl	(Uddin et al., 2017)

60% field capacity	Palmitic acid and C18:2ω6	(Saheri et al., 2020)
Fertilization and nutrition		
Organic and inorganic fertilizer interaction	SS, VC, TA, TC, TFl, C18:3ω3, C18:2ω6	(Yang et al., 2020)
N, P, K interaction	AAox, Chl *a* and *b*, *β*-C, TPh, FFl, VC and C18:3ω3, C18:2ω6	(Montoya-García et al., 2018)
Organic and inorganic fertilizer interaction	AAox, TFl and PUFA	(Hosseinzadeh et al., 2020 and 2021)
500 mg ZnO Nanoparticles and bulk L^{-1}	Chl *a* and *b*, TC, TPh, TFl, AAox	(Iziy et al., 2019)
10 mg Se L^{-1} biofortied	Chl *a* and *b*, TA, TC, TPh, TFl, AAox	(Puccinelli et al., 2021).
Cr (VI)-Spiked Soil	CAT, SOD, GP, and APX	(Thalassinos et al., 2021 and 2022; Akandi et al., 2022).
Foliar application 100 salicylic acid M and 50 mg CeO_2 L^{-1} nanoparticles	Chl *a* and *b*, TA, TPh, TFl, AAox, CAT	(Hassanpouraghdam et al., 2022).
1.3 mg Zn L^{-1} biofortification foliar	neoxanthin, lutein *β*-carotene, stearic acid, arachidic acid, behenic acid, myristic acid and oleic acid	(D'Imperio et al., 2022).

[1]Compounds that increased with the cultivation system; AA: amino acids; AAox: antioxidant activity; PUFA: polyunsaturated fatty acids; SS: soluble sugars; TA: total anthocyanins; β-C: β-carotene; Chl: chlorophyll; TC: total carotenoids; TPh: total phenols; TFl: total flavonoids; VC: vitamin C; TP: total protein; C18:2ω6: linoleic acid; and C18:3ω3: α-linolenic acid; CAT: catalase; GP: glutathione peroxidase, SOD: superoxide dismutase; APX: ascorbate peroxidase

Table 4. Influence of cultivar and abiotic factors in the metabolomic profile of *Portulaca oleracea L.*

Analytical metabolomics	Effect variation /country of origin	Name molecules	Reference
UHPLC-MS and UPLC	Greece and Bulgaria	**Phenolic compound**: vanillin, ellagic, gallic, gentisic, protocatechuic, 4-hydroxybenzoic, 2-hydroxybenzoic, *p*-coumaric, *m*-coumaric, *o*-coumaric, caffeic acid, ferulic acid, neochlorogenic acid, chlorogenic acids, rutin, and quercetin-3-O-glucoside	(Voynikov *et al*., 2019)
HPLC–DAD/ESI–MS	Spain	**Organic acid:** isocitric acid, citric acid, hydroxytyrosol hexoside, **Phenolic acids:** caffeoylglucaric acid, caffeic acid glucuronide isomer, caffeic acid-O hexoside, caffeic acid glucuronide isomer, ferulic acid-O-hexoside, sinapic acid-O-hexoside, ferulic acid derivative **Flavonoids:** cathechin, epicatechin, quercetin-O-hexoside isomer, kaempferol-O-hexoside, isorhamnetin-O-hexoside, quercetin-O-hexoside isomer	(Fernández-Poyatos et al., 2021).
UPLC–ESI–MS/MS	In three taxa of *P. oleracea* L. (*P. oleracea*, *P. rausii*, and *P. granulatostellulata*) / Egypt	**Phenolic acids**: protocatechuic acid hexoside, *O*-galloylquinic acid, protocatechuic acid, chlorogenic acid dimer, caftaric acid, **Organic acid**: (iso)citric acid **Amino acids**: proline, phenylalanine, *N*-acetylphenylalanine, tryptophan, *N*-(carboxyacetyl) phenylalanine **Peptide:** *N*- glutamylphenylalanine,	Farag & Shakour (2019)

	Alkaloid: aurantiamide, reticuline, **Lignan:** arabelline, **Saponin:** phytolaccoside a/b/d/e/g, diosgenin-*O*- di-deoxyhexosyl hexoside, diosgenin-*O*-di-hexosyl- deoxyhexoside, diosgenin-*O*-hexoside, dihydroxy-oleanenoic acid di-hexosyl deoxyhexoside, **Flavonoid**: quercetin-*O*-hexoside, myricetin-*O*-(*O*-galloyl)-hexoside, apigenin-6,8-*c*-dihexoside, apigenin-6-*c*-hexoside-8-*c*-pentoside, apigenin-6-*c*-pentoside-8-*c*-hexoside methyl ether, apigenin-8-*c*-hexoside-6-*c*-pentoside acetate, quercetin derivative, trihydroxymethoxyflavone-*o*-deoxyhexosyl-*o*-hexoside, apigenin-6,8-*c*-di-pentoside methyl ether, trihydroxymethoxyflavone-*o*-rutinoside, trihydroxymethoxyflavone-*o*-di-hexoside, α-mangostin, luteolin-6-*c*-hexoside, apigenin-6,8-di-*c*-pentoside, **Monoterpene glucosides:** portuloside B, **Isoflavone:** genistein, **Vitamin E:** desmethyl-tocopherol, delta tocopherol, **Fatty acid:** hydroxy-octadecadienoic acid, myristoleic acid, trihydroxy-octadecadienoic acid, trihydroxy-octadecaoic acid, tetrahydroxy-octadecanoic acid, dihydroxy-octadecenoic acid, ricinoleic acid, eicosapentaenoic acid, palmitic acid

HPLC	Wild and commercial cultivars / United Kingdom	**Amino acids**: glutamic acid, aspartic acid, leucine, and alanine, with smaller quantities of serine, proline, glycine, tyrosine, arginine, cysteine, threonine, valine, isoleucine, phenylalanine, lysine, histidine, and methionine	(Nemzer *et al*., 2020)
LC-HRAMS	38 accessions of purslane / Greece and Bulgaria	**Phenolic alcohols**: coniferyl alcohol, **Phenolic aldehydes**: (Z)-4-hydroxyphenyl-acetaldehyde oxime **Hydroxybenzoic acids:** protocatehuic acid-hexoside, **Phenolic compounds**: phenylacetic acid, acetophenone **Hydroxycinnamic acid and alcohols:** cinnamic acid, isoferulic acid, ferulic acid, o-coumaric acid, -coumaric acid -coumaric alcohol **Acylquinic acids:** chlorogenic acid, p-coumaroylquinic acid **Cinnamic acid amides**: N-caffeoyl-tyramine, N-feruloyl-octopamine, feruloyl-octopamine isomer, N-feruloyl-tyramine, N-feruloyl-3-methoxy-tyramine **Coumarin**: coumarin **Hydroxy- and methoxy- coumarins:** herniarin, umbelliferon, scaparon, 7,8-dihydroxy-coumarin **Flavonoids: q**uercetin, quercetin 3-glucoside, rutin **Lignans: s**esamolinol **Quinones:** deoxyshi-konin **Iridoid:** aucubin	(Balabanova *et al*., 2020)

		Terpenoids: geranyl isopentanoate **Amino acids and derivates: m**ethyltyramine, N-acetyltyrosine, guanine, agmatine, tyrosine, N-acetyltryptophan **Vitamines:** nicotinic acid, nicotinamide, N-methylnicotinamide **Índole derivates:** indole-3-carboxaldehyde **Tetrahydro-isoquinoline:** narcoclaurine **Others:** citric acid, isocitric acid, quinic acid	
RP-HPLC	Tunisian	Catechol, catechin hydrate, epigallocatechin, chlorogenic acid, epicatechin-3-gallate, caffeic acid, p-coumaric acid, sinapic acid, ferulic acid, myricitrin, luteolin-7-O-glucoside, rosmarinic acid, myricetin, isorhamnetin-3-O-glucoside, apigenin, lutolin, quercetin and kaempferol.	(Farhat et al., 2022).
UPLC/MS	Harvest time at 29, 43, and 52 days after planting / Budrio, Italy	**Fatty acid:** C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C16:1, C17:0, C18:0, C18:1n9c+t, C18:2n6c, C18:3n3, C20:0, C20:1CIS-11, C20:3n3+C21:0, C20:5n3, C22:0, C23:0 and C24:0 **Acids organics**: oxalic, quinic, malic, citric sinapic hexoside Caffeic acid and derivate **Tocopherols** (α- γ-, β- and δ-) **Sugars:** fructose, glucose, sucrose, and trehalose	(Petropoulos *et al*., 2019)
GC–MS	Ammonium/nitrate ratio in interaction with salinity	Chlorophyll, carotenoids, proteins **Sugars:** fructose, myo-inositol, galactinol, ononitol, raffinose, pinitol	Camalle *et al*. (2020)

	(NaCl) in two purslane cultivars / Israel	**Amino acids:** alanine, aspartic acid, glutamate, glycine, isoleucine, leucine, ornithine, phenylalanine, proline, serine, tyramine, urea **Organic acids**: oxalic, quinic, malic, citric, ascorbic	
GC–MS	Two purslane accessions / America and China	**Organic acids**: carbamate, lactic, glycolic, pyruvic, oxalic, hydracrylic, 3-hydroxybutyric, propanedioic, 3-hydroxyisovaleric, *α*-ketoisocaproic, benzoic, butanedioic, picolinic, glyceric, itaconic, 2-butenedioic, pentanedioic, 2,4-dihydroxybutanoic, dihydroxymalonic, D-(-)-citramalic, malic, L-threonic, *α*-ketoglutaric, L-(+)-tartaric, 2-aminoadipic, 2-keto-L-gulonic, 3-phosphoglycerate, citric, quininic, glucaric, pantothenic, D-gluconic, galactaric, *β*-D-glucopyranuronic, cis-coutaric **Amino acids**: L-norleucine, L-valine, L-alanine, L-leucine, 2-aminobutanoic acid, L-isoleucine, L-serine, L-threonine, glycine, pyrrole-2-carboxylic acid, L-methionine, *β*-alanine, 3-aminoisobutyric acid, l-5-oxoproline, L-aspartic acid, 4-aminobutanoic acid, L-proline, L-glutamic acid, L-phenylalanine, DL-ornithine, asparagine, L-glutamine, tyramine, L-lysine, L-tyrosine, L-tryptophan **Sugars:** d-(+)-xylose, d-arabinose, levoglucosan, d-(-)-rhamnose, d-fructose, fructose 6-phosphate, d-mannose, mannose 6-phosphate, D-galactose, D-glucose, D-allose, 2-O-glycerol-*α*-D-	(Zaman *et al*., 2020)

	galactopyranoside, glucose 6-phosphate, D-lactose, *β*-gentiobiose, D-(+)-turanose, maltose, D-trehalose, melibiose, sugar alcohols, ethylene glicol, propylene glicol, 1,3-butanediol, diethylene glicol, glycerol, l-threitol, xylitol, d-pinitol, D-glucitol, myoinositol, inositol monophosphate, phytol, glycerol 3-phosphate, galactinol **Amines**: hydroxylamine, cadaverine, ethanolamine, uracil, 5-methyl-4,6-pyrimidinediol, niacinamide, ammelide, putrescine, phosphorylethanolamine, 9h-purin-6-ol, dopamine, N-acetyl-d-glucosamine, uric acid, norepinephrine, (R)-, uridine, 2′-deoxyinosine, inosine, adenosine, cytidine, guanosine **Lipids and sterols**: palmitelaidic acid, palmitic acid, *α*-linolenic acid, oleic acid, (Z), 11-octadecenoic acid, (E), stearic acid, oleamide, 1-monopalmitin, 2-linoleoylglycerol, 1-monooleoylglycerol, glycerol monostearate, stigmasterol, stigmast-5-en-3*β*-ol, (24S)- **Others**: boric acid, phosphoric acid monomethyl ester, urea, phosphoric acid

Table 5. New phytochemicals reported in *Portulaca oleracea* L. with RMN.

Chemical classification	Assigned name in *P. oleracea* L.	Reference
Lignans	Oleralignans I, II, III, and IV.	(Ma et al., 2018)
	Oleralignan A, and six previously reported compounds loliolide **(1)**, isololiolide **(2)**, dehydrololiolide **(3)**, daphnetin **(4)**, esculetin **(5)**, and transcoumaric acid methyl ester **(6)**.	(Xu et al., 2022)
	Oleralignan B.	(Duan et al., 2021)
	Oleralignan C and D.	(Wang et al., 2022)
Homoisoflavonoids	Portulacanones A, B, C, and D.	(Yan et al., 2012)
	Oleracones J y K.	(Duan et al., 2020)
Flavonoids	Oleracones C, D, and E.	(Yang et al., 2018)
	Oleracone G.	(Duan et al., 2021)
Amide glycosides	Oleraciamide E and F.	(Liu et al., 2019)
Alkaloid	Portulacatone A and oleracein E.	(Gu et al., 2021)
	Oleracone L, portulacatone B, and portulacatal.	(Cui et al., 2021)
	Oleradacein.	(Liu et al., 2021)

	Oleracrylimide A, B, and C.	(Song et al., 2022)
Amide alkaloids	Oleraciamide G and oleraindole D.	(Xu et al., 2020)
	Oleraindole E and F.	(Lan et al., 2021)
Indole–alkaloids	Oleraindole A and B, and four previously reported [−]-neoechinulin A **(1)**, neoechinulin D **(2)**, isoechinulin A **(3)**, MT-6, and echinulin **(4)**.	(Zhao et al., 2019)
Isoindole alkaloids	Oleraisoindole, together with six known compounds, 7′-ethoxy-trans-feruloyltyramine **(1)**, N-trans-feruloyltyramine **(2)**, N-trans-feruloyl-3-methoxytyramine **(3)**, N-trans-p-coumaroyltyramine **(4)**, aurantiamide **(5)**, and ferulic acid methyl ester **(6)**.	(Jiang et al., 2018)
	Oleraisoindole A.	(Ma et al., 2019)
	Oleraurea **(1)** and 10 known compounds, p-hydroxybenzaldehyde **(2)**, p-hydroxybenzoic acid **(3)**, p-hydroxyacetophenone **(4)**, benzamide **(5)**, (E)-p-coumaramide **(6)**, (E)-ferulamide **(7)**, soyalkaloid A **(8)**, β-carboline-3-carboxylic acid **(9)**, 2, 3, 4, 9-tetrahydro-1H-pyrido [3, 4-b] indole-3-carboxylic acid **(10)**, (1S, 3S)-1-methyl-1, 2, 3, 4-tetrahydro-β-carboline-3-carboxylic acid **(11)**.	(Xiu et al., 2019)
Furan	Olerabenzofuran **(1)**, oleraindenone **(2)**, (+)-pinoresinol **(3)**, (-)-syringaresinol **(4)**, (+)-diasyringaresinol **(5)**, (+)-	(Tian et al., 2022)

	episyringaresinol **(6)**, (2 S)− 1-[2-(furan-2-yl)− 2-oxoethyl]− 5-oxopyrrolidine-2-carboxylic acid **(7)**, methyl (2 S)− 1[2-(furan-2-yl)− 2-oxoethyl]− 5-oxopyrrolidine-2-carboxylate **(8)**, drynaran **(9)**, and 2-furoic acid **(10)**.	
Organic acids	Oleraceacid A and B.	(Liu et al., 2021)
Esters	Portulacatone A **(1)** with six known compounds Oleracein E **(2)**, 6,7-dihdroxy-3,4-dihydro-2H-isoquinolin-1-one **(3)**, N-trans-p-coumaroytyramine **(4)**, 9H-carbazole **(5)**, isoaspergin **(6)** and flavoglaucin **(7).**	(Gu et al., 2022)

Figure 1. Commercial production and cultivars of *Portulaca oleracea* L. in Mexico.

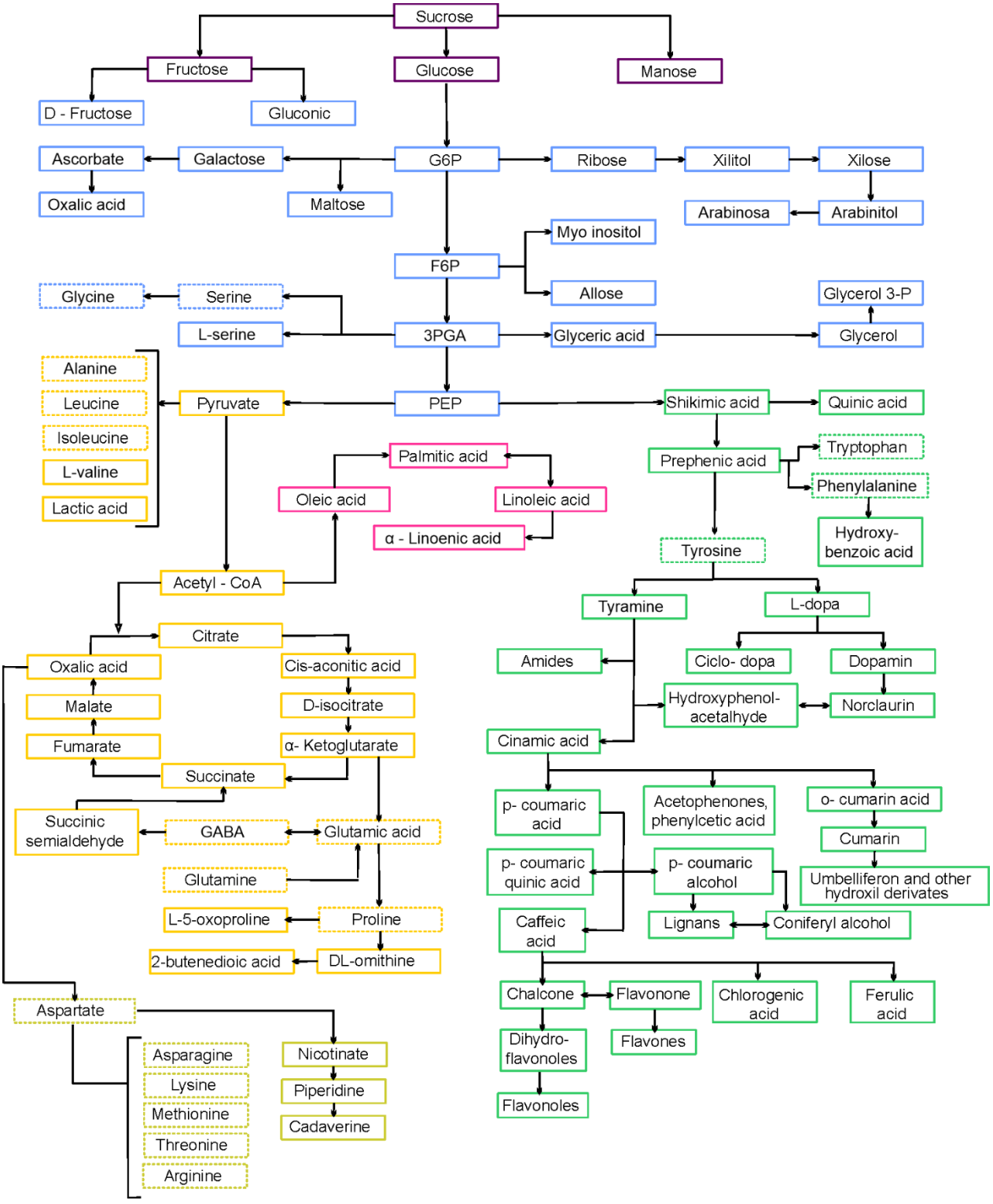

Figure 2. Primary and secondary metabolism routes of *Portulaca oleracea* L.

CAPÍTULO III

METABOLÓMICA BASADA EN RMN PARA DETERMINAR LA FLUCTUACIÓN DE METABOLITOS EN CULTIVARES DE VERDOLAGA EN HIDROPONIA EN DIFERENTES MOMENTOS DE COSECHA

PUBLICADO EN:
Montoya-García, C. O., García-Mateos, R., Magdaleno-Villar, J. J., Volke-Haller, V. H., Villa-Ruano, N., Zepeda-Vallejo, L. G., & Becerra-Martínez, E. (2023). NMR-based metabolomics to determine the fluctuation of metabolites in hydroponic purslane crops at different harvesting times. *Food Research International, 166*, 112489.

NMR-BASED METABOLOMICS TO DETERMINE THE FLUCTUATION OF METABOLITES IN HYDROPONIC PURSLANE CROPS AT DIFFERENT HARVESTING TIMES

César Omar Montoya-García [a], Rosario García-Mateos [a, *], J. Jesús Magdaleno-Villar [a],

Víctor Hugo Volke-Haller [b], Elvia Becerra-Martínez [c, *]

[a] Universidad Autónoma Chapingo - Departamento de Fitotecnia. Km. 38.5, Carretera México-Texcoco, 56230, Chapingo, Estado de México, México.

[b] Colegio de Postgraduados - Campus Montecillo. Km. 36.5, Carretera México-Texcoco, Montecillo, Texcoco, 56230, Estado de México, México.

[c] Centro de Nanociencias y Micro y Nanotecnologías, Instituto Politécnico Nacional, Av. Luis Enrique Erro S/N, Unidad Profesional Adolfo López Mateos, Zacatenco, Delegación Gustavo A. Madero, Ciudad de México, 07738, México.

*Corresponding authors:

Rosario García-Mateos, rosgar08@hotmail.com
Elvia Becerra-Martínez, elmartinezb@ipn.mx

ABSTRACT

Purslane (*Portulaca oleracea* L.) has a high content of nutrients and medicinal effects that depend on the genotype, harvesting time, and production system. The objective of the present research work was to elucidate the ^{1}H NMR-based metabolomics profiling of three native purslane cultivars from Mexico (Xochimilco, Mixquic, and Cuautla) grown under hydroponic conditions and harvested in three different times (32, 39, and 46 days after emergence). Five sugars, 15 amino acids, 7 organic acids, 3 campheoylquinic acids, as well as 2 alcohols and 3 nucleosides, were identified in these samples. Specifically, 37 compounds were detected in native purslane from Xochimilco and Cuautla, whereas 39 compounds were detected in purslane from Mixquic. Principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) separated the cultivars into three clusters. Mixquic cultivar had the highest number of differential compounds (amino acids and carbohydrates), followed by Xochimilco and Cuautla cultivars, respectively. Changes in the metabolome were observed in latest times of harvest for all the cultivars studied. The differential compounds were glucose, fructose, galactose, pyruvate, choline, and 2-hydroxysobutyrate. The results obtained in this investigation may contribute to selecting the best cultivar of purslane and the best time in which the levels of nutrients are optimal.

Keywords: *Portulaca oleracea* L.; metabolomics; nuclear magnetic resonance (^{1}H NMR); Principal component analysis (PCA); cultivars; hydroponic.

VII. INTRODUCTION

From a nutritional point of view, purslane (*Portulaca oleracea* L.) is an attractive plant due to its content of omega 3 and 6 fatty acids, phenolic compounds, vitamins, nutrients (which may help to complete the daily intake for humans). The plant is a short-cycle crop that can be harvested between 40 and 50 days. Also, it exhibits analgesic, anti-inflammatory, bronchodilator, neuroprotective, antiviral, antiproliferative, antidiabetic, and hypocholesterolemic properties. Purslane studies are mainly focused in determining the antioxidants and fatty acid fluctuation produced by abiotic factors (Montoya-García *et al*., 2018a and 2018b; Hosseinzadeh *et al*., 2020; Bekmirzaev *et al*., 2021; Farag *et al*., 2021).

Metabolomics is a reliable tool to identify a wide spectrum of substances with biological activity and to determine its concentration based in physiological, genetic, and/or environmental stimuli, in order to provide a holistic picture of the metabolism (Emwas *et al*., 2015; Saviano *et al*., 2019; Schmidtke *et al*., 2020; Calò *et al*., 2022). One of the main techniques for metabolomics is NMR, since it is useful to the simultaneously detection and quantification of primary metabolites (sugars, organic acids, and amino acids), and secondry metabolites (phenolic compounds, flavonoids, alkaloids, and terpenoids) (Sciubba *et al*., 2020; Marak *et al*., 2021; Managa *et al*., 2021; Yu *et al*., 2022).

NMR has successfully been applied for studying metabolic changes in plant genotypes in different times of harvest. Differences in the chemical composition of *Daucus carota* L., were detected under different times of collection suggesting that the main nutritional compounds are accumulated in latest ripening stages (Sciubba *et al*., 2020). Similarly, the metabolite content of the leaves from *Moringa oleifera* are influenced by environmental factors (Managa *et al*., 2021). The genotype and collection time of *Castanea crenata* influenced the composition of the primary metabolome, with changes in the concentration of amino acids, organic acids, and sugars (Yu *et al*., 2022). The fruits of *Solanum lycopersicum*, accumulate the highest concentration of sugars and organic acids in the latest ripening stages due to an increase in the photosynthetic activity that causes an increase in sucrose production (Abreu *et al*., 2019).

Several contributions have been made to determine the metabolome of purslane on the basis of the genotype. The main techniques used for this aim were high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and ^{1}H NMR. (Farag & Shakour, 2019; Balabanova *et al*., 2020; Camalle *et al*., 2020; Nemzer *et al*., 2020; Zaman *et al*., 2020). However, the content of bioactive molecules in native cultivars harvested at different times under hydroponic conditions has not been explored so far. Therefore, the objective of this study was to elucidate the metabolome of three native purslane cultivars, on the basis of the collection time (phenological stage) under greenhouse and hydroponic conditions.

VIII. MATERIALS AN METHODS

8.1 Chemicals used

The hydroponic nutrient solution was prepared using $(NO_3)_2$, NH_4Cl, KH_2PO_4, $MgSO_4$, KCl, K_2SO_4, $CaSO_4$, $CaCl_2$, $CaCO_3$, $FeSO_4$, EDTA-Mn, $CuSO_4+5H_2O$, $ZnSO_4+7H_2O$, H_3BO_3, $(NH_4)_6Mo_7O_{24}+4H_2O$, and H_2SO_4 as a nutrient stock. Deuterated solvents were supplied by Cambridge Isotope Laboratories, Inc. The internal standard 3-(trimethylsilyl) propionic acid-2,2,3,3-d4 sodium salt (TSP), ethylenediaminetetraacetic acid (EDTA), sodium azide (NaN_3), and mono-potassium phosphate (KH_2PO_4) were purchased from Sigma-Aldrich Co.

8.2 Site of study and conditions

The present study was carried out in the State of Mexico, Mexico (19.491260, -98.872207, 2215 masl), under greenhouse and hydroponic conditions in August and September 2021. The minimum and maximum temperatures were 18 °C and 30 °C, respectively, and the relative humidity ranged from 50 to 75 %.

8.3 Plant material and experimental design

The purslane cultivars evaluated were those cultured in Mexico (State of Morelos, State of Mexico, and Mexico City. These are commonly named as Cuautla, Mixquic and

Xochimilco cultivars, respectively). Three times of collection were performed in order to determine possible differences in the metabolome of purslane. These were the vegetative stage, first flowering, and second flowering at 32 (H1), 39 (H2), and 46 (H3) days after emergence (DAE), respectively. The experimental design was randomized with seven repetitions for each group.

8.4 Experiment setup and harvesting

A hydroponic system of floating rafts was used. Each experimental unit was developed in a 6 L high-density polyethylene container (external dimensions of 51 × 33 × 13 cm; and internal dimensions of 48 × 30.5 × 8 cm) coupled to an air pump to supply 10 mg L^{-1}dissolved oxygen. Sowing was done in polystyrene germination trays of 117 cells (22 mL volume per cell), containing vermiculite. The emergence of purslane seedlings occurred five days after planting. Then water was replaced by the hydroponic nutrient solution (HNS). The HNS was those recommended by Montoya-García *et al*. (2019) for purslane, with an ammonium/nitrate (NH_4^+/NO_3^-) ratio of 25/75 and an electrical conductivity of 2 dS m^{-1}. To prepare the HNS, well water and reagent grade salts were used (after chemical analysis, Table S1). The pH of the nutrient solution was adjusted to 5.5 with H_2SO_4 (97 %). For the harvests at 32 (H1), 39 (H2), and 46 (H3) DAE, the aerial biomass of a 0.03894 m^2 surface from each experimental unit was collected, and the height and aerial biomass yield were measured. For each cultivar and harvest, 7 samples were processed (n = 63). The fresh samples were subsequently lyophilized and ground for ^{1}H NMR analysis.

8.5 Preparation of the aqueous extract of the aerial biomass of purslane

Fifty milligrams of purslane powder were placed in an Eppendorf tube and mixed with 1 mL KH_2PO_4 buffer solution in D_2O (9 mM, pH 6.0), containing TSP (as internal standard, 0.7 mM), NaN_3 (0.2 mM), and EDTA (1 mM). The samples were sonicated for 20 min, and centrifuged at 10,000 rpm for 10 min. Finally, 600 µL of the supernatant were collected for ^{1}H NMR analysis (Becerra-Martínez *et al*., 2020).

