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IMPACT OF EXOGENOUS ARGININE ON BIOCHEMICAL TRAITS OF WHEAT PLANT UNDER H_2O_2 STRESS

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Abstract

Wheat plants were planted in pots of silt loam soil under the conditions of the green house. To examine the Interaction impact between H_2O_2 (0, 10, 20 and 40) mM and three levels of L-arginine (0, 5 and 10) mM. In antioxidant enzymes activity Superoxide dismutase-SOD, Peroxidase-POD, Catalase-CAT, Ascorbate peroxidase-APX, Glutathione peroxidase-GPX and Glutathione reductase-GR. It was measured in wheat leaves. The results showed that increasing H_2O_2 from 0 to 40 mM significant increased the SOD, POD, CAT, APX, GPX and RG activity. Under all concentrations of H_2O_2 increment in L-arginine levels from 0 to 10 mM promoted significant increment inSOD, POD, CAT, APX and GPX activity, except GR decreased. The impact of the association among H_2O_2 and L-arginine at 40 and 10 mM. Respectively, was sure given the most noteworthy qualities in antioxidant enzymes activity.

Key words: hydrogen peroxide, Arginine, Antioxidant enzymes, wheat plant.

Introduction

Plants are presented to various sorts of biotic and abiotic stresses, which cause what is known as oxidative stress because of the gathering of reactive oxygen species (ROS), for example H_2O_2, O_2^1, OH, $^1O_2$. These free radicals attack the membranes, proteins, pigment and DNA. which leads the cell to death. (Mittler, 2002). In order to remove the free radicals, the plant must develop its defensive mechanisms which are represented by the antioxidant system. This system includes two types, which are enzymatic antioxidants such as super oxide dismutase-SOD, catalase -CAT, ascorbate peroxidase- APX, guaiacol peroxidase- GPX and peroxidase- POD. and non-enzymatic antioxidants such as (Glutathione, Ascorbic acid, Tocopherol, Carotenoids, Polyamines) (Mittler *et al.,* 2004; Orabi *et al.,* 2017a, b, 2018; Noctor *et al.,* 2018).

Hydrogen peroxide is a rapier as in low concentrations it is a complex molecular signal that causes plant forbearance against environmental stresses. As for the high concentrations of hydrogen peroxide, it results in the release of stimulating factors for programmed cell death (Dat *et al.,* 2003). H_2O_2 contributes to many resistance mechanisms by strengthening the cell wall in the formation of a lignifications and improving resistance against disease infections (Dempsey and Klessig, 1995; Bozso *et al.,* 2005). It is also the regulatory key to a wide range of physiological processes such as a arraying of growth and plant sustainability (Cerny *et al.,* 2018). Senescence, photosynthesis, stomatal movement and cell cycl. H_2O_2 stimulates and activates many of the molecular signals responsible for Abscisic acid, Ethylene, Jasmonate, Salcylic acid, Potassium, Calcium and Nitric oxide (Noctor and Foyer, 1998; Liu *et al.,* 2004; Dessikan *et al.,* 2004; Mittler *et al.,* 2004; Peng *et al.,* 2005; Bright *et al.,* 2006).

Several studies present the role of H_2O_2 as a signal molecule regulating the enzymatic antioxidant system, (Orabi *et al.,* 2018) found that an increase in H_2O_2 from 0 to 2 mM is an increase activity of APX, CAT and PPO. (Sohag *et al.,* 2020) found Rice seeding soaked in H_2O_2 (5 and 10mM) in soil and hydroponic systems increases in activity of CAT, APX and GPOX. (Santhy *et al.,* 2014) noticed that spraying 80 mM of H_2O_2 cotton plants led to an increase in POD, CAT and Malate dehydrogenase.

