Current Plant Biology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Current Plant Biology

journal homepage: www.elsevier.com/locate/cpb

Growth enhancement and salt tolerance of Safflower (*Carthamus tinctorius* L.), by salicylic acid

Fatemeh Shaki, Hasan Ebrahimzadeh Maboud*, Vahid Niknam

Department of Plant Biology, School of Biology, College of Science, University of Tehran, Tehran, Iran

ARTICLE INFO

Keywords:
Safflower
Salinity
Salicylic acid
Resistance
PAL
Gene expression

ABSTRACT

It has been shown that salicylic acid acts as an endogenous signal molecule responsible for inducing stress tolerance in plants. The effect of SA (1 mM) and sodium chloride (0, 100, and 200 mM NaCl) on some biochemical and molecular responses of safflower plants was studied. Plants were harvested randomly at 21 days after the start of treatments. Results revealed that chlorophyll and total soluble protein contents decreased under salinity, however proline, glycine betaine, carbohydrate, total carotenoids, flavonoid, and anthocyanin contents, as well as PAL gene expression increased. The exogenous SA improved the response of safflower to salinity by increasing glycine betaine, total soluble protein, carbohydrates, chlorophylls, carotenoids, flavonoid, and anthocyanin contents. In addition, qRT-PCR analysis showed that exogenous SA induced expression of PAL gene in both salt-treated and untreated plants. Moreover, SA application under saline conditions decreased the levels of proline which could indicate successful acclimatization of these plants to saline conditions. Our data suggested that the exogenous SA played a key role in stress condition through regulation of osmotic adjustment and antioxidant system due to some compounds. These results provide novel insights about the physiological and molecular role of SA in salt resistance. Therefore, this component can be considered due to low price and availability.

1. Introduction

Salt poses particular challenges to global agriculture as it already affects 20% of cultivated and 33% of irrigated agricultural lands [1], with some predictions that salinization could impact 50% of arable lands by 2050 [2]. Salinity is also a significant problem in safflower production in many areas in the world [3].

Under salt condition, plants have adopted mechanisms to protect themselves by some strategies such as the accumulation of compatible solutes in cytosol, the production of reactive oxygen species (ROS), and accumulation of some secondary metabolites [4–7]. The accumulation of compatible solutes such as proline and glycine betaine (GB) is considered as a basic strategy for the protection of some plant species in response to salt stress [6,8]. Their accumulation occurs in the cytosol where it causes the osmotic adjustment [9,6]. Plant also protect themselves through reactive oxygen species (ROS) formation which can damage membrane lipids, nucleic acids, and proteins in cells [7,10,11]. On the other hand, specific levels of ROS are involved in antioxidative protection [7,12].

In stress condition, carbohydrates and proteins are accumulated in some plant tissues. These components are involved in osmotic adjustment in plant cells [13]. The major functions of carbohydrates are carbon storage, radical scavenging, and osmotic adjustment in cells [14]. The high carbohydrate concentrations, contributed to the prevention of oxidative damage and the maintenance of the structure of proteins under stress condition [15].

Salinity could change leaf pigments such as chlorophylls and carotenoids, and photosynthetic efficiency in many plant species [13]. Among the antioxidants present in the chloroplasts, carotenoids (Car) play an important role in the mechanisms protecting the photosynthetic apparatus against dangerous environmental factors [16]. They scavenge reactive oxygen species formed in stress condition and moderate the effect of stress in plants [17].

Flavonoids are a group of secondary metabolites that are synthesized via the phenylpropanoid pathway. They act as antioxidant agents by scavenging ROS and have a key role in stress protection in cells [18]. Their ability to act as antioxidants depends on the reduction potentials of their radicals [19]. Some kinds of water-soluble pigments derived from flavonoids via the shikimic acid pathway are anthocyanins. Plants produce anthocyanins as a protective mechanism in stress conditions. They accumulate in plant tissues under the influence of environmental stimuli [19].

* Corresponding author.
E-mail addresses: fatemeshaki373@ut.ac.ir (F. Shaki), ebizadeh@ut.ac.ir (H.E. Maboud).

https://doi.org/10.1016/j.cpb.2018.04.001
Received 6 December 2017; Received in revised form 25 March 2018; Accepted 1 April 2018

Phenylalanine ammonia-lyase (PAL) acts as a key enzyme in phenylpropanoid pathway, which is involved in the synthesis of secondary compounds such as salicylic acid, phenolics and lignin [7]. The phenylpropanoid pathway is an important pathway which placed across the border of primary and secondary metabolism in cells.

There are many agents in cells which are as signal transducers in response to stress conditions [6,7]. Salicylic acid (SA), considered as a phenolic compound which is able to reduce environmental stresses in plants [6,20,21]. It has a key role in defense system of plants and acts as a protector under environmental stresses [6,22]. These effects show that they could be ideal components in increasing the resistance against salinity.

Developing salt tolerant crops for the future will rely on identifying new mechanisms of tolerance. One of the most promising paths for identifying new mechanisms of salt tolerance is through studies of the genetic and physiological mechanism of plants [2].

Therefore, the purpose of the current study was to assess the impact of exogenous SA on some physiological parameters in safflower plants as well as the gene expression. The knowledge of alterations in these processes mediated by SA might provide a basis to enhance the growth and productivity of safflower in salt-affected areas.

