



Effects of Sodium Nitroprusside (SNP) on Kiwifruit Growth under Drought Stress

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ABSTRACT: Stress is one of the important factor that limits the growth of plant under different conditions mainly during drought. However, the natural mechanism of the plants prevent drought stress by increasing water uptake or decreasing water loss and water stress may cause oxidative damage. This may cause reactive oxygen species (ROS) to become active that could damage cell membranes. Different treatments were tested for drought resistance. However, sodium nitroprusside (SNP) can be noted as an external treatment in resistance to drought stress. It stimulates antioxidant enzymes that cause scavenging of ROS. The present study was conducted by taking two year old seedlings and were grown in pots. The seedlings were stressed at two levels at 100% FC and 40% FC and four SNP levels were used as a treatment in the form of 0, 0.1, 0.5, and 1 mM levels on kiwifruit seedlings. The different effects like stomatal conductance, RWC, Peroxidase (POD) enzyme amount, polyphenoloxidase (PPO) enzyme amount and proline amount of SNP were studied under drought stress in Kiwi fruit plant. The study concluded that under drought stress condition, SNP causes positive effects in the plant to cope with drought stress and this compound may be used as a healthy and cheap compound for improvement and productivity.

Keywords: sodium nitroprusside, stress, improvement and productivity

INTRODUCTION

Drought resistance by plants is an important mechanism to cope with different stresses under varied conditions. The different activities that may be evolved include decreased leaf size, stem extension and root proliferation. The plant starts to undergo some changes in molecular, physical and morphological basis through identified mechanisms. Water stress in Kiwi fruit alters the pattern of dry matter distribution to promote root growth. The growth inhibition induced by water deficits is a consequence of a reduction in both photosynthesis and photosynthates partitioning, which negatively affects leaf area development (Chartzoulakis, Noitsakis, & Therios, 1993). CO₂ assimilation in leaves is usually decreased by stomatal closure or by enhanced water uptake with deep root system can be seen under such condition. Osmolytes including glycine betaine, proline and other amino acids are crucial to stabilize cellular functions under drought (Farooq, *et al.*, 2009). Proline provides protection against stress by acting as an N-storage compound; as an osmolite, a hydrophilic protectant for enzymes and cellular structures; and as a free radical scavenger (Filippou *et al.*, 2013). The stress disrupts photosynthetic pigments and reduces the gas exchange

leading to a reduction in plant growth and productivity too.

The different studies in the field of stomata position under drought stress condition have shown stomatal closure and decreased stomatal conductance that is considered a natural phenomenon. Nitric oxide (NO) can focus on integral component of drought signaling network. It is argued that NO acts downstream of ABA in physiological events controlling stomatal transpiration and adaptive responses to water shortage. There is evidence that NO accumulation in guard cells in response to osmotic stress mediates the reorganization of actin microfilaments and then regulates the dynamics of vacuoles to induce stomatal closure (Arasimowicz *et al* 2009). This decreases the wastage of water and increases Relative Water Content (RWC) consequently in the plant. In both cases, this helps the plants to improve under drought stress condition. On the other hand, stress enhances ROS production in different cellular compartments such as in chloroplasts and mitochondria. Nitric oxide acts as an active signal molecule in the plant's vascular system, affecting most of the biological routes in the plant (Mata & Lamattina, 2001). The compound causes resistance in the plant under stress and disease conditions by affecting antioxidant enzymes and secondary metabolites.

Antioxidant system of the plant is activated in this case to cope with the condition, improving it by ROS scavenging. Among antioxidant enzymes are polyphenol oxidase (PPO) and peroxidase (POD) (Cruz de Carvalho, 2008). The objective of the study is to investigate the effects of the application of SND On Kiwifruit enzymes. Further, water relations of the plant and changes made in PPO and POD enzymes were also studied.

MATERIALS AND METHODS

Two-year-old seedlings of Highward Kiwifruit was obtained from Chaboksar, Iran. The experiment was conducted in the greenhouse of the faculty of Agriculture, University of Tabriz-Iran. The study was conducted in a factorial design completely randomised with four replications.

The kiwifruit seedlings were placed in the plastic pots with soil containing FC=25% at 7.8 PH. The plants were irrigated well two months before the experiment, and SNP as an external treatment was sprayed at four levels (0, 0.1, 0.5, 1) mM on the Kiwifruit plants. Two drought stress treatments at FC 40% and 100% were conducted at the same time, and were kept for about one month (July to August). Finally, the samples were evaluated.

