Universidad de Concepción
Dirección de Postgrado
Facultad de Agronomía
Programa de Doctorado en Ciencias de la Agronomía

Estrategias combinadas de elicitación y modelos predictivos en el estudio de la acumulación de fitoquímicos y propiedades antioxidantes en brotes de especies *Raphanus* crecidas a altas temperaturas

Combined strategies of elicitation and predictive models to study phytochemical accumulation and antioxidant properties in sprouts of *Raphanus* species grown under high temperatures

Tesis para optar al grado de Doctora en Ciencias de la Agronomía

MARÍA TRINIDAD TORO ROJAS

CHILLÁN-CHILE

2023

Profesora Guía: María Dolores López Belchí

Dpto. de Producción Vegetal, Facultad de Agronomía

Esta tesis ha sido realizada en el Departamento de Producción Vegetal de la Facultad de Agronomía, Universidad de Concepción

Profesora Guía — Dra. María Dolores López Belchí
Facultad de Agronomía
Universidad de Concepción

Profesor Co-Guía — Dr. José Becerra Allende
Facultad de Ciencias Naturales y Oceanográficas Universidad de Concepción

Comisión Evaluadora: — Dr. Nelson Zapata San Martín
Facultad de Agronomía
Universidad de Concepción

Dr. Jaime Ortíz Viedma
Facultad de Ciencias Químicas y Farmacéuticas
Universidad de Chile

Directora de Programa (s): — Dra. Marisol Vargas Concha
Facultad de Agronomía
Universidad de Concepción

DEDICATORIA

A mis hijos Phillip y Annette, quienes llegaron a este mundo durante este gran desafío. Son mi luz, mi fortaleza, mi fuerza e inspiración.

A mi esposo Roberto, por su apoyo incondicional y constante motivación, gracias por tu paciencia e inmenso amor.

A mis padres César y Ana María y mi hermano Andrés, gracias por guiarme e iluminar mi camino, todo lo que soy hoy es gracias a ustedes.

A todos mis viejitos que me cuidan desde el cielo.

A Dios, por darme la vida y las capacidades para realizarme profesionalmente, por darme la familia que tengo, por el amor y felicidad que me han dado.

AGRADECIMIENTOS

A la Dra. María Dolores López Belchí por su apoyo, guía y gestión para el desarrollo del presente trabajo. Gracias por ayudarme a ser una mejor profesional.

A los catedráticos del Doctorado en Ciencias de la Agronomía por compartir sus conocimientos y experiencias que complementaron mi formación dentro del Programa.

Al personal técnico de los Laboratorios de Análisis Químico de la Universidad de Concepción Campus Chillán y Concepción.

A los Dres. Jaime Ortiz, José Becerra y Nelson Zapata por su colaboración en la supervisión, edición y corrección del trabajo de Tesis.

Finalmente, esta investigación no hubiese sido posible sin el apoyo financiero de la Agencia Nacional de Investigación y Desarrollo, ANID, del gobierno de Chile. A través de la beca de DOCTORADO NACIONAL número ID 21181296 y del proyecto FONDECYT REGULAR 1201950.

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RESUMEN

Los brotes o germinados de plantas comestibles son una fuente de alimento que presenta un alto contenido de compuestos bioactivos en sus tejidos, que además pueden ser potenciados a través de mecanismos de elicitación. Evaluar los avances de las investigaciones relacionadas con esta temática se vuelven cruciales para identificar las tendencias actuales que apuntan a mantener una dieta rica en compuestos bioactivos o fitoquímicos provenientes de los germinados. Las técnicas para potenciar estos compuestos endógenos de las plantas durante el período de crecimiento nos traen nuevos desarrollos productivos e innovadores a nivel agronómico y comercial.

Existe un enorme aumento en la producción de publicaciones académicas, generando gran cantidad de información que puede resultar compleja para los lectores. El análisis de la información científica emergente y las tendencias pasadas conduce a la necesidad de abordar esta vasta información a través de métodos matemáticos y estadísticos, incluido el análisis bibliométrico. Así, el capítulo II brinda información sobre las características básicas de la literatura y evalúa el progreso y desarrollo de la investigación científica de la temática de interés. Primero se realizó una breve aproximación teórica que entrega información sobre la definición de germinados, formas de identificarlos, tiempos de cultivo y algunos ejemplos de diferentes elicitores y sus efectos en la composición de compuestos bioactivos. Además, este enfoque fue seguido por una sección de estadísticas descriptivas y finalmente se realizó el análisis bibliométrico. En éste último se identificaron algunos indicadores bibliométricos considerados de gran importancia en la evaluación de la investigación científica, como el análisis de productividad de países, autores, documentos y revistas, índices de colaboración y análisis de co-ocurrencia de palabras clave.

Debido a que no existen trabajos previos realizados con especies *Raphanus* (familia *Brassicaceae*) respecto a la respuesta de estrés abiótico como son las altas temperaturas, que pueden inducir o ser perjudiciales para la síntesis de compuestos bioactivos, y a la vez, combinando esta fuente de estrés con elicitores exógenos,

se realizó el trabajo presentado en el capítulo III. Este estudio utilizó como materia prima brotes de rábano silvestre y comestible de 7 días de edad, que fueron sometidos a un aumento de temperatura de crecimiento (30°C) combinado con la aplicación exógena de diferentes elicitores (MeJa, ácido cítrico, K_2SO_4 y quitosano) para activar los mecanismos de defensa. Se evaluaron rasgos de desarrollo, daño oxidativo, contenido de GSL y antocianinas, y capacidad antioxidante acompañado del desarrollo de un modelo predictivo. La acumulación de bioactivos fue altamente promovida por la aplicación de dos elicitores, K_2SO_4 y MeJa.

Ambas especies fueron susceptibles a la aplicación de elicitores más que a la alta temperatura. El análisis de correlación y el análisis de componentes principales (PCA) confirmaron que en términos de acumulación de GSL, el rábano comestible mejoró la síntesis de estos compuestos con K_2SO_4 y el elicitor MeJa, mientras que el aumento de temperatura de 10 °C no fueron tan decisivos. En relación a la acumulación de antocianos, el rábano comestible, a pesar de aumentar su contenido tras la aplicación de MeJa, no fue capaz de tolerar el aumento de temperatura y estos compuestos se redujeron días antes de la cosecha, a diferencia del rábano silvestre, que aumentó significativamente la síntesis de estos metabolitos secundarios con esta combinación. Además, el elicitor de MeJa combinado con alta temperatura incrementó el estrés oxidativo a través del contenido de MDA, situación generalmente asociada al mayor contenido de compuestos fenólicos y al aumento de la capacidad antioxidante (DPPH y ORAC).

Para finalizar, se desarrolló una red neuronal artificial para predecir el comportamiento del aumento de temperatura combinado con MeJa. La red generó una correlación entre los valores ajustados y observados de más del 90%, con alta precisión de predicción, permitiéndonos predecir el comportamiento de los brotes bajo estas condiciones. Por lo tanto, se demuestra que los elicitores en combinación con el análisis predictivo representan una herramienta eficaz para mejorar el valor nutricional de los brotes de especies de *Raphanus* en condiciones futuras de aumento de temperatura.

ABSTRACT

Sprouts of edible plants are a food source that has a high content of bioactive compounds in their tissues, which can also be enhanced through elicitation mechanisms. Evaluating the advances in research related to this topic becomes crucial to identify current trends that aim to maintain a diet rich in bioactive or phytochemical compounds from sprouts. The techniques to enhance these endogenous compounds of plants during the growth period bring us new productive and innovative developments at an agronomic and commercial level.

There is a huge increase in the production of academic publications, generating a large amount of information that can be complex for readers. The analysis of emerging scientific information and past trends leads to the need to address this vast information through mathematical and statistical methods, including bibliometric analysis. Thus, chapter II provides information on the basic characteristics of the literature and evaluates the progress and development of scientific research on the topic of interest. First, a brief theoretical approach was made that provides information on the definition of sprouts, ways to identify them, culture times and some examples of different elicitors and their effects on the composition of bioactive compounds. Furthermore, this approach was followed by a section on descriptive statistics and finally the bibliometric analysis was performed. In the latter, some bibliometric indicators considered of great importance in the evaluation of scientific research were identified, such as the analysis of productivity of countries, authors, documents and journals, collaboration indexes and analysis of co-occurrence of keywords.

Because there is no previous work carried out with *Raphanus* species (*Brassicaceae* family) regarding the abiotic stress response such as high temperatures, which can induce or be detrimental to the synthesis of bioactive compounds, and at the same time, combining this source of stress with exogenous elicitors, the work presented in chapter III was carried out. This study used 7-day-old edible wild radish sprouts as raw material, which were subjected to an increase in growth temperature (30°C) combined with the exogenous application of different

elicitors (MeJa, citric acid, K_2SO_4 and chitosan) to activate defense mechanisms. Developmental traits, oxidative damage, GSL and anthocyanin content, and antioxidant capacity accompanied by the development of a predictive model were evaluated. The accumulation of bioactives was highly promoted by the application of two elicitors, K_2SO_4 and MeJa.

Both species were susceptible to the application of elicitors rather than high temperature. Correlation analysis and Principal Component Analysis (PCA) confirmed that in terms of GSL accumulation, edible radish enhanced the synthesis of these compounds with K_2SO_4 and MeJa elicitor, while 10 °C temperature increase was not so decisive. In relation to the accumulation of anthocyanins, edible radish, despite increasing its content after the application of MeJa, was not able to tolerate the increase in temperature and these compounds were reduced days before harvest, unlike wild radish where the synthesis of these secondary metabolites was significantly increased with this combination. In addition, the MeJa elicitor combined with high temperature increased oxidative stress through the MDA content, a situation generally associated with a higher content of phenolic compounds and an increase in antioxidant capacity (DPPH and ORAC).

Finally, an artificial neural network was developed to predict the behavior of the temperature increase combined with MeJa. The network generated a correlation between the fitted and observed values of more than 90%, with high prediction accuracy, allowing us to predict the behavior of the sprouts under these conditions. Therefore, it is shown that elicitors in combination with predictive analysis represent an effective tool to improve the nutritional value of Raphanus species sprouts under future conditions of increased temperature.

CAPÍTULO I

INTRODUCCIÓN Y OBJETIVOS

1. INTRODUCCIÓN

1.1. El estudio bibliométrico y su importancia

La bibliometría es una parte de la cienciometría y según Pritchard (Pritchard, 1969) es la aplicación de métodos matemáticos y estadísticos para analizar el curso de la comunicación escrita o literatura de carácter científico, así como a los autores que la producen (Camps, 2008).

La bibliometría tiene como objetivo estudiar el estado o desarrollo de la investigación científica y el progreso de un campo de investigación en países, instituciones, centros de investigación, revistas y los propios científicos, detectando cambios a través de patrones e interacciones entre partículas, así como el análisis a través de un conjunto de métodos matemáticos y estadísticos (Durieux and Gevenois, 2010). Para ello se ayuda de leyes bibliométricas, basadas en el comportamiento estadístico regular que a lo largo del tiempo han mostrado los diferentes elementos que forman parte de la ciencia. Los instrumentos utilizados para medir estos aspectos son los indicadores bibliométricos; medidas que proporcionan información sobre los resultados de la actividad científica en cualquiera de sus manifestaciones (Bornmann et al., 2008).

Las investigaciones publicadas en revistas científicas o en sitios web son utilizadas por otros investigadores para sus estudios y citadas como referencias en sus artículos posteriores. Este enorme aumento en la producción de publicaciones académicas ha generado una gran cantidad de información que puede resultar compleja para los lectores. Por lo tanto, el análisis de la información científica emergente y las tendencias pasadas conduce a la necesidad de abordar esta vasta información a través de métodos matemáticos y estadísticos, incluido el análisis bibliométrico.

1.2. Terminología utilizada en bibliometría

Si bien la bibliometría es un campo de estudio bastante amplio, la terminología que se emplea para llevar a cabo este tipo de estudios lo es de igual forma. Sin embargo, para la presente investigación se tendrán en cuenta solo algunos de los conceptos más relevantes y aplicados en el presente estudio bibliométrico.

• ***Factor de impacto***

Es una medida que se refiere a la importancia de una publicación científica. Cada año es calculado por el Instituto para la Información Científica (Institute for Scientific Information o ISI) para aquellas publicaciones a las que da seguimiento, las cuales son publicadas en un informe de citas llamado Journal Citation Reports.

• ***Índice-h***

Este indicador es para la medición de la calidad profesional de científicos, en función de la cantidad de citas que han recibido sus artículos científicos. Un científico o investigador tiene índice h si ha publicado h trabajos con al menos h citas cada uno.

• ***Co-ocurrencia***

Hace referencia a una interconexión agrupada de términos, teniendo en cuenta su presencia en los documentos recuperados en la búsqueda inicial en la base de datos (Web of Science), estas redes son las que permiten identificar las tendencias de producción bibliográfica en los estudios bibliométricos.

• ***Producción:***

Este término hace referencia a la medición que se realiza de producción científica de los autores y las revistas. A partir de indicadores como la cantidad de documentos por año, idioma, revista, autor, área de conocimiento, entre otros.

• ***Calidad o impacto****:*

Se encarga de medir el indicador de citas. Eso es posible llevarlo a cabo a partir de las citas y las autocitas, el cuartil y el factor de impacto, el índice h, entre otros. (Spinak, 1996).

1.3. Bases de datos

Las bases de datos son conjuntos de información que se almacenan y se consultan con un sistema. Los gestores de bases de datos son un tipo de software que permite estructurar una base de datos, almacenar la información y consultarla. Las bases de datos habitualmente se encuentran organizadas sistemáticamente, de tal manera que el acceso a la información sea rápido y eficiente. A la hora de realizar el análisis bibliométrico de un campo de investigación, el primer paso es evaluar las bases de datos disponibles, su idoneidad y las consecuencias de utilizar una u otra. Son definidos por Luque (Martínez, 1995) como "*un conjunto de datos organizados en una secuencia lógica que permite un acceso sencillo, de modo que la información que contiene puede ser: actualizada, utilizada en cualquier momento por cualquier programa informático al que esté conectado y operados en todo momento según distintos criterios"*. Juegan un papel clave en la investigación bibliométrica, ya que permiten analizar la actividad científica que desarrollan los investigadores, instituciones, regiones y países e identificar tendencias en la investigación. La validez de un trabajo dependerá de la adecuada selección de la base, ya que debe cubrir suficientemente el área de estudio (Granda-Orive et al., 2013).

Existen algunas bases de datos como la web of science (WoS), Scopus, Scielo, etc. En particular, WoS de Clarivates Analytics es una de las plataformas más amplias utilizadas para la consulta de bases de datos del Institute for Scientific Information (ISI). Se dedica a recuperar, analizar y visualizar la investigación académica a través de diversos indicadores bibliométricos tales como: el Índice de Hirsch, las citas, temas de investigación y colaboración nacional e internacional, entre otros. De su colección principal toma dos índices de citación: Science Citation Index, Expanded (SCIE) y el Social Sciences Citation Index, (SCCI) para elaborar su ranking de revistas Journal Citations Reports (JCR), cuyo principal indicador bibliométrico es el Journal Impact Factor (JIF). Su objetivo no es suministrar el texto completo de los documentos, sino proporcionar herramientas de análisis que permitan valorar la calidad científica. La información suministrada por Web of

Science es de alta calidad, lo cual permite consultar nuevas tendencias, análisis de datos y contenido a través de herramientas programadas para localizar investigaciones de gran relevancia.

Web of Science proporciona acceso a las siguientes herramientas de análisis bibliométrico:

- ***Journal Citation Reports***

  Recurso fundamental para conocer el factor de impacto de las revistas académicas y científicas y su influencia en la investigación global. Proporciona datos estadísticos, basados en citas de artículos, que permiten evaluar la importancia de las revistas, comparándolas entre sí, dentro de su área temática.

- **Essential Science Indicators**

  Proporciona datos estadísticos, basados en las citas, que permiten ver el rendimiento de la investigación y hacer seguimiento de las tendencias en los distintos ámbitos de la ciencia.

- **InCites**

  InCites permite ver, analizar y evaluar la producción científica de los investigadores a diferentes niveles: individual, de publicaciones, institucional y regional. Entre otras cosas, sirve para crear perfiles generales de la Universidad, departamentos, de las distintas áreas del conocimiento y de los autores individuales (Pérez-Escoda, 2017).

1.4. Aplicación de la bibliometría en temática de interés

Es crucial identificar el conocimiento e investigación actual en el campo agroalimentario. Una de estas temáticas tiene relación con los mecanismos de elicitación en brotes de plantas y cómo se induce la síntesis de compuestos bioactivos en sus tejidos. Por lo tanto, el análisis bibliométrico va a proporcionar información de las tendencias desarrolladas por los investigadores del área. En relación con esto, se destacan las tendencias en las dietas ricas en compuestos bioactivos o fitoquímicos como los germinados. Las técnicas para potenciar estos compuestos endógenos de las plantas durante el período de crecimiento nos traen nuevos desarrollos productivos e innovadores a nivel agronómico y comercial porque los germinados se caracterizan por ser de rápido crecimiento y amigables con el medio ambiente. Por lo tanto, estos temas se han convertido en una prioridad de discusión por parte de los investigadores, proponiendo estrategias futuras para maximizar los beneficios de estas plantas. Por lo tanto, el análisis de la información científica emergente y las tendencias pasadas lleva a la necesidad de abordar esta vasta información a través de métodos matemáticos y estadísticos, incluido el análisis bibliométrico.

1.5. Familia Brassicaceae, aspectos generales y valor nutricional

La familia *Brassicaceae* también conocida como *Cruciferae*, es denominada así por el arreglo en cruz de los pétalos. Muchas de éstas tienen valor económico principalmente por ser plantas alimenticias, pero también pueden ser ornamentales o malezas. Esta familia incluye aproximadamente 338 géneros y 3709 especies, distribuidas ampliamente en los más diversos climas alrededor del mundo (Al-Shehbaz et al., 2006; Walters and Keil, 1996).

Los vegetales pertenecientes a la familia *Brassicaceae* son consumidas ampliamente alrededor del mundo y tienen un papel importante en la nutrición y salud humana, debido a su contenido en fitoquímicos que se pueden encontrar en sus tejidos, como vitaminas, minerales, glucosinolatos (GSL), compuestos fenólicos y antocianinas (Cartea and Velasco, 2008; Harbaum et al., 2007), todas ellas con un papel establecido en la prevención de varias enfermedades crónicas (Björkman et al., 2011; Drewnowski and Gomez-Carneros, 2000) (Fig.1) .

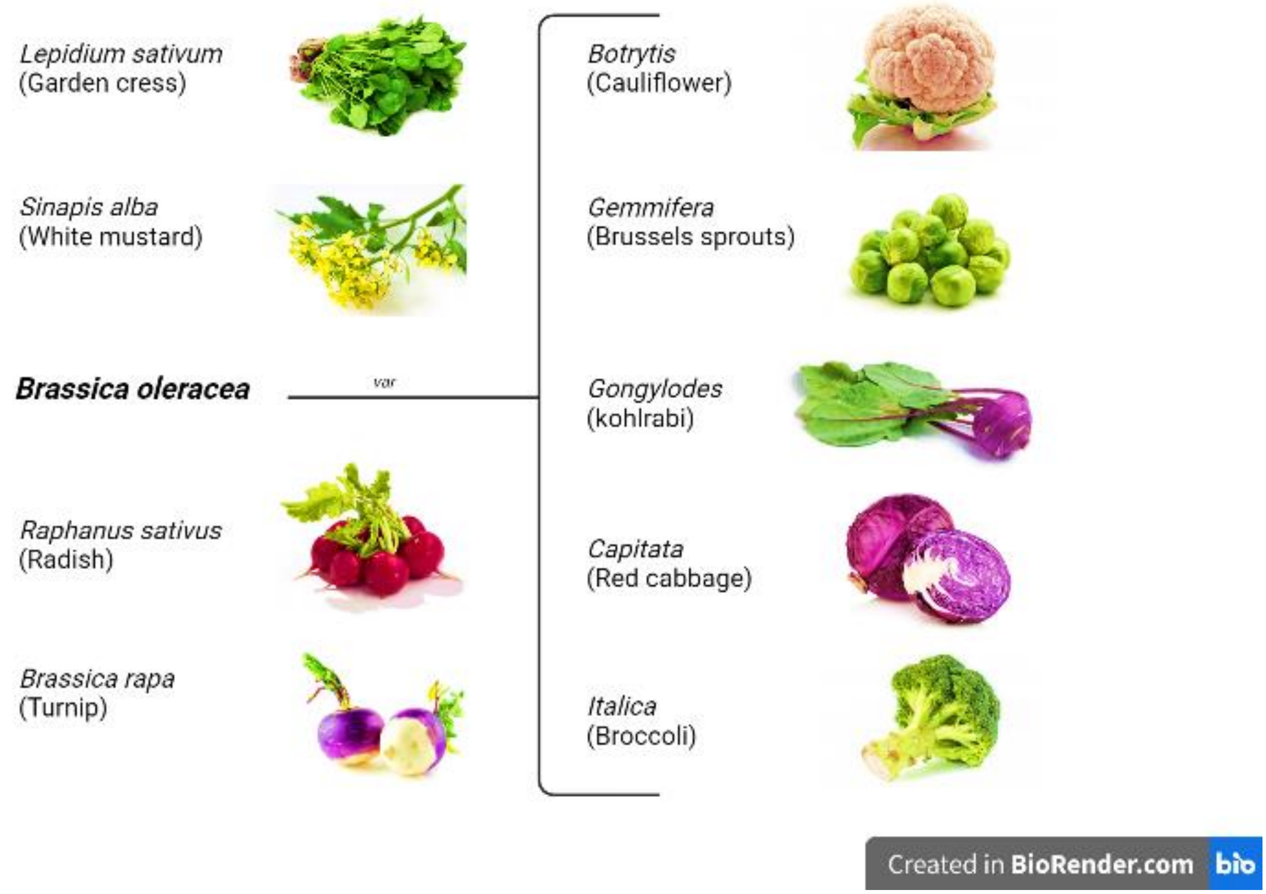


Figura 1. Verduras comunes de la familia Brassicaceae.

Las especies pertenecientes a esta familia se caracterizan por ser cosmopolitas, por lo que se encuentran ampliamente distribuidas a nivel mundial incluyendo todos los continentes excepto la Antártida. La mayor cantidad de géneros se encuentra en las zonas templadas del Hemisferio Norte, y su abundancia disminuye con la latitud. En los trópicos su distribución se ve reducida a zonas montañosas. El centro más importante de diversificación se encuentra en la región Irano-Turania (150 géneros y 900 especies), específicamente en las planicies de Turquía donde existe una extrema diversidad ecológica y geológica, por lo que se lo reconoce como el centro principal de origen (Koch and Kiefer, 2006; Lysak and Koch, 2011).

La importancia económica y social de las crucíferas es destacada ya que incluye especies que han sido cultivadas por siglos como fuente de aceites industriales y comestibles, condimentos y productos hortícolas. El cultivo oleaginoso más importante a nivel mundial que pertenece a esta familia es la colza, también llamada canola o raps (*Brassica napus* L.*)* (Warwick, 2011), la cual es una excelente materia prima para aceite comestible y biodiesel. Además, su harina es una fuente proteica para alimentación animal. Por otro lado, como condimentos, son utilizadas varias especies de mostazas, en especial *B. nigra* L*., B. juncea* L*.* y *Sinapis alba* L., otras son importantes cultivos hortícolas, principalmente los derivados de *B. oleracea* L*.* (repollos, coles, repollitos de Bruselas, coliflor). Varios miembros de esta familia presentan un elevado valor potencial como recurso para cultivos con destino alimentario, industrial, forraje o para la producción de biodiesel (por ejemplo *B. carinata Braun, Camelina sativa* L*., Crambe abyssinica, Eruca vesicaria* L*)*. Además, la especie *Arabidopsis thaliana* L*.* ha sido estudiada y adoptada como "organismo modelo" en estudios de genética vegetal (Warwick, 2011).

Uno de los grupos de especies de gran importancia son las pertenecientes al género *Raphanus*. Actualmente se considera que el género comprende dos especies básicas; *Raphanus sativus* L. (que integra todas las formas cultivadas, y *Raphanus raphanistrum* L. , que crece en estado silvestre (Schroeder, 1989; Warwick and Hall, 2009). *R. sativus* es una hortaliza de importancia económica cultivada en todo el mundo debido a su excelente adaptabilidad y alto valor nutricional de sus raíces engrosadas (Kaneko et al., 2011). El origen del cultivo es muy remoto y aunque no ha sido dilucidado por completo, las investigaciones recientes parecen indicar que fue domesticado independientemente en Europa y Asia (Snow and Campbell, 2005; Warwick, 2011).

Se encontró que el GSL glucorafenina (4-metilsulfinil-3-butenil) y dehidroerucina (4-metiltio-3-butenil, también conocida como glucorafasatina), son las predominantes en los brotes de rábano de 8 días (Baenas et al., 2016). Por otro lado, los compuestos de hidrólisis del GSL glucorafenina y dehidroerucina, de brotes de rábano, también mostraron inhibición de la fase I o inducción de enzimas metabolizadoras de xenobióticos de fase II (Barillari et al., 2007). El indol GSL, como la glucobrasicina, se hidroliza a indol-3-carbinol y su compuesto derivado 3,3′-

diindolymethane, tiene efectos potencialmente biológicos, incluida la actividad en el sistema enzimático metabolizador de carcinógenos (Aggarwal and Ichikawa, 2005).

Por otro lado, la planta silvestre comestible *R. raphanistrum* es conocida en la nutrición humana como alimento con fines medicinales (Hedge, 1965), en el tratamiento de afecciones hepatobiliares, problemas dispépticos, principalmente relacionados con discinesia biliar y afecciones bronquiales (Neto and Simões, 2007). Sin embargo, en Chile esta planta apenas se consume y se conoce como una maleza o planta invasora nociva. *R. raphanistrum* es nativa de las costas del Mediterráneo (Europa, el Oriente Medio y el norte de África) y se encuentra naturalizada en regiones templadas de los dos hemisferios. En especial, se encuentra ampliamente distribuida en Canadá, Estados Unidos, México y Australia (Warwick and Francis, 2005). Por otro lado, el consumo de *R. raphanistrum* ya sea de forma cruda o cocida también está asociado a ciertas propiedades beneficiosas, siendo utilizado tradicionalmente. Se ha reportado que el rábano silvestre es una fuente potencial de compuestos beneficiosos, incluida la vitamina E, ácidos grasos poliinsaturado (particularmente el ácido α-linolénico) y catorce compuestos fenólicos, siendo el más abundante el kaempferol-3,7-O-di-ramnósido, el cual ha demostrado un amplio espectro de bioactividades, incluyendo antioxidante, antifúngico, inhibición de glucosidasa , antitumoral, y actividades hipoglucémicas (Weizhun Yang et al., 2012). Por otro lado, Malik (Malik et al., 2010) evaluó la presencia de GSL en diferentes partes de la planta de *R. raphanistrum*. En total se identificaron diecisiete GSLs, de los cuales la glucoerucina, glucorafenina, glucobrassicina y gluconasturtiina contribuyeron del 90% al 100% del total de GSLs. Por otro lado, han especificado que las flores contenían las concentraciones más altas de GSLs.

1.6. Bioactividad de los fitoquímicos en Brassicaceae

Al igual que otras verduras, las *Brassicaceae* contienen una serie de nutrientes y fitoquímicos con propiedades quimiopreventivas para el cáncer, que incluyen ácido fólico, fibra, carotenoides, clorofila y una alta concentración de compuestos bioactivos fenólicos, sin embargo, las verduras crucíferas son únicas en el sentido de que son fuentes ricas en glucosinolatos que además son compuestos que contienen azufre, que casi están exclusivamente presentes en plantas de esta familia (Drewnowski and Gomez-Carneros, 2000; Pérez-Balibrea et al., 2008). Muchos estudios epidemiológicos vinculan una alta ingesta de vegetales de *Brassica* con una menor incidencia de diferentes tipos de cáncer (Verhoeven et al., 1997). También estas sustancias pueden actuar como antioxidantes indirectos al modular la actividad de las enzimas metabolizadoras xenobióticas (enzimas de fase I y fase II) que desencadenan la actividad antioxidante de larga duración (Vig et al., 2009), reduciendo el estado de estrés oxidativo responsable de desencadenar las enfermedades crónicas degenerativas (Verkerk et al., 2009). Está claro que la gama de actividades de estos compuestos es amplia y a muchos de ellos se les han atribuido grandes cualidades promotoras de la salud, sin embargo existen algunos que son perjudiciales para el consumo humano y animal (Real et al., 2010). Se cree que los productos de hidrólisis de glucosinolatos son responsables del característico olor y sabor picante de esta clase de verduras (Deng et al., 2015), sin embargo pueden tener efectos no deseados en los alimentos debido a su acidez y actividad bociogénica (Durham and Poulton, 1990; Tookey et al., 1980). La progoitrina ha demostrado ser potencialmente bociogénica en animales. Sin embargo, no hay evidencia de ningún efecto bociogenético en humanos por el consumo de *Brassica* (Mithen, 2001). El contenido de GSL en las plantas de *Brassica* es de alrededor del 1% de la materia seca. El consumo diario estimado de GSL varía entre 12 y 300 mg (Ciska and Kozłowska, 2001). Hasta la fecha, no hay informes sobre los efectos nocivos de la salud de los GSL en humanos que consumen cantidades normales de verduras, berros, ensalada de rúcula y rábano. Por el contrario, no se pueden excluir los efectos beneficiosos (Verkerk et al., 2009).

Varios estudios *in vitro* e *in vivo* sugieren que los fitoquímicos derivados de la *Brassica* pueden contrarrestar las vías inflamatorias y exhibir actividad quimiopreventiva (Wagner et al., 2013). La quimioprevención es una estrategia preventiva del cáncer para inhibir, retrasar o revertir la carcinogénesis humana, utilizando agentes químicos sintéticos o naturales (Sporn et al., 1976). Sin embargo, estos efectos quimiopreventivos asociados a los GSL no están directamente mediados por ellos, ya que estos no son biológicamente activos, por lo que estas cualidades están determinadas directamente a través de los ITC (Fahey et al., 2001; Navarro et al., 2011), cuya presencia se manifiesta tanto en la microbiota intestinal humana (Shapiro et al., 2006) como en las células de las plantas que contienen GSL (Rosa, 1999). No obstante, la variación en el contenido de GSL y sus productos bioactivos de hidrólisis, dependen tanto de la genética del vegetal, como del medioambiente, en el que se incluyen las prácticas de cultivo, la cosecha y el almacenamiento, el procesado y la elaboración de los alimentos por el consumidor (Cartea and Velasco, 2008; Moreno et al., 2006). Asimismo, se ha demostrado que todos los derivados de GSL no son iguales en su potencial biológico (Kusznierewicz et al., 2013). En consecuencia, la identificación, pero también la cuantificación y determinación de glucosinolatos individuales en plantas y tejidos vegetales es extremadamente importante.

Las cualidades bioactivas de las *Brassicas* están asociadas principalmente a los ITC, que son el producto de la hidrólisis de GSL mediado por la acción de la enzima mirosinasa. Los productos alimentarios que contienen mirosinasa activa, como los brotes de *Brassica* y las verduras de *Brassica* maduras poco cocinadas presentan una mayor biodisponibilidad de los ITC (Verkerk et al., 2009). Se ha demostrado que el tratamiento térmico podría inactivar la mirosinasa (Van Eylen et al., 2006). Los germinados, como productos recién cortados con una vida útil corta, pueden ser susceptibles a pérdidas de GSL debido a una alta actividad de mirosinasa que también puede verse afectada por las condiciones de germinación (por ejemplo, tiempo de germinación, exposición a la luz, cosecha y almacenamiento) (Aires et al., 2012).

Por ejemplo, el brócoli es una buena fuente de glucorafanina, el precursor del glucosinolato de sulforafano. Numerosos estudios han demostrado el efecto positivo del sulforafano en diferentes etapas de los procesos del cáncer. Otro de los compuestos presentes en el brócoli es la glucobrassicina, el precursor del indol-3-carbinol. Éste último ha demostrado suprimir la proliferación de varias líneas celulares de cáncer al dirigirse a un amplio espectro de vías de señalización que regulan la homeostasis hormonal, la progresión del ciclo celular y la proliferación celular (Zhang, 2004).

Los rábanos rojos (*Raphanus sativus* L.) son una rica fuente de antocianinas naturales. Se informó que la mayoría de las antocianinas en la naturaleza, incluidas las de los rábanos rojos, comprenden principalmente pelargonidina-3-soforosido-5-glucósidos con una unión de ácidos *p* -cumárico, ferúlico y cafeico (Jing et al., 2012). Además, la unión de glucósidos y ácidos puede conducir a la reducción significativa de las capacidades antioxidantes y antiproliferativas (Rice-Evans et al., 1996).

Además, se ha encontrado en especies de *Brassica* una gran cantidad de glucósidos flavonoides; entre ellos, los glucósidos de kaempferol y quercetina, sus derivados en combinación con ácidos hidroxicinámicos, así como los derivados del ácido sináptico, son los compuestos fenólicos más importantes presentes en esta especie (Martínez-Villaluenga et al., 2008). Se sabe que Kaempferol es un antioxidante fuerte y la quercetina también es un potente eliminador de radicales libres y se considera que protege contra las enfermedades cardiovasculares (Francisco et al., 2009).

1.7. Estructura, clasificación e hidrólisis de Glucosinolatos

Como se menciona anteriormente las cualidades promotoras de la salud de las *Brassicaceae* son atribuidos a la presencia de compuestos fenólicos y sobre todo a los GSL. Los GSL son metabolitos secundarios de las plantas y constituyen una clase bien definida de productos naturales aniónicos, conteniendo azufre, siendo esta cualidad casi exclusiva en plantas de la familia *Brassicaceae*, hallándose en cualquier parte de la planta, y encontrándose en diferentes perfiles y concentraciones. Existen algunos GSL que comúnmente son encontrados en la familia *Brassicaceae*, estos se pueden observar en la Tabla 1**.** Los GSL Poseen la capacidad de formar isotiocianatos (ITC) con la estructura común R–N═C═S y otros compuestos después de la hidrólisis (Blažević et al., 2020; Navarro et al., 2011; Velasco et al., 2007; Verkerk et al., 2009). Los GSL son N-hidroxisulfatos de β-tioglicósido (también conocidos como ésteres de (Z) -N-hidroximinosulfato o S-glucopiranosil tiohidroximatos) con una cadena lateral R y un azufre vinculado β-D–glucopiranosa. Existen tres categorías de GSL que se diferencian según la cadena lateral que posean. Éstas pueden clasificarse en GSL de origen alifático derivados de metionina, GSL indol o indólico de fuente de triptófano, y GSL aromáticos formados a partir de fenilalanina (por ejemplo, indol) (cada uno de ellos con espectros UV característicos y factores de respuesta aproximados a HPLC-UV) (Agerbirk and Olsen, 2012; Fahey et al., 2001; Halkier and Gershenzon, 2006; Verkerk et al., 2009; Yan and Chen, 2007).

