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The effect of water stress on phytochemical accumulation, bioactive compounds and expression of key genes involved in flavonoid biosynthesis in *Chrysanthemum morifolium* L.

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ABSTRACT

The present study was designed to evaluate water-induced alterations in non-enzymatic antioxidants as evidenced by the qualitative and quantitative properties of polyphenols and the molecular mechanism of phenylpropanoid pathway by the expression patterns of key genes (*pal*, *chi*, and *f3 h*) in two *Chrysanthemum morifoilum* cultivars. In addition, certain physiological alternations due to water stress were evaluated. Considerable differences were observed in leaf DPPH antioxidant activity as well as phenolic, flavonoid, and anthocyanin contents between the control and water-stressed plants in both cultivars. Expression profiles of the genes related to polyphenolic metabolism showed environment and cultivar-dependent responses as well as gene × environment interactions. Combining the data with HPLC analysis led to identification of six phenolic compounds (namely, chlorogenicacid, rutin, ferulic acid, quercetin, apigenin, and luteolin) whose values generally increased with increasing water stress. Correlations were also established between certain compounds and the genes studied, which appears to be useful for further study of the flavonoid biosynthetic pathway. Moreover, a strong correlation was detected between inhibition percentage by DPPH assay and luteolin and chlorogenic acid, suggesting their contributions to the antioxidant capacity of the *Chrysanthemum* plant. Water stress stimulated the synthesis of luteolin, quercetin, and rutin in "Taraneh" cultivar as well as luteolin and apigenin in "Azita" cultivar through up regulating expression of *chi* gene. Finally, the current work deploys differential responses of cultivars to water stress toward further insight into how the pharmaceutical quality of *Chrysanthemum* in terms of its biochemical compounds may be enhanced.

1. Introduction

Plant secondary metabolites have been used as natural sources toward pharmaceutical and food applications. Recently, there has been a growing interest in various elicitors employed to induce the synthesis of secondary metabolites (Akula and Ravishankar, 2011). Environmental stresses, especially water stress, have been considered as the main factors responsible for the elevated metabolite content in plants (Gharibi et al., 2016). Water deficit or osmotic stress leads to enhanced formation of reactive oxygen species (ROS), which can be detrimental to cellular components at high concentrations (Hernandez et al., 2004). On the other hand, antioxidants –both enzymatic (peroxidases, superoxide dismutase, and catalase) and non-enzymatic (phytochemicals) – could play a defensive role to diminish the activities of free radicals in plant (Oh et al., 2010). Nonetheless, a great interest has been shown to the exploitation of natural antioxidants available in medicinal plants or in other natural sources for their therapeutic and pharmaceutical properties.

Medicinal plants have been nowadays identified as natural sources of such non-enzymatic antioxidants as phenolic compounds (Salami et al., 2016). Amongst them, Chrysanthemum (*Chrysanthemum morifolium* Ramat.) is an important one (Zhu et al., 2013) for the bioactive compounds in its flowers and leaves with a variety of biological properties (Roein et al., 2014). Moreover, it contains significant amounts of polyphenols, mainly as chlorogenic and ferulic acids. Flavonols (quercetin and rutin) and flavones (luteolin and apigenin) have also been obtained from *Chrysanthemum* extract (Wang et al., 2008; Xie et al., 2012).

Included among secondary metabolites with a high antioxidant capacity are polyphenol/flavonoid compounds that have received increasing attention not only for their beneficial effects on human health but also for the protection they provide against oxidative injury in

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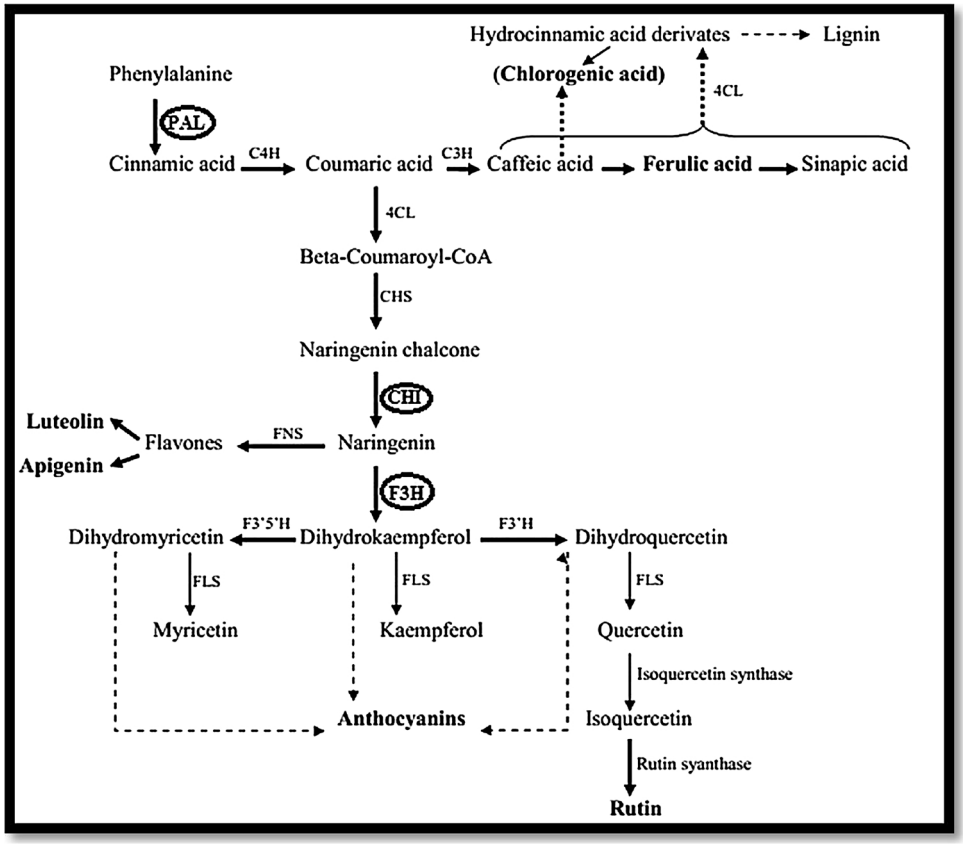