A. Stomatal Conductance (gs)

Leaf stomatal conductance (gs) was measured with the promoter. We were calibrating device before use. During the water regime period, five fully expanded leaves from the middle part of branches were selected and then they were used for stomatal conductance (Aganchich *et al.*, 2007).

B. Relative water content

RWC was evaluated by Yamasaki and Dillenburg method (Yamasaki & Dillenburg, 1999). For measuring RWC, we selected young fully developed leaves from top and measured fresh masses (FM). After 48 h their turgid mass (TM) was measured, and then the samples were placed in an oven under 80°C for 48 hours, and they were evaluated by mass (DM) with the use of the following relation:

$$\text{RWC}\% = [(FM-DM)/(TM-DM)] \times 100$$

Proline. The proline was measured by ninhydrin method. Fully expanded leaves from plants were sampled. Purified proline was used to standardize the procedure for quantifying sample values. Acid-ninhydrin was prepared by warming 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6M phosphoric acid. Kept cool (stored at 4°C) the reagent remains stable 24 hours. Approximately 0.5g of plant material was homogenized in 10 ml of 3% aqueous sulfosalicylic

acid. Two ml of filtrate was reacted with 2 ml acldninhdrin and 2 ml of glacial acetic acid in a test tube for 1 hour at 100°C, and the reaction terminated in an ice bath. The reaction mixture was extracted with 4 ml toluene, mixed vigorously with a test tube stirrer for 15-20 sec. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance read at 520 nm using toluene for a blank (Bates, Waldren, & Teare, 1973).

Enzymatic Assay. Each sample was taken from newly developed shoots. The total soluble protein concentration of enzyme was determined using BSA as a standard, POX activity was determined by Bacon (Bacon *et al.*, 1997) that mixture POX contained 1 ml of potassium phosphate buffer 0.1M, 500µl guaiacol 0.01M and 200µl of enzyme extract. The reaction was started by adding 300µl of H₂O₂ 0.03%. Then the absorbency of the solutions was measured by spectrophotometer at 470 nm. PPO activity was assayed according to Tonejia and Sachar (1974). The reaction mixture contained 200 µl of enzyme extract, 500 µl of catechole 1M and 1300µl of potassium phosphate buffer 0.1%. Absorbency was measured by spectrophotometer at 430 nm.

RESULTS AND DISCUSSION

The results of this study indicated that application of SNP on the Kiwifruit plant under stress condition has positive effects on plant's coping with stress. Stomatal closure and decreased stomatal conductance under drought stress are among the most important searchable issues and these quantities evaluation in the study, also, indicated a direct effect of SNP application on gs and RWC. Progressive decrease of plant water potential, however, causes xylem pressure to decrease to very adverse values, thus increasing the likelihood of embolism induction in xylem vessels and resulting hydraulic failure. Under such conditions, stomatal closure plays an important role in preventing hydraulic failure by regulating the rate of water loss and limiting the xylem pressure drop (Tyree & Sperry 1989).

Application of different levels of SNP on the Kiwifruit plant indicated that gs (stomatal conductance) was decreased with the increase of the treatments amount. This shows stomatal closure under stress condition. Similar results were obtained in the study conducted by Neill *et al* (2008). Leaf stomata could display temporal and spatial harmonized behavior or complex spatial and temporal dynamics, depending on environmental and physiological parameters. This type of dynamics could be related to stomatal heterogeneity at several spatial and temporal scales (Mott and Buckley, 1998).

RWC in the study conducted by Garcia *et al* 2001) indicated significant increase after application of SNP, which is in balance with findings of the current study. So increase of RWC after stomatal conductance inhibits seems completely logical. In this study, proline amount did not indicate a noteworthy difference in the data variance analysis with application of different levels of SNP. It seems that since kiwifruit can not tolerate drought and dry conditions, so proline amount has not a significant difference, but this amount will be significantly different in the plants which do osmoregulation. The Impact of sodium nitroprusside (SNP) on Kiwifruit growth under drought stress is represented in Table 1 and Fig. 1 & Fig. 2.

SNP is peroxidase and polyphenol oxidase stimulant that decreases the enzyme activity after application. In the same way after application of different levels of SNP with an increase of SNP amount, these enzymes will also increase accordingly.

Similar studies were reported by Thipyapong *et al*, 2004 on tomato under drought stress condition which indicated an increase of PPO amount after application of SNP. Zhang *et al* (1994) in their study on wheat species found that POD of the plant was increased after application of SNP, preparing the plant to cope with stress.

Table 1: Impact of sodium nitroprusside (SNP) on Kiwifruit growth under drought stress.

