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Modulating Effect of Salicylic Acid in Tomato Plants in Response to Waterlogging Stress

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Abstract

The present investigation to investigate ameliorative effect of salicylic acid (SA) on waterlogged situation was carried out as pot culture experiment in tomato variety H-86 (Kashi Vishesh) in poly house with nine treatments including control in three replications under short term stress. The response of various morphological and biochemical attributes progressively reduced with the increased duration of waterlogged condition. Seeds treated with SA (50 and 100 ppm) or without SA, and foliar application of SA were given alone or in combination. Treated seeds significantly promoted seedling growth and ameliorated waterlogged stress induced responses as confirmed by the changes in growth pattern and several morphological and biochemical attributes. Among treatments, SA (50 ppm seed treatment combined with100 ppm foliar spray) produced better results in terms of shoot length, leaf number, chlorophyll a, chlorophyll b, sugar and protein content, number of flower, number of fruits and fruit weight per plant as compared to waterlogging control plant.

Highlights

- Reduced growth of tomato and its productivity under waterlogging stress was observed.
- SA is an important signaling plant molecule which ameliorated waterlogging stress induced responses in tomato plants

Keywords: Waterlogging stress, Salicylic acid, Tomato

Tomato is one of the most important vegetable crops having rich source of minerals, vitamins, organic acids, sugar and protein content. It is very sensitive to waterlogged condition leading to a pronounced decrease of biomass production and overall negative impact on crop productivity. Waterlogging results in visible yellowing and senescence of leaves that causes decrease in leaf area, plant dry matter, relative water content and chlorophyll content in leaves and membrane stability index in both roots and leaves (Yiu *et al*., 2009). It also leads to reduced gas exchange between the plant tissue and atmosphere which causes initially hypoxia condition (low oxygen) and then leads to anoxia (complete absence of oxygen). The deleterious effects associated with hypoxia and anoxia as well as accumulation of toxic metabolites and reactive oxygen species (ROS) include decrease in cellular energy and lowering cytoplasmic pH which are responsible for the reduced growth and yield of many crops related to agriculture (Subbaiah and Sachs 2003). If waterlogging prolongs in soil, it causes inhibition of root respiration and reduces energy production because oxygen is required for terminal electron acceptor in electron transport chain in mitochondria which produces energy (ATP) that changes plant metabolic activity. One of the metabolic features affected by waterlogging conditions is the antioxidant system. It generates oxidative stress and promotes the production of

ROS including superoxide (O_2^-), singlet oxygen ($_1O^2$), hydroxyl anion (OH^-) and hydrogen peroxide (H_2O_2) that can be detrimental to proteins, lipids and nucleic acids. Salicylic acid (SA) is a phenolic compound widely distributed in plants with concentration differing among species (Raskin *et al.*, 1990). It acts as an important signaling molecule which mitigates plant responses to environmental stresses (Shakirova *et al.*, 2003). Increasing evidence showed that SA plays important roles in diverse physiological processes in plants through induced level of cytokinin, gibberellin and also nitric oxide under waterlogging condition which can mediate stomatal movement (Garcia-Mata and Lamattina, 2007). Besides plant developmental processes, SA can also be involved in the regulation of plant defence mechanism in responses to biotic and abiotic stresses. With these considerations, the present investigation was directed towards studying effect of SA on morphological and biochemical parameters in tomato (*Lycopersicon esculentum* Mill. var. Kashi Vishesh) under waterlogging condition, at vegetative and reproductive stages with a view to establish optimum doses of SA in terms of seed treatment and foliar spray and/or in the combination of these in pot culture.

Materials and Methods

The present experiment was carried out as pot culture in tomato plant variety H-86 (Kashi Vishesh) in poly house, Department of Plant physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi under waterlogged situation. Seeds of tomato cultivar H-86 (Kashi Vishesh), were procured from the Indian Institute of Vegetable Research (IIVR), Varanasi, Utter Pradesh. The experiment was laid out in complete randomized block design (CRD), which consisted of 9 treatments, 3 replications for each treatment, and tomato seeds were subjected to first seed treatment with SA (50 ppm and 100 ppm) for 4 h in petriplates then sowing was done in nursery bed. After 30 days, seedlings were transplanted in pots. The waterlogging stress was given at 15 (45 DAS) and 45 days (75 DAS) after transplanting, respectively for 12 h and after that, treatment with different concentrations of SA as foliar spray was applied. Morphological and biochemical parameters were recorded at different growth periods.