8.6 ^{1}H NMR spectroscopy

The ^{1}H NMR spectra were obtained in a 750 MHz Bruker spectrometer (Bruker Biospin, Rheinstetten, Germany) equipped with a 5 mm TXI probe, and using the NOESYPR1D pulse sequence to suppress the water signal. The purslane aqueous extracts were measured at 298.1 ± 0.1 K. The acquisition parameters were set as follow: scan number = 128, FID size = 64 K, spectral width = 10.00 ppm, receiver gain = 86.0, acquisition time = 2.18 s, relaxation time = 10 s, mixing time = 100 ms, and FID resolution = 0.45 Hz (Becerra-Martínez *et al*., 2020).

8.7 ^{1}H NMR signal assignation

To identify mutually coupled hydrogens in the spectrum, COSY (2D ^{1}H - ^{1}H, correlation spectroscopy) was used whereas correlations between protons and their neighboring carbons, was estimated with HSQC (2D ^{1}H - ^{13}C, heteronuclear single quantum coherence). For correlating ^{1}H and ^{13}C cores with two, three, or four bonds, HMBC (2D ^{1}H – ^{13}C, multibonded heteronuclear correlation) was used. Finally, to identify the metabolome, the HMDB (http://www.hmdb.ca/) and BMRB (https://bmrb.io/) databases were used.

8.8 ^{1}H NMR data processing

^{1}H NMR data from free induction decay (FID) were processed with the MestReNova software (version 6.0.2; MestReC, Santiago de Compostela, Spain). The baseline was corrected and the internal standard was calibrated to 0.0 ppm. Small variations in chemical shifts within ^{1}H NMR spectra were aligned using a peak search algorithm. The spectrum binning was divided into spaced windows called bins or buckets and the peak area from each bin was integrated. Normalization was applied to remove variations between samples (Shuzhao, 2014).

8.9 Metabolite quantification

The molar concentration of the metabolites was done following the methodology proposed by Villa-Ruano *et al*. (2019), which is based on the signal intensity of the ^{1}H NMR

spectrum, using TSP as an internal standard. The following equation was used to obtain the molar concentrations of the metabolome:

$$m_T\ M_T\ \times\ \frac{I_T}{I_{St}}\ \times \frac{X_{St}}{X_T}\ \times\ C_{St}\ \times\ V_{St}$$

where: m_T: mass of target compound [µg] in the solution used for ^{1}H NMR measurement; M_T: molecular weight of the target compound [g/mol]; I_T: relative integral value of the ^{1}H NMR signal of the target compound; I_{St}: relative integral value of the ^{1}H NMR signal of the standard compound; X_{St}: number of protons belonging to the ^{1}H NMR signal of the standard compound; X_T: number of protons belonging to the ^{1}H NMR signal of the target compound. C_{St}: concentration of internal standard (TSP) in the solution used for the ^{1}H NMR measurement [mmol/L]; V_{St}: volume of the solution used for the ^{1}H NMR measurement [mL].

8.10 Statistical analysis

Principal component analysis (PCA) was performed, followed by supervised orthogonal partial least squares discriminant analysis (OPLS-DA) of latent structures. In order to obtain information and to identify the differential metabolites among groups, a Hotelling T2 region was used (ellipse in the model plots, with 95% confidence interval). The quality of the model was defined on the values of R^2X and Q^2. The variable importance parameter (VIP) was considered to measure the contribution of the different variables in the model (Blasco *et al*., 2019; Becerra-Martinez *et al*., 2020). The validation of the OPLS-DA model was carried out using permutations (Rådjursöga *et al*., 2019), with the SIMCA 14.1 software for Windows.

To determine the single effect of each factor in the metabolome related with yields and height of purslane, t-Student test, analysis of variance (ANOVA), and Tukey's test (α= 0.05) were performed with the SAS 9.0 software.

IX. RESULTS AND DISCUSSION

9.1 Purslane yield and height

Significant differences were observed in the aerial biomass production of purslane according to the time of collection. This biomass at 32 DAE was 7.14 - 8.17 kg m^{-2}, at 39 DAE was 7.89 - 9.63 kg m^{-2} and at 46 DAE was 9.90 - 11.52 kg m^{-2}. Only in the third harvesting was statistically significant. Mixquic purslane (11.52 kg m^{-2}) produced the highest yield of aerial biomass followed by Xochimilco (11.23 kg m^{-2}) and Cuautla (9.90 kg m^{-2}) (Fig. 1A). Significant differences were found in the plant height at the latest harvest. Also, significant differences were observed by cultivar, where Xochimilco (30.2, 33.5, and 36.0 cm/plant) cultivar was differentiated from the Cuautla cultivar (26.5, 29.2, and 29.6 cm/plant) and Mixquic cultivar (26.7, 30.2, and 35.0 cm/plant) (Fig. 1B). Previous studies sustain that purslane cultivars from Xochimilco, Mexico, weighted 35 - 50 g and sized 25 - 27 cm/plant after treatment with 25/75 nitrate/ammonium ratio under hydroponic conditions at 32 days after transplantation (dat) (Montoya-García *et al*., 2019). On the other hand, the Mixquic cultivar yielded 28 - 130 t ha^{-1}, and sized 19-38 cm/plant with nitrogen treatments of 65 t ha^{-1}, at 27, 34, and 42 DAE, respectively (Montoya-García *et al*., 2017).

The hydroponic substrate caused a differential effect on crop yield of purslane from Irbid Jordan in five harvesting times with 3.8, 4.8, 5.8, 6.2, and 6.0 kg m^{-2} and heights of 45, 53, 59, 67, and 59 cm/plant with tuff:peatmos (2:1 v:v) (Alu'datt *et al*., 2019). Saheri *et al*. (2020) observed effects on the height of purslane from Iran, with 34.9 and 19.2 cm/plant at 45 dat using 90 and 30% water capacity in the field. Previous studies state that fertilization did not influence the height of purslane from China, with values between 19 and 24 cm at 80 dat (Yang *et al*., 2020). Under hydroponic conditions, the height of purslane from Pakistan and China were 40.1 and 21.3 at 28 dat (Zaman *et al*., 2019). The differences in height and yield observed in the present study could be explained mainly based in the cultivar assayed and in the time of harvest which denotes the phenological stage of the plant. Interestingly in latest harvesting purslane plants could accumulate higher amounts of biomass reflected in an increase in foliage and height.

9.2 Metabolomics profiling of purslane

Based on the ^{1}H NMR, ^{13}C NMR, and 2D NMR assays obtained at 750 MHz, several compounds were identified in the aqueous extract of aerial parts of purslane. Most of them derived from the primary metabolism (Figs. S1 and S2). The ^{1}H NMR spectrum was divided into three regions. The first region contained aliphatic compounds (0.80 to 3.00 ppm), the second regions contained sugars (3.00 to 5.50 ppm) and the third region contained aromatic compounds (6.00 to 9.5 ppm). In the first region, the highest intensities corresponded to citric acid (2.81 and 2.70 ppm) and malic acid (2.53 and 2.76 ppm). This latter region also contained aliphatic amino acids such as glutamine (2.12 and 2.44 ppm), GABA (1.89 and 2.28 ppm), alanine (1.47 ppm), aspartic acid (2.70 and 2.83 ppm), asparagine (2.85 and 2.94 ppm), proline (2.01 and 2.30 ppm), leucine (0.94 and 0.95 ppm), threonine (1.32 ppm), isoleucine (0.93 ppm) (Table 1; Fig. S1B). In the second region, the highest intensities corresponded to α and β-glucose (5.22 and 4.63 ppm) and fructose (3.99 and 4.01ppm). Other sugars identified in this region were galactose (4.55 ppm), myo-inositol (3.27 ppm), and sucrose (4.20 and 5.40 ppm) (Table 1; Fig. S1C). The third region presented higher intensities associated with tyrosine (7.18 and 6.88 ppm) and fumaric acid (6.55 ppm) but, amino acids such as phenylalanine and tryptophan were also observed as well as the nucleosides adenosine, guanosine, and uridine. Trigonelline and caffeoylquinic acids were in this region (Table 1; Fig. S1D). These phenolic acids ameliorate hyperglycemia and dyslipidemia, are considered beneficial for human health, mainly due to their anti-inflammatory and antioxidant properties (Wu *et al*., 2014).

UPLC-ESI-MS/MS has been previously used to determine the metabolite profiling in *P. oleracea*, *P. rausii*, and *P. granulatostellulata* from Egypt, these studies revealed the presence of amino acids, phenolic compounds, alkaloids, and fatty acids. The present study suggest that purslane cultivars are rich in proline, citric acid, phenylalanine, and tryptophan (Farag & Shakour, 2019). In wild and cultivated purslane plants from the United Kingdom, 17 amino acids were detected by HPLC. The bioactive compounds detected were aspartic and glutamic acid, alanine, leucine, glycine, valine, lysine, and arginine (Nemzer *et al*., 2020). In purslane cultivars from Bulgaria and Greece, 50 different compounds were identified with LC-HRAMS. These included phenols, flavonoids, terpenoids, lignin

quinones, cyclo-dopa amines, amino acids and vitamins (Balabanova *et al*., 2020). Camalle *et al*. (2020) reported that purslane from Israel contained fructose, myo-inositol, alanine, glutamate, isoleucine, leucine, phenylalanine, proline, and aspartic, citric and malic acid; the different compounds were galactitol, ononitol, raffinose, pinitol, glycine, serine tyramine, urea, and ascorbic acid. Zaman *et al*. (2020) detected 132 compounds in purslane genotypes from China and America through GC-MS, mainly organic acids, amino acids, sugars, and amines. At least 20 compounds coincided with those observed in the previous study.

9.3 Multivariate analysis of the purslane metabolome based on harvesting time

PCA and OPLS-DA models were applied to determine differences in the metabolome in function of the specific cultivar (Xochimilco, Mixquic, and Cuautla) and time of collection. In the PCA analysis, a separation was observed with statistical values of R^2X = 0.867 and Q^2 = 0.669 (Fig. S3). The model exhibited aberrant points that were eliminated according to Student's t-test. Therefore, modeling with OPLS-DA was useful to adjust four predictive components and one orthogonal component (Fig. 2A). This model was validated with the random permutation test (200 times) (Fig. S4A). The OPLS-DA model indicated differences between cultivars in the metabolite content of the aerial parts from purslane, but not between time of collection. The statistical values of the model were R^2X = 0.792, R^2Y = 0.355, and Q^2 = 0.272 (Fig. 2A). The differential metabolites were determined in the loading plots of the OPLS-DA model between the Mixquic cultivar versus Xochimilco and Cuautla cultivars (Fig. 2B). The Mixquic cultivar contained high concentrations of aspartic acid, pyruvic acid, O-phosphocholine, isoleucine, tyrosine, valine, galactose, sucrose, and proline. On the other hand, the Xochimilco cultivar had higher concentrations of citric acid, and histidine whereas the Cuautla cultivars stored glucose, fructose, asparagine, and ethanol (Fig. 2B).

The heatmap revealed differences between harvests in each cultivar (Fig. 3). In the first time of collection, purslane from Xochimilco had higher concentration of guanosine, myo-inositol, phenylalanine, tryptophan, leucine, proline, aspartic acid, and some organic acids such as citric, succinic, fumaric, malic, 4-O-caffeoylquinic, and 2-hydroxyisobutyric, while harvests 2 and 3 had a similar behavior (Fig. 3A). The third harvest of the Mixquic

cultivar contained high concentrations of sucrose, leucine, glutamic acid, adenosine, proline, alanine, phenylalanine, uridine, and citric, succinic, fumaric and 3-O-caffeoylquinic acids (Fig. 3B). The Cuautla cultivar mainly contained valine, fructose, tryptophan, glucose, ethanol, aspartic acid, asparagine, isoleucine, histidine, tyrosine, alanine, phenylalanine, proline, threonine, citric and succinic acids (Fig. 3C).

The analysis of variance indicated significant differences, depending on the harvesting time for galactose, pyruvic acid, choline, and 2-hydroxyisobutyric acid, with the highest concentrations at 32 DAE (H1). An increasing trend was observed in the third harvest (46 DAE, H3) for the signals detected from 2.5 to 5.5 ppm. These signals corresponded to α- and β-glucose, fructose, citric acid and malic acid. Sucrose and adenosine were only detected in the third harvest of the Mixquic cultivar (Table 2). To the best of our knowledge, there are not studies regarding the metabolomics profiling of purslane related to the time of harvest.

Some studies performed in other species such as *Solanum lycopersicum*, presented high concentration of amino acid in early phenological stages (Abreu *et al*., 2019). A similar case was observed for the leaves of *Camellia sinensis* during early spring (Zeng *et al*., 2019). Stems of *Daucus carota* increased their levels of primary metabolites in fall and winter (Sciubba *et al*., 2020). The present study suggests that the concentration of amino acids in the three purslane cultivars was the same in early and late harvests. This tendency is probably related to the short life cycle of the plant (32 to 46 DAE). However, this observation differed in the chemical profiling of the senescence stage of *Castanea crenata*, in which the amino acid content increased (Yu *et al*., 2022). It is known that amino acids are energetic precursors and are involved in redox regulation, and in the synthesis of secondary metabolites such as phenylpropanoids (Häusler *et al*., 2014).

In late harvests, photosynthetic activity is increased, which is translated into a higher content of carbohydrates and organic acids production (Abreu *et al*., 2019). Similar results were obtained in purslane native to Italy, which showed higher concentrations of fructose, glucose, sucrose and trehalose in stems than in leaves (Petropoulos et al., 2019). A similar tendency was observed in the fruits of *S. lycopersicum* (Abreu et al., 2020) and the leaves of *C. sinensis*, collected in late spring (Zeng et al., 2019). Interestingly, the concentration of

sucrose, glucose and fructose was higher in cultures of *Phyllanthus niruri* and *Phyllanthus urinaria* after 8 and 12 weeks (Mediani et al., 2015). These results contrasted with those reported for the fruits of *Castanea crenata* (Yu *et al*., 2022) whereas in the roots of *D. carota*, the content of sucrose, glucose, and fructose did not change depending on the harvesting time (Sciubba *et al*., 2020).

9.4 Multivariate analysis of metabolome content in purslane cultivars

To observe the effect of harvesting time in the metabolome of purslane cultivars, the OPLS-DA model was used (Figs. 4A, 4C and 4E). These analyses were performed with two predictive components and one orthogonal component for each time of harvest (Table S2). The results from Xochimilco, Mixquic, and Cuautla cultivars were validated with the random permutation test (200 times). The results are presented in Figs. S4B, S4C, and S4D. With these models, a clear separation of the Mixquic cultivar from the other two cultivars is observed (Figs. 4A, 4C and 4E). In the first and second harvests, the most abundant metabolites had similarities in their concentration in all cultivars. These metabolites were fructose, glucose, alanine, proline, guanosine, galactose, isoleucine, sucrose, choline, threonine, methanol, and valine for the Mixquic cultivar. For the Cuautla cultivar, the main metabolites were ethanol, malic acid, and asparagine. Finally, for the Xochimilco cultivar histidine, citric, formic, and aspartic acid were observed (Figs. 4B and 4D). In the third harvest, the Mixquic cultivar exhibited higher amount of differential metabolites than the Xochimilco cultivar, which mainly contained histidine, citric and formic acid. The Cuautla obtained a higher content of fructose, galactose, ethanol, and malic acid (Fig. 4F). In the third harvest, the Mixquic cultivar had higher concentration of galactose (0.38 mM) and myo-inositol (0.55 mM), whereas the Cuautla cultivar presented higher concentrations of fructose (10.9 - 12.2 mM) and glucose (α, β isomers (10.3 - 11.4 mM). The Xochimilco cultivar presented the lowest levels of carbohydrates (Table 2; Fig. S5). The concentrations of amino acids were different among cultivars. The Mixquic cultivar contained high concentrations of 15 amino acids, followed by Xochimilco with 5, and Cuautla with 4. The differential amino acids from Mixquic, were alanine (0.71 mM), isoleucine (0.086 mM), and proline (0.31 mM) during the first harvest. In the same context, aspartic acid (0.53 mM), isoleucine (0.087 mM), and tyrosine (0.068 mM) where the differential metabolites in the second harvest whereas in

the third one these were isoleucine (0.086 mM) and tyrosine (0.062 mM) (Table 2; Fig. S6). Regarding the organic acid composition, the Mixquic cultivar exhibited high concentration of pyruvic acid (0.09 - 0.10 mM), 2-hydroxyisobutyric acid (0.04 mM), acetic acid (0.09 mM), succinic acid (0.27 mM), 4-O-caffeoylquinic acid (0.018 mM), and 5-O-caffeoylquinic acid (0.049 mM). The levels for the Xochimilco cultivar, was citric acid (6.02 mM), whereas for the Cuautla cultivar the main metabolite was malic acid (13.85 mM) (Table 2; Fig. S7). The concentration of alcohols, nucleosides, and other metabolites was high in the Mixquic cultivar. Also, high amounts of methanol (0.49 mM), guanosine (0.02 mM), uridine (0.06 mM), choline (0.59 mM), and O-phosphocholine (0.12 mM) were observed. Trigonelline (0.05 mM) was the main metabolite for the Xochimilco cultivar whereas ethanol was the main metabolite for the Cuautla cultivar (0.03 mM) (Table 2; Fig. S8).

Camalle *et al*. (2020) observed variations in the content of carbohydrates, organic acids, and amino acids in two ecotypes of purslane from Israel. Nemzer *et al*. (2020) reported changes in the concentration of amino acids and organic acids in native purslane from the United Kingdom. The purslane cultivars contained higher concentrations of alanine, leucine, and aspartic, glutamic, malic, citric, and oxalic acids. Zaman *et al*. (2020) detected metabolite variations in cultivars from China and America which were related with their content of organic acids, amino acids, sugars, and alcohols. Clear metabolic differences have been described among 38 purslane cultivars from Bulgaria and Greece. These differences have been observed in the content of phenolic aldehydes, cyclodopa amides, and amino acids (Balabanova *et al*., 2020). Similarly, purslane cultivars from Egypt and showed differences in the content of flavonoids, glycosides, alkaloids, amino acids, fatty acids, and vitamins (Farag & Shakour, 2019).

X. CONCLUSIONS

Metabolite profiling by ^{1}H NMR revealed that primary metabolites significantly varied in the three cultivars studied. The OPLS-DA analysis successfully separated the three cultivars, being Mixquic cutivar with the highest concentration of amino acids and carbohydrates. The Xochimilco cultivar was rich in citrate and the Cuautla cultivar had the

highest concentration of glucose and fructose during the third harvest. The results obtained in this investigation could contribute to the selection of purslane cultivars according to their optimal harvesting time and nutritional content.

REFERENCES

Abreu, A.C., Marín, P., Aguilera-Sáez, L.M., Tristán, A.I., Peña, A., Oliveira, I., Simões, M., Valera, D., & Fernández, I. (2019). Effect of a shading mesh on the metabolic, nutritional, and defense profiles of harvested greenhouse-grown organic tomato fruits and leaves revealed by NMR metabolomics. *Journal of agricultural and food chemistry*, *67*(46), 12972-12985. https://pubs.acs.org/doi/abs/10.1021/acs.jafc.9b05657

Alu'datt, M.H., Rababah, T., Alhamad, M.N., Al-Tawaha, A., Al-Tawaha, A.R., Gammoh, S., Ereifej, K.I., Al-Karaki, G., Hamasha, H.R., Tranchant, C.C., & Kubow S. (2019). Herbal yield, nutritive composition, phenolic contents and antioxidant activity of purslane (*Portulaca oleracea* L.) grown in different soilless media in a closed system. *Industrial Crops and Products*, *141*(1), 111746. https://doi.org/10.1016j.indcrop.2019.111746

Balabanova, V., Hristov, I., Zheleva-Dimitrova, D., Sugareva, P., Lozanov, V., & Gevrenova, R. (2020). Bioinformatic insight into *Portulaca oleracea* L. (Purslane) of bulgarian and Greek origin. *Acta Biologica Cracoviensia Series. Botanica*, *62*(1), 7-21. https://doi.org/: 10.24425/abcsb.2020.131662

Becerra-Martínez, E., Pacheco-Hernández, Y., Lozoya-Gloria, E., Betancourt-Jiménez, M. G., Hidalgo-Martínez, D., Zepeda-Vallejo, L.G., & Villa-Ruano, N. (2020). ^{1}H NMR metabolomics profiling of recombinant tobacco plants holding a promoter of a sesquiterpene cyclase. *Phytochemical Analysis*, *31*(4), 480-487. https://doi.org/10.1002/pca.2911

Bekmirzaev, G., Ouddane, B., Beltrao, J., Khamidov, M., Fujii, Y., & Sugiyama, A. (2021). Effects of Salinity on the Macro-and Micronutrient Contents of a Halophytic Plant Species (*Portulaca oleracea* L.). *Land*, *10*(5), 481. https://doi.org/10.3390/land10050481

Blasco, H., Błaszczyn, J., Billautf, J.C., Nadal-Desbarats, L., Pradat, P.F., Devos, D., Moreau, C., Andres, C.R., Emond, P., Corcia, P., & Słowin, R. (2015). Comparative analysis of targeted metabolomics: Dominance-based rough set approach versus orthogonal partial least square-discriminant analysis. *Journal of Biomedical Informatics*, *53*, 291-299. https://doi.org/10.1016/j.jbi.2014.12.001

Calò, F., Girelli, C.R., Wang, S.C., & Fanizzi, F.P. (2022). Geographical origin assessment of extra virgin olive oil via NMR and MS combined with chemometrics as analytical approaches. *Foods*, *11*(1), 113. https://doi.org/10.3390/foods11010113

Camalle, M., Standing, D., Jitan, M., Muhaisen, R., Bader, N., Bsoul, M., Yvonne Ventura Y., Soltabayeva, A., & Sagi, M. (2020). Effect of Salinity and Nitrogen Sources on Leaf Quality, Biomass, and Metabolic Responses of Two Ecotypes of *Portulaca oleracea*. A*gronomy*, *10*(5), 656. https://doi.org/10.3390/agronomy10050656

Emwas, A.H., Luchinat, C., Turano, P., Tenori, L., Roy, R., Salek, R.M., Ryan, D., Merzaban J. S., Kaddurah-Daouk, R., Zeri, A.C., Gowda, N., Raftery D., Wang, Y., Brennan, L., & Wishart, D.S. (2015). Standardizing the experimental conditions for using urine in NMR-based metabolomic studies with a particular focus on diagnostic studies: a review. *Metabolomics*, *11*(4), 872-894. https://doi.org/10.1007/s11306-014-0746-7

Farag, M.A., & Shakour, Z.T.A. (2019). Metabolomics driven analysis of 11 Portulaca leaf taxa as analysed via UPLC-ESI-MS/MS and chemometrics. *Phychemestry*, *161*, 117-129. https://doi.org/10.1016/j.phytochem.2019.02.009

Farag, O.M., Abd-Elsalam, R.M., Ogaly, H.A., Ali, S.E., El Badawy, S.A., Alsherbiny, M.A., Li, C.G., & Ahmed, K. A. (2021). Metabolomic profiling and neuroprotective effects of purslane seeds extract against acrylamide toxicity in rat's brain. *Neurochemical Research*, *46*(4), 819-842. https://link.springer.com/article/10.1007/s11064-020-03209-6

Häusler, R.E., Ludewig, F., & Krueger, S. (2014). Amino acids–a life between metabolism and signaling. *Plant Science*, *229*, 225-237. https://doi.org/10.1016/j.plantsci.2014.09.011

Hosseinzadeh, M.H., Ghalavand, A., Mashhadi-Akbar-Boojar, M., Modarres-Sanavy, S.A. M., & Mokhtassi-Bidgoli, A. (2020). Increased medicinal contents of purslane by nitrogen and *Arbuscular Mycorrhiza* under drought stress. *Communications in Soil Science and Plant Analysis*, *51*(1), 118-135. https://doi.org/10.1080/00103624.2019.1695828

Managa, L.R., du Toit, E.S., & Prinsloo, G. (2021). NMR-based metabolomic analyses to identify the effect of harvesting frequencies on the leaf metabolite profile of a Moringa Oleifera cultivar grown in an open hydroponic system. *Molecules*, *26*(8), 2298. https://doi.org/10.3390/molecules26082298

Marak, S., Shumilina, E., Kaushik, N., Falch, E., & Dikiy, A. (2021). Effect of different drying methods on the nutritional value of hibiscus sabdariffa calyces as revealed by NMR metabolomics. *Molecules*, *26*(6), 1675. https://doi.org/10.3390/molecules26061675

Mediani, A., Abas, F., Khatib, A., Tan, C.P., Ismail, I. S., Shaari, K., Ismail, A., & Lajis, N. H. (2015). Phytochemical and biological features of *Phyllanthus niruri* and *Phyllanthus urinaria* harvested at different growth stages revealed by ^{1}H NMR-based metabolomics. *Industrial Crops and Products*, *77*, 602-613. https://doi.org/10.1016/j.indcrop.2015.09.036

Montoya-García, C.O., Volke-Haller, V., Santillán-Ángeles, A., López-Escobar, N.F., & Trinidad-Santos, A. (2019). Relación NH4+/NO3- en la producción de biomasa y el contenido nutrimental de *Portulaca oleracea* L. *Agrociencia*, *53*, 521-533. https://agrociencia-colpos.mx/index.php/agrociencia/article/view/1825

Montoya-García, C.O., Volke-Haller, V., Trinidad-Santos, A., & Villanueva-Verduzco, C. (2018a). Change in the contents of fatty acids and antioxidant capacity of purslane in relation to fertilization. *Scientia Horticulturae*, *234*, 152-159. https://doi.org/10.1016/j.scienta.2018.02.043

Montoya-García, C.O., Volke-Haller, V., Trinidad-Santos, A., & Villanueva-Verduzco, C. (2018b). Concentración nutrimental de la verdolaga (*Portulaca oleracea* L.) en respuesta a la fertilización con NPK. *Agrociencia*, *52*, 241-254.

http://www.scielo.org.mx/scielo.php?pid=S1405-31952018000200241&script=sci_abstract

Montoya-García, C.O., Volke-Haller, V., Trinidad-Santos, A., Villanueva-Verduzco, C., & Sánchez-Escudero, J. (2017). Respuesta de la verdolaga (*Portulaca oleracea* L.) a la fertilización con NPK. *Revista de Fitotecnia Mexicana*, *40*(3), 325-332. https://doi.org/10.35196/rfm.2017.3.325-332

Nemzer, B., Al-Taher, F., & Abshiru, N. (2020). Phytochemical composition and nutritional value of different plant parts in two cultivated and wild purslane (*Portulaca oleracea* L.) genotypes. *Food Chemistry*, *320*, 126621. https://doi.org/10.1016/j.foodchem.2020.126621

Petropoulos, S.A., Fernandes, Â., Dias, M.I., Vasilakoglou, I.B., Petrotos, K., Barros, L., & Ferreira, I.C. (2019). Nutritional value, chemical composition and cytotoxic properties of common urslane (*Portulaca oleracea* L.) in Relation to Harvesting Stage and Plant Part. *Antioxidants*, *8*(8), 293. https://doi.org/10.3390/antiox8080293

Rådjursöga, M., Lindqvist, H.M., Pedersen, A., Karlsson, G.B., Malmodin, D., Brunius, C., Ellegård, L., & Winkvist. A. (2019). The ^{1}H NMR serum metabolomics response to a two meal challenge: a cross-over dietary intervention study in healthy human volunteers. *Nutrition Journal*, *18*(25), 2-12. https://doi.org/10.1186/s12937-019-0446-2

Saheri, F., Barzin, G., Pishkar, L., Boojar, M.M.A., & Babaeekhou, L. (2020). Foliar spray of salicylic acid induces physiological and biochemical changes in purslane (*Portulaca oleracea* L.) under drought stress. *Biologia*, *75*(12), 2189-2200. https://link.springer.com/article/10.2478/s11756-020-00571-2

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), 1-13. https://link.springer.com/article/10.1007/s11306-019-1566-6

Schmidtke, L.M., Antalick, G., Šuklje, K., Blackman, J.W., Boccard, J., & Deloire, A. (2020). Cultivar, site or harvest date: the gordian knot of wine terroir. *Metabolomics*, *16*(5), 1-17. https://link.springer.com/article/10.1007/s11306-020-01673-3

Sciubba, F., Tomassini, A., Giorgi, G., Brasili, E., Pasqua, G., Capuani, G., Aureli, W., & Miccheli, A. (2020). NMR-based metabolomic study of purple carrot optimal harvest time for utilization as a source of bioactive compounds. *Applied Sciences*, *10*(23), 8493. https://doi.org/10.3390/app10238493

Shuzhao, L., Computational methods and data analysis for metabolomics, methods in molecular biology, Vol. 2104, Springer Science+Business Media, LLC, part of Springer Nature 2020. https://doi.org/10.1007/978-1-0716-0239-3_5

Wu, C., Zhang, X., Zhang, X., Luan, H., Sun, G., Sun, X., Wang, X., Gou, P., & Xu, X. (2014). The caffeoylquinic acid-rich Pandanus tectorius fruit extract increases insulin sensitivity and regulates hepatic glucose and lipid metabolism in diabetic db/db mice. The *Journal of nutritional biochemistry*, *25*(4), 412-419. https://doi.org/10.1016/j.jnutbio.2013.12.002

Yang, Y., Syed, S., Mao, S., Li, Q., Ge, F., Lian, B., & Lu, C. (2020). Bioorganic-mineral fertilizer can remediate chemical fertilizer-oversupplied soil: Purslane planting as an example. *Journal of Soil Science and Plant Nutrition*, *20*, 892-900. https://link.springer.com/article/10.1007/s42729-020-00175-4

Yu, J. M., Nam, M., & Kim, M.S. (2022). Metabolite profiling of chestnut (*Castanea crenata*) according to origin and harvesting time using ^{1}H NMR spectroscopy. *Foods*, *11*(9), 1325. https://doi.org/10.3390/foods11091325

Zaman, S., Bilal, M., Du, H., & Che, S. (2020). Morphophysiological and comparative metabolic profiling of purslane genotypes (*Portulaca oleracea* L.) under salt stress. *Biomedical Research International*, *2020*, 1-17 https://doi.org/10.1155/2020/4827045

Zaman, S., Hu, S., Alam, M. A., Du, H., & Che, S. (2019). The accumulation of fatty acids in different organs of purslane under salt stress. *Scientia Horticulturae*, *250*, 236-242. https://doi.org/10.1016/j.scienta.2019.02.051

Zeng, C., Lin, H., Liu, Z., & Liu, Z. (2020). Metabolomics analysis of *Camellia sinensis* with respect to harvesting time. *Food Research International*, *128*, 108814. https://doi.org/10.1016/j.foodres.2019.108814

Zhu, D., Hayman, A., Frew, R., Kebede, B., Chen, G., & Stewart, I. (2022). Milk powder extraction: optimization of conditions for the water-soluble metabolites by proton nuclear magnetic resonance (^{1}H NMR). *Analytical Letters*, *55*(1), 1-10. https://doi.org/10.1080/00032719.2021.1907588

TABLE

Table 1. ^{1}H NMR signals used to identify metabolome in purslane (*Portulaca oleracea* L.) cultivars at three harvest times.

