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EFFECT OF NUTRIENT SPRAY ON GROWTH AND ANTIOXIDANT STATUS OF *BRASSICA JUNCEA*

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ABSTRACT

Experiments were carried out in order to study the effect of Hi-foliar nutrient foliar spray on the growth profile and redox status of *Brassica junce*a, under field conditions. 0.3% of Hi-Foliar nutrient spray was applied in *Brassica juncea* plants at different stages of growth cycle i.e. 30 days after sowing (DAS), 45 DAS, 60 DAS and 30+45 DAS, 30+60 DAS,45+60 DAS and 30+45+60 DAS to assess the changes in growth, photosynthesis and antioxidant status. Various growth and yield related parameters such as plant height stem diameter, pod number, number of leaves per plant and grain weight per plant and activities of nitrate reductase, photosynthesis were positively affected by nutrient spray. Application of nutrient spray also improved the redox status of the treated plants by increasing activities of antioxidant enzymes such as ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, glutathione reductase and decreasing content of hydrogen peroxide and malondialdehyde. In majority of cases, a remarkable change occurred during 45 DAS (days after sowing) treatment. The changes in growth and antioxidant status were found to be closely related to the time of spray i.e. at proper growth stage.

Keywords: photosynthesis, nutrient spray, *Brassica juncea*, ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, glutathione reductase, malondialdehyde.

INTRODUCTION

Nutrients are important and crucial elements, which are required for the growth and development of plants. Nutrients can be applied to plants either through soil route or by foliar spray. Soil mode of application of nutrients or fertilizers has several limitations like leaching, antagonism between certain nutrients, heterogenic soils conditions, comparatively higher dosages etc [22]. Therefore to overcome these problems, effective foliar feeding is mostly preferred. Traditionally, majority of the foliar spray studies were conducted on nutrient deficient plants and foliar fertilizers were used to overcome

punctual nutrient deficiencies. However, there is an increasing trend to apply foliar sprays in the absence of deficiency symptoms, at least as it refers to elements with little phloem mobility such as Calcium (Ca), Boron(B),Iron(Fe),Manganese (Mn) or Zinc(Zn) [8] . Several synthetic macro and micro nutrients have been reportedly used as nutrient solution(s) for improving crop productivity. Studies have provided evidence that application of amino acids before as well as during the imposed stress, supply the plants with carbon skeleton and energy which is directly related to stress alleviation physiology and thus have a preventing and recovering effect [20]. Foliar applications of urea in root of N-deficient plants increased the total nitrogen and anthocyanin concentration over control. Nitrogen deficiency induced oxidative stress but foliar application of urea altered this response, significantly diminishing catalase and ascorbate peroxidase activity [5]**.** The foliar application of potassium silicate stimulated antioxidant superoxide dismutase activity and increased photosynthetic capacity and chlorophyll content in bentgrass, especially under a high fertilizer regime [19]**.** In view of the above, present experiments were designed to explore and elucidate the efficacy of nutrient foliar spray, a mixture of amino acids and other cell lysates on growth and antioxidant potential of *Brassica juncea.*

MATERIALS AND METHODS

Preparation and spraying of foliar applicant:

Seeds of *Brassica juncea* (cv. pusa jaikisan) were obtained from Indian Agricultural Research Institute, New Delhi. The seeds were washed with a mild detergent and sown in field, as per the recommended agronomic practices. Nutrient solutions were prepared in distilled water @ 0.3%w/v. Along with the nutrient mixture; an adjuvant @ .025% is also added. To determine the effect of nutrient spray on the growth and yield of *B. juncea*, under actual field conditions, *B. juncea* plants were sprayed with nutrient spray at different combinations of 30 DAS, 45DAS, 60DAS and 30+45 DAS, 30+60 DAS,45+60 DAS and 30+45+60 DAS respectively.

Growth profile measurement:

Different growth and yield related parameters like leaf number, plant height, stem diameter, pod number and total grain yield were recorded at maturity.

Chlorophyll content:

Chlorophyll contents in the leaves of treated plants was measured by the method of Hiscox and Israelstam; [13].The total chlorophyll (mg g^{-1} FW) concentrations in the leaf tissues were calculated according to the following equations: Total Chlorophyll = $[(20.2 \times A_{645}) + (8.02 \times A_{663})]$/ (wt. in g x 1000)

Nitrate reductase activity:

The nitrate reductase (NR) activity was estimated *in-vivo* in freshly harvested leaves by using the method described by Hageman and Huckelsby; [11]. The absorbance was measured in 540nm.