Some the studies that were conducted to reduce the stress of hydrogen peroxide in the use of organic compounds and in this study amino acids were used specifically from the arginine acid because of their positive roles in increasing vegetative growth and the area of the leaf surface and the leaf content of the chlorophyll

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and antioxidant system. L-Arginine is one of the important amino acids in plant organelles and the main substance of glutamine, polyamines, Agmatine, proline and nitric oxide (Liu *et al.,* 2006; Chen *et al.,* 2004). Several studies indicate the effect of L-arginine in some antioxidant enzymes and for various plant crops. (Nasibi *et al.,* 2011) found that treatment with L-arginine at a concentration of 10 Mm for tomato plants prone to drought stress is an increase in enzymes of SOD, APX and GR. while the activity of CAT and GPX decreased. (Kabiri *et al.,* 2016) likewise demonstrated that the utilization of various concentrations of L-arginine (0, 10 and 20 µM) caused an increase in activity of (Calatase, Ascorbate peroxidase and Guaiacol peroxidase). While (Nasibi et al. 2013) mentioned pretreated with (10 and 20) µM arginine the activity of GPX, CAT and APX decreased. (Nejadalimoradi *et al.,* 2014) and others indicated that the addition of arginine (1 and 5) mM gave a significant increase to the activity of catalase and ascorbate peroxidase in root and leaves of sunflower plants.

The point of this investigation is to assess the job of L-arginine in reducing the oxidative stress resulting by adding H_2O_2 as well as stimulating the enzymatic antioxidant system.

Materials and Methods

Wheat plants were planted in pots under the conditions of the green house during the growing seasons of 2019. In silt loam soil and placed in a plastic pots capacity of 5Kg. wheat (*Triticum aestivm*) variety buhooth (22), Seeds were soaked in different concentration of H_2O_2 (0, 10, 20 and 40 mM) for 48 h. all pot was treated with various L-arginine acid levels (0, 5 and 10mM) The experiment was designed according to the of Randomize Complete Block Design (RCBD) as global experience and three replicates. A foliar splash of Arg multiple times (at10-days intervals) was subject to the plants. The last gather was carry out 40 days after the beginning of handling. Every single plastic pot were inundated with faucet water till 65% of field capacity after week till the finish of trial.

Preparation of enzyme extracts:

Freshly cut sample (0.5g) is dissolve in 10 ml of (0.1) M potassium phosphate buffer (pH7.5) containing (0.5) mM EDTA and separated through cheese cloth. The homogenate is centrifuged at 15000xg for 15 minutes at 0-4^{0}C. The supernatant is collected and used as enzyme extract.

Superoxide dismutase (SOD)

SOD activity was assessed by account the decline in absorbance of NBT color (Dhindsa *et al.,* 1981). 25 mM of nitrobluetetrazolium chloride (NBT), 13 mM of methionine solution, 50 mM of phosphate buffer (pH 7.8), 50 mM of sodium carbonate, 0.1 mM of EDTA solution, and 0.1 ml enzyme extract. The reaction was begun by including 2l M riboflavin and setting the sample tubes below two 15 watt fluorescent lights for 15 min. The total response blend without enzyme, Which resulted in the maximum color, went about as the control. The reaction was halted by turning off the light and put the pipe in the dark. A non-lighted reaction mixture filled in as a clear. The activity is expressed as units mg $^{-1}$protein.

Catalase (CAT)

Aebi (1984) method is followed for measurement of Catalase activity.The test blend contained 100 µl of enzyme extract, 0.1 mM potassium phosphate buffer (pH 7.5) consist of 0.1 M EDTA and (0.3%) H_2O_2 and the absorbance was estimated at 240 nm. Catalase activity was expressed as ΔOD 240 nm units mg^{-1} protein.

Peroxidase (POD)

Peroxidase activity was controlled by the oxidation of guaiacol within the sight of H_2O_2. The expansion in absorbance at 470 nm wasregistered for 1 min. The reaction mixture contained 0.1 ml crude enzyme, 28 mM guaiacol 500 µl, 50 mM potassium phosphate buffer (pH 7) 1.9 ml and 5 mM H_2O_2 500 µl, . POX activity of the extract was statement as units mg^{-1} protein (Ghanati *et al.,* 2002).