	Metabolite	Chemical shifts (ppm), J (Hz), multiplicity
Sugars		
1	Fructose	3.99 (m) CH-5, 4.01 (dd, *J* = 12.7, 1.3 Hz) CH_2-11
2	Galactose	4.55 (d, *J* = 7.9 Hz)
3	Glucose (α, β isomers)	5.22 (d, *J* = 3.7 Hz) CH-2, 4.63 (d, *J* = 7.9 Hz) CH-2
4	Myo-inositol	3.27 (t, *J* = 9.4 Hz) CH-2
5	Sucrose	4.20 (d, *J* = 8.8 Hz) CH-3, 5.40 (d, *J*= 3.8 Hz) CH-7
Amino acids		
6	Alanine	1.47 (d, *J* = 7.2 Hz) CH_3-6
7	Asparagine	2.85 (dd, *J* = 16.9, 7.8 Hz) CH_2-6, 2.94 (dd, *J* = 16.9, 4.2 Hz) CH_2-6
8	Aspartic acid	2.70 (dd, *J* = 17.5, 7.4 Hz) CH_2-6, 2.83 (dd, *J* = 17.5, 3.7 Hz) CH_2-6
9	GABA	1.89 (m) CH_2-5, 2.28 (t, *J* = 7.4 Hz) CH_2-4
10	Glutamic acid	2.14 (m) CH_2-6, 2.45 (m) CH_2-7
11	Glutamine	2.12 (m) CH_2-6, 2.44 (m) CH_2-7
12	Histidine	8.61 (m) CH-2, 7.36 (m) CH-5
13	Isoleucine	0.93 (t, *J* = 7.4 Hz) CH_3-8, 1.00 (d, *J* = 7.0 Hz) CH3-9
14	Leucine	0.94 (d, *J* = 6.2 Hz) CH_3-8, 0.95 (d, *J* = 6.2 Hz) CH_3-9
15	Phenylalanine	7.31 (d, *J* = 7.5) CH-3, 5, 7.36 (m) CH-4, 7.42 (t, *J* = 7.5 Hz) CH-2, 6
16	Proline	2.01 (m) CH2-6, 2.3 (m) CH_2-4
17	Threonine	1.32 (d, *J* = 6.6 Hz) CH_3-8
18	Tryptophan	7.52 (d, *J* = 8.0 Hz) CH-6, 7.72 (d, *J* = 8.0 Hz) CH-7
19	Tyrosine	7.18 (d, *J* = 7.18 Hz) CH-3, 5, 6.88 (d, *J* = 7.18 Hz) CH-2, 6
20	Valine	0.98 (d, *J* = 7.0 Hz) CH_3-8, 1.03 (d, *J* = 7.0 Hz) CH_3-7

Organic acids		
21	Acetic acid	1.95 (s) CH_3-1
22	Citric acid	2.67 (d, *J* = 15.6 Hz) CH_2-2, 5, 2.78 (d, *J* = 15.6 Hz) CH_2-2, 5
23	Formic acid	8.42 (s) CH-2
24	Fumaric acid	6.55 (s) CH-4, 5
25	Malic acid	2.53 (dd, *J* = 15.5, 9.9 Hz) CH_2-5, 2.76 (dd, J = 15.5, 3.2 Hz) CH_2-5
26	Pyruvic acid	2.36 (s) CH_3–1
27	Succinic acid	2.62 (s) CH_3–1
28	2-Hydroxyisobutyric acid	1.33 (s) CH_3–1
29	3-O-caffeoylquinic acid	6.47 (d, *J* = 15.9 Hz) H-8´
30	4-O-caffeoylquinic acid	6.53 (d, *J* = 15.9 Hz) H-8´
31	5-O-caffeoylquinic acid	6.50 (d, *J* = 15.9 Hz) H-8´
Alcohols		
32	Ethanol	1.17 (t, *J* = 7.1 Hz), 3.64 (c, *J* = 7.1 Hz)
33	Methanol	3.35 (s) CH_3-1
Nucleosides		
34	Adenosine	6.06 (d, *J* = 6.2 Hz) CH-3, 8.24 (s) CH-2, 8.35 (s) CH-1
35	Guanosine	5.90 (d, *J* = 5.7 Hz) CH-3, 7.99 (s) CH-2
36	Uridine	7.86 (d, *J* = 8.1 Hz) CH-1
Other compounds		
37	Choline	3.18 (s) CH_3-5, 6, 7
38	Trigonelline	8.88 (m) CH-4, 9.13 (s) CH-2
39	O-Phosphocholine	3.21 (s) CH_3-5, 6, 7

s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet

The numbering of the molecules was taken from the HMDB database (http://www.hmdb.ca).

Table 2. Analysis of variance of the metabolome of purslane (Portulaca oleracea L.) cultivars at three harvest times as determined by 1H NMR.

	Metabolite	Harvest	Harvest 1			Harvest 2			Harvest 3		
			Xc	Mq	Mr	Xc	Mq	Mr	Xc	Mq	Mr
Sugars											
1	Fructose	ns	b	a	a	b	a	a	c	b	A
2	Galactose	*	c	a	b	b	a	a	b	a	b
3	Glucose (α, β isomers)	ns	c	b	a	b	a	a	c	b	a
4	Myo-inositol	ns	ab	a	b	a	a	a	b	a	C
5	Sucrose	ns	nd	a	nd	nd	a	nd	nd	a	nd
Amino acids											
6	Alanine	ns	a	a	b	a	a	b	a	a	b
7	Asparagine	ns	b	ab	a	a	a	a	a	a	a
8	Aspartic acid	ns	a	a	a	a	a	b	a	a	a
9	GABA	ns	b	a	c	b	a	c	b	a	c
10	Glutamatic acid	ns	a	a	a	a	a	a	a	a	a
11	Glutamine	ns	b	a	b	b	a	b	b	a	b
12	Histidine	ns	a	a	a	a	b	b	a	b	a
13	Isoleucine	ns	c	a	b	c	a	b	b	a	a
14	Leucine	ns	b	a	b	b	a	b	b	a	a
15	Phenylalanine	ns	a	a	a	a	a	a	a	a	a
16	Proline	ns	b	a	c	b	a	c	b	a	b
17	Threonine	ns	b	a	c	b	a	b	b	a	b
18	Tryptophan	ns	a	a	a	a	a	a	a	a	a
19	Tyrosine	ns	c	a	b	c	a	b	b	a	a
20	Valine	ns	b	a	b	b	a	b	b	a	B
Organic acids											
21	Acetic acid	ns	b	a	b	b	a	c	b	a	B
22	Citric acid	ns	a	c	b	a	c	b	a	b	a
23	Formic acid	ns	a	a	a	a	a	a	a	a	a

24	Fumaric acid	ns	a	a	a	a	a	a	a	a	a
25	Malic acid	ns	b	b	a	b	b	a	b	b	a
26	Pyruvic acid	*	a	a	a	a	a	b	a	a	b
27	Succinic acid	ns	b	a	b	b	a	b	b	a	b
28	2-Hydroxyisobutyric acid	*	b	a	c	b	a	c	b	a	c
29	3-O-caffeoylquinic acid	ns	b	a	ab	a	a	b	ab	a	b
30	4-O-caffeoylquinic acid	ns	b	a	b	b	a	b	b	a	c
31	5-O-caffeoylquinic acid	ns	b	a	b	a	a	b	b	a	c
Alcohols											
32	Ethanol	ns	b	b	a	b	ab	a	b	ab	a
33	Methanol	ns	b	a	b	b	a	b	b	a	c
Nucleosides											
34	Adenosine	ns	nd	a	nd	nd	a	nd	nd	a	Nd
35	Guanosine	ns	b	a	c	b	a	c	b	a	c
36	Uridine	ns	b	a	b	b	a	c	b	a	c
Other compounds											
37	Choline	*	b	a	c	b	a	c	b	a	c
38	Trigonelline	ns	a	a	b	a	a	b	a	a	b
39	O-Phosphocholine	–	b	a	ab	b	a	b	b	a	b
Number of significant metabolites			11	32	13	15	33	12	12	31	16

Different letters in the same row indicate significant difference (Tukey α=0.05). Xc= Xochimilco, Mq= Mixquic, Mr= Morelos, ns = not significant, nd = not detected.

FIGURE

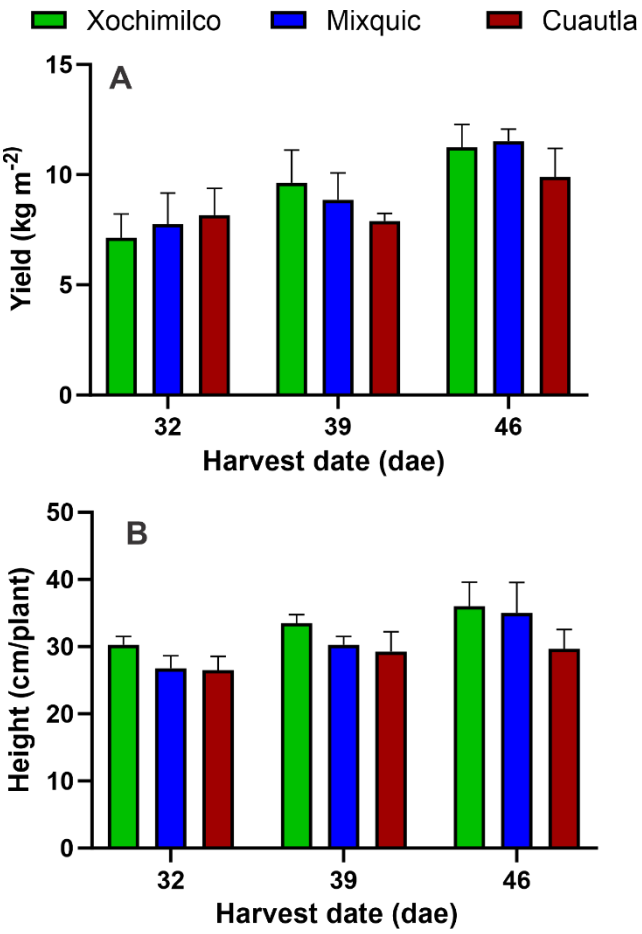

Figure 1. Yield and height of three purslane (*Portulaca oleracea* L.) cultivars at three harvesting times.

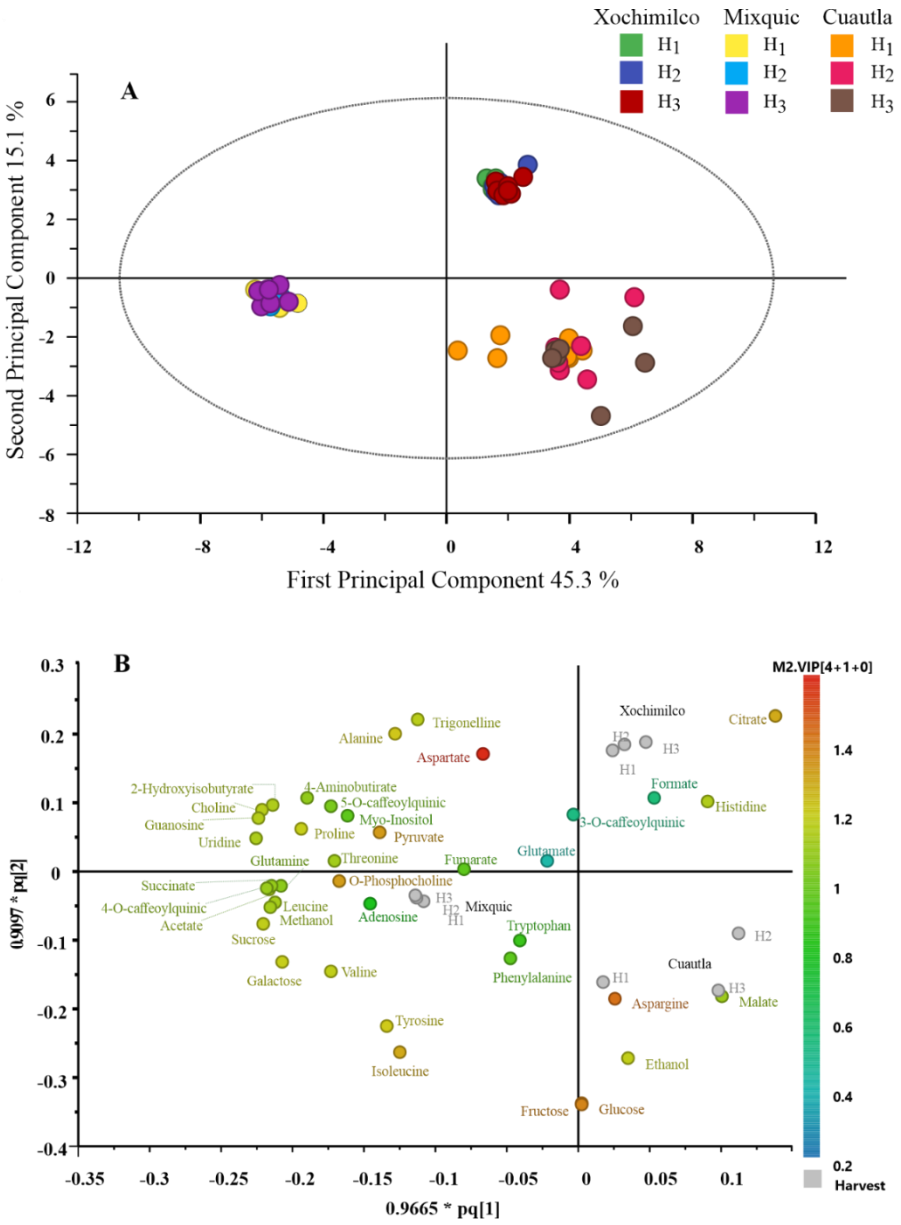

Figure 2. OPLS-DA score graphs generated from the 1H NMR spectra (750 MHz) of three purslane (*Portulaca oleracea* L.) cultivars at three harvesting times (H1: 32 days after emergence (DAE), H2: 39 DAE, and H3: 46 DAE) (A). The ellipse in A represents the Hotelling with 95% confidence. The corresponding load graphs that identify the discriminating metabolites between cultivars and harvesting times (B). Each circle represents a metabolite, and the color shows the importance of the metabolite variations between groups.

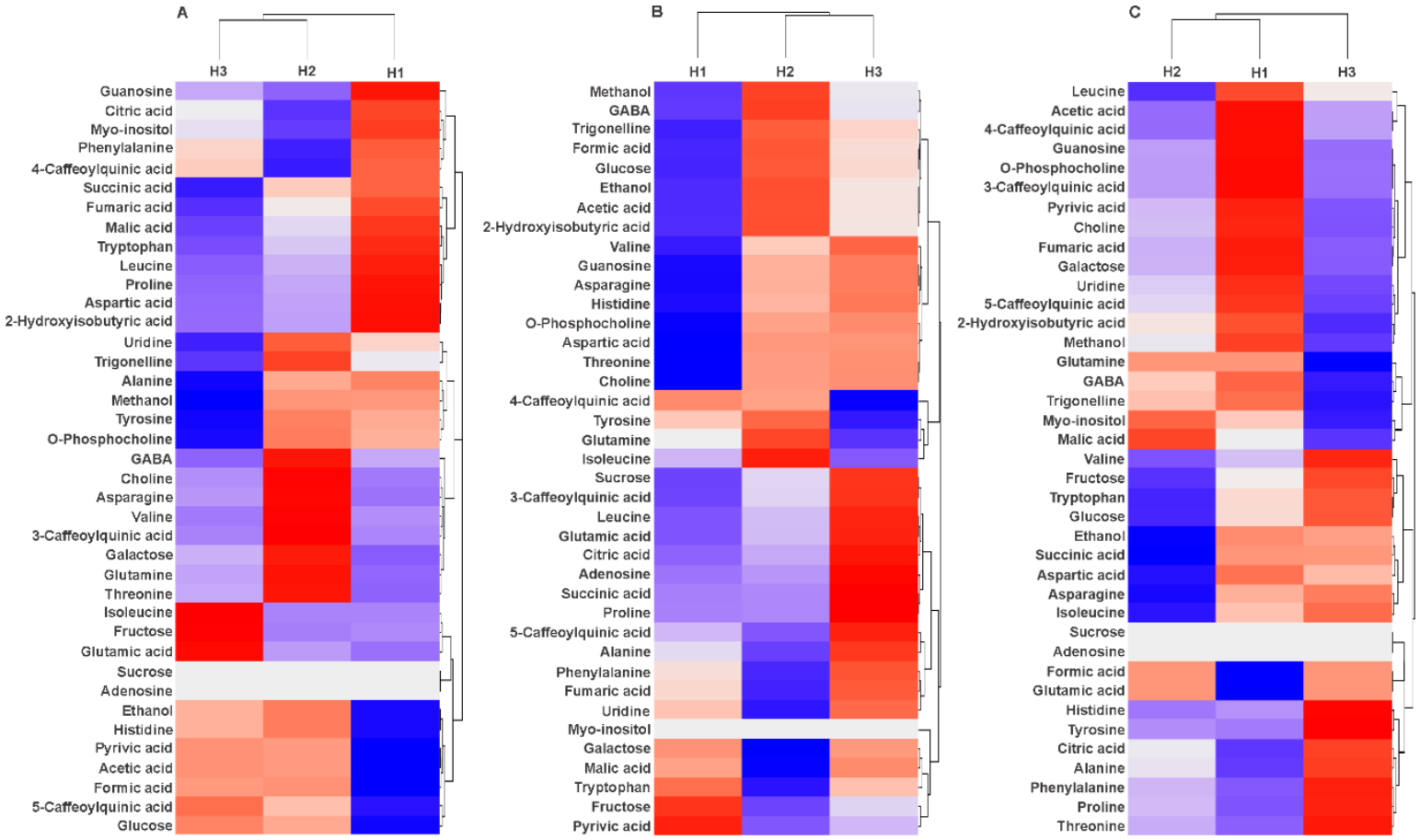

Figure 3. Heatmap of the metabolomics profiling of three purslane (*Portulaca oleracea* L.) cultivars: Xochimilco (A), Mixquic (B) and Cuautla (C) at three harvesting times (H1: 32 days after emergence (DAE); H2: 39 DAE; and H3: 46 DAE). The blue color represents a downward trend, and the red color represents upward trend.

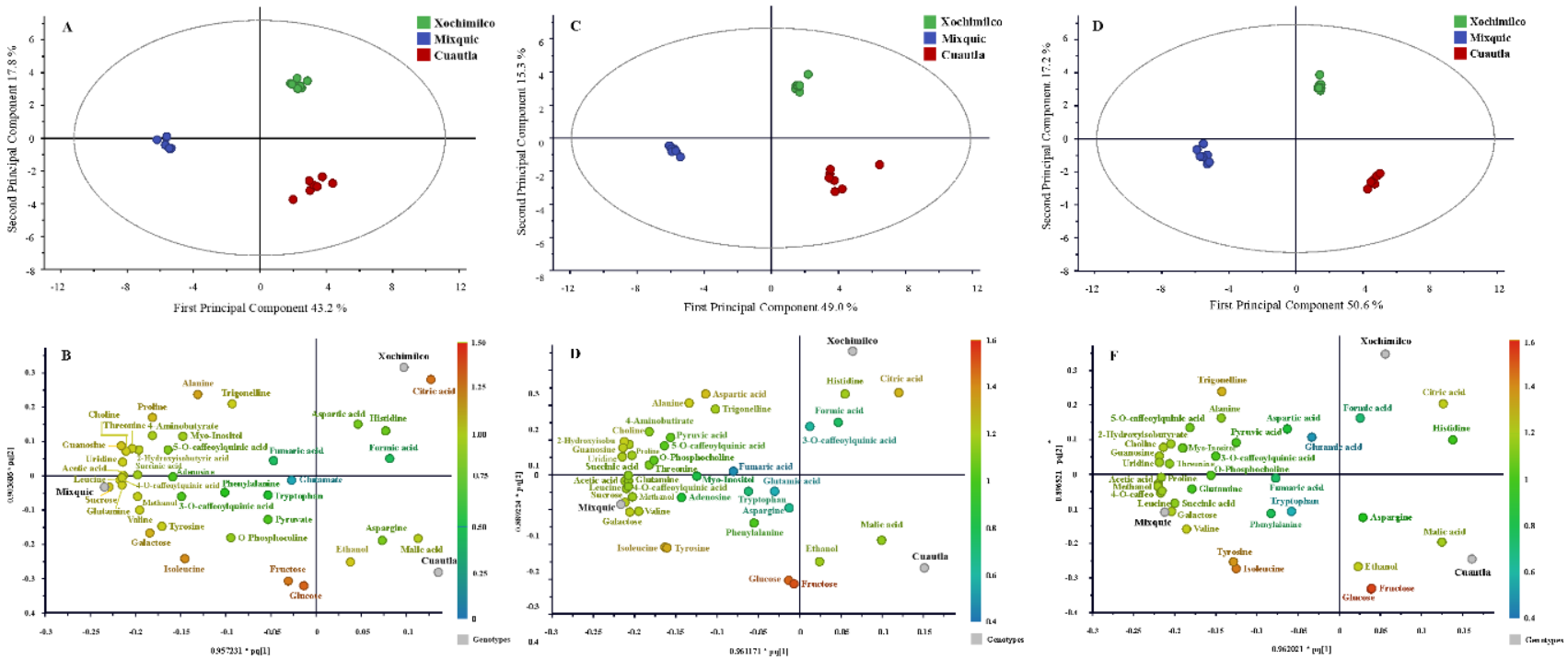

Figure 4. OPLS-DA score graphs generated from the 1H NMR spectra (750 MHz) of three independent purslane (*Portulaca oleracea* L.) cultivars for each harvesting time, 32 days after emergence (DAE) (A and B); 39 DAE (C and D); and 46 DAE (E and F). The ellipse in A, C, and E represents the Hotelling with 95% confidence. The corresponding load graphs that identify the discriminating metabolites between cultivars and harvesting times. Each circle represents a metabolite, and the color shows the importance of the metabolite variations between groups.

FIGURAS SUPLEMENTARIAS

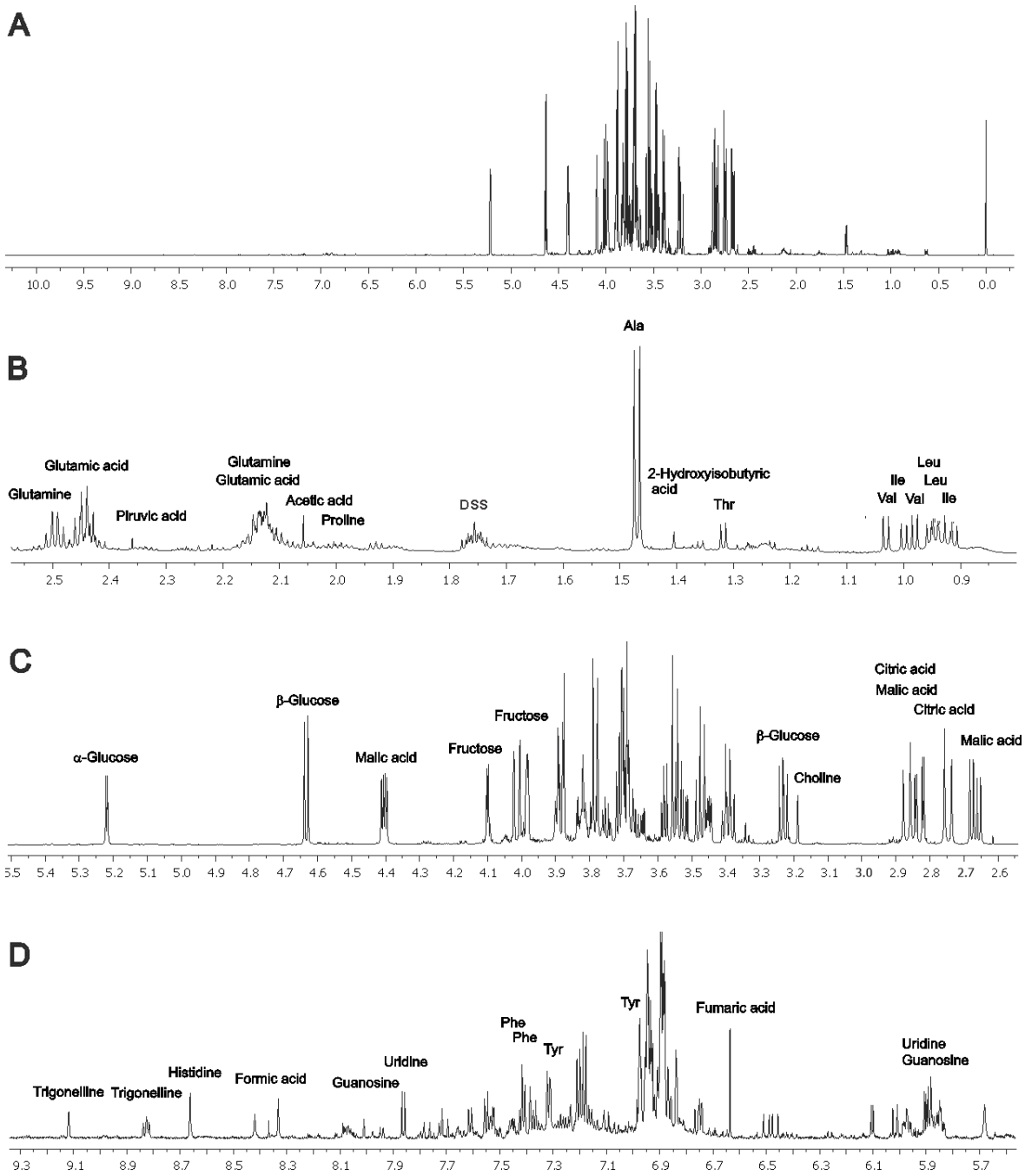

Figure S1. Characteristic ^{1}H NMR spectrum obtained at 750 MHz of aqueous extracts from the biomass of three purslane (*Portulaca oleracea* L.) cultivars at three harvesting times. (A) Full 1H NMR spectrum from 0.0 to 10.0 ppm; (B) Expanded ^{1}H NMR spectrum from 0.8 to 3.0 ppm; (C) Expanded 1H NMR spectrum from 3.2 to 5.5 ppm; (D) Expanded ^{1}H NMR spectrum from 5.8 to 9.6 ppm.

