Nitrogen Supplement Increase Antioxidants Enzymes and Seed Weight in Genotypes of Sunflower

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Abstract
A field experiment was carried out to improve the nitrogen tolerance traits of Sunflower using the 8 genotypes selected from Shamos and Local varieties compositions selected for the first electoral cycle based on three selection criteria (earliness of flowering, disc diameter and plant height) and sown in randomized complete block design with three replicates and in a split-plot arrangement with three nitrogen levels (50, 100, 200) kgNh1- represented the main plots and the genetic compositions represented the secondary plots. Results revealed that there was a significant between selected genotypes and the Nitrogen levels; the (SHD-selected) gave the highest peroxidase (POD) activity (34.23 U/mg). While, the SHC- control gave the lowest value of POD activity about (11.94 U/mg) under all levels of nitrogen fertilizers (50,100,200) kgNh1-. The nitrogen fertilizers caused increasing of superoxide dismutase (SOD) activity, whereas. The LD-selected gave the highest value of SOD under 200 kgNh1-, about (5.71 U/mg), compared with the LC genotype, which showed activity of SOD About (1.21 U/mg), under 50 kgNh1-. The change in nitrogen levels caused a difference in the activity of enzymes (POD, SOD, and CAT) and led to increasing of 300 seed weight. The LD gave the highest seed weight (48.07 gm and 50.40 gm) under two levels of nitrogen fertilizers (100) and (200) kgNh1-, respectively, while the SHC- control gave the lowest of 50 kgNh1- seed weights about (39.10 gm). We can conclude that increasing of nitrogen level will increase the concentration of anti-oxidant enzymes (POD, SOD, and CAT), and allow to enhance the improvement of productivity in genotypes of sunflower, because of Nentire in the structure of enzymes and protect the cell from deficient of elements and serve stress.

Keywords: Sunflower, Antioxidant enzymes, Nitrogen Fertilizer, Selected.

Introduction

The sunflower (Helanthus Annus L.) Scientific name is one of the oil crops that contributes to the increase of edible oil, which is a source of vitamins, thiamine, magnesium, and phosphorus; it also contains protein and sugar for hydrolysis [1]. The importance of cereal including sunflower cultivation is land reclamation because it tolerates salinity and has a rapid growth rate [2,3]. The cultivated area in Iraq is estimated at about 20 thousand hectares [4]. This is less than what it used to be twenty years ago; this decrease is due to the increase of biotic and abiotic stresses on the crop [5,6,7] as well as the lack of factories manufacturing oil and increase of the cost of nitrogen fertilizers. Nitrogen is one of the essential elements [8,9,10] of vegetative growth. An increase in nitrogen fertilizer causes the lodging of plants in addition to a decrease in leaf area [5]. A decrease in the quantity of nitrogen reduces dry matter. Moreover, it reduces the interception of sunlight on the surfaces of plant leaves [5]. Also, varieties may differ in their ability to

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absorb nitrogen due to the ability of plants to stimulate different mechanisms in nutrient acquisition and transport [11]. The low intensity of nitrogen composting causes the irregular functioning of oxidation enzymes, ROS, to produce the plant in several cellular organelles, such as mitochondria, which perform the respiration process, chloroplast photosynthesis, and photorespiration [12]. Nitrogen has the role of increasing oxidation of plant hormones as well as increasing salicylic acid, jasomonic acid, and gibberlic acid in the cell and regulates antioxidant enzymes [1,13]. The evolution of the sunflower progenies and the improvement of varieties to endure drought, disease, and chemical fertilizer levels depends on genetic variation and the ability of gene variability to produce. The new cultivated varieties depend on their content of genes expressing the quality below the level of stress exposed to that plant [10]. This study aims to derive genotypes from the Sunflower that tolerate high and low nitrogen levels and relate to oxidation enzymes in the plant cell.

Materials and Methods

A field experiment was carried out at the University of Baghdad to improve the tolerance traits of Sunflower to Nitrogen levels using the two varieties, Shamos, and common varieties, according to selection criteria: early flowering, plant height, and disk diameter under 10% selection intensity and three Nitrogen levels (50, 100, 200) kg.h-1.

Peroxidase enzymes (POD):

The efficacy of POD activity was measured according to [14]. The guaiacol was prepared with 1.36 mL of guaiacol and diluted to 250 mL by using distilled water, and then the guaiacol was mixed with 1mL from hydrogen peroxide, and the data were measured via spectrophotometer at wavelength 420 nm; the formula was calculated according to [15].