Tabla 1. Nombres comunes y químicos de GSL comúnmente encontrados en la familia *Brassicaceae* (Verkerk et al., 2009).

Nombre común	**Nombres químicos de los grupos R**
GSLs Alifáticos	
Sinigrina	2-Propenilo
Gluconapina	3-Butenilo
Glucobrassicanapina	4-Pentenilo
Progoitrina	2(R)-2-Hidroxi-3-butenilo
Epiprogoitrina	2(S)-2-Hidroxi-3-butenilo
Gluconapoleiferina	2-Hidroxi-4-pentenilo
Glucoibervirina	3-Metiltiopropilo
Glucoerucina	4-Metiltiobutilo
Dehidroerucina	4-Metiltio-3-butenilo
Glucoiberina	3-Metilsulfinilpropilo
Glucorafanina	4-Metilsulfinilbutilo
Glucorafenina	4-Metilsulfinilo-3-butenilo
Glucoalisina	5-Metilsulfinilpentenilo
Glucoerisolina	4-Metilsulfonilbutilo
GSLs Indóles	
Glucobrassicina	3-Indolilmetilo

4-Hidroxiglucobrassicina	4-Hidroxi-3-indolilmetilo
4-Metoxiglucobrassicina	4-Metoxi-3-indolilmetilo
Neoglucobrassicina	1-Metoxi-3-indolilmetilo
GSLs aromáticos	
Glucotropaeolina	Bencilo
Gluconasturtiina	2-Feniletilo

Las plantas que contienen GSL generalmente poseen la glucohidrolasa tioglucósida o tioglucohidrolasa que comúnmente es conocida como mirosinasa y se encuentra ubicada aparte de los GSL en las llamadas células de mirosina o idioblastos (Durham and Poulton, 1990; Wagner et al., 2013). Esta enzima cataliza la hidrólisis o degradación de GSL, y logra activarse cuando las plantas están estresadas por factores bióticos y abióticos como por ejemplo un daño tisular o una pérdida de integridad celular durante la senescencia del producto (Real et al., 2010; Sánchez-Pujante et al., 2017; Verkerk et al., 2009). Además, esta enzima actúa hidrolizando los GSL a D-glucosa y una multitud de productos fisiológicamente activos (por ejemplo, ITC, tiocianatos, nitrilos orgánicos y oxazolidina2-thione) (Durham and Poulton, 1990) (Fig. 2), determinados por el pH ambiental y la presencia de proteínas especificadas (Halkier and Gershenzon, 2006).

Por lo general, la estabilidad y la actividad de la mirosinasa disminuyen durante el procesamiento y los tratamientos domésticos (Oerlemans et al., 2006; AP Vale et al., 2015), específicamente con el uso de calor y la aparición de disrupción celular, lo que afecta la ingesta y la biodisponibilidad de GSLs y sus productos de descomposición (Getahun and Chung, 1999).

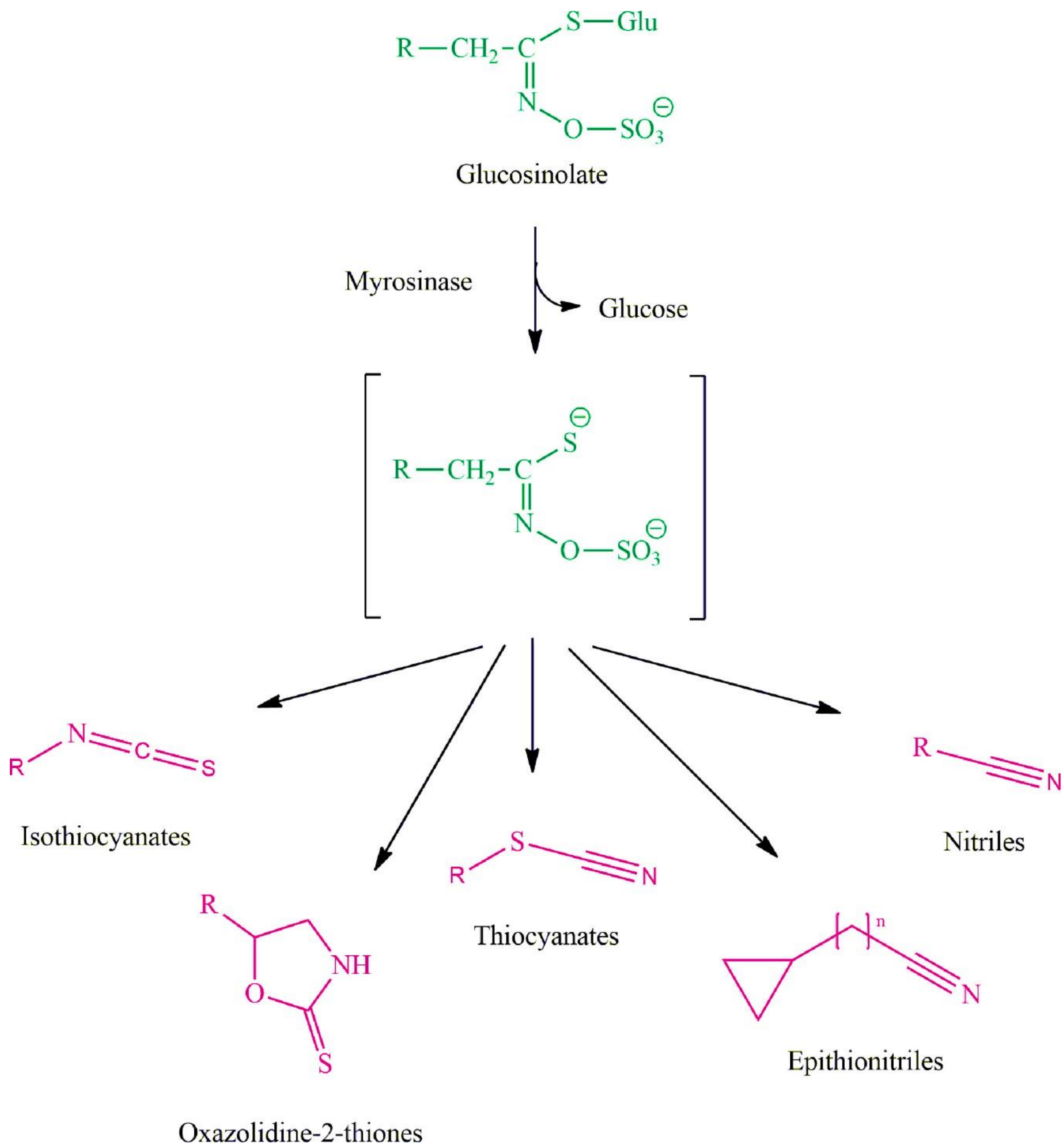


Figura 2. Estructura de glucosinolato e hidrólisis catalizada por mirosinasa (corchetes indican aglicona inestable) (Prieto et al., 2019).

Los ejemplos de estructuras de cadena lateral (R) se muestran como inserto) (Prieto et al., 2019; Radojčić Redovniković et al., 2008).

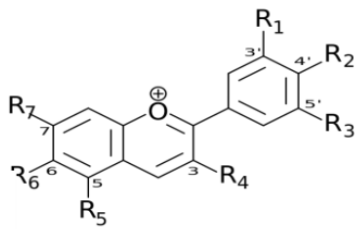
1.8. Antocianos

Si bien los alimentos de las crucíferas son reconocidos por su alto contenido en glucosinolatos, los alimentos de las *brasicáceas* también son ricos en compuestos fenólicos (flavonoles y antocianinas), carotenoides, vitaminas y minerales (Manchali et al., 2012).

Los antocianos, son los compuestos fenólicos más abundantes contenidos en la mayoría de las verduras y frutas, incluidos los rábanos, las uvas, las bayas y la col lombarda. Son muy apreciadas por sus atractivos colores y, especialmente, por sus numerosos beneficios para la salud (Araceli et al., 2009). Muchas investigaciones han indicado que los antocianos pueden prevenir o retrasar la aparición de enfermedades crónicas relacionadas con la edad debido a sus actividades antioxidantes (Feng et al., 2016). Además, se ha informado que los antocianos restringen la proliferación de células cancerosas debido a sus capacidades antiproliferativas (Sousa et al., 2016)

Dentro de las clases de compuestos bioactivos, los antocianos son flavonoides hidrosolubles que normalmente existen en las plantas en forma de glucósidos y forma acilada. Sus fracciones que no son carbohidratos (agliconas) se denominan antocianidinas. Hay muchos tipos de antocianinas, que se distinguen según el número y la posición de los grupos hidroxilo y metoxilo como sustituyentes en el anillo B, el tipo y el número de azúcares conjugados y la presencia o ausencia de un grupo acilo. Los seis tipos más importantes son pelargonidina (Pg), cianidina (Cy), delfinidina (Dp), peonidina (Pn), petunidina (Pt) y malvidina (Mv) (Jaakola, 2013) (Fig.3). Cy y sus derivados, que poseen dos grupos hidroxilo en el anillo B, son los más ampliamente distribuidos, seguidos por Dp y sus derivados (de Pascual-Teresa and Sanchez-Ballesta, 2008b). No solo son responsables de los colores rojo, azul y morado de muchas frutas, verduras, flores y semillas, sino que también protegen a las plantas contra diversos estreses bióticos y abióticos (Harborne and Williams, 2000).

A



B

Antocianidina	R1	R2	R3	R4	R5	R6	R7
Cianidina	-OH	-OH	-H	-OH	-OH	-H	-OH
Delfinidina	-OH	-OH	-OH	-OH	-OH	-H	-OH
Pelargonidina	-H	-OH	-H	-OH	-OH	-H	-OH
Malvidina	-OCH3	-OH	-OCH3	-OH	-OH	-H	-OH
Peonidina	-OCH3	-OH	-H	-OH	-OH	-H	-OH
Petunidina	-OH	-OH	-OCH3	-OH	-OH	-H	-OH

Figura 3. Estructuras químicas de los aglicones de antocianidina más comunes en frutas **(Brouillard, 1982) .**

Los glucósidos de cianidina se encuentran en la mayoría de las frutas y son las principales antocianidinas, por ejemplo, en las manzanas de piel roja. Las frutas de color oscuro como los arándanos y ciertas bayas de vid también pueden contener glucósidos de delfinidina, malvidina, peonidina y petunidina. En fresas, frambuesas y cerezas se encuentran glucósidos de pelargonidina además de glucósidos de cianidina (Jaakola, 2013).

1.9. Brotes de plantas

Los consumidores exigen hoy en día una gama diversificada de alimentos que brinden beneficios para la salud y además contribuyan a su bienestar. Es ahí donde los brotes comestibles aparecen para aportar con una fuente valiosa de diversos micronutrientes (vitaminas, minerales y aminoácidos), macronutrientes (proteínas, bajos en carbohidratos y alto contenido de fibra dietética) y metabolitos secundarios de las plantas (principalmente compuestos fenólicos y GSLs) (Gan et al., 2017). Los brotes crucíferos son alimentos vegetales frescos muy interesantes debido a que poseen niveles más altos de nutrientes y compuestos bioactivos en comparación con las plantas adultas (Benincasa et al., 2019). Los brotes crucíferos generalmente se recolectan y comercializan a los 7 u 8 días de edad después de la germinación, considerando que este estado fisiológico joven es óptimo para el consumo en términos de biomasa y tamaño, lo que permite la manipulación, así como su contenido de compuestos que promueven la salud, ya que como se dijo anteriormente los brotes tienen concentraciones significativamente mayores de fitoquímicos que las plantas maduras (10-100 veces), esto es debido a que durante el período de crecimiento ocurre una disminución de estos compuestos como resultado de un efecto de dilución de la expansión del tejido (Pérez-Balibrea et al., 2008).

Los brotes se forman a partir de semillas durante la germinación. Pueden crecer a partir de semillas de vegetales como el rábano, de granos como el arroz, de frijoles como la soya y de semillas de árboles como *Toona sinensis* y pimienta. Los brotes se han consumido como un alimento común en China durante más de 5000 años y luego se han extendido gradualmente a otros países del este. Como parte del cambio de estilo de vida en occidente hacia la comodidad y la salud, ha crecido el consumo de germinados en las poblaciones occidentales (Sikin et al., 2013). Los brotes han sido cada vez más populares entre personas de todo el mundo debido a sus valores nutricionales y beneficios para la salud.

Un gran número de estudios epidemiológicos han demostrado consistentemente que el consumo diario de alimentos de origen vegetal está asociado con la reducción de factores de riesgo de enfermedades crónicas, como enfermedades cardiovasculares, diabetes y obesidad (Raiola et al., 2017). Los efectos saludables de los alimentos de origen vegetal pueden estar relacionados con la presencia de varios compuestos bioactivos en las partes comestibles, como compuestos fenólicos , carotenoides , glucosinolatos , vitamina C y tocoferoles , que exhiben diversas propiedades biológicas (Barba et al., 2017). Numerosos estudios han demostrado que la germinación es una forma económica y eficaz de acumular compuestos bioactivos en semillas de legumbres, cereales, hortalizas, frutas, flores y plantas medicinales (Bartalné-Berceli et al., 2016; Gan et al., 2017). La germinación puede provocar el catabolismo y la degradación de los principales macronutrientes y la reducción de factores antinutricionales y no digeribles. La concentración de diferentes compuestos bioactivos aumentó durante la germinación de la semilla, proporcionando a los brotes muchas bioactividades, como actividades antioxidantes , antidiabéticas, antiinflamatorias, hipolipidémicas y anticancerígenas (Gan et al., 2017; Singh and Sharma, 2017). Por lo tanto, los germinados son buenos vegetales para la salud humana. De acuerdo con estas evidencias, muchos estudios se han centrado en estrategias dirigidas a aumentar el contenido de compuestos bioactivos en los germinados.

Las condiciones de germinación pueden afectar la síntesis y por ende el contenido de compuestos bioactivos en los germinados (HongKai Liu et al., 2016; López-Martínez et al., 2017). Por lo tanto, se pueden utilizar tratamientos específicos para aumentar la producción de metabolitos en la planta y mejorar su valor cualitativo para los productos frescos (Baenas et al., 2014a).

1.10. Efectos de la temperatura como inductor en la síntesis de compuestos bioactivos

Diferentes factores abióticos negativos presentes en el ambiente, como las altas temperaturas producen estrés en las plantas y se espera que las olas de calor sean más frecuentes, duraderas y aumenten en intensidad (Hoefgen and Nikiforova, 2008), lo que termina afectando el rendimiento de cultivos y biosíntesis y regulación de metabolitos secundarios. Algunas investigaciones muestran que los compuestos secundarios aumentan con temperaturas altas controladas (Verma and Shukla, 2015). El estrés por calor generalmente induce la acumulación de especies reactivas de oxígeno (ROS) y la activación de los sistemas de desintoxicación (Serrano et al., 2019). Si la temperatura es demasiado alta, desencadena explosiones oxidativas que conducen a un amplio espectro de respuestas, muchos procesos fisiológicos se ralentizan o se deterioran, lo que induce la acumulación de antioxidantes, que protegen la membrana celular de la descomposición y la peroxidación hasta la senescencia. Por lo tanto, es importante lograr un equilibrio entre el desarrollo del crecimiento de la planta y el mecanismo de defensa.

1.11. Uso de elicitores, mecanismo de acción y aplicación

Se sabe que los factores genéticos, ambientales, entre otros, afectan el rendimiento y la acumulación de compuestos bioactivos, por lo tanto es de suma importancia optimizar el desarrollo los brotes para mejorar su potencial biológico (Moreno et al., 2010; Ana Paula Vale et al., 2014).

Los inductores son sustancias que inducen cambios fisiológicos y morfológicos en el organismo vivo de la planta objetivo (Baenas et al., 2014a; Zhao et al., 2005). Estas sustancias al ser reconocidas por las plantas, dan lugar a la activación de sus mecanismos de defensa (Angelova et al., 2006; Delaunois et al., 2014). Las plantas cuentan con una serie de sistemas de defensa frente a herbívoros, insectos y microorganismos patógenos, que se pueden clasificar en mecanismos de defensa pasiva, presentes sin necesidad de que exista una infección previa y que ejercen una resistencia permanente, y mecanismos de defensa inducida, que se activan como respuesta al ataque de un determinado organismo (Beckers and Spoel, 2006). Las plantas al ser sometidas a factores de estrés reducen sus procesos fisiológicos y generan métodos constitutivos o inducidos para su defensa. La inducción de sustancias está a cargo de los elicitores, que aumentan la producción de compuestos fenólicos, alcaloides, terpenoides, aceites esenciales y glucosinolatos (Montoliu, 2010). Por lo tanto, los inductores se usan ampliamente para aumentar el rendimiento de metabolitos secundarios con bioactividades en células vegetales, tejidos o en toda la planta (Namdeo, 2007; Ramirez-Estrada et al., 2016; Meenakshi Thakur and Sohal, 2013) (Fig. 4)

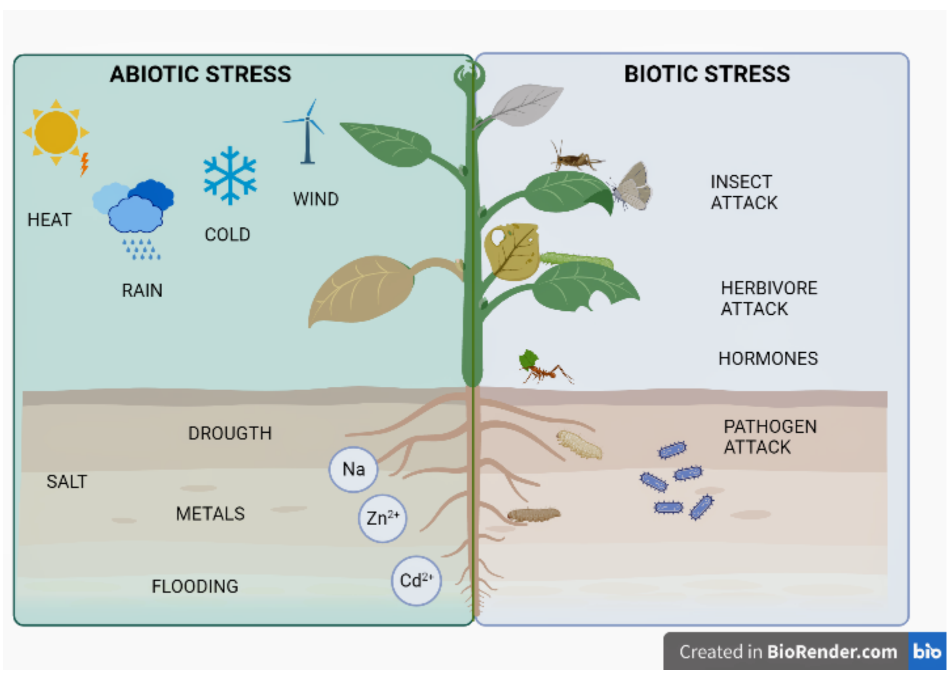


Figure 4. Fuentes de estrés en plantas (Azcón-Bieto and Talón, 2000).

En los sistemas de defensa de las plantas, las células han adquirido la capacidad de responder a los patógenos y al estrés ambiental mediante la creación de una respuesta de defensa. La respuesta de las plantas está determinada por varios factores, principalmente en función de sus características genéticas y estado fisiológico. El primer paso en la respuesta de la planta contra los elicitores es la percepción del estímulo por parte de los receptores localizados en las membranas plasmáticas de la célula vegetal, como las proteínas quinasas, que representan uno de los actores más importantes en la percepción de patógenos para una serie de elicitores fúngicos, o podrían estar localizados. dentro de la célula para iniciar procesos de señalización que activan las defensas de la planta, como ocurre con ciertos inductores bacterianos (Ebel and Mithöfer, 1998). La transducción de señales del elicitor es un área importante de investigación. Las plantas responden a los elicitores activando una variedad de mecanismos de defensa en la superficie de la membrana plasmática, inducción de proteínas relacionadas con la patogénesis (PR) y enzimas de protección contra el estrés oxidativo; respuestas hipertensivas caracterizadas por muerte celular rápida en las inmediaciones del punto de exposición al patógeno; producción de especies reactivas de oxígeno (ROS) y especies reactivas de nitrógeno (RNS); activación de genes relacionados con la defensa; cambios en el potencial de las células de la membrana plasmática y aumento de los flujos de iones (salida de Cl^- y K^+ y entrada de Ca^{2+}), responsables de una acidificación transitoria del citoplasma que puede actuar como una señal para la producción de metabolitos secundarios; cambios rápidos en la fosforilación y desfosforilación de proteínas (cambio de actividad enzimática, ubicación celular o asociación con otras proteínas) y oxidación de lípidos; barreras defensivas estructurales, como refuerzo y deposición de lignificación en la pared celular; y, la activación y biosíntesis de novo de factores de transcripción, los cuales regulan directamente la expresión de genes involucrados en la producción de metabolitos secundarios (Ferrari, 2010; Zhao et al., 2005) (Fig. 5).

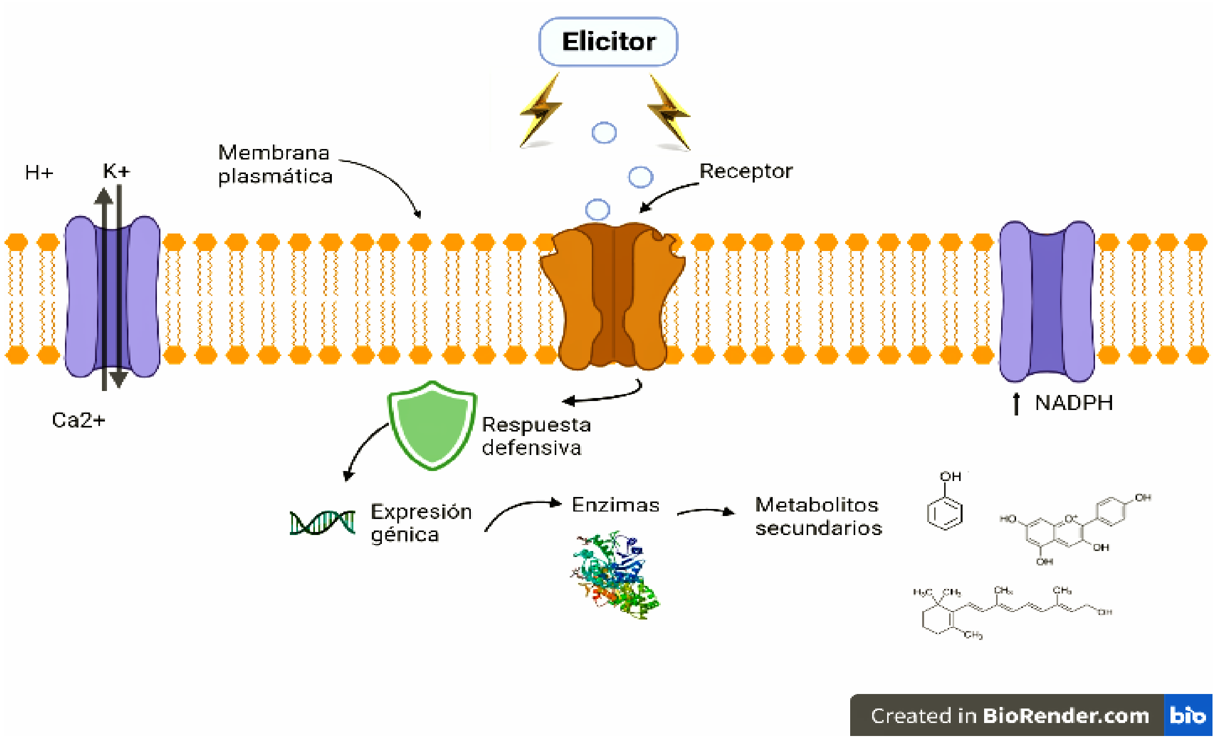


Figure 5. Mecanismo general después de la percepción del elicitor (Ferrari, 2010; Zhao et al., 2005).

Baenas (Baenas et al., 2014a) clasificó los elicitores en base a su origen según fueran hormonas vegetales (como el ácido jasmónico (JA), el jasmonato de metilo (MeJa), el etileno (ET) o el ácido salicílico (SA), de tipo biótico (de origen biológico, como quitosano o extractos de levaduras) o de tipo abiótico (de origen químico, como determinados iones metálicos, o de origen físico, como la radiación ultravioleta o el ozono). De esta forma, se han empleado una gran diversidad de elicitores en ensayos pre-cosecha en campo, en tratamientos post-cosecha, así como en cultivos de células vegetales in vitro (Namdeo, 2007).

Existen una serie de trabajos publicados sobre el efecto de diferentes elicitores en productos vegetales. Por ejemplo, Hassini (Hassini et al., 2019) concluyen que la aplicación foliar de metionina en brotes de brócoli aumentó significativamente la biomasa vegetal y mejoró la nutrición mineral. Además, establecen que el K_2SO_4 y la hormona vegetal MeJA dieron la mejor respuesta en la acumulación de GLS indol.

Además, el uso de Metionina (MET) y SA como inductores y el suministro de K_2SO_4 aumentaron la abundancia de compuestos fenólicos.

Hyun-Jin Kim (Kim et al., 2006), investigaron el efecto de rociar MeJA de manera exógena sobre el brote de rábano (*Raphanus sativus* L.). El tratamiento con MeJA aumentó significativamente el contenido de fenoles totales que resultó en una mayor capacidad de eliminación de radicales libres DPPH•(2,2-difenil-1-picrylhydrazyl). Además, la actividad PAL (fenilalanina amoniaco liasa) también aumentó en un 60% a las 24 h después del tratamiento con MeJA.

Baenas (Baenas et al., 2016), aplicaron dosis de tratamiento inductores, que fueron el MeJA, JA y MET, con el fin de encontrar un tratamiento exitoso y factible para producir brotes brócoli y de rábano con niveles mejorados de glucosinolatos que promueven la salud. Los resultados que se encontraron en estas especies fueron bastante interesantes debido a su alto contenido en GSL totales, siendo 302.84 y 379.71 mg/g en peso fresco, en brotes de brócoli y rábano, respectivamente. El MeJA 10 µM fue un tratamiento inductor altamente efectivo, causando aumentos significativos en la concentración de flavonoides y fenólicos totales, en un 31% y 23%, respectivamente, en brotes de brócoli de siete días de edad (Pérez-Balibrea et al., 2011).

Moléculas de señalización, como el SA y el MeJA (Cohen and Flescher, 2009; Horváth et al., 2007) y polímeros de carbohidratos naturales, como el quitosano (Cho et al., 2008), exhiben actividad inductora e inducen altamente las fitoalexinas de defensa de la planta, lo que sugiere que el tratamiento de plantas con inductores podría ser una forma factible de desencadenar la biosíntesis de metabolitos bioactivos (Ramos-Solano et al., 2010; Zhao et al., 2005). Además, en diferentes especies de *Brassica*, se indujeron flavonoides, fenilpropanoides y glucosinolatos de indol por aplicación exógena de SA y MeJA (Baenas et al., 2014a; Bennett and Wallsgrove, 1994; Brader et al., 2001; Kiddle et al., 1994; Liang et al., 2006; Natella et al., 2016).

El quitosano (0.01%) indujo aumentos significativos en el contenido de vitamina C de los brotes de brócoli, aumentando en un 54% y 44% después de 5 y 7 días de tratamiento, respectivamente (Pérez-Balibrea et al., 2011). Lee (Lee et al., 2005) indicaron que la aplicación de quitosano en brotes de soya aumentaron todos los parámetros de crecimiento medidos, incluida la longitud del hipocótilo, la longitud de la raíz y el grosor del hipocótilo. La aplicación de quitosano o biopolímeros derivados puede desempeñar un papel fundamental en la mejora en el crecimiento de las plantas principalmente a través de una mayor absorción de nitrógeno y nutrientes, esto debido a su actividad bioestimuladora que ha sido comprobada en varios cultivos, especialmente en especies vegetales que son más propensas a los factores estresantes. El quitosano puede usarse como una fuente adicional de carbono en los procesos biosintéticos de las planta (Mondal et al., 2013; Shahrajabian et al., 2021). Se ha demostrado anteriormente que el quitosano estimula el crecimiento y germinación en las plántulas, como los brotes de girasol y soja (Cho et al., 2008; Lee et al., 2005). Además , la aplicación de quitosano sobre las semillas de pearl Millet (Manjunatha et al., 2008), maní (YG Zhou et al., 2002), maíz (Guan et al., 2009), orquídeas (Kananont et al., 2010) mejoraron notablemente la germinación y el vigor de la plántula. La longitud de la raíz y la altura de la planta aumentaron significativamente con la aplicación foliar de quitosano en plantas de fresa (Rahman et al., 2018).

Sin embargo, los datos obtenidos sugirieron que la respuesta a la provocación depende de la especie (Scheuner et al., 2005).

1.12. Capacidad antioxidante y biomarcador de estrés oxidativo

Los polifenoles son los principales compuestos vegetales con capacidad antioxidante y también poseen las estructuras químicas ideales para la eliminación de radicales libres (Moure et al., 2001; Szajdek and Borowska, 2008). Los GSL y sus productos de hidrólisis son considerados antioxidantes indirectos, ya que no neutralizan los radicales libres directamente, sino que modulan la actividad de las enzimas metabolizadoras de xenobióticos (Vig et al., 2009). El ensayo ORAC permite expresar el aporte máximo de capacidad antioxidante de los glucosinolatos, con respecto a otros métodos de ensayo de capacidad antioxidante (Cabello-

Hurtado et al., 2012). Los métodos tradicionales para determinar capacidad antioxidante (DPPH, ORAC, FRAP, permiten conocer la capacidad antioxidante de los extractos, sin embargo, estos pueden ir acompañados de ensayos de medición de peroxidación de lípidos debido al afecto de las ROS, como son el ensayo del malondialdehído (MDA).

Durante la elicitación, el aumento de las actividades celulares conduce a la acumulación de especies reactivas de oxígeno (ROS) por encima de los niveles fisiológicos (Randhir et al., 2004). Las ROS causan daño a las moléculas celulares, por lo que desencadenan la activación de los sistemas de defensa antioxidantes, que juegan un papel clave para neutralizar las ROS y así evitar o reducir el posible daño oxidativo (Ampofo and Ngadi, 2020; Gao et al., 2019).

La sobreproducción de ROS puede conducir a la peroxidación de lípidos, que a menudo se controla midiendo el malondialdehído (MDA).

MDA se usa comúnmente como bioindicador de peroxidación bajo factores de estrés. Se ha demostrado que las antocianinas pueden mostrar capacidad antioxidante directa hacia ROS (Garcia and Blesso, 2021).) (Sajid Ali et al., 2019; Ayala et al., 2014).

2. HIPÓTESIS

1. El análisis bibliométrico brinda información sobre las características básicas de la literatura por medio de la evaluación del progreso y desarrollo de la investigación científica relacionada con las estrategias de elicitación para enriquecer brotes de plantas comestibles ricas en compuestos bioactivos.
2. El uso de alta temperatura y de inductores bióticos (quitosano, ácido cítrico, metil jasmonato y sulfato de potasio) por sí solos y en combinaciones de ellos mejoran los aspectos morfológicos, fisiológicos y compuestos bioactivos de los brotes de especies *Raphanus*.
3. La herramienta de análisis multivariado (Redes Neuronales Artificiales (ANNs)) nos permite predecir el comportamiento de algunas variables significativas frente a la acumulación de compuestos bioactivos.

3. OBJETIVOS GENERALES

1. Realizar un análisis bibliométrico de las publicaciones seleccionadas de la base de datos Web of Science (WoS).

2. Estudiar la estrategia de elicitación junto con modelos predictivos con el fin de aumentar la obtención de compuestos beneficiosos para la salud, como son los glucosinolatos y antocianos en brotes de rábano comestible y rábano silvestre cuando crecen a alta temperatura.

4. OBJETIVOS ESPECÍFICOS

1. Obtener información sobre la tendencia actual y futura en el desarrollo de la actividad científica relacionada con la elicitación en brotes de *Brassicaceae.*

2. Cultivar brotes de rábano comestible y rábano silvestre en condiciones controladas de cultivo y utilizando una alta temperatura por sí sola y en una combinación con elicitores bióticos (quitosano, ácido cítrico y metil jasmonato y sulfato de potasio).

3. Caracterizar la presencia y el contenido de bioactivos y nutrientes en los brotes mediante técnicas analíticas que incluyen cromatografía HPLC-DAD-ESI-MSn.

4. Estudiar los aspectos morfológicos, fisiológicos y compuestos bioactivos de los germinados como son el contenido de GSL y antocianinas totales e individuales.

5. Estudiar las actividades biológicas *in vitro* de los extractos en diferentes bioensayos de actividades antioxidantes (DPPH, ORAC), fenoles totales y evaluación del daño oxidativo inducido (ensayo MDA).

6. Aplicar una herramienta de análisis multivariado (Redes Neuronales Artificiales (ANNs)) para evaluar la respuesta de los brotes a altas temperaturas en combinación con la hormona metil jasmonato en cuanto a la capacidad antioxidante (DPPH y ORAC), contenido fenólico total (TPC) y estrés oxidativo (MDA).

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RESULTADOS Y DISCUSIÓN

CAPITULO II

Strategies of elicitation to enhance bioactive compound content in edible plant sprouts: A bibliometric study.

