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NATIONAL UNIVERSITY

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Раздел 3 Актуальные проблемы сохранение биологического разнообразия

CONTENTS

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INCREASING RESISTANCE OF WHEAT TO UNFAVORABLE ENVIRONMENTAL FACTORS BY PRE-SOWN PRIMING OF ITS GRAINS

Introduction

In this century the human being must face the challenges of producing enough to feed a growing population in a sustainable and environmentally friendly way. The yields are with increasing frequency affected by abiotic stresses such as salinity, drought, and high temperature or by new diseases and plagues [1] One of the most studied plant defense inducers and priming agents, the β-aminobutyric acid or BABA, has been used for investigating the transgenerational epigenetic basis of priming defense and the mechanistic of long-lasting induced resistance [2] . Interestingly, these authors found that BABA-IR can be detected up to 28 days after treatment of wild-type Arabidopsis through NPR1-dependent resistance but this effect disappear by 14 days after treatment when a NPR1-independent resistance is activated. Another work about BABA [3] included in this ebook is a commentary about a previously published paper which study the plant perception of BABA mediated by an aspartyl-tRNAsynthetase. Using BABA as priming agent in a screening for Arabidopsis mutants against the biotrophicoomycete *Hyaloperonosporaarabidopsidis*, authors identify an impaired in BABA-induced Immunity 1 (*IBI1*) gene, encoding an aspartyl-tRNAsynthetase (AspRS). This mutation seems to block both priming SA-dependent or SA-independent responses to BABA, indicating unilateral control of BABA-induced resistance by IBI1 [4].

Plants are able to respond to biotic or abiotic stresses through a complex network involving phytohormones, a potent secondary metabolism and secondary messengers like calcium, and stress receptors. Light also plays a key role in plant resistance. Protein kinase/phosphatase cascades are another important component of this network. Rasool and co-workers study the effects of the light on these proteins using light-grown *Arabidopsis thaliana* wild type and in mutant lines defective in several protein phosphatase regulatory subunits on aphid fecundity and susceptibility to *P. syringae* infection [5].

Nitrogen fertilization influences plant-pathogen interactions and elevated levels of nitrogen can promote susceptibility against biotrophs as well as can influence in plant resistance. The disruption of an ammonium transporter involved in the plant immune system, the ammonium transporter AMT1.1, alters basal defenses generating resistance against *Pseudomonas syringae* and *Plectosphaerellacucumerina* . In this work their authors study the role of this ammonium transporter on the basal defenses and the resistance against *P. syringae* and *P. cucumerina* demonstrating that it is a negative regulator of Arabidopsis defense responses [6].

Cross-talk between different signaling pathways has been reported to generate both synergistic and antagonistic defense responses. In some cases this cross-talk might contribute to fine-tune defense responses against some pathogens according to its mode of infection. Using some resistance elicitors such as acibenzolar-S-methyl (ASM), β-aminobutyricacid (BABA), cis-jasmone (CJ), and a combination of the three compounds, which involve SA and/or JA-dependent signaling pathways, study if these treatments are capable to control infection of spring barley by *Rhynchosporium commune* under field conditions [7].

Borges and co-workers propose priming crops as a way for controlling biotic and abiotic stresses and focus on the effect of the water-soluble vitamin K3 derivative, known as menadione sodium bisulphite (MSB), as a novel priming agent and as a tool for studying priming mechanisms. The work review the priming phenomenon and the importance of reactive oxygen species (ROS) as key signaling molecules that contribute to control of plant development as well as to the sensing of the external environment and priming induction [8].This method and their potential applications provide a new sustainable approach to crop protection. This technology currently can offer promising molecules capable to provide new long lasting treatments for crop protection against biotic or abiotic stresses.

One of the most promising approaches to improving plant resistance to unfavorable environmental factors is pre-primed seeds. During priming in order to increase stress tolerance in the plant that is of prime agricultural interest.