The activity of POD (U/ mg protein)=(SOD inhabition%)/(50%)×DF/(V(ML))

Super oxide dismutase (SOD):

The activity of SOD was measured according to [14]. Below are the solutions used for this experiment:

a-K2HPO4 (82.4 mmol) and EDTA-2Na (3.588 µmol) dissolved with distilled water(DW) and volume completed to 250 mL

b- K2HPO4 (82.4 g) and 0.0154 g of EDTA-2Na dissolved in DW and completed the volume 250 mL c- potassium phosphate buffer prepared by supplied solution (1), and amended the pH=7.8 .

d-The solution content 150 gm of L-methionine in 5 mL of distilled water

e-The solution content 0.1 mL of Triton X -100 in the 10 mL of distil water

f-the solution content NBT 14.4 and diluted it in the 10 mL of distil water

g. Nitro blue titrazolum (NBT) =14.4 mg ad dissolved in 10 mL of distilled water and then all solutions were mixed with volume of 18.35 mL of first solution .we also dissolved 0.0018 gm from riboflavin in 100 mL DW. We took 40 microliter of riboflavin dye and mixed it with 100 microliter from mixed of solutions with 100 microliter from sample and read at 540 nm . the activity of SOD was calculated as follows:

1-inhabition of enzyme (SOD)=((A2B-A1B)-(A2S-A1S))/((A2B-A1B))×100

A1B=Blank before illumination

A2B=Blank after illumination

A1S= the sample before illumination

A2S= the sample after illumination

1-The SOD activity (U/mL-1)=% inhibition/ dilution factor(1:10)

The activity of SOD according formula [16]

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The activity of SOD(U/ mg protein)=(SOD inhabition%)/(50%)×DF/(V(ML))

Catalase enzymes (CAT):

The activity of (CAT) was estimated according to method [15], the following of solution were used : we are diluted 1.36 g of KH2PO4 in 200 mL of DW and 1.7420 g K2HPO4 in the 200 mL of DW , and then the hydrogen peroxide (H2O2) used 0.34 mL of (H2O2=30%) to complete the volume 100 mL of DW. 0.1 mL of sample mixed with 1.9 mL of buffer solution, and 1.0 mL of hydrogen peroxide to assess the activity of CAT using the following calculation [16].

Activity of CAT (U/mg)=((Δ reading)/Δ)/(0.01× 0.1 ×concentration of protien 〖 mg〗^(-1))

Results and Discussion

Peroxidase enzymes (POD)

The activity of peroxidase plays a role in resistant abiotic- stress, shortage of nutrient elements, plant disease, and heat stress [8,17], the importance of selection in plant breeding method is used to get new genotypes [17]. The result in Table (1) shows that there were significant differences between the genotypes in the activity of POD; the LD selected gave the highest (28.97 U/mg), compared with all progenies and their parents while, the progeny (SHC- control) gave lower value in the activity (11.94 U/mg). The cultivars are one of the determinants of plant responsibility for nitrogen quantity supplied, where the improving process will include the growth of anti-oxidant enzymes [2,9]. A significant difference was observed between nitrogen levels in the activity of POD, and the activity increases with the increasing quantity of nitrogen fertilizers, and the nitrogen level 200 kgNh1- gave (29.11 U/mg) compared with 50 kgNh1- gave (17.96 U/mg). The available N in the soil will be overcome by Reactive oxygen species (ROS) by increasing the anti-oxidant enzymes [10].The significant differences were found in the interaction between genotypes and nitrogen levels in the activity of POD, the difference due to differences in the responsibility of genotypes, where the LD selected with 200 kgNh1- gave (42.76 U/mg). In comparison, SHC chose with 50 kgNh1- gave (7.91 U/mg) the differing activity in the seeds because of the genetic variation between varieties [2].

Table 1. Effect of nitrogen fertilizers on peroxidase activity (U/mg) in some selected genotypes from sunflower

Genotypes	50 kgNh^{1-}	100 kgNh^{1-}	200 kgNh^{1-}	Mean
Lf-selected	24.58	30.13	32.23	28.97
LD-selected	17.90	21.72	42.75	27.46
LS-selected	20.80	22.23	23.95	22.33
Lc-control	17.97	18.20	21.69	19.29
SHF-selected	9.27	17.32	25.41	17.33
SHD-selected	30.08	34.22	38.40	34.23
SHS-selected	15.14	15.53	31.50	20.72
SHC- control	7.91	10.96	16.96	11.94
L.SD 5%	**1.18**			**0.68**
Mean	17.96	21.28	22.33	
LSD 5%	**0.73**			

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Super oxide dismutase (SOD(

The activity of SOD is related to nitrogen fertility by increasing the amino acid in the structure of the enzyme. The result of Table (2). Appear that were significant differences between the genotypes in the activity of SOD. The genotype (LD- selected) gave the highest SOD activity (4.06 U/mg), while the genotype (L.C Origin) gave the lowest SOD (1.57 U/mg). The variant of action is because of the difference in the DNA sequence caused by the gene expression [18]. There are significant between nitrogen levels, where the nitrogen 200 kgNh1-gave the highest level of value (3.67 U/mg), but the nitrogen level 50 kgNh1- gave the lowest value(2.03 U/mg), the increasing of nitrogen caused an increasing SOD activity [16,19]. The interaction between nitrogen levels and genotypes was significant; the LD-Selected gave the highest value of activity (5.71 U/mg) under 200 kgNh1-, compared with the LC- control, which gave the lowest value (1.21 U/ mg) under 50 kgNh1-. The selection method is produced new progenies depending on the genetic variation that will be tolerated biotic and abiotic stress via the content of increasing anti-oxidant enzymes like; CAT, POD, and SOD [20,21.[

