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FLAVONOIDE MORINA DIMINUI O CRESCIMENTO E REDUZ ATIVIDADE FOTOSSINTÉTICA DE PLANTAS DE MILHO

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Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas (Área de concentração – Biologia Celular e Molecular) da Universidade Estadual de Maringá, para obtenção do grau de Doutor em Ciências Biológicas.

Orientador: Prof. Dr. Osvaldo Ferrarese-Filho

Coorientador: Prof. Dr. Rogério Marchiosi

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BIOGRAFIA

Renata Costa Sinzker nasceu em 02 de agosto de 1989, em Curitiba, Paraná. Possui graduação em Bacharelado em Ciências Biológicas pelo Centro universitário de Maringá - UniCesumar (2011) e graduação em Bioquímica pela Universidade Estadual de Maringá (2015). Em 2016 iniciou o curso de Mestrado no Programa de Pós-graduação em Ciências Biológicas (Biologia Celular e Molecular) também pela Universidade Estadual de Maringá, concluindo-o em maio de 2019. Em julho de 2019 iniciou o curso de doutorado pelo mesmo programa de pós-graduação que cursou o mestrado desenvolvendo o projeto intitulado "Flavonoide Morina Diminui o Crescimento e Reduz Atividade Fotossintética de Plantas de Milho". Ao longo da trajetória acadêmica desenvolveu pesquisa nas áreas de fisiologia animal, bioquímica e fisiologia vegetal.

Dedico este trabalho aos meus pais José e Cilmara (in memoriam), pelo tempo e dinheiro investidos em meus estudos e pelo carinho e amor dedicados ao meu crescimento. Ao meu esposo Marcos, meu filho Gabriel e minha irmã Roberta por serem meu porto seguro em momentos de dificuldade, por sempre acreditarem e enaltecerem meu potencial.

Sumário

Apresentação

Esta Tese é composta por dois capítulos. O capítulo I trata-se de um artigo de revisão, intitulado: **"Flavonoids: Structure, biosynthesis and biological roles in plants".** O capítulo II compreende um artigo sobre os efeitos no crescimento e em parâmetros fotossintéticos em plantas de milho cultivadas na presença do flavonoide morina. Este artigo foi intitulado "**Flavonoide Morina Diminui o Crescimento e Reduz Atividade Fotossintética de Plantas de Milho".** De acordo com o regulamento do Programa de Pós-Graduação em Ciências Biológicas (PBC), os artigos foram redigidos seguindo as normas das revistas as quais serão submetidas, conforme descrito abaixo:

Capítulo I – Sinzker, R. C.; Ferrarese-Filho, O.; Marchiosi, R. Flavonoids: Structure, biosynthesis and biological roles in plants. Será submetido ao Periódico *Acta Physiologiae Plantarum*

Capítulo II – Sinzker, R. C.; Ferrarese-Filho, O.; Marchiosi, R.; Flavonoide Morina Diminui o Crescimento e Reduz Atividade Fotossintética de Plantas de Milho. Será submetido ao periódico *Plant Physiology and Biochemistry*

RESUMO GERAL

INTRODUÇÃO – Este trabalho conta com uma revisão geral sobre flavonoides em plantas destacando seu papel em alguns estresses abióticos e como aleloquímicos. Sua composição estrutural é caracterizada por um esqueleto de carbono C6-C3-C6, formado de dois anéis benzênicos (A e B) conectados por uma ponte de três carbonos (C). Diferentes estados de oxidação do anel e padrões de substituição geram diferentes classes, sendo elas: flavanóis, antocianinas, flavonóis, flavonas, flavanonas e isoflavonas. O papel biológico dos flavonoides é bastante variado podendo atuar agente pigmentante, ou regulando o crescimento e desenvolvimento, ou atraindo polinizadores, ou protegendo contra a radiação UV ou ainda como antioxidante protegendo as plantas de danos dos radicais livres. Como sequência em nossos estudos, os efeitos do flavonóide morina sobre o crescimento e fotossíntese *de Zea mays,* uma espécie de planta C4, foram avaliados. A morina (3,5,7,2',4'-pentahidroxiflavona) foi selecionada devido sua similaridade estrutural com inibidores já conhecidos da PPDK. Os resultados revelaram que a morina diminuiu o crescimento radicular e a fotossíntese, especialmente a taxa fotossintética e o teor de clorofila (dados não publicados).

MATERIAIS E MÉTODOS - Sementes de milho (cv. IPR 164) foram germinadas a 25 ºC por 72 h, e posteriormente cultivadas ppoor 14 dias em hidroponia, utilizando-se solução nutritiva comercial, contendo 0, 50 µM e 300 µM do composto morina (Sigma-Aldrich). Os parâmetros de trocas gasosas e fluorescência da clorofila *a* foram obtidos a partir de plantas cultivadas por 14 dias, sendo analisados por meio da combinação entre medições de trocas gasosas e fluorescência multifase através do sistema portátil de fotossíntese com fluorômetro acoplado Li-6800-F2 (Li-Cor Inc., Lincoln, NE, USA).

RESULTADOS E DISCUSSÃO - O comprimento das raízes das plântulas cultivadas em hidroponia por 14 dias com 50 µM de morina foi inibido em 21,01%, já as plântulas tratadas com 300 µM apresentaram uma inibição do comprimento de 64,29% (Figura 1A). Registramos reduções significativas na assimilação (A; 36,71%), na transpiração (E; 38,34%), na condutância estomática (gsw; 42,59%), na eficiência quântica fotoquímica efetiva (F_v/F_m; 8,72%), no rendimento quântico máximo do PSII no escuro (Fv'/Fm'; 5,14%), na taxa fotossintética máxima (Pn; 32,53%), na taxa de saturação máxima (I_{max}; 22,09%) e na taxa de saturação equivalente a 50% de Pn (I_{sat50}; 13,51%) das plantas tratadas com 300 µM de morina. O cultivo de plântulas de milho também afetou drasticamente o crescimento, a morfologia das raízes e diminuiu o teor de clorofila. O efeito primário da luz é permitir a fotossíntese, o que leva à produção de açúcares (sacarose) que são transportados pelo floema até as raízes e as permitem crescerem e se desenvolverem. Por conseguinte, uma vez que houve redução na fotossíntese da planta, a produção de açucares também é reduzida. As raízes, por sua vez, com menor disponibilidade de substrato energético, também podem ter seu crescimento prejudicado.

CONCLUSÃO - Nossos resultados confirmaram que, de fato, a morina afetou os processos fotossintéticos, com destaque para redução de diversos parâmetros fotossintéticos (A, E, gsw, Fv/Fm, Fv'/Fm', Pn, Imax e $Imax_{50}$) e do teor de clorofila nas folhas. Tais alterações sugerem que uma exposição prolongada das plantas à morina poderia levar a danos estruturais nos fotossistemas. Além disso, a inibição da fotossíntese pode ter limitado a produção de açúcares que servem como substrato energético para estruturas não fotossintetizantes das plantas, como as raízes, inibindo seu crescimento. Estudos futuros irão revelar outros efeitos da morina nas plantas, bem como se ocorre realmente a inibição da enzima PPDK, confirmando o uso da morina como um potencial herbicida.

GENERAL ABSTRACT

INTRODUCTION – This work has a general review on flavonoids in plants highlighting their role in some abiotic stresses and as allelochemicals. Its structural composition is characterized by a C6-C3-C6 carbon skeleton, formed of two benzene rings (A and B) connected by a three-carbon bridge (C). Different ring oxidation states and substitution patterns generate different classes, namely: flavanols, anthocyanins, flavonols, flavones, flavanones and isoflavones. The biological role of flavonoids is quite varied and can act as a pigmenting agent, or regulating growth and development, or attracting pollinators, or protecting against UV radiation or even as an antioxidant protecting plants from free radical damage. As a follow-up in our studies, the effects of the flavonoid morina on the growth and photosynthesis of *Zea mays*, a C4 plant species, were evaluated. Morin (3,5,7,2',4'-pentahydroxyflavone) was selected due to its structural similarity with known PPDK inhibitors. The results revealed that morina decreased root growth and photosynthesis, especially photosynthetic rate and chlorophyll content (unpublished data).

MATERIALS AND METHODS –Maize seeds (cv. IPR 164) were germinated at 25 ºC for 72 h, and then cultured ppoor 14 days in hydroponics, using commercial nutrient solution, containing 0, 50 μM and 300 μM of the morin compound (Sigma-Aldrich). The parameters of gas exchange and chlorophyll a fluorescence were obtained from plants cultivated for 14 days, being analyzed by combining gas exchange measurements and multiphase fluorescence through the portable photosynthesis system with coupled fluorometer Li-6800-F2 (Li-Cor Inc., Lincoln, NE, USA).

