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THE INFLUENCE OF 24-EPIBRASSINOLIDE ON HEAT RESISTANCE AND PRODUCTIVITY OF MILLET (*Panicum miliaceum*) PLANTS

A. A. Vayner[1], N. N. Miroshnichenko[1], Yu. E. Kolupaev[1], T. O. Yastreb[1], V. A. Khripach[2] , Yu. A. Sotnikov[1]

[1]V.V. Dokuchaev Kharkiv National Agrarian University
(Kharkiv, Ukraine)
[2]Institute of Bioorganic Chemistry
National Academy of Sciences of Belarus
(Minsk, Belarus)

The effects of pre-sowing treatment of seeds with the solutions of 24-epibrassinolide (24-EBL) on productivity of millet plants (*Panicum miliaceum L.*) and their resistance to high temperature were investigated. An increase in the resistance of seedlings grown from seeds treated with 24-EBL to potentially lethal heating was shown. Also an increase in thermal resistance under the influence of phytohormone in leaves of adult plants grown in the field conditions was observed. The leaves of millet plants, grown from seeds treated with 24-EBL indicated higher chlorophyll content and decreased level of lipid peroxidation product malondialdehyde as compared to control. It was assumed that these effects are caused by the positive influence of the 24-EBL on the functioning of the antioxidant system of millet plants. It was also shown that pre-sowing treatment of seeds with 24-EBL increased the grain productivity of millet plants by 10-14%.

Key words: *Panicum miliaceum L., 24-epibrassinolide, heat resistance, stress-protective systems, productivity*

Brassinosteroids (BS) are phytohormones with a wide range of stress-protective effects. Possibility for the industrial synthesis of BS provides conditions for their practical use as stress-protective compounds. Currently, preparations based on brassinolide, 24-epibrassinolide (EBL), 28-homobrassinolide, 28-norbrassinolide and other BS under different trade names are produced in China, Japan, India, Russia, Belarus and other countries (Kamuro, Takatsuto, 1999; Zhao, Chen, 2003; Budykina et al., 2012). One important advantage of BS is their environmental safety and ability to induce physiological effects at lower compared to other phytohormones concentrations (Efimova et al., 2014).

Positive effect of exogenous BS on plant resistance to stressors is established in many studies. Thus the treatment with BS caused increase in resistance of cucumber, cabbage, corn and other species to low temperatures (Fariduddin et al., 2011; Jiang et al., 2013).

Under the influence of BS the tolerance of plants of different taxonomic groups to high temperatures was also induced (Kagale et al., 2007; Divi et al., 2010). An increase in the survival of plants after potentially lethal effect of hyperthermia (Singh, Shono, 2005; Mazorra et al., 2011), as well as their maintenance of ability to grow under sublethal effects of high temperatures (Ogweno et al., 2008) after treatment with BS was registered.

On a number of plant species an increased resistance to osmotic stress under influence of BS was shown (Kagale et al., 2007; Li et al., 2012). BS contributed to keeping close to the normal water content in tissues under conditions of water stress (Yuan et al., 2010), positively influenced the photosynthetic activity of plants (Li et al., 2012).

A positive effects of 24-EBL on seed germination, growth and chlorophyll content under the influence of salt stress in plants of *Brassica* genus were observed (Kagale et al., 2007; Ali et al.,

Adress for correspondence: A. Vayner, V.V. Dokuchaev Kharkiv National Agrarian University, p/o"Communist-1", Kharkiv, 62483, Ukraine;
e-mail: plant_biology@mail.ru

2008; Efimova et al., 2014). Under salinity conditions in *Cajanus cajan* plants an increase in nitrate metabolization, elevated levels of amino acids and protein were registered under the influence of BS (Dalio et al., 2013). At early stages of plant development an increased mitotic activity of meristematic root cells and enhanced growth of the aerial part of wheat seedlings under salinity were observed (Avalbaev et al., 2010).

Stress-protective and growth effects of BS determine their positive influence on plant productivity (Khripach et al., 2003). At the same time, the physiological effects of the BSs depend on species and even cultivar of studied plant (Shahbaz et al., 2008). In this regard, the investigation of specific features of BS action on current plant has to precede the practical application of these phytohormones.

