Abstract

Chilli is a spice crop with great medicinal value of its biochemical ingredients. Its high antioxidant value along with good nutritional properties make its extensive use in pharmaceutical industries. Foliar application of Salicylic Acid may stimulate various plant physiological parameter i.e., stomatal activity, ions uptake, seed germination, leave membrane response to electrolytes and growth rate. Salicylic Acid (SA) is known as molecule that is participated in physiological processes in chilies, plant tolerance and resistance to abiotic and biotic stresses. Ascorbic acid possesses antioxidant properties, has positive impact on flowering and expounds effective plant defense system against pesticide toxicity. Moreover, ascorbic acid is effective chemical to mitigate the destructive impacts of salt stress. The responses of different chilli varieties to ascorbic acid, salicylic acid and its combination were recorded in various traits i.e Cat, Sod, Pod, protein, carbohydrates, fiber, proline, height of plant, weight of fruit, length of fruit and thickness of pericarp. The aim of this research to demonstrate the role of ascorbic acid and salicylic acid improved the morphological and biochemical traits of hybrid chilies. The utilization of both Salicylic Acid and Ascorbic acid exhibited improved results in all plant attributes studied.

Key words: Cat, Sod, Pod, Ascorbic Acid, Salicylic Acid.

Introduction

Chili (Capsicum annuum L.) is a member of Salicaceae family and cultivated all over the globe due to its nutraceutical and economic value (Rahman et al., 2013). It is enriched in vitamins (A, D3, E, C, K, B2 and B12), lipids, minerals (Ca, P, Fe, K), proteins, and volatile compounds i.e., capscinoids (Chigoziri and Ekefan, 2013). The amount of vitamin C is found in higher magnitude in fresh green chili as compare to citrus fruits and more vitamin A than in comparison with carrots (Chigoziri and Ekefan, 2013). There is multiple consumption of Chili pepper as salad in green fgresh form, in various culinary recipes and value adding products like sauces, stews, soups and usually and a flavoring agent (Reyes-Escogido et al., 2011; Amruthraj et al., 2014).

Capsaicinoids are non-volatile compounds like capsaicin, nor capsaicin dihydrocapsaicin, homodihydrocapsaicin and nordihydrocapsaicin, (Nwokem et al., 2010; Al Othman et al., 2011). which give chilies its hot taste, (Orobiyi et al., 2015) and are international sought by pharmaceutical industries for their therapeutic charateristics (Liljana et al., 2013; Reddy and Sasikala, 2013).

Ascorbic acid (As A) can play important role to stress resistance in plants and enhance plant growth which help into increase yield. (Zhou et al. 2016). It increases water retention ability of plants during drought stress. (Noman et al. 2015). Another investigation revealed that As A is important tool to control various steps involved from germination of seeds like plant growth and development, response to biotic and abiotic stress stimuli till the plant maturity accompanied by yield enhancement (Latif et al.2016).

Salicylic acid is considered a signaling molecule for production of phenolic compounds and natural plant growth regulator or phytohormone. The physiochemical and metabolic mechanisms in plants are regulated by synthesis of Salicylic Acid by independent and dependent pathways in plants (Dempsey and Klessig, 2017) and (Jayakannan et al., 2015; Vicente and Plasencia, 2011). Salicylic acid has positive influence on flower production, electrolyte movements in plants, chlorophy enhancement and rate of photosynthesis. (Amanullah et al., 2010; Sahu, 2013; Souri and Tohidloo, 2019).