(Artículo publicado en Plants 2021, 10(12), 2759, https://doi.org/10.3390/plants 10122759)

Abstract

Vegetable sprouts are a food source that presents high content of bioactive compounds which can also be enhanced through elicitation mechanisms. To better understand the scientific production and research trends on this topic, a bibliometric analysis by means of the Web of Science database was carried out. The results showed significant growth in research on elicitation of edible plants sprouts. The three most productive journals were the Journal of Agricultural and Food Chemistry, followed by Food Chemistry and LWT-Food Science and Technology. The co-occurrence of keyword analysis of the different authors showed that the main research topics in this domain were 'germination', 'antioxidant activity', 'sprouts', 'glucosinolates' and 'phenolics'. The countries with the highest number of scientific publication were China, followed by India and USA. The productivity patterns of the authors conformed to Lotka's law. This study provides an overview of research on elicitation to enrich bioactive compounds in sprouts, and the needed to review and update the trends on this subject.

Keywords: phytochemicals; germinated; antioxidants; elicitation; bibliometric analysis

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1. Introduction

Plants are an indispensable part of our diet since they are a contribution of vitamins or minerals, essentials for a correct physiological function (Menaka Thakur et al., 2019). In recent decades, special attention has been given to a large group of biologically active phytochemicals used in a wide range of industrial applications (Alvarado et al., 2019; Ramirez-Estrada et al., 2016).

Synthesis of bioactive compounds is carried out through secondary metabolism, generally at low concentrations (< 1 % dry weight) and integrated from primary metabolites as common precursors at specific physiological and developmental stages. Therefore, yields of bioactive compounds can be affected by genetic, environmental, agronomic, or geographical factors and impurities (Halder et al., 2019).

Recently, there is an increased knowledge and understanding of the benefits and nutritional contributions provided by the bioactive compounds. However, researchers have gone further and have wondered how to carry out enrichment of bioactive compounds in plants, given that chemical synthesis of secondary metabolites becomes complicated due to the complexity and specificity of their structures, in addition to their high production costs (Halder et al., 2018).

In addition, currently there are a great interest in the market of bioactive compounds such as polyphenols and glucosinolates due to the wide range of uses in the food, pharmaceutical and cosmetic industries. However, obtaining large amounts of phytochemicals to develop new ingredients involves production cost, supply or seasonality problems and takes a long extraction times. All these factors must be considered to introduce products based on phytochemicals into the market (Patra et al., 2018). The cost of polyphenols of winemaking by-products is well known since grape trade dominate the polyphenol market, exceeding USD 700 million in 2015 (Sridhar and Charles, 2021). Although there are some studies on economic impact related to essential oils or phenolic compounds, the commercialization of isolated bioactive compounds is still scarce. However, elicitation techniques are a tool to accumulate bioactive compounds in plants obtaining an enriched raw material previously to extraction. Thus, different biotechnological tools have been used to increase the synthesis of secondary metabolites in plants, such as the optimization of culture media, cell cultures, micropropagation, hairy root cultures, elicitation, precursor feeding, or biotransformation. Among all these, elicitation strategy stands out as a powerful technique to increase bioactive compound synthesis manipulating metabolic pathways (Halder et al., 2019). An elicitor can be a chemical substance or physical factor capable of generating defensive morphological and physiological responses that increase or induce the synthesis of bioactive compounds (HongKai Liu et al., 2019; Menaka Thakur et al., 2019).

Depending on the type of elicitor, the process involves the exogenous application of stressors or inducers, for example, through radiation sources, ultrasound, and foliar irrigation, to the soil or the growing medium (HongKai Liu et al., 2019; HongKai Liu et al., 2021). This occurs by activating specific receptors in the plant cell membrane and initiating a signaling cascade ending in the expression of genes and transcription factors in the secondary metabolite synthesis pathway, increasing its concentration and accumulation. Thus, the plant is allowed to survive adverse conditions and adapt to te environment (Menaka Thakur et al., 2019).

Various studies have shown that sprouts grown from seeds during germination have great nutritional, biological, and medicinal values, evidencing an increase in the antioxidant, antidiabetic, anti-inflammatory, hypolipidemic, and anticarcinogenic activities of plant extracts (Baenas et al., 2014a; Singh and Sharma, 2017; Michał Świeca, 2016). Sprouts and micro-vegetables have higher bioactive compound content than mature plants (Halder et al., 2019; Menaka Thakur et al., 2019), due to the dilution of the phytochemicals resulting from the tissue expansion of a plant in the later development stage (AP Vale et al., 2015) (Figure 1).

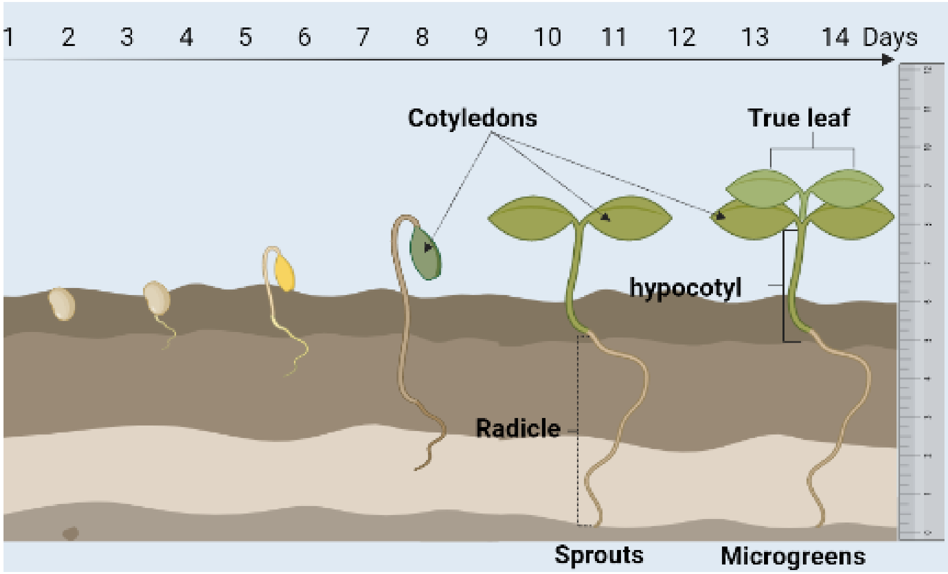

Figure 1. Developmental differences between sprouts and microgreens.

Evaluating advances in research on elicitation mechanisms in plant sprouts and how the synthesis of bioactive compounds is induced is crucial to identifying current food-related knowledge. As mentioned, trends in diets rich in bioactive compounds or phytochemicals as sprouts are highlighted. Techniques to enhance these endogenous compounds of plants during the growth period bring us new productive and innovative developments at the agronomic and commercial level because sprouts are characterized by being fast-growing and environmentally friendly. Therefore, these topics have become a priority for discussion by researchers, proposing future strategies to maximize the benefits of these plants (Table 1).

Published research in scientific journals or on websites are used by other researchers for their studies and cited as references in their subsequent articles. This enormous increase in the production of academic publications has generated a large amount of information that can be complex for readers. Therefore, the analysis of the scientific information emerging and past trends, leads to the need to address this vast information through mathematical and statistical methods, including bibliometrics analysis (Briner and Denyer, 2012; Broadus, 1987; Durieux and Gevenois, 2010; Rehn et al., 2008).

Bibliometrics can be defined as a sub-branch of informetrics, which is aimed to study the status or development of scientific research and the progress of a research field in countries, institutions, research centers, journals, and scientists themselves, detecting changes through patterns and interactions between articles, as well as analyzing through a set of mathematical and statistical methods (Broadus, 1987; Cobo et al., 2015; Durieux and Gevenois, 2010; Hui Liu et al., 2020). Since there is no organization and compilation of information regarding the studies on the elicitation mechanisms in sprouts, a bibliometric analysis of the selected publications of the Web of Science (WoS) database was carried out in the present study. This study was aimed to provide information on the basic characteristics of the literature and evaluate the progress and development of scientific research. A brief theoretical approach was first carried out to provide information on the definition of sprouts, ways to identify them, cultivation times, and some examples of different elicitors and their effects on the composition of bioactive compounds. In addition, this approach was followed by a descriptive statistics section. Finally, the bibliometric analysis was carried out, identifying some bibliometric indicators considered of great importance in the evaluation of scientific research, such as productivity analysis of countries, authors, documents and journals, collaboration indexes, and keyword co-occurrence analysis, all of them were analyzed with the support of RStudio software and visualized through the biblioshiny interface.

Therefore, it is necessary to review and update the research carried out, who directs them, what work networks exist, and what is the focus of the research in everything related to elicitation strategies to enrich edible plant sprouts with bioactive compounds since it is a topic that will continue to be a trend in the next years.

Table 1. Biotic and abiotic elicitors used in sprouts and the observed changes in bioactive compound contents after application.

Species	Bioactive Compound	Type of Stress	Dose	Variation	Authors
Kale	Phenolic Acid	Selenium	0.38 mM	+ 138 %	(Zagrodzki et al., 2020)
Yellow maize	Zeaxanthin	NaCl	300 mM	+ 21 %	(He et al., 2020)
Radish	Glucosinolates	Methyl Jasmonate	0.25 mM	+ 73 %	(Baenas et al., 2016)
Broccoli	Glucosinolates	Sucrose	176 mM	+ 82 %	(Natella et al., 2016)
Wheat	Phenolic Acid	Salix daphnoides extract	1 ml L^{-1}	+ 4 %	(Gawlik-Dziki et al., 2016)
Peanut	Resveratrol	Ultrasound	240 W	+ 940 %	(Yu et al., 2016)
Lentil	Phenolic content	Mannitol	0.0006 mM	+ 48 %	(M. Świeca, 2015)
Buckwheat	Phenolic content	Tyrosine	0.1 mM	+ 30 %	(M. Świeca, 2015)
Kidney Bean	Phenolic compounds	Glutamic acid	5 mM	+ 16 %	(Limón et al., 2014)
Lentil	Flavonoids	Phenylalanine	2,410 mg Kg^{-1}	+ 160 %	(Michał Świeca and Baraniak, 2014)
Broccoli	Vitamin C	Salicylic acid	0.2 mM	+ 26 %	(Pérez-Balibrea et al., 2011)
Mung bean	Antioxidant activity	Oregano extract	1 ml L^{-1}	+ 40 %	(Randhir et al., 2004)

The variations indicate the percentage increase in the synthesis of the bioactive compound after elicitation.

2. Results and discussion

2.1. Descriptive statistics and exploration of spatial-temporal data

To describe and provide an overview of the general characteristics of the dataset of the research field, a descriptive analysis was performed. After using the keywords presented in the methodology, a total of 787 studies indexed between 1992 and March 2021 in WoS were selected. During the study period, 2,248 Keywords Plus were identified among 2,738 authors, with an average of 3.48 authors per paper. The average number of coauthors (not first authors) per article was 4.54. A summary of the main information of the related articles is presented in Table S1. The first article in the selected data set was published in 1992 and since then, the research field has had an average annual growth rate of 43% over the 29 years, with an average production of 26.82 articles per year. The number of publications showed a gradual increase with some oscillations (Figure 2). From 2014 (where there is a leap in the number of publications) until 2020, the total number of articles increased sporadically reaching more than 120 publications, with an average growth rate of 25%. During the first quarter in 2021, 120 articles related to the topic have been published, highlighting the growing interest in the use of elicitors in plant sprouts. The increase in research output on the topic within that period can also be attributed to the need for researchers to find new sources of plant-derived bioactive compounds rapidly and efficiently, given the importance of these compounds in human health. In spite of this, safety issues related with bioactive compounds can be controversial due to the great potential they have for rapid auto-oxidation, and trigger high levels of active enzymes (Vilas-Boas et al., 2021).

For example, some authors have mentioned that experimental results demonstrated that elicitors were effective in inducing the production of phytochemicals (phenols, glucosinolates (Figure S1), anthocyanins, etc.) in sprouts, and these compounds were capable of inducing apoptosis in cancer cells (Amer et al., 2021; Ampofo and Ngadi, 2020; Viacava and Roura, 2015).

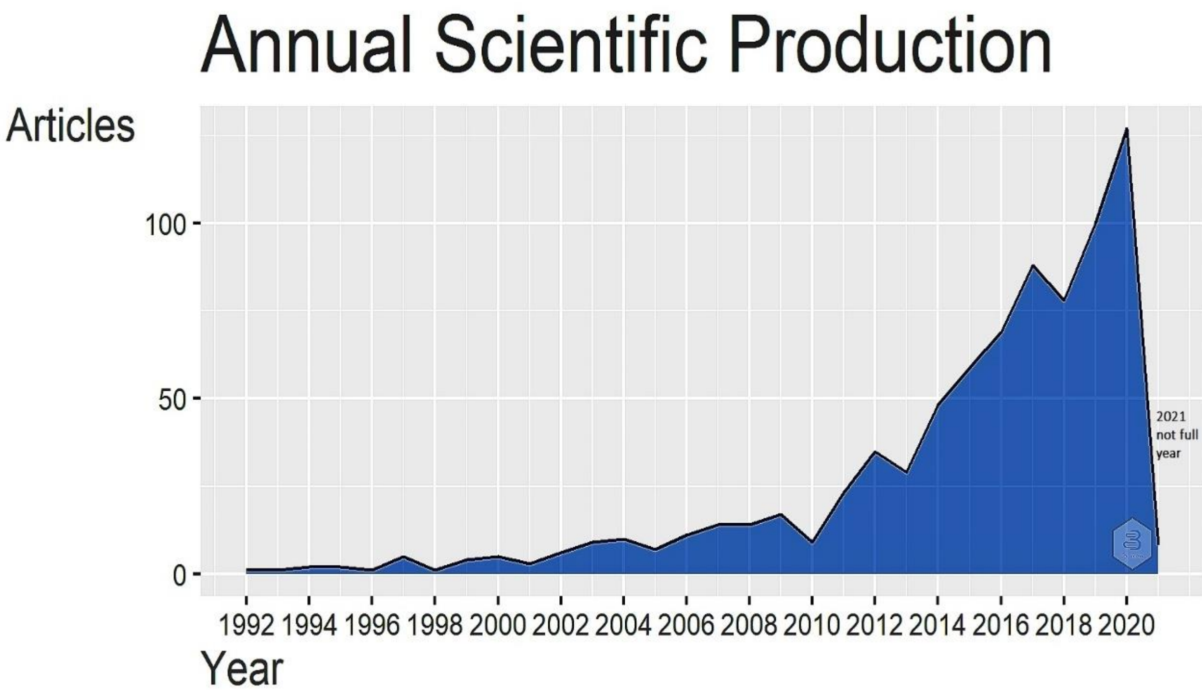

Figure 2. Number of the articles published on elicitation of sprouts in the WoS database between 1992 and 2020.

2.2. Bibliometric analysis

2.2.1. Production source

The great advances of this century have resulted in an evident development of human knowledge, which has been exposed to the world through journals that store the production of researchers. Scientific publication is an essential aspect of the progress or culmination of research, project, or thesis. This acquired knowledge allows the generation of academic exchanges a good communication of knowledge. In addition it must be useful, precise, valid, novel, and reproducible (Chen et al., 2021; El-Omar, 2014).

The origin and choice of a journal allow readers to rapidly understand the main content and conclusions of the research related in this case to the mechanisms of elicitation in sprouts and bioactive compounds. The articles obtained from the WoS database were distributed across 246 journals in total (Table S2). The top ten journals according to productivity indicators are shown in Table 2. *Journal of Agricultural and Food Chemistry* (*n*=42), followed by *Food Chemistry* (*n*=39), *LWT-Food Science and Technology* (*n*=32), *Journal of the Science of Food and Agriculture* (*n*=30) and *Journal of Food Science and Technology-Mysore* (*n*=23) were the journals with the highest number of publications on the subject. All of them coincide in their topics related to chemistry and biochemistry in the specific area of food and agriculture, also involving advances in methods and analytical approaches to research. The cumulative number of articles in some journals increased dramatically over time (Figure 2), highlighting the increase produced by the j*ournal of LWT-Food Science and Technology* from 2012 where a radical increase in the number of publications was observed. The journals with the most cited articles were *Food Chemistry* (*n*=2232), followed by *Journal of Agricultural and Food Chemistry* (*n*=1280) *LWT-Food Science and Technology* (*n*=399), *Journal of the Science of Food and Agriculture* (*n*=393) and *Journal of Food Science and Technology-Mysore* (*n*=233).

Table 2. Characteristics of the top 10 research journals according to some productivity indicators (*h-index, g-index* and *m-index*). TC represents total citations; NP number of publications, TC total citations; and PY_start is years of scientific activity.

Sources	*h-index*	*g-index*	*m-index*	TC	NP	PY_start
Journal of Agricultural and Food Chemistry	22	42	0.7333	2232	42	1992
Food Chemistry	22	35	1.4667	1280	39	2007
LWT-Food Science and Technology	12	19	1.2000	399	32	2012
Journal of the Science of Food and Agriculture	12	19	0.4800	393	30	1997
Journal of Food Science and Technology-Mysore	11	14	0.5500	233	23	2002
International Journal of Food Science and Technology	9	13	0.8182	202	20	2011
Molecules	10	18	1.0000	337	19	2012
Journal of Food Processing and Preservation	5	6	0.7143	57	17	2015
Scientia Horticulturae	9	16	0.8182	304	16	2011
Food Science and Biotechnology	5	10	0.3125	122	15	2006

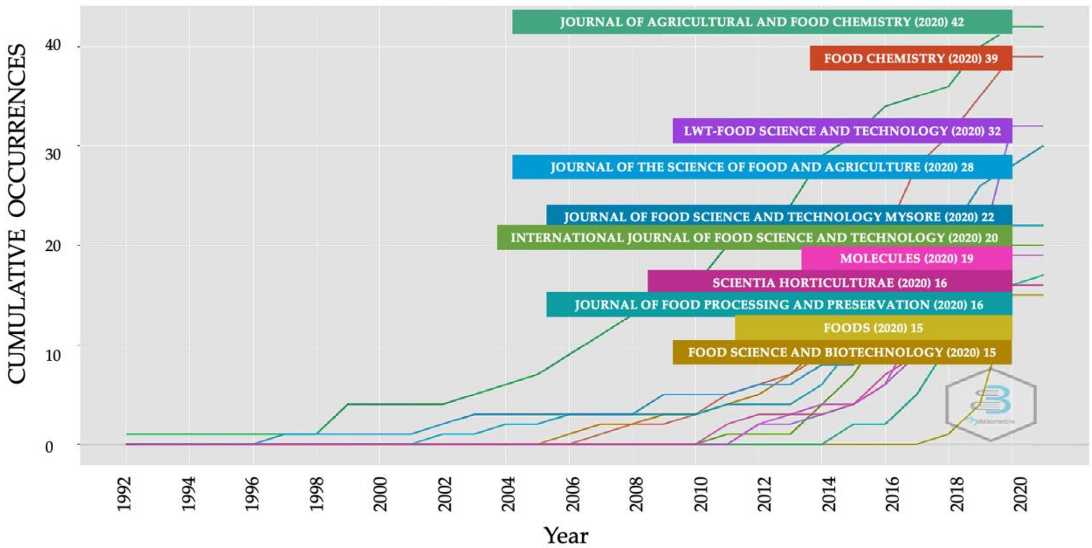

Figure 3. Cumulative occurrences of journals overtime.

2.2.2. Co-occurrence of author’s keyword

Keywords symbolize the primary units of research topics in a specific field and the central content of articles. In addition, they facilitate the search for authors and can provide better visualization of the dynamic structure of knowledge. On the other hand, keyword analysis entails a study of the critical points and predictions of future research trends of the most cited articles. Keyword co-occurrence analysis is referred to publications in which the same keywords (terms specified by the author) are listed in the same article. With co-occurrence analysis, a network can be mapped. Each node in the network represents a keyword and the link between the nodes represents the co-occurrence of the keywords (Azevedo et al., 2019; George et al., 2021; Su and Lee, 2010; Xu et al., 2021).

The results of the co-occurrence analysis of the top 50 author’s keywords are shown in a network visualization (Figure 4), using Louvain's clustering algorithm. The author’s keywords have a size proportional to the number of times expressed in terms of the number of occurrences. The colors represent the macro-area group in which the different items are agglomerated and the distance between the keywords is proportional to the relationship between the items. The link between the keywords represents the co-occurrence between them (i.e., keywords that co-occur or occur together), the thickness of the link indicates the occurrence of co-occurrences between keywords (i.e., the number of times keywords co-occur or occur together), the thicker the link between the nodes, the higher the co-occurrences between keywords.

Among the 2,252 keywords, the first 50 with the highest co-occurrence threshold have been studied. The most numerous clusters with orange color and the largest keyword were ‘germination’ (Figure 4), at the top of the list with *n*=468 co-occurrences and a high total linkage strength (588.4). It was followed by ‘antioxidant activity’ (*n*=275), ‘antioxidant’ (*n*=215), ‘sprouts’ (*n*=177), ‘glucosinolates’ (*n*=162), ‘phenolics’ (*n*=162), ‘phenolic compounds’ (*n*=120), ‘antioxidants’ (*n*=116), ‘phytochemicals’ (*n*=115), ‘sprouting’ (*n*=111), ‘seed germination’ (*n*=97), ‘elicitation’ (*n*=95) ‘soybean’ (*n*=94), ‘antioxidant capacity’ (*n*=92), ‘flavonoids’ (*n*=88), ‘broccoli’ (*n*=78) ‘glucosinolate’ (*n*=74), ‘activity’ (*n*=47), ‘broccoli sprouts’ (*n*=72) and ‘phenolic’ (*n*=71).

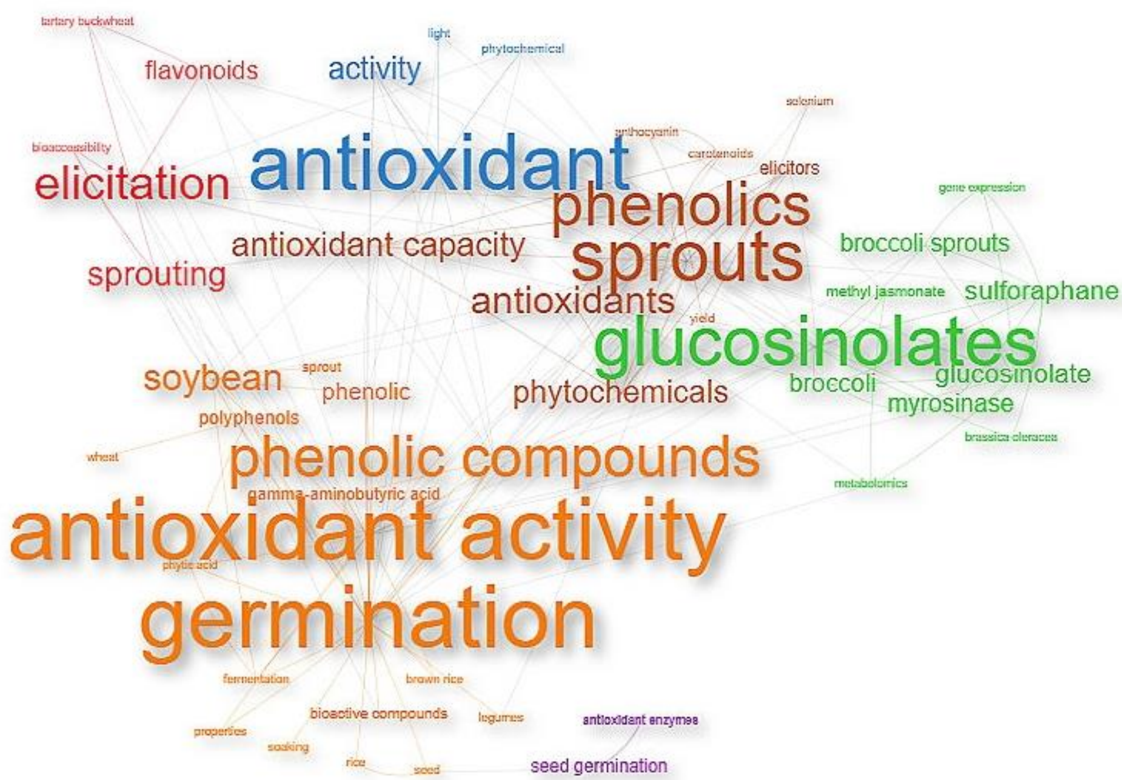

Figure 4. Co-occurrence of author’s keyword manifested on clusters.

This corresponded to a great indicator about the topic in areas related to agronomy, biochemistry, biomedical and food science, and technology.

It is possible to note the connection of the word ‘germination’ with many keywords related to the determination of the antioxidant activity and bioactive compounds of some species, though with less intensity with the word ‘elicitation’ or ‘elicitor’. This is reflected in Figure 4, where the terms are separated by macro-area. This suggests that, although many investigations resort to germination as a technique to obtain plant material, they do not necessarily use elicitors as a mechanism to promote the synthesis of bioactive compounds, (the objectives of their research do not merit it), demonstrating that the sprouts elicitation is a little-explored and fruitful field. It is important to note that a frequent topic studied by most of the articles was related to the determination of the antioxidant activity of sprouts or germinated, in addition to the quantification and determination of bioactive compounds, such as phenols, glucosinolates, or anthocyanins (Table S3). Mainly the growing interest in the determination of the antioxidant qualities of different plant sources has gained more attention from 1995 to the present, due to the known health benefits of consuming fruits and vegetables that contain high amounts of nutrients and phytochemicals (Hanbo Wang et al., 2017). These benefits are related to improved physiological functions or reduced risk of cardiovascular and neurodegenerative diseases, obesity, and cancer (Jincheng Wang et al., 2015). Nowadays, great efforts are being made to improve and enhance the synthesis of these compounds from plant sources. One of them is through elicitation mechanisms. Keywords related to the topic are listed in the same article several times.

Figure 5 shows the evolution of the main author’s keywords and the relationships that appear between them over time. The width of the streamlines is proportional to the number of keywords shared by the connected topics and reflects the degree to which they are related. Here, the new keywords that are changing, such as phytochemicals or glucosinolates are shown. The word ‘elicitation’ begins to be used strongly in the second period, appearing in subsequent periods together with words such as ‘sprouts’ or ‘seed germination’, the latter being the word with the highest outgoing flow count.

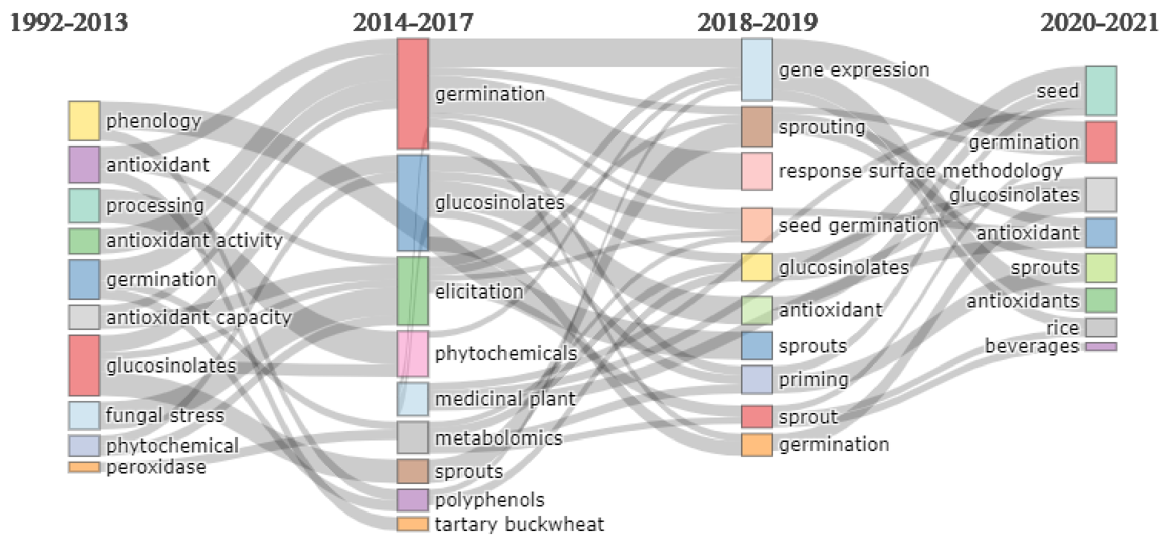

Figure 5. Thematic evolution of the author’s keywords.

In addition, the conceptual structure of the field was mapped using Multiple Correspondence Analysis (MCA) (Figure 6). MCA is a multivariate exploratory technique for the graphic and numerical analysis of multivariate categorical data (Aria and Cuccurullo, 2017). A two-dimensional graphic map was prepared with the 50 most relevant author’s keywords, considering the similarities in the distribution of them. The results are interpreted based on the relative positions of the points and their distribution along the dimensions. The more similar these words are and the closer they are to the map, the better they will be represented (Ampah et al., 2021; Aria and Cuccurullo, 2017). The broadest cluster corresponds to blue color and represents the keywords belonging to the disciplines of a central theme that have been the object of greater attention and research, though the red cluster shows the areas that have received less investigation. The closer the points are on the chart, the more similar the distribution of keywords will be, involving repeated coexistence in articles. On the other hand, the keywords closest to the central point indicate that they are outstanding in the field of study, whereas the furthest ones are more related to other research topics.

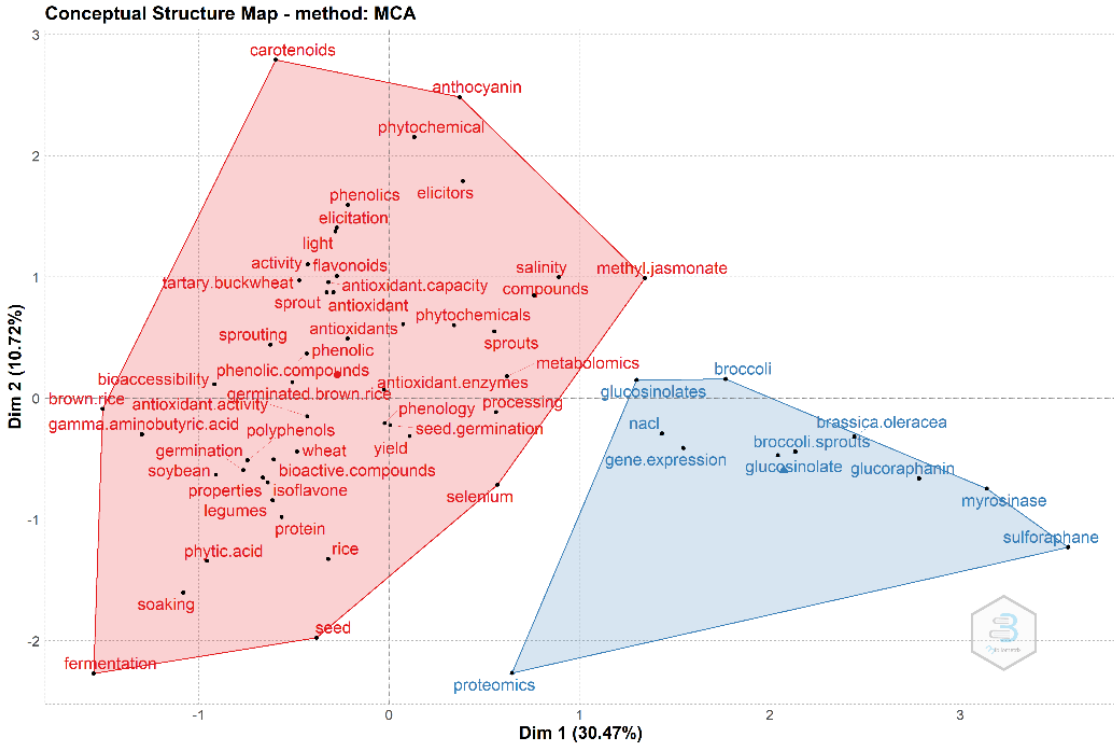

Figure 6. Conceptual structure plot using Multiple Correspondence Analysis (MCA).

2.2.3. Most relevant publications

Imported records represent a collection that can be classified according to the number of local references and citations (LC, within the knowledge domain) and also by the number of global citations (GC, within and outside the knowledge domain) (Garfield, 1999). The top 10 documents in terms of LC and GC are presented in Table 2. The average publication time of these articles was 18 years, where the shortest is 6 years, i.e. Lucía Plaza (Plaza et al., 2003) with 15 LC and 100 GC, and Montserra Dueñas (Dueñas et al., 2015) with 14 LC and 49 GC. The most-cited article with 40 LC and 152 GC was from the US researcher Reena Randhir in collaboration with researchers from the same country, published in Process Biochemistry (Randhir et al., 2004), they investigated and evidenced that the phenylpropanoid pathway was stimulated in mung bean sprouts by natural elicitors such as fish protein hydrolysates, lactoferrin and oregano extract. In addition, they also found that elicitation significantly improved the phenolic, antioxidant and antimicrobial properties of mung bean sprouts. The second most-cited article with 25 LC and 72 GC was international collaborative research conducted by Patricio J Cáceres from Ecuador and Martínez-Villaluenga C, Amigo L and Frias J, these last three Spanish researchers, published in *Food Chemistry* (Cáceres et al., 2014). They investigated the phytochemical content and antioxidant activity of Ecuadorian brown rice sprouts at different temperatures and hours of darkness. This research group found that germination improved γ-aminobutyric acid, total phenolic compounds, and antioxidant activity in all cultivars. The top 10 most-cited articles agree that elicitation or stress induced in edible sprouts improves the biological activity and boosts the levels of bioactive compounds. These sprouts can be consumed directly as 'ready to eat food' or incorporated to staple foods. Moreover, sprouts have been widely used in the prevention of chronic and non-communicable diseases (Bahadoran et al., 2012; López-Chillón et al., 2019).

The differences between GC and LC could be the result of the relationship that these articles have with other themes or subjects beyond that of elicitation in edible sprouts, such the endogenous variations of bioactive compounds (glucosinolates, phenols, etc.) and the implications of the Anti-Tumor-Promoter effect of these compounds. In essence, this shows that Global citation more related to external themes than internal ones.

Table 3. The 10 most locally (LC) and globally (GC) cited articles.

