Journal of Engineering and Applied Research (JEAR)
Technical and Vocational University
Winter & Spring 2024, Vol. 1, No. 1, p. 25-38
Journal Homepage: https://jear.tvu.ac.ir/
:10.48301/JEAR.2024.194111

Evaluation of Potassium and Nitrogen on Poutresin Content in Wheat against FHB

Nasibeh Tavakoli Hasanaklou[1*], Ali Ebadi[2], Hourieh Tavakoli Hasanaklou[3], Khadijeh Gholampour Ahanghar Kalayi[4]

[1]Department of Plant Molecular Physiology, Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran.
[2]Membrane Agriculture Faculty of University of Mohaghegh Ardabili, Ardabil, Iran.
[3]Crop Science Department, Agricultural Institute of Slovenia, Ljubljana, Slovenia.
[4]MSc Degree, Department of Pasturage and Watershed Management and Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran.

ARTICLE INFO

Article Type:
Original Research

Received: 02.19.2024
Revised: 03.17.2024
Accepted: 04.08.2023

Keyword:
Antioxidant Enzyme Activity
FHB
Lysine
Methionine
Proline
Wheat

***Corresponding Author:**
Nasibeh Tavakoli Hasanaklou
Email:
nasibehtavakoli93@gmail.com

ABSTRACT

To assess the effects of potassium and different forms of nitrogen on antioxidant enzyme activity and physiological changes in wheat affected by Fusarium Head Blight (FHB), a factorial experiment was conducted using a completely randomized design with three replications. The treatments included two disease levels (control and infected), three potassium levels (80, 100 and 120 kg.ha^{-1}) and three nitrogen application rates (ammonium sulphate, calcium nitrate and a compound treatment consisting of 75% calcium nitrate + 25% ammonium sulphate). The results showed an increase in the levels of superoxide dismutase (SOD) and putrescine under FHB, particularly with the application of potassium and nitrate. The highest content of putrescine (0.43 µmol/FW) was observed in Fusarium × ammonium × potassium (80 kg). However, protein yield decreased under FHB disease, particularly with ammonium application, whereas potassium and nitrate application led to an increase in protein yield up to (33.7 mg.plant^{-1}). Methionine content decreased to (0.17 mg.g^{-1} FW) under disease conditions. Fusarium head blight of wheat increased the activity of antioxidant enzymes. The application of potassium and nitrate had a greater effect on reducing the adverse effects of the disease than potassium alone. This effect could be attributed to their capability of reducing osmolyte levels and modulating the activity of antioxidant enzymes.

E-ISSN: 3060-6357

Introduction

Fusarium head blight (FHB) of wheat is one of the most important and destructive diseases in Iran and throughout the world. In regions with warm and humid weather, it appears at the flowering stage of wheat. In the past, it was observed in scattered locations in Iran, but now it is one of the significant diseases of wheat in Mazandaran, Golestan, and Fars Provinces, and in Dasht-e-Moghan in Ardabil Province. Various species of the genus Fusarium are involved in the development of this disease, but the *Fusarium graminearum* species complex is known as the main causal agent of FHB of wheat in most regions [1]. Fusarium can produce mycotoxins, the most important of which is deoxynivalenol (DON), and the degree of its pathogenicity depends on how much DON it produces [2]. Deoxynivalenol is a low molecular weight inhibitor of protein synthesis with cell membrane and hemolytic activities [3]. Moreover, it contributes to cell damage caused by Fusarium in cereals, including chloroplast and the cell membrane permeability, toxic effects on ribosomes, stimulation of H_2O_2 production, and cell death. Low pH values, osmotic stress, oxidative stress, and high polyamine levels stimulate the synthesis of DON by Fusarium [4]. Various compounds including arginine, ornithine, agmantine, citrulline, and putrescine, which are precursors of polyamine synthesis, stimulate synthesis of DON by Fusarium. It has not been completely established yet whether plants or fungi synthesize polyamines. However, the quantity of putrescine produced conforms to gene expression patterns of genes in plants. Polyamines are a group of polycations produced in eukaryotes and prokaryotes, and their role in the regulation of biological processes has been acknowledged. Deficiency of polyamines reduces fungal growth, while their accumulation in fungi makes them toxic [5]. Under in vitro conditions, the effects of polyamines on DON induction are much greater compared to those of hydrogen peroxide and sugars. Gardiner et al. (2009) put forth the hypothesis in which they suggested Fusarium probably uses its special receptors to sense the amount of polyamine that exists and then produces DON accordingly [6].