Ascorbate peroxidase (APX)

APX activity is determined following the method as described by Nakano and Asada (1987). It is a spectrophotometric method where the average of decline in absorbance value of ascorbate through its oxidation measured at 290nm wavelength. APX activity was expressed as µmol ascorbate oxidized units mg^{-1} protein.

Glutathioneperoxidase(GPX)

GPX was estimated as portrayed by Elia *et al.,* (2003) with slight adjustment utilizing H_2O_2 as a substrate. The oxidation of NADPH was recorded at 340 nm for 1 min and the activity was resolved using the extinction coefficient of 6.62 mM^{-1}cm^{-1}.

Glutathione Reductase (GR)

The method, described by Smith *et al.,* (1988), can be used for assay of the enzyme glutathione reductase. It depends on the rate of increment in absorbance at 412 mm within the sight of substrate for example oxidized glutathione. The test blend includes 0.5 ml of 3mM DNTB arranged in phosphate buffer 0.1 M PH7.5 ,10 µl of 200 ¼M potassium phosphate support (PH 7.5) containing 100 µM EDTA), 200 µl of 200 µM NADPH and 200 µl of enzyme extract. The last bulk is made up to (2.9) ml

with distilled water. The expansion in absorbance at 412 nm is estimated for a time of 10 minutes following including GSSG. Glutathione reductase activity is statement as ΔA412 min^{-1}. mg^{-1} protein.

Protein estimation

The method developed by Bradford (1976) is largely followed for the estimation of soluble protein.

Statistical Analysis

The results were analyzed statistically according to the design used and conducted the statistical analysis according to the SAS program. The test used the least significant difference at the 5% probability level to compare the arithmetic averages. (SAS, 2001).

Results

The H_2O_2 stress and exogenous L-arginine and their interaction, demonstrate the significant effect on the activity of enzymatic antioxidants. As when H_2O_2 increased, the activity of a SOD enzyme increased significantly especially at 40 mM Fig. 1 and the activity of the SOD increased with increased concentrations of arginine, Whereas, the interaction treatment 40mM H_2O_2 and 10mM arginine gave the highest values with a percentage of displacement 121.52%. When the H_2O_2 concentration was raised from 0 to 40 mM Fig. 2, the CAT activity increased significantly and the same trend increased with the increase in arginine concentration Whereas, the interaction treatment 40 mM H_2O_2 and 10 mM arginine gave the highest values. We observed from the results of Fig. 3 a high increase in the POD with an increase in the H_2O_2 concentration as well as an enzyme activity expanded with an expansion in the concentration of L-arginine, The interference treatment was achieved between 40 mM H_2O_2 and 10 mM arginine the highest value. Fig. 4 shows the role of H_2O_2 in increasing the activity of APX, as well as the role of L-arginine in increasing APX activity The interference treatment was achieved between 40 mM H_2O_2 and 10 mM arginine the highest value Under the conditions of the experiment Fig. 5, the activity of the GPX increased as the H_2O_2 concentration increased and the same increase was recorded with the arginine concentration, The 40 mM H_2O_2 and 10 mM arginine treatment recorded the highest

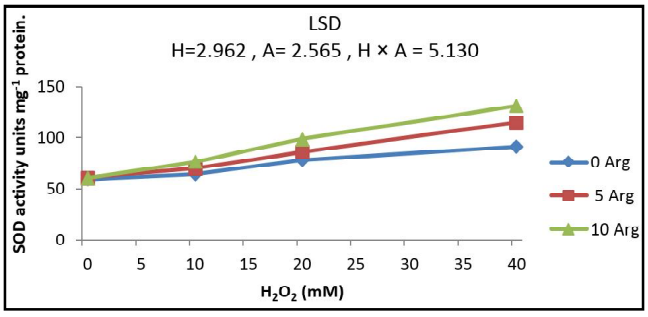

Fig. 1: Effect of H_2O_2 and Arginine treatments and their interaction on the activity of superoxide dismutase (SOD) units mg^{-1} protein.