Position	Publication year	Author's name	Title	Source	Country	LC	GC	Authors keywords
1	2004	Randhir R, Lin Y-T, Shetty K.	Stimulation of phenolics, antioxidant and antimicrobial activities in dark germinated mung bean sprouts in response to peptide and phytochemical elicitors.	*Process Biochemistry*	USA	40	152	Mung vean (Vigna radiata); PPP (pentose phosphate pathway); ElicitorsFPH (fish protein hydrosylates); Lactofertinoregano extractG6PDH (glucose-6-phosphate dehydrogenase); GPX (guaiacol peroxidase); Sprouts, Phytochemicals, Peptides; Phenolics, Antioxidants, Antimicrobials, Helicobacter pylori.
2	2014	Cáceres PJ, Martínez-Villaluenga C, Amigo L, Frias J.	Maximising the phytochemical content and antioxidant activity of Ecuadorian brown rice sprouts through optimal germination conditions.	*Food Chemistry*	Ecuador	25	72	Brown rice; Germination; gamma-Aminobutyric acid; Phenolic compounds; Antioxidant activity; Response surface methodology.
3	2012	Lim J-H, Park K-J, Kim B-K, Jeong J-W, Kim H-J.	Effect of salinity stress on phenolic compounds and carotenoids in	*Food Chemistry*	Republic of Korea	23	102	Antioxidant; Buckwheat sprout; Carotenoid; Fagopyrum esculentum; NaCl;

			buckwheat (*Fagopyrum esculentum M*.) sprout.					Phenolic compound; Salinity stress.
4	2014	Baenas N, García-Viguera C, Moreno DA.	Elicitation: A Tool for Enriching the Bioactive Composition of Foods.	*Molecules*	Spain	18	118	Elicitor; Phytochemicals; Health; Phenolics; Glucosinolates; Activity.
5	2003	Plaza L, de Ancos B, Cano PM.	Nutritional and health-related compounds in sprouts and seeds of soybean (Glycine max), wheat (*Triticum aestivum.L*) and alfalfa (Medicago sativa) treated by a new drying method.	*European Food Research and Technology*	Spain	15	100	Sprouting; Alfalfa; Soybean; Wheat; Vitamins; Minerals; Phytoestrogens.
6	2004	Kaukovirta-Norja A, Wilhelmson A, Poutanen K.	Germination: a means to improve the functionality of oat.	*Agricultural and Food Science*	Finland	15	68	Oat; Germination; Processing; Bioactivity.
7	2007	Khattak AB, Zeb A, Bibi N, Khalil SA, Khattak MS.	Influence of germination techniques on phytic acid and polyphenols content of chickpea (*Cicer arietinum L*.) sprouts.	*Food Chemistry*	Pakistan	15	69	Chickpea; Germination time; Illuminations; Phytic acid; Polyphenols.

8	2014	Świeca M, Sęczyk Ł, Gawlik-Dziki U.	Elicitation and precursor feeding as tools for the improvement of the phenolic content and antioxidant activity of lentil sprouts.	*Food Chemistry*	Poland	15	34	Elicitation; Bioaccessibility; Precursor feeding; Sprouting; Antioxidant Activity; Phenolic overproduction.
9	2015	Dueñas M, Martínez-Villaluenga C, Limón RI, Peñas E, Frias J	Effect of germination and elicitation on phenolic composition and bioactivity of kidney beans.	*Food Research International*	Spain	14	49	Kidney beans; Germination; Elicitors; Phenolic compounds; Flavonoids; ACE inhibition activity; Antioxidant activity
10	2006	Fernandez-Orozco R, Piskula MK, Zielinski H, Kozlowska. H, Frias J, Vidal-Valverde C.	Germination as a process to improve the antioxidant capacity of *Lupinus angustifolius L.* var. Zapaton.	*European Food Research and Technology*	Poland	13	58	Lupin; Germination; Antioxidant capacity; Vitamin C; Vitamin E; Polyphenols.

2.2.4. Productivity analysis and collaboration networks between countries

The productivity analysis in terms of the total number of publications per country indicated that China (*n*=150) is the leading country in terms of the total number of publications, accounting for 19.31% of the total number of articles, followed by India (*n*=85) and USA (*n*=60). The list of the 10 most productive countries and international collaborations according to different ranges of MCP (Multiple Country Publications) and SCP (Single Country Publication) is presented in Table S4. The percentage of MCP for China was only 16.7 % and in the case of India was 8.2 % showing that there is little collaboration between researchers from different countries. However, it is possible to highlight the participation of Spain, where 35% of its publications were in collaboration with other countries. The distribution of countries according to the number of publications produced in collaboration or not with other countries is shown in Figure 7A.

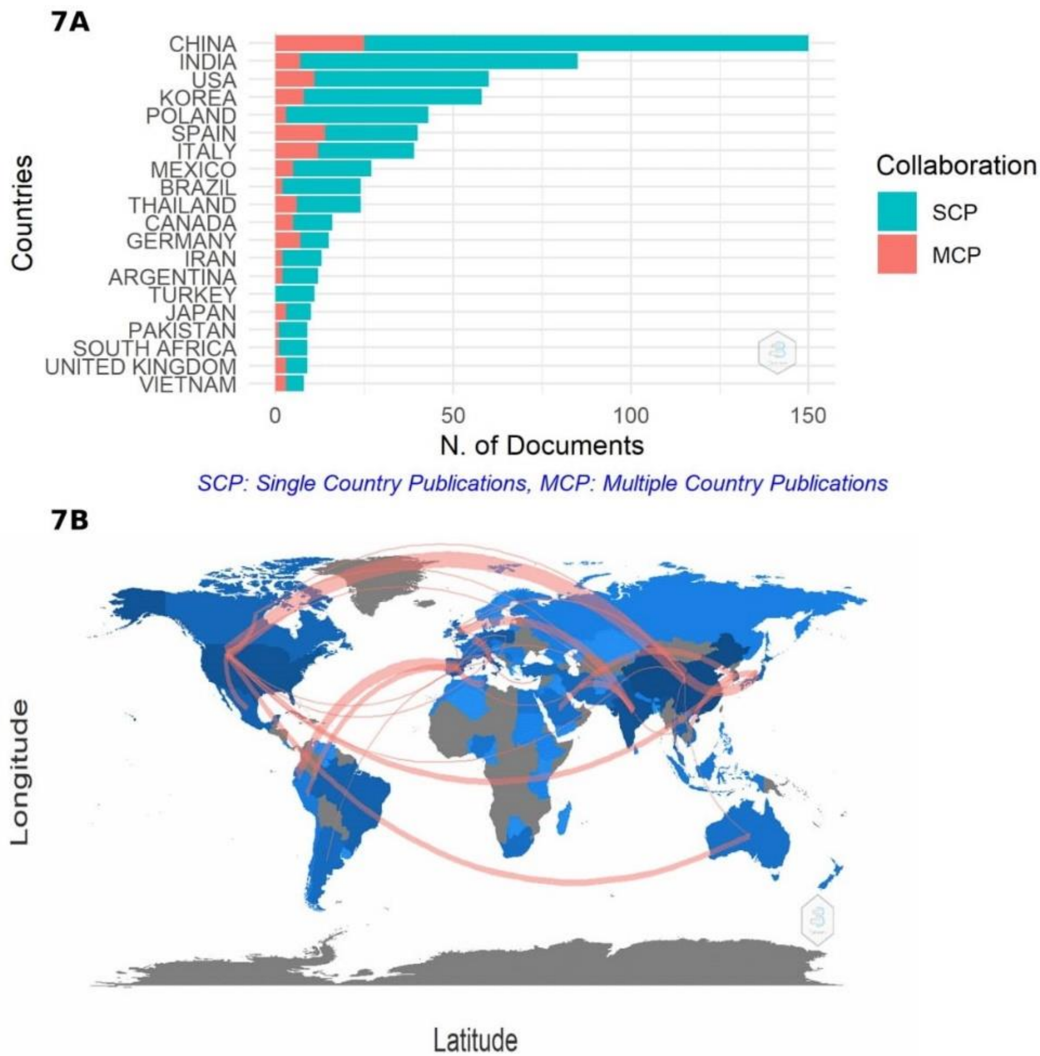

Figure 7. Distribution of the number of publications from single and multiple countries (A). Frequency collaboration network between countries working with elicitation in plant sprouts (B).

(The red links represent the collaborative associations between different countries. The blue color represents the number of publications. The darker the color, the greater the number of publications).

Based on the citation number, the countries with the highest number of publications in the world related to elicitation in sprouts were China (*n*=2604), followed by the USA (*n*=2537), and Spain (*n*=1076).

From the collaboration map (Figure 7B) it can be emphasized that the highest rate of collaboration takes place from USA to China (10 articles) followed by Spain to Ecuador (6 articles) and Japan to China (5 articles). Collaborative associations between developed countries and other countries are becoming more frequent, strengthening, and accelerating the rates of progress of developing countries, although these are still low according to the graphs. In addition, it is observed that institutions from developing economies tend to choose co-authors from institutions of international excellence. This suggests that for future research the authors should consider joint research with universities or entrepreneurial institutions in such contexts and increase collaborative networks.

2.2.5. Analysis of productivity in the authors

The authors were categorized under different performance indexes. A total of 2,738 researchers authored these 787 articles. Only the 10 most productive authors wrote a total of 123 articles, whereas the rest of the 664 articles were written by other authors.

In general, few authors are responsible for most of the articles and association can be modeled by using Lotka's Law. Lotka's Law, one of the basic laws on informetrics was given by Alfred Lotka in 1926. It explains the scientific productivity of authors, which indirectly can help to calculate the publication frequency of researchers (authors) in each area. In simple words, it states "the frequency of publications by authors in a research domain" (Lotka, 1926).

The empirical and theoretical frequencies for the total authors who published the selected articles shows in Figure 8. Consequently, data conform to Lotka's law. To validate the observation, a beta coefficient β=2.550, a constant C=0.424, and a statistical verification with a Kolmogorov-Smirnoff goodness-of-fit test was obtained resulting in 0.911 and a p-value of 0.076. Lotka's Law applied to the productivity of elicitation research in sprouts and the outcome showed how this issue will continue to be a topic of interest. 85.1% of the authors contributed to only 1 article (*n*=2331), whereas the percentage of authors contributing to 2, 3 and 4 articles was 9.6% (*n*= 262), 2.3% (*n*= 62) and 0.9% (*n*= 25) respectively.

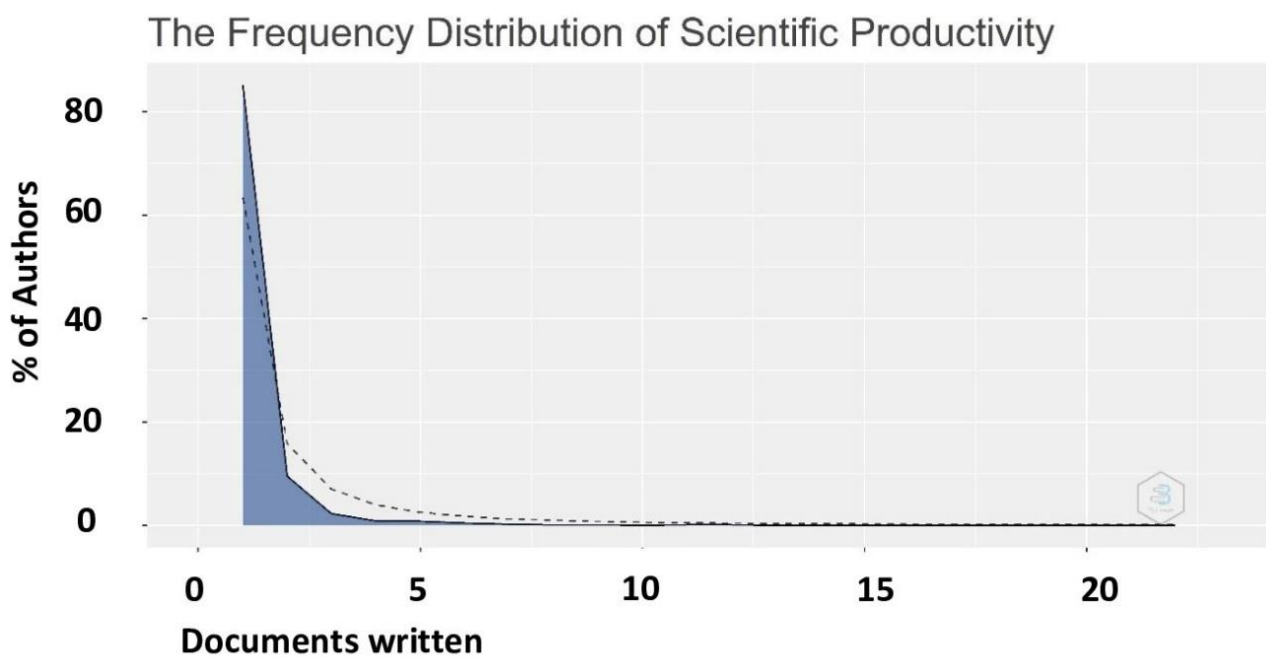

Figure 8. Frequency of authors publishing a given number of articles. The blue area corresponds to the empirical or observed data and the dotted line represents modeled or theoretical relationship according to Lotka's Law.

According to Van Raan AF (Van Raan, 2006), several indicators in different aspects of performance and to provide a more adequate and multifaceted picture of reality are needed. Therefore, creating an analysis of the scientific performance or productivity of an author in each area of study according to his/her scientific productivity based on the number of publications may not be representative of the overall quality of the researcher. Hence, other indicators are used jointly to evaluate the relevance of an author for the scientific community, such as the impact indicators of publications according to the number of citations and indicators such as the dominant factor in a defined area (Aria and Cuccurullo, 2017; Massaro et al., 2016).

Accordingly, the *h-index* factor can be used to evaluate the productivity and impact of the researcher. The *h-index*, or Hirsch index (Hirsch, 2005), indicates the number of publications that have received at least h citations. The number of times a publication is cited is a viable indicator that has been used to represent its influence in a research community and a subject area (Olisah and Adams, 2021; Tahim et al., 2016).

The *h-index* is considered a robust, stable, and suitable indicator for assessing productivity (Vanclay, 2007). It is easy to calculate and is capable of evaluating researchers with a single number (Cabezas-Clavijo and Delgado-López-Cózar, 2013). It has great simplicity of calculation based on the WoS database, so it has been accepted and admitted by the scientific community (Bornmann and Daniel, 2007). In addition, the *h-index* evaluates the relevance of publications over time (Vanclay, 2007) through the articles published and the citations received by the articles. Therefore, the values of *h* increase over time (Bornmann and Daniel, 2007). However, the *h-index* has certain disadvantages, mainly its inability to discriminate between active and inactive (or retired) scientists and its weakness in distinguishing between papers that were significant in the past and papers that are "trendy" today (Egghe, 2007). Therefore, it is evident that the *h-index* of a scientist depends on the scientific age of the author (Glänzel, 2006). Moreover, it is also inappropriate when comparing authors from different research fields (Kelly and Jennions, 2006).

Since the *h-index* factor is susceptible to some limitations, evaluations are often supplemented with *g-index*, *m-index*, and other varieties of *h-index*. The *g-index* proposed by Egghe (Egghe, 2006, 2007), considers the performance of the top articles (Bornmann et al., 2008). Thus, the e *m-index* is a variant of the *h-index* and is defined as the *h-index* of an individual divided by the number of years since his or her first publication (Choudhri et al., 2015).

Another way to calculate scientific productivity is according to the dominance factor. The dominance factor (DF) is a ratio that indicates the fraction of articles by several authors in which an academic appears as the first author

Since the main authors have contributed the maximum work in the selected area, their works have been reviewed to understand the dimensions and trends of the field, taking into account the general results according to the different productivity evaluation criteria.

The main contributions of the most influential first author in co-authored with the second most influential author deal with the effects of elicitation on the main health-promoting compounds. The results of one of his articles showed that the phenolic content and the antioxidant potential of lentil sprouts can be improved by treating under abiotic stress conditions using UV-B as an elicitor, without having any negative influence on the nutritional quality of the lentil sprouts (Michał Świeca et al., 2014). Other biotic elicitors used by the author were H_2O_2, mannitol, NaCl, arachidonic acid, jasmonic acid, and abscisic acid (M. Świeca, 2015). Other more influential authors they investigated the effects of $CaCl_2$ on glucosinolate metabolism and isothiocyanate formation, as well as the antioxidant capacity of broccoli sprouts. The results showed that the treatment with $CaCl_2$ increased the biosynthesis of glucosinolates, as well as proimoting the gene expression of myrosinase, triggering an increased formation of isothiocyanates. As a consequence, an increase in the antioxidant capacity of the sprouts was observed (Runqiang Yang et al., 2016). In another of their articles, they evaluated the effect of growth temperature on glucoraphanin and sulforaphane activity in broccoli sprouts. The results established that the sprouts grown at 25 °C had higher glucoraphanin content and sulforaphane formation than those grown at 20 and 30 °C (Guo et al., 2014). The effect of glucosinolate activation in sprouts depends on the effects of activation of genes that enable glucosinolate and myrosinase biosynthesis (HongKai Liu et al., 2019).

Finally, several authors have documented the efficacy and importance of elicitation in sprouts to enhance secondary metabolites biosynthesis and accumulation. Fo instance, the effectiveness of the use of UV-B radiation as an elicitor in the accumulation of vitamin C, phenolic compounds, and flavonoids in mung bean (Vigna radiata) sprouts was shown (Hanbo Wang et al., 2017). The increase in phenolic compounds could be attributed to de novo synthesis and transformation. Inducers could stimulate the biosynthetic pathway of phenolic compounds by triggering the accumulation of phenolic compounds in sprouts, through the participation of the enzyme phenylalanine ammonialase (PAL) (HongKai Liu et al., 2019).

Other authors determined that sucrose was an effective elicitor to induce the synthesis and accumulation of glucosinolates, phenolic compounds, flavonoids, anthocyanins, and vitamin C in broccoli (*Brassica oleracea L*. var. botrytis subvar. cymosa) sprouts (Natella et al., 2016). On the other hands, it was found that an ultrasound treatment combined with germination can be an effective method of producing resveratrol-enriched peanut sprouts (Yu et al., 2016).

Consequently, elicitation emerges as a powerful tool to increase and accumulate both primary essential and secondary metabolites with high protective potential for the plant, also important for humans, since these molecules can act by reducing the oxidative stress state responsible for triggering chronic degenerative diseases. In addition, it is environmentally friendly, allows reducing transgenic technology, and is an alternative to conventional cultivation techniques, whose purpose is to enhance the synthesis of phytochemicals with protective effects for health and improve bioactivity in edible plants (Baenas et al., 2016; M. Świeca, 2015).

2.2.6. Limitations and future perspectives

The present research gives us a new insight of mechanisms of elicitation in edible sprouts, providing an overview of evolutionary thematic directions and research trends, however, there are some limitations. The literature used provides a wide range of research and topics on scientific articles published in the last three decades and indexed in WoS, although other scientific databases could be revised such as Scopus and Google Scholar.

The effect of elicitation on changes in the concentrations of bioactive compounds needs further investigation, due to the rapid development of omics sciences. Hence it would be possible to apply robust and novel techniques to study the effects of elicitation on bioactive compounds in sprouts and at the same time contribute more information in the elaboration of metabolic networks of inducers in sprouts.

The importance of bioactive compounds is due to the strong relation between ingestion of fruits and vegetables rich in these compounds and the decreased risk of developing chronic degenerative diseases (e.g. diabetes, neurodegenerative diseases, cancer, etc.). However, other investigations are need for understand how foods confer beneficial effects and the complexity developed by the exposome itself and by the interaction of human metabolisms and microbiota.

The optimal conditions of elicitors for each type of sprouts and bioactive compound require novel studies due to the specificity between inducers and sprouts. In addition possible synergistic effects for these compounds can be analyzed by data analytical techniques and the use of AI-based algorithms.

3. Materials and Methods

3.1. Theoretical framework for the inclusion or exclusion of terms

To determine the inclusion or exclusion criteria it is important to previously define some concepts, including the concepts of sprout and microgreens since the terms are often confused. Sprouts are the product obtained from the germination of seeds and are harvested before the development of their true leaves. They can be consumed completely, including their seeds. On the other hand, microgreens are the product of seedlings whose cotyledon and true leaves have fully expanded (Benincasa et al., 2019). Both sprouts and microgreens are in a developmental stage where they contain low amounts of antinutrients. On the contrary, they are rich in nutrients such as essential amino acids, carbohydrates, and fatty acids from the enzymatic breakdown of macromolecules as well as an elevated amount of bioactive compounds (Benincasa et al., 2019; Galieni et al., 2020). For instance, mung beans can be grown in approximately 5 days obtaining mature sprouts of approximately 5 cm in length. Sprouts up to 8-9 cm long can be grown in 8 days, but longer growth of more than 10 cm should be avoided since they acquire a bitter taste in some species (Bass et al., 1988). On the other hand, Brassicaceae sprouts are generally harvested and marketed at 7-8 days of age after germination, considering that this young physiological state is optimal for consumption in terms of biomass and size. It allows manipulation, as well as concentrates a higher content of health-promoting compounds since sprouts have significantly greater concentrations of phytochemicals than mature plants (10-100 times) (Cevallos-Casals and Cisneros-Zevallos, 2010; Hanlon and Barnes, 2011; Pérez-Balibrea et al., 2008).

In contrast, it is also important to know and define the concept of elicitor. An elicitor can be a chemical substance or physical factor capable of generating defensive morphological and physiological responses, based on an increase in secondary metabolite synthesis.

They can be classified according to their nature, in biotic and abiotic elicitors; and according to their origin in exogenous and endogenous elicitors (Namdeo, 2007). The first biotic elicitors were described in the early 1970s (Keen, 1975). The biotic elicitor classification can present an undefined composition such as crude extracts of yeasts, fungi, and bacteria, and a defined composition such as purified polysaccharides, glycoproteins, chitosan, or alginate. Among the nature of abiotic elicitor, the variety is wide, for example, salts of metals such as $Ag_2S_2O_3$, $CuCl_2$, $NiSO_4$; osmotic stressors as mannitol, NaCl, KCl, polyvinylpyrrolidone; gases such as nitric oxide and ethylene; physical stressors like UV radiation, temperature, or drought and internal signaling molecules as jasmonic acid, methyl jasmonate or salicylic acid (Halder et al., 2019). On the other hand, elicitors of exogenous origin are found outside the plant cell. Glucomannose, polyamines, polygalacturonase, arachidonic acid belong to this group. Instead, the endogenous origin is formed via secondary reactions induced by biotic or abiotic signals. Hepta-β-glucosides or alginate oligomers belong to this group (Namdeo, 2007).

3.2. Database and software

In this section, the methodological techniques and software tools used in this study is explained. For this purpose, a set of publications indexed in Web of Science© (WoS) (https://webofknowledge.com/), from Clarivate Analytics related to the investigation of mechanisms of elicitation of secondary metabolite synthesis in sprouts has been collected, refined, processed, and analyzed. WoS covers more than 15,000 journals and more than 90 million documents (Merigó et al., 2015), and allows searching using keywords or co-occurrence analysis of terms (Reuters, 2006) tools.

This bibliometric analysis was developed with the support of RStudio software v.4.0.2, applying a quantitative analysis, structural, performance, and quality indicators (Durieux and Gevenois, 2010; Team, 2013). RStudio is one of the most widely used tools by researchers, data analysts, and analytical practitioners to perform statistical analyses. R integrates several packages and is updated almost daily, becoming a useful tool for performing meta-analyses such as bibliometric analyses (Forliano et al., 2021). Within RStudio, the Bibliometrix 2.2.1 package was applied since it is a useful R tool for the analysis of complete scientific maps and was developed by Aria and Cuccurullo (Aria and Cuccurullo, 2017). Recently, the Bibliometrix package has obtained increasing attention from academics and researchers from different areas and disciplines (Andreo-Martínez et al., 2020; Han et al., 2020; Radanliev et al., 2020; Yataganbaba et al., 2017). It provides tools that allow them to perform descriptive analyses from bibliographic data platforms. This package also provides several functions to facilitate the understanding and interpretation of network patterns, including the analysis of the different architectures of a bibliographic collection through conceptual, intellectual, and social structures (Durieux and Gevenois, 2010). The biblioshiny interface was applied, which is interrelated with Bibliometrix, facilitating its main functions, including the size of the labels and the color palette for better legibility of the graphics and images.

3.3. Data collection and extraction

The database used to obtain the scientific output information of the present bibliographic review was the WoS. The temporary search period was from inception 1975 to the end of December 2020. Limitations on the year were established to optimize comparability during the bibliometric analysis since the most recent studies have not had time to obtain an appropriate number of citations (Massaro et al., 2016). This study was carried out by an advanced search in WoS, in all languages and all document types. The searching syntax used was the Boolean operators' "TS" to indicate de topics, "AND" to link the two fields, and "OR" to combine the building and construction fields in searching the topics, and "NOT" for excluding a topic. Figure 9 provides graphical evidence of the words used in the search and the different phases of the data extraction activity). The citation indexes selected from the WoS main collection were Science Citation Index Expanded (SCI-Expanded), Book Citation Index-Science (BKCI-S) and Emerging Sources Citation Index (ESCI). This first selection included a total of 1,045 articles, filtered to avoid possible inconsistencies or errors. This was performed by identifying thematic areas of interest and a detailed examination of the abstracts to check if the articles identified in the first stage were suitable for analysis subsequently. The articles obtained with a complete record and cited references derived from data collection were exported in BibTeX format for subsequent filtering and analysis, where the duplicate documents were finally eliminated, leaving a final sample of 787 articles.

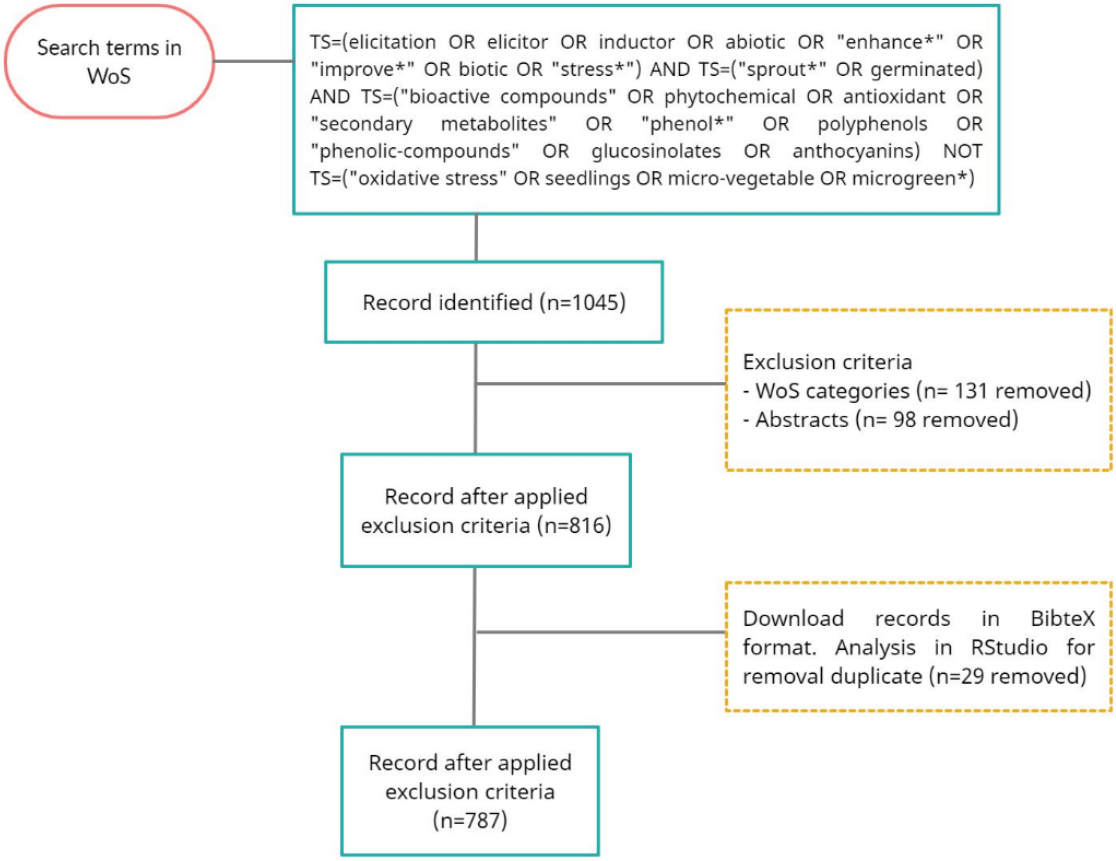

Figure 9. Data collection flow diagram.

3.4. Evaluation of scientific productivity using Lotka's law

To evaluate the distribution of the number of scientific articles published by the various authors in this study, Lotka's law: $F = c/N^{\beta}$ was used, which is commonly known as the scientific productivity law. This law describes the frequency F of authors who publish N articles in each field, where β and c are constants. Using the Lotka function, the coefficient β of the bibliographic collection can be estimated and evaluated through a Kolmogorov-Smirnov statistical test whether there is a similarity of this empirical distribution with the theoretical one (Aria and Cuccurullo, 2017; Lotka, 1926). R package Bibliometrix (Aria and Cuccurullo, 2017) was used for this analysis.

4. Conclusions

In this review, a description and characterization of plant sprouts and bioactive compounds was carried out, specifying how to differentiate them or identify their stage of growth and harvest as well as information on the classification of the different types of elicitors. It is noteworthy that this field of research is interesting and prominent since these enriched and ready-to-eat sprouts can be used in various clinical trials, due to their potential protective and preventive effects against inflammatory and oxidative processes. Secondly, a descriptive analysis of the selected dataset in WoS was performed, followed by a bibliometric analysis, which was carried out during the last 29 years, a period that has allowed generating knowledge and at the same time build a broad vision of many publications on the subject. Most of the selected publications correspond to widely referenced scientific articles. On the other hand, the topic has had a notable increase in the rate of publication in recent years, demonstrating that it is a preponderant and contingent topic. The journals with the highest productivity concerning the total number of articles were the *Journal of Agricultural and Food Chemistry*, followed by *Food Chemistry and LWT-Food Science and Technology*, being *Food Chemistry* the most-cited journal. These journals coincide in their topics related to chemistry and biochemistry in food and agriculture, showing that publications on the subject focus on specific issues rather than broader or more general topics. The research topics were identified from the perspective of keywords, according to the co-occurrence of the author's keyword analysis. The top five research topics in this domain were 'germination', 'antioxidant activity', 'antioxidant', 'sprouts' and 'glucosinolates'. However, words such as 'germination' and 'elicitation' were found with a lower intensity of connection and separated from the macro area. However, this id is not capable to suggest that they were not equally related. This study identifies the most influential publications through the analysis of the Local Citation and Global Citation, which provides unique insight for understanding the development trajectory and intellectual dynamics of the research of the mechanisms of elicitation in edible plants sprouts. The analysis of productivity by country showed that China is the leading country in the total number of publications, followed by India and the USA. However, even though scientific collaboration between countries has increased considerably throughout the 20th century, there are still few collaboration networks. On the other hand, it was found that the productivity patterns of the authors conformed to Lotka's law, demonstrating that the subject will continue to be of interest, and it is very likely that the number of publications will increase significantly in the coming years. In addition, productivity assessment indicators were used to address different aspects of performance and thus provide a more adequate and multifaceted picture of the reality, allowing to identify the research areas of the main authors. As world are facing serious food crisis and over-exploitation of natural supplies because of demographic and economic development, it is necessary greater focus on international collaboration between developed and less developed countries, to create knowledge on new nutritional alternatives. Finally, this study presents new insights in the research of elicitation in plant sprouts and its potential effects in improving the synthesis of bioactive compounds. Moreover, there are significant results of the research executed by the main researchers, which provides justification for future intensive research in aspects of food technology and science, considering its potential to ensure nutrition and protection of human health.

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CAPITULO III

Title: Enhancement of antioxidant responses and phytochemical accumulation combining elicitors and predictive models in Raphanus species growth under high temperature. (Artículo enviado a *Scientia Horticulturae*, 11/03/2023)

Abstract

Crop production is being affected by higher temperatures which can decrease food products or threat human nutrition. In the current study, edible and wild radish sprouts were subjected to temperature rising of growth combined with exogenous application of different elicitors to active defending mechanisms. Development traits, oxidative damage, glucosinolates and anthocyanin content, and antioxidant capacity accompanied by the development of a predictive model were evaluated. Accumulation of bioactives was highly promoted by the application of two elicitors, K_2SO_4 and methyl jasmonate (MeJa). High temperature combined with MeJa largely activated oxidative mechanisms. Hence, an artificial neural network was developed to predict the behavior of MeJa and temperature, building an adequate projection on plant growth responses. It is shown that the use of elicitors and predictive analytics represent an effective tool to study the response and improve the nutritional value of *Raphanus* species sprouts under future conditions of increased temperature.

Keywords: Antioxidant mechanisms, Methyl jasmonate, Artificial neural network, Abiotic stress.

1. Introduction

The genus *Raphanus*, belonging to the *Brassicaceae* family, consists of two species: *Raphanus sativus* L. (edible radish) and *Raphanus raphanistrum* L. (wild radish) (Schroeder, 1989). *R. sativus*, is an economically important vegetable that is widely cultivated throughout the world due to its excellent climatic adaptability and high nutritional value. On the other hand, the wild edible plant *R. raphanistrum* is known in human nutrition both as food and for medicinal purposes (Hedge, 1965). The *Brassicaceae* family stands out as unique because they are rich sources of glucosinolates (GSL), secondary metabolites containing nitrogen and sulfur compounds, which are almost exclusively present in plants of this family and involved in plant defense (Baenas et al., 2014a; Pérez-Balibrea et al., 2008). GSL and their hydrolysis products, isothiocyanates (ITC), have been reported to play an important role in cancer chemoprevention in a variety of cellular and animal models, probably due to their ability to induce phase 2 detoxification enzymes (Natella et al., 2016; Plumb et al., 1996). In plant cells, the GSL are separated from the myrosinases, enzymes responsible for their hydrolysis and act when there is an alteration of the tissue, for example, because of the chewing of fresh plants by both animals and humans. The enzyme *β*-D-thioglucosidase present in the intestinal microbiota is largely responsible for converting ingested GSL into its ITC and related indoles, biologically active molecules to which health benefits are attributed (Baenas et al., 2016; Dinkova-Kostova and Kostov, 2012). In addition, the *Brassicaceae* family contains a variety of nutrients and phytochemicals, including folic acid, vitamins (C and D), fiber, carotenoids, chlorophyll and a high concentration of bioactive phenolic compounds such as anthocyanins, all of which have an established role in the prevention of various chronic diseases (Björkman et al., 2011). In particular, anthocyanins belong to the flavonoid family, are pigments that are present in different plant organs, such as fruits, flowers and leaves. *Anthocyanins function as antioxidants, confer tolerance to different biotic and abiotic stresses.* In *Brassica*, the anthocyanins are mainly acylated cyanidins. Acylation of the anthocyanin molecule improves its stability through intramolecular and/or intermolecular copigmentation and self-association reactions (de Pascual-Teresa and Sanchez-Ballesta, 2008a), allowing vegetables rich in acylated anthocyanins to be a source of stability for applications in the food industry.