Rep	SNP	gs	RWC	POX	PPO	prolin
1	1	88.2	77.8	0.3	0.04	94.4
1	2	87	84.3	1.1	0.07	95.7
1	3	83.5	87.3	2.5	0.09	96
1	4	82.7	89.5	2.9	0.11	100.1
2	1	83.8	80.1	0.7	0.05	113.3
2	2	82.7	87.9	0	0.08	114.8
2	3	79.3	89	2.3	0.1	115.2
2	4	78.6	90.1	2.7	0.12	120.1
3	1	90	79.5	0.6	0	85
3	2	88.7	80.2	1.2	0.08	86.1
3	3	85.2	81.2	2.7	0.11	86.4
3	4	84.4	85.3	3.1	0.13	90.1

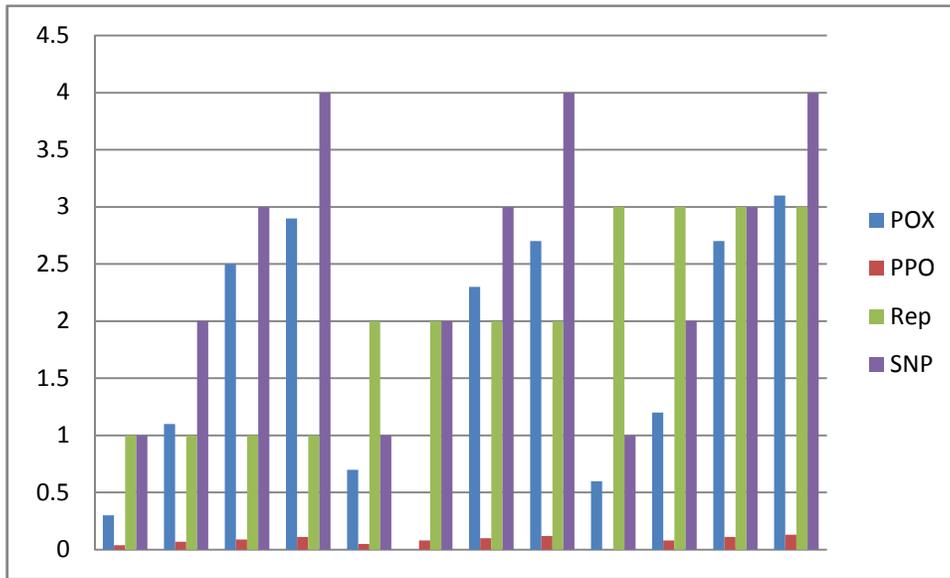


Fig. 1. POX, PPO, Rep and SNP concentration.

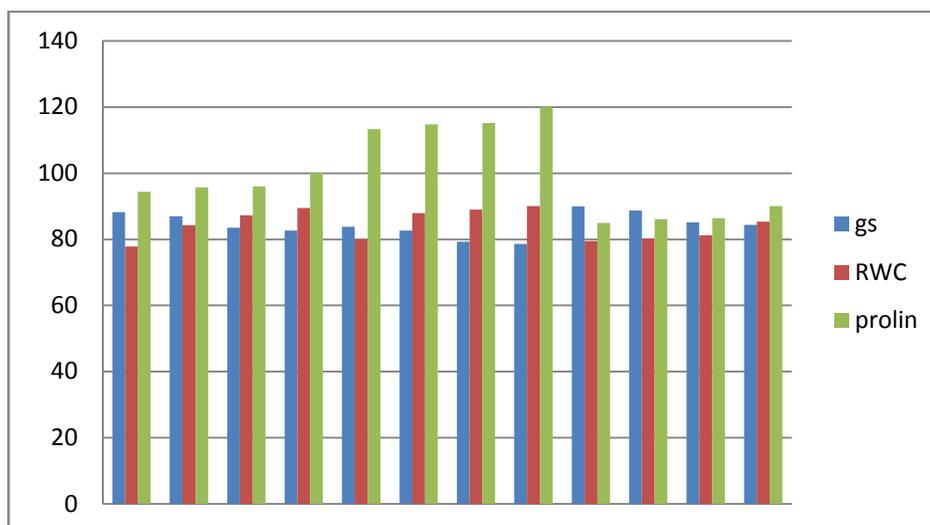


Fig. 2. gs, RWC and Prolin concentration.

CONCLUSION

Considering the importance of the plant's coping way with stress and with regard to the effects of SNP as an external treatment under drought stress condition and improvement of antioxidant system of the plant under such conditions by SNP. It can be concluded that that the compound can be used as a healthy, economical and effective compound in coping with drought stress condition.

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