Material and Method

Plant material

The study was conducted at Vegetable Research Institute (VRI), Ayub Agricultural Research Institute Faisalabad under field conditions in Pakistan in 2020-2021. Vigorous and uniform seedlings were transplanted into the open field under tunnel with plastic sheet. We maintained the distance between one row to other 75 cm and plant to plant distance is 45 cm, 40 days after sowing (DAS). Experiment was carried out without the application of insecticides and fungicides. Weeds were kept under control by hand removing. All the plots received regular and even cultural practices. There were 3 replicates per treatment and 10 plants per replicates. Foliar application of salicylic acid, ascorbic acid and their combination were applied 25 days after transplanting with 10 days' interval at the stages of planting, initiation of flowering and fruiting. The different levels of salicylic acid and ascorbic acid such as T1 (Control) T2 (SA 1mM), T3 (SA 2mM), T4 (AA 1mM), T5 (AA 2mM), T6 (SA 1mM + AA 1mM), T7 (SA 1mM + AA 2mM), T8 (SA 2mM + AA 1mM) and T9 (SA 2mM + AA 2Mm) used in four hybrid varieties V1=SV8233-HD (G1), SV5232-HY (G2), SV8883-HA (G3) and golden heart.

Vegetative growth parameter

Vegetative growth parameters i.e., Plant height (cm), Fruit weight (g), yield (g) root weight (g) shoot weight (g), Fruit width (mm) fruit length (mm), and pericarp thickness (mm) were estimated from five guarded plants in every treatment and their means was calculated.

Estimation of CAT activity

A reaction mixture containing 15 mM phosphate buffer (pH 7.0) and 15 mM H_2O_2 was used to estimate Catalase Enzyme Activity (CAT) using procedure described by Dhindsa et al. (1981). The absorbance fluctuations were taken at 240 nm after 40 second. The amount of enzyme activity equivalent to amount of enzyme required to reduce absorption from reaction mixture at 240 nm within one minute was equivalent to one unit of CAT.

Estimation of SOD activity

1000 ll of enzymatic extracts with 2.50 ml of 55.0 M methionine, 100 m M of phosphate buffer (pH 7.8), 300 ml nitro blue tetrasolium 0.75 mM and 60 ll of 0.1 mM riboflavin were kept in a test tube and incubated for 10 min in fluorescence light (40 lmol m-2 s-1) using method performed by

the Xu et al. (2008. The absorbing solution read to 560 nm by a UV/visible spectrometer. One unit of SOD enzyme was determined as the amount of enzyme that inhibits 50% of photo-reduction NBT.

Estimation of POD activity

POD activity was calculated from 50 ml of enzyme extract placed into reaction mixture, 0.4% H2O2, potassium phosphate buffer to pH 6.1 and 1% guaiacol using by the Zhou and Leul (1998) method with absorbance at 470 nm.

Estimation of proline amount

A step wise procedure developed by Bates et al. (1973) was adopted to find out the amount of proline. Firstly, a homogenized leave sample of 0.2 grams obtained through centrifugation at 18,000 g for 15 minutes was obtained. 2 ml of leave sample taken in a test tube was mixed with 2 ml glacial acetic acid and freshly prepared acid nin-hydrin solution (1.25 g nin-hydrin dissolved in 20 ml 6 m orthophosphoric acid and 30 ml glacial acetic acid). Heating of test tube solution at 100°C for 1 hour and cooled at 25°C. Then, 4 ml of toluene were poured into the contents of the test tube in phase separation stage placing test tubes at vertical position for 10 min. The absorbance reading was observed at 520 nm and content of proline was amounted in lg g^{-1} on weight basis.

Estimation of proximate

Protein, carbohydrates, fat, crude fiber, ash content were measured by AOAC (1990).

Result and Discussion

Results

Plant height (cm)

Analysis of variance showed significant differences between four varieties of chilies subjected to various levels of salicylic acid and ascorbic acid and their combination. However significant differences were observed in V4 plant height growth in response to different applications of salicylic acid and ascorbic acid. Out of nine salicylic acids, ascorbic acid and their combination, maximum plant growth was given by T9 (80.3cm) whereas minimum plant growth in 33cm were observed in T9 and T1 in chilies varieties. In variety V3 at the treatment T9 (58 cm) was observed in chilies i.e minimum plant height growth was observed in T1 (34cm). Moreover, maximum plant height at T9 (69cm) less growth of plant height was observed (45cm) in variety V2. Whereas the highest plant height in T9 (51.6cm) and lowest

plant height (33cm) in variety of V1. It is depicted that different levels of salicylic acid, ascorbic acid and their combination strains significantly affected the
plant height in chilies (Table 1).