Some researchers have stated that methionine has a minute role in DON induction. However, the combination of amino acid and polyamine (putrescine and methionine) intensifies DON induction. Production of putrescine in spikes occurs faster than DON induction [7]. FHB damages the cell walls of seeds by destroying their starch and protein reserves and reduces the baking quality of the flour [8]. The viscoelastic properties of stored proteins in wheat grains are necessary for baking bread with suitable quality [9]. Gluten-containing proteins absorb water and form a network during the preparation of dough that results in the formation of the elastic structure of the dough.

Accumulation of putrescine due to potassium deficiency [10], ammonium application [11], osmotic stress [12], and acidic stress [13] have previously been reported. Under conditions of potassium deficiency, a large quantity of putrescine, amounting to more than 1% of total dry weight, is accumulated in plants [10]. It seems a potassium deficiency is the main reason for increases in putrescine content, which compensates for the deficiency of potassium by performing its physiological functions in plants [14]. Potassium is one of the macronutrients of plants and plays an important role in protecting them against various stresses. Potassium mainly plays a catalytic role in plants, and its deficiency reduces their resistance to pests and diseases [15]. Potassium deficiency results in an imbalance between the cations and anions in minerals, which leads to lower cell pH [16], and reduction in pH values results in increased synthesis of DON by the fungus [4]. In addition to potassium deficiency, accumulation of ammonium also leads to increased putrescine accumulation. Increased ammonium accumulation accelerates increases in ethylene content, and ethylene acts as a signal of the occurrence of stress, which leads to greater putrescine content in plants. Consequently, the putrescine content in plants treated with ammonium was higher compared to those treated with nitrate; in addition, the spermidine content in plants treated with nitrate was greater than the spermine and putrescine contents [17]. Lovatt (1990) showed that ammonium accumulation in plants in response to environmental stresses was one of the main factors contributing to changes in intermediate compounds and even in plants treated with nitrate under stress conditions, a large quantity of ammonium accumulated compared to the time there was no stress [18]. Considering the spread of FHB of wheat in some regions of Iran, and the occurrence of cancers and digestive diseases resulting from the use of wheat grains infected with DON, it is necessary that physiological strategies should be developed to reduce the accumulation of this compound in wheat.

Methodology

To study the effect of potassium and nitrogen forms on wheat physiological changes under FHB, an experiment was conducted as a factorial in a completely randomized design with three replications in the experimental greenhouse of Mohaghegh Ardabili University. Treatments included two levels of disease (polluted and control), three levels of potassium (80, 100, and 120 kg.ha^{-1}), and three forms of nitrogen (ammonium sulfate, calcium nitrate, and compound treatment included 75% calcium nitrate + 25% ammonium sulfate). Firstly, three wheat cultivars (Gonbad, Morvarid and Chamran) were cultivated, and then the Gonbad cultivar showed the highest sensitivity to FBH and was selected as the control cultivar. The recommended nutrients were applied based on soil test results and pots filled with 10 kg of soil (Table 1).

Table 1. The characteristics of the soil used in the soil experiment.

Texture	Sand (%)	Silt (%)	Clay (%)	K (mg.kg^{-1})	P (mg.kg^{-1})	N (mg.kg^{-1})	OC (%)	pH	EC (dsm^{-1})
Loamy sand	84	14	2	170	8.5	0.06	0.62	7.88	0.625

Disease inoculation

In this study, isolates of the fungus *F. graminearum* with international code 130951 CBS and accession code 118867 JX obtained from Fusarium Head Blight (FHB)-infected wheat spikes in the Moghan region were isolated and identified using morphological and molecular methods. The isolates were selected from the fungal collection of the Department of Plant Pathology and grown on potato dextrose agar (PDA) medium in an incubator at 25°C. After approximately one week, the fungus covered the entire surface of the medium. To prepare the inoculum, 2.5 g of wheat straw powder and 2.5 g of barley straw powder were placed in 25 ml Erlenmeyer flasks and 125 ml of water was added to each flask. After autoclaving (at 1 atm pressure and 125°C), the flasks were placed under a laminar flow hood. The fungal culture medium in the Petri dishes was finely chopped with a sterile scalpel, and a portion of approximately one square centimetre in size was added to each of the Erlenmeyer flasks. The flasks were then incubated in a shaking incubator at 25°C and 120 rpm for 96 hours. The liquid culture medium in the flasks was then covered with aluminium foil using sterile cotton swabs. Samples were taken from the covered medium, which appeared as a dark brown suspension. The macroconidia were then counted using a haemocytometer and adjusted to a concentration of 10×10^6 conidia per ml, and the resulting suspension was sprayed onto the clusters using a hand sprayer. After 14 days, samples from plants showing symptoms on the clusters were taken to the laboratory and stored in a refrigerator at -80°C.