Hydrogen peroxide content:

Hydrogen peroxide was measured by the method of Alexieva *et al*; [1]. Leaf tissue was homogenized in10 ml of 0.1 % (w/v) aqueous tri-chloro-acetic acid (TCA) and centrifuged at 10,000xg for 30 min at 4 °C. The reaction mixture containing the supernatant, potassium phosphate buffer and KI reagent was incubated for 1 h in dark and subsequently the absorbance was measured at 390 nm. The concentration of H_2O_2 was calculated using a standard curve of H_2O_2.

Malondialdehyde content:

Procedure of Heath and Packer; [12] was followed for measuring the malondialdehyde (MDA) content. Leaf tissue was homogenized in 10 ml of 0.25 % TBA (w/v) prepared in 10 % TCA. The homogenate was heated at 95 °C for 30 min and centrifuged at 10,000xg for 30 min. Absorbance of the supernatant was measured at 532 nm and 600 nm. Absorbance at 600 nm was subtracted from the absorbance at 532 nm for non-specific absorbance. The concentration of MDA was calculated by using an extinction coefficient of 155 mM^{-1} $cm^{-1.}$

Antioxidant enzyme activity:

All antioxidant enzymes were extracted according to Grace and Logan; [10] with slight modification. All Enzyme activities were expressed as enzyme units per milligram of protein. Ascorbate peroxidase activity was determined as described by Nakano and Asada; [16]. The decrease in absorbance was measured at 290 nm. The enzyme activity of ascorbate peroxidase was quantified using an extinction coefficient of 2.8 mM^{-1} cm^{-1}.Monodehydroascorbate reductase activity measurement given by Miyake and Asada [15]. The decrease in absorbance was measured at 340 nm. The enzyme activity of monodehydroascorbate reductase was quantified using an extinction coefficient of 6.2 mM^{-1} cm^{1}. Dehydroascorbate reductase activity was determined as described by Dalton *et al;* [4]. The increase in absorbance was measured at 265 nm. The enzyme activity was calculated using an extinction coefficient of 14 $mM^{-1}cm^{-1}$.Glutathione reductase activity was determined by Grace and Logan [10]. The decrease in absorbance was measured at 340nm.The enzyme activity was calculated using an extinction coefficient of 6.2mMcm.

Statistical Analysis:

The data obtained from field trials during the course of present investigation were analyzed statistically by using the random block design (RBD) and the laboratory experiments were analyzed by completely randomized designs (CRD). All experiments were carried out three times, with three replicates each. One way ANOVA was carried out to determine significant differences ($P \leq 0.05$) between the means. The experimental data are expressed as mean ± SE.

RESULTS

The growth profile of *Brassica juncea* (plant height, stem diameter, pod number, number of leaves per plant and grain weight per plant) was significantly affected by nutrient spray treatment [Table 1]. The treatment of plants with nutrient spray at 45 DAS increased the plant height and stem diameter by 20% compared to control. The increase in leaf number per plant varied from 21% to 74% and the maximum increase of 74% was recorded at 30+45 DAS. Treatment of Brassica seedlings with nutritional spray treatment showed a significant increase ($P \leq 0.05$) in pod number as compared to the untreated (control) plants. A 62% increase in grain weight per plant was also recorded with 45DAS treatment. Other treatments also showed a significant increase in grain weight varying from 31 to 50%.Total chlorophyll contents was increased by 28% at 45 days after sowing treatment .However, no statistically significant ($P \leq 0.05$) difference in total chlorophyll contents was recorded among various treatments. The treatment of nutrient spray at different growth stages induced a significant decrease in nitrate reductase activity, over that of control plants. Maximum decrease of 58% in nitrate reductase activity was recorded at 45 DAS treatment [Table 2]. The level of hydrogen peroxide and malondialdehyde was found to decrease in the treated plants as compared to control. Maximum decline of 44% in malondialdehyde (MDA) and 28% in Hydrogen peroxide (H_2O_2) levels was recorded at 45DAS treatment [Table 3].The specific activities of antioxidant enzymes (i.e. ascorbate peroxidase, monodehydroascorbate reductase and dehydro ascorbate reductase and glutathione reductase) were significantly enhanced by nutrient spray treatments [Table 4]. Control plants possess the minimum values. At 45 DAS treatment maximum increase in the activity of enzymes was recorded.