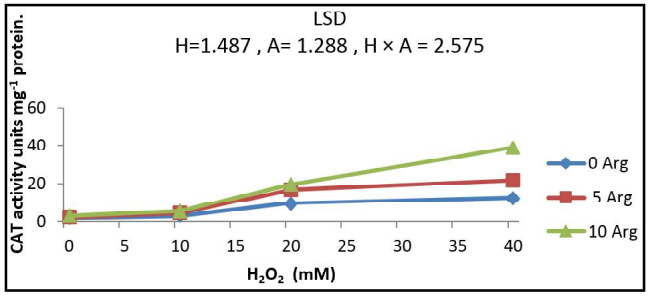

Fig. 2: Effect of H_2O_2 and Arginine treatments and their interaction on the activity of catalase (CAT) units mg^{-1} protein.

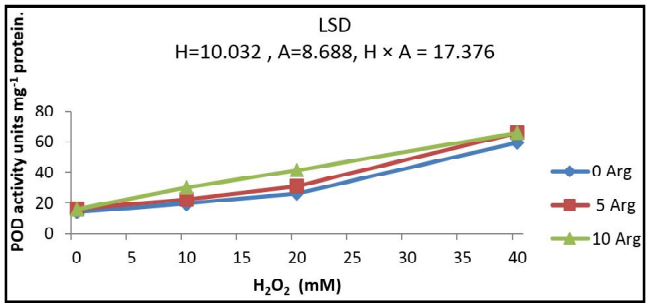

Fig 3: Effect of H_2O_2 and Arginine treatments and their interaction on the activity of peroxidase (POD) units mg^{-1} protein.

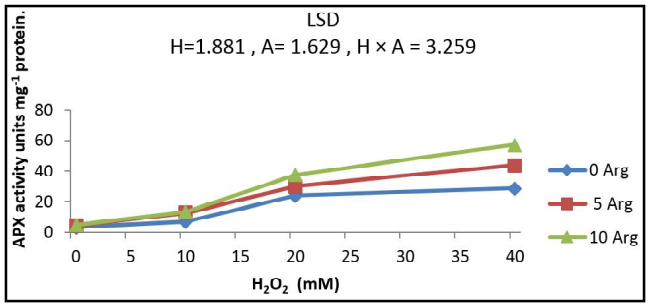

Fig 4: Effect of H_2O_2 and Arginine treatments and their interaction on the activity of Ascorbate peroxidase (APX) units mg^{-1} protein.

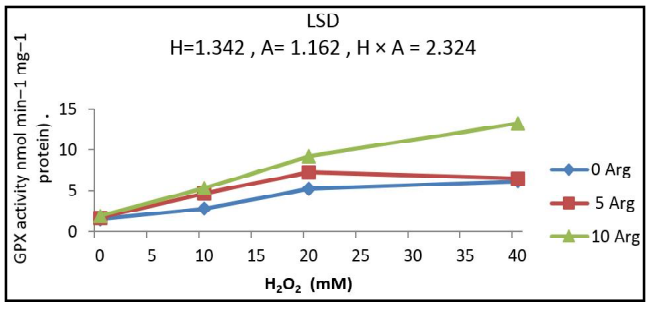

Fig 5: Effect of H_2O_2 and Arginine treatments and their interaction on the activity of glutathione peroxidase (GPX) units mg^{-1} protein.

values compared to the control treatment. GR activity also increased as H_2O_2 levels were raised and exogenous L-arginine was effective decreased of GR at (10 mM), The increase was 50% at the binary interference between 40 mM H_2O_2 and 10 mM arginine Fig. 6.

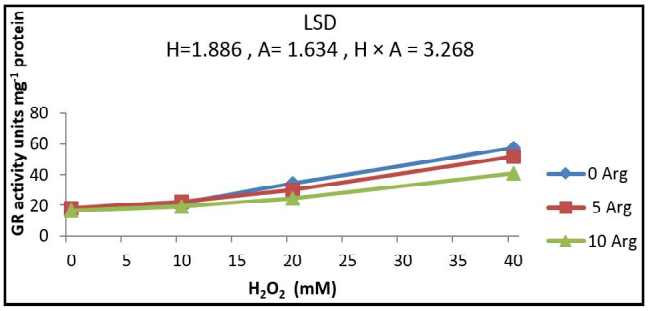

Fig. 6: Effect of H_2O_2 and Arginine treatments and their interaction on the activity of glutathione reductase in (GR)units mg^{-1} protein.