Ascorbate peroxidase

Ascorbate peroxidase activity was extracted by [19]. 2ml buffer phosphate (0.05 M, pH=7) was mixed with 40 µl H_2O_2 5 mM, then 0.2 ml protein extract was added. After that, 20 µl Ascorbate (50 µM) was added, and measurement of ascorbate peroxidase was done by spectrophotometer (model UV- 160A- SHIMADZO, Japan) the absorbance rate at 290 nm [19].

Measuring the methionin content

For extraction of methionine, first, leaf samples were well grounded in a mortar containing 0.1% HCl. Then, to quantify methionine, 10 ml of the filtered solution was combined with 4 ml of 5-normal sodium hydroxide in addition to 2 ml each of aqueous glycine and aqueous sodium nitro ferricyanide dehydrate solution and HCl (1:1). The resulting mixture was incubated for 10 minutes at 40 degrees Celsius. Subsequently, 5 millilitres of 1:1 hydrochloric acid were introduced, followed by filtration, and the absorbance was measured at 510 nm using a spectrophotometer (model UV- 160A- SHIMADZO, Japan) [20].

Putrescine assay

Putrescine was measured by a spectrophotometer (model UV- 160A- SHIMADZO, Japan) and calculated by peroxidase reaction. 5-10 M peroxidase was considered as reactive [21].

Protein yield

Total protein content was determined through the application of the Bradford method, as detailed in Reference [22]. In this procedure, 0.1 g of leaves were pulverized with 1 ml of extraction buffer. Subsequently, the samples underwent centrifugation for 21 minutes using a centrifuge spinning at 11,500 revolutions per minute at 4 degrees Celsius. Following centrifugation, each test tube received 5 millilitres of Bradford's solution and 990 microliters of extraction buffer. Then, 10 microliters of extract were introduced into each tube, and thoroughly mixed, and the absorbance was measured at 595 nm.

Following this determination, the protein yield was calculated using the following formula:

Protein yield= Total protein × Seed yield

Seed yield and harvest index

Ten plants were randomly selected from each pot (each pot included 25 plants), and then the plants were threshed using a stationary combine to determine the seed yield.

(Harvest Index= seed yield/Total biomass *100)

Statistical analysis

Statistical combined analysis was performed using SAS software. Mean comparisons were also performed using Duncan’s multiple range test at $P \leq 0.05$.

Results and discussion

Putrescine

Results showed that the interaction of nitrogen, potassium, and disease was significant on putrescine (Table 2). The highest content of putrescine (0.43 µmol/FW) was observed in Fusarium × ammonium × potassium (80 kg) and the lowest putrescine concentration (0.015 µmol/FW) was attributed to the application of potassium (100 and 120 kg) in the control treatment. Increasing the application of potassium led to a significant decrease in the amount of polyamine under disease conditions. Furthermore, the application of nitrate was more influential compared to ammonium in decreasing putrescine (Figure 1). Application of ammonium led to an increase in polyamines amount, such as putrescine under potassium deficit [23]. It appears that the potassium deficit is the main reason for the putrescine increase [14].

Table 2. Effect of nitrogen, potassium and disease on the physiological characteristics and yield in wheat.

	DF	**Yield**	**Harvest Index**	**Protein yield**	**Putressin**	**Methionine**	**Ascorbate peroxidase**
Disease(D)	1	0.68^{**}	3079^{**}	1640^{**}	0.08^{**}	0.011^{**}	0.56^{**}
Potassium(P)	2	0.05^{**}	302^{**}	899^{**}	0.23^{**}	0.0006^{ns}	0.08^{**}
Nitrogen (N)	2	0.001^{ns}	11^{ns}	93^{**}	0.21^{**}	0.0002^{ns}	0.01^{**}
D×P	2	0.028^{**}	125^{**}	31^{**}	0.14^{**}	0.00001^{ns}	0.15^{**}
D×N	2	0.005^{ns}	23^{ns}	30^{**}	0.18^{**}	0.0001^{ns}	0.019^{**}
P×N	4	0.004^{ns}	20^{ns}	15^{**}	0.22^{**}	0.0002^{ns}	0.012^{**}
D×P×N	4	0.001^{ns}	7^{ns}	11^{**}	0.7^{**}	0.0008^{ns}	0.007^{*}
Error	36	0.004	17	3	0.0002	0.0004	0.002
CV (%)	-	20	18	12	11	10	12.6

* and ** represent significance levels at 5 and 1% respectively. ns represent nonsignificant.

