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Salicylic acid supplementation improves photosynthesis and growth in mustard through changes in proline accumulation and ethylene formation under drought stress

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ABSTRACT

Drought stress is becoming a major threat to plant productivity loss in agricultural system. The present study was carried out to evaluate the physiological and biochemical alterations induced by salicylic acid (SA) in mustard plant under moderate drought stress conditions. Therefore, a pot culture experiment was conducted to test whether SA application at concentration of 0.5 mM through foliar spray could protect the mustard (*Brassica juncea* L.) cultivar Pusa Jai Kisan subjected to drought stress on the basis of growth and photosynthesis. The treatments were as follows: (i) 100% FC + 0 mM SA, (ii) 50% FC + 0 mM SA, (iii) 100% FC + 0.5 mM SA and (iv) 50% FC + 0.5 mM SA. The control treatment received 100% field capacity (FC) irrigation, whereas moderate drought stress corresponded to 50% field capacity. Plants subjected to drought stress caused significant reduction in growth and photosynthetic parameters, activity of ribulose 1,5-bisphosphate carboxylase (Rubisco), nitrate reductase (NR), ATP-sulfurylase (ATPS) which accounted for decreased nitrogen (N) and sulfur (S) assimilation. Whereas, a pronounced increase was observed in proline metabolism. Exogenously applied 0.5 mM SA alleviated the stress by increasing the proline production through the increase in γ-glutamyl kinase (GK) and decrease in proline oxidase (PROX) activity. In addition SA application restricted the ethylene formation by inhibiting the 1-aminocyclopropane carboxylic acid synthase (ACS) activity more conspicuously under moderate drought stress than no stress. These findings reflect that SA application alleviates the drought-induced decrease in growth and photosynthesis through increased proline content. Higher proline content was a result of increased N and S assimilation and increased synthesis of proline synthesizing enzyme which lowers the oxidative stress in mustard.

1. Introduction

Drought stress is one of the main environmental factors limiting plant growth and yield and it is the most prevalent cause of crop yield loss due to an increase in temperature and a decrease in water availability respectively, that deviates from the optimal condition for plant life (Larcher, 2003). Drought stress is most difficult to tackle because of the strong link between transpiration and photosynthesis (Posch and Bennett, 2009).

Indian mustard (*Brassica juncea* L.) constitutes an important group of oilseed crop predominantly grown on nearly 70% of the cultivated land area (Ram et al., 2012). It is the third most important source of vegetable oil in the world after soybean and palm oil. Nearly 37% of the total rapeseed-mustard area is rain-fed, where the crop is severely affected by drought, resulting in acute yield losses particularly in the drought prone areas of Eastern and Western India. Thus, there is an urgent need to develop varieties that can maintain optimum yield under the stress. High water use efficiency (WUE), the ability of the plant to produce dry matter/unit of water is a genetic character, dependent on photosynthetic rate and transpiration rate which are very crucial for the productivity of the crop under drought stress.

The major physiological effects of drought stress results in decreased photosynthesis could be attributed to the perturbations of biochemical processes, such as decrease in ribulose bisphosphate carboxylase (Rubisco) activity and decreases in ribulose-1,5-bisphosphate (RuBP) or Pi regeneration capacity (Delfine et al., 1998). Flexas and Medrano (2002) reported that limitation of plant growth forced by low water availability is mainly due to the reduction of plant carbon balance, which is dependent on photosynthesis. The decrease in intercellular CO_2 concentration results in stomatal or diffusional limitation to

Abbreviations: ACS, 1-aminocyclopropane carboxylic acid synthase; ATPS, ATP sulfurylase; DAS, days after sowing; FC, field capacity; GK, γ-glutamyl kinase; N, nitrogen; NR, nitrate reductase; NUE, nitrogen use efficiency; PROX, proline oxidase; PSII, photosystem II; ROS, reactive oxygen species; Rubisco, ribulose 1,5-bisphosphate carboxylase; RuBP, ribulose 1,5-bisphosphate; SA, salicylic acid; S, sulfur; SUE, sulfur use efficiency; TBARS, thiobarbituric acid reactive substances