RESULTS AND DISCUSSION - The root length of seedlings grown in hydroponics for 14 days with 50 μM of morin was inhibited in 21.01%, while seedlings treated with 300 μM showed a length inhibition of 64.29% (Figure 1A). We found significant reductions in assimilation (A; 36.71%), transpiration (E; 38.34%), stomatal conductance (gsw; 42.59%), effective photochemical quantum efficiency (Fv/Fm; 8.72%), in the maximum quantum yield of PSII in the dark (Fv'/Fm'; 5.14%), in the maximum photosynthetic rate (Pn; 32.53%), in the maximum saturation rate (Imax; 22.09%) and in the saturation rate equivalent to 50% of Pn ($Isat_{50}$; 13.51%) of plants treated with 300 μM of morine. The cultivation also drastically affected the growth, root morphology and decreased chlorophyll content. The primary effect of light is to allow photosynthesis, which leads to the production of sugars (sucrose) that are transported by the phloem to the roots and allow them to grow and develop. Therefore, since there has been a reduction in the photosynthesis of the plant, the production of sugars is also reduced. The roots, in turn, with less availability of energy substrate, may also have their growth impaired.

CONCLUSION - Our results with confirmed that, in fact, morina affected the photosynthetic processes, with emphasis on the reduction of several photosynthetic parameters (A, E, gsw, Fv/Fm, Fv'/Fm', Pn, Imax and Imax50) and the chlorophyll content in the leaves. Such alterations suggest that prolonged exposure of plants to morin could lead to structural damage to photosystems. In addition, inhibition of photosynthesis may have limited the production of sugars that serve as an energy substrate for non-photosynthesizing plant structures, such as roots, inhibiting their growth. Future studies will reveal other effects of morin on plants, as well as whether inhibition of the PPDK enzyme actually occurs, confirming the use of morin as a potential herbicide.

CAPÍTULO I

ARTIGO:

Flavonoids: Structure, biosynthesis and biological roles in plants

Este artigo será submetido ao Acta Physiologiae Plantarum

Flavonoids: Structure, biosynthesis and biological roles in plants

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Abstract

Flavonoids are a group of different polyphenolic compounds that are extensively represented in plants and have multiple cellular roles. Here is an overview of flavonoids in plants, including their chemistry, biosynthesis, and biological roles. Flavonoids are characterized by their C6-C3-C6 carbon skeleton consisting of two benzene rings (A and B) connected by a three-carbon bridge (C). According to the ring oxidation state and substitution pattern, they are further divided into six classes: flavanols, anthocyanins, flavonols, flavones, flavanones, and isoflavones. Flavonoid biosynthesis occurs through the shikimate chorismate pathway. The pathway begins with the condensation of one molecule of *p*-coumaroyl-CoA and three molecules of malonyl-CoA to form chalcones, precursors to other flavonoids. Flavonoids have been shown to have various biological roles, including pigmentation, regulation of growth and development, the attraction of pollinators, protection from UV radiation, and antioxidant activity that can protect plants from free radical damage. This mini-review highlights the role of flavonoids in some abiotic stresses and their role as allelochemicals.

Keywords: abiotic stress, allelochemicals, antioxidants, flavonoid, reactive oxygen species.

Structure and classification of flavonoids

Following terpenoids (30000) and alkaloids (12000), the third-largest group of natural products is represented by flavonoids, comprising nearly 9000 compounds. Known as an important class of low molecular weight plant secondary metabolites, flavonoids have a polyphenolic structure (Fig. 1). The canonical core structure present in all flavonoids is the C6-C3-C6 carbon skeleton (Symonowicz and Kolanek, 2012). Flavonoids are present as derivatives of 2-phenyl-benzo-γ-pyrone, and the carbon atoms are formed by two benzene rings (A and B), which are connected by an oxygen bonded to the pyrene ring (C). Rings A and B can condense to form a compound termed chalcone. This can undergo cyclization by an isomerase forming a flavonone – the initial component for the formation of other groups of flavonoids (Brodowska, 2017). Flavonoids are present in free form as aglycones or bound

as glycosides and methylated derivatives (Havsteen, 2002; Veitch and Grayer, 2008; Kumar and Pandey, 2013).

Flavonoids are classified in various ways, including their plant origin, biological role, and chemical structure. Some flavonoids are known for their biological roles, such as antioxidant, anti-inflammatory, and antitumor activities. Flavonoids can also be classified according to their plant origin, such as fruits, vegetables, herbs, and spices. However, the most common classification is based on chemical structure (Fig. 1). Due to structural differences, flavonoids can be classified into six classes: a) flavanols, b) anthocyanidins, c) flavonols, d) flavones, e) flavanones, and f) isoflavones (Shen et al., 2022). The classes of flavonoids differ in their level of oxidation and C-ring substitution, whereas the individual component within a class differs in an A– and B–ring substitution pathway (Kumar and Pandey, 2013). The multiplicity in chemical structures of flavonoids has been attributed to secondary modifications by glycosylation, prenylation, methylation and acylation that affect the bioactive role, transport and accumulation of the resulting compounds (Volp et al., 2008; Kumar and Pandey, 2013; Mbaveng et al., 2014; Santos et al., 2017).

a. Flavanols

Flavanols or flavan-3-ols comprise a complex group characterized by a carbon skeleton with a hydroxyl group in position 3 of the C ring (Fig. 2). They are usually present in fruits derivatives and teas (de Pascual-Teresa et al., 2010).

The main representative of flavanols is catechin (Fig. 2), which forms the building blocks of tannins. Catechins usually occur as aglycones or are esterified with gallic acid (Erlund, 2004). There are several types of catechins, such as catechin 3-gallate, gallotocatechin 3-gallate, epicatechin (Fig. 2), epigallocatechin, epicatechin 3-gallate and epigallocatechin 3-gallate (Brodowska, 2017).

b. Anthocyanidins

Anthocyanidins correspond to a group of phytochemicals that constitute the natural pigments responsible for the blue, red, purple and orange colors of flower petals, fruits and vegetables,

and certain special varieties of grains (Tsao, 2010). Anthocyanidins are found in the vacuoles in the form linked to sugars, which provide greater stability and solubility for the compound (de Pascual-Teresa et al., 2010; Oancea and Oprean, 2011) and in the aglycone form; its derived compounds depend on the number and position of hydroxyl and methoxyl groups present at different positions in the basic structure (Fig. 1).

The main anthocyanidins found in fruits and vegetables are cyanidin (Fig. 2), pelargonidin (Fig. 2), delphinidin, malvidin, petunidin and peonidin (Brodowska, 2017). More than 500 types of anthocyanidins have been described. These compounds have attracted great interest in the technological field due to their impact on the sensory characteristics of food products and on human health due to their biological activities (de Pascual-Teresa et al., 2000; de Pascual-Teresa et al., 2010).

c. Flavonols

Flavonols (3-hydroxyflavones) are one of the most analyzed groups due to their antioxidant and biological properties. These polyphenolics are present in many vegetables and fruits (Brodowska, 2017). The main flavonols found are quercetin (Fig. 2), kaempferol (Fig. 2), and myricetin. Quercetin is the main representative flavonol, and it is one of the major dietary flavonoids. It is present in various plant parts, including fruits, vegetables, and beans. Kaempferol is found in fruits, vegetables, and especially broccoli. Myricetin is a major plant secondary metabolite, and it is commonly found in the whole plant kingdom and in the majority of human foods, *i.e.*, different fruits, berries, grapes, herbs, vegetables, and many other plants (Rashid et al., 2019).

d. Flavones

Flavones have three functional groups, including hydroxyl, carbonyl and conjugated double bonds (Fig. 1). Flavones can react in several ways, including reduction reactions, degradation in the presence of base, oxidation, substitution, addition and condensation (Singh et al., 2014; Brodowska, 2017). The main flavones found are apigenin and luteolin (Fig. 2). Apigenin is

found in onions, teas and chamomile, while luteolin is usually found in vegetables and fruits such as broccoli and apples.

e. Flavanones

Flavanones are widespread in 42 plant families, including Asteraceae, Fabaceae and Rutaceae. Depending on the type of plant, flavanones can be found in all parts, from the vegetative to the reproductive organs (Brodowska, 2017). According to Khan (2014), it is estimated that approximately 350 flavanones are in the aglycone form and 100 in the form of glycosides.

Naringenin and hesperetin are the flavanones of greatest interest due to their high prevalence in foods (Khan, 2014). Naringenin (Fig. 2) is found in high concentrations in citrus fruits such as lemon and orange and in low concentrations in tomatoes and their products (Erlund, 2004). Naringenin can be found both in the form of aglycones and glycosides (Khan, 2014). The aglycone is the least dominant form in nature, prevailing in glycosidic forms (Goulas and Manganaris, 2012; Khan, 2014). Like naringenin, hesperetin (Fig. 2) and its glycosides are also present in citrus fruits.

f. Isoflavones

Isoflavones comprise a group of flavonoids consisting of heterocyclic phenols (Yu et al., 2016). They are widely produced by plant families known as Faboideae and Fabaceae, especially soybean (Shen et al., 2022), whose content varies from 26 to 381 mg/100 g of biomass (Yu et al., 2016). Genistein and daidzein (Fig. 2) are the most common isoflavones, and they exist in chemical structures as aglycones, 7-O-glycosides, 6'-O-acetylglucosides and 6'-O-malonylglycosides (Yeung and Yu, 2003).