Presowing priming involves soaking seeds under controlled conditions until complete saturation with water or essential components for the further development of plants, seeds, followed by drying. It was found that the priming of seeds leads to early pipping seeds, increase the percentage of germination, synchronized, rapid growth of seedlings, improving the growth of plants in the growing season and during seed maturation, increased seed weight, and thus increases the yield and resistance to abiotic and biotic stress [9].It has been shown that the priming increases the synthesis of proteins, RNA, DNA in seeds, also increases the activity of antioxidant enzymes catalase, superoxide dismutase, peroxidase, ascorbate peroxidase, glutathione reductase[10, 11]..

The research on *Induced Resistance for Plant Defense* focuses on the understanding the mechanisms underlying plant resistance or tolerance since these will help us to develop fruitful new agricultural strategies for a sustainable crop protection.To date, insufficiently studied the molecular and biochemical mechanisms involved in the process of priming. We have been shown the regulation of antioxidant enzymes and aldehyde oxidase – an enzyme, carrying out the biosynthesis of plant hormone ABA, in terms of priming is of great importance in the prevention of pre-harvest sprouting seeds. ABA is a plant growth regulator involved in various processes, including response to environmental stress and seed maturation and dormancy [12]. Dormancy is a mechanism to prevent germination during unfavorable ecological conditions, when the probability of seedling survival is low [13] . A dormant seed is one that is unable to germinate for a specific period of time under a combination of environmental factors that are normally suitable for the germination of the non-dormant seed [14] . In cereal crops, an optimum balance between dormancy and non-dormancy is desirable. Dormancy at harvest is desired because it prevents the germination of the physiologically mature grain (i.e. PHS)in the head prior to harvest, a phenomenon that considerably lowers grain quality and is especially common in cool, moist environments. ABA regulates a number of key events during seed development, such as the deposition of storage reserves, prevention of precocious germination, acquisition of desiccation tolerance, and induction of primary dormancy [15].

In higher plants, ABA is derived from an epoxy-carotenoid precursor that is oxidatively cleaved to produce xanthoxin [16] . It is known that AO is involved in ABA biosynthesis, converting abscisic aldehyde to ABA, and the by-product of this reaction is superoxide.Following the cleavage, xanthoxin is converted to ABA by a series of ring modifications to yield abscisic aldehyde, which is oxidized to ABA by AO (EC 1.2.3.14), a molybdenum-containing enzyme [17].

This paper presents the results of the impact of pre-sowing grain priming maintenance plant hormone ABA, the activity of aldehyde oxidase (AO) and antioxidant enzymes in two different varieties of wheat.

Materials and methods

In our studies using wheat seeds are sterilized for 5 min in 1% NaClO, then washed thoroughly with distilled water.

Priming of wheat seeds was carried out according to the method of Rose [18] in our modification. Seeds of wheat, cultivar – NAZ and Saratovskaya – 29 were soaked in a solution of 50 mM Na2MoO4, 100 mM and 200mM within a day (24 hours) and then the seeds were dried at 25 ° C t = for 25-30 h.

Native electrophoresis of aldehyde oxidase performed in alkaline tris-glycine buffer system using 1 mm plates, 7.5% PAGE at 4 ° C for 4.5 hour at a constant current of 35 mA per gel. As a substrate used benzaldehyde and indole 3-aldehyde. [19].Electrophoresis division for glutathione reductase (GR) were carried out as for the AO; gel staining was performed on Pinhero et.al [20]. The intensity of the color bands of enzymes was determined by electronic program Scion Image.

Endogenous ABA content was carried out using a mono-clonal antibody from Sigma (USA) according to the attached methodological instructions.

The experiments were performed in 3-4-fold repetition.

Results and discussion

In our studies, we hypothesized that the priming of seeds with a relatively high concentration of molybdenum can lead to optimal saturation of seeds of wheat this element and to prevent molybdenum deficiency of the plant.

Accordingly, experiments to optimize the conditions of priming were performed optimum time of seed hydration were selected in Na2MoO4 salt solution,allowing them to absorb molybdenum to saturation and the optimal concentration of molybdate solution.

Optimal concentrations of soluble molybdenum salt Na2MoO4, which did not inhibit germination of the wheat seeds were selected. Seeds of wheat two varieties: resistent cultivar- NAZ and unstable – Saratovskaya – 29, soaked in a solution Na2MoO4 50 mM, 100 mM and 200 mm during the day -24 hour (Figure 1.).