Chlorophyll Content (mg g^{-1} fresh weight): The Chlorophyll content in the leaf samples was estimated according to the method of Arnon (1949) using 80% acetone solution and the absorbance was recorded at 645nm and 663 nm using spectrophotometer.

Estimation of Total Sugar Content: Total sugar content in the plant samples was measured following method proposed by Morris (1948) with slight modification. In this method, one gm of fully expended tomato leaf was homogenised in 10 mL ethanol and centrifuged at 4000 rpm for 15 min. 2 mL of supernatant was added to 6 mL 0.2% anthrone which was prepared in 98% sulphuric acid. After that, mixed sample was placed for 3 min in

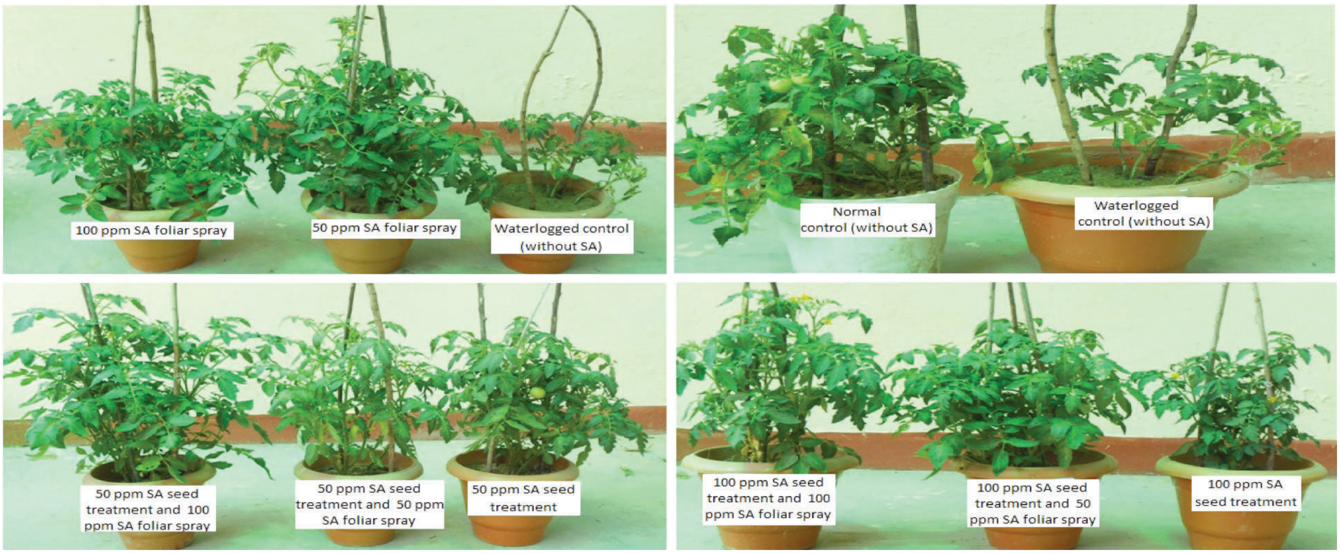

Fig. 1: Effect of different concentrations of salicylic acid (SA) on tomato plants under waterlogging stress

boiling water bath and absorbance was recorded at 620 nm.

Estimation of Protein content: Protein content present in the plant sample was determined according to the method of Bradford (1976). Protein binding dye Coomassie Brilliant Blue G-250 was used and developed blue colour was recorded at absorbance 660 nm using spectrophotometer.

Statistical analysis: The experiment was laid out in complete randomized block design which consisted of 9 treatments with 3 replications. The statistical significance was determined by Analysis of variance (ANOVA) and data expressed as Mean ± SEM (P<0.05).

Results and Discussion

World-wide waterlogging stress is one of the major abiotic stresses affecting plant growth and productivity. Keeping this in mind, the extent of waterlogging stress-induced morphological and biochemical responses in tomato (*Lycopersicon esculentum* Mill.) at different growth stages was assessed. In view of knowing the extent of its response, stress tolerance and adaptation by the plants, it was thought worthwhile to critically find out the most suitable concentrations of SA to induce certain morphological and biochemical activities influencing growth and/or sustaining plants to combat waterlogging stress conditions.