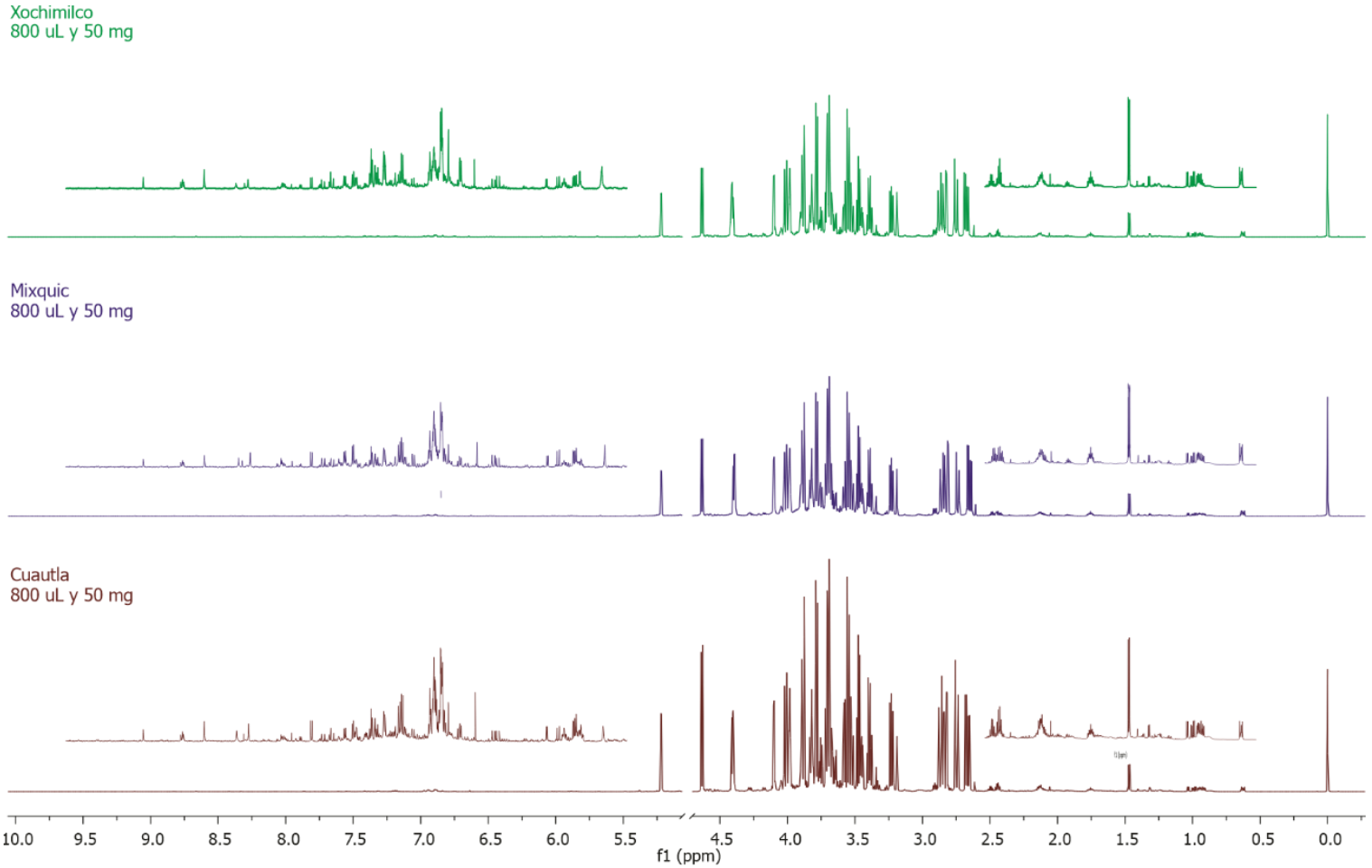

Figure S2. Representative 750 MHz ^{1}H NMR spectra of three purslane (*Portulaca oleracea* L.) cultivars at three harvesting times are shown in the range of δ -0.5 to 10.0 ppm, and the expansions for the range of δ 6.5 to 9.0 ppm. Green represents Xochimilco

cultivar, blue is Mixquic cultivar, and red is Cuautla cultivar. The spectra obtained at 750 MHz were scaled in accordance with TSP used as the internal standard and ^{1}H chemical shift in parts per million.

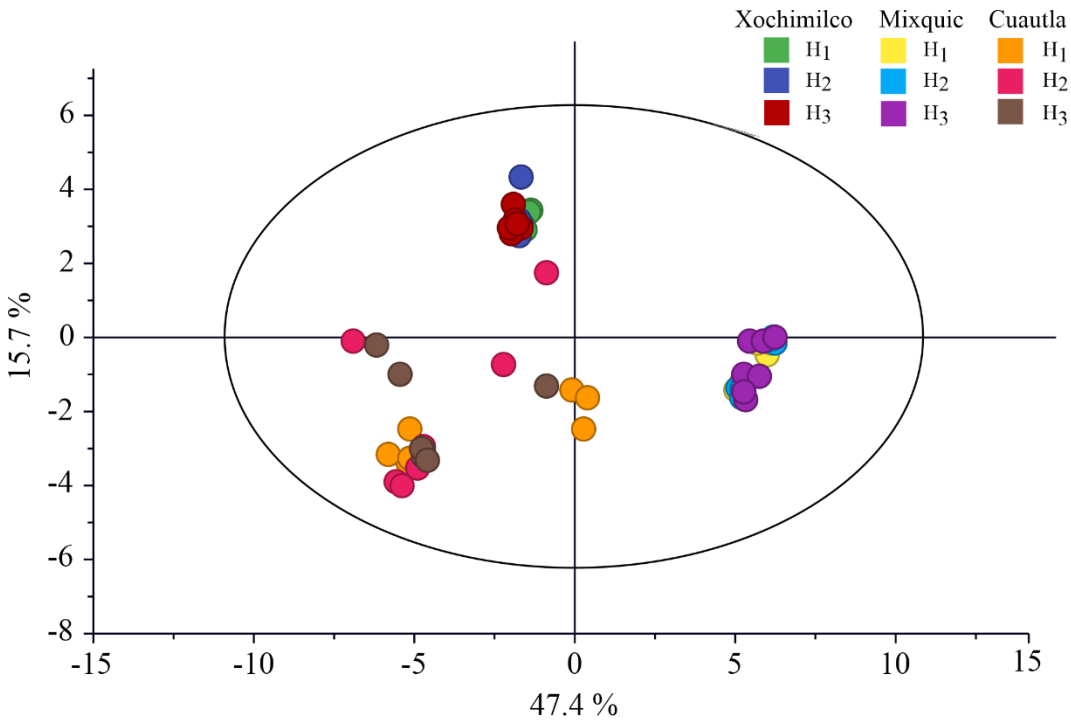

Figure S3. Las puntuaciones del análisis de componentes principales (ACP) de la parcela general de tres cultivares de verdolaga (*Portulaca oleracea* L.) en tres épocas de cosecha. El componente 1 muestra el 47.4% de la variación, mientras que el componente 2 muestra el 15.7% de la variación.

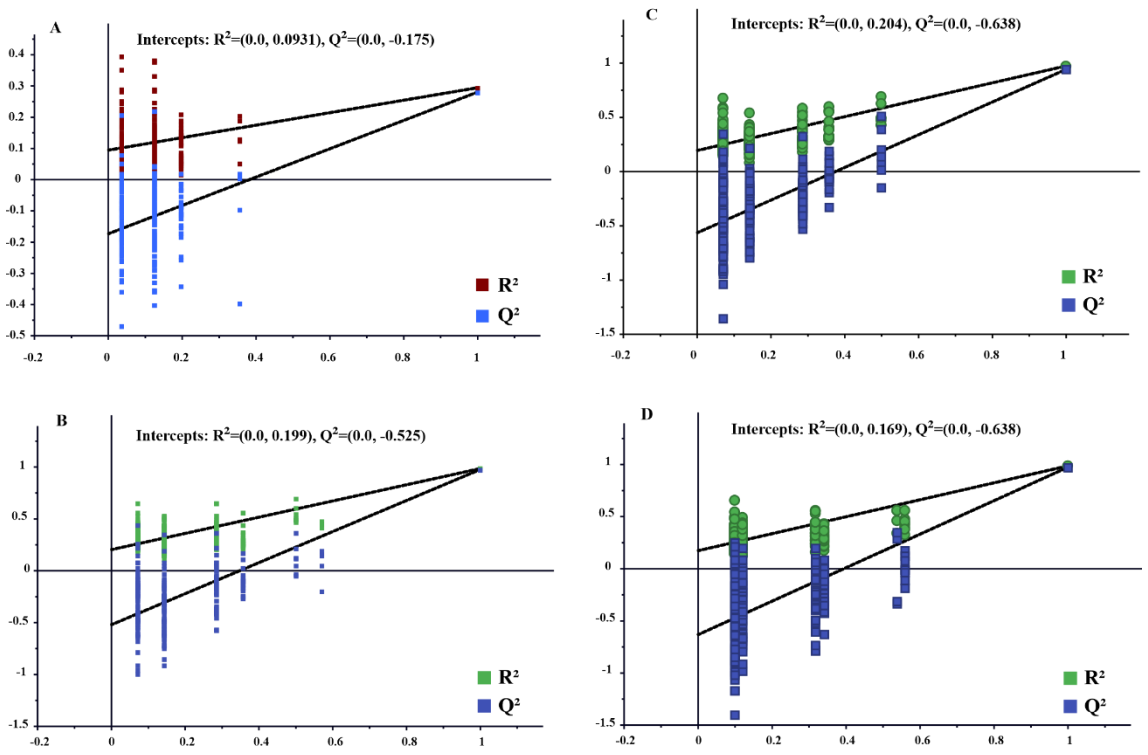

Figure S4. General permutation analysis for three purslane (*Portulaca oleracea* L.) cultivars at three harvesting times (A). For independent cultivars per time of harvest, 32 days after emergence (DAE) (B); 39 DAE (C); and 46 DAE (D).

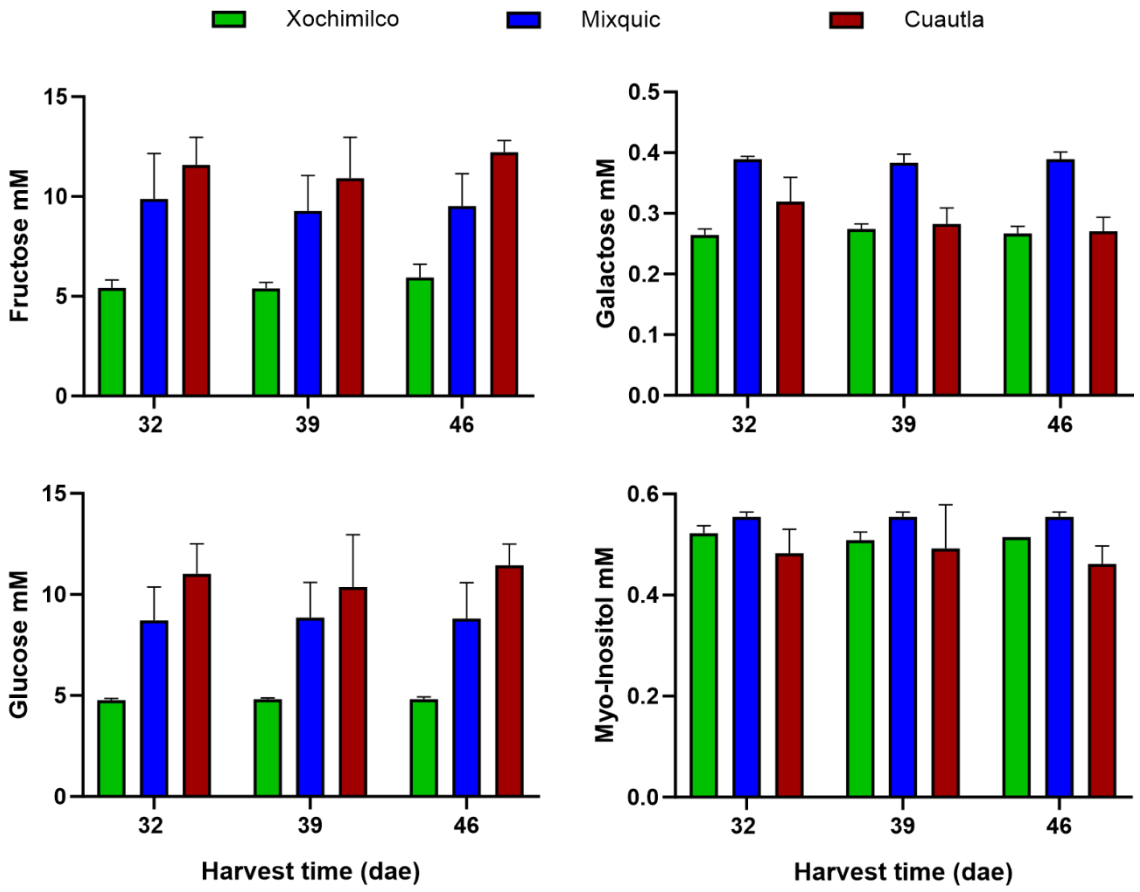

Figure S5. Relative concentrations of carbohydrates metabolites estimated by the ^{1}H NMR method in the three purslane (*Portulaca oleracea* L.) cultivars at three harvesting times. The data are expressed as the mean ± standard error calculated using seven replicates.

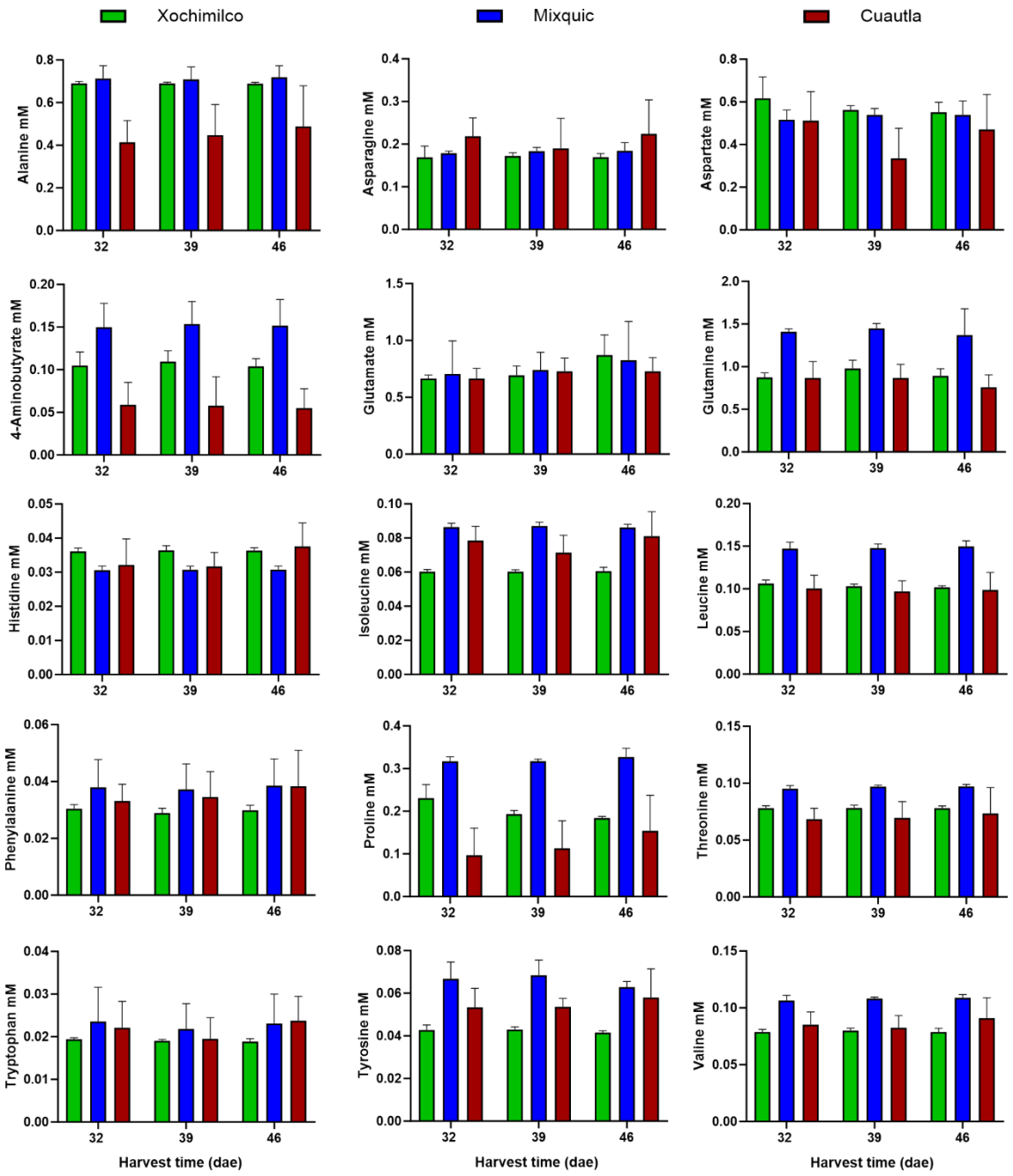

Figura S6. Relative concentrations of amino acids metabolites estimated by the ^{1}H NMR method in the three purslane (*Portulaca oleracea* L.) cultivars at three harvesting times. The data are expressed as the mean ± standard error calculated using seven replicates.

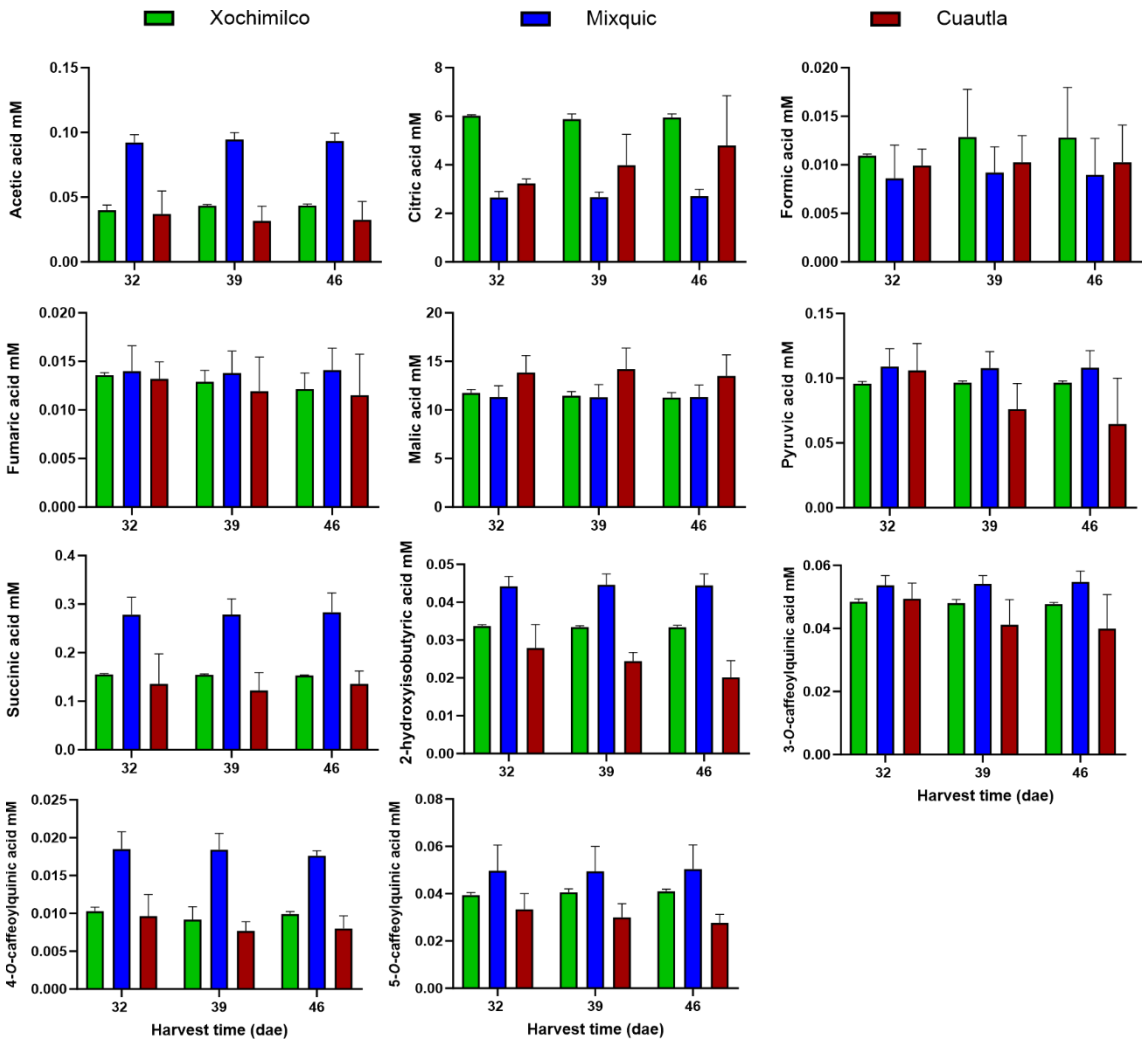

Figura S7. Relative concentrations of organic acids metabolites estimated by the ^{1}H NMR method in the three purslane (*Portulaca oleracea* L.) cultivars at three harvesting times. The data are expressed as the mean ± standard error calculated using seven replicates.

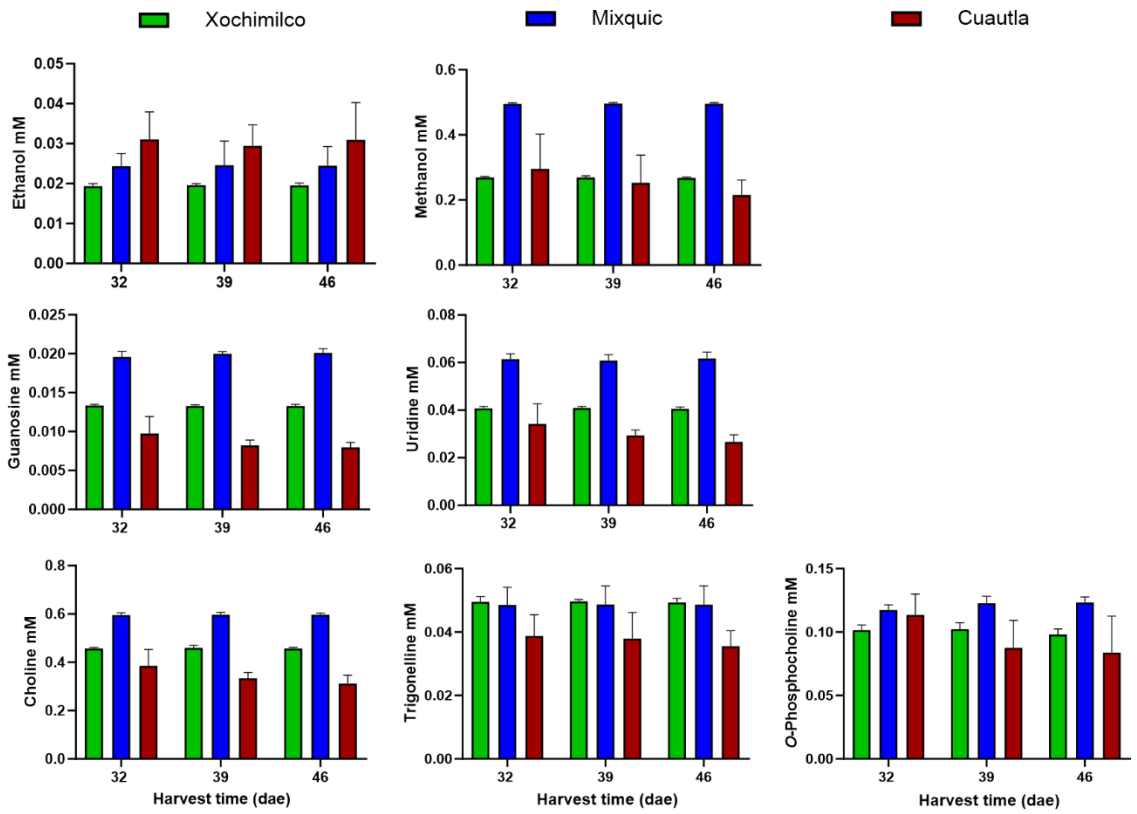

Figura S8. Relative concentrations of alcohols, glycosides, and other compounds estimated by the ^{1}H NMR method in the three purslane (*Portulaca oleracea* L.) cultivars at three harvest times. The data are expressed as the mean ± standard error calculated using seven replicates.

TABLE SUPPLEMENTARY

Table S1. Characteristics of irrigation water and hydroponic nutrient solution applied for purslane (Portulaca oleracea L.) production.

	NO_3^-	$H_2PO_4^-$	SO_4^{-2}	Cl^-	HCO_3	CO_3^-	NH_4^+	Na^+	Ca^{+2}	K^+	Mg^{+2}	pH	EC
	– mEq L^{-1} –												
Water	0.013	0.003	0.107	0.085	1.725	0.300	0	1.098	0.454	0.102	0.520	8.07	0.23
HNS	9	1	9	4	0	0	3	0	9	7	4	5.5	3.1

EC: electrical conductivity; HNS: hydroponic nutrient solution

Table S2. Q^2 and R^2 values with their corresponding CV-ANOVA and obtained from model OPLS-DA for genotypes purslane (*Portulaca oleracea* L.) in three harvesting times.

Harvest (dde)		R^2X	R^2Y	Q^2	PC1 (x)	PC2 (y)	CV-ANOVA
					– – – – – % – – – – –		
1	32	0.750	0.986	0.954	43.2	17.8	7.421E–16
2	39	0.779	0.977	0.949	49.0	15.3	1.261E–14
3	46	0.820	0.990	0.982	50.6	17.2	8.134E–19

CAPÍTULO IV

INFLUENCIA DE LA FERTILIZACIÓN NITROGENADA EN HIDROPONÍA, EN EL PERFIL METABOLÓMICO DE VERDOLAGA BASADO EN RMN

INFLUENCIA DE LA FERTILIZACIÓN NITROGENADA EN HIDROPONÍA EN EL PERFIL METABOLÓMICO DE VERDOLAGA BASADO EN RMN

Montoya-García, César Omar[1*]; García-Mateos, Rosario[1]; Magdaleno-Villar, J. Jesús[1]; Volke-Haller, Víctor Hugo [2]; Becerra-Martínez, Elvia[3*]

[1]Universidad Autónoma Chapingo - Departamento de Fitotecnia. Km. 38.5, Carretera México-Texcoco, 56230, Chapingo, Estado de México, México.

[2]Colegio de Postgraduados - Campus Montecillo. Km. 36.5, Carretera México-Texcoco, Montecillo, Texcoco, 56230, Estado de México, México.

[3]Centro de Nanociencias y Micro y Nanotecnologías, Instituto Politécnico Nacional, Av. Luis Enrique Erro S/N, Unidad Profesional Adolfo López Mateos, Zacatenco, Delegación Gustavo A. Madero, Ciudad de México, 07738, México.

*Autor para correspondencia: Rosario García-Mateos, rosgar08@hotmail.com; Elvia Becerra-Martínez, elmartinezb@ipn.mx

RESUMEN

El estrés abiótico por nutrición puede influir en la concentración del metaboloma de verdolaga (*Portulaca oleracea* L.), este factor afecta la síntesis de los compuestos del metabolismo primario y secundario de las plantas. El objetivo de la presente investigación fue elucidar el metaboloma de verdolaga de tres cultivares nativos, por la aplicación de nitrógeno en condiciones controladas de hidroponía, con la finalidad de obtener información del perfil y las concentraciones óptimas metabolómicas en función del nitrógeno que promuevan, favorezcan o activen la biosíntesis del metaboloma. Se evaluaron tres cultivares nativos de México, y cinco concentraciopnes de nitrógeno en hidroponía (0.5, 4.0, 8.0, 12.0 y 16.0 mM N L^{-1}), en un diseño factorial (3 × 5), en bloques completos al azar, con siete repeticiones. Se identificaron por RMN, 41 metabolitos, incluidos 6 carbohidratos, 15 aminoácidos, 11 ácidos orgánicos, 2 alcoholes, 4 nucleótidos, colina, O-fosfocolina y trigonelina. El análisis de componentes principales (PCA) y el análisis discriminante de mínimos cuadrados parciales (OPLS-DA) permitieron separar los cultivares en tres clusters; (i) 8 y 16 mM N; (ii) 0.5 y 4 mM N; y 12 mM N. Los modelos de regresión individuales para cada metabolito permitieron observar un aumento de concentración de algunos metabolitos en función de la concentración de nitrógeno con excepción del ácido cafeoilquínico. Las concentraciones óptimas metabolómicas se observaron entre la aplicación de 11 y 13 mM N, lo que produjo un mayor rendimiento y altura de la planta. El cultivar que produjo mayor concentración de metabolitos con la menor dosis de nitrógeno fue el cultivar Mixquic, mientras que el cultivar Xochimilco produjo mayor rendimiento a la concentración 12 mM de nitrógeno. Estos resultados indicaron que el estudio metabolómico puede proporcionar indirectamente información sobre las rutas metabólicas más favorecidas en función de los factores abióticos nutrimentales. Con la información generada mediante la regresión se logró identificar la dosis optima metabolómica de nitrógeno para producir la mayor concentración de los metabolitos de interés económico y nutrimental en la verdolaga, contribución única e importante para productores, consumidores e investigadores.