Fig. 1. Schematic representation of phenylpropanoid biosynthetic pathway in plants. PAL, phenylalanine ammonia lyase; C4H, cinnamate 4-hydroxylase; C3H, ρ-coumarate 3-hydrolase; 4CL, 4-hydroxy cinnamoyl COA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; FNS, flavones synthase; F3H, flavanone-3-hydroxylase; F3′H, flavonoid-3′-hydroxylase; F3′5′H, flavonoid-3′5′-hydroxylase; FLS, flavonol synthase.

plants under abiotic stresses (Andre et al., 2009; Qiu et al., 2013). Terpenes and phenolics are the more important secondary metabolites because of their structural properties contributing to plant stress tolerance (Moharramnejad et al., 2015). Accumulated polyphenols and flavonoids have been reported in response to biotic/abiotic stresses (Kasote et al., 2015; Rossi et al., 2016). This is, however, contradicted by reports in the literature indicating reduced phenolic compounds during water stress (Krol et al., 2014). Finally, anthocyanins have been observed to accumulate under water deficit conditions to contribute to drought resistance in plants (Moharramnejad et al., 2015).

Formation of polyphenolic compounds is initiated by the phenylpropanoid biosynthetic pathway, which is known as the starting point for the synthesis of a large family of low molecular weight plant secondary metabolites such as lignin, phenolic acids, flavonoids, stilbenes, and lignans (Hernandez et al., 2004; Andre et al., 2009). In this pathway, some major flavonoids and phenolic acids can be produced by key genes including phenylalanine-ammonia-lyase (*pal*), cinnamate 4-hydroxylase (*c4 h*), chalcone synthase (*chs*), Flavanone 3-hydroxylase (*f3 h*), and chalconeisomerase (*chi*) (Fig. 1) (Mouradov and Spangenberg, 2014).

As the phenylpropanoid pathway is a gateway for the synthesis of various secondary metabolites and triggers a cascade of biochemical reactions (Jadhav et al., 2013), investigation of the expression patterns of the genes involved in the generation of these metabolites under water stress can provide new insights for a deeper understanding of the polyphenols accumulation mechanism towards water stress. Previous studies demonstrated the effects of water stress on the expression of certain key genes in the phenylpropanoid pathway in different plant species including potato (Andre et al., 2009), lettuce (Oh et al., 2010), and Kacip Fatimah (Jaafar et al., 2012). Most previous studies were focused on the phytochemical characteristics of *Chrysanthemum* in normal conditions (Wang et al., 2008). Our literature review, however, revealed no study to have investigated the effects of water stress on the phytochemical characteristics of this particular species under water stress conditions.

In order to determine the mechanism involved in the response to dehydration stress and the mode of polyphenolic accumulation in this species, the current study, therefore, conducted at the following objectives: 1) to assess the effects of water deficit on the amount of polyphenolic compounds based on HPLC analysis and on the anthocyanin content and its antioxidant activity; 2) to evaluate the response of *Chrysanthemum* cultivars to water stress with respect to certain physiological characteristics; and 3) to assess the expression profiles of three major genes involved in the phenylpropanoid pathway under water deficit conditions.

2. Materials and methods

2.1. Plant material and experimental conditions

Chrysanthemum cultivars “Taraneh” (purple flower color) and “Azita” (red flower color) were obtained from the Iranian Research Center for Ornamental Plants, Mahallat, Iran. Both dehydration and control plants were grown in pots including clay garden soil under greenhouse conditions with an 8-h light/16-h dark cycle at 25 °C. Uniform plants at the vegetative stage were selected for the water stress experiment. The experimental plants were subjected to water stress over a period of seven days after watering had been stopped and soil moisture measured.

The experiment was conducted at Isfahan University of Technology, Iran, using a completely randomized factorial design with three replicates. Each replicate consisted of three plants. Leaf samples were harvested at 0, 3, 5, and 7 days after exposure to water stress conditions.

2.2. Relative water content (RWC)

To determine leaf relative water content, samples of fresh leaves