Biosynthesis of flavonoids

All plant cells can produce flavonoids, and their biosynthesis evolves metabolites of the shikimate, phenylpropanoid, and acetate pathways (Fig. 3). The two major precursors

originate from the shikimate pathway, providing ring A and ring B, respectively, with chain linkages forming ring C (Fig. 1). Ring A is generated from malonyl-CoA synthesized by carboxylation of acetyl-CoA via the acetate pathway; however, ring B along with the linking chain (ring C) is synthesized from *p*-coumaroyl-CoA via the shikimate pathway (Shah and Smith, 2020).

The shikimate pathway provides phenylalanine. In this way, erythrose-4-phosphate and phosphoenolpyruvate are converted to chorismate in seven metabolic steps. Chorismate is the precursor of the aromatic amino acids tryptophan, tyrosine, and phenylalanine. *p*-Coumaroyl-CoA is generated directly from the amino acid phenylalanine by three enzymatic reactions of the phenylpropanoid pathway. The first reaction is the deamination of phenylalanine to *t*-cinnamate, which is catalyzed by the enzyme phenylalanine ammonia-lyase (PAL). In the second reaction, *t*-cinnamate is converted to *p*-coumarate by the activity of cinnamate 4-hydroxylase (C4H). Finally, by the action of *p*-coumarate-CoA ligase (4CL), *p*-coumarate is converted to *p*-cumaroil-CoA(Marchiosi et al., 2020). From the reaction of acetate toward acetyl-CoA (AcylCoA synthetase), the acetate pathway provides malonyl-CoA (ACCase). By action of chalcone synthase (CHS), three molecules of malonyl-CoA and one molecule of *p*-coumaryl-CoA are condensed to form naringenin chalcone, which is the entry of the flavonoid biosynthesis pathway. CHS is the main rate-limiting enzyme in this biosynthetic pathway (Shah and Smith, 2020) (Figure 3).

Flavanones are synthesized from naringenin chalcone by chalcone isomerase (CHI), which causes intramolecular cyclization of chalcone to form heterocyclic ring C. Flavanones produce flavanols by dihydroflavonol reductase (DFR) and isoflavonoids by isoflavonoid synthase (IFS) activity. After conversion of flavanone to dihydroflavonol by flavanone 3-hydroxylase (F3H), dihydroflavonol reductase (DFR) converts dihydroflavonol into leucoanthocyanidins, which are converted into anthocyanidins by the anthocyanidin synthase (ANS) enzyme. Flavanones also produce flavones by the action of flavone synthase (FNS), while flavonol synthase (FLS) converts dihydroflavonols into flavonol (Shah and Smith, 2020) (Figure 3).

In regulating flavonoid formation, transcriptional control is essential. The MBW complex, which comprises WD40, bHLH, and MYB proteins, is the central transcriptional regulator in flavonoid biosynthesis. The MYB domain at the N-terminus of MYB transcription factors

(TFs) is needed for DNA binding and interaction with other proteins. According to the amount and location of MYB domain repeats, these proteins are categorized into 3R-MYB, 4R-MYB, R2R3-MYB, and 1R-MYB/MYB-related proteins, being that R2R3-MYB members are the main regulators of flavonoid metabolism (Nagar et al., 2022). A complementary review of the regulation of flavonoid biosynthesis can be seen in Shen et al. (2022).

Biological roles

Environmental stresses such as biotic and abiotic stresses are serious threats to agricultural production. Most notably, UV-B radiation, heat, cold, air pollution, mechanical wounding, nutritional deficiency, salinity, water stress, drought, and heavy metals result in a global reduction in crops, which leads to worldwide economic costs (Nakabayashi and Saito, 2015).

Flavonoids can protect plant cells against oxidative damage caused by free radicals, leading to disease and premature aging. They handle the vibrant colors of many flowers and fruits, attracting pollinators and aiding in seed dispersal. Some flavonoids can help protect plants against pathogens, including bacteria, fungi, and viruses. Flavonoids play an essential role in floral coloration, pollinator attractiveness, UV protection, and regulation of auxin movement and catabolism. They can also regulate plant growth and development, including cell differentiation and organ formation. Certain flavonoids can influence soil composition, increasing plant nutrient availability and promoting soil health. Flavonoids play an essential role in floral coloration, pollinator attractiveness, UV protection, and regulation of auxin movement and catabolism. Moreover, flavonoids can also act as allelochemicals, influencing the growth and development of neighboring plants (Nagar et al., 2022; Shen et al., 2022; Nabil-Adam et al., 2023).

Because flavonoids perform a wide variety of biological roles, their responses under most abiotic stresses have been described here. In addition, their roles as allelochemicals have also been emphasized.

a. Flavonoids in response to abiotic stresses

Plants adopt different strategies to survive abiotic stresses at the molecular, metabolomic, physiological, and morphological levels. In general, abiotic stress increases the production of reactive oxygen species (ROS), and the scavenging of these reactive species is the main goal of plants. Since flavonoids have antioxidant properties that effectively scavenge ROS, the increase in flavonoid production is a quick, immediate response to improve plant tolerance (Shen et al., 2022).

The response of flavonoids to abiotic stress may vary depending on the plant species, the intensity and duration of the stress, and the type of flavonoid. The differential response of flavonoids to abiotic stress is thought to be mediated by multiple factors, including changes in the activities of enzymes, expression of genes, and availability of substrates required for flavonoid biosynthesis. Additionally, the response of flavonoids to abiotic stress can vary depending on the stage of plant development, with some flavonoids accumulating more in early developmental stages while others accumulate more in later stages (Shomali et al., 2022).

Several studies have reported changes in the level of flavonoid content under different abiotic stresses, which are summarized in Table 1. For example, flavonoids play a critical role in protecting plants from UV radiation by absorbing it, scavenging ROS, and reducing oxidative stress. In general, UV-B radiation exposure can lead to the accumulation of flavonoids in tissues of leaves, stems, and other plant organs. In some plants, such as *Medicago sativa* (Gao et al., 2019), *Kalanchoe pinnata* (dos Santos Nascimento et al., 2015), *Ligustrum vulgare* (Gourlay et al., 2022), and *Populus tremula* (Khalil et al., 2022), the levels of flavonoids increase after UV-B radiation.

Salinity stress occurs when the salt concentration in the soil exceeds the optimal level for plant growth and can result in a range of physiological and biochemical changes in plants, including oxidative stress and damage to cellular membranes. In general, salinity stress can lead to the accumulation of flavonoids in plant species, such as *Arabidopsis thaliana* (Zhang et al., 2021), *Amaranthus tricolor* (Sarker and Oba, 2018), *Amaranthus lividus* (Hossain et al., 2022), and *Apocynum venetum* (Xu et al., 2020).

Water stress occurs when plants experience a shortage of water, either due to drought or other factors that limit water availability. Water stress can lead to the accumulation of certain

flavonoids, such as rutin, quercetin, apigenin, and luteolin, in the tissues of *Chrysanthemum morifolium* (Hodaei et al., 2018).

Drought stress occurs when plants experience a prolonged period of low water availability. Flavonoids play a crucial role in mitigating the harmful effects of drought stress in plants by reducing oxidative stress and modulating the expression of stress-responsive genes. Drought stress can lead to the accumulation of quercetin and cyanidin in *Arabidopsis thaliana* (Rao et al., 2020), flavonols in *Zea mays* (Li et al., 2021), and total flavonoids in *Triticum aestivum* (Ma et al., 2014).

Temperature stress can occur when plants experience temperatures outside of their optimal range, either due to cold or heat stress. High-temperature stress can lead to the accumulation of certain flavonoids (quercetin, luteolin, cyanidin, kaempferol, myricetin, and rutin) in tissues of *Lactuca sativa* (Pérez-López et al., 2018) and *Solanum lycopersicum* (Muhlemann et al., 2018).

Heavy metal stress, such as exposure to cadmium, copper, and zinc, can significantly impact plant growth and development. Heavy metal stress can lead to the accumulation of certain flavonoids in plant tissues. For example, cadmium stress can lead to an increase in total flavonol levels in *Solanum lycopersicon* (Ahmad et al., 2018), copper stress increases several types of flavonoids in *Belamcanda chinensis* (Zhu et al., 2020), and copper plus zinc increases total flavonoids in *Lycopersicon esculentum* (Badiaa et al., 2020).

b. Flavonoids as allelochemicals

Allelochemicals are secondary metabolites produced by plants and are present in the soil at concentrations ranging from 10^{-5} to 10^{-6} M (Gniazdowska and Bogatek, 2005). Despite these low concentrations, allelochemicals can positively or negatively affect the growth and development of neighboring plants (Marchiosi et al., 2020). A complex network of biochemical signaling mediates this biological process, as allelopathic plants release root or leaf exudates and volatile molecules or leachate from plant litter decomposition (Ghitti et al., 2022).