Under normal conditions, the synthesis of secondary metabolites in plants is limited, however, under stress conditions they accumulate a large amount of these compounds which can be increased or triggered through elicitors which induce physiological and morphological changes related to defense mechanisms (Baenas et al., 2014b; HongKai Liu et al., 2019; Verma and Shukla, 2015). Different negative abiotic factors present in the environment, such as high temperatures produce stress in plants and heat waves are expected to be more frequent, last longer and increase in intensity (Hoegh-Guldberg et al., 2018), which ends up affecting the yield of crops and biosynthesis and regulation of secondary metabolites. Some research shows that secondary compounds increase with controlled high temperatures (Verma and Shukla, 2015). Heat stress generally induces reactive oxygen species (ROS) accumulation and activation of detoxification systems (Serrano et al., 2019). If the temperature is too high, it triggers oxidative bursts that lead to a wide spectrum of responses, many physiological processes are slowed down or impaired, which induces the accumulation of antioxidants, which protect the cell membrane from breakdown and peroxidation until senescence. Therefore it is important to reach a balance between plant growth development and the mechanism defense. Researches have demonstrated that GSL content and other bioactive compounds in *Brassica* species increased at higher temperatures (Choi et al., 2014; Sim et al., 2022). Hence, the purpose of this study was to evaluate the influence of different elicitors (chitosan, citric acid, methyl jasmonate and potassium sulfate) in edible radish and wild radish sprouts, at a higher growth temperature (up to 10 °C above), on the morphological, physiological aspects and bioactive compounds of the sprouts such as the content of total and individual GSLs and individual anthocyanins, total phenolic compounds and capacity antioxidant, followed by an evaluation of the oxidative damage induced by the MDA assay, since it is one of the most used indicators of lipid peroxidation. Finally, considering the complexity of the interaction, a principal component analysis (PCA) to assess the conditions (type of elicitor) capable of providing the most significant overall effect on biomass and the phytochemical composition of edible radish and wild radish sprouts was used. Also, in order to optimize the elicitation process, a multivariate analysis tool (Artificial Neural Networks (ANNs)) was applied to evaluate the response of the sprouts to high temperatures in combination with the hormone methyl jasmonate in terms of the capacity antioxidant (DPPH and ORAC), total phenolic content (TPC) and oxidative stress (MDA). This research seeks to innovate in the agri-food industry and explore the behavior of *Raphanus* species exposed to high temperature conditions and foliar application treatments, according to the production and accumulation of phytochemicals framed in the issue of food safety.

2. Materials and methods

2.1. Plant material and treatments of sprouts

Edible radish (ER) seeds from Semillería San Alfonso SL (Santiago, Chile) and wild radish (WR) seeds from Lautaro city, Araucanía Region, Chile (Latitude: - 38.5167, Longitude: -72.45 38° 31′ 0″ South, 72° 27′ 0″ West) were used in the study. Seeds were soaked in sodium hypochlorite and distilled water, then weighed and spread in trays with coconut fiber as substrate. Four replicates per treatment were placed in a controlled environment chamber with a 16 h light to 8 h dark cycle and air temperatures of 20/15 °C (optimum) or 30/24 °C (higher), with 60 % RH during the day and 80 % RH at night, and PAR of 400 μmol m^{-2} s^{-1}. Chitosan, citric acid, potassium sulfate, and methyl jasmonate were used as chemical elicitors in combination with high or optimum temperature. Chitosan from crab shells was acquired from Sigma-Aldrich (Sigma–Aldrich, St. Louis, MO, USA). Citric acid, potassium sulfate (K_2SO_4) and methyl jasmonate (MeJa) were purchased from Merck (Merck, Darmstadt, Germany). Each elicitor was applied at a specific concentration by exogenous spraying (Hassini et al., 2019; Mahdavian, 2021; Pérez-Balibrea et al., 2011) with two controls at 20 °C (C_1) and 30 °C (C_2). Samples were collected 7 days after germination and frozen for analysis.

2.2. Physical parameters and sprouting potential

To evaluate the influence of elicitors on the physical properties of ER and WR sprouts, at the end of the experiment, fresh weight (g) and germination rate (%) were measured, the latter according to Ling (Ling et al., 2014). Radicle length (cm) was determined by measuring from the tip of the radicle to the base of the hypocotyl with a measuring ruler. Hypocotyl length (cm) of sprouts was determined by measuring the length of the hypocotyl with a Vernier caliper, the latter two were determined each day.

2.3. Sprout stress response to elicitors

Fresh germinated (100 mg) were crushed with a mortar. The homogenized tissue powder was suspended in 1.5 mL of 0.1 % TCA and centrifuged at 12000 g for 20 min at 4 °C. 0.5 mL of supernatant was mixed with 0.5 mL of a solution of 20 % TCA and 0.5 % thiobarbituric acid (TBA). The mixture was heated at 95°C in a constant temperature water bath for 30 min and then cooled in ice to ambient temperature. After centrifuging at 12000 g for 15 min, the supernatant was used to measure the absorbances at 440 nm, 532 nm, and 600 nm. The MDA concentration was calculated using its extinction coefficient 155 mM^{-1} (Heath and Packer, 1968; Hodges et al., 1999). The assays were performed using 96-well micro in a Synergy H1 hybrid multi-mode reader (Biotek, Winooski, VT, US). All the reagents were acquired from Merck (Darmstadt, Germany). Lipid peroxidation rate equivalents were expressed as nmol MDA mg^{-1} FW (fresh weigh).

2.4. Extraction of bioactive compounds

Bioactive compounds (GSL and anthocyanins) were extracted according to López (MD López et al., 2022), using different methods. GSL were extracted from freeze-dried samples (50 mg) using 1 mL of 70 % (v/v) boiling methanol. Then, they were heated at 70 °C for 30 min in a heating bath with shaking every 5 min. The extraction was stopped by placing the reaction mixture into an ice-water bath for 5 min. Finally, the extracts were centrifuged (17,500 g, 5 min). The supernatants were collected and filtered (0.45 μm PVDF). All samples were stored at −20 °C before analysis. To extract anthocyanins, an amount of 0.5 g of each sample was first poured into 5 mL of 25:24:1 (methanol: water: formic acid), stirred or vortexed for 5 min, and then placed in an ultrasonic bath for 1 h and kept overnight at 4 °C. Then, samples were centrifuged at 10,000 rpm for 10 min and filtered through a 0.22 μm PVDF membrane (Millex V13, Millipore, Bedford, MA, USA), before placing the extracts in amber vials for chromatographic analysis. All the solvents used in the extractions were of analytical grade and obtained from Merck (Darmstadt, Germany).

2.5. Analysis of glucosinolates and anthocyanins

GSLs were identified by using standards and following their UV–Vis spectra, retention times by HPLC-DAD, according to previous work by Baenas (Baenas et al., 2016) where the identification of their fragmentation patterns (M^- and MSn) had been carried out. Chromatograms were recorded at 227 nm, and intact GSL were quantified using glucoerucin and glucobrassicin as external standards for aliphatic and indolic GSLs, respectively (Sigma Aldrich, St. Louis, MO, USA). The analysis was carried out in triplicate and the results were expressed as mg 100 g^{-1} DW (dry weigh).

For anthocyanin analysis, identification was also performed in the HPLC-DAD-ESI-MSn system using previously established conditions for these compounds in cruciferous sprouts (Baenas et al., 2015). The extracted samples were quantified by a Hitachi HPLC-DAD system (Hitachi technologies, MERCK, Darmstadt, Germany) using the same chromatographic conditions, recording chromatograms at 520 nm, and using cyanidin 3-*O*-glucoside as external standard (Sigma-Aldrich, St. Louis, MO, USA). The analysis was carried out in triplicate and the results were expressed in mg 100 g^{-1} DW.

2.6. Antioxidant properties

2.6.1. Total phenolic contents

The total phenolic content (TPC) of the extracts was analyzed according to Singleton and Rossi (Singleton and Rossi, 1965). In microplate reader, Folin-Ciocalteu reagent, 0.5N, was mixed with extract solutions and purified water. It was shacked 30 seconds and incubated 5 min at 25°C in the dark. Finally, 10 % Na_2CO_3 was added, shacked for 30 s and incubated for 1 hour at 25°C in the dark. Then its absorbance at 750 nm was measured. The TPC of the extract were expressed as mg GAE g^{-1}.

2.6.2. Antioxidant capacity

The oxygen radical absorbance capacity ($ORAC_{FL}$) assay (María D López et al., 2018), as well as the 2,2-diphenyl-1-picrylhydrazyl (DPPH·) method (Mena et al., 2011) were used to measure free radical scavenging activities. Briefly, the antioxidant capacity for $ORAC_{FL}$ was evaluated by measuring the variation in fluorescence after 120 min of reaction with the radical. DPPH was evaluated by measuring the variation in absorbance at 515 nm after 30 min of reaction with the radical. The assays were performed using microplate reader previously described. The results were expressed as μmol Trolox g^{-1}. Four replications were carried out.

2.7. Statistical Analysis

To analyze the results collected for the two species, an analysis of variance (ANOVA) was used to observe significant differences. Before this analysis, the conformity of the data at each collection point with a normal distribution was examined using the Shapiro-Wilk test. In addition, Tukey's post hoc test was performed. The results were considered significant at $p < 0.05$. To elucidate the correlation between variables and discriminate the contents of GSLs, anthocyanins and other characteristics under different elicitation conditions in the two species, principal component analysis (PCA) was used through mean-centered data, using R software using the ggplot2 package for plotting. To better predict the behavior of the variables antioxidant capacity (DPPH and ORAC), the total phenolic content (TPC) and the behavior of the oxidative stress biomarker malondialdehyde (MDA), a three-layer neural network (ANN) was applied: an input layer, an output layer, and a middle or hidden layer. In an ANN model each neuron aggregates the weighted inputs from different paths and then applies a transfer function to the sum; the resulting value is then directed via output paths to other neurons. A series of layers are formed with neurons called multilayer perceptrons (Idris et al., 2022). This array consists of an input layer representing the number of input parameters (ie, temperature, *Raphanus* species, and the MeJa elicitor). All statistical analyzes were performed using the R software (v. 4.0.5).

3. Results and discussion

3.1. Sprouts growth performance and malonyl-dialdehyde status affected by stress

Morphological parameters were evaluated during 7-day germination, including radicle and hypocotyl growth, fresh weight, and germination index (supplementary Tables S1, Table S2, Fig. S1. and Fig S2.). All treatments showed radicle emergence and significant elongation over time. After 7 days of sprouting, improving these parameters, an increase of 45.83 % and 6.46 % was observed up to the length of the radicle (ER and WR, respectively) (Fig. S1A and Fig. S1C) and an increase of 66.83 % and 38.54 % up in the hypocotyl (ER and WR, respectively) (Fig. S1B and Fig. S1D) compared to C_2. Applying chitosan with high temperature (30°C) resulted in greater hypocotyl and radicle length from day 5 of sprouting, with a significant interaction of indicators ($p<0.05$) in both species. Chitosan can improve plant growth and nutrient uptake since can be used as an additional source of carbon in plant biosynthetic processes, but response depends on the species (Mondal et al., 2013; Shahrajabian et al., 2021). Citric acid elicitor at high temperature (30°C) significantly affected plant biomass and germination index, with total biomass production 3 times greater in ER species (Malik et al., 2010) (Fig. S2A, Fig. S2B, Fig. S2C and Fig. S2D). The ER species obtained high germination rates (greater than 70 %) in all treatments. Citric acid had positive effects beyond pH modulation, and higher temperature had positive effects on the morphological characteristics of the sprouts(Tahjib-Ul-Arif et al., 2021).

During elicitation, increased cellular activities lead to the accumulation of reactive oxygen species (ROS) above physiological levels (Randhir et al., 2004). MDA was used as an indicator of the degree of membrane lipid peroxidation, which is caused by higher levels of reactive oxygen species (ROS)(Sajid Ali et al., 2019; Ayala et al., 2014). The treatments with MeJa/30 °C and citric acid at 20 °C and 30 °C had a significant effect on MDA compared to C_1 and C_2 in ER ($p<0.05$) (Fig. 1) being approximately 40.02 % higher than the controls. In the case of WR only the MeJa/30 °C treatment showed a significant effect, where the MDA content was 66.91 % higher compared to C_2 (Fig. 1). Sakamoto and Suzuki (Sakamoto and Suzuki, 2019) found that MDA concentration increased with foliar MeJa application on radish sprouts. This situation is explained due to MeJa plays a key role in ameliorating oxidative stress, promoting enzyme activities and increasing the expression levels of defense-related genes in plants(Rui Liu et al., 2022). According to Swieca (Michał Świeca et al., 2014), the exposure of plant cells to elevated temperatures (30°C) induces oxidative stress through peroxidation of polyunsaturated fatty acids to harmful aldehydes such as MDA.

The results obtained in this study suggested that the application of elicitors caused oxidative stress in sprouts of ER and WR, causing the increase of active oxygen species. To avoid to destroy cell membrane structure due to increased ROS the elicitation has to be controlled. Analysis of variance showed that the interaction between elicitor and temperature had a significant effect ($p<0.05$) on MDA content in both species.

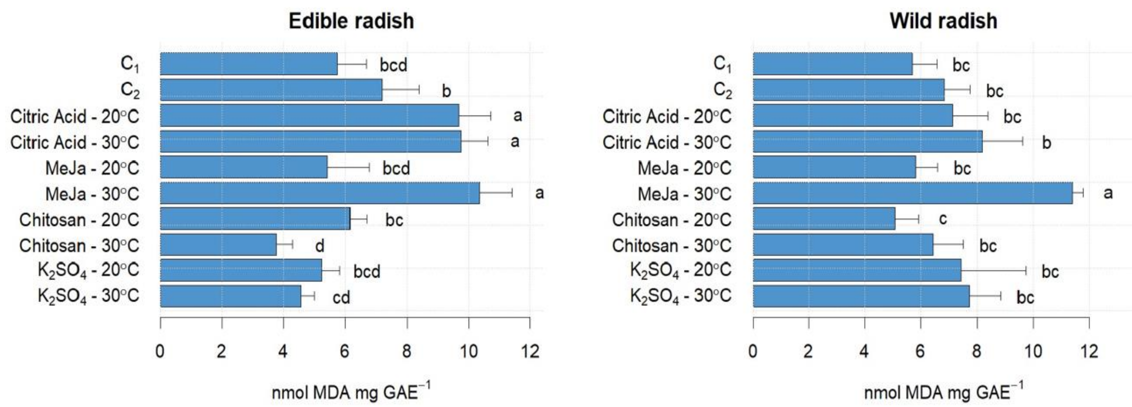

Figure 1. Oxidative stress (MDA content) in sprouts of ER and WR.

3.2. Bioactive compounds in ER and WR sprouts

3.2.1. Glucosinolates

The 7-day-old radish sprouts showed different GSL profiles (Table 1). GSL ranged from 51.01 to 81.77 mg 100 g^{-1} DW, and from 44.82 to 103.54 mg 100 g^{-1} DW in ER and WR, respectively.

Table 1. Comparison of the content of GSLs of ER and WR after foliar application of the combination of different elicitors and high temperatures.

Edible radish								
Treatments		Glucosinolates (mg 100 g^{-1} DW)						
Elicitor	Temperature	GRE	4-HGB	DER	GB	MGB	TGLs
C_1	20°C	32.17 ± 7.016cd	5.20 ± 0.928bc	7.52 ± 0.627ab	2.93 ± 0.473e	3.19 ± 0.545a	51.01 ± 9.588de
C_2	30°C	34.92 ± 8.018cd	4.92 ± 0.589bcd	8.49 ± 0.990a	4.41 ± 0.285d	0.78 ± 0.303c	53.44 ± 9.233de
Citric acid	20°C	34.56 ± 3.285cd	5.17 ± 0.918bc	5.34 ± 0.147d	2.85 ± 0.377e	2.81 ± 0.115a	50.72 ± 4.842de
Citric acid	30°C	43.38 ± 3.421bc	3.96 ± 0.091cd	7.19 ± 0.743abc	2.46 ± 0.324e	1.56 ± 0.212bc	58.55 ± 4.791cd
MeJa	20°C	41.05 ± 4.536bc	3.01 ± 0.793d	7.63 ± 0.499ab	8.03 ± 0.585b	1.85 ± 0.312ab	61.56 ± 6.725bcd

MeJa	30°C	43.45 ± 3.595bc	7.84 ± 0.969a	6.58 ± 0.418bcd	10.22 ± 0.400a	1.01 ± 0.325c	69.10 ± 5.707abc
Chitosan	20°C	26.69 ± 3.540d	3.44 ± 1.189cd	5.35 ± 0.501d	2.09 ± 0.408e	2.418 ± 0.719ab	39.98 ± 6.358e
Chitosan	30°C	49.92 ± 8.414b	3.40 ± 0.581cd	7.15 ± 0.812abc	2.18 ± 0.190e	1.57 ± 0.267bc	64.24 ± 6.765bcd
K_2SO_4	20°C	54.243 ± 2.804ab	3.05 ± 0.766d	8.03 ± 0.611ab	6.29 ± 0.931c	2.46 ± 0.300ab	74.08 ± 5.412ab
K_2SO_4	30°C	64.43 ± 3.062a	6.78 ± 1.017ab	5.83 ± 0.262cd	2.25 ± 0.312e	2.47 ± 0.251bc	81.77 ± 4.904a
Significance							
Temperature (A)		***	***	NS	NS	***	***
Elicitor (B)		***	**	***	***	***	***
A×B		**	***	***	***	***	*

Wild radish

Treatments		Glucosinolates (mg 100 g^{-1} DW)					
Elicitor	Temperature	GRE	4-HGB	DER	GB	MGB	TGLs
C_1	20°C	46.56 ± 0.265def	0.95 ± 0.239d	2.52 ± 0.252c	2.50 ± 0.565e	0.73 ± 0.404d	53.27 ± 1.705d
C_2	30°C	36.17 ± 6.361f	0.86 ± 0.093d	1.13 ± 0.272d	5.33 ± 0.173cd	1.33 ± 0.231cd	44.82 ± 7.113d
Citric acid	20°C	57.16 ± 5.697bcd	3.57 ± 0.722a	1.53 ± 0.601d	5.93 ± 0.382c	1.22 ± 0.355cd	69.42 ± 7.740c
Citric acid	30°C	41.92 ± 3.859ef	1.07 ± 0.510d	4.58 ± 0.330a	2.98 ± 0.746de	0.92 ± 0.304cd	51.47 ± 5.732d
MeJa	20°C	54.65 ± 2.474cde	1.94 ± 0.520cd	4.43± 0.129a	17.64 ± 0.876a	1.74 ± 0.643bcd	80.01 ± 4.873bc
MeJa	30°C	50.97 ± 7.603cde	1.45 ± 0.680cd	4.18 ± 0.229ab	16.41 ± 0.876a	1.56 ± 0.613bcd	74.57 ± 9.002bc
Chitosan	20°C	60.88 ± 5.338bc	1.25 ± 0.360cd	2.61 ± 0.092c	5.67 ± 1.073cd	2.90 ± 0.603a	73.30 ± 7.453c
Chitosan	30°C	60.81 ± 5.547bc	3.83 ± 0.447a	2.71 ± 0.566c	4.08 ± 0.336cde	1.49 ± 0.502bcd	72.92 ± 7.372c
K_2SO_4	20°C	89.22 ± 3.996a	3.37 ± 0.327ab	3.45 ± 0.415bc	4.92 ± 0.927cde	2.59 ± 0.436ab	103.54 ± 6.073a
K_2SO_4	30°C	68.60 ± 6.657b	2.31 ± 0.273bc	3.70 ± 0.414ab	12.37 ± 2.051b	2.03 ± 0.592abc	89.01 ± 9.988ab
Significance							
Temperature (A)		***	*	**	*	**	***

Elicitor (B)	***	***	***	***	***	***
A×B	**	***	***	***	**	*

C_1: represent control 1 (20°C without elicitor) and C_2 represent control 2 (30°C without elicitor). Mean separation within a column followed by different letters are significantly different according to Tukey test at $p \leq 0.05$.

NS, *, **, and *** indicate non-significant, significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively. GRE is glucoraphenin; 4-HGB is hydroxyglucobrassycin; DER is dehydroerucin; GB is glucobrassycin; MGB is 4-methoxyglucobrassycin.

In general, all inductors improved the content of total glucosinolates (TGLs). However, the significant effect ($p < 0.05$) of the elicitors K_2SO_4 and MeJa stands out at both temperatures (Table 1). In addition, the application of both increased the abundance of indole and aliphatic GSL. Similar results were found by Hassini in broccoli seedlings (Hassini et al., 2019). The highest total GSLs content was found by using K_2SO_4/30 °C and K_2SO_4/20 °C. ER and WR, increased about 53.01 % and 88.81 %, compared to C_2 and C_1, respectively. The predominant GSL was glucoraphenin (GRE), which accounted for 70.28 % and 79.55 % in ER and WR, respectively, of the total GSLs content after 7 days of sprouting. This finding was similar to the results reported by other authors (Baenas et al., 2016; MD López et al., 2022; Chenguang Zhou et al., 2013). In terms of GSL composition, there were different responses (Fig. 2). In general, aliphatic GSL were in the majority (81.70 % and 83.88 % ER and WR, respectively) and indole GSL were lower (18.30 % and 16.12 % ER and WR, respectively).

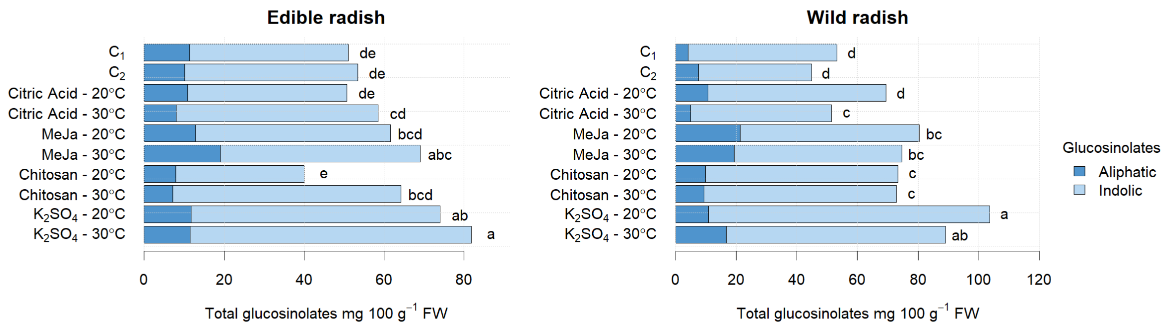

Figure 2. GSLs (aliphatic and indole glucosinolates) in sprouts of ER and WR under the application of different elicitors.

In both species, K_2SO_4, chitosan, and citric acid were the elicitors that mostly induced the synthesis of aliphatic GSLs, mainly glucoraphenin (GRE). Meanwhile, MeJa appeared to be more efficient for indole GSLs, mainly glucobrassicin (GB). Regarding the application of K_2SO4, metabolomic and transcriptomic studies have demonstrated that it plays a direct role in GSL biosynthesis. Sulfur deficiency in plants leads to reduced expression of all major GSL biosynthetic genes, which results in a decrease in GSL production. In Brassica species, sulfur nutrition plays a vital role in determining yield components and seed quality due to their high sulfur requirements throughout the growing season (d'Hooghe et al., 2014). Also, studies showed that the abundance of sulfur-containing metabolites, including cysteine, glutathione, sulpholipids, and GSL (aliphatic, indole, and aromatic), decreased due to reduced sulfur availability (Falk et al., 2007). Therefore, since sulfur is one of the characteristic constituents of GSLs (Baenas et al., 2014a), an optimal supply could modulate GSLs metabolism. However, the demand for sulfur in plants depends on the types of species and stages of development (Narayan et al., 2022). For example, during early vegetative growth, a greater amount of sulfur is required

(Borpatragohain et al., 2019). Other authors have reported that fertilization with methionine (sulfur-containing amino acid) in 7-day-old broccoli sprouts increased up to 32 % GSLs content (Baenas et al., 2016). As sulfur is a constituent of amino acids, utilizing it could potentially yield a beneficial outcome for the synthesis of GSLs (Hoefgen and Nikiforova, 2008).

On the other hand, MeJa was another elicitor that had significant effects on TGLs synthesis in both species. In ER, the application of MeJa/30 °C increased about 29.30 % compared to C_2 and WR the application of MeJa/20 °C increased about 50.91 %, compared to C_1. Results found in the literature showed that MeJa caused GSLs accumulation in tissues, achieving GSLs contents higher than 22 %, applying 10 μM and 25 μM MeJA treatments on 7-day-old broccoli sprouts (Pérez-Balibrea et al., 2011). Under MeJA treatment (50-250 μM), there was a statistically significant. TGLs in broccoli and radish sprouts increased after exogenous application of the elicitor in cotyledons (Baenas et al., 2016). As mentioned, MeJa was more efficient for indole GSLs production, but at the same time induced a slight decrease in aliphatic GSLs compared to the other elicitors. The latter was also reported by Hassini (Hassini et al., 2017), where MeJa treatment significantly increased total GSLs in red and white cabbage sprout varieties, mainly due to the increase in indole GSLs content up to five times. However, MeJa induced a decrease in aliphatic GSLs in white cabbage. Jasmonates are known for their role in biotic and abiotic stress management in plants and also play an important role in several other growth and developmental responses, in trichome formation, reproduction, senescence promotion, photomodulation, and fiber initiation and elongation (Barket Ali, 2021).

The endogenous jasmonate pathway in plants is triggered by herbivore attack, activating defense mechanisms and a series of physiological events, one of these responses being GSLs biosynthesis. It has been established that exogenous application of MeJA simulates herbivore attack and initiates the jasmonate pathway in plants such as broccoli (Chiu et al., 2019). Several pathway genes have been shown to be demonstrated after phytohormone application in *Arabidopsis thaliana*, where the IQD1 protein, the OBP2 transcription factor, and the ATR1/MYB34 and HIG1/MYB51 genes were overexpressed and found to be regulators with respect to increased concentrations of major indole GSLs (Baenas et al., 2014b; Berger, 2007). Therefore, the application of K_2SO_4 and MeJa significantly improved the accumulation of TGLs and natural antioxidants, including acylated anthocyanins and phenolic compounds, in the sprouts of both species, which was also reflected in the values of the peroxidation bioindicator against stress.

Chitosan also influenced TGLs accumulation in both species. In ER chitosan application increased by 20.21 % compared to C_2 and in WR it increased by 37.60 % compared to C_1. Chitosan had a greater impact on the synthesis of aliphatic GSLs. It is primarily used in agriculture for its ability to trigger the biosynthesis of protective biomolecules against pests and pathogens, and it also acts as a positive regulator of defensive genes (Shahrajabian et al., 2021). Seom Sin (Sim et al., 2022) found that foliar application of chitosan resulted in higher GSL content in kimchi cabbage plants subjected to extremely high temperature treatment compared to plants without chitosan application. Citric acid slightly increased the accumulation of TGLs. In the case of ER increased only 9.56 % compared to C_2 and in WR increased 30.32

% compared to C_1. This represents a novelty data since to date, there are almost no reports on the effect of citric acid fertilization on GSLs content in vegetable crops.

Temperature had a significant effect ($p<0.05$) on the accumulation of GSLs. Regarding the accumulation of TGLs, different responses were observed. The use of high temperatures led to a greater accumulation of TGLs in ER; however, WR was negatively impacted by this increase, suggesting that WR may have poor tolerance to temperature increases when it comes to TGL accumulation. Conversely, the content of aliphatic GSLs such as GRE, which are known to exhibit a potent cytotoxic effect against some cell lines (Manivannan et al., 2019), increased due to the temperature rise in both species. Additionally, a synergistic effect was observed between the elicitor and the temperature increase, as applying both stressors simultaneously led to an efficient increase in the synthesis of GRE. Previous studies have shown that GSL contents, especially aliphatics, increase at higher temperatures (30°C) (Choi et al., 2014; Ishida et al., 2014). Seom Sin (Sim et al., 2022) found that the application of moderately high temperatures (30/24 °C) on kimchi cabbage plants markedly improved the content of indole GSLs and aliphatics. In general, abiotic stresses can change the GSL profile. Plants under stress situation can enhance the synthesis of secondary messengers in the cytosol, leading to the release of GSLs from the vacuole into the cytoplasm, contacting the enzyme myrosinase, which hydrolyzes them and converts them to isothiocyanates, which are signaling molecules that promote heat tolerance in plants (Ishida et al., 2014; Sim et al., 2022). In addition, the content of GSL degradation products is promoted with the increase in myrosinase activity, which could enhance the defensive function of the plant against stress (Textor and Gershenzon, 2009). However, it has been reported that in radish sprouts treated with ultraviolet light, only about 6 % of the GRE compound was converted to sulforaphene (the most important ITC present in radish) (Martínez-Zamora et al., 2021). This suggests that some GSL are metabolized into compounds that do not contribute to ITC synthesis (Hanlon and Barnes, 2011). Hence, elicitation increased total GSLs content, making ER and WR sprouts a prominent source of sulforaphene.

3.2.2. Anthocyanins

Contents of individual anthocyanins were consistent with data from the literature. The total anthocyanin content (TAC) in sprouts of ER ranged between 6.94 and 0.62 mg 100 g^{-1} of DW and between 5.64 and 1.39 mg 100 g^{-1} DW in sprouts of WR (Table 2).

Table 2. Anthocyanin content (mg 100 g^{-1} DW) in ER and WR sprouts after different treatment with elicitors.

Edible radish										
Treatments		Anthocyanins (mg 100 g^{-1} DW)								
Elicitor	Temperature	1-Cy	2-Cy	3-Cy	4-Cy	5-Cy	6-Cy	7-Cy	8-Cy	TAC
C_1	20°C	1.00 ± 0.045a	0.51 ± 0.030ab	0.42 ± 0.039b	1.80 ± 0.152b	0.27 ± 0.036bc	0.11 ± 0.012bc	0.16 ± 0.011b	0.05 ± 0.012ab	4.33 ± 0.302c
C_2	30°C	0.08 ± 0.032d	0.25 ± 0.132cd	0.12 ± 0.012b	0.92 ± 0.144cd	0.69 ± 0.493a	0.17 ± 0.056b	NP	NP	2.22 ± 0.768de
Citric acid	20°C	0.36 ± 0.042bcd	0.36 ± 0.062bc	0.34 ± 0.020b	1.13 ± 0.296bc	0.16 ± 0.073bc	0.08 ± 0.029bc	0.14 ± 0.039b	0.05 ± 0.029ab	2.61 ± 0.624d
Citric acid	30°C	0.12 ± 0.031d	0.12 ± 0.011d	0.26 ± 0.096b	0.21 ± 0.062de	0.03 ± 0.011c	0.01 ± 0.004c	0.03 ± 0.022d	NP	0.78 ± 0.364f
MeJa	20°C	0.71 ± 0.101ab	0.48 ± 0.086ab	0.82 ± 0.060a	3.85 ± 0.489a	0.27 ± 0.021bc	0.38 ± 0.057a	0.33 ± 0.053a	0.07± 0.010a	6.91 ± 0.500a
MeJa	30°C	0.31 ± 0.056cd	0.57 ± 0.017a	0.40 ± 0.141b	3.44 ± 0.521a	0.40 ± 0.113ab	0.34 ± 0.108a	0.02 ± 0.012d	0.01 ± 0.011cd	5.49 ± 0.696b

Elicitor	Temperature	1-Cy	2-Cy	3-Cy	4-Cy	5-Cy	6-Cy	7-Cy	8-Cy	TAC
Chitosan	20°C	0.57 ± 0.153bc	0.16 ± 0.043d	0.24 ± 0.031b	1.09 ± 0.282bc	0.11 ± 0.017bc	0.08 ± 0.013bc	0.14 ± 0.032b	0.04 ± 0.006abc	2.42 ± 0.391d
Chitosan	30°C	0.17 ± 0.020d	0.11 ± 0.010d	0.10 ± 0.018b	0.14 ± 0.010de	0.04 ± 0.013c	0.03 ± 0.013c	0.03 ± 0.007d	NP	0.62 ± 0.067f
K_2SO_4	20°C	0.13 ± 0.032d	0.25 bue± 0.095cd	0.24 ± 0.081b	0.78 ± 0.291cde	0.09 ± 0.037bc	0.05 ± 0.024c	0.11 ± 0.029bc	0.02 ± 0.015bcd	1.67 ± 0.393def
K_2SO_4	30°C	0.04 ± 0.015d	0.13 ± 0.022d	0.17 ± 0.033b	0.51 ± 0.091cde	0.15 ± 0.022bc	0.10 ± 0.009bc	0.05 ± 0.010cd	NP	1.13 ± 0.098ef
Significance										
Temperature (A)		***	***	**	***	NS	NS	***	***	***
Elicitor (B)		***	***	***	***	***	***	***	**	***
A×B		**	***	NS	NS	*	*	***	NS	*

Wild radish

Treatments		Anthocyanins (mg 100 g^{-1} DW)								
Elicitor	Temperature	1-Cy	2-Cy	3-Cy	4-Cy	5-Cy	6-Cy	7-Cy	8-Cy	TAC
C_1	20°C	0.04 ± 0.017d	0.08 ± 0.025bc	0.39 ± 0.077b	1.47 ± 0.342bc	0.02 ± 0.021d	0.09 ± 0.011cd	0.01 ± 0.006bc	0.06 ± 0.021ab	2.16 ± 0.426bcd
C_2	30°C	0.28 ± 0.043ab	0.12 ± 0.033ab	0.57 ± 0.106ab	1.86 ± 0.571b	0.09 ± 0.01bcd	0.07 ± 0.004cd	0.04 ± 0.015ab	0.03 ± 0.004abc	3.06 ± 0.676b
Citric acid	20°C	0.14 ± 0.011cd	0.04 ± 0.009c	0.30 ± 0.055b	1.22 ± 0.276bcd	0.05 ± 0.014cd	0.11 ± 0.018bcd	0.02 ± 0.006bc	0.01 ± 0.001cd	1.87 ± 0.367cd
Citric acid	30°C	0.35 ± 0.063a	0.17 ± 0.011a	0.70 ± 0.145a	0.86 ± 0.155cd	0.15 ± 0.019b	0.18 ± 0.067ab	0.04 ± 0.013ab	0.03 ± 0.027abc	2.50 ± 0.303bc
MeJa	20°C	0.14 ± 0.029cd	0.08 ± 0.025bc	0.70 ± 0.168a	3.61 ± 0.360a	0.12 ± 0.028bc	0.20 ± 0.056a	0.07 ± 0.022a	0.04 ± 0.006ab	4.96 ± 0.500a
MeJa	30°C	0.11 ± 0.015cd	0.08 ± 0.013bc	0.75 ± 0.138a	4.14 ± 0.571a	0.23 ± 0.086a	0.22 ± 0.052a	0.05 ± 0.02ab	0.06 ± 0.015a	5.64 ± 0.649a
Chitosan	20°C	0.33 ± 0.039a	0.12 ± 0.011ab	0.42 ± 0.054b	0.44 ± 0.093d	0.05 ± 0.006cd	0.02 ± 0.006d	NP	NP	1.39 ± 0.060d
Chitosan	30°C	0.27 ± 0.079ab	0.13 ± 0.047ab	0.71 ± 0.120a	0.74 ± 0.159cd	0.07 ± 0.008cd	0.14 ± 0.005abc	0.02 ± 0.005bc	0.03 ± 0.01bcd	2.11 ± 0.274bcd
K_2SO_4	20°C	0.19 ± 0.029bc	0.08 ± 0.020bc	0.42 ± 0.047b	1.45 ± 0.197bc	0.08 ± 0.017bcd	0.04 ± 0.005d	0.03 ± 0.017abc	NP	2.29 ± 0.231bcd
K_2SO_4	30°C	0.05 ± 0.021d	0.08 ± 0.011bc	0.36 ± 0.058b	1.38 ± 0.114bc	0.08 ± 0.013bcd	0.19 ± 0.046ab	0.06 ± 0.025a	NP	2.22 ± 0.079bcd
Significance										
Temperature (A)		***	***	***	NS	NS	NS	*	NS	*
Elicitor (B)		***	**	***	***	***	***	***	***	***
A×B		***	***	**	*	***	***	**	**	**

Mean separation within a column followed by different letters are significantly different according to Tukey test at p ≤ 0.05. NS, *, ** and *** indicate non-significant. Significant at p ≤ 0.05., p ≤ 0.01, and p ≤ 0.001, respectively. NP means not present.