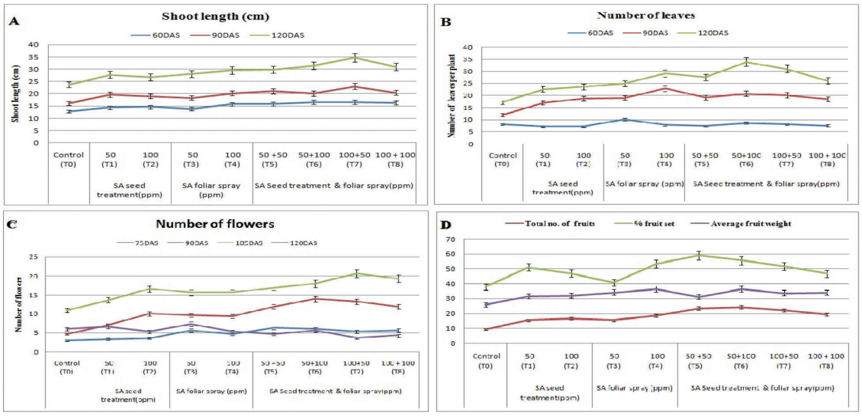

Fig. 2: Effect of Salicylic acid on length of shoot (A), number of leaves (B), number of flowers (C) and total number of fruits, percent fruit set, average fruit weight per plant (D) at different growth periods in tomato under waterlogged condition

Shoot length (cm): The data pertaining to shoot length at different treatments under different growth periods (60, 90 and 120 DAS) are presented in (Fig 2A). There was a reduction in shoot length with waterlogged condition, but significantly less reduction was observed with SA treatment. In control, without SA, maximum reduction in shoot length was observed in waterlogged control (T0). There was a significant increasing effect among treatments with SA (100 ppm seed treatment and 50 ppm foliar spray), which recorded maximum 46.83% highest shoot length (34.80 cm) at 120 DAS with treatment T7, when all 9 treatments were considered over the same growth periods in waterlogged stress condition. These findings were supported by Walter (2004) who reported that tomato plant height and number of leaves differed significantly in flooded plant as compared to control. In other crops, plant height was reduced in response to flooding in soybean (Githiri *et al.*, 2006). Similar reduction in plant height due to flooding was reported by Kuo *et al.* (1980). In tomato and other plants, flooding stress causes deleterious symptoms such as epinasty, leaf chlorosis and necrosis, senescence of leaves and retarded growth of shoot. Plant growth and number of leaves were negatively affected by flooding condition. Hayat (2012) reported that the treatment of stressed plant with SA increased shoot length per plant compared to those grown without salicylic acid. Foliar application at low concentration of SA also promoted and influenced growth, development, differentiation of cells and tissues of plants and enhanced the plant growth parameters (Helgi *et al.*, 2005).

Number of Leaves per plant: A significant decrease in number of leaves was observed under waterlogged condition but it increased significantly when treated with SA (Fig 2B). The waterlogged control plants recorded 8.14,12 and 17.22 number of leaves at 60, 90 and 120 DAS, respectively. Among different growth periods, maximum 49.17% increase in number of leaves was recorded in T6 (33.88) at 120 DAS followed by 42.55% in T6 (20.89) at 90 DAS and 19.56% in T3 (10.12) at 60 DAS as compared to respective waterlogging controls. These findings are supported by Kramer (1951) who reported that yellowing and death of the lower leaves of tomato plants may be due to desiccation, but most likely resulting from poisoning by toxic substances moving up from the affected roots that are associated with reduction of leaf number. In addition, the most common effect of flooding stress is a reduction in leaf transpiration i.e., an increase in stomatal closure (Blanke and Cooke, 2004; Yetisir *et al.*, 2006; Atkinson *et al.*, 2008). Foliar application of salicylaldehyde at 10^{-5}M stimulated different morphological and growth criteria of tomato plants, and which also increased cytokinin and auxin causing increased number of formed branches, leaves and improved photosynthetic pigment content in tomato plants (Kord and Hathout, 1992). SA showed synergetic effect with auxin and gibberellins (Datta and Nanda, 1985).