Since levels of polyamines increase considerably when FHB spreads, finding a way to reduce these levels can prove effective in reducing the quantities of fungal toxin produced by Fusarium. In this research, potassium application could reduce the amount of putrescine and increase the activity of antioxidant enzymes. The potassium appeared to be effective in lowering the levels of polyamines, and nitrate application prevented increases in putrescine (which is one of the stimulators of DON synthesis). The degree of pathogenic of Fusarium depends on the amount of DON it produces [5]. Deoxynivalenol is a low molecular weight inhibitor of protein synthesis, affects cell membrane permeability, and has hemolytic activity [3]. Moreover, it contributes to cell damage caused by Fusarium through chloroplast and cell membrane permeability, toxic effects on ribosomes, stimulation of H_2O_2 production, and cell death. Low pH values, osmotic stress, oxidative stress, and high levels of polyamines stimulate DON synthesis by Fusarium [4]. In the present study, polyamine content decreased when potassium was applied. Thus, when potassium is deficient, levels of polyamines such as putrescine increase to perform the physiological role potassium plays [23] and, when the levels of polyamines increase, the causal agent of FHB senses the quantity of the present fungus and produces DON accordingly. Considering the results of the present research, the application of potassium and nitrate might reduce the number of fungal toxins produced by lowering the levels of polyamines.

Methionine

The effects of FHB of wheat on methionine content were significant at the 1% probability level (Table 2). The findings revealed that the control group exhibited the highest methionine content (0.2 $mg.g^{-1}$ FW) while the ill plants demonstrated the lowest levels of methionine (0.17 $mg.g^{-1}$ FW) (Figure 2). Researchers believe Fusarium senses polyamine levels during the development of FHB by its special sensors and produces DON accordingly [6]. Therefore, the reduction in methionine content could be due to its conversion into polyamines, which eventually increases the quantity of Fusarium mycotoxin produced. However, methionine is also a precursor of ethylene, which is a volatile messenger for plant defence responses, and an endogenous plant hormone involved in cell growth and development processes such as germination, senescence, and responses to biotic and abiotic stresses [24]. Researchers noticed ethylene application reduced the transcription of PR genes [25] while sequencing the cDNA clone in wheat infected with Fusarium spp. showed that Fusarium increased PR-genes transcription [26]. PR genes associated with diseases are expressed when diseases attack plants and make plants resistant to pathogens. Therefore, not only does ethylene not have a positive effect on Fusarium disease, but it also prevents the expression of resistant genes.

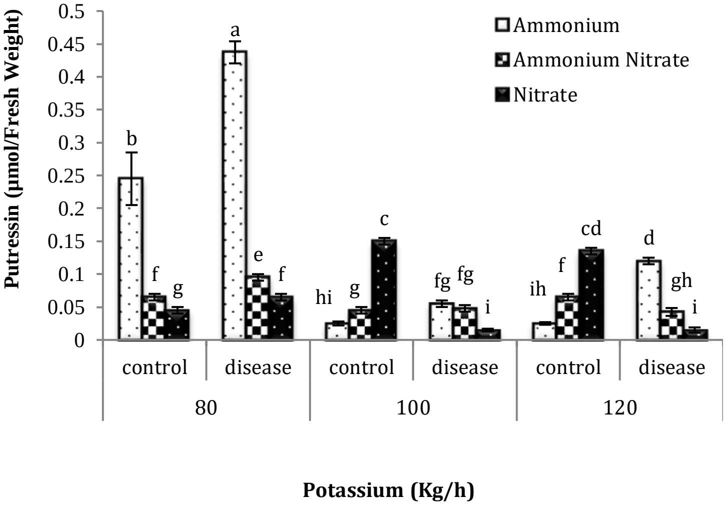

Figure 1. Effect of potassium on the Putresin content under FHB disease.

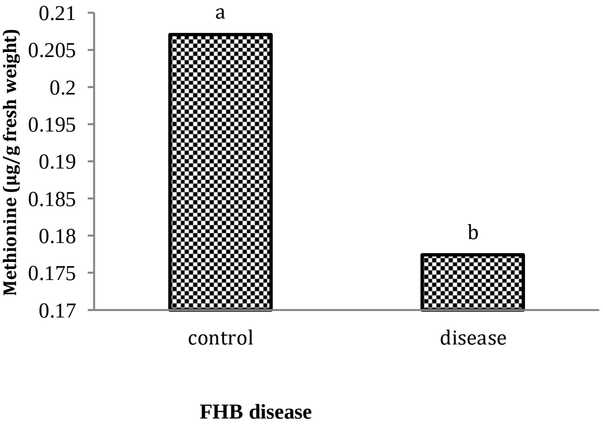

Figure 2. Effect of FHB disease on methionine.