The physiological effects of BS on millet (*Panicum miliaceum L.*) have been poorly studied. Recently, laboratory experiments have shown a positive effect of BS on the resistance of this species to salt stress (Vayner et al., 2014). The aim of this work was to investigate the effect of pre-sowing treatment with 24-epibrassinolide (24-EBL) on the resistance of millet plants to hyperthermia and their productivity in model field experiments.

MATERIALS AND METHODS

Before experiments the surface of millet seeds (cv. Konstantinovskoe) was decontaminated by the immersion into 5% hydrogen peroxide solution for 15 min. Thereafter, seeds were rinsed with distilled water. Pre-sowing treatment was performed by 1-day immersion of seeds into the solution of 24-EBL, control variants were treated with distilled water. Then they were dried on sieves during the day.

In first experimental series the effect of 24-EBL on thermal resistance of four-day (at the start of experiments) millet seedlings in the lab was investigated. The heat stress was created by the warming up of seedlings in ultrathermostat bath at a temperature of 47°C for 10 min. One day after the heating seedlings were exposed to ambient light (3-4 klux) and after another 5 days were evaluated for their survival.

The effect of BS on millet plant productivity was investigated in model field experiments in 2014. There were two independent experiments conducted on unfertilized areas. The first one was carried out on plots located on the Experimental Field of V.V. Dokuchaev Kharkiv National Agrarian University. The soil type was chernozem typical heavy loamy which contents 5,47% humus, amount of available phosphorus and potassium 135 and 89 mg/kg respectively in the arable layer, pH_{KCl} – 5,30 (high level of fertility). The second experiment was carried out on the site with anthropogenically disturbed soil layer, characterized by somewhat lower humus content (3,55%), much lower amount of available phosphorus (61 mg/kg) and alkaline pH_{KCl} values (7,38). Available potassium content was 100 mg/kg. The area of every sample plot in both experiments was 2,5 m^2.

Weather conditions during plant vegetation have been relatively favorable. Precipitation amount was 1,2 times higher than the multiyear averages value. The temperature was 3 degrees above the multiyear averages value.

Chlorophyll content (Shlyk, 1971) and the product of lipid peroxidation (LPO), malondialdehyde (MDA) (Merzlyak et al, 1978) were determined in June and July in second leaves from the top of plants grown in the field.

Heat resistance of plants was estimated using modified Matskov method (Matskov, 1976). 5 cm segments of second (from top) leaf laminae were warmed up at 40°C in water ultrathermostat bath for 30 min with a consequent increasing of the heating temperature for 5°C every 10 min up to 55°C. After cooling samples were placed into 0,2 n HCl solution for 20 minutes for the manifestation of damage. Determination of lamina injury included imaging segments with a scanner, the spectral analysis of color of obtained image, its evaluation by analyzing the frequency distribution function of the intensity of the red component of leaf color by its decomposition into two Gaussian components, characterizing the affected and unaffected fraction of the surface, using the software (Pat. № 47439).

Laboratory experiments were carried out in 4-6 replicates, each experiment was repeated at least three times independently, field experiments had 4 replicates. The average means and their standard errors or least significant differences ($LSD_{0,05}$) are shown.

RESULTS AND DISCUSSION

The treatment of seeds with 24-EBL solutions of wide concentration range (from nanomolar to micromolar) caused an increase in the survival of millet seedlings after potentially lethal heating (Fig. 1). The most effective concentration was 20 nM. It was used for the following investigations of 24-EBL effects in model field experiments.