Number of flower per plant: Data showed reduction in number of flower per plant with waterlogged condition but significantly increased number of flower per plant was observed with SA treatment (Fig 2C). Number of flowers was recorded at 15 days intervals from 75 DAS to 120 DAS. In waterlogged control, plants recorded 3.0, 4.67, 11.0 and 6.0 number of flowers at 75, 90, 105 and 120 DAS, respectively. Among different growth periods, maximum 66.64% increase in number of flowers in T6 (14.0) at 90 DAS was recorded followed by 52.60% in T5 (6.33) at 75 DAS, 46.78% in T7 (20.67) at 105 DAS and 18.14% in T3 (7.33) at 120 DAS as compared to respective controls. These findings are supported by Rachie and Roberts (1974) who reported that growth of lateral branches and average leaf size was greatly reduced in waterlogged plants because of premature senescence, abscission of older leaves and also influenced by minerals and water uptake that cause reduction of flower in crop plants. The inhibition of yield and yield components in tomato genotype was pronounced and this is consistent with Kozlowski's work (1997) where flooding of soil often prevented flower bud initiation, anthesis, fruit set and fruit enlargement in flood intolerant species. Similarly, SA stimulated flowering in Lemna and Impatiens and inhibited biosynthesis of ethylene, stomatal closure and promoted root uptake (Raskin *et al.*, 1990; Raskin, 1992). Among the morphogenetic processes affected by SA are flowering and tuberization, for example, it hastens flower initiation in *Phaseolus* (Lagoa and Pereira, 1991) and induces tuberization in potato (Koda *et al.*, 1992).

Number of fruit, percent fruit set and average fruit weight per plant: There was a reduction in number of fruit, percent fruit set and average fruit weight per plant under waterlogged condition (Fig 2D). There was 61.65% increase in number of fruits in T6 (24.33) as compared to control (9.33). In case of percent fruit set, maximum 36.09% was recorded in T5 (59.18) as compared to waterlogged control (37.82). Maximum increase by 29.48% in average fruit weight was observed in case of T6 (36.60) as compared to control (25.81). These findings are supported by the work of Subbaiah and Sachs (2003) who found deleterious effects associated with hypoxia and anoxia including accumulation of toxic substances that decreased cellular energy, lowered cytoplasmic pH, which are responsible for the reduction of crop productivity. Similarly, flooding causes significant reduction of yield in crop plants e.g. 24 h of flooding can reduce the yield of flood sensitive garden pea (*Pisum sativum*) by 50% (Taiz and Zeiger, 2010). Tomato plants sprayed with SA at 100 ppm increased vegetative growth, yield and its components (Ali *et al.*, 2009). Similar results were obtained with SA in broad bean (Sanaa *et al.*, 2001). Using 150 ppm of SA as a foliar application produced the highest increment in number of pods and green pod's yield of snap bean (Kmal *et al.*, 2006).

Chlorophyll a content (mg g^{-1} fresh weight): A significant reduction in Chlorophyll a content was observed under waterlogged condition, however, SA treatment led to an increment in chlorophyll a content (Fig 3A). Among the treatments, maximum 45.76% increase in chlorophyll a content was observed in treatment T3 (0.59 mg g^{-1} fresh weight) as compared to waterlogged control (0.32 mg g^{-1} fresh weight) at 50 DAS (Fig 3A). In this connection, reduction in chlorophyll content in leaf tissues has been observed under waterlogged condition in tomato (Else *et al.*, 2009). Similarly, after imposing waterlogging stress, reduction in the chlorophyll content was evident in maize and SA increased its content (Singh *et al.*, 1995; Yan *et al.*, 1996).

Chlorophyll b content (mg g^{-1} fresh weight): There was a significant reduction in chlorophyll b content under waterlogged condition, however, loss of chlorophyll due to waterlogging was significantly minimized by SA treatment (Fig 3B). Among different growth periods, maximum 57.57%

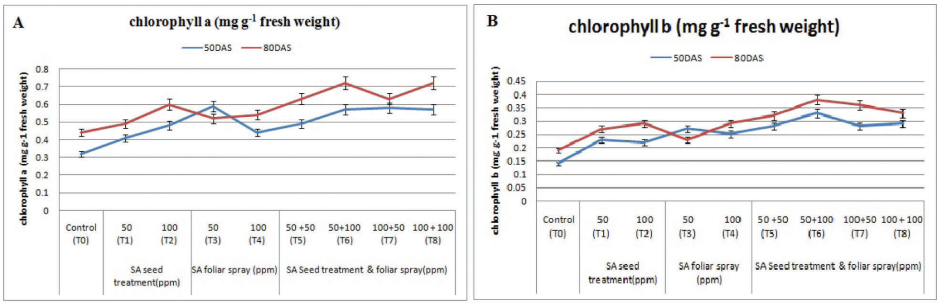

Fig. 3: Effect of Salicylic acid on chlorophyll a (A) and chlorophyll b (B) content in tomato plants at different growth periods under waterlogged condition