Root weight (g) and Shoot weight (g)

The data regarding chilies plant growth responses to ascorbic and salicylic acid presented in Table 1 revealed that foliar of AA and SA applications significantly improved plant dry weight (g) and shoot weight (g) compared- to control plants. The maximum value of shoot weight observed in T9 (161g) and the minimum value of (71g) in variety V4. The highest shoot weight of T9 (123.3g) and the lowest weight in T1 (60.33g) in variety V3. The shoot weight greater in T9 (141.0g) and lower in control treatment of V2 as followed by V1 variety of chilies. Whereas the highest value of root weight in treatment of T9 (47.16 g) as compared to T1 (20.10g) variety of V4. In variety V3 treatment T9 showed root weight (32.43g) and minimum value in T1 (18.03g). The values of root weight T9 (39.10g) and T1 (16.10g) in V2 as the same the highest value T9 (36.33g) and lowest value T1 (13.46). The highest values of growth parameters were recorded, ascorbic and salicylic acid applied together with all concentrations as foliar with respect either individual or control plants.

Fruit width and Fruit length (mm)

Different concentrations of SA and Ascorbic acid on different chilies varieties resulted significant differences on fruit length constituting range of fruit length from 96.6 mm to 81. 0mm.The maximum fruit length (96.6mm) was measured in T9 treatment for variety V3 followed by, 99.0mm for V2 and (95.0mm) for V1 respectively as compared to control. Similarly, greater fruit width(30.13mm) was observed in treatment T9 and lowest (17.10mm) in T1 for variety V4 which was statistically similar with variety V3, V2 and V1 of chilies.

Peri-carp thickness (mm)

The maximum value of peri-carp thickness in T9 (1.99mm) and minimum (1.62mm) for variety V4 was calculated. Moreover, in variety V3 the value was measured in T9 (1.89mm) and T1 (1.48mm). The greater value of peri-carp thickness of V2, V1 was showed (1.85mm) and (2.10mm) respectively as compared to control.

Fruit weight (g)

Significant impacts of SA and AA was estimated for fruit weight and maximum magnitude of the trait under study was observed in T9 (10.2 g) for V4 followed by (8.4 g) in V3. However, the highest fruit weight (11.6 g), (16.6 g) was recorded in treatment T9 in V2 and V1 plants as compared to T1 treatment.

Fat (%)

Fat percentage was also influenced significantly through foliar spray of SA and AA with the greatest value of T1 (2.7%) in variety of V4. The fat (2.5%) of T1 variety V3 was recorded in plants. However, the lowest fat concentration (1.3%) at T9 in V3 and V1 was recorded. The amount of fat % present in treatment T9 (1.6%) in V2 as compared to T1 treatment.

Ash (%)

A significant mean differences for ash percentage was estimated for different concentrations of foliar spray of SA and AA. The greatest value of T9 (9.2%), (8.2%) in varieties of V4 and V3 was recorded in plants. However, the greatest value of ash content (7.8%), (7.6%) was recorded in treatment T9 in V2 and V1 plants as compared to T1 treatment.

Protein (%) Highest crude protein was found in T9 (23.48%) of V1 followed by (8.8 %) in V4. Lowest protein was recorded in V3, V2 in treatments in T9 (8.4 %) and (7.9%).

Carbohydrate (%) The present results revealed high carbohydrate levels. Highest carbohydrates were found in T9 (57.27 %), followed by T9 (52.49%) in varieties of V4 and V3 as compared to control. In varieties V2 and V1 (51.26 %), (48.87%) was measured in treatment T9 respectively.

Fiber (%) The investigation showed that highest value (34.12%) fiber was depicted in treatment T9 in both varieties of V3, V1. The fiber content of treatment T9 (32.78%) in V2, while the T9 (31.78%) was found in variety of V4 as compared to control.

Proline (%)

The data regarding proline content at various SA and AA foliar application revealed the mean differences for treatments and chilies varieties. Maximum proline contents of 19.1% was found in

V2 followed by V1 having value of 18.5%. Furthermore, in treatment T9 maximum value of proline (%) was observed in V3 (17.65%) and V4 (17.85%)respectively.