Discussion

Hydrogen peroxide was appeared to initiate overproduction of reactive oxygen species causing oxidative stress. The results showed that antioxidant enzymes activities (SOD, POD, CAT, APX, GPX and GR) significantly increased in wheat leaves with increasing Hydrogen peroxide from 0 to 40 mM. At whatever point a plant is presented to H_2O_2 superoxide dismutase (SOD) assumes a protective job in changing over superoxide to hydrogen peroxide.. Superoxide dismutase (SOD) isozymes are compartmentalized in higher plants and assume a noteworthy role in fighting oxygen radical interceded harmfulness. A few reports with different plants gave proof of upgraded activities of SOD by H_2O_2 treatment (Orabi and Sadak, 2015; Asaad, 2013). As saw in the present examination, it was recommended that the increment in H_2O_2 treatment related to an increment in explicit activity of POD. Furthermore, other examination refered to that the acceptance of peroxidase by the illumination would be one of the defense systems initiated ROS-interceded cell signaling. (Jaleel *et al.,* 2008) represent POD assumes a key role in diminishing H_2O_2 content gathering, dispensing with MDA coming about peroxidation of membrane and keeping up layer lipids integrity. This finding was in agreement with those of (Santhy *et al.,* 2014). Ascorbate peroxidase is suppose to assume the most basic act in suppression ROS and securing cells in plants, euglena and algae, and different living beings (Bahari *et al.,* 2015). Ascorbate peroxidaseis engaged with rummaging of H_2O_2 in water-water and ascorbate-glutathione cycles and uses glutathione as the electron donor (Gill and Tuteja, 2010). GPX enzyme can the reduction of hydrogen peroxide and lipid peroxides by making use of glutathione as electron donor. GR is found in the two eukaryotes and prokaryotes It is a potential protein of the ASH-GSH cycle and assumes a fundamental job in protection system against oxidative stress by proceeding with the diminished of glutathione. (Romero-Puertas *et al.,* 2006), H_2O_2 is changed over to O_2 and H_2O by CAT and POX which utilize AsA as the hydrogen giver. L-arginine assumes a noteworthy job in abiotic stress continue and is related with sign transduction method of biotic pressure opposition. The results of a few investigations appeared exogenous utilization of L-arginine can to create defensive impacts in plant reaction to abiotic stress factors.

The results showed spraying of L-arginine increased the activity of antioxidant enzymes (SOD, POD, CAT, APX and GPX) except GR decreased and the concentration of 10 mM was more effective than others. The present results showed Fig. 1 that arginine induced SOD activation under H_2O_2 lead to production of ROS, SOD is one of the status compounds antioxidant system prevention agent of free radical, changes over two superoxide anions into O_2 and H_2O_2 (Manafi *et al.,* 2015; Qaiser *et al.,* 2010). Activities of POD enzyme was likewise influenced by arginine treatment Fig. 2. It has been discovered that arginine has a defensive role against free radical and goes about as an actuated antioxidant system and restrain free radicals. APX activity altogether increased under various H_2O_2 and increasingly prompted by arginine treatment. APX in charge of expelling H_2O_2 in water-water and ascorbate-glutathione cycles and uses glutathione as the electron giver (Gill and Tuteja, 2010). Both APX and GPX increased significantly with the addition of arginine. While the GR showed in Fig. 6 significantly lowered.

Conclusion

We conclude from the current study when raising the levels of hydrogen peroxide led to an expansion in the activity of antioxidant enzymes and this proves the action of oxidized H_2O_2 within plant cells. As for the addition of arginine, it was necessary to reduce oxidative stress due to its role in increasing the enzymatic antioxidant activity of wheat.

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