1-cy is Cy-3-O-(CA)soph-5-O-(MA)glu; 2-Cy is Cy-3-O-(CA-FE)soph-5-O-(MA)glu; 3-Cy is Cy-3-O-(pCoA)soph-5-O-(MA)glu+ Cy-3-O-(FE-SI)diglu-5-O-glu; 4-Cy is Cy-3-O-(pCoA)soph-5-O-(MA)glu + Cy-3-O-(FE)soph-5-O-(MA)glu + Cy-3-O-(SI)soph-5-O-(MA)glu; 5-Cy is Cy-3-O-(diSI)soph-5-O-(MA)glu + Cy-3-O-(CA-FE)soph-5-O-(MA)glu; 6-Cy is Cy- 3-O-(FE-SI)soph-5-O-(MA)glu + Pg-3-O-(FE)soph-5-O-(MA)glu; 7-Cy is Cy-3-O-(CA-SI)soph-5-O-(MA)glu + Cy-3-O-(CA-FE)soph-5-O-(MA)glu; 8-Cy is Cy-3-O-(diFE)soph-5-O-(MA)glu.

Cy = cyanidin; glu = glucoside; SI = sinapoyl; CA = caffeoyl; soph = sophoroside; *p*CoA = *p*-coumaroyl; FE = feruloyl; MA = malonyl.

Not all elicitors resulted in improved TAC. However, the addition of MeJa significantly increased the abundance of TAC. The highest TAC was found by using MeJa/20°C and MeJa/30°C, ER and WR, increasing by 59.58 % and 161.11 %, compared to C_1 and C_2, respectively. Similar results were found by Baenas (Baenas et al., 2016) where the application of MeJA (25 μM) increased TAC by 23 % in radish sprouts. In both species the most abundant anthocyanin was made up of three compounds, cyanidin 3-*O*-(p-coumaroyl) sophoroside-5-*O*-(malonyl) glucoside), cyanidin 3-*O*-(feruloyl) sophoroside-5-*O*- (malonyl) glucoside) and cyanidin 3-*O*-(sinapoyl) sophoroside-5-*O*-(malonyl) glucoside, which represent 55.76 % and 73.40 % TAC in ER and WR in the optimum conditions, respectively. These anthocyanins present three different aromatic groups (p-coumaroyl, feruloyl and synapoyl) in the diglycosidic substituent C-3 while an aliphatic group (malonic acid) in the C-5 sugar. These data are consistent with those found in China rose radish sprouts (Baenas et al., 2015), red cabbage (Park et al., 2014), and sango radish sprouts (Matera et al., 2012).

In both species, the increase in temperature and the interaction with the elicitors resulted in a significant effect ($p<0.05$) on the accumulation of TAC. This shows that the two species were susceptible to the increase in temperature and therefore the source of stress caused the sprouts to show changes in the synthesis of anthocyanins. However, in this case ER showed less resistance to temperature rise, showing greater accumulation of TAC at 20°C. On the other hand, WR increased TAC synthesis with increasing temperature, being more susceptible to this change.

The increase of phenolic compounds in elicitor-treated sprouts can be attributed to de novo synthesis and transformation. One of the pathways for the synthesis of phenolic compounds is through the phenylpropanoid route (HongKai Liu et al., 2019). Anthocyanin biosynthesis is part of the general phenylpropanoid pathway. Most steps in anthocyanin formation are inducible by jasmonates, which induce the enzyme phenyl alanine ammonium lyase (PAL) (EC 4.3.1. 5), which is key in the formation of secondary compounds and more sensitive to any stress (Wasternack and Strnad, 2019). Therefore, PAL activity induced by MeJa treatment could favor gene expression of phenolic biosynthesis (Pérez-Balibrea et al., 2011), triggering the accumulation of these secondary metabolites in ER and WR sprouts.

These results agree with those observed in the malondialdehyde (MDA) assay, since as mentioned above, MDA is commonly used as a bioindicator of peroxidation under stress factors. It has been demonstrated that anthocyanins can show direct antioxidant capacity toward ROS (Garcia and Blesso, 2021). Therefore, the results obtained suggest that MeJa increased ROS which triggered anthocyanin synthesis.

3.3. Antioxidant properties ER and WR of sprouts

The increase in anthocyanin content observed after MeJa elicitor application in both ER and WR sprouts coincided with the total phenolic content (TPC) and antioxidant capacity assays (Fig. 3). All elicitors improved the antioxidant properties of the sprouts in both species. In the case of the TPC assay, the application of MeJa significantly improved its content. It increased by 71.61 % (4622.30 mg GAE 100 g^{-}

^{1}) compared to C_1 (2693.55 mg GAE 100 g^{-1}) in ER whereas WR was increased by 63.00 % (4747.57 mg GAE 100 g^{-1}), compared to C_1 (2912.66 mg GAE 100 g^{-1}). The application of K_2SO_4 also increased the average TPC content in both species. However, high temperature had no significant effect on TPC ($p>0.05$). The same results were found by De Pascale in *Brasica rapa* species (De Pascale et al., 2007).

On the other hand, the DPPH antioxidant capacity assay showed higher values after application of MeJa/30 °C in both species. In ER was observed a increase around 48.96 % (4502.76 µmol Trolox 100 g^{-1}) compared to C_2 (3064.034 µmol Trolox 100 g^{-1}) whereas it increased by 34.33 % (5000.57 mg µmol Trolox 100 g^{-1}) compared to C_2 (3722.63 µmol Trolox 100 g^{-1}) in WR. As mentioned above, several investigations have shown that MeJa treatment activates the phenylpropanoid pathway inducing the accumulation of phenolic compounds (HongKai Liu et al., 2019). Polyphenols are the main plant compounds with antioxidant capacity and also possess the ideal chemical structures for free radical scavenging (Moure et al., 2001). In this study, high concentrations of phenolic compounds were related to a subsequent increase in the antioxidant potential of the sprouts, which suggest that these compounds act as effective antioxidants. The application of MeJa and K_2SO_4 at 30 °C had no significant difference ($p>0.05$) for the ORAC assay in ER, increasing 35.72 % (38,324.47 µmol Trolox 100 g^{-1}) compared to C_2 (28,237.60 µmol Trolox 100 g^{-1}). Higher values were shown when K_2SO_4/30 °C was applied in WR, increasing by 34.04 % (36,939.03 µmol Trolox 100 g^{-1}), compared to C_1 (27,555.40 µmol Trolox 100 g^{-1}).

Antioxidant properties were mainly related to total phenolic compounds, since these chemicals present a high antioxidant activity. On the other hand, GSL and their hydrolysis products are considered indirect antioxidants, since they do not neutralize free radicals directly, but modulate the activity of xenobiotic metabolizing enzymes (Vig et al., 2009). Some *Brassica oleracea* studies have concluded that the maximum contribution to antioxidant capacity attributable to GSL was only about 2 % (Cabello-Hurtado et al., 2012). Therefore, it could be considered that GSLs contribute only a small part to the total antioxidant capacity of these extracts and the phenolic compounds such as anthocyanins would contribute to the effect observed. However, further research is needed.

Finally, despite the differences, the use of elicitors was more notable than the increase of 10 degrees of temperature in terms of sprouts growth and bioactive compounds, being more efficient in improving the physical parameters of the sprouts elicitors such as chitosan and citric acid, and more effective in the accumulation of phytochemicals and increases in antioxidant capacity the elicitors K_2SO_4 and MeJa, so the decision to apply them depends on the objectives that are taken.

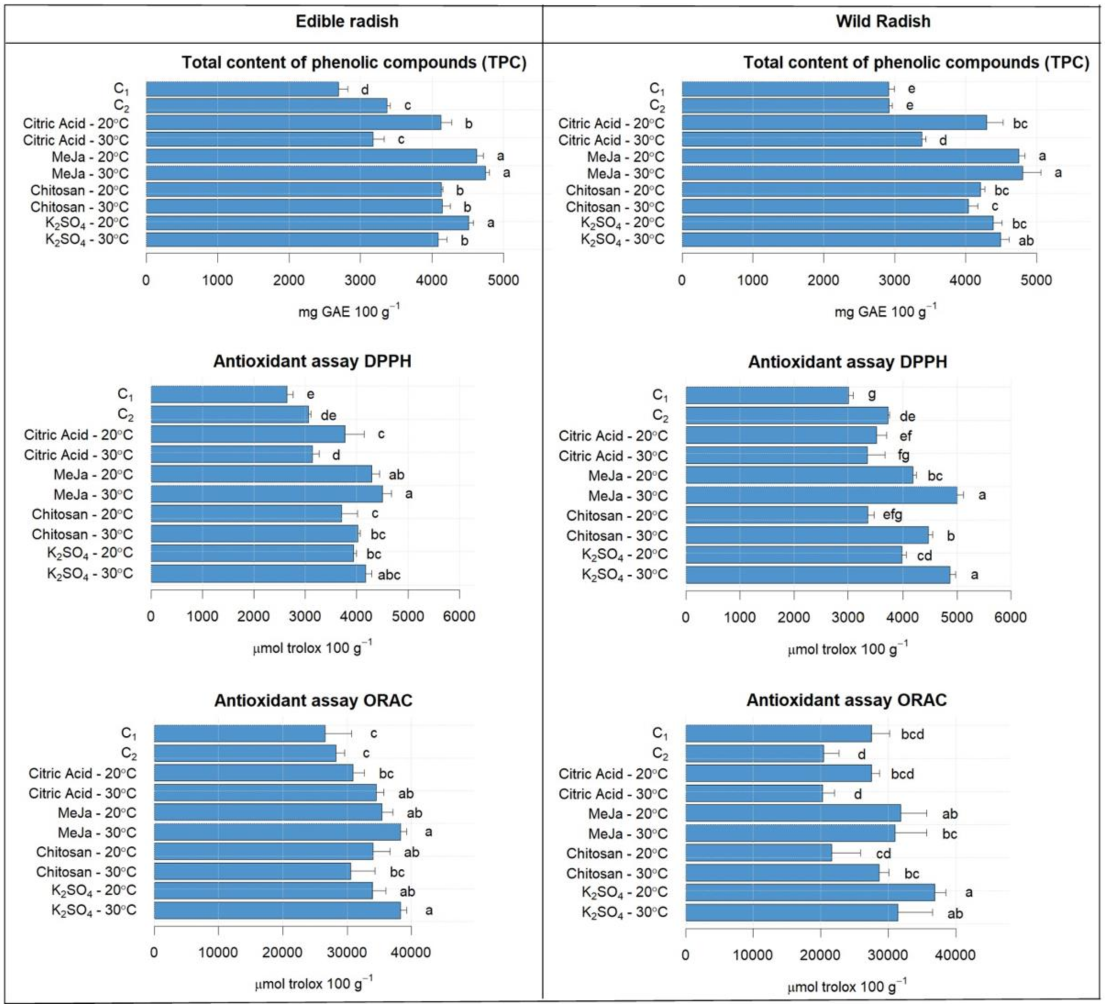

Figure 3. Total phenol content (TPC) and antioxidant capacity (DPPH and ORAC) assays in ER and WR sprouts.

3.4. Interaction of elicitors with phytochemical content: PCA analysis at 30 °C

To study all the results globally, a correlation matrix was made. Fig. 4 showed the correlations that exist after applying a combination of elicitors with high temperatures (30 °C). It can be seen the TPC and antioxidant capacity (DPPH and ORAC) showed a positive correlation. This is attributed to stress which can promote the production of phenolic compounds and consequently increase antioxidant capacity. Also, in both species a positive correlation was observed between the TAC and GB (glucobrassicin). Both secondary metabolites were positively correlated, likewise, GB with DPPH. In addition, GRE (glucoraphenin) had a high correlation with GSLs since it is the GSL that predominates in both species.

The correlation analysis at 30 °C showed that in both species there was a negative correlation between some parameters, for example, the lower the diameter of the hypocotyl (HYP), the radicle (RAD) or the fresh weight (FW), the higher MDA and the content of GSLs in particular 4-HGB (hydroxyglucobrassicin) and also the TAC was. Abiotic stress negatively affects the growth and development of plants by altering biochemical and physiological processes such as photosynthesis, respiration, transpiration, etc. Stressed plants increase production of secondary metabolites because of growth is often inhibited more than photosynthesis, and fixed carbon is assigned predominantly to secondary metabolites (Kumar and Sharma, 2018).

On the other hand, in both species a positive correlation was observed between GB and TAC, which is consistent with the high correlation that exists between GB, TPC and DPPH. Some studies have shown the relationship between GSLs and antioxidant capacity, concluding that GB could contribute a greater proportion to the total antioxidant capacity (Akula and Ravishankar, 2011). A positive correlation between TAC and MDA also stands out in WR at 30°C. Otherwise, it can be observed in the supplementary material (Fig. S3), that the variables had lower correlation in WR at 20°C, confirming that the high temperatures in combination with the application of the elicitor caused a great oxidative stress in WR and therefore there was a significant accumulation of anthocyanins.

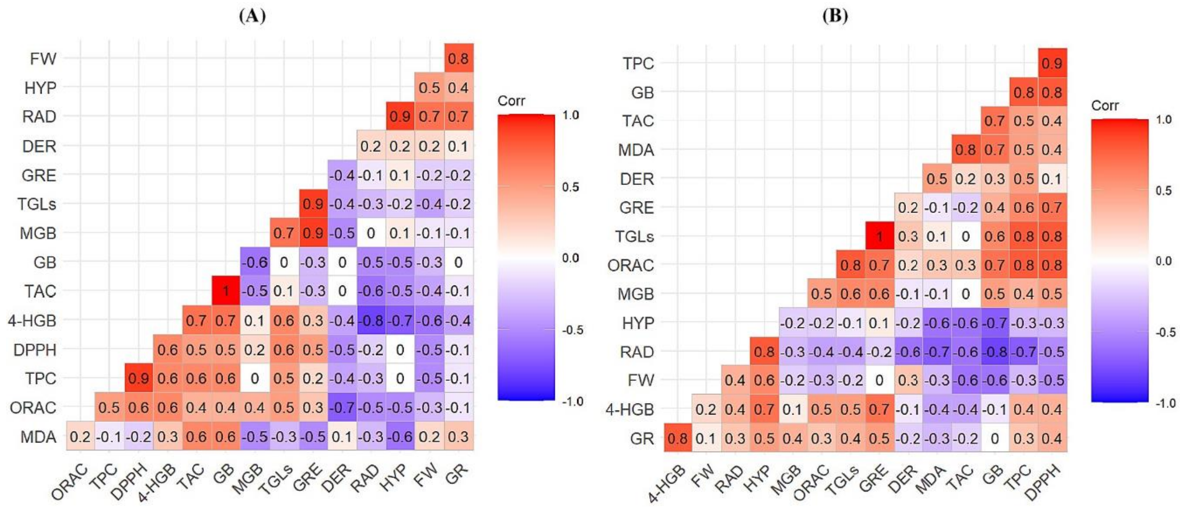

Figure 4. Correlation matrix between different variables at 30°C. (A) correspond to ER and (B) correspond to WR.

RAD: Length of the radicle; HYP: Hypocotyl length, FW: weight sprouts; GR: germination rate; MDA: Malondialdehyde Assay; TGLs: Total Glucosinolates; TAC: Total anthocyanins; GRE: Glucoraphenin; 4-HGB: Hydroxyglucobrassicin; DER: Dehydroerucine; GB: Glucobrassicin; MGB: 4-methoxyglucobrassicin; TPC: Total phenolic content; DPPH: DPPH assay; ORAC: ORAC assay for ER and WR.

Given the large number of variables under study and the correlations seen between them in the correlation matrix, an individual statistical analysis would not be the most appropriate, since this would imply leaving out relevant information that can be used to understand the processes underlying the experiment, therefore, a PCA analysis was performed in this study.

To study the way in which the original variables are related to the created components, the biplot graph is studied at 30°C (Fig. 5), where the first two components or dimensions (Dim) that explain the variables can be seen. Carrying out this rotation, fifteen principal components are obtained, as many as the original variables, both for the ER and for the WR.

In the case of ER at 30 °C (Fig. 5A), Dim1 retained 40.51 % and Dim2 26.44 %. The variables most positively correlated with Dim1 were DPPH, TPC, ORAC and 4-HGB. On the other hand, the most negatively correlated variables were FW, RAD and HYP. For ER, a PCA was also obtained at 20 °C (Fig. S4A), which highlights that GSL 4-HGB had a negative correlation with Dim1, meaning that when Dim1 increases, 4-HGB decreases. This behavior is also explained in the correlation matrix. Meanwhile, the variables most positively correlated with Dim2 are MGB and GRE in ER, and the most negatively correlated was MDA, implying that when one increases the others decrease. However, the increase in temperature induced oxidative stress in ER and WR sprouts. This response was related to MDA, antioxidant capacity and anthocyanin content, while GSL decreased when dealing with the WR species.

For WR at 30 °C (Fig. 5B), Dim1 and Dim2 retained 44.83 % and 28.00 % respectively. The variables most positively correlated with Dim1 were TPC, DPPH, ORAC and GB, and those negatively correlated were RAD and FW. While the variables most correlated with Dim2 were 4-HGB and GR. No significant variables were found since were negatively related to Dim2. The PCA provided at 20 °C for WR (Fig. S4) highlighted that the variables positively correlated to Dim2 were MDA and 4-HGB, and negatively the TAC variable. Therefore, at optimum temperature (20 °C) there was no significant activity oxidative stress, based on a greater accumulation of anthocyanins, although there was an increase in the synthesis of GSLs.

In both species, the first three components concentrated 80.5 % (Fig. 5C) and 83.8 % (Fig. 5D) respectively, so it is possible to reduce the problem to these three variables, which explain a large percentage of what occurs with the fifteen original variables of the study.

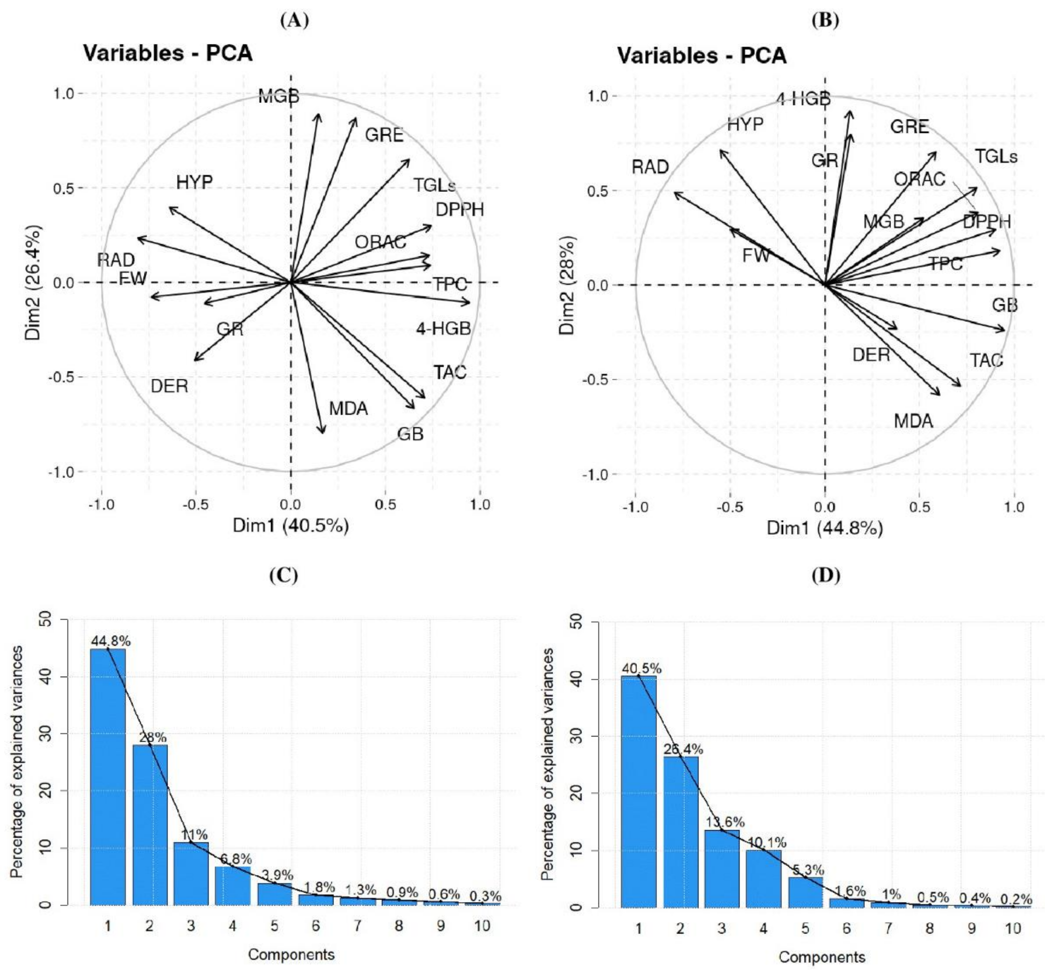

Figure 5. Principal component analysis (PCA) at 30°C. Letter (A) represent the PCA of edible radish, letter (B) represent the PCA of wild radish, letter (C) represent the percentage of explained of edible radish and letter (D) represent the percentage of explained of wild radish.

RAD: Length of the radicle; HYP: Hypocotyl length, FW: weight sprouts; GR: germination rate; MDA: Malondialdehyde Assay; TGLs: Total Glucosinolates; TAC: Total anthocyanins; GRE: Glucoraphenin; 4-HGB: Hydroxyglucobrassicin;

DER: Dehydroerucine; GB: Glucobrassicin; MGB: 4-methoxyglucobrassicin; TPC: Total phenolic content; DPPH: DPPH assay; ORAC: ORAC assay for ER and WR.

3.5. ANNs modelling to predict the effect of high temperatures and exogenous of MeJa

In the results obtained, the elicitation strategies manage to increase the content of bioactives and consequently the biological activity of the sprouts of the *Raphanus* species, for which the MeJa elicitor has been selected to evaluate the optimal conditions that achieve the highest accumulation of bioactive compounds, through mathematical modeling tools. Thus, in recent years, multivariate modeling techniques have been used as data analysis tools with the aim of analyzing, describing and, in general, interpreting multidimensional observations. Among them, ANNs (Artificial neural network) stand out since this technique allows very fast and simple simulations (Soares et al., 2013). Given this background, an experiment was established to evaluate the response of edible and wild radish sprouts to elevated temperature in combination with the hormone methyl jasmonate (MeJa) (input variables) on the antioxidant capacity (DPPH and ORAC), the of total phenols (TPC) and the behavior of the oxidative stress biomarker malondialdehyde (MDA) as output variables.

Fig. 6 shows the architecture of the developed neural network, which consisted of an input layer with 4 neurons, three intermediate layers with 15-10-5 neurons, and an output layer with 4 neurons. The adjustment of the weights of the intermediate layers was carried out using the 'back-propagation' algorithm. The network was trained with the experimental data obtained previously. The neural network generated a correlation between fitted and observed values of more than 90 % for the variables DPPH, ORAC, TPC and MDA, with small error values and high prediction accuracy.

The best concordance for the validation of the ANNs model was obtained for the TPC ($R^2_{validation}$=0.989) followed by antioxidant activity measured by the DPPH method ($R^2_{validation}$= 0.975), and the MDA test ($R^2_{validation}$= 0.957). The lowest value for the correlation coefficient for validation was obtained for the ORAC antioxidant activity assay ($R^2_{validation}$ = 0.851) (Fig. S5). This allows us to demonstrate that ANNs can accurately and precisely predict the best biological properties of *Raphanus* sprouts due to the high correlation coefficient between the observed and predicted variables, depending on the growth temperature, the species and the applied elicitor giving greater reliability to future predictions.

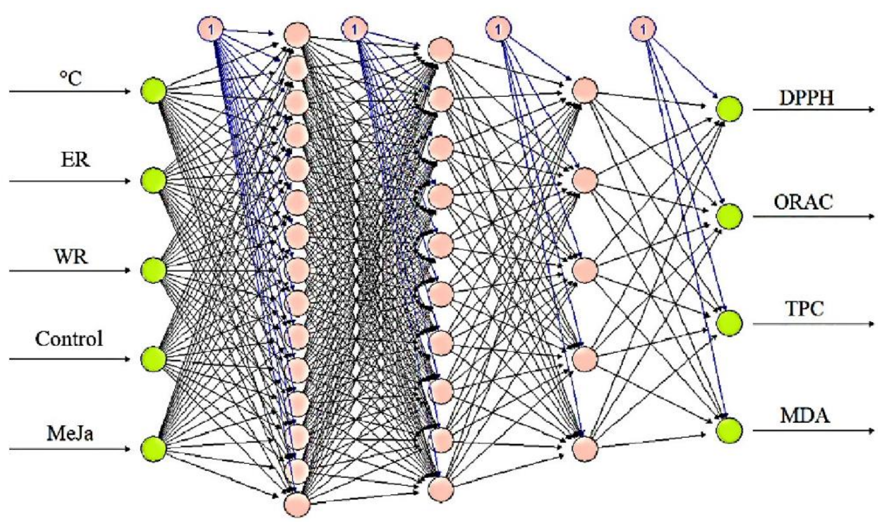

Figure 6. Structure of the ANNs model for the prediction of antioxidant capacity by the DPPH and ORAC method, content of phenolic compounds (TPC) and oxidative stress biomarker malondialdehyde (MDA).

4. Conclusions

The selected species were edible radish (ER) and wild radish (WR), which are family *Brassicaceae*, characterized by being almost unique sources rich in glucosinolates. To date, no studies have examined the impact of foliar application of elicitors in combination with high temperatures on the secondary metabolite content of *Raphanus* species as well as the use of predictive models. The application of K_2SO_4 and MeJa significantly improved the accumulation of TGSLs and natural antioxidants, including acylated anthocyanins and phenolic compounds, in the sprouts of both species, which was also reflected in the values of the peroxidation bioindicator against stress. However, the elicitors chitosan and citric acid were more efficient in improving the physical parameters of the sprouts, so the decision to apply them depends on the intentions in crop production. Despite the differences, the use of elicitors was more notable than the increase of 10 degrees of temperature in terms of sprouts growth. The correlation matrices and the PCA analysis allowed us to correlate the variables and to reduce to three the variables that explain a large percentage of what happens with the fifteen original variables of the study. The artificial neural network is a tool that allowed us to predict the behavior of the DPPH, ORAC, TPC and MDA variables, depending on the species, the application of MeJa and the increase in temperature improved the behavior of antioxidant activity and increased oxidative damage, limiting the production to a temperature range of 20 to 30°C. To achieve greater understanding and improve predictions at other temperatures, we must keep the network in constant training.

This study emphasizes the need for further research in this area. Furthermore, due to interest for bioactive compounds with biological potential, variation in phytochemical content must be taken into account. It has also been shown that all derivatives of bioactive compounds are not equal in their biological potential. Consequently, the identification, but also the quantification and individual determination of them in plants and plant tissues is extremely important. Finally, this research seeks to innovate in the agri-food industry and explore the behavior of *Raphanus* species exposed to high temperature conditions and foliar application treatments, according to the production and accumulation of phytochemicals framed in the issue of food security. Furthermore, it is demonstrated that elicitors in combination with predictive analysis represent an effective tool to improve the nutritional value of sprouts of *Raphanus* species under future conditions of increased temperature.

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CONCLUSIONES GENERALES

Esta investigación realizó en primera instancia una descripción y caracterización de los brotes de plantas y compuestos bioactivos, especificando cómo diferenciarlos o identificar su etapa de crecimiento y cosecha, así como información sobre la clasificación de los diferentes tipos de elicitores. En segundo lugar, se realizó un análisis descriptivo del dataset seleccionado en WoS, seguido de un análisis bibliométrico, el cual se realizó durante los últimos 29 años, período que ha permitido generar conocimiento y a la vez construir una visión amplia de muchas publicaciones sobre el tema.

Los resultados demostraron que el tema ha tenido un incremento notable en el ritmo de publicación en los últimos años. Se identificaron las revistas con mayor productividad respecto al total de artículos, las cuales coinciden en sus temas relacionados con la química y la bioquímica en la alimentación y la agricultura. Por otro lado, se encontró que los patrones de productividad de los autores se ajustaron a la ley de Lotka, demostrando que el tema seguirá siendo de interés, y es muy probable que el número de publicaciones aumente significativamente en los próximos años. Además, se utilizaron indicadores de evaluación de la productividad para abordar diferentes aspectos del desempeño y así brindar un panorama más adecuado y polifacético de la realidad, permitiendo identificar las áreas de investigación de los principales autores. El análisis de colaboración entre países demuestra que es necesario un mayor enfoque en la colaboración internacional entre países desarrollados y menos desarrollados, con el fin de crear conocimiento sobre nuevas alternativas nutricionales.

El impacto de la aplicación foliar de elicitores en combinación con altas temperaturas en el contenido de metabolitos secundarios de las especies de *Raphanus* concluyó que la aplicación de K_2SO_4 y MeJa mejoró significativamente la acumulación de TGSLs y antioxidantes naturales, incluyendo antocianinas aciladas y compuestos fenólicos, en los brotes de ambas especies, lo que también se reflejó en los valores del bioindicador de peroxidación frente al estrés. Sin embargo, los elicitores quitosano y ácido cítrico fueron más eficientes en mejorar los parámetros físicos de los brotes, por lo que la decisión de aplicarlos depende de las intenciones en la producción del cultivo.

A pesar de las diferencias, el uso de elicitores fue más notorio que un aumento de 10 grados de temperatura en cuanto al crecimiento de los brotes. Las matrices de correlación y el análisis PCA nos permitieron correlacionar las variables y reducir a tres las variables que explican un gran porcentaje de lo que ocurre con las quince variables originales del estudio. La aplicación de ANNs es una herramienta que nos permitió predecir el comportamiento de las variables DPPH, ORAC, TPC y MDA, dependiendo de la especie. La aplicación de MeJa y el aumento de temperatura mejoró el comportamiento de la actividad antioxidante y aumentó el daño oxidativo, limitando la producción a un rango de temperatura de 20 a 30°C. Para lograr una mayor comprensión y mejorar las predicciones a otras temperaturas, debemos mantener la red en constante entrenamiento.

Las investigaciones realizadas en el presente estudio buscan innovar en la industria agroalimentaria y explorar el comportamiento de las especies pertenecientes a la familia *Brassicaceae* expuestas a condiciones de alta temperatura y tratamientos de aplicación foliar, según la producción y acumulación de fitoquímicos enmarcados en el tema de la seguridad alimentaria. Además, se demuestra que el análisis predictivo representan una herramienta eficaz para mejorar el valor nutricional de los brotes de especies de *Raphanus* en condiciones futuras de aumento de temperatura.

Finalmente, estos estudios presentan nuevos conocimientos en la investigación de la elicitación en brotes de plantas y sus efectos potenciales en la mejora de la síntesis de compuestos bioactivos con potencial biológico. Dado que existen resultados significativos de las investigaciones realizadas por los principales investigadores se justifican futuras investigaciones intensivas en aspectos de tecnología y ciencia de los alimentos, con el fin de evaluar el potencial biológico de las plantas, con el fin de asegurar una buena nutrición y protección de la salud humana.

GENERAL CONCLUSIONS

This research carried out in the first instance a description and characterization of plant sprouts and bioactive compounds, specifying how to differentiate them or identify their stage of growth and harvest, as well as information on the classification of the different types of elicitors. Secondly, a descriptive analysis of the selected WoS dataset was carried out, followed by a bibliometric analysis, which was carried out during the last 29 years, a period that has allowed generating knowledge and at the same time building a broad vision of many publications on the subject.