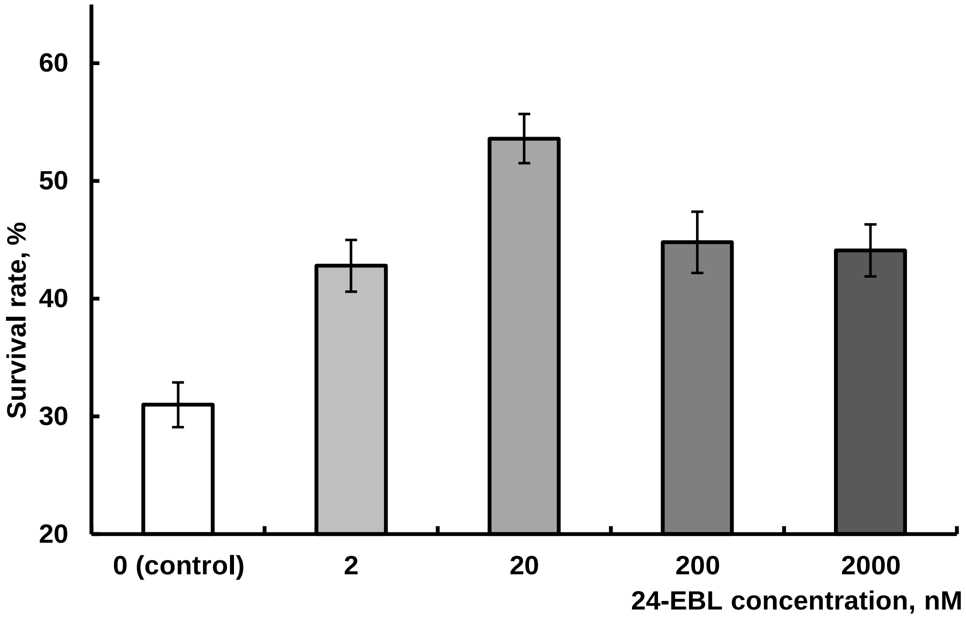

Fig. 1. Effect of 24-EBL on the survival of millet seedlings (%) after damaging heating (47°C, 10 min)

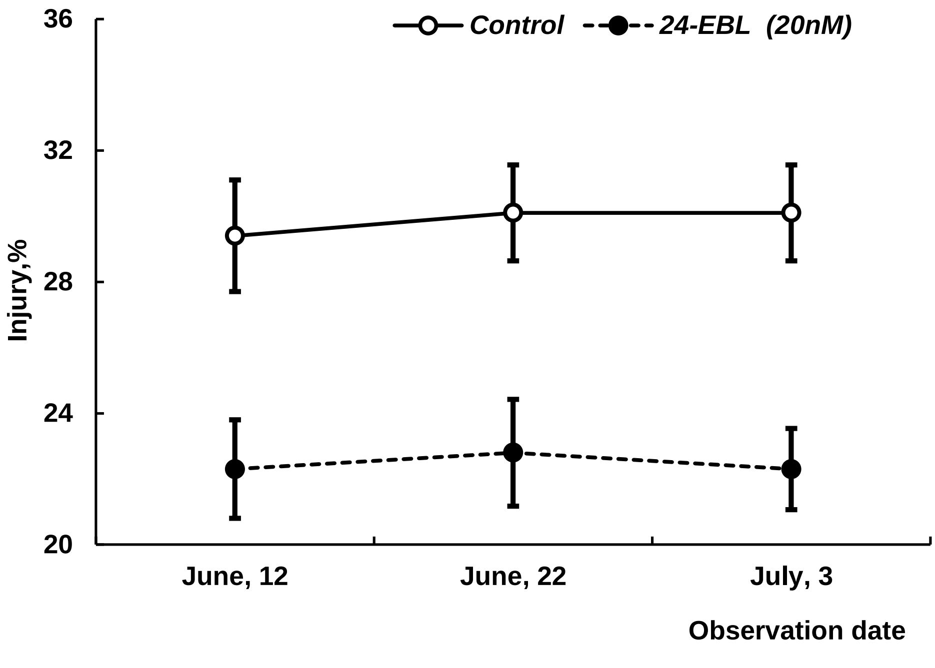

Fig. 2. Injury of leaf tissue of millet plants after heating (% from control without warming-up)
Note. Here, and in Fig. 3 and 4 the results of experiments conducted on plots with anthropogenically disturbed soil are shown (see section "Methods").

Seed pre-treatment with 24-EBL caused prolonged physiological effects. For example, in the experiment conducted in the area with anthropogenically disturbed soil layer in June and July heat resistance of leaves from pretreated plants was significantly higher than in ones from control (Fig. 2). Similar results were obtained on plants grown in more favorable conditions in the experimental field (data are not shown).