CONCLUSION

Nutrient spray treatment increased growth parameters (plant height, stem diameter, pod number and number of leaves per plant and grain weight per plant progressively in a time dependent manner, compared with the control [Table 1]. Application of nutritional spray increased the average plant height as compared to the untreated plants. Since plant height is dependent on internodal elongation, the

observed phenomenon might be attributed to an increased level of gibberellic acid (GAs), as GA is mainly responsible for shoot elongation [21]. It has been reported Nagasubramaniam *et al;* [17] that foliar application of salicylic acid (100ppm) increased the plant height of baby corn. Nutritional spray treatment also caused increase in leaf number compared to control. Leaf number as well as leaf size is regulated by a complex interaction of various genes whose expression is also modulated by growth hormones Gonzalez *et al;* [9]. Therefore, it can be safely concluded that nutrient foliar spray treatment potentially alters the activity of specific genes that are involved in growth regulation.The stem diameter of treated plants did not show much variation among various treatments. In a study done by El-Nemr *et al*; [7] the effect of application of 'Hormovill', a foliar compound containing extracts of yeast, actinomyces, *Bacillus subtilius* and organic matter on broccoli (*Brassica oleracea var*. *Italica*) was studied, and no significant change in the diameter of treated plants was recorded. Treatment of *Brassica juncea* with *foliar* nutritional spray treatment also showed a significant increase in pod number and grain weight. These results are in agreement with those of El-Ghamry *et al;* [6] who reported that humic acid significantly increases number of pods in Faba bean plants. The possible reason put forth by the authors that humic acid constitute a stable fraction of carbon, thus regulating the carbon cycle and release of nutrients including nitrogen, phosphorus, and sulphur which are needed for plant growth. In the present experiments, nutrient foliar spray which is a mixture of various molecules originating from plant hydrolysate, containing carbon skeleton (sugars) molecular along with other growth promoting essential elements may help in enhancing the productivity of *Brassica* plants. Nitrate reductase (NR) is the first enzyme involved in the metabolic route of NO_3 assimilation in higher plants. The different combination of foliar nutrient spray treatments induced a significantly decrease in nitrate reductase activity, over that of control plants [Table2]. Maximum decrease in nitrate reductase activity was recorded for 45 DAS treatment. As the nutrient foliar spray contains appreciable amounts of available nitrogen, it might have caused the suppression of nitrate reductase activity. Ruiz *et al;* [18] have studied the decrease of the NR activity to the foliar application of amino acids and micronutrients in Pepper plants. Total chlorophyll contents were increased in 45 days after sowing treatment [Table2]. A previous study by Blanke *et al*; [3] recorded a 21% increase in chlorophyll content of ammonium fertilizer treated kohlrabi plants. Higher chlorophyll contents in the treated plants might have caused an enhancement in the photosynthetic efficiency in the foliar nutrient spray treated plants, resulting in higher photosynthate production and better growth and productivity. Reactive oxygen species (ROS) accumulation is determined by balance between production and scavenging. ROS scavenging is due to synergistic action of antioxidant system [23]. In our experiment, levels of malondialdehyde and hydrogen peroxide declined significantly in nutrient foliar spray treated plants as compared to the controls. Maximum decline of MDA and H_2O_2 levels was recorded at 45 days after sowing treatment [Table3]. Malondialdehyde (MDA) is a product of lipid peroxidation and has been extensively used as an index to measure the extent of membrane damage caused by reactive oxygen species [14]. Low level of MDA indicates reduced oxidative damage in the cell. So it can be inferred that nutrient foliar spray treatment is inducing specific alterations/optimization in the cellular metabolism leading to a decrease oxidative damage to cell. Further, the level of H_2O_2, one of the most damaging forms of reactive oxygen species, also decreased in the treated seedlings. This decrease in H_2O_2 is due to the induction of H_2O_2 metabolizing enzyme like ascorbate peroxidase,monodehydroascorbate reductase, dehydroascorbate reductase, glutathione reductase as noted in the experiments [Table 4]. Specific activity

of all these antioxidant enzymes increased continuously with nutrient spray treatment. A previous study was also reported by Xu *et al*; [23] where callus protoplast exhibited a higher antioxidant activity as compared with the mesophyll protoplast during *in vitro* culture. This is consistent with the lower levels of ROS accumulation, higher antioxidant enzyme activity and lower lipid peroxidation in callus protoplast. In conclusion, it can be said that nutrient foliar spray treatment has an ability to induce/improve the photosynthetic capacity, metabolism and plant growth in mustard plants. Nutrient spray treatment elevated the anti-oxidative status of the treated plants, thereby decreasing the damage done by reactive oxygen species produced as a result of various metabolic reactions involving energy transduction, in different forms.