The results showed that the subject has had a notable increase in the rate of publication in recent years. The journals with the highest productivity with respect to the total articles were identified, which coincide in their topics related to chemistry and biochemistry in food and agriculture. On the other hand, it was found that the productivity patterns of the authors were adjusted to Lotka's law, demonstrating that the topic will continue to be of interest, and it is very likely that the number of publications will increase significantly in the coming years. In addition, productivity evaluation indicators were used to address different aspects of performance and thus provide a more adequate and multifaceted panorama of reality, allowing the identification of the research areas of the main authors. The analysis of collaboration between countries shows that a greater focus on international collaboration between developed and less developed countries is necessary, in order to create knowledge about new nutritional alternatives.

The impact of foliar application of elicitors in combination with high temperatures on the content of secondary metabolites of Raphanus species concluded that the application of K_2SO_4 and MeJa significantly enhanced the accumulation of TGSLs and natural antioxidants, including acylated anthocyanins and phenolic compounds, in the sprouts of both species, which was also reflected in the values of the peroxidation bioindicator against stress. However, the chitosan and citric acid elicitors were more efficient in improving the physical parameters of the sprouts, so the decision to apply them depends on the crop production intentions.

Despite the differences, the use of elicitors was more noticeable than the 10 degree temperature increase in terms of growth sprouts. The correlation matrices and the PCA analysis allowed us to correlate the variables and reduce to three the variables that explain a large percentage of what happens with the fifteen original variables of the study. The application of ANNs is a tool that allowed us to predict the behavior of the variables DPPH, ORAC, TPC and MDA, depending on the species, the application of MeJa and the increase in temperature improved the behavior of antioxidant activity and increased oxidative damage, limiting production to a temperature range of 20 to 30°C. To gain a better understanding and improve predictions at other temperatures, we must keep the network in constant training.

The investigations carried out in this work seek to innovate in the agri-food industry and explore the behavior of species belonging to the Brassicaceae family exposed to high temperature conditions and foliar application treatments, according to the production and accumulation of phytochemicals framed in the theme of food safety. In addition, it is shown that predictive analysis represents an effective tool to improve the nutritional value of *Raphanus* species sprouts in future conditions of increased temperature.

Finally, these studies present new insights into the investigation of elicitation in plant sprouts and its potential effects on enhancing the synthesis of bioactive compounds with biological potential. Given that there are significant results of the investigations carried out by the main researchers, future intensive investigations in aspects of technology and food science are justified, in order to evaluate the biological potential of plants, in order to ensure good nutrition. and protection of human health.

V. DIVULGACION DE RESULTADOS

Publicaciones

- Toro, M. T., Ortiz, J., Becerra, J., Zapata, N., Fierro, P., Illanes, M., & López, M. D. (2021). Strategies of Elicitation to Enhance Bioactive Compound Content in Edible Plant Sprouts: A Bibliometric Study. Plants, 10(12), 2759. https://doi.org/10.3390/plants10122759.
- María-Trinidad Toro, Roberto Fustos, Jaime Ortiz, José Becerra, Nelson Zapata, María-Dolores López (2023). Enhancement of antioxidant responses and phytochemical accumulation combining elicitors and predictive models in Raphanus species growth under high temperature. (Artículo enviado a *Scientia Horticulturae*, 11/03/2023)

Participación en otras publicaciones durante el periodo doctoral

- López, M. D., Toro, M. T., Riveros, G., Illanes, M., Noriega, F., Schoebitz, M., ... & Moreno, D. A. (2022). Brassica sprouts exposed to microplastics: Effects on phytochemical constituents. Science of The Total Environment, 153796. Artículo. https://doi.org/10.1016/j.scitotenv.2022.153796
- Romero ME., Toro T., Noriega F., López MD., Wellness ingredients and functional food. En, The Role of Alternative and Innovative Food Ingredients and Products in Consumer Wellness (book). ELSEVIER. 2019, The role of alternative and innovative food ingredients and products in consumer wellness, ELSEVIER, 2019, 36-60. Capítulo de libro.
- Sharifi-Rad, J., Rajabi, S., Martorell, M., López, M. D., Toro, M. T., Barollo, S., ... & Pezzani, R. (2020). Plant natural products with anti-thyroid cancer activity. Fitoterapia, 104640. Review.https://doi.org/10.1016/j.fitote.2020.104640.
- Uquiche, E. L., Toro, M. T., & Quevedo, R. A. (2019). Supercritical extraction with carbon dioxide and co-solvent from Leptocarpha Rivularis. Journal of Applied Research on Medicinal and Aromatic Plants, 14, 100210. https://doi.org/10.1016/j.jarmap.2019.100210.

Congresos, seminarios y conferencias

Comunicaciones orales

- VII Jornadas de Ciencia y Tecnología de la Facultad de Cs Agrarias UNR. Título: Aplicación foliar de K_2SO_4 y Na_2SO_4 en brotes de rábano y brócoli: Efectos en la capacidad antioxidante. Toro, M.T & López, M.D. Jun-2022. Rosario, Argentina y Concepción, Chile.
- Workshop Jóvenes investigadores en las Ciencias Agronómicas. Título: "Efectos de temperaturas altas y metil jasmonato exógeno sobre el crecimiento y la actividad antioxidante de los brotes de *Brassicaceae*". Autores: Toro, M.T & López, M.D. Ene-2022. Concepción, Chile.
- Workshop Jóvenes investigadores en las Ciencias Agronómicas. Título: Efectos de la aplicación de K_2SO_4 y Na_2SO_4 sobre el contenido de glucosinolatos en brotes de rábano y brócoli. Autores: Toro, M.T., López, M.D & Fierro, P. Dic-2020. Concepción, Chile.
- Charla: "Mecanismos de elicitación en brotes vegetales". Curso de Avances en propiedades físicas y químicas de alimentos, asignatura del programa de magíster en ciencias de los alimentos. Universidad de Chile. Santiago, Chile.
- Charla: "Brotes, el alimento del futuro". Explora Conicyt. Iniciativa 1000 científicos 1000 aulas. Comunidad Educativa Kümelén. Concepción, Chile.

Posters

- Plant Biology Conference (PB22). Título: Artificial neural network modelling to predict the effect of high temperatures and exogenous methyl jasmonate on growth and biological potential in Brassicaceae sprouts. Autores: M.T. Toro*; M.D. López; J.Ortiz. Jul-2022. Portland, Estados Unidos.
- Plant Biology Conference (PB22). Título: Role of elicitors in broccoli sprouts: defense, production, and enrichment in bioactive compounds Autores: M.D. López; M.T. Toro, M. Illanes; S. Fischer; N. Zapata; K. Henríquez; A. Pinto; M. Schoebitz; C. Gracía-Viguera; D.A. Moreno.Portland. Estados Unidos.
- XVI Spanish Portuguese Congress of Plant Physiology. Título: Response of the wild radish (Raphanus raphanistrum L.) sprouts to different elicitors to improve the accumulation of bioactive molecules. Autores: López, M.D, Toro, M.T., Fustos, R., Fierro, P., Zapata, N., Becerra, J., Ortíz, J. Jun-2019, Pamplona.

VI. GLOSARIO

GSL, glucosinolates

WoS, web of science

TC, total citations

NP, number of publications

PY,_start years of scientific activity

MCA, Multiple Correspondence Analysis

LC, local citation

GC, global citation

MCP, Multiple Country Publications

SCP, Single Country Publication

SCI-Expanded, Science Citation Index Expanded

BKCI-S, Book Citation Index-Science

ESCI, Emerging Sources Citation Index

MeJa, methyl jasmonate

ITC, isothiocyanate

ROS, reactive oxygen species

MDA, malondialdehyde

PCA, principal component analysis

ANN, artificial neural network

DPPH, 2,2-diphenyl-1-picrylhydrazyl

ORAC, oxygen radical absorbance capacity

TPC, total phenolic content

TAC, total anthocyanin content

TGSLs, total glucosinolates

ER, edible radish

WR, wild radish

TCA, trichloroacetic acid

TBA, thiobarbituric acid

PVDF, polyvinylidene difluoride

HPLC-DAD-ESI-MSN, high performance liquid chromatography equipped with photodiode array detection-mass

DW ,dry weight

FW, fresh weight

HYP, hypocotyl

RAD, radicle

GAE, Gallic acid equivalent

GRE, glucoraphenin

GB, glucobrassicin

PAL, phenyl alanine ammonium lyase

4-HGB, hydroxyglucobrassicin

VII. ANEXOS

MATERIAL SUPLEMENTARIO

Tablas complementarias artículo 1

Table S1. Main investigations of elicitation mechanisms in the synthesis of bioactive compounds in sprouts.

Description	Results
MAIN INFORMATION ABOUT DATA	
Timespan	1992:2020
Sources (Journals, Books, others)	246
Documents	787
Average years from publication	6
Average citations per documents	16.98
Average citations per year per doc	2.076
References	26,166
DOCUMENT TYPES	
article	722
article; book chapter	7
article; early access	1
article; proceedings paper	10
editorial material	1
meeting abstract	1
review	37
review; book chapter	1
DOCUMENT CONTENTS	
Keywords Plus (ID)	2,248
Author's Keywords (DE)	2,228
AUTHORS	
Authors	2,738
Author Appearances	3,576
Authors of single-authored documents	13
Authors of multi-authored documents	2725
AUTHORS COLLABORATION	
Single-authored documents	17
Documents per Author	0.287
Authors per Document	3.48
Co-Authors per Documents	4.54
Collaboration Index	3.54

Table S2. The 246 journals that contain the articles extracted from the database.

Nº	Sources	Articles
1	JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY	42
2	FOOD CHEMISTRY	39
3	LWT-FOOD SCIENCE AND TECHNOLOGY	32
4	JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE	30
5	JOURNAL OF FOOD SCIENCE AND TECHNOLOGY-MYSORE	23
6	INTERNATIONAL JOURNAL OF FOOD SCIENCE AND TECHNOLOGY	20
7	MOLECULES	19
8	JOURNAL OF FOOD PROCESSING AND PRESERVATION	17
9	SCIENTIA HORTICULTURAE	16
10	FOOD SCIENCE AND BIOTECHNOLOGY	15
11	FOODS	15
12	FRONTIERS IN PLANT SCIENCE	13
13	JOURNAL OF FOOD BIOCHEMISTRY	12
14	ACTA PHYSIOLOGIAE PLANTARUM	11
15	CEREAL CHEMISTRY	10
16	INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES	10
17	JOURNAL OF CEREAL SCIENCE	10
18	JOURNAL OF FOOD MEASUREMENT AND CHARACTERIZATION	10
19	JOURNAL OF FOOD SCIENCE	10
20	JOURNAL OF PLANT PHYSIOLOGY	10
21	FOOD RESEARCH INTERNATIONAL	9
22	HORTSCIENCE	8
23	JOURNAL OF FUNCTIONAL FOODS	8
24	AGRONOMY-BASEL	7
25	ENVIRONMENTAL AND EXPERIMENTAL BOTANY	7
26	FOOD SCIENCE \& NUTRITION	7
27	INDUSTRIAL CROPS AND PRODUCTS	7
28	BMC PLANT BIOLOGY	6
29	TRENDS IN FOOD SCIENCE \& TECHNOLOGY	6
30	ACTA SCIENTIARUM POLONORUM-HORTORUM CULTUS	5
31	EUROPEAN FOOD RESEARCH AND TECHNOLOGY	5

32	FOOD AND BIOPROCESS TECHNOLOGY	5
33	FOOD CONTROL	5
34	HORTICULTURE ENVIRONMENT AND BIOTECHNOLOGY	5
35	INTERNATIONAL FOOD RESEARCH JOURNAL	5
36	JOURNAL OF THE AMERICAN SOCIETY FOR HORTICULTURAL SCIENCE	5
37	PLANT BIOLOGY	5
38	ANNALS OF BOTANY	4
39	CEREAL RESEARCH COMMUNICATIONS	4
40	CYTA-JOURNAL OF FOOD	4
41	FOOD \& FUNCTION	4
42	FOOD BIOSCIENCE	4
43	FOOD SCIENCE AND TECHNOLOGY	4
44	FOOD SCIENCE AND TECHNOLOGY INTERNATIONAL	4
45	HORTICULTURAE	4
46	MOLECULAR NUTRITION \& FOOD RESEARCH	4
47	PLANT CELL AND ENVIRONMENT	4
48	PLANT GROWTH REGULATION	4
49	PLANTS-BASEL	4
50	PROCESS BIOCHEMISTRY	4
51	SOUTH AFRICAN JOURNAL OF BOTANY	4
52	AUSTRALIAN JOURNAL OF BOTANY	3
53	FOOD SCIENCE AND TECHNOLOGY RESEARCH	3
54	GLUCOSINOLATES	3
55	INTERNATIONAL JOURNAL OF FOOD PROPERTIES	3
56	JOURNAL OF CHEMISTRY	3
57	JOURNAL OF ENVIRONMENTAL BIOLOGY	3
58	JOURNAL OF FOOD COMPOSITION AND ANALYSIS	3
59	JOURNAL OF FOOD PROCESS ENGINEERING	3
60	JOURNAL OF PROTEOMICS	3
61	KOREAN JOURNAL FOR FOOD SCIENCE OF ANIMAL RESOURCES	3
62	NOTULAE BOTANICAE HORTI AGROBOTANICI CLUJ-NAPOCA	3
63	PLANT PHYSIOLOGY AND BIOCHEMISTRY	3
64	POSTHARVEST BIOLOGY AND TECHNOLOGY	3
65	SCIENTIFIC REPORTS	3
66	AGRICULTURAL AND FOOD SCIENCE	2
67	BIOLOGIA PLANTARUM	2
68	BIOMED RESEARCH INTERNATIONAL	2

69	BIOSCIENCE RESEARCH	2
70	BOTANY	2
71	CURRENT RESEARCH IN NUTRITION AND FOOD SCIENCE	2
72	EUPHYTICA	2
73	FOOD BIOTECHNOLOGY	2
74	FRESENIUS ENVIRONMENTAL BULLETIN	2
75	FUNCTIONAL FOODS IN HEALTH AND DISEASE	2
76	INTERNATIONAL JOURNAL OF PLANT SCIENCES	2
77	INVASIVE PLANT SCIENCE AND MANAGEMENT	2
78	ITALIAN JOURNAL OF AGRONOMY	2
79	JOURNAL OF APPLIED RESEARCH ON MEDICINAL AND AROMATIC PLANTS	2
80	JOURNAL OF CHROMATOGRAPHY A	2
81	JOURNAL OF EXPERIMENTAL BOTANY	2
82	JOURNAL OF FOOD QUALITY	2
83	JOURNAL OF HORTICULTURAL SCIENCE \& BIOTECHNOLOGY	2
84	JOURNAL OF INTEGRATIVE PLANT BIOLOGY	2
85	JOURNAL OF MICROBIOLOGY BIOTECHNOLOGY AND FOOD SCIENCES	2
86	JOURNAL OF PHYTOPATHOLOGY	2
87	JOURNAL OF PLANT NUTRITION	2
88	NUTRITION \& FOOD SCIENCE	2
89	PAKISTAN JOURNAL OF AGRICULTURAL SCIENCES	2
90	PAKISTAN JOURNAL OF BOTANY	2
91	PHILIPPINE AGRICULTURAL SCIENTIST	2
92	PHYSIOLOGIA PLANTARUM	2
93	PHYTOCHEMISTRY	2
94	PHYTON-ANNALES REI BOTANICAE	2
95	PLANT FOODS FOR HUMAN NUTRITION	2
96	PLANT MOLECULAR BIOLOGY	2
97	PLANTA	2
98	POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES	2
99	QUALITY ASSURANCE AND SAFETY OF CROPS \& FOODS	2
100	RSC ADVANCES	2
101	SEED SCIENCE AND TECHNOLOGY	2
102	SEED SCIENCE RESEARCH	2
103	SPROUTED GRAINS: NUTRITIONAL VALUE PRODUCTION AND APPLICATIONS	2
104	UKRAINIAN FOOD JOURNAL	2

105	ABSTRACTS OF PAPERS OF THE AMERICAN CHEMICAL SOCIETY	1
106	ACTA ALIMENTARIA	1
107	ACTA BIOLOGICA CRACOVIENSIA SERIES BOTANICA	1
108	ACTA BOTANICA BRASILICA	1
109	ACTA SCIENTIARUM POLONORUM-TECHNOLOGIA ALIMENTARIA	1
110	ACTA SOCIETATIS BOTANICORUM POLONIAE	1
111	AFRICAN JOURNAL OF BIOTECHNOLOGY	1
112	AGRICULTURAL RESEARCH	1
113	AGRICULTURAL SYSTEMS	1
114	AGRICULTURE-BASEL	1
115	AGRONOMY JOURNAL	1
116	ALLELOPATHY JOURNAL	1
117	AMERICAN JOURNAL OF POTATO RESEARCH	1
118	ANALYST	1
119	ANALYTICAL BIOCHEMISTRY	1
120	ANALYTICAL CHEMISTRY	1
121	ANNALS OF AGRICULTURAL SCIENCE	1
122	ANNALS OF THE UNIVERSITY DUNAREA DE JOS OF GALATI FASCICLE VI-FOOD TECHNOLOGY	1
123	APPLIED ECOLOGY AND ENVIRONMENTAL RESEARCH	1
124	APPLIED FOOD BIOTECHNOLOGY	1
125	ARCHIVES OF AGRONOMY AND SOIL SCIENCE	1
126	ASIA PACIFIC JOURNAL OF CLINICAL NUTRITION	1
127	AUSTRALIAN JOURNAL OF EXPERIMENTAL AGRICULTURE	1
128	AUSTRALIAN JOURNAL OF PLANT PHYSIOLOGY	1
129	BIOCHIMIE	1
130	BIOLOGIA	1
131	BIOLOGIA FUTURA	1
132	BIOORGANICHESKAYA KHIMIYA	1
133	BIORESOURCE TECHNOLOGY	1
134	BIOSCIENCE JOURNAL	1
135	BIOTECNIA	1
136	BMC GENOMICS	1
137	BOTANICAL SCIENCES	1
138	BOTANICAL STUDIES	1
139	BRAZILIAN ARCHIVES OF BIOLOGY AND TECHNOLOGY	1
140	CANADIAN JOURNAL OF PLANT SCIENCE	1

141	CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY	1
142	CENTRAL EUROPEAN JOURNAL OF BIOLOGY	1
143	CHEMISTRY CENTRAL JOURNAL	1
144	CIENCIA RURAL	1
145	COGENT FOOD \& AGRICULTURE	1
146	CURRENT TOPICS IN NUTRACEUTICAL RESEARCH	1
147	CZECH JOURNAL OF FOOD SCIENCES	1
148	EGYPTIAN JOURNAL OF BOTANY	1
149	EMIRATES JOURNAL OF FOOD AND AGRICULTURE	1
150	ENVIRONMENTAL PROTECTION STRATEGIES FOR SUSTAINABLE DEVELOPMENT	1
151	ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH	1
152	EUROPEAN JOURNAL OF AGRONOMY	1
153	FOOD ANALYTICAL METHODS	1
154	FOOD AUSTRALIA	1
155	FOOD ENGINEERING REVIEWS	1
156	FOOD PACKAGING AND SHELF LIFE	1
157	FOOD PROCESSING: METHODS TECHNIQUES AND TRENDS	1
158	FOOD QUALITY AND SAFETY	1
159	FOOD SECURITY	1
160	FOOD TECHNOLOGY AND BIOTECHNOLOGY	1
161	FREE RADICAL RESEARCH	1
162	FRUITS	1
163	GAYANA BOTANICA	1
164	GENETIC RESOURCES AND CROP EVOLUTION	1
165	GRASAS Y ACEITES	1
166	INDIAN JOURNAL OF EXPERIMENTAL BIOLOGY	1
167	INDIAN JOURNAL OF TRADITIONAL KNOWLEDGE	1
168	INTERNATIONAL JOURNAL OF BIOLOGY AND CHEMISTRY	1
169	INTERNATIONAL JOURNAL OF FOOD SCIENCES AND NUTRITION	1
170	INTERNATIONAL JOURNAL OF GASTRONOMY AND FOOD SCIENCE	1
171	INTERNATIONAL JOURNAL OF PHYTOREMEDIATION	1
172	INTERNATIONAL JOURNAL OF PLANT PRODUCTION	1
173	ISRAEL JOURNAL OF PLANT SCIENCES	1

174	JOURNAL INTERNATIONAL DES SCIENCES DE LA VIGNE ET DU VIN	1
175	JOURNAL OF AGRICULTURAL SCIENCE AND TECHNOLOGY	1
176	JOURNAL OF AGRICULTURAL SCIENCES-TARIM BILIMLERI DERGISI	1
177	JOURNAL OF ANALYTICAL METHODS IN CHEMISTRY	1
178	JOURNAL OF AOAC INTERNATIONAL	1
179	JOURNAL OF APPLIED BOTANY AND FOOD QUALITY	1
180	JOURNAL OF BIOSCIENCES	1
181	JOURNAL OF ESSENTIAL OIL RESEARCH	1
182	JOURNAL OF FOOD AND NUTRITION RESEARCH	1
183	JOURNAL OF FOOD SCIENCE AND TECHNOLOGY-UKRAINE	1
184	JOURNAL OF FUNDAMENTAL AND APPLIED SCIENCES	1
185	JOURNAL OF INTEGRATIVE AGRICULTURE	1
186	JOURNAL OF OLEO SCIENCE	1
187	JOURNAL OF PLANT BIOCHEMISTRY AND BIOTECHNOLOGY	1
188	JOURNAL OF PLANT BIOLOGY	1
189	JOURNAL OF PLANT GROWTH REGULATION	1
190	JOURNAL OF TEXTURE STUDIES	1
191	JOURNAL OF THE CHILEAN CHEMICAL SOCIETY	1
192	JOURNAL OF THE CHINESE INSTITUTE OF ENGINEERS	1
193	JOURNAL OF THE JAPANESE SOCIETY FOR FOOD SCIENCE AND TECHNOLOGY-NIPPON SHOKUHIN KAGAKU KOGAKU KAISHI	1
194	JOURNAL OF ZHEJIANG UNIVERSITY-SCIENCE B	1
195	KOREAN JOURNAL OF HORTICULTURAL SCIENCE \& TECHNOLOGY	1
196	KVASNY PRUMYSL	1
197	LEGUME RESEARCH	1
198	MEAT SCIENCE	1
199	MOLECULAR \& CELLULAR PROTEOMICS	1
200	MOLECULAR PLANT PATHOLOGY	1
201	NUSANTARA BIOSCIENCE	1
202	NUTRIENTS	1
203	OXIDATIVE MEDICINE AND CELLULAR LONGEVITY	1
204	PEERJ	1
205	PESQUISA AGROPECUARIA BRASILEIRA	1

206	PHILIPPINE JOURNAL OF CROP SCIENCE	1
207	PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B-BIOLOGICAL SCIENCES	1
208	PHOTOSYNTHETICA	1
209	PHYSIOLOGY AND MOLECULAR BIOLOGY OF PLANTS	1
210	PHYSIOLOGY INTERNATIONAL	1
211	PHYTON-INTERNATIONAL JOURNAL OF EXPERIMENTAL BOTANY	1
212	PHYTOPATHOLOGY	1
213	PLANT AND SOIL	1
214	PLANT BREEDING	1
215	PLANT CELL TISSUE AND ORGAN CULTURE	1
216	PLANT JOURNAL	1
217	PLANT METHODS	1
218	PLANT PHYSIOLOGY	1
219	PLANT SCIENCE TODAY	1
220	PLOS ONE	1
221	POLISH JOURNAL OF ENVIRONMENTAL STUDIES	1
222	POTATO RESEARCH	1
223	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA	1
224	PROCESSES	1
225	PROTEOME SCIENCE	1
226	PROTEOMICS	1
227	QUIMICA NOVA	1
228	RESEARCH JOURNAL OF BIOTECHNOLOGY	1
229	REVISTA BRASILEIRA DE CIENCIA DO SOLO	1
230	REVISTA BRASILEIRA DE CIENCIAS AGRARIAS-AGRARIA	1
231	REVISTA BRASILEIRA DE FRUTICULTURA	1
232	REVISTA DE BIOLOGIA TROPICAL	1
233	REVISTA DE LA FACULTAD DE AGRONOMIA DE LA UNIVERSIDAD DEL ZULIA	1
234	REVISTA DE LA FACULTAD DE CIENCIAS AGRARIAS	1
235	REVISTA MEXICANA DE INGENIERIA QUIMICA	1
236	REVISTA VIRTUAL DE QUIMICA	1
237	RICE SCIENCE	1
238	ROMANIAN BIOTECHNOLOGICAL LETTERS	1
239	RUSSIAN JOURNAL OF PLANT PHYSIOLOGY	1
240	SCIENCE OF THE TOTAL ENVIRONMENT	1
241	SEMINA-CIENCIAS AGRARIAS	1
242	SOIL SCIENCE	1

243	SOUTH AFRICAN JOURNAL OF ENOLOGY AND VITICULTURE	1
244	SWEET POTATO: POST HARVEST ASPECTS IN FOOD FEED AND INDUSTRY	1
245	TROPICAL PLANT BIOLOGY	1
246	ZEMDIRBYSTE-AGRICULTURE	1

Table S3. The top 50 author keywords found in the highest number of articles

N° keywords	Author Keywords	Frequency	%
1	germination	142	14
2	antioxidant activity	84	8
3	antioxidant	53	5
4	glucosinolates	48	5
5	sprouts	45	4
6	phenolics	36	4
7	phenolic compounds	33	3
8	elicitation	27	3
9	antioxidants	25	2
10	sprouting	23	2
11	antioxidant capacity	22	2
12	phytochemicals	22	2
13	soybean	22	2
14	flavonoids	20	2
15	glucosinolate	19	2
16	broccoli sprouts	18	2
17	seed germination	18	2
18	activity	17	2
19	sulforaphane	17	2
20	broccoli	16	2
21	phenolic	15	1
22	myrosinase	14	1
23	bioactive compounds	13	1
24	polyphenols	13	1
25	sprout	13	1
26	antioxidant enzymes	12	1
27	seed	12	1
28	yield	12	1
29	elicitors	11	1

30	gamma-aminobutyric acid	11	1
31	methyl jasmonate	11	1
32	phytic acid	11	1
33	brassica oleracea	10	1
34	gene expression	10	1
35	metabolomics	10	1
36	rice	10	1
37	wheat	10	1
38	brown rice	9	1
39	carotenoids	9	1
40	fermentation	9	1
41	legumes	9	1
42	light	9	1
43	phytochemical	9	1
44	properties	9	1
45	selenium	9	1
46	soaking	9	1
47	tartary buckwheat	9	1
48	anthocyanin	8	1
49	bioaccessibility	8	1
50	compounds	8	1

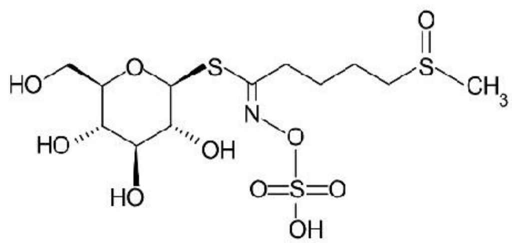
Table S4. The most productive countries concerning publications on elicitation in sprouts.

Rank	Country	Articles	Frequency (%)	SCP	MCP	MCP Ratio (%)
1	China	150	0.1931	125	25	0.1667
2	India	85	0.1094	78	7	0.0824
3	USA	60	0.0772	49	11	0.1833
4	Korea	58	0.0746	50	8	0.1379
5	Poland	43	0.0553	40	3	0.0698
6	Spain	40	0.0515	26	14	0.3500
7	Italy	39	0.0502	27	12	0.3077
8	Mexico	27	0.0347	22	5	0.1852
9	Brazil	24	0.0309	22	2	0.0833
10	Thailand	24	0.0309	18	6	0.2500

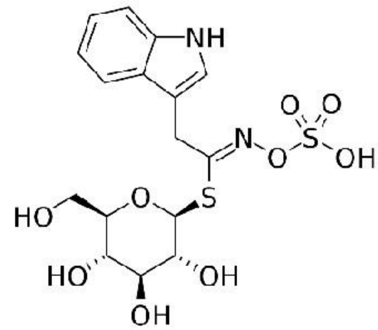
Freq: Frequency; SCP: Single Country Publications; MCP: Multiple Country Publications.

Figuras complementarias artículo I

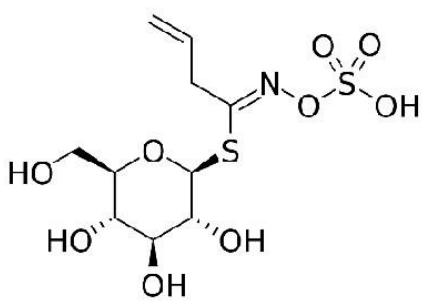
Figure S1. Chemical structures of the main glucosinolates.



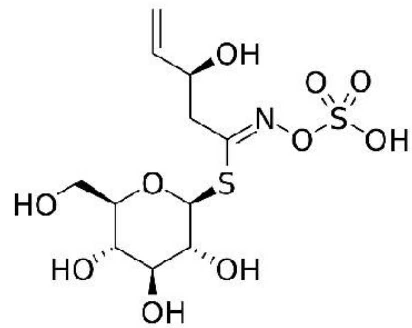
Glucoraphanin



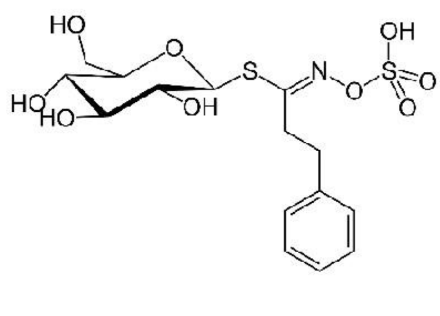
Glucobrassicin



Sinigrin



Progoitrin



Gluconasturtiin

Tablas suplementarias artículo II

Table S1. Development of radicle in edible and wild radish sprouts of 7-days-old.