The amount of lipid peroxidation product MDA in leaves of plants from control group during observation period fluctuated a bit, which could be connected with age-related changes in leaves, as well as with the natural changeability in conditions of field experiment (Fig. 3). At the same time at all stages of the experiment in leaves of plants grown from seeds treated with 24-EBL, MDA content was lower than in the control ones. Experi-

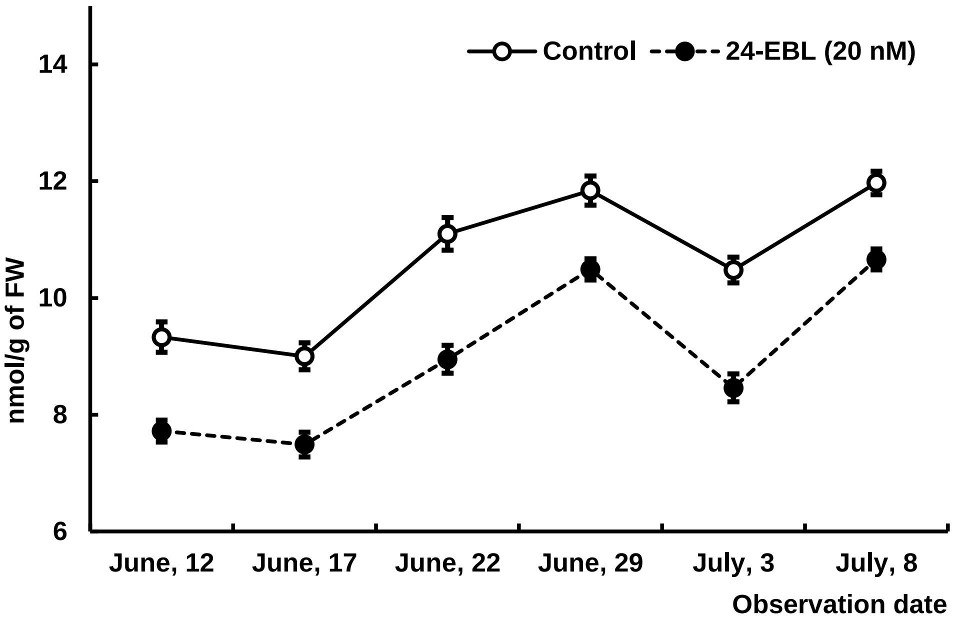

Fig. 3. Content of MDA in millet leaves (nmol/g of fresh weight)

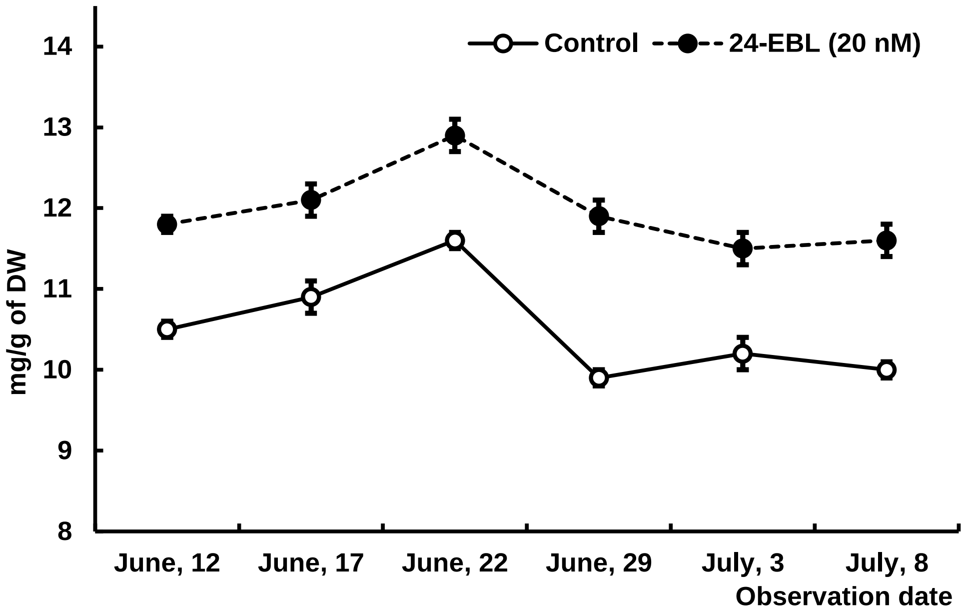

Fig. 4. Total chlorophyll (*a* and *b*) content in millet leaves (mg/g of dry weight)