Palabras clave: *Portulaca oleracea* L.; rutas metabólicas; resonancia magnética nuclear (^{1}H-RMN); concentración optima metabolómica.

I. INTRODUCCIÓN

El crecimiento, desarrollo y rendimiento de las plantas, específicamente en el momento de producción depende de diversos factores, principalmente del estrés abiótico, , debido a que diferentes tipos de estrés (temperatura, intensidad lumínica, agua, salinidad, disponibilidad nutrimental) alteran las rutas de síntesis de los metabolitos primarios y secundarios, como respuesta a las necesidades de la planta (Giménez et al., 2021; He et al., 2021; Hnilickova et al., 2021; Montoya-García et al., 2018a).

El nitrógeno (N) es uno de los elementos esenciales que su disponibilidad en el suelo o en los sistemas hidropónicos influye en la síntesis de compuestos del metabolismo primario y secundario principalmente en aquellos que en su estructura contienen N (ácidos nucleicos, clorofila, aminoácidos, proteínas y algunos lípidos) (Chowdhury et al., 2021; Li et al., 2021). Con la finalidad de disminuir los efectos por deficiencias de N en campo e hidroponía, en ocasiones, los agricultores realizan aplicaciones excesivas de fertilizantes nitrogenados de manera empírica, lo que puede causar en las plantas la reducción en la eficiencia del uso de N y en el ambiente efectos perjudiciales (Ghafoor et al., 2021). Al contrario, las deficiencias de N, pueden afectar directa o indirectamente las vías metabólicas de las plantas, influyendo en la concentración de su metaboloma, por lo tanto, las funciones estructurales o bioquímicas (Shi et al., 2019).

En algunas de las especies estudiadas se ha señalado que éstas responden de forma diferente a las diferentes concentraciones de nitrógeno, debido a su variabilidad genética. Al respecto, se ha reportado que la limitación de nitrógeno causa diferencias en el perfil del metaboloma de *Cichorium spinosum* L., con la disminución de la concentración de carbohidratos, y un aumento de la de aminoácidos en función de la fuente de N (NH_4^+ o NO_3^-) (Chatzigianni et al., 2020). En contraste, la dosis intermedia de N (85 g N por planta) en *Lycium barbarum* L. causó incrementos en las concentraciones de algunos amininoácidos (serina, asparagina, fenilalanina y lisina); otros 49 metabolitos fueron afectados por la insuficiencia de N (Shi et al., 2019). En el caso de *Camellia sinensis*, la deficiencia de N se reportó íntimamente ligada a la producción de aminoácidos y ácidos orgánicos; sin embargo, se incrementó la concentración de azucares (Lin et al., 2021). En plántulas de *Isatis indigotica* se observó un aumento proporcional de aminoácidos en función de la dosis de N aplicada,

mientras que la dosis intermedia de N indujo un incremento específicamente de aminoácidos aromáticos como fenilalanina y otros metabolitos (quercetina, indigo e indirubina) (Cao et al., 2020).

Por otra parte, en las últimas décadas un cultivo que ha despertado gran interés en su producción, comercialización y consumo es la verdolaga (*Portulaca oleracea* L.), por su aporte sustancial de minerales, polisacáridos, ácidos grasos, proteínas, alcaloides, terpenoides, esteroles, vitaminas, lignanos, compuestos fenólicos, flavonoides, homoisoflavonoides, homoisoflavones, glucosidos de amida, alcaloides de amida e isoindol, y oleraceínas (Balabanova et al., 2020; Camalle et al., 2020; Farag & Shakour, 2019; Fernández-Poyatos et al., 2021; Nemzer et al., 2020; Zaman et al., 2019; Zaman et al., 2020). Estos compuestos se han identificado en diversos cultivares del mundo; sin embargo, la información sobre los efectos del estrés abiótico por deficiencia de nitrógeno sobre el metaboloma de la verdolaga es limitado; el estudio metabolómico por RMN de la verdolaga en función de diferentes dosis de nitrógeno podría contribuir a su conocimiento (Montoya-García et al., 2023).

Con el uso de la metabolómica por GC–MS, Camalle et al. (2020) observaron los efectos de la aplicación de NaCl en la concentración del metaboloma de verdolaga proveniente de Israel, identificaron que a mayor estrés causado por la alta concentración de NaCl causo un incremento en la concentración de pigmentos, proteínas, ácidos grasos, aminoácidos, ácidos orgánicos y carbohidratos; resultados similares observaron Zaman et al. (2020) en 132 metabolitos en dos cultivares de verdolaga provenientes de EE.UU. y China. En verdolaga proveniente de México, la concentración de compuestos fenólicos y flavonoides se incrementaron a bajas y altas dosis de N en campo (0 y 300 kg N ha^{-1}), mientras que los ácidos grasos poliinsaturados incrementaron en proporción a la dosis de N aplicada (Montoya-García et al., 2018a).

Una comprensión más detallada de los cambios similares o diferentes en los cultivares nativos de verdolaga en función del nitrógeno aplicado, proporcionará amplia comprensión de la respuesta nutrimental e indirectamente información de esta especie sobre las rutas metabólicas favorecidas. Por lo anterior, el objetivo del presente estudio fue elucidar el metaboloma de verdolaga de tres cultivares nativos por la aplicación de nitrógeno en

condiciones controladas de hidroponía, con la finalidad de obtener las concentraciones optimas metabolómicas en función del nitrógeno.

II. MATERIALES Y MÉTODOS

2.1 Químicos empleados

La solución nutritiva hidropónica se elaboró con sales grado reactivo: $Ca(NO_3)_2$; NH_4Cl; KH_2PO_4; $MgSO_4$; KCl; K_2SO_4; $CaSO_4$; $CaCl_2$; $CaCO_3$; $FeSO_4$; EDTA-Mn; $CuSO_4+5H_2O$; $ZnSO_4+7H_2O$; H_3BO_3; $(NH_4)_6Mo_7O_{24}+4H_2O$; y H_2SO_4.

Los disolventes deuterados, el estándar interno Sal sódica de ácido 2,2,3,3-d(4)-3-(trimetilsilil) propiónico (TSP), ácido etilendiaminotetraacético (EDTA), azida de sodio (NaN_3) y fosfato mono-patasico (KH_2PO_4) se adquirieron en Cambridge Isotope Laboratories, Inc. Sigma-Aldrich Co.

2.2 Sitio y condiciones de estudio

El estudio se llevó a cabo en el Estado de México, México (19.491260, -98.872207, 2215 msnm), en condiciones de invernadero. Las temperaturas mínimas y máximas promedio registradas fueron 18 y 30 °C, respectivamente, y la humedad relativa varió entre 50 a 75 %.

2.3 Material vegetal, diseño experimental

Se evaluaron tres cultivares de zonas de alta productividad de México de los estados de Morelos, Estado de México y Ciudad de México, denominados por su zona de producción: Cuautla (V_C), Mixquic (V_M) y Xochimilco (V_X), respectivamente. Se evaluaron cinco niveles de nitrógeno en hidroponía (0.5, 4.0, 8.0, 12.0 y 16.0 mM N L^{-1}), y la cosecha se efectuó a los 46 d después de la emergencia (dde). El diseño tratamientos fue factorial (3 × 5), y el diseño experimental fue en bloques completos al azar, con siete repeticiones.

2.4 Conducción del experimento y cosecha

Se empleó un sistema hidropónico de balsas flotantes, donde cada cultivar, nivel de nitrógeno y su repetición se encontraron aislados; las unidades experimentales consistieron en un contenedor de polietileno de alta densidad de 6 L (medidas externas de 51 × 33 × 13 cm; e internas de 48 × 30.5 × 8 cm) y una bomba de aireación (10 mg L^{-1} de oxígeno disuelto). La siembra se realizó en charolas de germinación de poliestireno de 117 cavidades (22 mL

de volumen por cavidad), con sustrato de vermiculita, con 4 semillas por cavidad, y, éstas se colocaron dentro de los contenedores con agua donde se mantuvieron flotando.

La solución nutritiva hidropónica (NSH) se colocó a los 5 d después de la siembra, para este día la emergencia de las plántulas ya había ocurrido. La NSH se preparó con una relación amonio/nitrato (NH_4^+/NO_3^-) de 25/75, conductividad eléctrica de 2 dS m^{-1} y un valor de pH de 5.5 (Montoya-García et al., 2019). Se empleó agua de pozo (previo análisis químico (Tabla S1)) y sales grado reactivo para elaborar la NSH.

A la cosecha (46 dde), de cada unidad experimental, se recolectó toda la biomasa área de una superficie de 0.03894 m^2, se midió el rendimiento, así como la altura de la biomasa área. Las muestras en fresco se guardaron en ultracongelador (-80°C). Posteriormente, se liofilizaron y se molieron para su análisis por ^{1}H-NMR. Se eligieron siete muestras procesadas (n = 105) por cultivar y nivel de nitrógeno.

2.5 Preparación del extracto acuoso de la biomasa aérea de verdolaga

El polvo liofilizado y finamente molido (50 mg) de cada muestra de verdolaga se colocó en un tubo eppendorf con 1 mL de solución buffer KH_2PO_4 en D_2O (9 mM, pH 6.0), más el estándar interno (TSP; 0.7 mM), NaN_3 (0.2 mM) y EDTA (1 mM). Se aplicó ultrasónido a la muestra por 20 min, posteriormente, se centrifugó a 10 000 rpm por 10 min. Se separó el sobrenadante (600 µL) para el análisis de ^{1}H-RMN.

2.6 Espectroscopia de ^{1}H-RMN

Los espectros de ^{1}H-RMN se obtuvieron con un espectrómetro Bruker de 750 MHz (Bruker Biospin, Rheinstetten, Alemania) equipado con una sonda TXI de 5 mm, se usó secuencia de pulsos NOESYPR1D para suprimir la señal de agua. Los extractos acuosos de la verdolaga se midieron 298.1 ± 0.1 K. Los parámetros de adquisición se establecieron como: número de escan = 128, tamaño del FID = 64 K, ancho espectral = 10.00 ppm, ganancia del receptor = 86.0, tiempo de adquisición = 2.18 s, tiempo de relajación = 10 s, tiempo de mezclado = 100 ms y resolución del FID = 0.45 Hz (Becerra-Martínez et al., 2020).

2.7 Asignación de señales de ^{1}H-RMN

Para identificar (i) hidrógenos acoplados entre sí en el espectro, se utilizó COSY (2D ^{1}H - ^{1}H, espectroscopia de correlación); (ii) correlaciones entre los protones y sus carbonos

vecinos, con HSQC (2D ^{1}H – ^{13}C, coherencia cuántica única heteronuclear); (iii) correlacionar los núcleos ^{1}H y ^{13}C con dos, tres o cuatro enlaces, se usó HMBC (2D ^{1}H - ^{13}C, correlación heteronuclear de enlaces múltiples); y, (iv) para identificar el metaboloma se emplearon las bases de datos de HMDB (http://www.hmdb.ca/) y BMRB (https://bmrb.io/).

2.8 Procesamiento de datos de ^{1}H-RMN

Con el software MestReNova (versión 6.0.2; MestReC, Santiago de Compostela, España) se procesaron los datos de inducción de caída libre (FID) de ^{1}H-RMN. Se corrigió la línea base y la fase, el estándar interno se calibró a 0.0 ppm. Se alinearon las pequeñas variaciones en los desplazamientos químicos dentro de los espectros de ^{1}H-RMN con el uso de un algoritmo de picos. El binning del espectro se dividió en ventanas espaciadas llamadas bins o buckets y se integró el área del pico en cada bin. Se aplicó la normalización para eliminar la variación entre las muestras (Shuzhao, 2014).

2.9 *Cuantificación de los metabolitos*

La cuantificación del metaboloma se realizó siguiendo la metodología propuesta por Villa-Ruano et al. (2019), que considera como principio la intensidad de la señal en el espectro de ^{1}H-RMN proporcional a la concentración molar de los metabolitos mediante una integración de las señales utilizando TSP como patrón interno. Se utilizó la siguiente ecuación para obtener las concentraciones molares del metaboloma:

$$m_T\, M_T \times \frac{I_T}{I_{St}} \times \frac{X_{St}}{X_T} \times C_{St} \times V_{St}$$

donde: m_T: masa del compuesto objetivo [µg] en la solución utilizada para la medición de ^{1}H-RMN; M_T: peso molecular del compuesto objetivo [g/mol]; I_T: valor integral relativo de la señal de ^{1}H-RMN del compuesto objetivo; I_{St}: valor integral relativo de la señal de ^{1}H-RMN del compuesto patrón; X_{St}: número de protones pertenecientes a la señal de ^{1}H-RMN del compuesto estándar; X_T: número de protones pertenecientes a la señal de ^{1}H-RMN del compuesto objetivo. C_{St}: concentración de patrón interno (TSP) en la solución utilizada para la medición de ^{1}H-RMN [mmol/L]; V_{St}: volumen de la solución utilizada para la medición de ^{1}H-RMN [mL].

2.10 Análisis estadístico

Se realizó análisis de componentes principales (PCA) seguido de la proyección ortogonal supervisada para un análisis discriminante de estructuras latentes (OPLS-DA); se empleó la metodología de Blasco et al. (2019) modificada por Becerra-Martínez et al. (2020) que consiste en obtener información e identificar los metabolitos diferenciales entre los diversos grupos de estudio; se utilizó una región T2 de Hotelling (elipse en los gráficos del modelo, con intervalo de confianza de 95%), y la calidad del modelo se definió con base en los valores de R^2X y Q^2. Se consideró el parámetro de importancia de la variable (VIP) como medida de la contribución de las diferentes variables en el modelo. La validación del modelo OPLS-DA se realizó mediante permutaciones (Rådjursöga et al., 2019), con el software SIMCA 14.1 para Windows.

Para determinar el efecto aislado de cada factor de estudio sobre el metaboloma, rendimiento y altura de verdolaga se generaron modelos de regresión en función del nitrógeno aplicado y cultivar que se consideró como variable auxiliar (Xochimilco: $V_X = 0$, $V_M = 0$, $V_C = 0$; Mixquic: $V_X = 0$, $V_M = 1$, $V_C = 0$; Cuautla: $V_X = 0$, $V_M = 0$, $V_C = 1$). Para estimar los modelos se usó el método descrito por Volke (2008), el cual consiste en especificar un modelo inicial con una o pocas variables, a partir de la relación gráfica entre las variables respuesta y los factores de estudio, e incorporar variables al modelo, con base en la relación gráfica entre los residuos y los factores aún no incluidos en el modelo que muestren alguna tendencia de respuesta, hasta obtener un modelo con menor cuadrado medio del error (CME). Los modelos de regresión se obtuvieron con SAS 8.2 para Windows; y las gráficas, en función del nitrógeno aplicado y cultivares se generaron con los valores estimados por los modelos.

III. RESULTADOS Y DISCUSIÓN

3.1 Rendimiento y altura de verdolaga

Las diferencias entre cultivares y concentraciones de N se aprecian en la Figura 1. Los modelos de regresión generados para rendimiento (Cuadro 1) indicaron diferencias entre los cultivares, así como el efecto de las concentraciones aplicadas de N. El cultivar Xochimilco (V_X) presentó mayor incremento de rendimiento (entre 8 y 12 mM N), seguido

de los cultivares Mixquic (V_M) y Cuautla (V_C). El rendimiento máximo fue de 10.2, 10.4 y 9.0 kg m^{-2} con 14, 16 y 12.5 mM N para V_X, V_M y V_C, respectivamente. Se observó un descenso en la producción de biomasa aérea a partir de la dosis de 14.5 y 13.0 mM N para V_X y V_C, respectivamente (Figura 2).

El modelo de regresión de altura (Cuadro 1), indicó que los cultivares V_X y V_M presentaron un comportamiento similar a la aplicación de las dosis crecientes de nitrógeno, seguido de V_C; los tres cultivares presentaron un comportamiento cuadrático, con tendencia de disminución después de 12 mM N (Figura 2). Con base en estos resultados, se estima que con 11.5 mM N se podría obtener la mayor altura en los cultivares V_C (33.2 cm) y, V_X y V_M (36.8 cm).

Determinadas concentraciones de nitrógeno aseguran el crecimiento y desarrollo del cultivo, lo que explica la aplicación necesaria para aumentar el rendimiento de las plantas; sin embargo, el decremento observado a partir de 12.5 mM N en rendimiento y altura se podría atribuir a una posible toxicidad, donde la planta no puede asimilar la dosis excesiva de nitrógeno llegando a un punto crítico. Los rendimientos reportados en otras partes del mundo en cultivares de verdolaga en condiciones hidropónicas fueron inferiores a los obtenidos en el presente estudio; en España, 1.27, 2.44 y 3.84 kg m^2 (Cros et al., 2007; Fontana et al., 2006; Franco et al., 2011); Turquía, 1.68 y 1.52 kg m^2, (Kiliç et al., 2008; Kaymak, 2013); Argentina, Jordán e Irán de 1.56, 3.88 y 3.31 kg m^2, respectivamente (Harris et al., 2013; Alu'datt et al., 2019; Petropoulos et al., 2015); Lebanon, UK e Italia de 1.19, 1.97 y 0.50 kg microgreens m^2, respectivamente (Corrado et al., 2021; Giménez et al., 2021; Puccinelli et al., 2021).

Los resultados reportados por Montoya -García et al. (2017) del cultivar Mixquic, coinciden con los obtenidos en el presente estudio; los autores destacaron la mejor respuesta al N aplicado en campo hasta la tercera fecha de cosecha, donde el tallo principal se bifurcó y se generó la segunda floración (42 dde); además, el cultivo de verdolaga produjo 109 t ha^{-1} de biomasa fresca (10.9 kg m^2) con 0 kg N ha^{-1}, e incrementos hasta de 300 kg N ha^{-1} con 125 t ha^{-1} (12.5 kg m^2), y altura entre 36 y 42 cm/planta. Montoya -García et al. (2017) reportaron que el cultivar nativo de Xochimilco fue susceptible a la una concentración superior de 25 % de amonio en la solución nutritiva con respecto al nitrato; las alturas

máximas se reportaron de 27 cm/planta y producción de biomasa de 45 g/planta; similares a los observados en este estudio.

3.2 Identificación de metabolitos en los extractos acuosos de verdolaga

Los espectros de ^{1}H-RMN, ^{13}C RMN, y 2D RMN obtenidos a 750 MHz para los tres cultivares nativos de verdolaga revelo una huella digital particular, dependiente del origen de la planta y la concentración de nitrógeno en la solución nutritiva hidropónica (NSH) (Figuras S1 y S2). El análisis por ^{1}H-RMN permitió la identificación de 41 metabolitos distribuidos en tres regiones de los espectros: alifática (0.80 – 3.0 ppm), anomérica (3.0–5.5 ppm) y aromática (6.0 – 10.0 ppm) (Cuadro S2). En la región correspondiente entre 0.80 – 3.0 ppm, se detectaron aminoácidos alifáticos (alanina, asparagina, arginina, acido glutámico, glutamina, γ-aminobutírico, isoleucina, leucina, treonina y valina), prolina (no alifático), ácidos orgánicos (acético, cítrico, málico, pirúvico, succínico y 2-hidroxiisobutírico) y etanol (Figura S1 y S2). En la región anomérica, se observaron colina (3.18 ppm), O-fosfocolina (3.21 ppm) y metanol (3.35 ppm); además de seis carbohidratos (fructosa, galactosa, myo-inositol, sacarosa, xilosa, α- y β-glucosa); predominando fructosa (4.01 ppm), α-glucosa (5.22 ppm) y β-glucosa (4.63 ppm). En la región aromática, se identificaron al alcaloide trigonelina (8.88 ppm); aminoácidos aromáticos como la histidina, fenilalanina, triptófano y tirosina; en esta región también se observaron los ácidos: fórmico (8.42 ppm), fumárico (6.55 ppm), y los isómeros 3-O cafeoilquínico (6.47 ppm), 4-O cafeoilquínico (6.53 ppm) y 5-O cafeoilquínico (6.47 ppm); los nucleósidos adenosina (6.06 ppm), citidina (6.03 ppm), guanosina (5.90 ppm) y uridina (7.86 ppm) (Figura S1 y S2).

3.3 Análisis multivariado del contenido metaboloma de verdolaga por el nitrógeno aplicado

Con la finalidad de identificar variaciones en el metaboloma de la biomasa área de verdolaga causado por la aplicación de nitrógeno en NSH y en cada cultivares nativo, se realizaron modelos de OPLS-DA ajustados con cuatro componentes predictivos y un componente ortogonal (Figura 3); éste se validó con la prueba de permutación aleatoria (200 times) para los cultivares Xochimilco (Figura S3A), Mixquic (Figura S3B) y Cuautla (Figura S3C). El modelo de OPLS-DA indico diferencias entre los niveles de nitrógeno en NSH, para los tres cultivares (Figura 3) y se determinaron los metabolitos diferenciales en el gráfico de

distribución del modelo OPLS-DA con base en las variables de importancia en la proyección VIP (> 1) y p (< 0.05); los valores estadísticos para los modelos de OPLS-DA de cada cultivar se observan en la Cuadro S3. En la verdolaga **V_X** se observó una tendencia similar entre 8 y 16 mM N; el metabolito con mayor concentración fue el ácido fórmico (Figura 3B); en la verdolaga **V_M** los metabolitos con mayor concentración fueron xilosa, citidina, etanol y metanol correspondientes a los niveles de 8 y 16 mM N, mientras que en 12 mM N la mayor concentración fue sacarosa, acido pirúvico y adenosina (Figura 3D); y en la verdolaga **V_C** los niveles de 8 y 16 mM N se comportaron similares con mayor concentración de ácido fumárico, sacarosa y adenosina, para la dosis 0.5 mM N se cuantificó una mayor concentración el ácido fórmico (Figura 3F).

Cabe aclarar que cuatro metabolitos fueron dependientes de la dosis de N y el cultivar; (i) la sacarosa, solo se observó en las dosis de 16 mM N para los tres cultivares, y en 12 mM N en V_M; (ii) xilosa, en V_M y V_C con 16 mM N; (iii) adenina, en V_M con 12 y 16 mM N; y V_C con 16 mM N; (iv) citidina, en V_M con 16 mM N.

Con el uso del mapa de calor se observó que en cada cultivar se identificó un perfil y concentración diferente de los metabolitos dependiendo de la concentración de nitrógeno; la verdolaga V_X a la de 16 mM N presentó mayor concentración de metabolitos como sacarosa, asparagina, fenilalalanina, histidina, isoleucina, threonina, tirosina, prolina, guanosina, O-fosfocolina, ácido glutámico; seguidos de 8 mM N, pero el mismo cultivar de la verdolaga producida con 12 mM N mostró mayor concentración de otros metabolitos como: el ácido acético, ácido fumárico y metanol; los ácidos cafeoilquínico (4-O-CQA y 5-OCQA) (Figura 4A).

En la verdolaga V_M se observaron mayores concentraciones del metaboloma con 16 mM N, los metabolitos que destacan son: sacarosa, xilosa, alanina, histidina, tirosina, isoleucina, valina, treonina, fenilalanina y ácido glutámico; con 12 mM N se obtuvieron concentraciones altas en 4-O-CQA, 5-OCQA, adenosina, ácido pirúvico, glutamina, 2-hydroxyisobutirato, acido succínico y O-fosfocolina; en las dosis más bajas de N, se observaron concentraciones elevadas de 3-O-CQA para 0.5 mM N; y, etanol y metanol con 4 mM N (Figura 4B).

La verdolaga V_C tuvo un comportamiento similar a los dos cultivares antes mencionados; las concentraciones del metaboloma se vieron favorecidas con la concentración de 16 mM N, los metabolitos que destacan son fructosa, glucosa, myo-inositol, sacarosa, galactosa, xilosa, alanina, asparagina, ácido aspártico, γ-aminobutírico, ácido glutámico, glutamina, histidina, isoleucina, leucina, fenilalanina, prolina, treonina, triptófano, tirosina, valina; y en 0.5 mM N los ácidos cafeoilquínico (3-O-CQA y 5-OCQA) (Figura 4C).

3.4 Análisis de regresión del contenido metabolómico de los cultivares de verdolaga en función de nitrógeno aplicado

Con la finalidad de estimar las dosis óptimas de nitrógeno para identificar la mayor concentración de metabolitos y su función de respuesta, se desarrollaron modelos de regresión para cada uno de los compuestos identificados por ^{1}H-RMN; a continuación, se describen los modelos obtenidos para cada grupo y su metaboloma.

3.4.1 Carbohidratos

Se determinaron modelos por cada carbohidrato identificados mediante ^{1}H-RMN y por cultivar en función de la concentración de nitrógeno (Cuadro 2). En Figura 5 se observaron las tendencias graficas determinadas mediante regresión y los precursores de las posibles rutas metabólicas pertenecientes a los azúcares. Se observó la presencia de sacarosa en los tres cultivares solo con la aplicación de 16 mM N; este disacárido se hidroliza en glucosa y fructosa, donde la concentración de 13 mM N promovió la mayor concentración de estos metabolitos en V_X y V_M. En galactosa y myo-inositol se obtuvo la mayor concentración con 16 mM de N. Se observó xilosa en los cultivares V_M y V_C con 16 mM de N. En términos generales a partir de 12 mM de N se puede incrementar la concentración de determinados carbohidratos en los tres cultivares.

Los modelos de regresión para fructosa (FRU), indican que la máxima concentración se obtuvo con 13 mM N para V_X (13.8 mM FRU) y V_M (13.5 mM FRU); posterior a 13.5 mM de N la concentración disminuyó; mientras que, en el cultivar V_C, con 16 mM de N se obtuvo la mayor concentración (14.7 mM FRU). Los modelos de regresión de galactosa (GAL) indican que la mayor concentración se obtuvo con 12 mM de N en V_X (0.475 mM GAL); con 16 en V_M (0.864 mM GAL) y V_C (0.621 mM GAL). Los modelos de regresión

para glucosa (GLC), se observó que el cultivar V_M fue superior a V_X; la mayor concentración se obtuvo con 15 mM de bN para V_X (10.51 mM GLC); con 13 mM de N para V_M (11.52 mM GLC); y con 16 mM de N para V_C (13.91 mM GLC). Los modelos de regresión para myo-inositol (Myo) indican que con 16 mM de N se obtuvieron los máximos incrementos, en V_X (0.64 mM Myo); en V_M (0.74 mM Myo); y, en V_C (0.64 mM Myo). (Figura 5; Cuadro 2).

Un suministro adecuado de nitrógeno promueve el desarrollo y crecimiento de los cultivos, además de proporcionar una relación carbono/nitrógeno suficiente para la síntesis de carbohidratos, elementos que funcionan como fuente de energía en el proceso de fotosíntesis. Los azúcares de bajo peso molecular se translocan desde las hojas hasta los sitios de demanda, debido a que representan el principal almacén de energía para la construcción de biopolímeros esenciales, lo cual genera una adecuada división celular y por ende el incremento de biomasa (Fettke and Fernie, 2015; Huang et al., 2018; Hamed et al., 2022). Varios carbohidratos como la sacarosa, rafinosa, maltosa y glucosa se acumulan con frecuencia en las células de las plantas que sufren estrés abiótico, con la finalidad de brindar protección a través del ajuste osmótico del daño oxidativo (Hong et al., 2016).

En condiciones de estrés por deficiencia nutrimental, los azúcares solubles en verdolaga disminuyen, por el contrario, si se combina fertilizante orgánico con inorgánico se elevan la concentración de estos metabolitos (Yang et al., 2020); pero el estrés hídrico en el suelo los azúcares tienden a incrementarse principalmente en plántulas de verdolaga (Saheri et al., 2020). En genotipos de verdolaga proveniente de Irán y Grecia reportaron los azúcares fructosa, glucosa, sacarosa y trealosa (Petropoulos et al., 2015). Petropoulos et al. (2019) determinaron que la concentración de azúcares en tallos y hojas de verdolaga son mayores en cosechas tempranas y tardías, respectivamente; mientras que la concentración de carbohidratos en semillas se incrementa si se procesan por cocción (Petropoulos et al., 2020).