Table 2. Effect of nitrogen fertilizers on super oxide dismutase activity (U/mg) in some selected genotypes from sunflower

Genotypes	50 kgNh^{1-}	100 kgNh^{1-}	200 kgNh^{1-}	Mean
Lf-selected	2.65	2.58	3.64	2.95
LD-selected	2.41	4.08	5.71	4.06
LS-selected	1.96	2.70	3.68	2.78
Lc-control	1.21	1.39	2.13	1.57
SHF-selected	2.65	3.62	3.52	3.26
SHD-selected	2.05	2.39	5.23	3.22
SHS-selected	1.87	2.47	2.71	2.35
SHC- control	1.47	2.29	2.72	2.16
L.SD 5%	**0.32**			**0.18**
Mean	2.03	2.69	3.67	
LSD 5%	**0.09**			

Catalase enzymes (CAT(

The enzyme (CAT) is present in the peroxisome in the cell. Its biochemical defense system in cells from reactive oxygen species (H2O2, O2-, OH-) from oxidative damage [22]. Table (3) shows that there were significant differences between the genotypes in the activity of catalase activity. The genotype (LF-selected) gave the highest catalase activity (9.32 U/mg), compared with the genotype (LC – control), which showed the lowest activity (2.82 U/mg). The movement of catalase increases depending on the ability of the genotype to produce it [23]. There are significant between nitrogen levels, where the nitrogen 200 kgNh1-gave the highest level of value (8.73U/mg), but the nitrogen level of 50 kgNh1- showed the lowest value (3.03 U/mg), the increase of nitrogen

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caused increasing CAT activity [24]. The interaction between nitrogen levels and genotypes was significant; the LF-Selected gave the highest value of activity (18.01 U/mg) under 200 kgNh1-, compared with the SHC- control, which gave the lowest value (1.65 U/ mg) under 50 kgNh1-. The increase of CAT in the cells of plants because of nitrogen fertilizers produced anti-oxidant enzymes [25]. These results support the previous results that connect the genetic variation with the stress susceptibility in plants[26,27.[

Table 3. Effect of nitrogen fertilizers on catalase (U/mg) activity in some selected genotypes from sunflower

Genotypes	50 kgNh^{1-}	100 kgNh^{1-}	200 kgNh^{1-}	Mean
Lf-selected	4.80	5.17	18.01	9.32
LD-selected	2.33	8.77	9.88	6.99
LS-selected	5.21	6.09	9.16	6.82
Lc-control	3.07	3.20	4.81	3.69
SHF-selected	4.80	5.17	18.01	9.32
SHD-selected	5.21	6.09	9.16	6.82
SHS-selected	2.33	8.77	9.88	6.99
SHC- control	1.73	2.30	3.16	2.40
L.SD 5%	**0.53**			**0.31**
Mean	3.03	4.76	8.73	
LSD 5%	**0.33**			

seed weight

The seed weight is present in seed growth and embryo size. It is related to the photosynthesis system, a pathway in the cell of plants [22]. Table (4) shows significant differences between the genotypes in the seed weight. The genotype (LD -selected) gave the highest seed weight (48.65 gm), compared with the genotype (SHC -selected gave the lowest seed weight (40.28 gm). The increase in seed weight is because of the improved photosynthesis system [28,29]. There are significant between nitrogen levels, where the nitrogen 200 kgNh1-gave the highest value (45.63 gm), but the nitrogen level 50 kgNh1- gave the lowest value (42.40 gm). The increased nitrogen quantity caused an increase in seed weight [8,19]. The interaction between nitrogen levels and genotypes was significant; the gave the lowest weight under 50 kgNh1-, about (39.10gm), compared with the SHC LD-Selected gave the highest value (50.40 gm) under 200 kgNh1-. The increasing of nitrogen level in the cell caused improvement between source to sink [13.[

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Table 4. Effect of nitrogen fertilizers on 300 seed weight (g) in some selected genotypes from sunflower

Genotypes	50 $kgNh^{1-}$	100 $kgNh^{1-}$	200 $kgNh^{1-}$	Mean
Lf-selected	45.60	46.53	48.48	46.87
LD-selected	47.49	48.07	50.40	48.65
LS-selected	42.00	43.42	47.97	44.46
Lc-control	41.33	41.79	43.57	42.23
SHF-selected	39.47	40.03	42.50	40.67
SHD-selected	44.17	45.07	45.95	45.05
SHS-selected	40.03	41.57	43.87	41.82
SHC- control	39.10	39.43	42.30	40.28
LSD 5%	**N.S**			**1.52**
Means	42.40	43.24	45.63	
L.SD5%	**1.69**			

Conclusion

This study showed that the nitrogen level enhanced the activity of antioxidant enzymes (POD, SOD, and CAT) and eventually led to a significant increase in 300 seed weight. Also, the genotype SHC gave the highest seed weight under two levels of nitrogen fertilizer. We also showed that increase in Nitrogen level has led to an increase in the concentration of antioxidant enzymes and improved the productivity of the selected genotypes.

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