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Tables:

Treatment (DAS)	Plant height	Stem diameter	Number of leaves/plant	Pod number	Grain weight
Control	129.1±3.89	10.25±0.73	21.4±1.08	82.14±3.09	6.67±0.36
30	148.51±4.45	10.52±0.65	26±1.56	130.14±4.55	8.77±0
45	155.9±5.68	12.34±1.03	29±2.01	169.71±6.64	10.82±0.53
60	141.7±4.55	10.74±0.82	31.1±2.73	174.57±7.57	9.95±0.51
30+45	135.1±3.89	10.74±0.82	37.4±3.09	157.00±5.52	9.70±0.41
30+60	126±4.43	9.95±0.53	33.9±2.99	154.14±6.91	9.20±0.21
45+60	138.8±4.92	9.70±1.01	29.2±3.11	155.71±5.28	9.98±0.56
30+45+60	136±4.74	10.53±1.29	32.4±2.84	120.86±4.54	9.96±0.41
CD at 5%	5.26	1.83	2.13	8.56	1.23

Table 1. Effect of nutrient spray on plant height (cm),stem diameter(mm), pod number, number of leaves per plant and grain weight(g) per plant of *Brassica juncea* at different days after sowing (± standard error)

Treatment (Days after sowing)	Nitrate reductase	Total chlorophyll
Control	4.62±.0.12	4.17±0.057
30	5.48±0.21	2.87±0.051
45	5.92±0.18	1.74±0.042
60	5.72±0.16	2.99±0.035
30+45	5.88±0.20	2.87±0.027
30+60	5.85±0.11	2.99±0.036
45+60	5.40±0.15	3.23±0.025
30+45+60	5.45±0.14	2.98±0.041
CD at 5%	0.576	0.62

Table 2. Effect of nutrient spray in leaf nitrate reductase activity (nmol $NO_2 \cdot kg^{-1}$(leaf fresh mass)$\cdot s^{-1}$) and chlorophyll (µg/g of fresh weight) of *Brassica juncea* at different days after sowing (± standard error)

Treatment (Days after sowing)	Malondialdehyde	Hydrogen peroxide
Control	2.99±0.19	2270.34±33.04
30	2.77±0.16	1990.50±22.26
45	2.07±0.24	1625.38±18.89
60	2.79±0.23	1843.29±19.52
30+45	2.74±0.29	2011.81±21.69
30+60	2.63±0.16	2030.53±12.85
45+60	2.56±0.19	2015.41±17.48
30+45+60	2.84±0.17	2018.83±15.97
CD at 5%	0.553	28.25

Table 3. Effect of nutrient spray in malondialdehyde (µM/g fresh mass), hydrogen peroxide (µM/g fresh mass) of *Brassica juncea* at different days after sowing (± standard error)

Treatment(DAS)	Ascorbate peroxidase	Monodehydroascorbate reductase	Dehydroascorbate reductase	Glutathione reductase
Control	40.18±1.49	24.20±1.1	10.72±0.75	24.19±0.95
30	39.86±1.57	24.02±1.87	10.63±0.81	24.04±1.24
45	53.68±2.08	32.32±2.13	14.31±1.11	32.33±1.85
60	44.55±1.07	26.83±1.68	11.88±0.87	26.83±1.07
30+45	46.77±1.86	28.15±1.79	12.47±1.05	28.17±1.18
30+60	47.49±2.11	28.58±1.31	12.66±1.01	28.58±1.31
45+60	55.91±1.99	33.66±2.07	14.91±1.21	33.65±.1.79
30+45+60	44.58±2.25	23.82±1.99	11.02±0.83	25.08±1.57
CD at 5%	5.01	2.65	2.67	3.71

Table 4. Effect of nutrient spray in and specific activity of ascorbate peroxidase (EU/mg of protein), monodehydroascorbate reductase (EU/mg of protein) and dehydroascorbate reductase (EU/mg of protein) and glutathione reductase (EU/mg of protein) of *Brassica juncea* at different days after sowing (± standard error)