3.4.2 Aminoácidos

Se determinaron modelos por cada aminoácido por cultivar (Cuadro 3). En la Figura 6 se observan las tendencias graficas determinadas mediante regresión y las posibles rutas metabólicas pertenecientes a los aminoácidos, identificados mediante ^{1}H-RMN. Los aminoácidos se agrupan en cuatro familias derivadas de glutamato, piruvato, aspartato y del

ácido shikimico CITA (Taiz et al, 2015). Los aminoácidos esenciales para humanos que se identificaron fueron alanina, asparagina, aspartato, fenilalanina, glutamato, histidina, isoleucina, leucina, lisina, metionina, treonina, triptófano y valina; además de glutamina y prolina, estos últimos se encuentran en concentraciones adecuadas en alimentos de origen animal (Trovato et al., 2021).

Los modelos de regresión para alanina, aspártico, γ-aminobutírico, glutamina, histidina, isoleucina leucina, fenilalanina, prolina, treonina, tirosina y valina indican incrementos de concentración en función de la dosis de nitrógeno aplicada, con máximos incrementos a la dosis de 16 mM. El comportamiento de los cultivares fue diferente en relación a las concentraciones aplicadas de nitrógeno, lo que favoreció la síntesis y concentración de algunos aminoácidos por cultivar; por ejemplo, la concentración de alanina, aspártico, asparagina y glutamina fue mayor en el cultivar V_M; triptófano fue en V_X (Figura 6; Tabla 3).

Después de asimilar y transformar el nitrógeno inorgánico a orgánico por la ruta glutamina sintetasa (GS) y glutamato sintasa (GOGAT), el nitrógeno es redistribuido por las aminotransferasas hacia los aminoácidos más activos (acido glutámico y glutamina). En condiciones de estrés es posible que el N se recicle hacia la síntesis de aminoácidos aromáticos (e.g. fenilalanina) por la ruta del ácido shiquímico, ácido también precursor de metabolitos secundarios (compuestos fenólicos) (Figura 6). Por lo tanto, las concentraciones de glutamato y glutamina para los tres cultivares guardan una relación estrecha en la asimilación y movilización de nitrógeno dependientes del N aplicado. Los niveles de algunos aminoácidos, como glutamato, prolina, γ-aminobutírico, alanina, leucina, isoleucina y valina se incrementaron en función del N aplicado, por lo tanto, la asimilación y fijación del N en estos aminoácidos pueden ser precursores de varias rutas metabólicas que favorecen de forma importante la biosíntesis de lípidos o metabolitos secundarios (alcaloides, compuestos fenólicos, flavonoides y otros metabolitos que contienen nitrógeno) (Taiz et al., 2015). (Figura 6).

Diversos estudios realizados en verdolaga con técnicas metabolómicas han indicado la presencia de diversos aminoácidos (AA), algunos coinciden con los identificados en el presente estudio. Un estudio en Egipto en tres taxa de verdolaga (*P. rausii, P. oleracea* y *P.*

granulatostellulata) tres aminoácidos (prolina, fenilalanina y triptófano); sin embargo, en otra investigación realizada en cultivares de verdolaga de Bulgaria y Grecia reporta seis AA y sus derivados, donde únicamente tirosina fue similar a nuestro estudio (Balabanova et al., 2020). En dos ecotipos de verdolaga de Israel se determinaron 11 AA, donde solamente siete fueron similares (alanina, isoleucina, acido aspártico, leucina, glutamato, fenilalanina y prolina), los últimos tres se incrementaron durante el estudio con la aplicación de amonio con relación en nitrato en la solución nutritiva (Camalle et al., 2020). En verdolaga cultivada y silvestre del Reino Unido, se observaron 17 AA, de los cuales 12 AA fueron similares (ácido aspártico, treonina, ácido glutámico, prolina, alanina, valina, isoleucina, leucina, tirosina, fenilalanina, histidina, metionina) (Nemzer et al., 2020); En EEUU y China se reportaron 26 AA en verdolaga, en este caso coincidieron 14 AA, con excepción de histidina (Zaman et al., 2019).

3.4.3 Ácidos orgánicos

Se determinaron modelos de regresión por cada ácido orgánico por cultivar (Cuadro 4). En la Figura 7 se observan las tendencias graficas determinadas mediante regresión y las posibles rutas metabólicas pertenecientes a los ácidos orgánicos identificados mediante ^{1}H-RMN. Los metabolitos con mayor concentración fueron el ácido málico y el ácido cítrico; el ácido fórmico no cambio su concentración por el nitrógeno aplicado en los tres cultivares evaluados.

Los modelos de regresión para los ácidos fumárico, málico y pirúvico indicaron que no hay diferencia entre los cultivares evaluados, los mayores incrementos se obtuvieron a las concentraciones de 16, 13.5 y 11 mM de N, respectivamente. El modelo de regresión del ácido acético para V_M indicó una respuesta cuadrática con mayor respuesta a 9 mM de N; seguido de V_C y V_X, donde el incremento se observó hasta 16 mM de N. Los modelos para el ácido cítrico señalaron que V_X y V_C su respuesta fue mayor que en el cultivar V_M antes de 3 mM de N; posterior a esta dosis de N la verdolaga V_M se comportó superior a V_X y V_C; después de 12 mM de N las concentraciones disminuyeron en los tres cultivares. El modelo de regresión de ácido succínico reveló que la mayor concentración se obtuvo con 14, 13 y 16 mM de N para V_X, V_M y V_C, respectivamente. El modelo de regresión para acido 2-hidroxiisobutírico (2-HIBA) demostró que la concentración se incrementó por el N aplicado,

donde V_M fue superior en 0.00665 mM 2-HIBA que V_X y V_C. Las concentraciones de los ácidos 3-O-caffeoylquinico y 5-O-caffeoylquinico disminuyeron en función del N aplicado; en ambos ácidos la concentración de V_M fue superior a V_X y V_C. El ácido 4-O-caffeoylquinico se incrementó por el N aplicado donde V_M y V_C fueron superiores en 0.003 y 0.0015 mM que V_X (Figura 7; Cuadro 4).

El C inorgánico como CO_2, durante la fotosíntesis se convierte en C orgánico para la biosíntesis de diversos metabolitos, su concentración es fundamental para la síntesis de aminoácidos, ácidos grasos y proteínas. Como parte de la respiración celular, durante la glicólisis se genera el ácido pirúvico que es trasportado a través de la membrana mitocondrial para llevar a cabo el ciclo TCA, donde se sintetizan los ácidos cítrico, succínico, fumárico y málico (Taiz et al., 2015). Por otro lado, en la síntesis de los compuestos fenólicos participan los precursores la ertitrosa 4-fosfato y nuevamente el fosfoenolpiruvato (PEP) para dar origen al ácido shikímico, por esta ruta se obtienen los aminoácidos aromáticos; pero principalmente en condiciones de estrés se puede disparar la síntesis de compuestos fenólicos, como los isómeros de los ácidos cafeoilquínico (O-CQA); se observaron los tres isómeros de los ácidos cafeoilquínico (O-CQA); en niveles de deficiencia por N, las concentraciones de 3- O-CQA y 5 O-CQA se incrementaron. Se conoce que estos ácidos, en las dietas humanas, tienen efectos biológicos en la circulación sanguínea, propiedades antiobesidad y propiedades antioxidantes (Claude et al., 2021).

Nemzer et al. (2020) indicaron que en verdolaga silvestres y cultivada se observaron diferencias en la concentración de los ácidos orgánicos, en la verdolaga cultivada reportaron mayor concentración de ácido málico, cítrico y oxálico. En genotipos de verdolaga provenientes de Irán y Grecia se identificaron los ácidos: oxálico, málico, cítrico y fumárico (Petropoulos et al., 2015). El ácido orgánico que reportaron Balabanova et al. (2020), Farag & Shakour (2019) y Fernández-Poyatos et al. (2021) fue el ácido cítrico en diversas accesiones de verdolaga. En relación de la proporción nitrato/amonio de 100/0 en la solución nutritiva, se observó un incremento de ácido cítrico, málico y ascórbico en verdolaga producida en hidroponía (Camalle et al., 2020). Zaman et al. (2020) identificaron 35 ácidos orgánicos en cultivares provenientes de China y EE. UU., destacando los ácidos pirúvico, málico y cítrico similares a los que observamos por ^{1}H-NMR; es importante señalar que el

ácido oxálico tiene un efecto negativo en la salud humana, se ha reportado en altas concentraciones en verdolaga en otras partes del mundo (China, EE.UU., UK); sin embargo, en los cultivares estudiados no se observó, lo que podría ser un atributo de calidad prometedor para el consumidor.

3.4.4 Otros metabolitos

Se determinaron modelos de regresión para cada uno de los metabolitos de los tres cultivares (Cuadro 5). En la Figura 8 se observan las tendencias graficas determinadas mediante regresión y las posibles rutas metabólicas, identificados también por ^{1}H-RMN.

Las concentraciones de etanol no cambiaron por la diferentes dosis de nitrógeno aplicado en los tres cultivares; así como el metanol para V_X y V_C. Si bien los ajustes de los modelos de regresión presentaron valores bajos de R^2 (<0.50), las ecuaciones mostraron CV relativamente bajos (< 30) y Pr.F. aceptable (<0.01) (Cuadro 5). Por lo tanto, los R^2 indicaron una ligera respuesta de metanol y de la trigonelina, a los factores estudiados. Los modelos de regresión para guanosina, uridina, colina, trigonelina y O-fosfocolina muestraron una respuesta similar entre los cultivares V_X y V_C; sin embargo, V_M presentó mayor concentración en los metabolitos anteriormente mencionados; los contenidos más altos se obtuvieron con la dosis de 16 mM de N.

IV. CONCLUSIONES

El perfil metabolómico de verdolaga mediante RMN reveló que las concentraciones de metabolitos primarios y secundarios en verdolaga fueron diferentes entre los cultivares nativos, y también por las dosis de nitrógeno en la solución nutritiva aplicada. El análisis estadístico con OPLS-DA mostró clusters pertenecientes a las dosis de nitrógeno en la solución nutritiva para los tres cultivares Xochimilco (V_X), Mixquic (V_M) y Cuautla (V_C), donde se observaron tres grupos con comportamientos similares: (i) 8 y 16 mM de N; (ii) 0.5 y 4 mM de N; y 12 mM de N. Los análisis de regresión mostraron incrementos en todos los metabolitos detectados en función de la concentración de nitrógeno, con excepción del ácido fórmico, etanol y metanol; en términos generales la concentración que promueve la mayor concentración de metabolitos es con 12 mM de N Con la información generada mediante

regresión se puede identificar la dosis optima metabolómica de nitrógeno para producir la mayor concentración de los metabolitos de interés económico y nutrimental de la verdolaga.

REFERENCIAS

Becerra-Martínez, E., Pacheco-Hernández, Y., Lozoya-Gloria, E., Betancourt-Jiménez, M. G., Hidalgo-Martínez, D., Zepeda-Vallejo, L. G., & Villa-Ruano, N. (2020). ^{1}H-NMR metabolomics profiling of recombinant tobacco plants holding a promoter of a sesquiterpene cyclase. *Phytochemical Analysis*, 31 (4), 480-487. https://doi.org/10.1002/pca.2911

Blasco, H., Błaszczyn, J., Billautf, J.C., Nadal-Desbarats, L., Pradat, P.F., Devos, D., Moreau, C., Andres, C.R., Emond, P., Corcia, P., & Słowin, R. (2015). Comparative analysis of targeted metabolomics: Dominance-based rough set approach versus orthogonal partial least square-discriminant analysis. *Journal of Biomedical Informatics*, 53, 291-299. https://doi.org/10.1016/j.jbi.2014.12.001

Camalle, M., Standing, D., Jitan, M., Muhaisen, R., Bader, N., Bsoul, M., et al. (2020). Effect of Salinity and Nitrogen Sources on Leaf Quality, Biomass, and Metabolic Responses of Two Ecotypes of *Portulaca oleracea*. https://www.preprints.org/manuscript/202004.0044/v1

Cao, Y. W., Qu, R. J., Tang, X. Q., Sun, L. Q., Chen, Q. Q., & Miao, Y. J. (2020). UPLC-Triple TOF-MS/MS based metabolomics approach to reveal the influence of nitrogen levels on *Isatis indigotica* seedling leaf. *Scientia Horticulturae*, 266, 109280. https://doi.org/10.1016/j.scienta.2020.109280

Chatzigianni, M., Aliferis, K. A., Ntatsi, G., & Savvas, D. (2020). Effect of N supply level and N source ratio on *Cichorium spinosum* L. metabolism. *Agronomy*, 10(7), 952. https://doi.org/10.3390/agronomy10070952

Chowdhury, N. B., Schroeder, W. L., Sarkar, D., Amiour, N., Quilleré, I., Hirel, B., ... & Saha, R. (2022). Dissecting the metabolic reprogramming of maize root under nitrogen-deficient stress conditions. *Journal of Experimental Botany*, 73(1), 275-291. https://doi.org/10.1093/jxb/erab435

Claude, S. J., Park, S., & Park, S. J. (2021). Transcriptome-Wide Identification and Quantification of Caffeoylquinic Acid Biosynthesis Pathway and Prediction of Its Putative BAHDs Gene Complex in A. spathulifolius. *International journal of molecular sciences*, 22(12), 6333. https://doi.org/10.3390/ijms22126333

Corrado, G., El-Nakhel, C., Graziani, G., Pannico, A., Zarrelli, A., Giannini, P., ... & Rouphael, Y. (2021). Productive and morphometric traits, mineral composition and secondary metabolome components of borage and purslane as underutilized species for microgreens production. *Horticulturae*, 7(8), 211. https://doi.org/10.3390/horticulturae7080211

D'Imperio, M., Durante, M., Gonnella, M., Renna, M., Montesano, F. F., Parente, A., ... & Serio, F. (2022). Enhancing the nutritional value of *Portulaca oleracea* L. by using soilless agronomic biofortification with zinc. *Food Research International*, 155, 111057. https://doi.org/10.1016/j.foodres.2022.111057

Farag, M.A., Shakour, Z.T.A., 2019. Metabolomics driven analysis of 11 *Portulaca* leaf taxa as analysed via UPLC-ESI-MS/MS and chemometrics. *Phychemestry* 161, 117–129. https://doi.org/10.1016/j.phytochem.2019.02.009.

Fernández-Poyatos, M.D.P., Llorent-Martínez, E.J., Ruiz-Medina, A., 2021. Phytochemical composition and antioxidant activity of *Portulaca oleracea*: influence of the steaming cooking process. *Foods* 10 (1), 94. https://doi.org/10.3390/foods10010094.

Fettke, J., & Fernie, A. R. (2015). Intracellular and cell-to-apoplast compartmentation of carbohydrate metabolism. *Trends in Plant Science*, 20(8), 490-497. https://doi.org/10.1016/j.tplants.2015.04.012

Ghafoor, I., Habib-ur-Rahman, M., Ali, M., Afzal, M., Ahmed, W., Gaiser, T., & Ghaffar, A. (2021). Slow-release nitrogen fertilizers enhance growth, yield, NUE in wheat crop and reduce nitrogen losses under an arid environment. *Environmental Science and Pollution Research*, 28(32), 43528-43543. https://link.springer.com/article/10.1007/s11356-021-13700-4

Giménez, A., Martínez-Ballesta, M. D. C., Egea-Gilabert, C., Gómez, P. A., Artés-Hernández, F., Pennisi, G., Orsini, F., Crepaldi A., & Fernández, J. A. (2021). Combined

effect of salinity and LED lights on the yield and quality of purslane (*Portulaca oleracea* L.) microgreens. *Horticulturae*, 7(7), 180. https://doi.org/10.3390/horticulturae7070180

Hameed, M. K., Umar, W., Razzaq, A., Aziz, T., Maqsood, M. A., Wei, S., ... & Chang, L. (2022). Differential metabolic responses of lettuce grown in soil, substrate and hydroponic cultivation systems under NH_4^+/NO_3^- application. *Metabolites*, 12(5), 444. https://doi.org/10.3390/metabo12050444

He, J., You, X., & Qin, L. (2021). High salinity reduces plant growth and photosynthetic performance but enhances certain nutritional quality of C4 halophyte *Portulaca oleracea* L. grown hydroponically under LED lighting. *Frontiers in Plant Science*, 12, 651341. https://doi.org/10.3389/fpls.2021.651341

Hnilickova, H., Kraus, K., Vachova, P., & Hnilicka, F. (2021). Salinity stress affects photosynthesis, malondialdehyde formation, and proline content in *Portulaca oleracea* L. *Plants*, 10(5), 845. https://doi.org/10.3390/plants10050845

Hong, J., Yang, L., Zhang, D., & Shi, J. (2016). Plant metabolomics: an indispensable system biology tool for plant science. *International journal of molecular sciences*, 17(6), 767. https://doi.org/10.3390/ijms17060767

Huang, L., Li, M., Zhou, K., Sun, T., Hu, L., Li, C., & Ma, F. (2018). Uptake and metabolism of ammonium and nitrate in response to drought stress in Malus prunifolia. *Plant Physiology and Biochemistry*, 127, 185-193. https://doi.org/10.1016/j.plaphy.2018.03.031

Li, D., Liu, J., Zong, J., Guo, H., Li, J., Wang, J., Li, L. & Chen, J. (2021). Integration of the metabolome and transcriptome reveals the mechanism of resistance to low nitrogen supply in wild bermudagrass (*Cynodon dactylon* (L.) Pers.) roots. *BMC plant biology*, 21(1), 1-14. https://link.springer.com/article/10.1186/s12870-021-03259-0

Lin, Z. H., Chen, C. S., Zhong, Q. S., Ruan, Q. C., Chen, Z. H., You, X. M., ... & Li, X. L. (2021). The GC-TOF/MS-based Metabolomic analysis reveals altered metabolic profiles in nitrogen-deficient leaves and roots of tea plants (*Camellia sinensis*). *BMC Plant Biology*, 21(1), 1-13. https://bmcplantbiol.biomedcentral.com/articles/10.1186/s12870-021-03285-y

Montoya-García, C. O., García-Mateos, R., Becerra-Martínez, E., Toledo-Aguilar, R., Volke-Haller, V. H., & Magdaleno-Villar, J. J. (2023). Bioactive compounds of purslane (*Portulaca oleracea* L.) according to the production system: A review. *Scientia Horticulturae*, 308, 111584. https://doi.org/10.1016/j.scienta.2022.111584

Montoya-García, C. O., Volke-Haller, V., Santillán-Ángeles, A., López-Escobar, N.F., & Trinidad-Santos, A. (2019). Relación NH_4^+/NO_3^- en la producción de biomasa y el contenido nutrimental de *Portulaca oleracea* L. *Agrociencia* 53: 521-533. https://agrociencia-colpos.mx/index.php/agrociencia/article/view/1825

Montoya-García, C. O., Volke-Haller, V., Trinidad-Santos, A., & Villanueva-Verduzco, C. (2018a). Change in the contents of fatty acids and antioxidant capacity of purslane in relation to fertilization. *Scientia Horticulturae* 234: 152-159. https://doi.org/10.1016/j.scienta.2018.02.043

Montoya-García, C. O., Volke-Haller, V., Trinidad-Santos, A., & Villanueva-Verduzco, C. (2018b). Concentración nutrimental de la verdolaga (*Portulaca oleracea* L.) en respuesta a la fertilización con NPK. *Agrociencia* 52: 241-254. http://www.scielo.org.mx/scielo.php?pid=S1405-31952018000200241&script=sci_abstract

Montoya-García, C.O., Volke-Haller, V., Trinidad-Santos, A., Villanueva-Verduzco, C., & Sánchez-Escudero, J. (2017). Respuesta de la verdolaga (*Portulaca oleracea* L.) a la fertilización con NPK. *Revista de Fitotecnia Mexicana*, 40 (3), 325-332. https://doi.org/10.35196/rfm.2017.3.325-332

Nemzer, B., Al-Taher, F., Abshiru, N., 2020. Phytochemical composition and nutritional value of different plant parts in two cultivated and wild purslane (*Portulaca oleracea* L.) genotypes. *Food Chemistry*. 320, 126621 https://doi.org/10.1016/j.foodchem.2020.126621

Petropoulos, S. A., Fernandes, Â., Arampatzis, D. A., Tsiropoulos, N. G., Petrović, J., Soković, M., ... & Ferreira, I. C. (2020). Seed oil and seed oil byproducts of common purslane (*Portulaca oleracea* L.): A new insight to plant-based sources rich in omega-3 fatty acids. *LWT*, 123, 109099. https://doi.org/10.1016/j.lwt.2020.109099

Petropoulos, S.A., Fernandes, ˆA., Dias, M.I., Vasilakoglou, I.B., Petrotos, K., Barros, L., Ferreira, I.C, 2019. Nutritional value, chemical composition and cytotoxic properties of common purslane (*Portulaca oleracea* L.) in relation to harvesting stage and plant part. *Antioxidants*, 8 (8), 293. https://doi.org/10.3390/antiox8080293.

Petropoulos, S.A., Karkanis, A., Fernandes, ˆA., Barros, L., Ferreira, I.C., Ntatsi, G., Petrotos, K., Lykas, C., Khah, E., 2015. Chemical composition and yield of six genotypes of common purslane (*Portulaca oleracea* L.): an alternative source of omega-3 fatty acids. *Plant Foods for Human Nutrition*, 70 (4), 420–426. https://link.springer.com/article/10.1007/s11130-015-0511-8.

Puccinelli, M., Pezzarossa, B., Pintimalli, L., & Malorgio, F. (2021). Selenium biofortification of three wild species, *Rumex acetosa* L., *Plantago coronopus* L., and *Portulaca oleracea* L., grown as microgreens. *Agronomy*, 11(6), 1155. https://doi.org/10.3390/agronomy11061155

Rådjursöga, M., Lindqvist, H.M., Pedersen, A., Karlsson, G.B., Malmodin, D., Brunius, C., Ellegård, L., & Winkvist. A. (2019). The ^{1}H NMR serum metabolomics response to a two meal challenge: a cross-over dietary intervention study in healthy human volunteers. *Nutrition Journal*, 18 (25), 12-12. https://doi.org/10.1186/s12937-019-0446-2

Shi, Z., Wei, F., Wan, R., Li, Y., Wang, Y., An, W., Qin, K., Dai, G., Cao, Y., & Feng, J. (2019). Impact of nitrogen fertilizer levels on metabolite profiling of the *Lycium barbarum* L. *Fruit. Molecules*, 24(21), 3879. https://doi.org/10.3390/molecules24213879

Li, Shuzhao, ed. Computational methods and data analysis for metabolomics. Totowa, NJ, USA, *Humana Press*, 2020. https://doi.org/10.1007/978-1-0716-0239-3_5

Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015). Plant physiology and development (No. Ed. 6). *Sinauer Associates Incorporated*.

Trovato, M., Funck, D., Forlani, G., Okumoto, S., & Amir, R. (2021). Amino Acids in Plants: Regulation and Functions in Development and Stress Defense. *Frontiers in Plant Science*, 12. https://doi.org/10.3389/fpls.2021.772810

Volke, H.V.H., 2008. Estimación de funciones de respuesta para información de tipo no experimental, mediante regresión. *Colegio de Postgraduados, Montecillo, México State, México*, pp. 113 p.

Zaman, S., Bilal, M., Du, H., & Che, S. (2020). Morphophysiological and comparative metabolic profiling of purslane genotypes (*Portulaca oleracea* L.) under salt stress. *BioMed research international*, 2020. https://doi.org/10.1155/2020/4827045

TABLAS

Tabla 1. Análisis de regresión del rendimiento y altura de tres cultivares de verdolaga (*Portulaca oleracea*) en función de la dosis de nitrógeno en hidroponía.

Factor	Modelos de regresión	R^2	CV	Pr.F.
Rendimiento	$V_X = 0.316 + 1.349\ N - 0.0458\ N^2$	0.884	20.54	0.0001
	$V_M = 0.613 + 1.111\ N - 0.031\ N^2$	0.861	20.52	0.0001
	$V_C = 1.264 + 1.169\ N - 0.044\ N^2$	0.772	24.10	0.0001
Altura	$= 9.953 + 4.623\ N - 0.199\ N^2 - 3.598\ V_C$	0.830	15.92	0.0001

Tabla 2. Análisis de regresión de la concentración de azúcar determinada por ^{1}H-RMN de tres cultivares de verdolaga (*Portulaca oleracea*) en función de la dosis de nitrógeno en hidroponía.

Metabolite	Regression model	R^2	CV	Pr. F
Fructose				
	$V_X = 2.138 + 1.786\ N - 0.068\ N^2$	0.784	24.3	0.0001
	$V_M = 1.426 + 1.788\ N - 0.066\ N^2$	0.842	22.2	0.0001
	$V_C = 2.653\ N^{0.5} + 0.259\ N$	0.979	18.9	0.0001
Galactose				
	$V_X = 0.120 + 0.056\ N - 0.0022\ N^2$	0.673	28.2	0.0001
	$V_M = 0.213\ N^{0.5}$	0.879	39.1	0.0001
	$V_C = 0.205 + 0.026\ N$	0.875	15.4	0.0001
Glucose (α, β isomers)				
	$V_X = 1.338 + 1.210\ N - 0.0399\ N^2$	0.791	26.4	0.0001
	$V_M = 1.731\ N - 0.065\ N^2$	0.956	25.0	0.0001
	$V_C = 1.765 + 0.759\ N$	0.860	25.9	0.0001
Myo-inositol				
	$V_X = 0.400 + 0.015\ N$	0.492	17.9	0.0001
	$V_M = 0.273 + 0.1157\ N^{0.5}$	0.557	23.4	0.0001
	$V_C = 0.343 + 0.0217\ N$	0.728	16.3	0.0001

Tabla 3. Análisis de regresión de la concentración de aminoácidos determinada por ^{1}H-RMN de tres cultivares de verdolaga (*Portulaca oleracea*) en función de la dosis de nitrógeno en hidroponía.

Metabolite	Regression model	R^2	CV	Pr. F
Alanine	$V_X = 0.150\ N - 0.00431\ N^2$	0.955	26.0	0.0001
	$V_M = 0.148 + 0.139\ N - 0.0044\ N^2$	0.845	23.1	0.0001
	$V_C = 0.254 + 0.0621\ N$	0.828	24.3	0.0001
Asparagine	$V_X = 0.027 + 0.018\ N - 0.000363\ N^2$	0.887	21.0	0.0001
	$V_M = 0.035 + 0.034\ N - 0.00123\ N^2$	0.788	27.4	0.0001
	$V_C = 0.048 + 0.014\ N$	0.780	30.8	0.0001
Aspartic acid	$0.162 + 0.27\ N + 0.0614\ V_M + 0.045\ V_C$	0.731	22.6	0.0001
GABA	$V_X = 0.026\ N - 0.00069\ N^2$	0.882	47.1	0.0001
	$V_M = 0.05622\ N^{0.5}$	0.920	32.0	0.0001
	$V_C = 0.0368\ N - 0.0015\ N^2$	0.937	30.1	0.0001
Glutamic acid	$V_X = 0.077\ N$	0.952	26.9	0.0001
	$V_M = 0.141\ N - 0.00423\ N^2$	0.886	39.7	0.0001
	$V_C = 0.1806 + 0.0588\ N$	0.822	238	0.0001
Glutamine	$V_X = 0.204 + 0.1023\ N - 0.00343\ N^2$	0.853	17.0	0.0001
	$V_M = 0.187\ N - 0.00734\ N^2$	0.943	28.1	0.0001
	$V_C = 0.199 + 0.0755\ N - 0.0019\ N^2$	0.800	20.6	0.0001
Histidine	$0.00968 + 0.00398\ N + 0.0038\ V_M$	0.951	13.9	0.0001
Isoleucine	$0.04177 + 0.00731\ N + 0.0079\ V_M$	0.848	19.2	0.0001
Leucine	$0.07 + 0.00947\ N + 0.0296\ V_M$	0.718	22.9	0.0001
Phenylalanine	V_X and $V_C = 0.0334 + 0.00388\ N$	0.838	16.5	0.0001
	$V_M = 0.0274 + 0.0118\ N - 0.00047\ N^2$	0.712	24.0	0.0001
Proline	$0.157 + 0.0127\ N + 0.01802\ N^{0.5}\ V_M$	0.713	21.2	0.0001
Threonine	$0.056 + 0.0061\ N + 0.00403\ N^{0.5}\ V_M$	0.820	18.0	0.0001
Tryptophan	$V_X = 0.02651 + 0.000338\ N$	0.115	19.6	0.09
	V_M and $V_C = 0.02166 + 0.00517\ N^{0.5}$	0.378	23.7	0.0001
Tyrosine	$V_X = 0.0228 + 0.00481\ N$	0.905	16.0	0.0001
	$V_M = 0.0197 + 0.0225\ N^{0.5}$	0.747	22.2	0.0001

	$V_C = 0.02146 + 0.0192\ N^{0.5}$	0.839	15.6	0.0001
Valine	V_X and $V_C = 0.06 + 0.0091\ N$	0.899	14.3	0.0001
	$V_M = 0.0326 + 0.043\ N^{0.5}$	0.816	20.0	0.0001

Tabla 4. Análisis de regresión de la concentración de ácidos orgánicos determinada por ^{1}H-RMN de tres cultivares de verdolaga (*Portulaca oleracea*) en función de la dosis de nitrógeno en hidroponía.