increment in chlorophyll b content was found in the treatment T6 (0.33 mg g^{-1} fresh weight) at 50 DAS as compared to waterlogged control T0 (0.14 mg g^{-1} fresh weight), and same trend followed at 80 DAS in T6 (0.38 mg g^{-1} fresh weight) as compared to waterlogging control T0 (0.19 mg g^{-1} fresh weight). These findings are supported by Percival *et al.* (2005) who reported reduction in chlorophyll content in leaf tissues under waterlogged condition in tomato. Stimulative effect of SA might be due to its antioxidative nature protecting chloroplasts and preventing chlorophyll degradation from the toxic reactive oxygen radicals (Bowler *et al.*, 1992; Aono *et al.*, 1993).

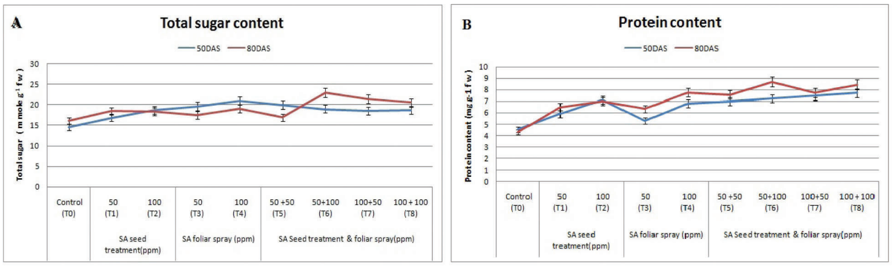

Fig. 4: Effect of Salicylic acid on sugar (A) and protein (B) content in tomato plants at different growth periods under waterlogged condition

Total Sugar content (mg g^{-1} fresh weight): Data showed that total sugar content slightly decreased in waterlogging condition, but significantly increased when SA was supplied (Fig 4A). Among both the growth periods (50 DAS and 80 DAS), in 50 DAS, maximum 30.62% sugar content was observed in T4 (20.97 mmol g^{-1} fresh weight) as compared to waterlogged control (14.55 mmol g^{-1} fresh weight), while 80 DAS, maximum sugar content was found in T6 (22.96 mmol g^{-1} fresh weight) as compared to control (16.04 mmol g^{-1} fresh weight). Total sugar content decreased in waterlogged stress because it causes disturbance to photosynthetic machinery which decreases rate of photosynthesis and its partitioning, proper utilization of stem reserves for cellular functioning under stress. Khodary (2004) pointed out that SA application increased total sugar content in maize. Similarly, Amin *et al.* (2007) reported that SA regulates sugar contents (translocation from source to sink) and causes significant increase of total soluble sugar content as SA alleviates the oxidative stress by increasing the activities of antioxidant enzymes under waterlogging situation.

Protein content (mg g^{-1} fresh weight): The data revealed that there was a reduction in protein content under waterlogged condition which was found to significantly increase with SA treatment under waterlogged stress (Fig 4B). Among treatments, maximum 50.46% increment of protein content was observed in treatment T6 (8.70 mg g^{-1} fresh weight) as compared to waterlogged control (4.31 mg g^{-1} fresh weight) at 80 DAS followed by 41.32% in treatment T8 at 50 DAS as compared to respective controls. These findings showed that there was a decrease in total protein content under stress but higher protein content observed with SA treatment could be attributed to synthesis of anaerobic stress protein (ASPs) induced by root hypoxia (Blom and Voesenek, 1996; Subbaiah and Sachs, 2003). Similarly, El-Tayeb (2005) observed that increased protein content induced by SA might be helping in maintaining osmolarity in the cells during abiotic stress.

Conclusion

Significant morphological and biochemical differences were observed in tomato in relation to its performance under waterlogging stress at vegetative and reproductive growth stages; there was a significant effect of these traits on almost all the parameters evaluated under waterlogged condition, and plants treated with SA as a seed treatment or foliar spray or both together ameliorated the effects of waterlogged stress induced responses. Data showed that plants exposed to waterlogging stress were highly affected in terms of morphological and biochemical attributes such as shoot length, number of leaf, chlorophyll content, sugar and protein content, number of flower per plant and number of fruits per plant. Treatment, T6 (50 ppm seed treatment and 100 ppm foliar spray) with SA performed best significantly enhancing plant growth and development and leading to a pronounced increase of biomass production and overall positive impact on its productivity.

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