Antioxidant enzymatic activity. SOD (ug-1Fw), POD (ug-1Fw), CAT (ug-1Fw).

The maximum value of sod influenced by the SA and AA application. The results showed that maximum sod content present in treatment (T9) (1.40 ug-1Fw) V4 (1.39 ug-1Fw) V3. The highest value of sod in T9 (1.28 ug-1Fw), (1.12 ug-1Fw) V2 and V1 respectively. The maximum pod contents were recorded in treatments T9 (17.9 ug-1Fw), (17.75 ug-1Fw), (17.65 ug-1Fw) and (17.30 ug-1Fw) of varieties V4, V2, V3 and V1 respectively. The highest value of Cat contents in the treatment of T9 (6.15 ug-1Fw) variety of V4. Least value of cat contents in pepper plant was seen in (5.70 ug-1Fw), (5.53 ug-1Fw) and (5.19 ug-1Fw) varieties of V1, V3, V4 respectively.

Yield (g) The yield of pepper varieties were estimated by the foliar application of SA, AA and their combination. The largest yield of T9 (217.66 g) in variety V3 as compared to T1 treatment. The yield was measured (215.33g) in V4 of T9. The highest value of yield estimated in (208.6g) and (205.3g) V1 and V2 as compared to control. It cleared that ascorbic acid and salicylic acid at their different levels of applications significantly increased all varieties yield compared to control.

Discussion

Plant height (cm)

The foliar application of SA mitigated the growth reducing effects constituted due to various abiotic stresses thus, improved plant height, number of leaves plant per, chlorophyll content, fruit quality and yield as compared to control (Table 1). Such inferences were in accordance with the result findings of Zaghlool et al. (2006) who concluded additive association with plant growth and fruit yield parameters with the application of SA. Moreover, in addition to increased plant growth parameters like plant height, stem diameter, number of branches, number of leaves, root length as well fresh and dry weight the nitrogen, phosphorus, potassium uptake percentages was also increased with increasing concentrations of ascorbic acid as foliar application (Mazhar et al., 2011).

Carbohydrate:

Carbohydrates contents have positive relation with the foliar application of ascorbic acid due to reducing effects of plant stress on plant growth. The massive increase in total contents of

carbohydrates due to foliar application of ascorbic acid in our case was resembled with Mazhar et al. (2011).

Proline

The increase in proline content to combat the reducing effects of abiotic plant stresses matched the results with Nazi et al., 2016 who conducted experiment on cucumber in arid conditions and found As A-induced stimulation in the chlorophyll a, proline, relative water content and Ci concentration.

Yield

The impact of different formulation of SA significantly improved plant growth and becomes cause of increase in yield of the plant. Results showed that foliar spraying of ascorbic acid at 100 mg/l increased yield and improved plant characteristics such as, number of total and folded leaves, fresh weight of total and folded leaves, leaf area per plant, plant fresh and dry weight and leaves dry matter and total soluble solid (Jerry et al., 2011; Nafeesa et al., 2019).

Protein

Application of Ascorbic acid at concentration of 200 ppm (T5) provided maximum yield per plant and remained superior over Acetyl Salicylic acid concentrations. Highest values of protein content were noted with the treatment application of Acetyl Salicylic Acid at concentrations 200 ppm (T3) and was observed to be superior to Ascorbic Acid concentrations (Saha et al., 2020).

Fruit width (mm) and Fruit length (mm)

The foliar applications of SA indicated positive influence on some fruit characteristics, earliness, yield, chlorophyll contents in leaves and plant growth. However, SA treatments had no effect on pH, AA and TA of tomato (Yildrim and Dursun, 2009).

Enzymatic Activities:

The use of Ascorbic Acid along with 0.03% Zn combination enhanced photosynthetic pigmentation, vegetative growth, ion uptake, antioxidant activity in lettuce (Noreen et al., 2021). In broad beans, foliar application of SA enhanced the activities of antioxidants enzymes and all growth parameters. (Azooz et al., 2011).

CHILLI

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