There are many secondary compounds that act as allelochemicals. However, only a few works have reported the precise mechanism of how flavonoids participate in the

allelopathic process. Overall, the roles of flavonoids as allelochemicals in plants are complex and diverse. Flavonoids act as growth inhibitors and stress triggers for neighboring plants. In addition to allelopathic effects, flavonoids can also affect plant-rhizosphere microbial interactions. Flavonoids act as signaling molecules, attracting beneficial microorganisms such as nitrogen-fixing bacteria and mycorrhizal fungi while repelling pathogenic microorganisms (Weston et al., 2013). Moreover, flavonoids can act as light catalysts for photosynthesis or as regulators of channels involved in pho phosphorylation. They are also involved in energy transfer and photosensitization (Pietta et al., 2000). Furthermore, they may act with plant growth hormones in controlling respiration, photosynthesis, and morphogenesis (Middleton and Teramura, 1993; Harborne and Baxter, 1999).

By and large, the effects are related to the inhibition of cell growth, disturbances in the production and action of the plant hormone auxin, and the production of ROS, which activates a calcium cascade causing the death of the root system. Therefore, these effects have aroused great interest in agriculture, as these interactions can combat weeds that harm crops (Mierziak et al., 2014; Palma-Tenango et al., 2017). There are some reports addressing this promising subject. For example, catechins excreted by *Centaurea maculosa* inhibit the germination and growth of *Centaurea diffusa* and *Arabidopsis thaliana* (Kong et al., 2004). Additionally, the root death of neighboring plants was observed in *Centaurea maculosa*-infested fields, an effect caused by a ROS wave induced by (-)-catechins released by weeds (Bais et al., 2003; 2010). Application of (-)-catechin triggers programmed cell death that spreads from the root zone to steles, presumably by triggering ROS-induced calcium waves that alter cellular ion homeostasis and cellular pH imbalance (Bais et al., 2003).

Allelopathic rice cultivars accumulated two flavone *O*-glycosides in the root tissue, which were rapidly transformed into aglycones in soil. These glycosides were more resistant to microbial degradation and less mobile in rice soil, enhancing the inhibitory effect on the paddy field weed *Echinochloa crus-galli* (Kong et al., 2007). A methanolic extract (20%) of *Artemisia santolinifolia* caused severe oxidative stress and persistent wilting in large weeds, an effect that appears to be due to the phenolics and flavonoids (rutin and quercetin) present in the plant extract (Anwar et al., 2021).

A bioassay-guided fractionation of root extracts of *Stellera chamaejasme*, a toxic and ecologically threatening weed, led to the isolation of six flavonoids with intense phytotoxic activity against *Arabidopsis thaliana* seedlings. The isolated flavonoids reduced seedling growth and disrupted root development (Yan et al., 2014).

Isolated from *Cynara cardunculus* methanolic extract, the flavonoids myricitrin, naringenin, and quercetin inhibited germination and seedling growth and caused necrosis or chlorosis in *Trifolium incarnatum* (Kaab et al., 2020). The authors designed a novel herbicide composition to improve the post-emergence activity of the methanolic extract. The formulation containing the *C. cardunculus* crude methanolic extract showed a similar herbicidal effect as an industrial bioherbicide containing pelargonic acid, suggesting that this plant species may be a suitable source of natural compounds potentially usable as natural herbicides. Similarly, a methanolic extract of *Plantago major* containing flavonoids and other secondary metabolites was tested on the germination and early seedling growth of *Portulaca oleracea* (Al-obaidi, 2020). The methanolic extract inhibited germination and root and plumule growth. Although the compounds were not tested alone, this study suggests that *Plantago major* could be used as a source of possible natural herbicides.

As may be noted, the allelopathic effects of flavonoids on neighboring plants make them a promising target for developing natural herbicides and for the genetic engineering of plants with improved allelopathic tolerance. However, the precise mechanism by which flavonoids participate in allelopathy is still unknown (Mierziak et al., 2014).

As an alternative to classic herbicides, allelochemicals have been isolated from different plant species (Vyvyan, 2002) based on investigations of allelopathic phenomena and mechanisms of action (Macías et al., 2020; Hoang Anh et al., 2021) or chemical synthesis to obtain new molecules with the core of natural products to produce herbicides (Araniti et al., 2020; Sparks and Duke, 2021). Despite advances in this research field, no new site of action for herbicides has been proposed in recent decades. Based on these gaps, a new approach has been carried out in our laboratory: the use of bioinformatics tools to prospect inhibitors for pyruvate orthophosphate dikinase (PPDK). This enzyme catalyzes the conversion of pyruvate to phosphoenolpyruvate, and its activity is essential to maintain photosynthetic CO_2 assimilation in C_4 plants. A decrease in PPDK activity limits the photosynthetic rate and

reduces plant growth, and thus, the enzyme may be a promising target for the development of selective herbicides for C_4 weeds (Constantin et al., 2021).

The flavonol morin (2′,3,4′,5,7-pentahydroxyflavone) has structural similarity with known PPDK inhibitors. Morin is a yellow-colored naturally occurring substance in *Maclura tinctoria* and *Maclura pomifera* wood and from *Psidium guajava*. Morin has also been shown to have anti-inflammatory and anticancer activities in humans and animals. However, the extent to which these activities translate to plants is poorly understood (Thakur et al., 2020). In our recent studies, the effects of morin on the growth and photosynthesis of *Zea mays*, a C4 plant species, were evaluated. The results revealed that morin decreased root growth and photosynthesis, especially the photosynthetic rate and chlorophyll content (data not published). Overall, while the use of morin as an herbicide is a promising area of research, further studies are needed to fully understand its mechanisms of action and to address the challenges associated with its use as an herbicide.

Conclusions

This review describes how flavonoids are classified and biosynthesized and their biological roles in abiotic stress and as allelochemicals. Significant knowledge gaps remain in our understanding of how flavonoids act as allelochemicals because of the complexity and diversity of their effects on plants. The role of flavonol morin as an inhibitor of PPDK and photosynthesis has been briefly described, and the results suggest a target for the development of selective herbicides for C4 weeds. Additional accomplishments will bring new insights and likely start a new step toward obtaining flavonoid-based herbicidal agents with potential applications in agriculture.

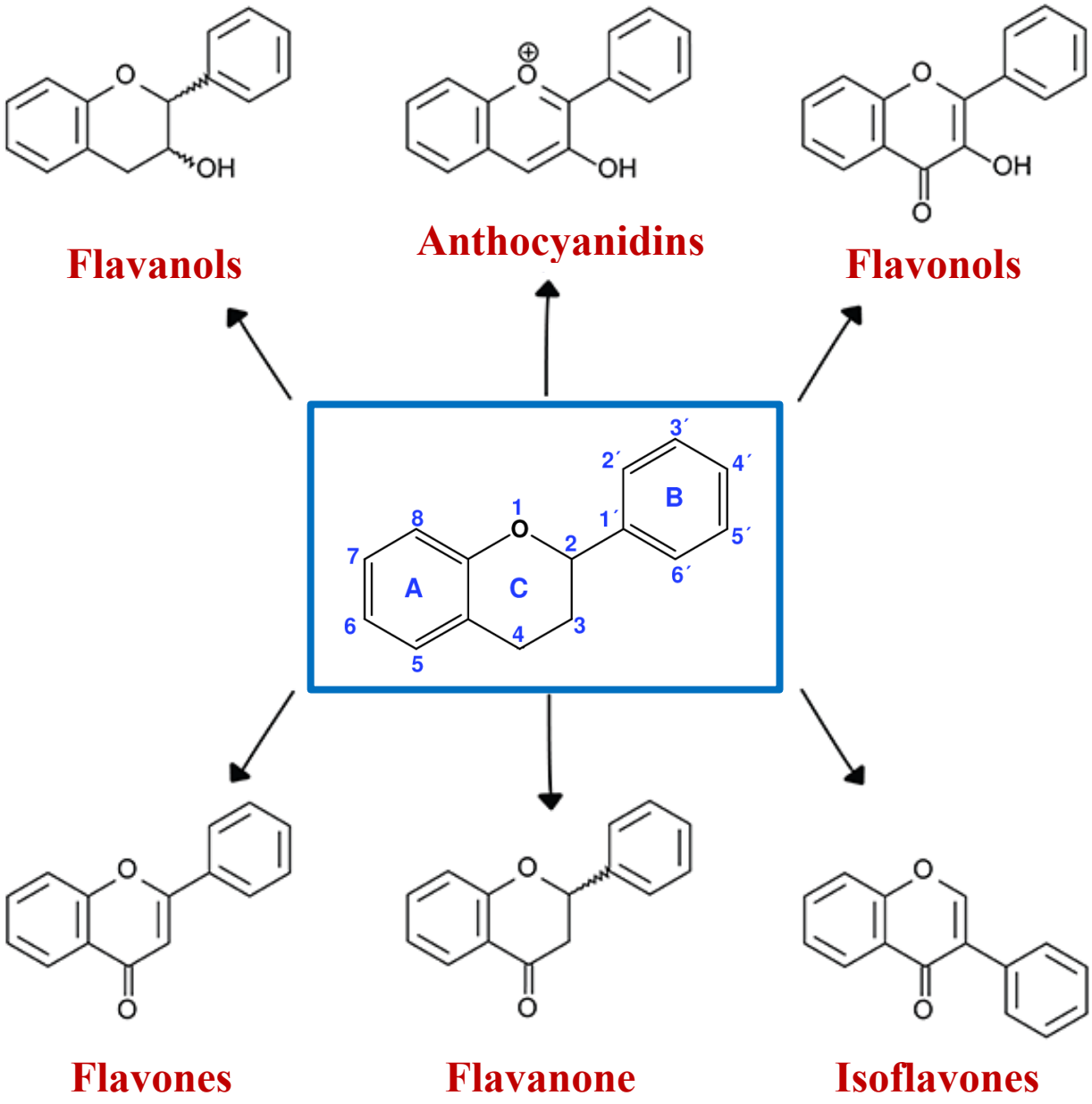

Fig. 1.: Canonical core structure (formed by two benzene rings A and B connected by an oxygen bonded to the pyrene ring - C) present in all types of flavonoids.