Ascorbate peroxidase

There was a significant difference between FHB, potassium, and the style of nitrogen regarding Ascorbate peroxidase (Table 2). Ascorbate peroxidase activity was higher under disease conditions and potassium compared to control. Moreover, nitrate had more influence on the activity of this enzyme in comparison with ammonium. The maximum ascorbate peroxidase activity (67.717) was observed in nitrate application and potassium (120 kg) under disease conditions, and the minimum amount (18.89) was attributed to nitrate ammonium and potassium (80 kg). Plants use different biochemical and molecular mechanisms to delay the growth and development of pathogens [27] so that an incompatible interaction between a plant host and a pathogen results in plant defence responses signalled through a signal pathway that includes the production of various types of active oxygen, nitric acid, salicylic acid, and jasmonic acid [28]. This signal pathway activates a set of responses that control or eliminate the pathogen [29]. Ascorbate peroxidase is a special type of antioxidant enzyme encoded by a multigene family that includes numerous isoenzymes, and it has been reported that these isoenzymes acquire their abiotic resistance through detoxifying H_2O_2 into H_2O and oxidizing substrates such as ascorbate [30]. Ascorbate peroxidase reduces damage caused by the production of Fusarium toxins. Therefore, the application of potassium and nitrate can increase plant tolerance to the disease by increasing the activity of ascorbate peroxidase.

Protein yield

The interaction of FHB × potassium × nitrogen had a significant influence on protein yield (Table 2). The disease led to a decline in protein and protein yield was greater in the control treatment compared to stress conditions. An increase in the application of potassium and nitrate caused a rise in protein yield under disease and control conditions. The control plants exhibited the highest protein content when treated with ammonium nitrate (33.7 $mg.plant^{-1}$)(Figure 5). Improved protein yield caused by the application of potassium and nitrate might be due to increases in some proteins involved in stress because proteins, including probenazole-induced protein (PBZ), that are involved in stresses are expressed when plants are attacked by pathogens. This protein is an effective inducer of host resistance against stress and an inducer of cell apoptosis [31]. Marschner also believes potassium is one of the elements activating enzymes that cause the accumulation of small molecular compounds, which results in the production of large molecules like those of starch and proteins. Therefore, when the potassium content of plants decreases, protein and starch levels decline and, hence, under conditions of stress, potassium and nitrate application can increase proteins that help plant defence systems against pathogens [23].

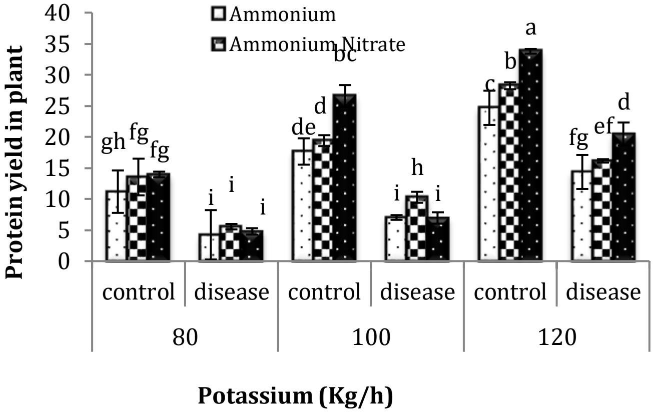

Figure 3. Effect of potassium application and nitrogen source on the protein yield in plants under FHB disease.