Figure 1 shows that seeds of variety NAZ, hydrated in a solution of 50 mM Na2MoO4, showed high activity of the enzyme, indicating that the maximum saturation molybdenum enzyme. From electrophoregrameAO grain unstable cultivar-Saratovskaya- 29 shows that in the spectrum of this sort is not isozyme AO1. Since the hydration of seeds of both varieties in a 50 mM solution Na2MoO4 showed high (80-85%), germination of seeds, so the concentration of the solution was Na2MoO4 optimal hydration of seeds of both varieties.

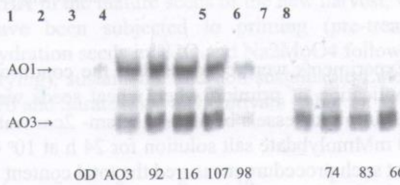

Figure 1 – AO activity in wheat grain NAZ (1 to 4) and 29 Saratovskaya (5 to 8), hydrated in a solution of Na2MoO4: 1 and 5 – Control (H2O); 2 and 6 – 50 mM Na2MoO4; 3 and 7 – 100 mM Na2MoO4; 4 and 8 -200 mM Na2MoO4.

In the next experiment we studied the activity of AO NAZ varieties of seeds in water and hydrated at 50 mM Na2MoO4 solution for 1 hour, 12 hour, 24 hour, 30 hour and 36 hour. (Figure 2).

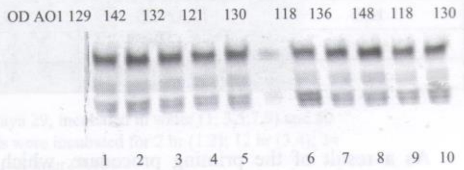

Figure 2 – Activity AO1 NAZ wheat grain, hydrated in H2O (1 to 5) and 50 mM Na2MoO4 solution (6 to 10)

Maximum activity was observed in seeds AO1 of NAZ cultivar, hydrated through water 12hours after soaking, and the maximum activity of the enzyme from corn, soaked in 50 mM Na2MoO4 solution was observed after 24 hours, i.e. maximum saturation molybdenum. Thus, it was shown that it is advisable to carry out the hydration of the seeds in 50 mM Na2MoO4 solution for 24 hours, as these optimal conditions hydrating seed will increase in ABA content of the caryopsis. The same conditions for seed hydration have been shown and for the variety Saratovskaya 29 (Figure 3).

Figure 3 shows that the seed varieties Saratovskaya 29, hydrated in water AO3 observed a decrease in activity, but the seeds of this variety shows high activity of AO 24 hours after soaking in a solution of 50 mM Na2MoO4.

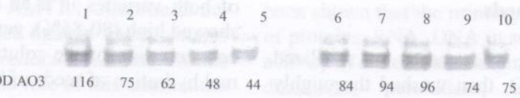

Figure 3 – activity AO3 grain wheat Saratov-29 hydrated in H2O (C1 to 5) and 50 mM Na2MoO4 solution (6 to 10)

Experiments were conducted in the conditions of application of priming when wheat seeds were placed into the vessels beneath the 1sm- 2cm water or 50 mMmolybdate salt solution for 24 h at 10° C. After t such procedure measured the total content of ABA in the embryo and endosperm of wheat were untreated control seeds (Table 1).

Excessive ROS formed in the conditions of application of priming, in embryonic tissues during hypoxia induces the synthesis of ABA and then cause the expression of protective genes, inducing the synthesis of antioxidant enzymes. Table 1 shows that the increase in ABA content of 10.5 times in seeds during hydration in 50 mM Na2MoO4 solution and 5.2 times by soaking the seeds in water varieties lutescens-70 compared to controls. Saratovskaya-29 showed that increased ABA content in 8 times and 4.5 times, respectively, in 50 mM Na2MoO4 and H2O solution and compared with controls.