Edible radish					
20°C			28°C		
Elicitor	Day	Radicle length (cm)	Elicitor	Day	Radicle length (cm)
Control	1	0.883	Control	1	0.650
Control	1	0.655	Control	1	0.578
Control	1	0.786	Control	1	0.544
Citric Acid	1	0.567	Citric Acid	1	0.58
Citric Acid	1	0.542	Citric Acid	1	0.796
Citric Acid	1	0.489	Citric Acid	1	0.871
MeJa	1	0.387	MeJa	1	0.642
MeJa	1	0.607	MeJa	1	0.523
MeJa	1	0.599	MeJa	1	0.589
Chitosan	1	0.482	Chitosan	1	0.482
Chitosan	1	0.392	Chitosan	1	0.495
Chitosan	1	0.554	Chitosan	1	0.590
K_2SO_4	1	0.733	K_2SO_4	1	0.612
K_2SO_4	1	0.835	K_2SO_4	1	0.633
K_2SO_4	1	0.872	K_2SO_4	1	0.714
Control	2	1.235	Control	2	2.245
Control	2	1.123	Control	2	2.550
Control	2	1.282	Control	2	2.322
Citric Acid	2	1.243	Citric Acid	2	2.044
Citric Acid	2	1.145	Citric Acid	2	1.581
Citric Acid	2	1.163	Citric Acid	2	2.179
MeJa	2	0.968	MeJa	2	2.164
MeJa	2	1.396	MeJa	2	1.258
MeJa	2	1.215	MeJa	2	1.160
Chitosan	2	0.603	Chitosan	2	2.703
Chitosan	2	0.776	Chitosan	2	2.785
Chitosan	2	0.772	Chitosan	2	2.655
K_2SO_4	2	1.584	K_2SO_4	2	2.326
K_2SO_4	2	1.509	K_2SO_4	2	1.945
K_2SO_4	2	2.033	K_2SO_4	2	2.012
Control	3	1.650	Control	3	3.533
Control	3	1.583	Control	3	3.680
Control	3	1.575	Control	3	3.744

Citric Acid	3	2.255	Citric Acid	3	2.842
Citric Acid	3	2.773	Citric Acid	3	2.800
Citric Acid	3	2.687	Citric Acid	3	3.077
MeJa	3	1.032	MeJa	3	2.378
MeJa	3	1.181	MeJa	3	2.271
MeJa	3	1.103	MeJa	3	2.377
Chitosan	3	0.931	Chitosan	3	3.531
Chitosan	3	1.080	Chitosan	3	3.850
Chitosan	3	1.136	Chitosan	3	3.821
K_2SO_4	3	1.881	K_2SO_4	3	2.855
K_2SO_4	3	1.954	K_2SO_4	3	2.745
K_2SO_4	3	2.062	K_2SO_4	3	2.698
Control	4	2.101	Control	4	4.144
Control	4	2.029	Control	4	4.212
Control	4	2.205	Control	4	4.254
Citric Acid	4	2.987	Citric Acid	4	4.874
Citric Acid	4	3.032	Citric Acid	4	4.590
Citric Acid	4	3.033	Citric Acid	4	4.798
MeJa	4	2.658	MeJa	4	3.321
MeJa	4	2.443	MeJa	4	3.476
MeJa	4	2.581	MeJa	4	3.276
Chitosan	4	1.349	Chitosan	4	4.349
Chitosan	4	1.657	Chitosan	4	4.235
Chitosan	4	1.232	Chitosan	4	4.182
K_2SO_4	4	2.864	K_2SO_4	4	3.021
K_2SO_4	4	2.965	K_2SO_4	4	2.987
K_2SO_4	4	2.732	K_2SO_4	4	3.210
Control	5	5.657	Control	5	4.550
Control	5	5.788	Control	5	4.412
Control	5	5.667	Control	5	4.355
Citric Acid	5	3.565	Citric Acid	5	5.237
Citric Acid	5	3.451	Citric Acid	5	5.197
Citric Acid	5	3.478	Citric Acid	5	5.403
MeJa	5	3.265	MeJa	5	3.876
MeJa	5	3.850	MeJa	5	4.061
MeJa	5	3.002	MeJa	5	5.609
Chitosan	5	2.737	Chitosan	5	5.737
Chitosan	5	2.570	Chitosan	5	5.490
Chitosan	5	2.593	Chitosan	5	5.609
K_2SO_4	5	3.324	K_2SO_4	5	3.230
K_2SO_4	5	3.012	K_2SO_4	5	3.451

K_2SO_4	5	3.265	K_2SO_4	5	3.822
Control	6	5.855	Control	6	4.744
Control	6	5.798	Control	6	4.656
Control	6	5.874	Control	6	4.612
Citric Acid	6	3.955	Citric Acid	6	5.237
Citric Acid	6	3.987	Citric Acid	6	5.391
Citric Acid	6	4.004	Citric Acid	6	5.253
MeJa	6	3.754	MeJa	6	4.365
MeJa	6	3.633	MeJa	6	4.474
MeJa	6	3.486	MeJa	6	4.371
Chitosan	6	3.051	Chitosan	6	6.351
Chitosan	6	3.455	Chitosan	6	6.699
Chitosan	6	3.045	Chitosan	6	6.598
K_2SO_4	6	3.874	K_2SO_4	6	3.633
K_2SO_4	6	3.532	K_2SO_4	6	3.766
K_2SO_4	6	3.724	K_2SO_4	6	3.644
Control	7	6.022	Control	7	4.832
Control	7	6.012	Control	7	4.988
Control	7	6.212	Control	7	4.798
Citric Acid	7	5.251	Citric Acid	7	6.265
Citric Acid	7	5.487	Citric Acid	7	6.312
Citric Acid	7	5.368	Citric Acid	7	6.213
MeJa	7	4.076	MeJa	7	4.620
MeJa	7	3.965	MeJa	7	4.590
MeJa	7	4.130	MeJa	7	4.723
Chitosan	7	4.745	Chitosan	7	7.100
Chitosan	7	4.854	Chitosan	7	7.021
Chitosan	7	4.733	Chitosan	7	7.189
K_2SO_4	7	4.462	K_2SO_4	7	4.586
K_2SO_4	7	4.168	K_2SO_4	7	4.590
K_2SO_4	7	4.356	K_2SO_4	7	4.638

Wild radish					
20°C			28°C		
Elicitor	Day	Radicle length (cm)	Elicitor	Day	Radicle length (cm)
Control	1	0.682	Control	1	1.255
Control	1	0.533	Control	1	1.124
Control	1	0.781	Control	1	1.036
Citric Acid	1	0.570	Citric Acid	1	1.254
Citric Acid	1	0.426	Citric Acid	1	1.152

Citric Acid	1	0.391	Citric Acid	1	1.332
MeJa	1	0.532	MeJa	1	0.643
MeJa	1	0.607	MeJa	1	0.446
MeJa	1	0.589	MeJa	1	0.564
Chitosan	1	0.376	Chitosan	1	0.556
Chitosan	1	0.489	Chitosan	1	0.577
Chitosan	1	0.408	Chitosan	1	0.699
K_2SO_4	1	0.432	K_2SO_4	1	0.644
K_2SO_4	1	0.656	K_2SO_4	1	0.555
K_2SO_4	1	0.533	K_2SO_4	1	0.421
Control	2	0.529	Control	2	2.765
Control	2	1.274	Control	2	2.845
Control	2	1.002	Control	2	2.754
Citric Acid	2	0.898	Citric Acid	2	2.623
Citric Acid	2	0.973	Citric Acid	2	2.565
Citric Acid	2	0.002	Citric Acid	2	2.632
MeJa	2	1.484	MeJa	2	1.164
MeJa	2	1.232	MeJa	2	1.258
MeJa	2	1.550	MeJa	2	1.160
Chitosan	2	0.854	Chitosan	2	1.033
Chitosan	2	0.837	Chitosan	2	1.237
Chitosan	2	0.672	Chitosan	2	1.261
K_2SO_4	2	0.883	K_2SO_4	2	1.895
K_2SO_4	2	1.244	K_2SO_4	2	1.488
K_2SO_4	2	1.105	K_2SO_4	2	1.633
Control	3	1.780	Control	3	3.012
Control	3	1.850	Control	3	3.324
Control	3	1.736	Control	3	3.114
Citric Acid	3	1.333	Citric Acid	3	2.842
Citric Acid	3	1.243	Citric Acid	3	2.763
Citric Acid	3	2.048	Citric Acid	3	2.852
MeJa	3	1.667	MeJa	3	2.878
MeJa	3	1.720	MeJa	3	2.682
MeJa	3	1.721	MeJa	3	2.512
Chitosan	3	1.032	Chitosan	3	2.791
Chitosan	3	1.281	Chitosan	3	2.882
Chitosan	3	1.273	Chitosan	3	2.755
K_2SO_4	3	1.783	K_2SO_4	3	2.612
K_2SO_4	3	1.555	K_2SO_4	3	2.845
K_2SO_4	3	1.305	K_2SO_4	3	2.780
Control	4	2.773	Control	4	4.966

Control	4	2.550	Control	4	5.021
Control	4	2.636	Control	4	5.111
Citric Acid	4	2.435	Citric Acid	4	4.874
Citric Acid	4	2.051	Citric Acid	4	4.712
Citric Acid	4	2.683	Citric Acid	4	4.764
MeJa	4	1.918	MeJa	4	3.320
MeJa	4	2.319	MeJa	4	3.112
MeJa	4	2.088	MeJa	4	3.212
Chitosan	4	1.667	Chitosan	4	4.225
Chitosan	4	1.863	Chitosan	4	4.256
Chitosan	4	1.960	Chitosan	4	4.087
K_2SO_4	4	2.164	K_2SO_4	4	3.114
K_2SO_4	4	1.883	K_2SO_4	4	3.247
K_2SO_4	4	2.453	K_2SO_4	4	3.304
Control	5	4.970	Control	5	6.020
Control	5	4.863	Control	5	5.988
Control	5	4.655	Control	5	6.140
Citric Acid	5	3.677	Citric Acid	5	5.237
Citric Acid	5	4.054	Citric Acid	5	5.343
Citric Acid	5	3.954	Citric Acid	5	5.454
MeJa	5	2.204	MeJa	5	3.822
MeJa	5	2.372	MeJa	5	3.756
MeJa	5	2.502	MeJa	5	3.732
Chitosan	5	2.662	Chitosan	5	4.500
Chitosan	5	2.722	Chitosan	5	4.471
Chitosan	5	2.836	Chitosan	5	4.572
K2SO4	5	2.643	K_2SO_4	5	3.547
K2SO4	5	2.722	K_2SO_4	5	3.805
K2SO4	5	2.665	K_2SO_4	5	3.845
Control	6	5.044	Control	6	6.647
Control	6	5.112	Control	6	6.487
Control	6	4.988	Control	6	6.544
Citric Acid	6	5.872	Citric Acid	6	5.621
Citric Acid	6	5.502	Citric Acid	6	5.532
Citric Acid	6	5.633	Citric Acid	6	5.843
MeJa	6	2.598	MeJa	6	4.212
MeJa	6	2.632	MeJa	6	4.418
MeJa	6	2.754	MeJa	6	4.271
Chitosan	6	3.154	Chitosan	6	5.781
Chitosan	6	3.002	Chitosan	6	5.612
Chitosan	6	3.040	Chitosan	6	5.589

K_2SO_4	6	3.354	K_2SO_4	6	3.877
K_2SO_4	6	3.754	K_2SO_4	6	4.405
K_2SO_4	6	3.565	K_2SO_4	6	4.134
Control	7	5.422	Control	7	7.210
Control	7	5.390	Control	7	7.221
Control	7	5.530	Control	7	7.321
Citric Acid	7	6.207	Citric Acid	7	6.471
Citric Acid	7	6.306	Citric Acid	7	6.512
Citric Acid	7	6.336	Citric Acid	7	6.321
MeJa	7	3.473	MeJa	7	4.787
MeJa	7	3.235	MeJa	7	4.634
MeJa	7	3.029	MeJa	7	4.834
Chitosan	7	4.814	Chitosan	7	7.665
Chitosan	7	4.960	Chitosan	7	7.726
Chitosan	7	5.051	Chitosan	7	7.764
K_2SO_4	7	4.332	K_2SO_4	7	5.760
K_2SO_4	7	4.464	K_2SO_4	7	5.531
K_2SO_4	7	4.212	K_2SO_4	7	4.620

Table S2. Development of hypocotyl in edible and wild radish sprouts of 7-days-old.

Edible radish					
20°C			28°C		
Elicitor	Day	Hypocotyl length (cm)	Elicitor	Day	Hypocotyl length (cm)
Control	1	0	Control	1	0
Control	1	0	Control	1	0
Control	1	0	Control	1	0
Citric Acid	1	0	Citric Acid	1	0
Citric Acid	1	0	Citric Acid	1	0
Citric Acid	1	0	Citric Acid	1	0
MeJa	1	0	MeJa	1	0
MeJa	1	0	MeJa	1	0
MeJa	1	0	MeJa	1	0
Chitosan	1	0	Chitosan	1	0
Chitosan	1	0	Chitosan	1	0
Chitosan	1	0	Chitosan	1	0
K_2SO_4	1	0	K_2SO_4	1	0
K_2SO_4	1	0	K_2SO_4	1	0

K_2SO_4	1	0	K_2SO_4	1	0
Control	2	0.602	Control	2	0.850
Control	2	0.707	Control	2	0.865
Control	2	0.556	Control	2	0.843
Citric Acid	2	0.563	Citric Acid	2	0.927
Citric Acid	2	0.482	Citric Acid	2	0.692
Citric Acid	2	0.550	Citric Acid	2	0.817
MeJa	2	0.519	MeJa	2	0.634
MeJa	2	0.627	MeJa	2	0.613
MeJa	2	0.733	MeJa	2	0.648
Chitosan	2	0.713	Chitosan	2	0.550
Chitosan	2	0.742	Chitosan	2	0.558
Chitosan	2	0.683	Chitosan	2	0.633
K_2SO_4	2	0.398	K_2SO_4	2	0.612
K_2SO_4	2	0.724	K_2SO_4	2	0.787
K_2SO_4	2	0.633	K_2SO_4	2	0.888
Control	3	0.981	Control	3	1.020
Control	3	1.003	Control	3	1.109
Control	3	0.906	Control	3	1.043
Citric Acid	3	1.132	Citric Acid	3	1.194
Citric Acid	3	1.273	Citric Acid	3	1.292
Citric Acid	3	1.383	Citric Acid	3	1.371
MeJa	3	0.833	MeJa	3	1.182
MeJa	3	0.779	MeJa	3	1.061
MeJa	3	0.681	MeJa	3	0.921
Chitosan	3	1.233	Chitosan	3	1.891
Chitosan	3	1.102	Chitosan	3	1.783
Chitosan	3	1.038	Chitosan	3	1.955
K_2SO_4	3	1.005	K_2SO_4	3	1.176
K_2SO_4	3	1.002	K_2SO_4	3	1.065
K_2SO_4	3	0.881	K_2SO_4	3	1.186
Control	4	1.473	Control	4	1.303
Control	4	1.632	Control	4	1.275
Control	4	1.344	Control	4	1.321
Citric Acid	4	1.614	Citric Acid	4	1.944
Citric Acid	4	1.806	Citric Acid	4	1.954
Citric Acid	4	1.432	Citric Acid	4	2.191
MeJa	4	1.400	MeJa	4	1.375
MeJa	4	1.139	MeJa	4	1.252
MeJa	4	1.437	MeJa	4	1.309
Chitosan	4	1.716	Chitosan	4	2.572

Chitosan	4	1.694	Chitosan	4	1.954
Chitosan	4	1.718	Chitosan	4	2.201
K_2SO_4	4	1.636	K_2SO_4	4	2.082
K_2SO_4	4	1.226	K_2SO_4	4	1.912
K_2SO_4	4	1.476	K_2SO_4	4	1.823
Control	5	1.746	Control	5	1.531
Control	5	1.806	Control	5	1.465
Control	5	1.788	Control	5	1.554
Citric Acid	5	1.790	Citric Acid	5	2.459
Citric Acid	5	2.012	Citric Acid	5	2.376
Citric Acid	5	2.213	Citric Acid	5	2.297
MeJa	5	1.773	MeJa	5	1.506
MeJa	5	1.622	MeJa	5	1.481
MeJa	5	1.767	MeJa	5	1.372
Chitosan	5	1.919	Chitosan	5	2.408
Chitosan	5	1.987	Chitosan	5	2.284
Chitosan	5	1.847	Chitosan	5	2.668
K_2SO_4	5	1.858	K_2SO_4	5	2.224
K_2SO_4	5	1.953	K_2SO_4	5	2.186
K_2SO_4	5	1.973	K_2SO_4	5	2.246
Control	6	1.854	Control	6	2.364
Control	6	1.901	Control	6	2.298
Control	6	1.835	Control	6	2.325
Citric Acid	6	2.107	Citric Acid	6	2.587
Citric Acid	6	2.022	Citric Acid	6	2.501
Citric Acid	6	1.988	Citric Acid	6	2.482
MeJa	6	1.797	MeJa	6	2.024
MeJa	6	1.800	MeJa	6	2.192
MeJa	6	1.847	MeJa	6	1.871
Chitosan	6	2.238	Chitosan	6	3.425
Chitosan	6	2.355	Chitosan	6	3.245
Chitosan	6	2.203	Chitosan	6	2.944
K_2SO_4	6	2.221	K_2SO_4	6	2.364
K_2SO_4	6	2.398	K_2SO_4	6	2.361
K_2SO_4	6	2.432	K_2SO_4	6	2.321
Control	7	2.099	Control	7	2.507
Control	7	1.978	Control	7	2.413
Control	7	2.086	Control	7	2.402
Citric Acid	7	2.109	Citric Acid	7	2.684
Citric Acid	7	2.552	Citric Acid	7	2.721
Citric Acid	7	2.077	Citric Acid	7	2.697

MeJa	7	1.844	MeJa	7	2.232
MeJa	7	2.016	MeJa	7	2.354
MeJa	7	1.918	MeJa	7	2.198
Chitosan	7	2.304	Chitosan	7	4.067
Chitosan	7	2.115	Chitosan	7	4.082
Chitosan	7	2.232	Chitosan	7	4.061
K_2SO_4	7	2.653	K_2SO_4	7	2.507
K_2SO_4	7	2.632	K_2SO_4	7	2.413
K_2SO_4	7	2.707	K_2SO_4	7	2.321

Wild radish

20°C			28°C		
Elicitor	Day	Hypocotyl length (cm)	Elicitor	Day	Hypocotyl length (cm)
Control	1	0	Control	1	0
Control	1	0	Control	1	0
Control	1	0	Control	1	0
Citric Acid	1	0	Citric Acid	1	0
Citric Acid	1	0	Citric Acid	1	0
Citric Acid	1	0	Citric Acid	1	0
MeJa	1	0	MeJa	1	0
MeJa	1	0	MeJa	1	0
MeJa	1	0	MeJa	1	0
Chitosan	1	0	Chitosan	1	0
Chitosan	1	0	Chitosan	1	0
Chitosan	1	0	Chitosan	1	0
K_2SO_4	1	0	K_2SO_4	1	0
K_2SO_4	1	0	K_2SO_4	1	0
K_2SO_4	1	0	K_2SO_4	1	0
Control	2	0.592	Control	2	0.522
Control	2	0.489	Control	2	0.402
Control	2	0.532	Control	2	0.586
Citric Acid	2	0.771	Citric Acid	2	0.395
Citric Acid	2	0.848	Citric Acid	2	0.794
Citric Acid	2	0.852	Citric Acid	2	0.817
MeJa	2	0.554	MeJa	2	0.710
MeJa	2	0.690	MeJa	2	0.361
MeJa	2	0.738	MeJa	2	0.519
Chitosan	2	0.748	Chitosan	2	1.235
Chitosan	2	0.551	Chitosan	2	1.136
Chitosan	2	0.633	Chitosan	2	1.156

K_2SO_4	2	0.821	K_2SO_4	2	0.545
K_2SO_4	2	0.745	K_2SO_4	2	0.702
K_2SO_4	2	0.590	K_2SO_4	2	0.586
Control	3	0.883	Control	3	1.034
Control	3	0.850	Control	3	1.101
Control	3	0.905	Control	3	0.943
Citric Acid	3	1.232	Citric Acid	3	1.486
Citric Acid	3	1.236	Citric Acid	3	1.393
Citric Acid	3	1.373	Citric Acid	3	1.290
MeJa	3	0.763	MeJa	3	1.283
MeJa	3	0.888	MeJa	3	1.261
MeJa	3	0.812	MeJa	3	0.971
Chitosan	3	1.003	Chitosan	3	1.741
Chitosan	3	1.153	Chitosan	3	1.836
Chitosan	3	1.033	Chitosan	3	1.905
K_2SO_4	3	1.232	K_2SO_4	3	0.932
K_2SO_4	3	1.105	K_2SO_4	3	0.891
K_2SO_4	3	1.053	K_2SO_4	3	0.876
Control	4	1.245	Control	4	1.306
Control	4	1.395	Control	4	1.387
Control	4	1.230	Control	4	1.421
Citric Acid	4	1.971	Citric Acid	4	1.929
Citric Acid	4	1.658	Citric Acid	4	1.954
Citric Acid	4	1.712	Citric Acid	4	1.886
MeJa	4	1.175	MeJa	4	1.381
MeJa	4	1.279	MeJa	4	1.283
MeJa	4	1.285	MeJa	4	1.454
Chitosan	4	1.362	Chitosan	4	2.432
Chitosan	4	1.465	Chitosan	4	2.003
Chitosan	4	1.315	Chitosan	4	2.321
K_2SO_4	4	1.435	K_2SO_4	4	1.286
K_2SO_4	4	1.593	K_2SO_4	4	1.386
K_2SO_4	4	1.366	K_2SO_4	4	1.575
Control	5	1.446	Control	5	1.844
Control	5	1.378	Control	5	1.734
Control	5	1.507	Control	5	1.898
Citric Acid	5	1.678	Citric Acid	5	3.396
Citric Acid	5	1.688	Citric Acid	5	3.281
Citric Acid	5	1.901	Citric Acid	5	3.100
MeJa	5	1.371	MeJa	5	1.783
MeJa	5	1.602	MeJa	5	1.682

MeJa	5	1.481	MeJa	5	1.752
Chitosan	5	1.336	Chitosan	5	2.704
Chitosan	5	1.502	Chitosan	5	2.321
Chitosan	5	1.515	Chitosan	5	2.486
K_2SO_4	5	1.501	K_2SO_4	5	1.864
K_2SO_4	5	1.522	K_2SO_4	5	1.712
K_2SO_4	5	1.607	K_2SO_4	5	1.738
Control	6	1.577	Control	6	2.837
Control	6	1.465	Control	6	2.687
Control	6	1.597	Control	6	2.754
Citric Acid	6	2.107	Citric Acid	6	3.571
Citric Acid	6	2.022	Citric Acid	6	3.171
Citric Acid	6	1.988	Citric Acid	6	3.482
MeJa	6	1.543	MeJa	6	1.865
MeJa	6	1.555	MeJa	6	1.852
MeJa	6	1.464	MeJa	6	1.901
Chitosan	6	1.786	Chitosan	6	2.976
Chitosan	6	1.803	Chitosan	6	2.604
Chitosan	6	1.899	Chitosan	6	2.792
K_2SO_4	6	1.745	K_2SO_4	6	2.032
K_2SO_4	6	1.688	K_2SO_4	6	2.344
K_2SO_4	6	1.801	K_2SO_4	6	2.176
Control	7	1.840	Control	7	3.137
Control	7	1.853	Control	7	3.212
Control	7	1.760	Control	7	3.253
Citric Acid	7	2.485	Citric Acid	7	3.775
Citric Acid	7	2.538	Citric Acid	7	3.589
Citric Acid	7	2.364	Citric Acid	7	3.597
MeJa	7	1.730	MeJa	7	2.882
MeJa	7	1.762	MeJa	7	2.791
MeJa	7	1.692	MeJa	7	2.940
Chitosan	7	2.452	Chitosan	7	4.450
Chitosan	7	2.573	Chitosan	7	4.490
Chitosan	7	2.607	Chitosan	7	4.360
K_2SO_4	7	2.287	K_2SO_4	7	2.995
K_2SO_4	7	1.750	K_2SO_4	7	3.188
K_2SO_4	7	1.888	K_2SO_4	7	3.086

Figuras complementarias artículo II

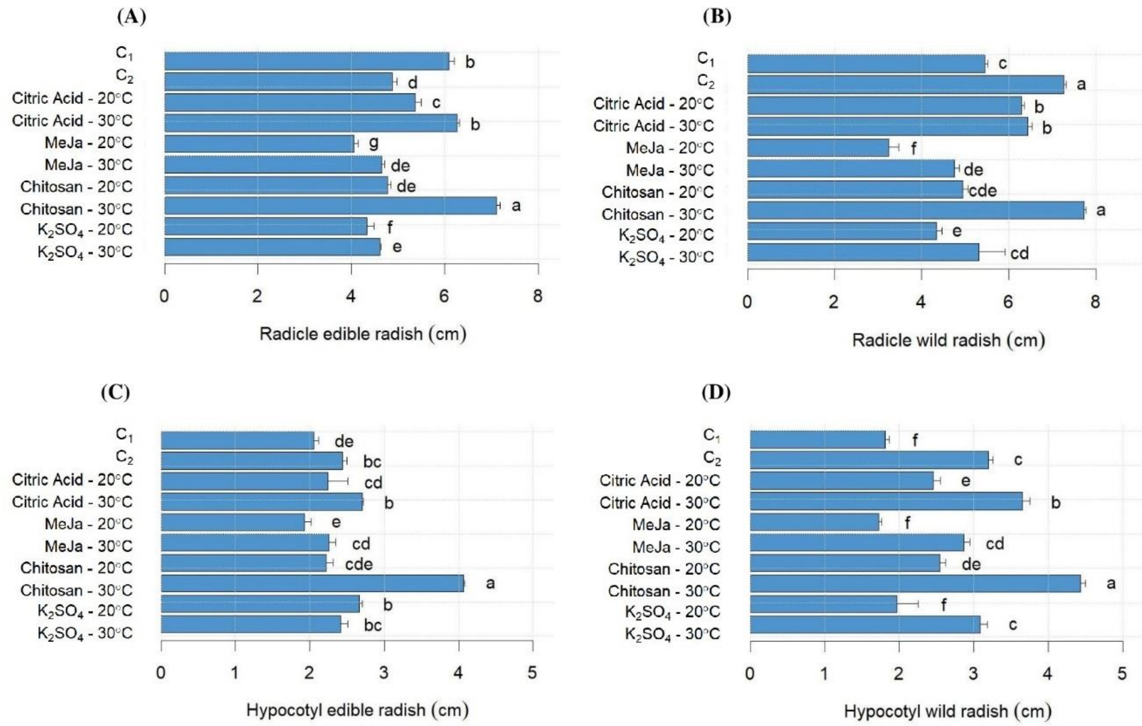


Figure S1. Effect of elicitors on radicle growth and hypocotyl growth in 7-day germinated sprouts. Letters A and B represent the Hypoctyl growth of ER and WR. Letters C and D represent the radicle growth of ER and WR. Results are the mean of four independent determinations ± standard error.

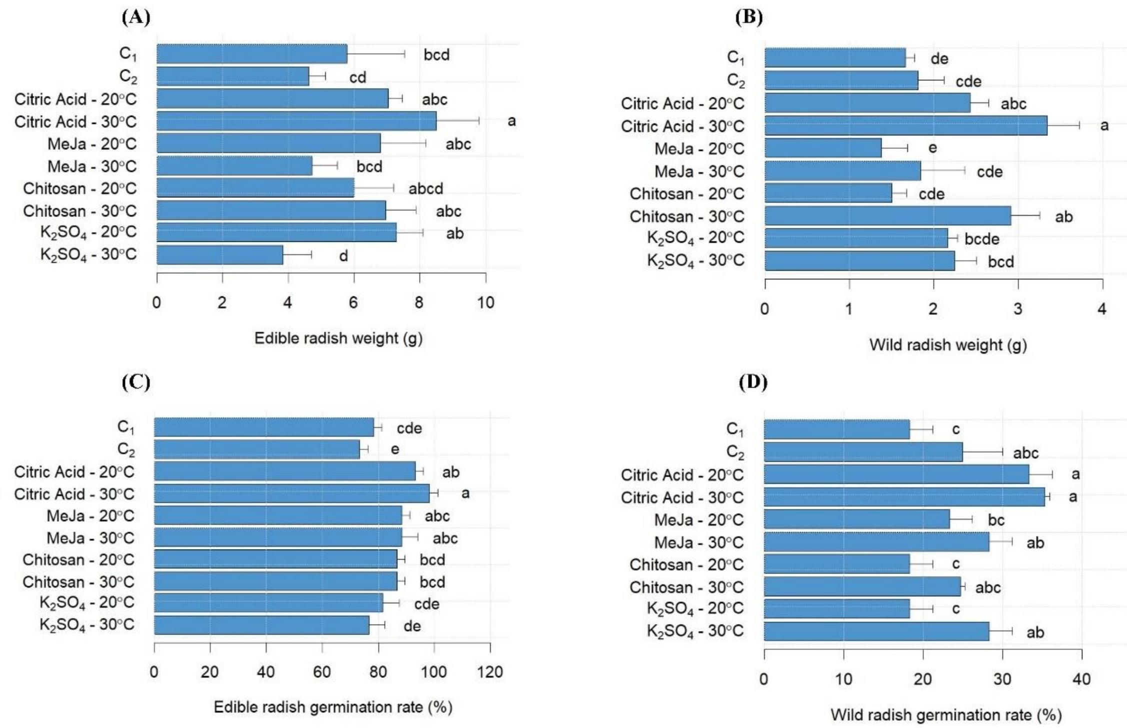


Figure S2. Effect of elicitors on fresh weight and germination rate in 7-day germinated sprouts**.** Letters A and B represent the fresh weight of ER and WR. Letters C and D represent the germination rate of ER and WR. Results are the mean of four independent determinations ± standard error.

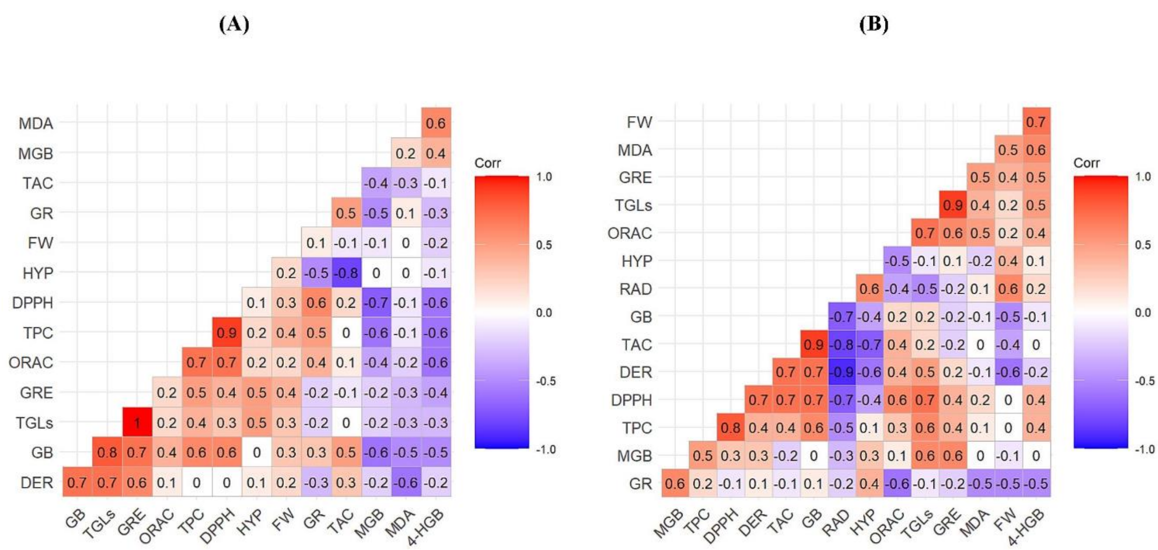


Figure S3. Correlation matrix between different variables at 20°C, (A) correspond to ER and (B) correspond to WR.

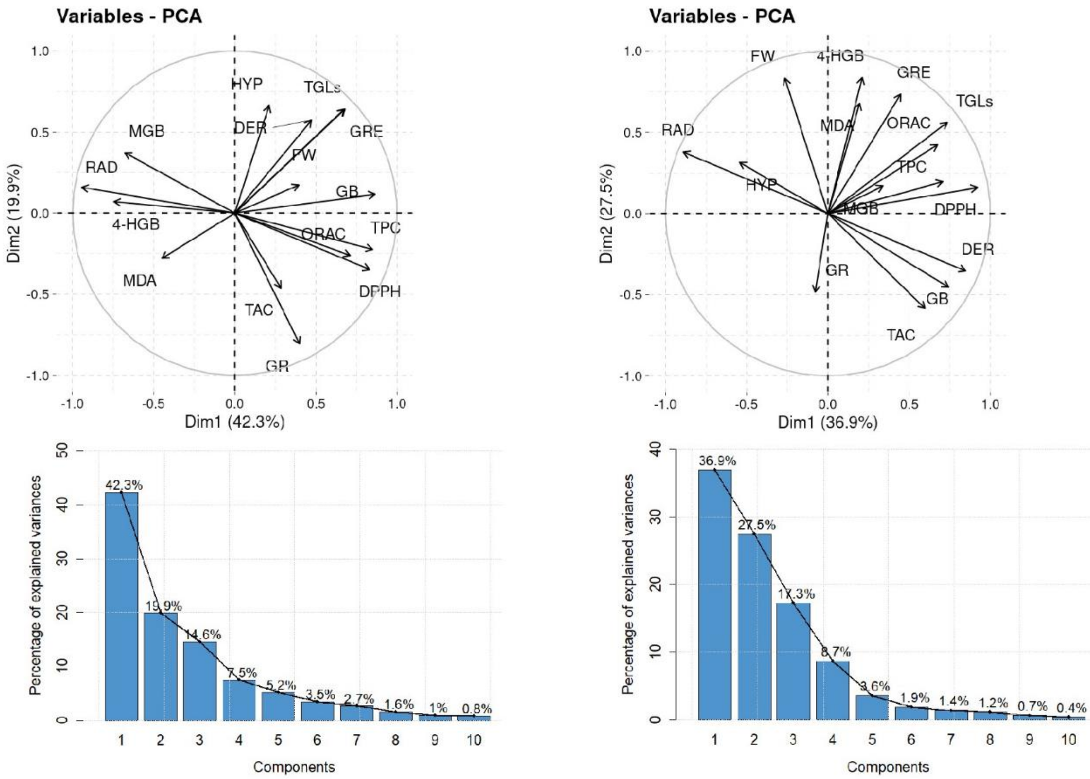


Figure S4. Principal component analysis (PCA) at 20°C. Letter (A) represent the PCA of edible radish, letter (B) represent the PCA of wild radish, letter (C) represent the percentage of explained of edible radish and letter (D) represent the percentage of explained of wild radish. RAD: Length of the radicle; HYP: Hypocotyl length, FW: weight sprouts; GR: germination rate; MDA: Malondialdehyde Assay; TGLs: Total Glucosinolates; TAC: Total anthocyanins; GRE: Glucoraphenin; 4-HGB: Hydroxyglucobrassicin; DER: Dehydroerucine; GB: Glucobrassicin; MGB: 4-methoxyglucobrassicin; TPC: Total phenolic content; DPPH: DPPH assay; ORAC: ORAC assay for ER and WR.

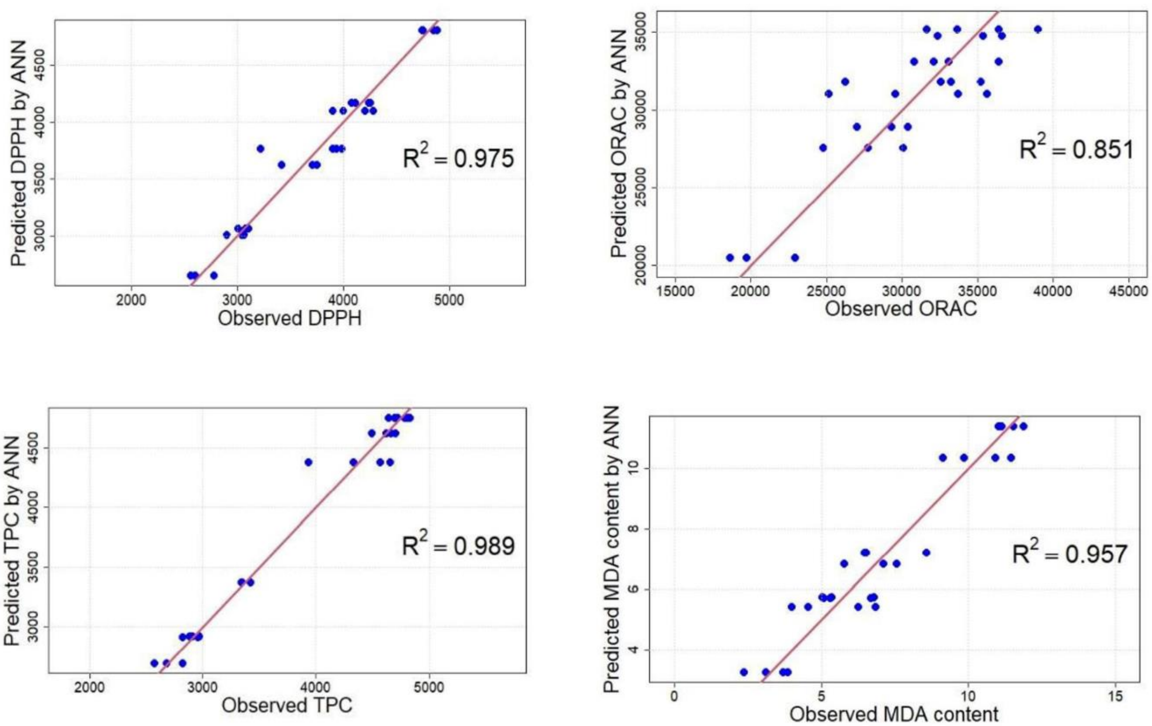


Figure S5. Variables predicted by ANNs v/s observed variables for DPPH, ORAC, TPC and MDA.