Seed yield and harvest index

The results showed that disease × potassium had a significant effect on seed yield and harvest index (Table 2). Results showed that FHB led to a decrease in seed yield and harvest index, while potassium decreased the rate of disease and increased seed yield and harvest index. The highest seed yield (0.49 g.plant^{-1}) belonged to optimal condition and potassium application (120 kg), while the lowest seed yield (0.15 g.plant^{-1}) was observed under potassium application (80 kg) and polluted wheat. The maximum harvest index (33.78) was attributed to potassium application (100 kg) under optimum conditions, and this treatment was in the same group as potassium application (80 kg) in polluted wheat (Figure 6). Since the causal agent of the disease attacks the economic part of the plant (the ears), a reduction in the harvest index that is caused by the disease cannot be avoided. The effects of potassium, indicating an increasing harvest index, might also be due to increased stress toleration in plants and reduced losses caused by fusarium. FHB mainly lowers wheat yield due to its prevalence in the early stages of plant growth and when the maturation of the immature spikes starts, it results in the production of wrinkled seeds, reduced quality of wheat flour, and lower seed yield [32]. Moreover, wheat plants need energy to increase their resistance to biotic and abiotic stresses, and this energy is provided through reducing yield so that the quantities of osmolytes such as proline and soluble sugars and the activity of antioxidant enzymes increase, and the plants can resist against the stress imposed on them. Results of this research also indicated the activity of antioxidant enzymes increases despite the reduction in crop yield caused by the disease.

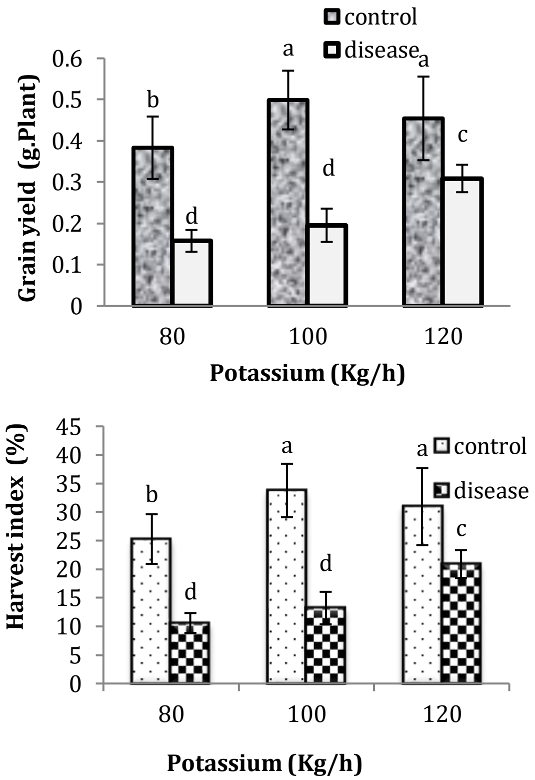

Figure 4. Effect of potassium and disease on the grain yield (a) and harvest index (b).

Conclusion

The findings of the present research illustrate that, as expected, FHB reduced seed yield and harvest index because the main target of this disease is the seeds, which are the economically important part of wheat plants. However, potassium and nitrate application led to an increase in protein yield by up to (33.7 $mg.plant^{-1}$). It seems the application of ammonium resulted in increased synthesis of DON and, eventually, had undesirable effects on cellular and molecular structures causing reductions in yield. Moreover, potassium and nitrate application increased plant resistance to Fusarium by enhancing defence mechanisms so that in the potassium treatment this element replaced putrescine and prevented Fusarium from using putrescine, which is one of the main factors that increased production of Fusarium toxins. The highest content of putrescine (0.43 μmol/FW) was observed in Fusarium × ammonium × potassium (80 kg). Methionine is one of the main precursors of various polyamines which decreased to (0.17 $mg.g^{-1}$ FW) under disease conditions. Although polyamines are known as one of the factors in abiotic stress tolerance, in some biotic stresses, particularly in the disease caused by Fusarium, they increase the production of fungal toxins so that even Fusarium itself produces DON according to the quantity of polyamines that exists in the plants. Therefore, reduction in the methionine content might be one of the factors that increase the intensity of this disease. Furthermore, the study of the activities of ascorbate peroxidase showed increases in their activities and improved plant resistance, and these enzymes were more active under the influence of potassium and when nitrate was applied as compared to potassium deficiency and application of ammonium.

Disclosure statement and funding

The authors declare no potential conflicts of interest. The present study received no financial support from any organization or institution.

Acknowledgment

We would like to give special thanks to all the participants in this study.