Table 1 – The content of ABA (pmol / ml) in the embryo and endosperm of two wheat varieties in terms of priming

variant	Lutescens-70		Saratovskaya 29	
	ABA(pmol / ml) embryo	ABA(pmol / ml) endosperm	ABA(pmol / ml) embryo	ABA(pmol / ml) endosperm
H_2O	15,3±0,39	5,8±0,87	10, 04±0,31	2,32±0,12
Na_2MoO_4	37,8±0,48	5,2 ±0,63	18,35± 0,39	3,5 ± 0, 08
control	2,94±0,07	1,14±0,54	1,75± 0,06	0,95± 0,04

As a result of the priming procedure, which is accompanied by the formation of excess free radicals induced defensive responses in plant cells. In order to control the level of free radicals and protecting cells during exercise stress, the plant tissue containing antioxidant enzymes such as superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, etc.

Glutathione reductase (GR) – an enzyme which participates in the conversion of oxidized glutathione in reduced form. The active participation of ascorbate system – glutathione cycle to neutralize ROS and maintaining the redox – balance shown in many examples [20].

We carried out a study of the activity of the GR in two varieties of winter wheat varieties NAE and spring wheat varieties Saratov 29 during priming.

Figure 4 shows the evolution of the activity of the GR NAZ wheat grains during different periods of incubation of seeds in water and a solution of 50 mM Na2MoO4.

On electrophoregram of spectrum GR shown that the activity of the GR in the seeds of wheat resistant variety to the the two forms of isoenzymes GR 1 and GR 2 for 12 hours of incubation, the seed activity practically unchanged and the value of enzyme activity are almost identical, indicating that the equilibrium balance reduced and oxidized glutathione. Incubation of seeds within 24hours and 32 hours during this period increased GR activity and almost no imbalance reduced and oxidized glutathione, wheat varieties NAZ. During 48 hours of incubation the seed, the enzyme activity decreases. Therefore NAZ variety seeds can be incubated for 32 h in this case will not occur oxidation of DNA, RNA and protein molecules important.

Figure 5 shows that Activityof GR Saratovskaya 29 lower than NAZ winter varieties and seed throughout the incubation period (48 h), gradually decreases. Also, as for the NAZ varieties, seed varieties Saratovskaya – 29 during the 12 hour incubation, the seeds show high activity and the activity of isoenzymes GR1 and GR2 content reduced and oxidized forms of glutathione almost equal. Incubation of the class of seed in water and a solution of molybdate reduces isoenzyme activity GR1 and GR2 activity increases slightly. This suggests that further hydrating the seeds for 32-48 hours results in a significant accumulation of ROS and GR activity decreases and thus accumulate oxidative radicals. Based on the data, seed varieties Saratovskaya 29-hydration is not recommended , to subject more than 24 hours in the salt solutions and H2O. Thus, seeds soaked in a solution of molybdenum in priming results in the formation of seeds enriched in molybdenum. This allows AO to increase the level of ABA in maturing seeds of wheat and thus producing stable seedlings to abiotic stresses.

In our experiments we measured the content of ABA in the mature seeds of the new harvest, which have been subjected to priming (pre-treatment hydration seeds in H2O and Na2MoO4 followed by drying), sustainable and non-sustainable Lutescens -70 and Saratovskaya29 cultivars (table 2).

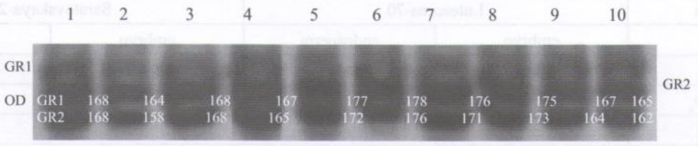

Figure 4 – GH activity in grain wheat NAZ incubated in water (1, 3,5,7,9) and a solution of 50 mM Na2MoO4 (2, 4, 6, 8, 10). The seeds were incubated for 2 hr (1.2); 12 hr (3.4); 24 h (5,6); 32 h (7,8).; 48 hour (9,10)