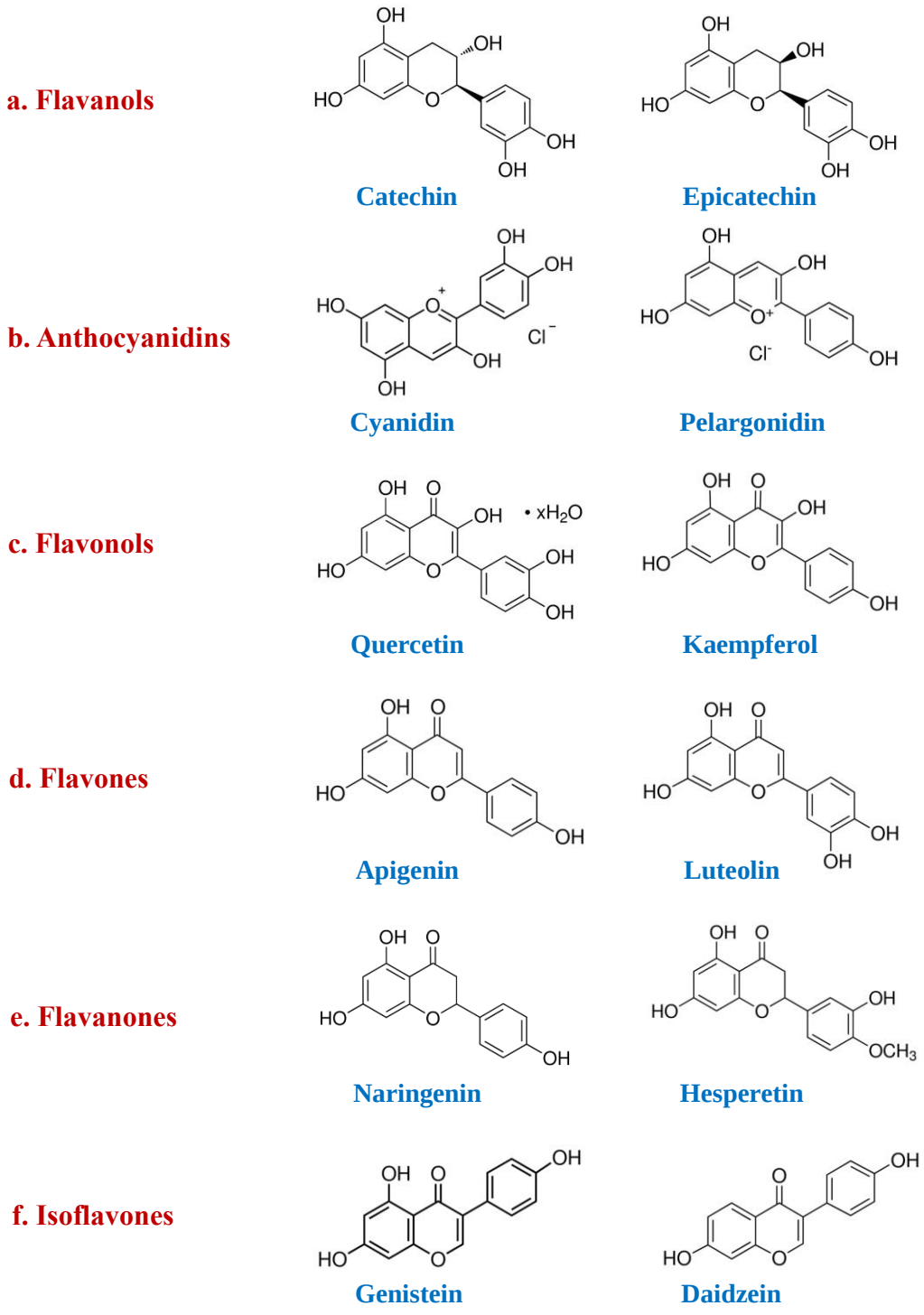

Fig. 2. Chemical structures of the most common flavonoids.

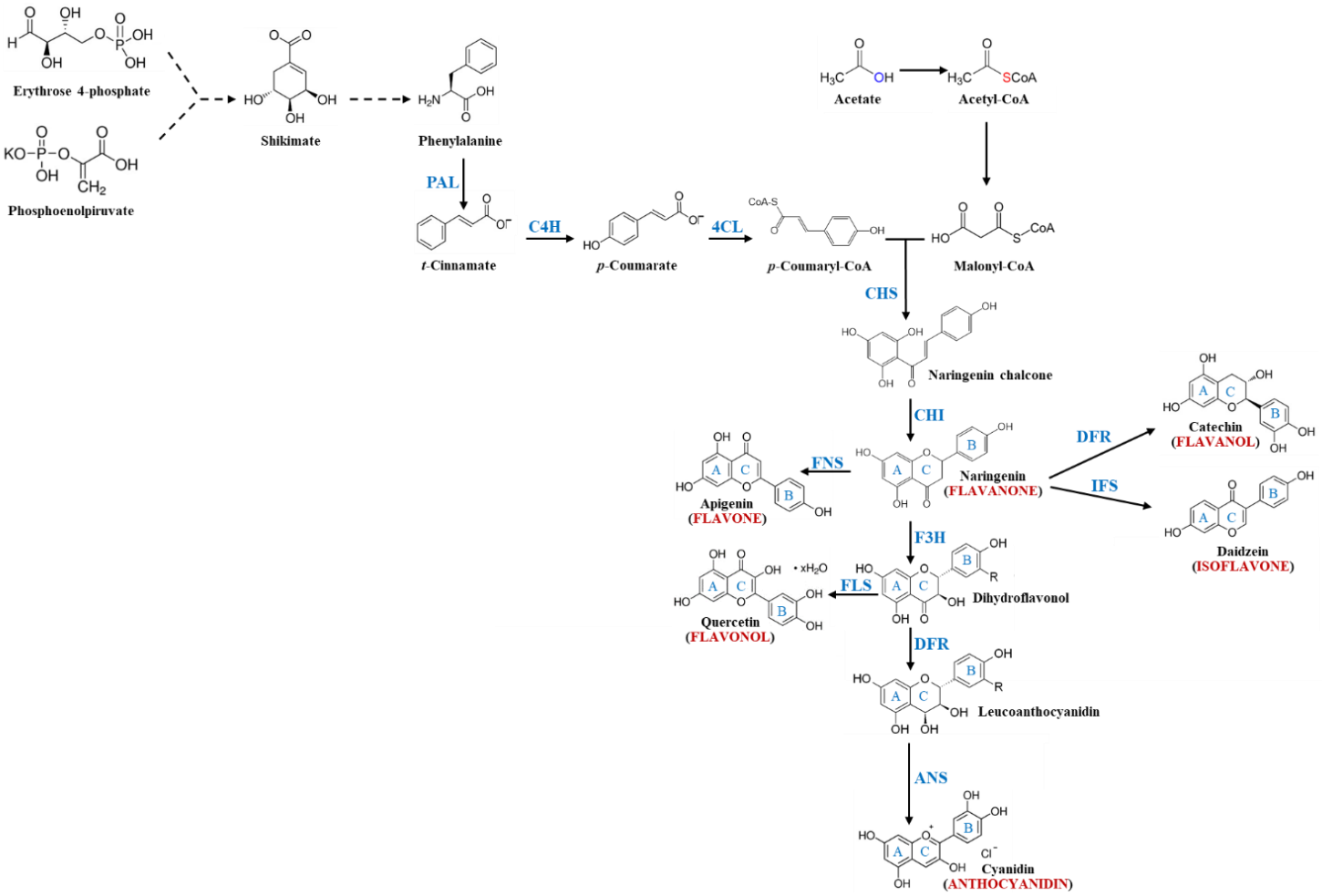

Fig. 3. Simplified overview of the flavonoid biosynthetic pathway with their subgroups. PAL, phenylalanine ammonia lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumaroyl CoA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; DFR, dihydroflavonol reductase; ANS, anthocyanidin synthase; FNS, flavone synthase; FLS, flavanol synthase; IFS, isoflavonoid synthase. Adapted from Shah and Smith (2020).