Metabolite	Regression model	R^2	CV	Pr. F
Acetic acid	$V_X = 0.036 + 0.0157\ N^{0.5}$	0.423	32.3	0.0001
	$V_M = 0.0436 + 0.032\ N - 0.00173\ N^2$	0.785	20.8	0.0001
	$V_C = 0.071 + 0.0025\ N$	0.335	23.5	0.0016
Citric acid	$V_X = 1.106 + 0.737\ N - 0.0288\ N^2$	0.777	21.0	0.0001
	$V_M = 1.0575\ N - 0.046\ N^2$	0.929	31.1	0.0001
	$V_C = 1.116 + 0.656\ N - 0.0289\ N^2$	0.695	25.0	0.0001
Formic acid	$V_X = 0.013$; $V_M = 0.022$; $V_C = 0.020$	–	–	–
Fumaric acid	$0.0103 + 0.0037\ N^{0.5}$	0.327	33.9	0.0001
Malic acid	$3.585 + 1.464\ N - 0.055\ N^2$	0.773	18.3	0.0001
Pyruvic acid	$0.0257 + 0.00544\ N - 0.000236\ N^2$	0.300	35.5	0.0001
Succinic acid	$V_X = 0.031\ N - 0.00106\ N^2$	0.906	37.7	0.0001
	$V_M = 0.0836 + 0.0258\ N - 0.000953\ N^2$	0.685	21.7	0.0001
	$V_C = 0.0118 + 0.00568\ N$	0.481	22.1	0.0001
2-Hydroxyisobutyric acid	$0.00422\ N - 0.000143\ N^2 + 0.00665\ V_M$	0.940	28.2	0.0001
Phenolic acid				
3-O-caffeoylquinic acid	$0.0577 - 0.00368\ N + 0.00014\ N^2 + 0.00442\ V_C$	0.483	20.0	0.0001
4-O-caffeoylquinic acid	$0.0058 + 0.000738\ N - 0.0000358\ N^2 + 0.003\ V_M + 0.0015\ V_C$	0.311	26.5	0.0001
5-O-caffeoylquinic acid	$0.0376 - 0.00253\ N^{0.5} + 0.0117\ V_M + 0.00753\ V_C$	0.282	24.3	0.0001

Tabla 5. Análisis de regresión de la concentración de diversos compuestos determinados por ^{1}H-RMN de tres cultivares de verdolaga (*Portulaca oleracea*) en función de la dosis de nitrógeno en hidroponía.

Metabolite	Regression model	R^2	CV	Pr. F
Alcohols				
Ethanol	$V_X = 0.050$; $V_M = 0.048$; $V_C = 0.030$	–	–	–
Methanol	$V_X = 0.498$; $V_C = 0.547$	–	–	–
	$V_M = 0.393 + 0.1614\ N - 0.0091\ N^2$	0.477	34.0	0.0003
Nucleosides				
Guanosine	$0.00579 + 0.000577\ N + 0.00595\ V_M$	0.723	21.1	0.0001
Uridine	V_X and $V_C = 0.0155 + 0.00212\ N$	0.674	25.6	0.0001
	$V_M = 0.0135 + 0.00853\ N - 0.000354\ N^2$	0.795	19.1	0.0001
Other compounds				
Choline	V_X and $V_C = 0.236 + 0.0185\ N$	0.721	16.8	0.0001
	$V_M = 0.188 + 0.0628\ N - 0.0024\ N^2$	0.831	14.5	0.0001
O-Phosphocholine	V_X and $V_C = 0.0407 + 0.0042\ N$	0.718	19.9	0.0001
	$V_M = 0.0395 + 0.00541\ N$	0.730	22.7	0.0001
Alkaloids				
Trigonelline	V_X and $V_C = 0.0352 + 0.00141\ N$	0.390	21.3	0.0001
	$V_M = 0.03 + 0.00612\ N^{0.5}$	0.488	16.1	0.0001

FIGURAS

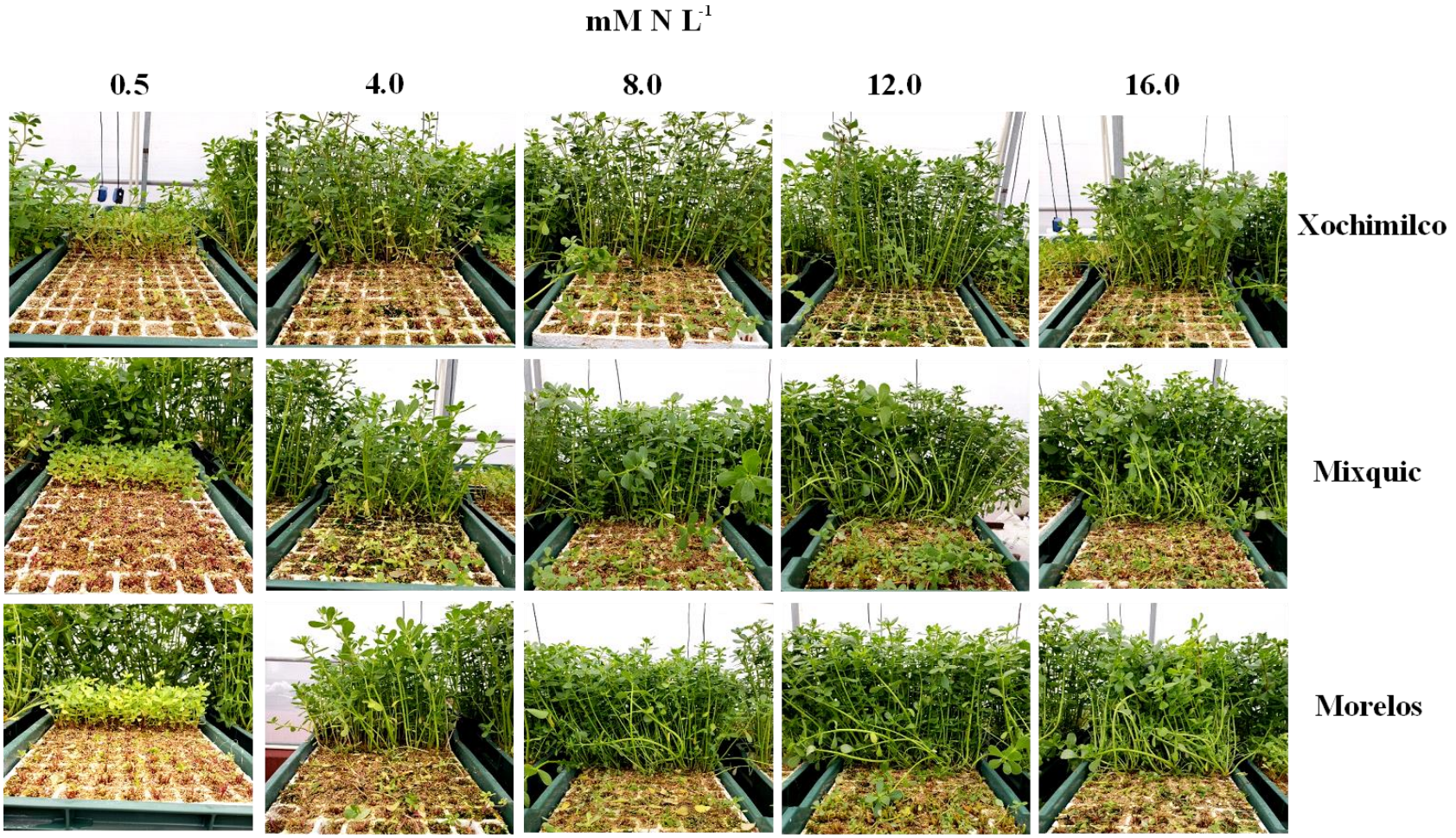

Figura 1. Comparación del rendimiento morfológico de tres cultivares de verdolaga (*Portulaca oleracea*) en función de la dosis de nitrógeno en hidroponía.

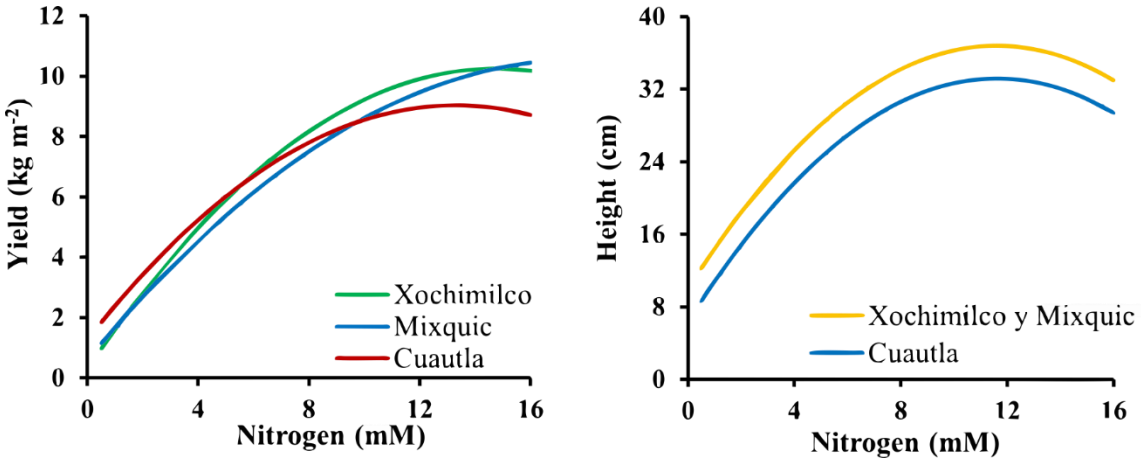

Figura 2. Rendimiento y altura de tres cultivares de verdolaga (*Portulaca oleracea*) en función de la dosis de nitrógeno en hidroponía. Las tendencias de los metabolitos se generaron con modelos de regresión.

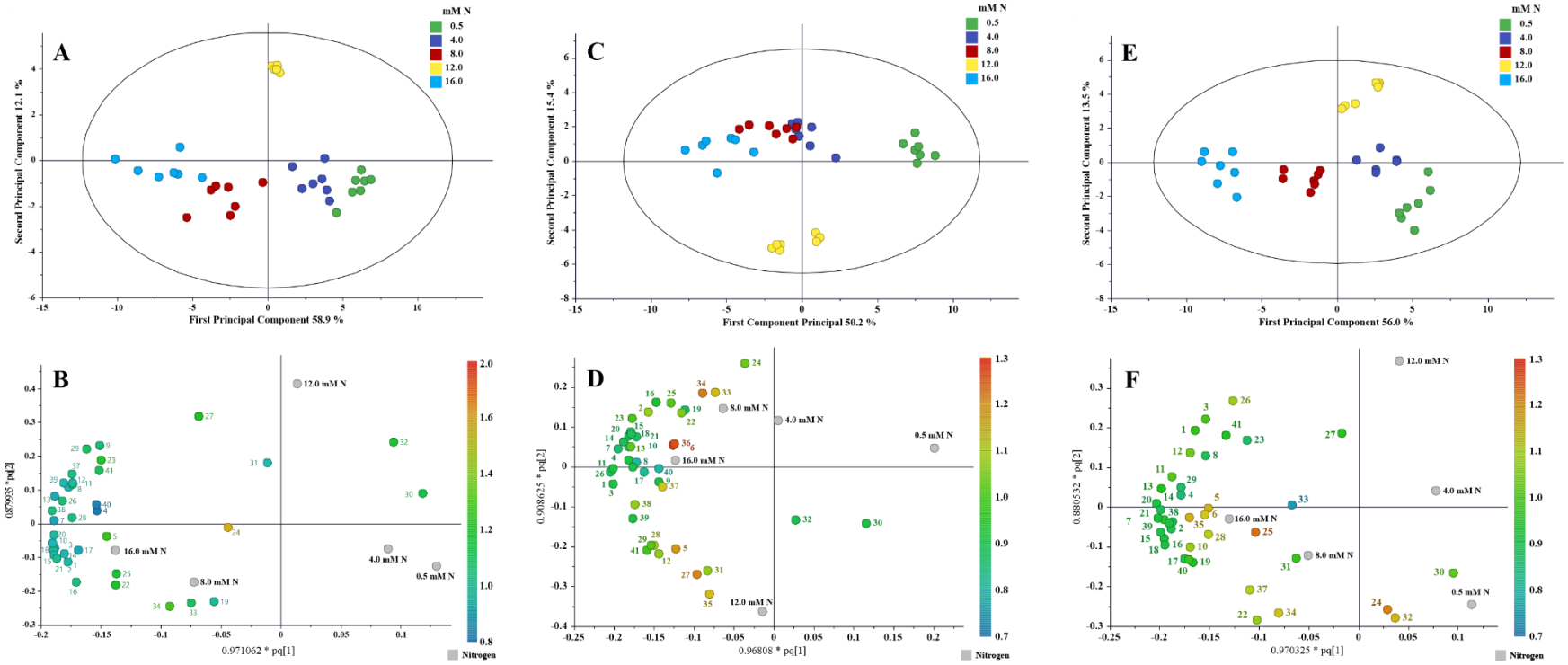

Figura 3. Gráficas de puntuación OPLS-DA generadas a partir de los espectros ^{1}H-RMN (750 MHz) de tres cultivares de verdolaga (*Portulaca oleracea*) en función de la dosis de nitrógeno en hidroponía: cultivar Xochimilco (A y B), Mixquic (C y D), y Cuautla (E y F). La elipse en A, C y E representa el Hotelling con 95% de confianza. Los gráficos de carga correspondientes que identifican los metabolitos discriminantes entre cultivares y dosis de N. Cada círculo representa un metabolito, y su color muestra la importancia de las variaciones de metabolitos entre grupos (metabolitos diferenciales). 1. Fructosa; 2. Galactosa; 3. Glucosa (isómeros α, β); 4. Mio-inositol; 5. Sacarosa; 6. Xilosa; 7. Alanina; 8. Asparagina; 9. Ácido aspártico; 10. γ-aminobutírico; 11. Ácido glutámico; 12. Glutamina; 13. Histidina; 14. Isoleucina; 15. Leucina; 16. Fenilalanina; 17. Prolina; 18. Treonina; 19. Triptófano; 20. Tirosina; 21. Valina; 22. Ácido acético; 23. Ácido cítrico; 24. Ácido fórmico; 25. Ácido fumárico; 26. Ácido málico; 27. Ácido pirúvico; 28. Ácido succínico; 29. Ácido 2-hidroxiisobutírico; 30. Ácido 3-O- cafeoilquínico; 31. Ácido

4-O- cafeoilquínico; 32. Ácido 5-O- cafeoilquínico; 33. Etanol; 34. Metanol; 35. Adenosina; 36. Citidina;37. Guanosina; 38. Uridina; 39. Colina; 40. Trigonelina; y 41. O-fosfocolina.

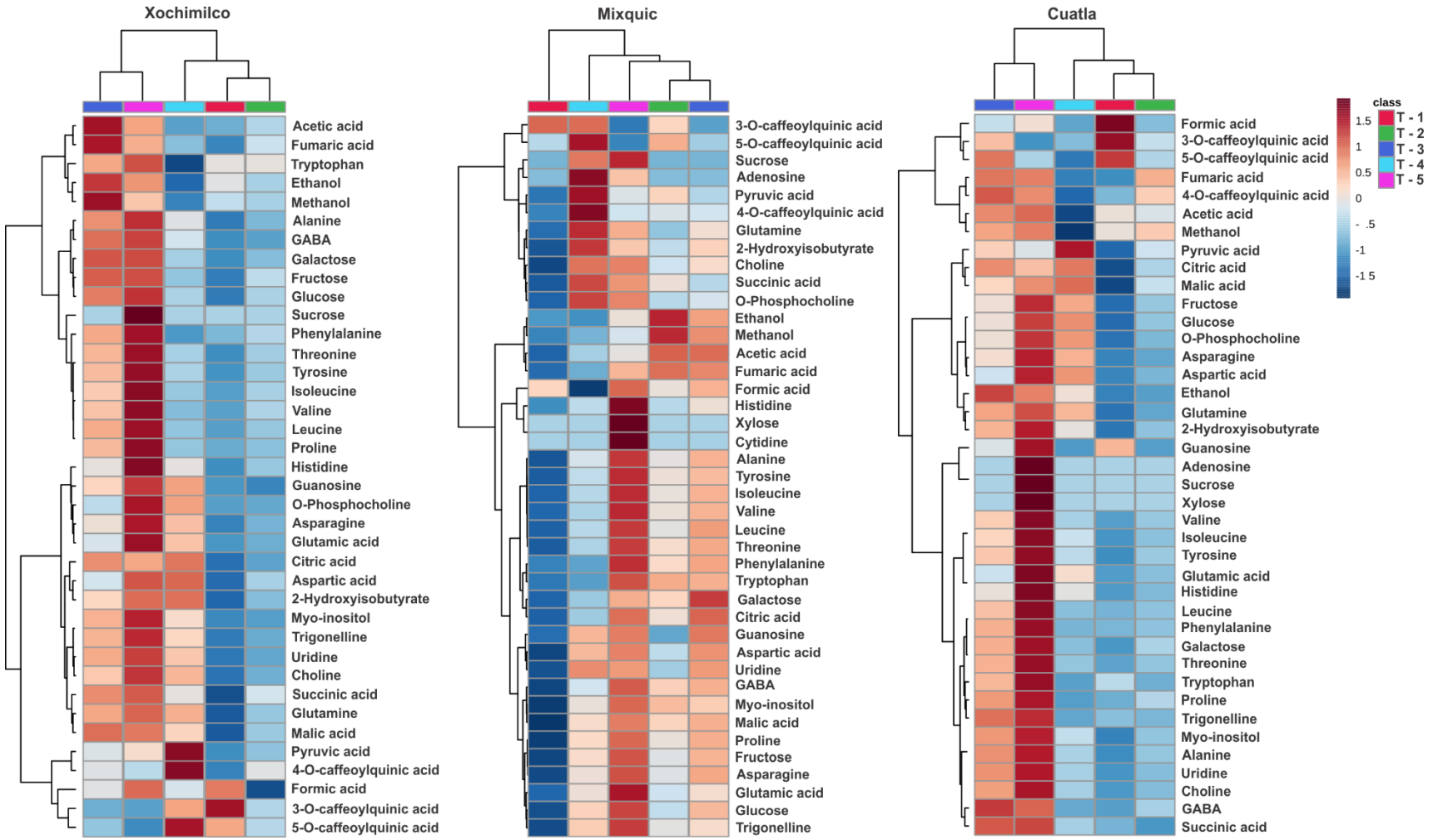

Figura 4. Mapa de calor del perfil metabolómico de tres cultivares de verdolaga (*Portulaca oleracea*) en función de la dosis de nitrógeno en hidroponía. El color azul representa una tendencia descendente y el rojo una tendencia ascendente. T-1: 0,5; T-2: 4,0; T-3: 8,0; T-4: 12,0; T-5: 16,0 mM N L^{-1}.

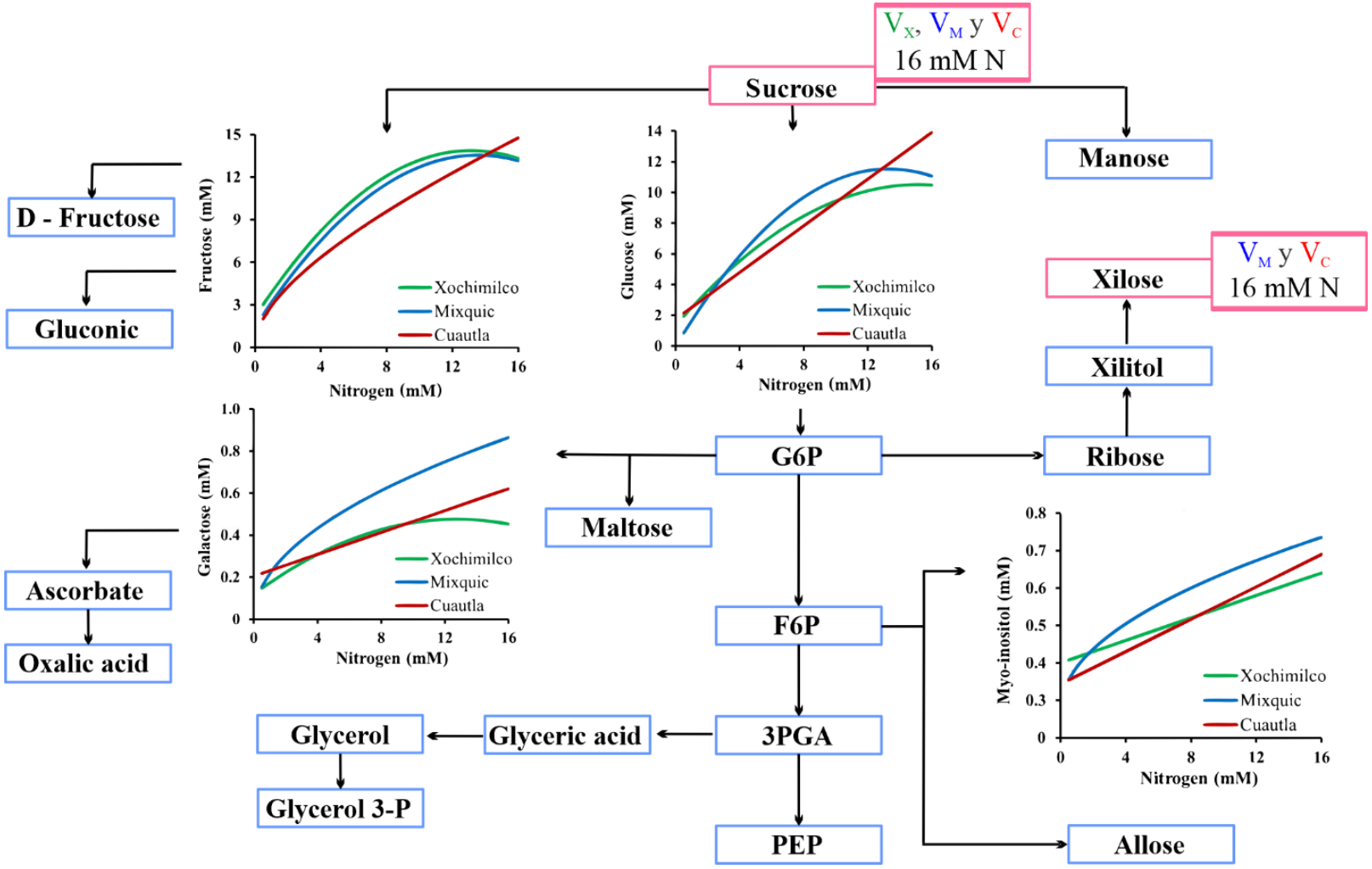

Figura 5. Principales reservas y distribución de carbono en el proceso de glucólisis. Concentración de carbohidratos determinada por ^{1}H-RMN de tres cultivares de verdolaga (*Portulaca oleracea*) en función de la dosis de nitrógeno en hidroponía. Las tendencias de los metabolitos se generaron con modelos de regresión. Los recuadros en rosa indican que los metabolitos se encontraron con la dosis de 16 mM de N en determinados cultivares de verdolaga.

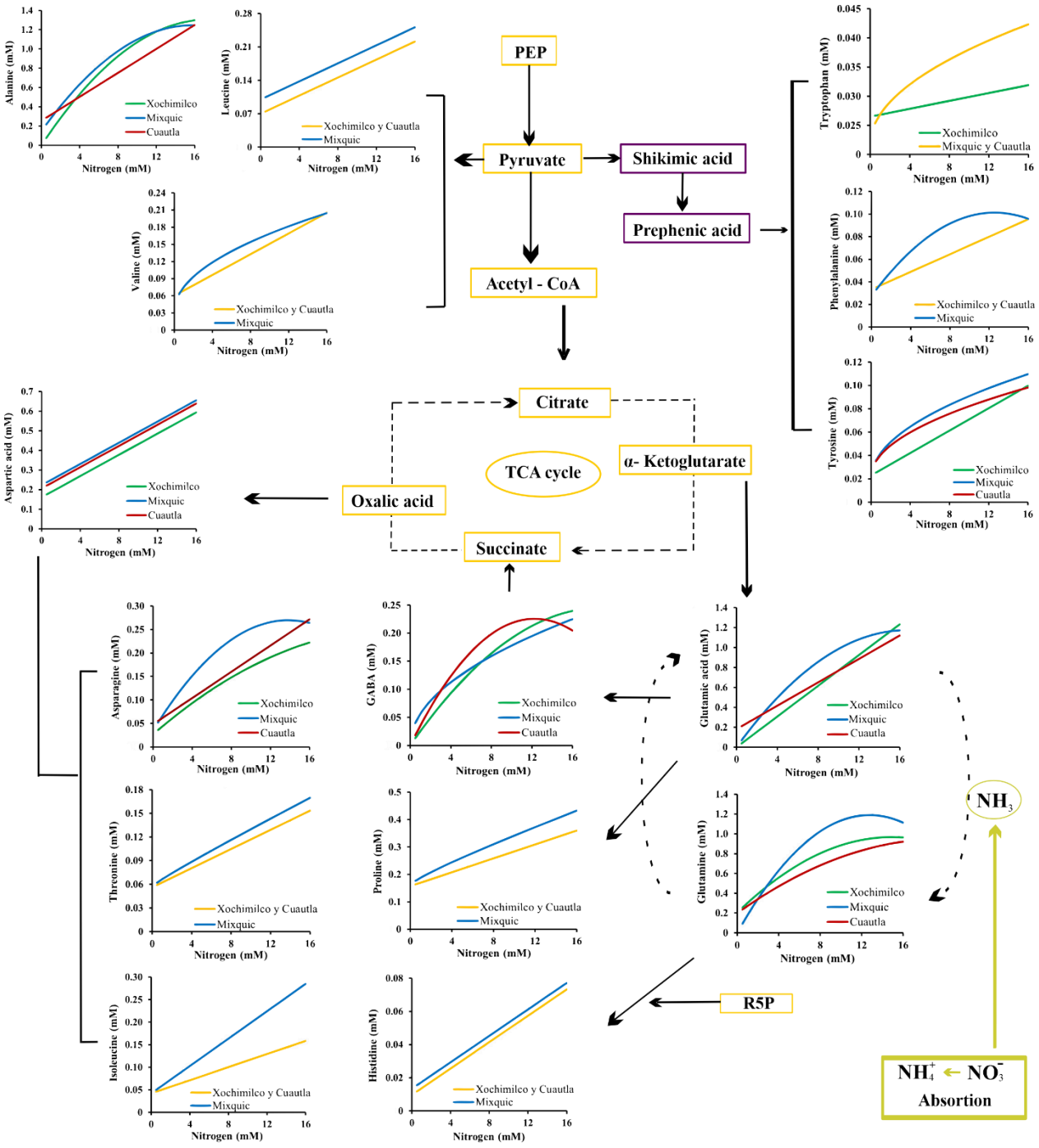

Figura 6. Principal asimilación de nitrógeno relacionada con la vía del metabolismo de aminoácidos. Metaboloma determinado por ^{1}H-RMN de tres cultivares de verdolaga (*Portulaca oleracea*) en función de la dosis de nitrógeno en hidroponía. Las tendencias de los metabolitos se generaron con modelos de regresión.