mental plants during the observation period also exhibited higher total chlorophyll content (Fig. 4).

Investigation of the effect of growth regulators on the integral indicator such as productivity provides information about the complex adaptive action on plants. Grain productivity of millet plants grown in the experimental field (under favorable conditions), in the pretreated with 24-EBL variants exceeded control values by 10% (table). At the same time the parameters of productive panicles number in plants and grain yield per panicle increased by 9-10%.

Productivity of plants grown in the area with athropogenic disturbance of soil was more than two-fold lower compared to plants cultivated in normal field conditions (Table). At the same treatment with 24-EBL increased grain productivity by 14%. This effect was achieved due to a significant (20%) increase in grain yield per panicle.

Thus, in general, pre-sowing treatment of millet seeds with 24-EBL positively influenced the resistance and productivity of plants. This effect was observed both under the estimation of resistance of seedlings to potentially lethal heating in

Effect of 24-EBL on grain productivity and yield structure of millet

Variant	Number of productive panicle on stem	Grain yield per panicle, g	Crop capacity, g/m^2
Experimental field (favorable conditions)			
Control	1,03	1,66	239,3
24-EBL, 20 nM	1,12 (109)	1,83 (110)	264,2 (110)
$LSD_{0,05}$	0,09	0,12	19,1
Antropogenically disturbed soil (adverse conditions)			
Control	1,11	0,54	103,2
24-EBL, 20 nM	1,12 (101)	0,65 (120)	117,3 (114)
$LSD_{0,05}$	0,12	0,10	11,5

Note. In brackets – % to control.

the lab (Fig. 1), and under evaluating of the heat tolerance of leaves of plants grown in the field (Fig. 2).

Antioxidant system can be one of the stress-protective mechanisms that are induced by BS and contributing to the decrease in damage which is caused by the action of hyperthermia on plants (Fariduddin et al., 2014). Thus, in a number of papers (Ogweno et al., 2008; Li et al., 2012; Talaat, Shawky, 2013) a positive effect of BS on the activity of superoxide dismutase (SOD), the only enzyme neutralizing superoxide anion radical, was established (Shao et al., 2008). In young millet plants the increase of SOD, catalase and guaiacol peroxidase activity under the influence of 24-EBL in physiologically normal conditions and under the influence of salt stress was shown (Vayner et al., 2014). The positive influence of pre-sowing treatment of seeds with 24-EBL on the functioning of the antioxidant system was manifested in adult plants grown in the field. This is evidenced by consistently lower MDA content in leaves of the experimental variant compared to the control (Fig. 3). A significant reduction of MDA levels under the influence of BS was shown in rape plants under salt stress conditions (Efimova et al., 2014).

The decrease in the intensity of lipid peroxidation in the variant with 24-EBL pretreatment through more efficient functioning of the antioxidant system can promote preservation of pool of photosynthetic pigments. This is indicated by our obtained data on the increase in the content of chlorophyll in leaves of plants pretreated with this phytohormone (Fig. 4).

Stress-protective effects of 24-EBL seem to be an important component of its positive effect on the productivity of millet plants (table). It should be noted that effects of pre-sowing treatment with BS were prolonged. It is possible that they are based on changes in the functioning of hormonal system of plants caused by the BS in the early phases of development. Such effects in the future may contribute to changes in ontogenetic programs in plants (Shakirova, 2001). It is known that exogenous BS can cause the accumulation of cytokinins in plants (Yuldashev, 2009). It was also shown that exogenous BS can induce the synthesis of other plant hormones of stress, in particular, jasmonic acid (Mussig et al., 2000) and ethylene (Avalbaev et al., 2010). Naturally, the significance of changes in the content of other plant hormones in the implementation of the prolonged influence of exogenous BS to millet plant requires special investigations.