Reference

[1] Davari, M., Wei, S., Babay-Ahari, A., Arzanlou, M., Waalwijk, C., Van Der Lee, T. A., Zare, R., Gerrits Van Den Ende, A., De Hoog, G., & Van Diepeningen, A. (2013). Geographic differences in trichothecene chemotypes of Fusarium graminearum in the Northwest and North of Iran. *World Mycotoxin Journal*, *6*(2), 137-150. https://doi.org/10.3920/ WMJ2012.1493

[2] Audenaert, K., Vanheule, A., Höfte, M., & Haesaert, G. (2014). Deoxynivalenol: A Major Player in the Multifaceted Response of Fusarium to Its Environment. *Toxins*, *6*(1), 1-19. https://doi.org/10.3390/toxins6010001

[3] Gilbert, J., & Tekauz, A. (2000). Review: Recent developments in research on fusarium head blight of wheat in Canada. *Canadian Journal of Plant Pathology*, *22*(1), 1-8. https:// doi.org/10.1080/07060660009501155

[4] Walter, S., Nicholson, P., & Doohan, F. M. (2010). Action and reaction of host and pathogen during Fusarium head blight disease. *New Phytologist*, *185*(1), 54-66. https://doi.org /10.1111/j.1469-8137.2009.03041.x

[5] Valdés-Santiago, L., Cervantes-Chávez, J. A., León-Ramírez, C. G., & Ruiz-Herrera, J. (2012). Polyamine Metabolism in Fungi with Emphasis on Phytopathogenic Species. *Journal of Amino Acids*, *2012*(7), 837932. https://doi.org/10.1155/2012/837932

[6] Gardiner, D. M., Kazan, K., & Manners, J. M. (2009). Nutrient profiling reveals potent inducers of trichothecene biosynthesis in Fusarium graminearum. *Fungal Genetics and Biology*, *46*(8), 604-613. https://doi.org/10.1016/j.fgb.2009.04.004

[7] Gardiner, D. M., Kazan, K., Praud, S., Torney, F. J., Rusu, A., & Manners, J. M. (2010). Early activation of wheat polyamine biosynthesis during Fusarium head blight implicates putrescine as an inducer of trichothecene mycotoxin production. *BioMed Central Plant Biology*, *10*(1), 289. https://doi.org/10.1186/1471-2229-10-289

[8] Siranidou, E., Kang, Z., & Buchenauer, H. (2002). Studies on Symptom Development, Phenolic Compounds and Morphological Defence Responses in Wheat Cultivars Differing in Resistance to Fusarium Head Blight. *Journal of Phytopathology*, *150*(4-5), 200-208. https://doi.org/10.1046/j.1439-0434.2002.00738.x

[9] Shewry, P. R. (2009). Wheat. *Journal of Experimental Botany*, *60*(6), 1537-1553. https:// doi.org/10.1093/jxb/erp058

[10] Smith, T. A. (1984). Putrescine and Inorganic Ions. In B. N. Timmermann, C. Steelink, & F. A. Loewus (Eds.), *Phytochemical Adaptations to Stress* (pp. 7-54). Springer. https:// doi.org/10.1007/978-1-4684-1206-2_2

[11] Corey, K. A., & Barker, A. V. (1989). Ethylene evolution and polyamine accumulation by tomato subjected to interactive stresses of ammonium toxicity and potassium deficiency. *Journal of the American Society for Horticultural Science*, *114*(4), 651-655. https:// worldveg.tind.io/record/10414/

[12] Flores, H. E., & Galston, A. W. (1982). Polyamines and Plant Stress: Activation of Putrescine Biosynthesis by Osmotic Shock. *Science*, *217*(4566), 1259-1261. https://doi.org/10. 1126/science.217.4566.1259

[13] Young, N. D., & Galston, A. W. (1983). Putrescine and Acid Stress 1: Induction of Arginine Decarboxylase Activity and Putrescine Accumulation by Low pH. *Plant Physiology*, *71*(4), 767-771. https://doi.org/10.1104/pp.71.4.767

[14] Coleman, R. G., & Richards, F. J. (1956). Physiological Studies in Plant Nutrition: XVIII. Some Aspects of Nitrogen Metabolism in Barley and other Plants in Relation to Potassium Deficiency. *Annals of Botany*, *20*(3), 393-409. https://doi.org/10.1093/oxfordjourna ls.aob.a083531

[15] Sardans, J., & Peñuelas, J. (2021). Potassium Control of Plant Functions: Ecological and Agricultural Implications. *Plants*, *10*(2), 419. https://www.mdpi.com/2223-7747/10 /2/419

[16] Aziz, A., Martin-Tanguy, J., & Larher, F. (1999). Salt stress-induced proline accumulation and changes in tyramine and polyamine levels are linked to ionic adjustment in tomato leaf discs. *Plant Science*, *145*(2), 83-91. https://doi.org/10.1016/S0168-9452(99)00 071-0

[17] Feng, J., & Barker, A. V. (1993). Polyamine concentration and ethylene evolution in tomato plants under nutritional stress. *HortScience*, *28*(2), 109-110. https://doi.org/10.2127 3/HORTSCI.28.2.109