Table 1. Flavonoid production against abiotic stress in plants (adapted from Shomali et al., 2022)

Stress	**Plant species**	**Increases in the levels of flavonoids**
UV-B radiation	*Medicago sativa*	Total flavonoids
UV-B radiation	*Kalanchoe pinnata*	Total flavonoids and quercetin content
UV-B/Drought	*Ligustrum vulgare*	Quercetin-3-O-rutinoside, luteolin 7-O-glucoside
UV-B/Drought	*Populus tremula*	Proanthocyanidins
Salinity	*Arabidopsis thaliana*	Total flavonoids
Salinity	*Amaranthus tricolor*	Total flavonoids
Salinity	*Apocynum venetum*	Flavonols (quercetin and kaempferol)
Salinity	*Amaranthus lividus*	Total flavonoids
Water stress	*Chrysanthemum morifolium*	Rutin, quercetin, apigenin, and luteolin
Drought	*Arabidopsis thaliana*	Quercetin 3-O-glucoside and cyanidin 3-O-glucoside
Drought	*Zea mays*	Flavonols
Drought	*Triticum aestivum*	Total flavonoids
Temperature	*Lactuca sativa*	Quercetin, luteolin, cyanidin, kaempferol, myricetin, rutin
Temperature	*Solanum lycopersicon*	Total flavonoids
Cadmium	*Belamcanda chinensis*	Flavonols
Copper	*Belamcanda chinensis*	Eleven kinds of flavonoids
Copper and zinc	*Lycopersicon esculentum Mill*	Total flavonoids

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CAPÍTULO II

ARTIGO:

Flavonoide Morina Diminui o Crescimento e Reduz Atividade Fotossintética de Plantas de Milho

Este artigo será submetido

ao Periódico *Plant Physiology and Biochemistry*

Flavonoide Morina Diminui o Crescimento e Reduz Atividade Fotossintética de Plantas de Milho

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INTRODUÇÃO

Plantas são capazes de sintetizar compostos orgânicos a partir de precursores inorgânicos (CO_2) e poder redutor. Tal processo é chamado de fotossíntese, que pode ser didaticamente dividido em duas fases: reações luminosas e de assimilação do carbono (Nelson & Cox, 2014). Devido a sua importância crucial no metabolismo das plantas, o aparato fotossintético constitui um dos principais sítios de ação de herbicidas, com aproximadamente 50% dos herbicidas comerciais afetando alguma etapa do processo fotossintético (Gao et al., 2018; Jablonkai, 2011). Os herbicidas que atuam sobre a fotossíntese afetam principalmente as reações luminosas, seja bloqueando o fluxo de elétrons através dos fotossistemas, atuando como aceptor artificial de elétrons ou inibindo a síntese de pigmentos absorvedores de luz (Oliveira Jr. & Inoue, 2011). Quando em busca de um novo composto com efeito herbicida a fotossíntese deve ser considerada uma prioridade a ser avaliada, estudos apontam os ácidos salicílico, cinâmico e benzoico (Gao et al., 2018), além de vários aleloquímicos, como moléculas promissoras de efeito herbicida (Marchiosi et al., 2016; Parizotto et al., 2016; Bortolo et al., 2018; Constantin et al., 2021; Foletto-Felipe, 2021).

É razoável supor que as reações de assimilação do carbono também possam ser alvos da ação de novos herbicidas devido a extrema importância desta via para a sobrevivência das plantas. Neste contexto, algumas enzimas em particular merecem destaque tais como a ribulose-1,5-bifosfato carboxilase/oxigenase (Rubisco) e a piruvato ortofosfato dicinase (PPDK), esta última uma enzima específica de plantas C4. A redução da atividade da PPDK, seja pela inibição de sua síntese ou por sua inativação, resulta em limitações na taxa fotossintética e no crescimento da planta, sugerindo que esta enzima é um bom alvo para ação de novos herbicidas (Constantin, 2018).

Neste trabalho, a morina (3,5,7,2',4'-pentaidroxiflavona) foi selecionada devido sua similaridade estrutural com inibidores já conhecidos da PPDK (dados não publicados), e teve seus efeitos testados sobre o crescimento, fotossíntese e teor de clorofila de plantas de milho. Morina é um flavonoide de coloração amarelada extraído de plantas pertencentes à família Moraceae. Ela pode ser encontrada em diversas preparações de origem vegetal e é recomendada no tratamento de diversas patologias por possuir efeitos antioxidantes,

antidiabéticos, anti-inflamatórios, antitumorais, anti-hipertensivos, bactericida e neuro protetor (Gopal, 2013; Caselli et al., 2016).

MATERIAIS E MÉTODOS

Germinação das sementes e crescimento das plantas

Sementes de milho (cv. IPR 164) foram sanitizadas com hipoclorito de sódio 2% por 5 min, lavadas abundantemente com água deionizada e distribuídas uniformemente em papel de germinação umedecido com água deionizada. As sementes foram encobertas com outra folha de papel, enroladas e acondicionadas em tubos apropriados para preservar a umidade, climatizados a 25 ºC em câmaras de germinação por 72 h.

Plântulas viáveis obtidas após a germinação foram selecionadas, medidas, e acondicionadas em suportes ajustáveis apropriados para o crescimento em hidroponia, utilizando-se solução nutritiva comercial, contendo 0, 50 µM e 300 µM do composto morina (Sigma-Aldrich). Os cultivos foram realizados por 14 dias, substituindo-se a solução nutritiva a cada dois dias. Ao final do experimento, foi determinado o comprimento final de cada raiz e a biomassa fresca de cada sistema. A biomassa seca das raízes foi determinada após secagem por 72 h em estufa a 60 ºC.

Análises combinada de trocas gasosas e fluorescência da clorofila *a*

Os parâmetros de trocas gasosas e fluorescência da clorofila *a* foram obtidos a partir de plantas cultivadas por 14 dias em ambiente com temperatura e umidade controlados. Todas as medidas foram realizadas no 14º dia na mesma porção foliar da 2ª folha com lígula expandida bem definida de no mínimo três plantas para cada tratamento.

Estes parâmetros foram analisados por meio da combinação entre medições de trocas gasosas e fluorescência multifase através do sistema portátil de fotossíntese com fluorômetro acoplado Li-6800-F2 (Li-Cor Inc., Lincoln, NE, USA). As medidas de trocas gasosas ocorreram entre às 7:00 e 15:00 h, porém os dados só foram considerados quando as variáveis analisadas dentro do mesmo grupo experimental apresentaram invariabilidade. A metodologia foi adaptada para plantas C_4 com base em Galazzi (2011), de Sousa (2012),

Loriaux *et al.* (2013), Moualeu-Ngangue, Chen & Stützel (2017), Moriwaki *et al.* (2019) e Zhou, Akçay & Helliker (2019).

As taxas de assimilação do CO_2 (*A*, μmol m^{-2} s^{-1}), a taxa de transpiração (E, mmol m^{-2} s^{-1}), a condutância estomática (g_s, mol m^{-2} s^{-1}) e a concentração intercelular de CO_2 (c_i, μmol mol^{-1}) foram mensuradas a partir da curva de resposta à luz [2000, 1900, 1800, 1700, 1600, 1500, 1400, 1200, 1000, 800, 600, 400, 200, 175, 150, 125, 100, 75, 50, 25 e 0 µmol m^{-2} s^{-1} de densidade de fluxo de fótons fotossinteticamente ativos (DFFFA)]. As condições da câmara foliar foram controladas para manter o CO_2 em concentração próxima à ambiente (400 µmol mol^{-1}) e em concentração saturante (2000 µmol mol^{-1}).

A partir da curva de resposta de *A* à DFFFA foi possível calcular o rendimento quântico da fotossíntese (α) [(µmol CO_2 m^{-2} s^{-1})/(µmol fóton m^{-2} s^{-1})], assimilação máxima de CO_2 ($A_{máx}$), o ponto de compensação da luz (P_{comp}), o ponto de saturação da luz (P_{sat}) e a taxa de respiração (Rd). Estes parâmetros foram estimados utilizando os modelos matemáticos linear e hiperbólico (Machado *et al.*, 2005; Moriwaki *et al.*, 2019).

Juntamente com as medições de trocas gasosas, as medidas de fluorescência da clorofila *a* foram realizadas utilizando a abordagem de flash multifásico descrita por Loriaux *et al.* (2013) com adaptações. O flash foi ajustado para luz vermelha saturante igual a 8000 µmol m^{-2} s^{-1}, com três fases de 300 ms de comprimento cada e profundidade da rampa de 25%. Após 10 h de adaptação ao escuro foram determinadas a fluorescência inicial (F_0), a fluorescência máxima (F_m) e o rendimento quântico máximo do fotossistema II (PSII)(F_v/F_m), onde F_v corresponde à diferença entre F_m e F_0 (Marchiosi *et al.*, 2016). Todas as avaliações foram realizadas utilizando uma cabeça sensora com câmara foliar de 2 cm^2, temperatura em 27 ºC e o déficit de pressão de vapor controlado entre o ar e a folha (DPV) em 0,2 KPa com vazão de 700 µmol s^{-1}.