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photosynthesis (Chaves et al., 2003). Rubisco is the main enzyme responsible for CO_2 fixation and its decreased activity decreases photosynthesis. Therefore, osmotic adjustment that allows stomata to remain partially open during severe water deficit is beneficial (Alves and Setter, 2004). Lunde et al. (2008) have shown that the process of sulfur (S) acquisition and assimilation plays an integral role with the plant metabolism, and its deficiency leads to a reduced chlorophyll content, photosystem II (PS II) efficiency and Rubisco content. Under drought stress, Xu and Zhou (2006) reported that the inhibition of photosynthesis was associated with reduced N and S metabolisms and decreased the availability of N to photosynthetic apparatus, which affects Rubisco protein and activity.

One of the mechanisms involved in acclimation seems to be an accumulation of proline a compatible solute and its concentration which has been suggested as a general indicator for drought tolerance (Liu et al., 2011). It plays a protective role in supporting osmotic adjustment or by stabilizing membranes, protection of cellular structures, and regulation of cellular redox potential (Hare et al., 1998; Szabados and Savouré, 2010) and functioning as oxygen radical scavengers (Ashraf and Foolad, 2007). Proline may also function as a protein compatible hydrotrope (Srinivas and Balasubramanian, 1995), alleviating cytoplasmic acidosis, and maintaining appropriate NADP+/NADPH ratios compatible with metabolism. The rapid breakdown of proline upon relief of stress may provide sufficient reducing agents that support mitochondrial oxidative phosphorylation and generation of ATP for recovery from stress and repairing of stress-induced damages (Hare et al., 1998). In plants, the precursor for proline biosynthesis is glutamic acid. γ-Glutamyl kinase (GK) and γ-glutamyl phosphate reductase are the two enzymes regarded as an enzyme complex called P5C synthetase because it catalyzes the end product glutamine γ-semialdehyde (GSA) which is non-enzymatically converted to pyrroline-5-carboxylate. Therefore, the regulation of proline biosynthesis is mainly controlled by the activity of P5C synthase (Boggess et al., 1976). The enzyme proline oxidase (PROX) also influences the level of proline accumulation as it degrades proline to glutamate. The protection of photosynthesis and tolerance to plants under drought stress could be stimulated by adopting the strategies to increase proline metabolism and investment of N to photosynthesis by increasing nitrogen use efficiency (NUE) and sulfur use efficiency (SUE).

Salicylic acid (SA) as a potent signaling molecule in plants is involved in defense mechanisms by regulating physiological and biochemical functions and has diverse effects on tolerance to biotic and abiotic stress factors (Gunes et al., 2007; Nazar et al., 2011). Previous studies have shown that SA provokes plant resistance to salinity (Nazar et al., 2011; Khan et al., 2014), drought (Yazdanpanah et al., 2011) and temperature (Wang et al., 2010). Exogenous SA application enhanced the growth and photosynthetic rate in wheat (Hussein et al., 2007) under water stress, and increases photosynthetic activity and stomatal conductance under drought stress (Habibi, 2012). In addition, it has been found that plants treated with SA generally exhibited better resistance to drought stress (Al-Hakimi and Hamada, 2001). Nazar et al. (2011) reported that higher allocation of N and S to leaf through the increase in the activity of NR and ATP-sulfurylase, respectively with 0.5 mM SA application under salt stress increased photosynthesis in mung bean cultivars suggesting a role of S in photosynthesis. Recently, Palma et al. (2013) reported that SA and its related compounds are important components for modulating redox balance and trigger an increase in total glutathione content. SA may also influence photosynthesis under drought stress by inhibiting ethylene synthesis. Salicylic acid treatment resulted in retarding ethylene synthesis, interfering with membrane depolarization, stimulating photosynthetic machinery, increasing the content of chlorophyll as well as blocking wound response in soybeans (Leslie and Romani, 1988). The inhibition of ethylene synthesis under drought stress by SA may result in increasing plant response to ethylene and influencing proline metabolism photosynthetic-NUE and -SUE and photosynthesis.