In general, pre-treatment of seeds with 24-EBL had a positive effect on the resistance and productivity of millet plants. The action of the plant hormone for grain productivity was more significant in adverse conditions - under cultivation of plants in anthropogenically disturbed soil (Table).

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ВПЛИВ 24-ЕПІБРАСИНОЛІДУ НА ЖАРОСТІЙКІСТЬ І ПРОДУКТИВНІСТЬ РОСЛИН ПРОСА (*Panicum miliaceum*)

А. О. Вайнер[1], М. М. Мірошниченко[1], Ю. Є. Колупаєв[1],
Т. О. Ястреб[1], В. О. Хрипач[2], Ю. О. Сотников[1]

[1]Харківський національний аграрний університет ім. В.В. Докучаєва
(Харків, Україна)
e-mail: plant_biology@mail.ru
[2]Інститут біоорганічної хімії
Національної академії наук Білорусі
(Мінськ, Білорусь)

Досліджували вплив передпосівного замочування насіння у розчинах 24-епібрасиноліду (24-ЕБЛ) на продуктивність рослин проса (*Panicum miliaceum* L.) та їх стійкість до високої температури. Показано підвищення стійкості проростків, вирощених з насіння, обробленого 24-ЕБЛ, до потенційно летального прогріву. Також виявлено підвищення під впливом фітогормону терморезистентності листків дорослих рослин, вирощуваних у польових умовах. У листках рослин проса, вирощених з насіння, обробленого 24-ЕБЛ, відзначався вищий порівняно з контролем вміст хлорофілів і знижений вміст продукту пероксидного окиснення ліпідів малонового діальдегіду. Висловлено припущення, що ці ефекти зумовлені позитивним впливом 24-ЕБЛ на функціонування антиоксидантної системи рослин проса. Показано, що передпосівна обробка насіння 24-ЕБЛ підвищувала зернову продуктивність рослин проса на 10-14 %.

Ключові слова: *Panicum miliaceum L., 24-епібрасинолід, жаростійкість, стрес-протекторні системи, продуктивність*

ВЛИЯНИЕ 24-ЭПИБРАССИНОЛИДА НА ЖАРОУСТОЙЧИВОСТЬ И ПРОДУКТИВНОСТЬ РАСТЕНИЙ ПРОСА (*Panicum miliaceum*)

А. А. Вайнер[1], Н. Н. Мирошниченко[1], Ю. Е. Колупаев[1],
Т. О. Ястреб[1], В. А. Хрипач[2], Ю. А. Сотников[1]

[1]Харьковский национальный аграрный университет им. В.В. Докучаева
(Харьков, Украина)
e-mail: plant_biology@mail.ru
[2]Институт биоорганической химии
Национальной академии наук Беларуси
(Минск, Беларусь)

Исследовали влияние предпосевного замачивания семян в растворах 24-эпибрассинолида (24-ЭБЛ) на продуктивность растений проса (*Panicum miliaceum* L.) и их устойчивость к высокой температуре. Показано повышение устойчивости проростков, выращенных из семян, обработанных 24-ЭБЛ, к потенциально летальному прогреву. Также выявлено повышение под влиянием фитогормона терморезистентности листьев взрослых растений, выращиваемых в полевых условиях. В листьях растений проса, выращенных из семян, обработанных 24-ЭБЛ, отмечалось более высокое по сравнению с контролем содержание хлорофиллов и пониженное содержание продукта пероксидного окисления липидов малонового диальдегида. Предполагается, что эти эффекты обусловлены положительным влиянием 24-ЭБЛ на функционирование антиоксидантной системы растений проса. Показано, что предпосевная обработка семян 24-ЭБЛ повышала зерновую продуктивность растений проса на 10-14 %.

Ключевые слова: *Panicum miliaceum L., 24-эпибрассинолид, жароустойчивость, стресс-протекторные системы, продуктивность*