2. Materials and methods

2.1. Plant cultivation and chemical treatments

Seeds from the Goldasht cultivar of safflower, *Carthamus tinctorius* L. were used in this study. Seeds were sown in Tref peat in a greenhouse with a 15 h light/9 h dark photoperiod at 27 ± 2 °C temperature for seed germination and subsequent growth. Following germination, plants were transplanted to plastic pots with perlite. Each pot, was considered as one replicate and there were four replicates of each treatment. Each pot was treated with different salinity concentration (0, 100, 200 mM NaCl) and salicylic acid (0 and 1 mM) [23] with 100 ml of half-strength Hoagland's nutrient solution, which served as a nutrient media at alternative days [24]. The nutrient solution was replaced every alternate day with fresh one. A foliar spray of SA three times (at 1-week intervals) [23] was applied to the plants. The final harvest was performed 21 days after the start of treatments. The two youngest pairs of fully expanded leaves were sampled from each plant. Leaves were immediately frozen in liquid nitrogen before being stored at −70 °C.

2.2. Determination of proline content

Free proline was quantified following the protocol of Bates et al. (1973). Approximately 0.5 g of plant tissue was homogenized in 5 ml of 3% aqueous sulphosalicylic acid. Two ml of the supernatant was heated with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid in a water bath at 100◦C for 1 h, and the reaction was stopped in an ice bath. For extraction, 4 ml of toluene was added, and samples were mixed vigorously for 15–20 s. Samples were then set aside to allow separation of the organic and aqueous phases. The absorbance was read at 520 nm in UV–vis spectrophotometer (UV-160, Shimadzu, and Tokyo, Japan) using toluene as a blank. Proline concentration was determined from a standard curve, and concentrations were calculated based on fresh weight.

2.3. Determination of glycine betaine content

Glycine betaine (GB) was quantified following the protocol of Greive and Grattan [65]. Finely ground plant tissue (0.5 g per sample) was shaken with 30 ml of deionized water for 48 h. The samples were then filtered and the filtrate was stored in a refrigerator (4 °C) until analysis. Thawed extracts were diluted 1:1 with sulfuric acid (3N). Aliquot (0.5 ml) was measured into test tube and cooled in ice water for 1 h. Cold potassium iodide-iodine reagent (0.3 ml) was added and the mixture was homogenized with a vortexer. The samples were stored at 4 °C for 16 h. After the end of the period samples were dissolved in 9 ml of 1, 2-dichloro ethane. After 3.0-3.5 h, the absorbance was measured at 365 nm. Reference standards of glycine betaine (0–200 μg ml^{-1}) were prepared in 3 N sulfuric acid and the procedure for sample estimation was followed and concentrations were calculated based on fresh weight.

2.4. Total carbohydrate content

Total carbohydrate content was quantified using phenol sulphuric method [25]. For determination of carbohydrates content, 0.1 g of dry leaf powder was extracted using 3 ml of ethanol: distilled water (8: 2; v/v), and supernatant was collected after centrifugation at 5000*g*. Then, 0.5 ml phenol solution (5%) and 2.5 ml sulphuric acid (98%) were added to each sample and the absorbance was read at 485 nm.

2.5. Protein extraction and determination

Plant leaf tissues were homogenized at 4 °C with a pestle and mortar in 1 M Tris-HCl (pH = 6.8) to quantify protein content. The Tris-HCl buffer contained 5 mM 1.4 dithiotheritol (DTT), 0.5 mM NaCl, and 5 mM ethylenediaminetetraacetic acid (EDTA). The homogenate was centrifuged at 13,0009*g* (J2–21 M, Beckman, Palo Alto, USA) for 30 min at 4 °C. The obtained supernatant was kept at −70 °C and used for protein determination. Proteins were determined according to Bradford [26], using bovine serum albumin (BSA) as standard. Five milliliters of the Bradford reagent and 50 μl of the each protein extract were mixed and then reaction mixtures were incubated at room temperature for 20 min. The absorbance values were measured at 595 nm.

2.6. Determination of chlorophyll and carotenoid contents

Primary leaf samples (0.1 g fresh weight) extracted in 80% acetone/water (v/v) and was used for the spectrophotometric determination of chlorophyll a, b and total carotenoids [27]. The absorbance was determined at 470, 646.8, and 663.2 nm using UV–vis spectrophotometer (UV-160, Shimadzu, Tokyo, Japan). The chlorophyll and carotenoid contents obtained using the following formulas.

$$\text{Chla (mg/ml)} = 12.25\,A_{663.2} - 2.79\,A_{646.8}$$

$$\text{Chlb (mg/ml)} = 21.50\,A_{646.8} - 5.10\,A_{663.2}$$

$$\text{Tchl (mg/ml)} = \text{chla} + \text{chlb}$$

$$\text{Cx} + \text{c} = (1000\,A_{470} - 1.8C_a - 1.8C_a - 85.02C_b)/198$$

In these formulas Chla, Chlb, Tchl, and Cx + c are chlorophyll a, chlorophyll *b*, total chlorophyll, and carotenoids (consist of Xanthophyll and carotene), respectively.

2.7. Determination of total flavonoid content

Flavonoid quantification was done using aluminum chloride colorimetric method [28]. Plant leaf tissue (0.1 g) was extracted in 2 ml of 80% methanol. The solution centrifuged at 8000 g for 10 min. Plant extracts (0.5 ml) were mixed with 2 ml of methanol, 0.1 ml of 10% aluminum chloride ($ALCL_3$), 0.1 ml of potassium acetate (1 M), and 2.8 ml of distilled water and kept at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm.

2.8. Determination of anthocyanin content

For anthocyanin quantification, plant leaf tissues (0.1 g fresh weight) were homogenized with a pestle and mortar in 10 ml of methanol (99% methanol, 1% HCL) [29]. The solution centrifuged at 6000 g for 10 min. The absorbance for each sample was read at 550 nm.