The reported research was conducted to explain that supplementation of SA protects photosynthesis and growth through enhanced proline metabolism, photosynthetic-NUE, SUE, together with inhibition of ethylene and alleviates adverse effects of drought stress in mustard (*B. juncea* L) cv. Pusa Jai Kisan.

2. Materials and methods

2.1. Plant material and growth conditions

Plants of mustard (*B. juncea* L.) cv. Pusa Jai Kisan were surface sterilized seeds and sown in 23 cm diameter earthen pots filled with reconstituted soil (peat and compost, 4:1, v/v, mixed with sand 3:1, v/v). Two plants per pot were maintained and were saturated alternate days with 250 ml of full strength Hoagland's nutrient solution for improving and accelerating the growth of plants. Plants grown in pots were kept in a greenhouse under natural day/night conditions with photosynthetically active radiation (PAR) 900 ± 28 μmol m^{-2} s^{-1} and average day/night temperature of $23/17 \pm 2$ °C in the Department of Botany, Jamia Hamdard University, Delhi, India. The control pots were maintained to field capacity (FC) during the entire growth period. Performance of drought stress was done at 15 days after sowing (DAS), all the pots were maintained at 100% FC (no stress) and 50% FC (moderate drought stress) during the treatment period. Computation was made of pots' weight and amount of water needed for the desired soil moisture regime. Rain-out shelters were used to maintain imposed stress during rainfall; they were pulled back so that pots received maximum sunlight. Furthermore, the pots were regularly covered with rain out shelters during night time. Using this system, the pots were protected from rainfall and any external moisture entry. SA was dissolved in absolute ethanol then added drop wise to water (ethanol/water: 1/1000 v/v) and was applied at 30 DAS on the foliage of plants at the concentrations of 0.5 mM on plants subjected to no stress (100% FC) or drought stress (50% FC) with a hand sprayer. The control plants were sprayed with ethanol/water: 1/1000 v/v. A surfactant teepol (0.5%) was added with the control and SA treatments solution. The volume of the spray was 25 ml per pot. The concentration of SA was selected based on our earlier findings (Nazar et al., 2011). The experiment followed a randomized complete block design and the number of replicates for each treatment was four. Measurements were done at 45 DAS and care was taken to select the same age of leaves for the determinations.

2.2. Estimation of proline

Proline content was determined spectrophotometrically by adopting the ninhydrin method of Bates et al. (1973). Fresh leaf samples weighing 300 mg were homogenized in 3 ml of 3% sulfosalicylic acid. The homogenate filtrate was reacted with 1 ml each of acid ninhydrin and glacial acetic acid for 1 h in a test tube placed in a water bath at 100 °C. The mixture was extracted with toluene and the absorbance was measured on a spectrophotometer (UV–vis L164, Elico) at 520 nm using L-proline as a standard.

To determine the activity of γ-glutamyl kinase activity (GK) and proline oxidase activity (PROX), enzyme extract was prepared by homogenizing 500 mg leaf sample in 0.1 M Tris–HCl buffer, pH 7.5, at 4 °C. The homogenate was centrifuged at 30,000 ×*g* for 30 min and pellet was collected and used as extract for assay of GK and PROX. For GK enzyme activity extract was kept in a freezer at −20 °C.

Activity of GK (EC 2.7.2.11) was assayed by the method of Hayzer and Leisinger (1980) with slight modification. The frozen sample was suspended in 10 ml of 0.1 M Tris–HCl buffer containing 1 mM 1, 4-dithiothreitol (DTT) to rupture the cell and centrifuged at 30,000 ×*g* for 30 min. The assay mixture contained 50 mM L-glutamate, 10 mM ATP, 20 mM $MgCl_2$, 100 mM hydroxylamine HCl and 50 mM Tris–HCl, pH 7.0 with 200 μl of desalted extract in a final volume of 500 μl. The