Nas condições adaptadas à luz, após 25 a 30 min de aclimatação por planta, foram mensurados em conjunto com as análises de trocas gasosas os seguintes parâmetros: eficiência quântica fotoquímica efetiva ($F_v´/F_m´$), rendimento quântico efetivo (ϕPSII), taxa de transporte de elétrons no PSII (ETR), *quenching* fotoquímico (qP) e *quenching* não-fotoquímico (NPQ) (Genty *et al.*, 1989; Galazzi, 2011). Estes parâmetros foram determinados sob DFFFA de 1400 µmol m^{-2} s^{-1} em com concentração de CO_2 mantida a 400 µmol mol^{-1} na câmara foliar. Além da fluorescência adaptada ao escuro e ao claro, foi

estimado o conteúdo de clorofila por meio do índice SPAD (SPAD-502, Konica Minolta, Ramsey, USA).

RESULTADOS

O comprimento das raízes das plântulas cultivadas em hidroponia por 14 dias com 50 µM de morina foi inibido em 21,01%, já as plântulas tratadas com 300 µM apresentaram uma inibição do comprimento de 64,29% (Figura 1A), ambos sendo estatisticamente significativos. As biomassas fresca e seca das raízes das plantas cultivadas com 50 µM de morina aumentaram em 51,50% e 32,86%, respectivamente (Figura 1B, C). Assim como vemos na figura 2, a mesma concentração de morina alterou a formação de raízes adventícias, interferindo positivamente no peso e negativamente no comprimento. Nos tratamentos com 300 µM as biomassas fresca e seca das raízes reduziram em 57,14% e 53,28%, respectivamente (Figura 1B, C). Fenotipicamente, as raízes cultivadas com esta concentração de morina tiveram suas raízes adventícias muito reduzidas (Figura 2C).

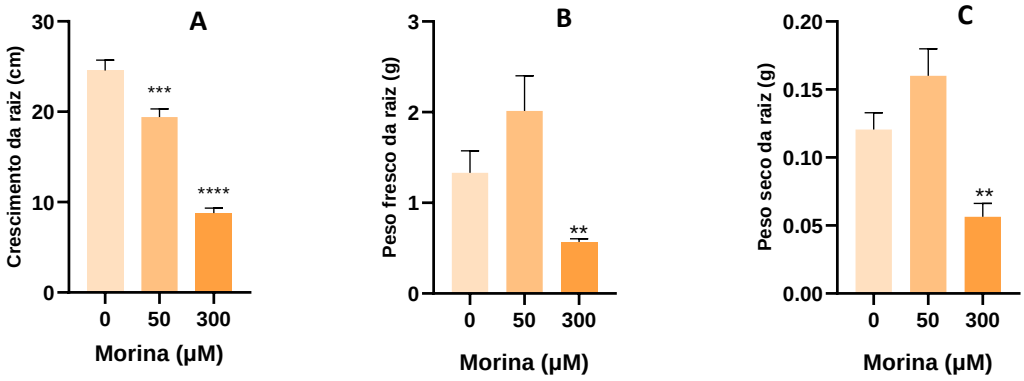

Figura 1. Efeitos da morina sobre o crescimento de raízes de plantas de milho. Medidas de comprimento (**A**), biomassa fresca (**B**) e seca (**C**) das raízes de plântulas de milho cultivadas por 14 dias na presença de 50 e 300 µM de morina. Valores médios ± EPM (n = 6) marcados com asterisco (*) diferem do controle de acordo com o teste de comparação múltipla de Dunnett (One-way ANOVA) com nível de significância 5% ($P \leq 0,05$).

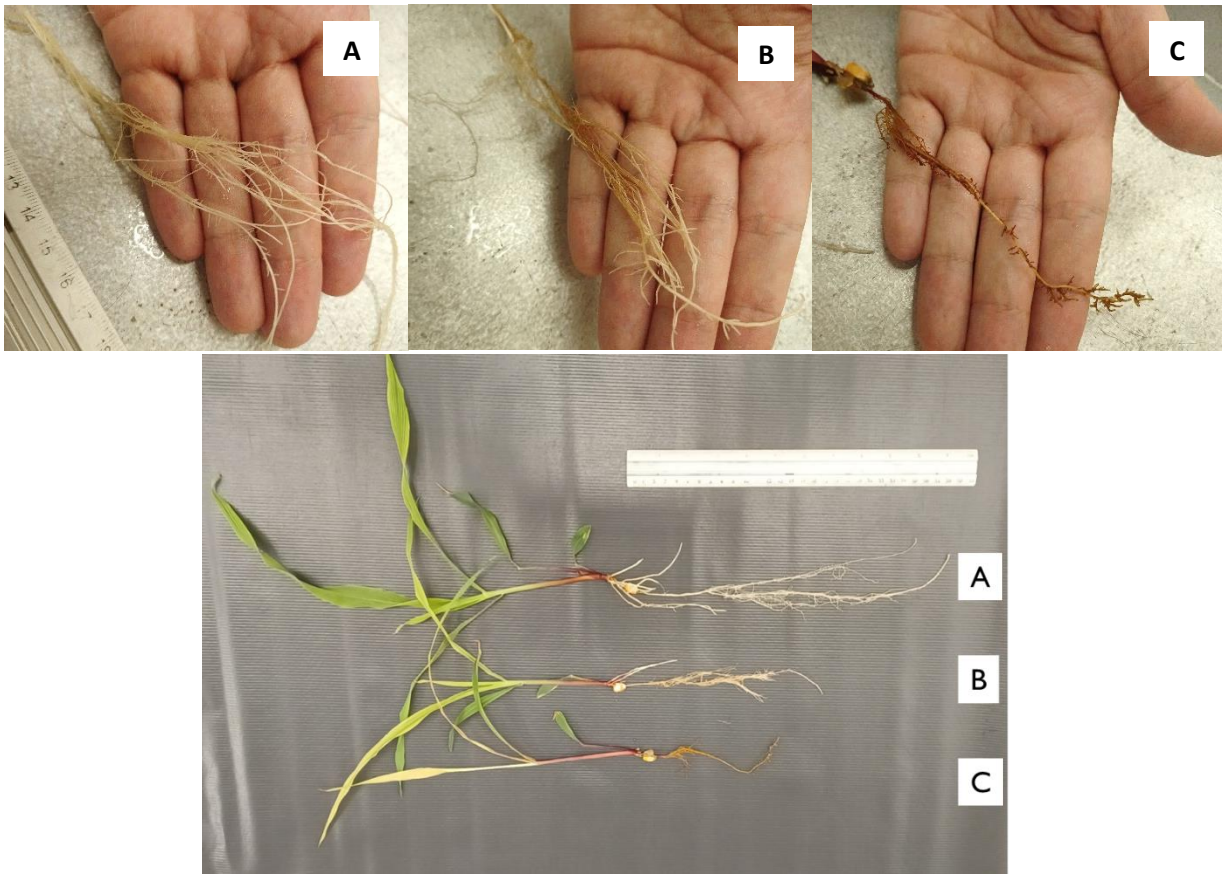

Figura 2. Raízes de plantas de milho após 14 dias de tratamento com 0 (**A**), 50 μM (**B**) e 300 μM (**C**) de morina.

O teor de clorofila foi significativamente reduzido em 17,44% e 39,98% nos tratamentos com 50 e 300 μM de morina respectivamente. Alterações de coloração nas folhas foram também observadas bem como redução do teor SPAD (Figura 4D). Concomitante a essas alterações, registramos reduções significativas no tratamento com 300 μM de morina na assimilação (A; 36,71%), na transpiração (E; 38,34%), na condutância estomática (gsw; 42,59%), na eficiência quântica fotoquímica efetiva (F_v/F_m; 8,72%), no rendimento quântico máximo do PSII no escuro (Fv'/Fm'; 5,14%), na taxa fotossintética máxima (Pn; 32,53%), na taxa de saturação máxima (I_{max}; 22,09%) e na taxa de saturação equivalente a 50% de Pn (I_{sat50}; 13,51%).