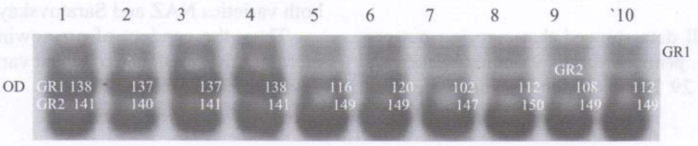

Figure 5 – GR activity in grain wheat Saratovskaya 29, incubated in water (1, 3,5,7,9) and 50 mM Na2MoO4 solution (2, 4, 6, 8, 10). The seeds were incubated for 2 hr (1.2); 12 hr (3.4); 24 h (5,6); 32 h (7,8).; 48 hour (9,10)

Table 2 – Content of ABA (pM / ml) in the grain of the new harvest, the resistant variety Lutescens 70 and unstable – Saratovskaya 29

Variants	Lutecsens-70 (grain)	Saratovskaya29 (grain)
H_2O	4, 07 ± 0,20	2,10±0,17
Na_2MoO_4	5,85 ± 0,28	3,61±0,19
control	2,93 ± 0,19	1,84±0, 12

The presented data show that endogenous ABA content of the resistant variety Lutescens-70 and sensitive varieties Saratov 29, raised grain of the new harvest of wheat in 2 times in comparison with the control variant. It is also shown that a stable grade than for endogenous phytohormone ABA content is 1.5 times higher than the grade unstable Saratov 29. In embodiments with a molybdenum content of ABA observed maximum grain varieties both compared with the control. These results show that the priming procedure significantly increases the level of stress hormone ABA in seeds in two contrasting varieties of wheat

The correlation between the activity of AO and ABA accumulation in the grain of wheat Lutescens -70 was confirmed conducted native electrophoresis, substrate for AO served aldehyde indole-3 (Figure 6).

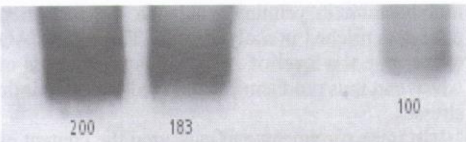

Figure 6 – Active AO in mature seeds of a new crop wheat Lutescens-70 variants: 1 – Na2MoO4; 2 – H2O; 3 – control

In the application of priming seeds of wheat increased resistance, by increasing the content of endogenous ABA and antioxidants. The main water-soluble antioxidants in plant cells are ascorbic acid and glutathione, and antioxidants zhirorast-vorimye – carotenoids, tocopherols and flavonoids. Our results showed that against oxidative stress in cells in priming conditions synthesized enzymatic and non-enzymatic antioxidants (Table 3)

Table 3 – The total content of water and fat-soluble antioxidants in mature wheat seed varieties Lutescens-70 and Saratovskaya – 29

Variants	Lutescens-70		Saratovskaya-29	
	embryo	endosperm	embryo	endosperm
H2O	139.7 ± 3.7	49.4 ± 5.3	95.1 ± 2.7	44.1 ± 4.5
Na_2MoO_4	153.5 ± 6.2	48.8 ± 3.2	107.9 ± 5.1	51.7 ± 3.8
Control	107.3 ± 2.5	46.7 ± 1.7	86.4 ± 2.8	53.2 ± 3.7

These data show a high antioxidant content in mature seeds of the new crop wheat, in both embodiments, compared with untreated wheat seeds (Control).

Views will data showed that carrying out pre-sowing seed priming both varieties NAZ and Saratovskaya 29 led to an increase in the content of endogenous ABA in 2 times and 1.5 times the antioxidants and as a result has led to an increase in resistance to stress (adverse environmental factors) both varieties NAZ and Saratovskaya-29.

Thus, the conduct of pre-sowing seed priming increases the resistance of wheat varieties to adverse environmental factors.

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2-бөлім

ҚОРШАҒАН ОРТА ЛАСТАУШЫЛАРЫНЫҢ БИОТАҒА ЖӘНЕ ТҰРҒЫНДАР ДЕНСАУЛЫҒЫНА ӘСЕРІН БАҒАЛАУ

Раздел 2

ОЦЕНКА ДЕЙСТВИЯ ЗАГРЯЗНИТЕЛЕЙ ОКРУЖАЮЩЕЙ СРЕДЫ НА БИОТУ И ЗДОРОВЬЕ НАСЕЛЕНИЯ

Section 2

ASSESSMENT OF ENVIRONMENTAL POLLUTION ON BIOTA AND HEALTH