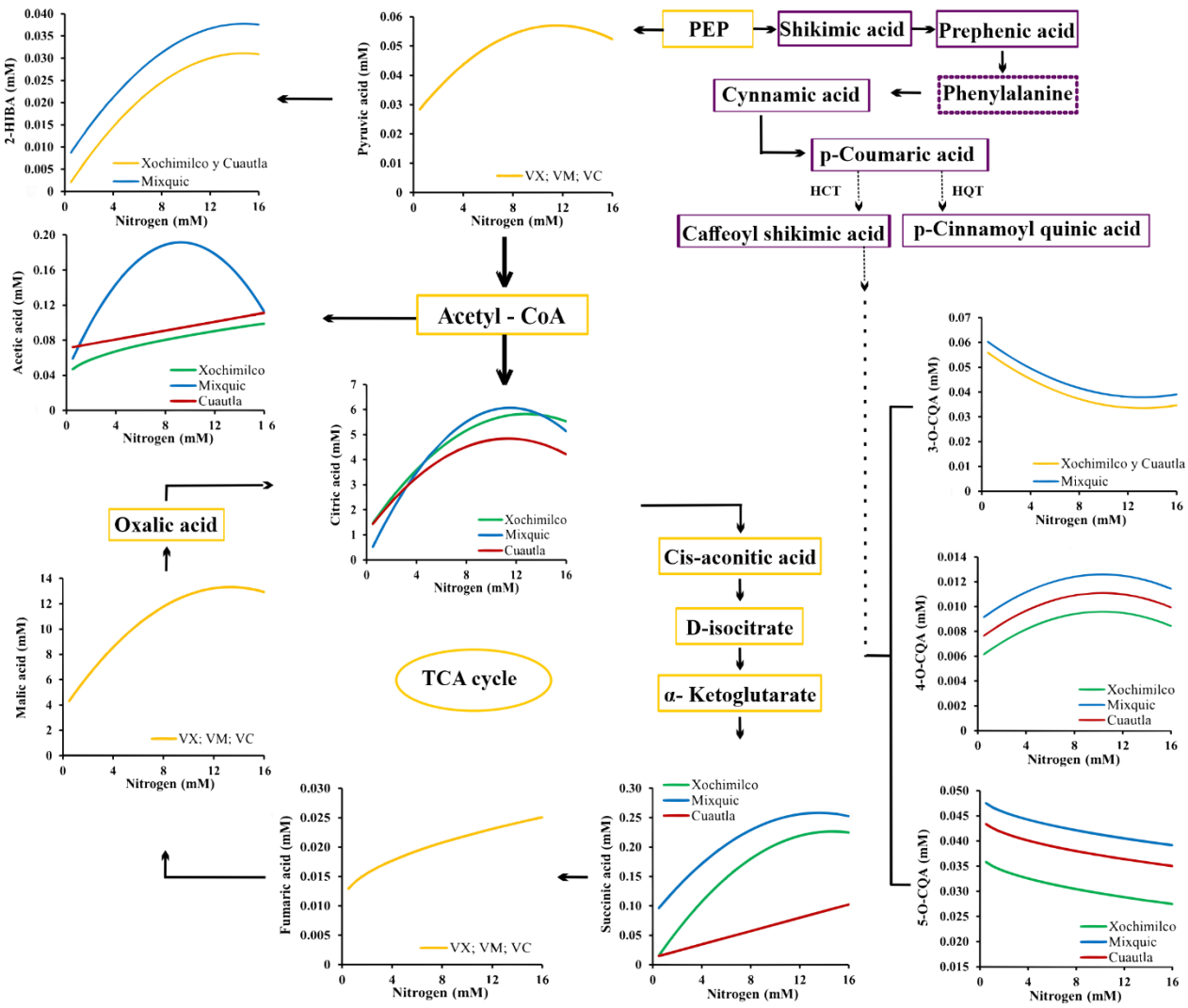

Figura 7. Concentración de ácidos orgánicos y vía metabólica, determinada por ^{1}H-RMN de tres cultivares de verdolaga (*Portulaca oleracea*) en función de la dosis de nitrógeno en hidroponía. Las tendencias de los metabolitos se generaron con modelos de regresión. 2-HIBA: ácido 2-hidroxiisobutírico; O-CQA: O- cafeoilquínico; HCT: hidroxicinamoil CoA shikimato hidroxicinamoil-transferasa; HQT: hidroxicinamoil CoA quinato hidroxicinamoil-transferasa.

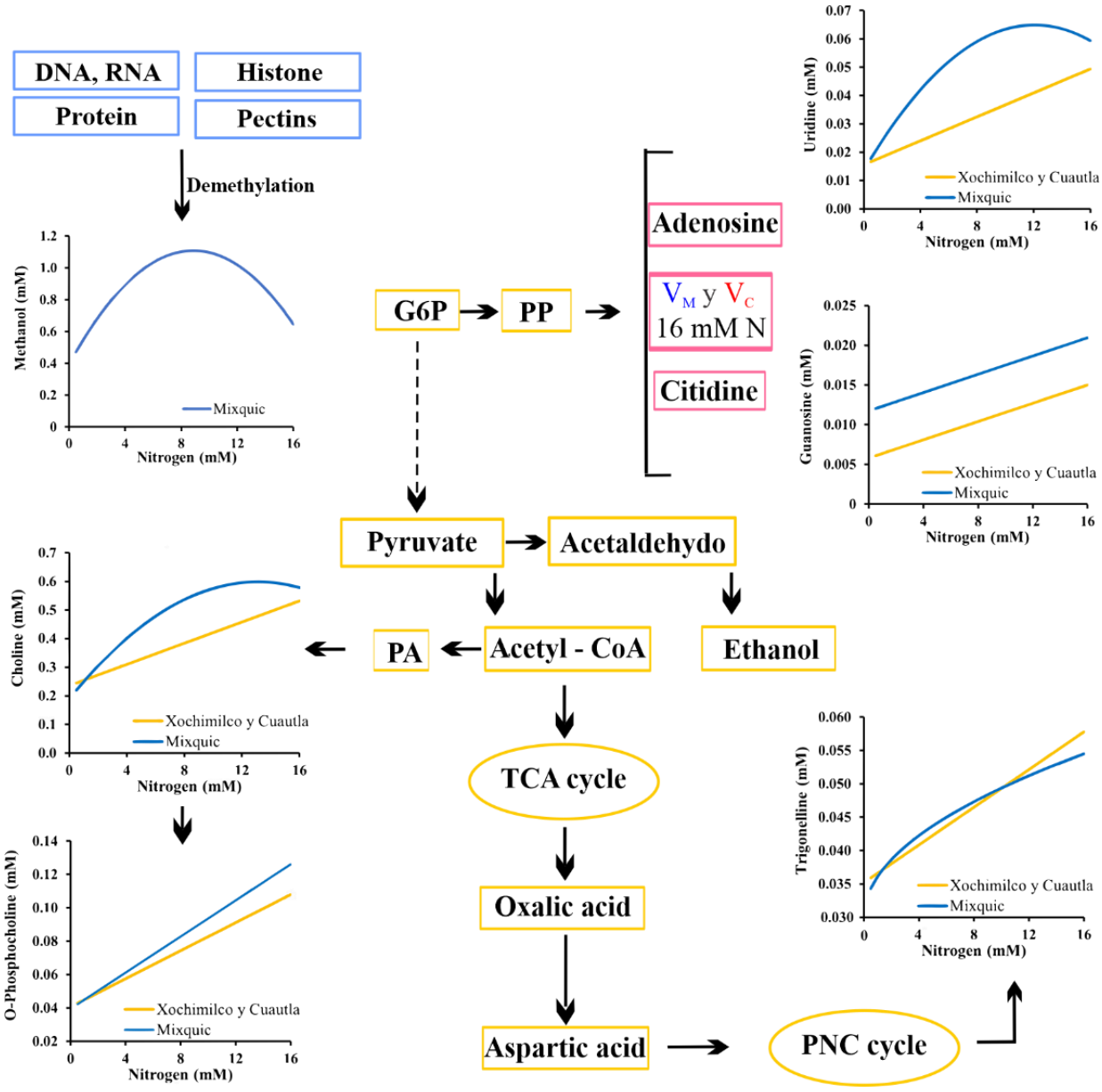

Figura 8. Ruta del metabolismo de alcoholes, nucleósidos, trigonelina, colina y O-fosfocolina, determinada por ^{1}H-RMN de tres cultivares de verdolaga (*Portulaca oleracea* L.) en función de la dosis de nitrógeno en hidroponía. Las tendencias de los metabolitos se generaron con modelos de regresión. PP: vía de las pentosas fosfato; PA: ácido fosfatídico; PNC: ciclo de los nucleótidos de piridina. Los recuadros en rosa indican que los metabolitos se encontraron con la dosis de 16 mM de N en determinados cultivares de verdolaga.

TABLAS SUPLEMENTARIAS

Tabla S1. Características del agua de riego y de la solución nutritiva hidropónica aplicadas en la producción de verdolaga (*Portulaca oleracea*).

N	NO_3^-	$H_2PO_4^-$	SO_4^{-2}	Cl^-	HCO_3	CO_3^-	NH_4^+	Na^+	Ca^{+2}	K^+	Mg^{+2}	pH	EC
					mM L^{-1}								
Water	0.013	0.003	0.0535	0.085	1.725	0.3	0	1	0.227	0.102	0.26	8.07	0.23
0.5	0.375	1	6.375	4	2	0	0.125	0	4.5	7	2	5.5	2.7
4.0	3	1	5.5	4	2	0	1	0	4.5	7	2	5.5	2.8
8.0	6	1	4.5	4	2	0	2	0	4.5	7	2	5.5	2.9
12.0	9	1	4.5	4	0	0	3	0	4.5	7	2	5.5	3.1
16.0	12	1	3.5	4	0	0	4	0	4.5	7	2	5.5	3.2

EC: electrical conductivity

Tabla S2. Características espectrales de ^{1}H-RMN utilizadas para identificar el metaboloma en cultivares de verdolaga (*Portulaca oleracea*) en función de la dosis de nitrógeno en condiciones de hidroponía.

	Metabolite	Chemical shifts (ppm), J (Hz), multiplicity
Sugars		
1	Fructose	3.99 (m) CH-5, 4.01 (dd, *J* = 12.7, 1.3 Hz) CH_2-11
2	Galactose	4.55 (d, *J* = 7.9 Hz)
3	Glucose (α, β isomers)	5.22 (d, *J* = 3.7 Hz) CH-2, 4.63 (d, *J* = 7.9 Hz) CH-2
4	Myo-inositol	3.27 (t, *J* = 9.4 Hz) CH-2
5	Sucrose	4.20 (d, *J* = 8.8 Hz) CH-3, 5.40 (d, *J*= 3.8 Hz) CH-7
6	Xylose	4.57 (d, J = 7.9 Hz)
Amino acids		
7	Alanine	1.47 (d, *J* = 7.2 Hz) CH_3-6
8	Asparagine	2.85 (dd, *J* = 16.9, 7.8 Hz) CH_2-6, 2.94 (dd, *J* = 16.9, 4.2 Hz) CH_2-6
9	Aspartic acid	2.70 (dd, *J* = 17.5, 7.4 Hz) CH_2-6, 2.83 (dd, *J* = 17.5, 3.7 Hz) CH_2-6
10	GABA	1.89 (m) CH_2-5, 2.28 (t, *J* = 7.4 Hz) CH_2-4
11	Glutamic acid	2.14 (m) CH_2-6, 2.45 (m) CH_2-7
12	Glutamine	2.12 (m) CH_2-6, 2.44 (m) CH_2-7
13	Histidine	8.61 (m) CH-2, 7.36 (m) CH-5
14	Isoleucine	0.93 (t, *J* = 7.4 Hz) CH_3-8, 1.00 (d, *J* = 7.0 Hz) CH3-9
15	Leucine	0.94 (d, *J* = 6.2 Hz) CH_3-8, 0.95 (d, *J* = 6.2 Hz) CH_3-9
16	Phenylalanine	7.31 (d, *J* = 7.5) CH_3, 5, 7.36 (m) CH-4, 7.42 (t, *J* = 7.5 Hz) CH-2, 6
17	Proline	2.01 (m) CH2-6, 2.3 (m) CH_2-4
18	Threonine	1.32 (d, *J* = 6.6 Hz) CH_3-8
19	Tryptophan	7.52 (d, *J* = 8.0 Hz) CH-6, 7.72 (d, *J* = 8.0 Hz) CH-7
20	Tyrosine	7.18 (d, *J* = 7.18 Hz) CH-3, 5, 6.88 (d, *J* = 7.18 Hz) CH-2, 6
21	Valine	0.98 (d, *J* = 7.0 Hz) CH_3-8, 1.03 (d, *J* = 7.0 Hz) CH_3-7
Organic acids		

22	Acetic acid	1.95 (s) CH_3-1
23	Citric acid	2.67 (d, *J* = 15.6 Hz) CH_2-2, 5, 2.78 (d, *J* = 15.6 Hz) CH_2-2, 5
24	Formic acid	8.42 (s) CH-2
25	Fumaric acid	6.55 (s) CH-4, 5
26	Malic acid	2.53 (dd, *J* = 15.5, 9.9 Hz) CH_2-5, 2.76 (dd, J = 15.5, 3.2 Hz) CH_2-5
27	Pyruvic acid	2.36 (s) CH_3–1
28	Succinic acid	2.62 (s) CH_3–1
29	2-Hydroxyisobutyric acid	1.33 (s) CH_3–1
30	3-O-caffeoylquinic acid	6.47 (d, *J* = 15.9 Hz) H-8´
31	4-O-caffeoylquinic acid	6.53 (d, *J* = 15.9 Hz) H-8´
32	5-O-caffeoylquinic acid	6.50 (d, *J* = 15.9 Hz) H-8´
Alcohols		
33	Ethanol	1.17 (t, *J* = 7.1 Hz), 3.64 (c, *J* = 7.1 Hz)
34	Methanol	3.35 (s) CH_3-1
Nucleosides		
35	Adenosine	6.06 (d, *J* = 6.2 Hz) CH-3, 8.24 (s) CH-2, 8.35 (s) CH-1
36	Cytidine	6.03 (d, J=7.50 Hz), 7.86 (d, J=7.5 Hz)
37	Guanosine	5.90 (d, *J* = 5.7 Hz) CH-3, 7.99 (s) CH-2
38	Uridine	7.86 (d, *J* = 8.1 Hz) CH-1
Other compounds		
39	Choline	3.18 (s) CH_3-5, 6, 7
40	Trigonelline	8.88 (m) CH-4, 9.13 (s) CH-2
41	O-Phosphocholine	3.21 (s) CH_3-5, 6, 7

s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet

The numbering of the molecules was taken from the HMDB database (http://www.hmdb.ca).

Tabla S3. Valores Q2 y R2 con sus correspondientes CV-ANOVA obtenidos del modelo OPLS-DA para el cultivar verdolaga (*Portulaca oleracea*) en función del nitrógeno aplicado.

Cultivar	R^2X	R^2Y	Q^2	PC1 (x)	PC2 (y)	CV-ANOVA
				––––– % –––––		
Xochimilco	0.900	0.890	0.763	58.9	12.1	9.235E–15
Mixquic	0.902	0.890	0.810	50.2	15.4	1.676E–14
Cuautla	0.859	0.846	0.749	56.0	13.5	8.134E–19

FIGURAS SUPLEMENTARIAS

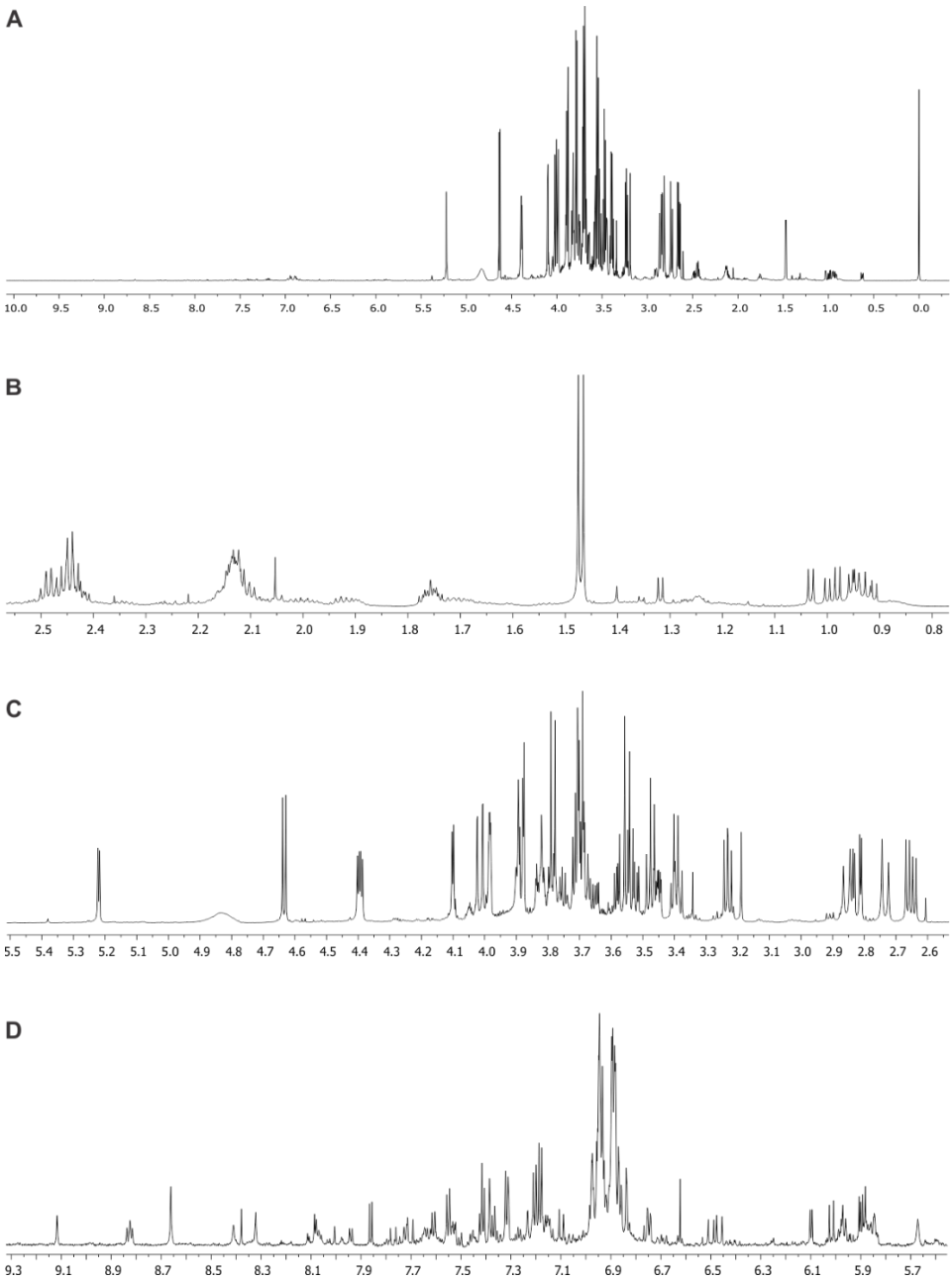

Figura S1. Espectro ^{1}H-RMN obtenido a 750 MHz de los extractos acuosos de la biomasa de tres cultivares de verdolaga (*Portulaca oleracea*) en función de la dosis de nitrógeno en hidroponía. (A) Espectro ^{1}H-NMR completo de 0.0 a 10.0 ppm; (B) Espectro ^{1}H-NMR expandido de 0.8 a 3.0 ppm; (C) Espectro ^{1}H-NMR expandido de 3.2 a 5.5 ppm; (D) Espectro ^{1}H-RMN expandido de 5.8 a 9.6 ppm.

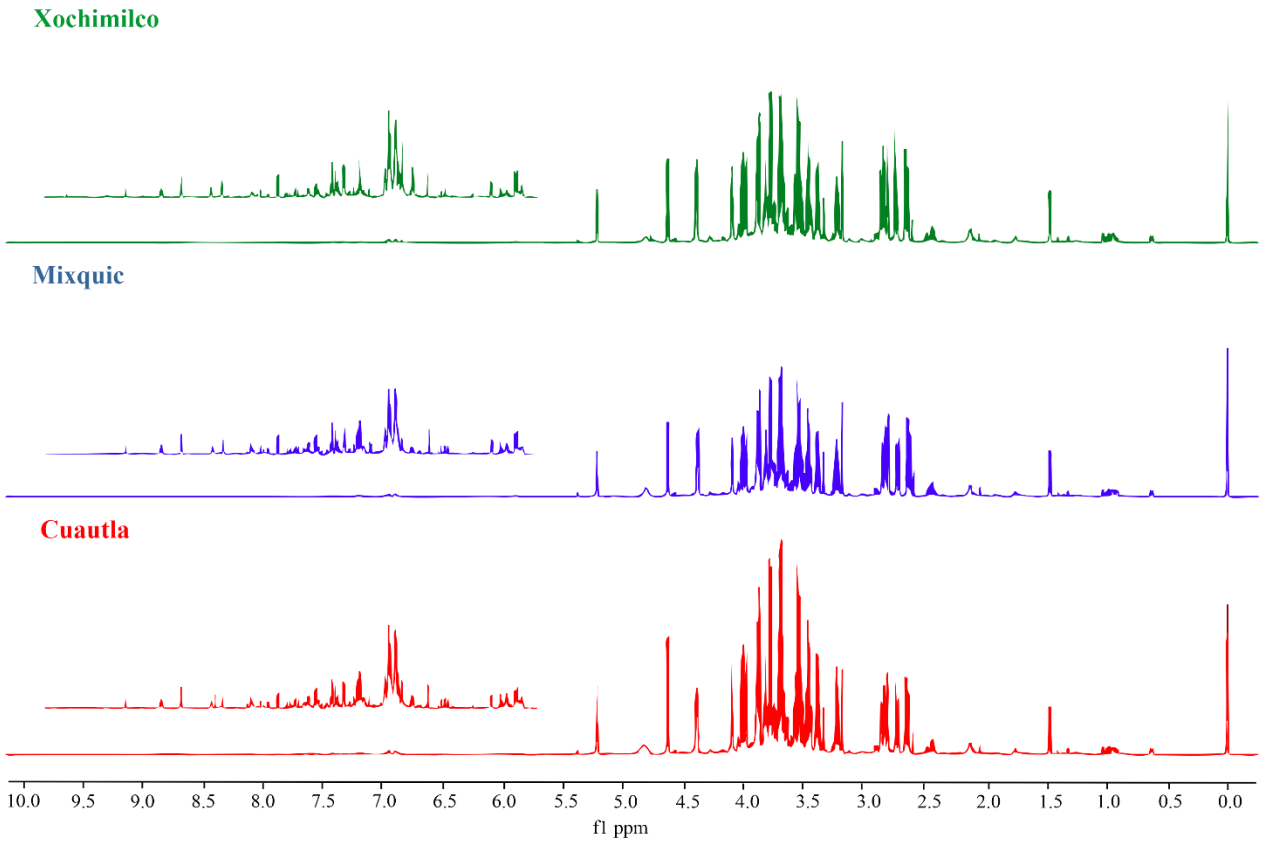

Figura S2. Espectros ^{1}H-RMN de 750 MHz representativos de tres cultivares de verdolaga (*Portulaca oleracea* L.) en función de la dosis de nitrógeno en hidroponía. Se utilizó la solución buffer de D_2O: CD_3OD KH_2PO_4 a pH 6.0 (70:30, v/v) en el intervalo de δ -0.5 a 10.0 ppm, y las expansiones para el rango de δ 6.5 a 9.0 ppm. Cultivares (A) Xochimilco, (B) Mixquic, y (C) Cuautla. Los espectros obtenidos a 750 MHz se escalaron de acuerdo con el TSP utilizado como patrón interno y el desplazamiento químico ^{1}H (ppm).

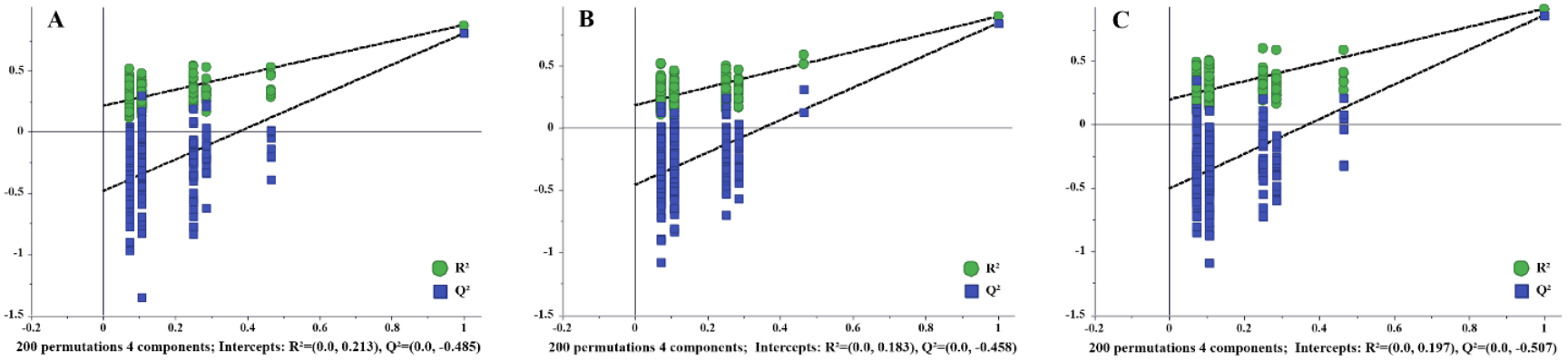

Figura S3. Análisis general de permutación de tres cultivares: (A) Xochimilco, (B) Mixquic, y (C) Cuautla de verdolaga (*Portulaca oleracea*) en función de la dosis de nitrógeno en hidroponía).

CONCLUSIONES GENERALES

(i) Con base a la revisión bibliográfica del estado del arte de la verdolaga, se concluye que las técnicas metabolómicas basadas en NMR han contribuido a dilucidar el metaboloma de la verdolaga en condiciones específicas de crecimiento (estrés, hidropónico, campo abierto y controlado), con reportes de nuevos compuestos de los grupos de los lignanos, flavonoides, homoisoflavonoides, homoisoflavonas, glucósidos amídicos, alcaloides, alcaloides amídicos e isoindólicos y alcaloides fenólicos como las oleraceínas.

(ii) La determinación del perfil de metabolitos mediante RMN reveló que los niveles de metabolitos primarios en verdolaga fueron significativamente diferentes según el cultivar y el momento de cosecha. Se identificaron 37 metabolitos en los cultivares de Xochimilco y Cuautla, y 39 metabolitos en cultivar de Mixquic; incluidos 5 azúcares, 15 aminoácidos, 7 ácidos orgánicos, 3 ácido cafeoilquínico, 2 alcoholes, y 3 nucleósidos, entre otros compuestos. Por el momento de cosecha, se observaron incrementos en la concentración de metabolitos en la segunda y tercera cosecha, los compuestos con mayor concentración fueron glucosa, fructosa, galactosa, piruvato, colina y 2-hidroxisobutirato. El análisis de OPLS-DA mostró clusters claramente separados para los tres cultivares evaluados, siendo Mixquic el cultivar que mostró mayor concentración de compuestos entre ellos aminoácidos y carbohidratos; Xochimilco, obtuvo mayor contenido de citrato en las tres cosechas; y Cuautla, tuvo concentraciones superiores de glucosa y fructosa en la tercera cosecha. El momento de cosecha influyó directamente en el rendimiento de verdolaga, a los 32 dde, el cultivar Cuautla fue superior; a los 39 dde, destaco el cultivar Xochimilco; mientras que a los 46 dde, el cultivar Mixquic alcanzo el máximo rendimiento

(iii) El perfil metabolómico de verdolaga mediante RMN reveló que las concentraciones de metabolitos primarios y secundarios en verdolaga fueron diferentes entre los cultivares nativos, y también por las dosis de nitrógeno en la solución nutritiva aplicada. Se observaron 41 metabolitos, incluidos 6 carbohidratos, 15 aminoácidos, 11 ácidos orgánicos, 2 alcoholes, 4 nucleótidos, colina, O-fosfocolina y trigonelina. Los análisis de regresión mostraron incrementos en todos los metabolitos detectados en función de la concentración de nitrógeno, con excepción del ácido fórmico, etanol y metanol; en términos generales después de 12 mM se observaron incrementos decrecientes por el nitrógeno aplicado. El mayor contenido de metabolitos correspondió a fructosa,

glucosa, alanina, acido glutámico, glutamina, ácido málico y ácido cítrico. El cultivar que produce mayor concentración de metabolitos con la menor dosis de nitrógeno es el cultivar V_M, seguido V_X y V_C. El rendimiento de biomasa fresca estuvo influenciado por las dosis crecientes de nitrógeno; para V_X presentó mayor incremento de rendimiento entre 8 y 12 mM N, seguido de V_M y V_C; con la dosis de 16 mM la verdolaga V_M fue superior a los cultivares restantes.

Con la información puede contribuir a seleccionar el cultivar y el momento de cosecha óptima para potencializar el contenido nutricional de la verdolaga, además de identificar la dosis optima metabolómica de nitrógeno para producir la mayor concentración de los metabolitos de interés económico y nutrimental de la verdolaga. Esta información contribuye en promover su cultivo y la aceptación por los productores, consumidores e investigadores.