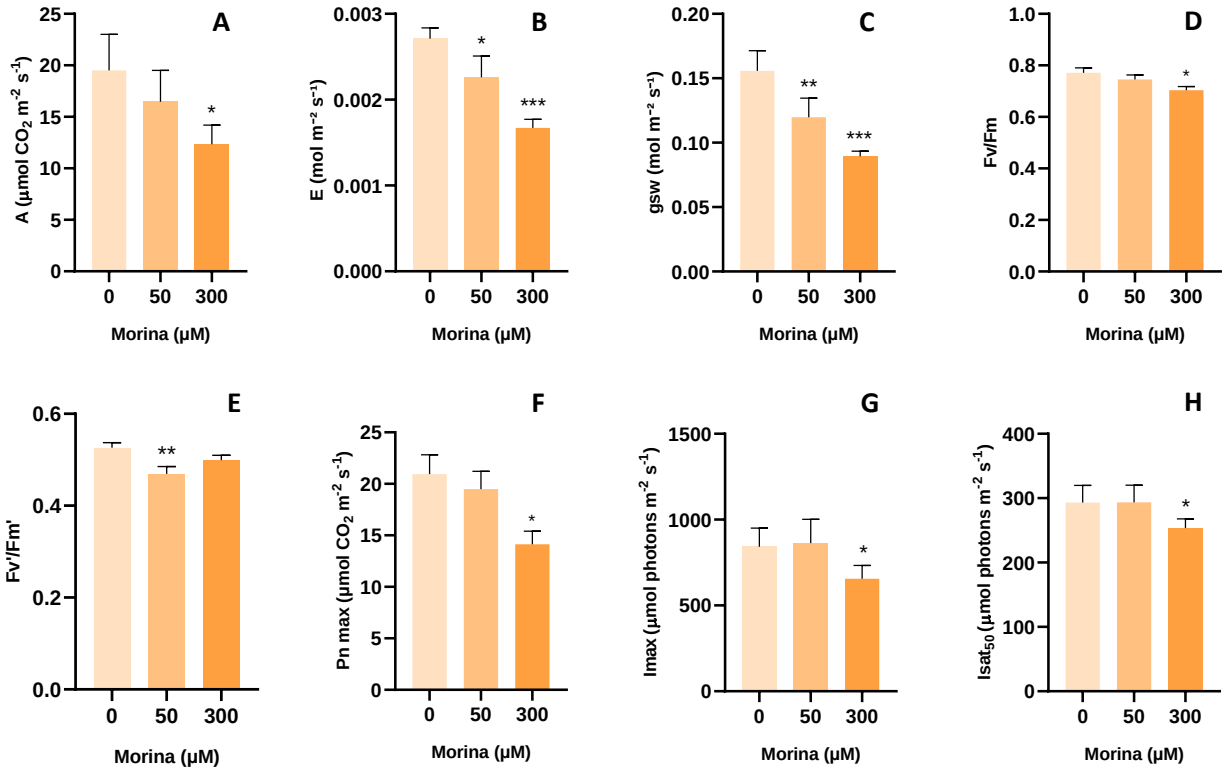

Figura 3: Efeitos da morina na assimilação (A), na transpiração (B), na condutância estomática (C), na eficiência quântica fotoquímica efetiva (D), no rendimento quântico máximo do PSII no escuro (E), na taxa fotossintética máxima (F), na taxa de saturação máxima (G) e na taxa de saturação equivalente a 50% de Pn (H), verificados após 10 h de adaptação ao escuro das plantas de milho cultivadas por 14 dias. Valores médios ± EPM (n = 5) marcadas com asterisco (*) diferem do controle de acordo com o teste de comparação múltipla de Dunnett (*One-way* ANOVA) com nível de significância de 5% ($P \leq 0,05$).

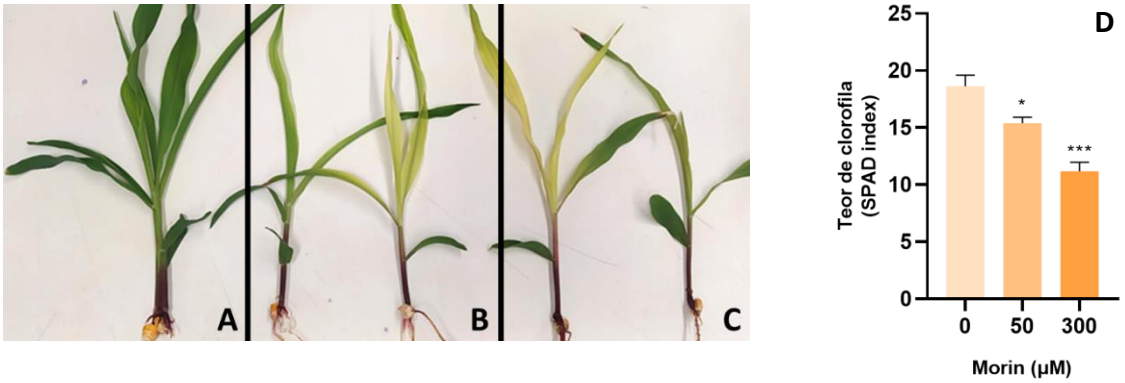

Figura 4. Efeitos da morina na coloração das folhas de milho após 14 dias de tratamento com 0 (**A**), 50 µM (**B**) e 300 µM (**C**) de morina. Efeitos da morina no teor de clorofila - SPAD (**D**). Valores médios ± EPM (n = 4) marcadas com asterisco (*) diferem do controle de acordo com o teste de comparação múltipla de Dunnett (One-way ANOVA) com nível de significância de 5% ($P \leq 0,05$).

DISCUSSÃO

De modo geral, o cultivo de plântulas de milho em hidroponia por 14 dias na presença de Morina nas concentrações de 50 e 300 µM afetou drasticamente o crescimento, a morfologia das raízes, diminuiu o teor de clorofila e reduziu os valores dos seguintes parâmetros fotossintéticos: A, E, gs, F_v/F_m, F_v'/F_m', Pn, Isat e Isat50. Curvas de resposta à luz e análise da fluorescência da clorofila *a* em plantas adaptadas à luz e ao escuro, indicam interferências da Morina sobre o transporte de elétrons fotossintético e nas reações de assimilação do carbono, sendo que valores de Fv/Fm e Fv'/Fm' nos fornecem uma estimativa da integridade estrutural do PSII (Lu *et al.*, 2009), e ambos foram reduzidos em 8,72% e 5,14% respectivamente.

A capacidade fotossintética (Pn) foi reduzida em 32,53% trazendo prejuízos para o crescimento, assim como a redução do teor de clorofila também indica menor capacidade em realizar a fotossíntese e consequentemente menor crescimento. O uso da variável Pn, para representar a taxa fotossintética máxima, nos fornece um valor mais realista da taxa fotossintética líquida máxima pois sua interpretação é direta e sua magnitude será sempre na faixa em que as medidas foram realizadas (Lobo et al., 2013). Relacionados também à capacidade fotossintética das plantas cultivadas, valores de assimilação e saturação máxima

foram reduzido, sugerindo possível dano ao fotossistema por serem alcançados em menor intensidade de luz. No escuro a respiração também foi reduzida em 26,91%.

É importante lembrar que a morina é possivelmente uma substância inibidora da PPDK. Esta enzima catalisa a formação de fosfoenolpiruvato (PEP) a partir de piruvato nas células do mesófilo (Carroll et al., 1990; Parsley & Hibberd, 2006). Por sua vez, o PEP é utilizado pela PEP carboxilase para a fixação primária do bicarbonato em oxaloacetato, o qual pode ser enviado para a célula envoltória do feixe vascular na forma de malato (Buchanan et al. 2015). Este é, então, descarboxilado pela enzima málica, liberando o CO_2 para a fixação secundária pela Rubisco (Buchanan et al. 2015). Dessa forma, a inibição da taxa fotossintética das plantas de milho expostas à morina pode estar relacionada com o papel inibitório deste flavonoide sobre a PPDK. Além disso, a biossíntese de clorofila também foi inibida pela morina, prejudicando a eficiência do aparato fotossintético, como evidenciado pela discreta redução encontrada em Fv/Fm. Corroborando nossos dados, a floretina, uma chalcona com estrutura molecular muito similar à morina e que também atua como inibidora da PPDK, inibiu drasticamente a Pn e a biossíntese de clorofila em plantas de milho (Constantin, 2022).

O crescimento radicular depende de alguns fatores que estão nas partes das plantas que estão acima do solo, como por exemplo, a fotossíntese, o aproveitamento de luz, hormônios e fatores móveis (van Gelderen et al., 2018). Valores reduzidos de condutância estomática também limitam a fotossíntese e consequentemente o crescimento da planta, uma vez que estômatos fechados diminuem a captação de CO_2. Possíveis danos também podem ocorrer na absorção de água pela morfologia alterada das raízes, confirmando a necessidade de manter os estômatos mais fechados e diminuindo também a transpiração.

O efeito primário da luz é permitir a fotossíntese, o que leva à produção de açúcares (sacarose) que são transportados pelo floema até as raízes e as permitem crescerem e se desenvolverem. Por conseguinte, uma vez que houve redução na fotossíntese da planta, a produção de açúcares também foi reduzida. As raízes, por sua vez, com menor disponibilidade de substrato energético, também podem ter seu crescimento prejudicado. Nesse sentido, a redução fotossintética causada pela morina pode ter levado a uma diminuição no crescimento das raízes das plantas.

CONCLUSÃO

Nossos resultados com milho confirmaram que, de fato, a morina afetou os processos fotossintéticos, com destaque para redução de diversos parâmetros fotossintéticos (A, E, gsw, Fv/Fm, Fv'/Fm', Pn, Imax e $Imax_{50}$) e do teor de clorofila nas folhas. Tais alterações sugerem que uma exposição prolongada das plantas à morina poderia levar a danos estruturais nos fotossistemas. Além disso, a inibição da fotossíntese pode ter limitado a produção de açúcares que servem como substrato energético para estruturas não fotossintetizantes das plantas, como as raízes, inibindo seu crescimento. Estudos futuros irão revelar outros efeitos da morina nas plantas, bem como se ocorre realmente a inibição da enzima PPDK, confirmando o uso da morina como um potencial herbicida.

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