[18] Lovatt, C. J. (1990). Stress alters ammonia and arginine metabolism. In H. Flores, R. Arteca, & J. Shannon (Eds.), *Polyamines and Ethylene: Biochemistry, Physiology, and Interaction* (pp. 166-179). Chemical Rubber Company Press. https://lovattresearch.ucr.edu/site s/default/files/2019-12/techsyp_lovatt_1990.pdf

[19] Webb, R. P., & Allen, R. D. (1995). Isolation and characterization of a cDNA for spinach cytosolic ascorbate peroxidase. *Plant Physiology*, *108*(3), 1325. https://doi.org/10.1104/pp.108.3.1325

[20] Lošák, T., Hlušek, J., Filipčík, R., Pospíšilová, L., Manasek, J., Prokeš, K., Bunka, F., Kracmar, S., Martensson, A., & Orosz, F. (2010). Effect of nitrogen fertilization on metabolisms of essential and non-essential amino acids in field-grown grain maize (Zea mays L.). *Plant, Soil and Environment*, *56*(12), 574-579. https://doi.org/10.172 21/288/2010-PSE

[21] Stevanato, R., Mondovi, B., Sabatini, S., & Rigo, A. (1990). Spectrophotometric assay for total polyamines by immobilized amine oxidases. *Analytica Chimica Acta*, *237*, 391-397. https://doi.org/10.1016/S0003-2670(00)83942-2

[22] Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1-2), 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

[23] Marschner, H. (1995). *Mineral nutrition of higler plants*. Academic Press. https://www.sciencedirect.com/book/9780124735439/marschners-mineral- nutrition-of-higher-pl ants#book-description

[24] Abeles, F. B., Morgan, P. W., & Saltveit Jr, M. E. (2012). *Ethylene in plant biology* (2 ed.). Academic press. https://books.google.com/books/about/Ethylene_in_Plant_Biolog y.html?id=-D7tvAvWpSQC

[25] Ohme-Takagi, M., & Shinshi, H. (1990). Structure and expression of a tobacco β-1,3-glucanase gene. *Plant Molecular Biology*, *15*(6), 941-946. https://doi.org/10.1007/BF00039434

[26] Li, W. L., Faris, J. D., Muthukrishnan, S., Liu, D. J., Chen, P. D., & Gill, B. S. (2001). Isolation and characterization of novel cDNA clones of acidic chitinases and β-1,3-glucanases from wheat spikes infected by Fusarium graminearum. *Theoretical and Applied Genetics*, *102*(2), 353-362. https://doi.org/10.1007/s001220051653

[27] Bowles, D. J. (1990). Defense-related proteins in higher plants. *Annual review of biochemistry*, *59*(1), 873-907. https://doi.org/10.1146/annurev.bi.59.070190.004301

[28] Lu, Z. X., Gaudet, D., Puchalski, B., Despins, T., Frick, M., & Laroche, A. (2005). Inducers of resistance reduce common bunt infection in wheat seedlings while differentially regulating defence-gene expression. *Physiological and Molecular Plant Pathology*, *67*(3-5), 138-148. https://doi.org/10.1016/j.pmpp.2005.12.001

[29] Veronese, P., Ruiz, M. T., Coca, M. A., Hernandez-Lopez, A., Lee, H., Ibeas, J. I., Damsz, B., Pardo, J. M., Hasegawa, P. M., Bressan, R. A., & Narasimhan, M. L. (2003). In Defense against Pathogens. Both Plant Sentinels and Foot Soldiers Need to Know the Enemy. *Plant Physiology*, *131*(4), 1580-1590. https://doi.org/10.1104/pp.102.01 3417

[30] Shigeoka, S., Ishikawa, T., Tamoi, M., Miyagawa, Y., Takeda, T., Yabuta, Y., & Yoshimura, K. (2002). Regulation and function of ascorbate peroxidase isoenzymes. *Journal of Experimental Botany*, *53*(372), 1305-1319. https://doi.org/10.1093/jexbot/53.372.1305

[31] Midoh, N., & Iwata, M. (1996). Cloning and Characterization of a Probenazole-Inducible Gene for an Intracellular Pathogenesis-Related Protein in Rice. *Plant and Cell Physiology*, *37*(1), 9-18. https://doi.org/10.1093/oxfordjournals.pcp.a028918

[32] Stack, R. W. (2000). Return of an old problem: Fusarium head blight of small grains. *Plant health progress*, *1*(1), 19. https://doi.org/10.1094/PHP-2000-0622-01-RV