PHYTOHORMONAL REGULATION OF GASES EXCHANGE IN *BETA VULGARIS* L. DURING WATER STRESS[*]

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The paper assesses the impact of abscisic acid (ABA) and cytokinin benzylaminopurine (BAP) on the control of exchange of gases in the leaves of sugar beet during water stress. The pot experiments with young plants of the Takt variety were carried out under controlled conditions in a greenhouse. ABA in the concentration of 100 µM and BAP in the concentration of 1 µM and 10 µM were applied to the leaves 3 days after the plants were divided into variants and after irrigation was interrupted. The relative water content (RWC) in leaves, the photosynthetic rate (P_N), the transpiration rate (E) and the water use efficiency (WUE) were measured just before the application of the growth regulators and then 2 hours and 1, 3, 5, 7 and 9 days after the application of the growth regulators. Throughout this period, the plants retained high RWC but there was a statistically significant decrease of P_N and E and also an improvement of WUE in comparison with the non-stressed plants. The application of ABA first decreased P_N and E in the stressed plants, and deterioration of WUE occurred (2 and 24 hours after the application). At later dates, the levels of P_N and E obtained by measurement were, conversely, higher and WUE was approximately the same in comparison with the plants that were stressed but not treated with ABA. The application of BAP on plants stressed by drought resulted, throughout the monitored period, in statistically significantly higher levels of P_N and E in comparison with the plants not treated by BAP. In the case of joint application of both substances, the identified levels of P_N and E were statistically provably higher than when ABA was applied alone; these levels were almost the same as the levels measured after the application of BAP alone.

sugar beet; water stress; photosynthetic rate; transpiration rate; abscisic acid; benzylaminopurine

INTRODUCTION

Drought is one of the most serious problems of agricultural production on the global scale. Sixty-one % of the area on Earth has precipitation lower than 500 mm (D e n g et al., 2005). Drought results in discordance between the intake and output of water by a plant and in disruption of the water balance and the plants' requirements for water during their ontogenesis. If the output of water through transpiration is higher than the intake through roots, water stress occurs. The plants fade and most of them close their stomata in order to reduce transpiration and prevent water loss. Nevertheless, this action also limits the entry of CO_2 into leaves. The concentration of CO_2 in chloroplasts decreases. In the relation between O_2 and CO_2, photorespiration is given the priority. As a consequence, the photosynthesis rate decreases (for example, F l e x a s et al., 2004, 2006).

Atmospheric drought has a direct effect on leaves and the stomatal apparatus. The soil drought occurs gradually. Gradual dehydration of the soil substrate makes a plant hyper-produce abscisic acid (ABA). It also brings about fast changes in the activity of enzymes, production of stress proteins called dehydrins, production of osmotically active substances, including the amino acid of proline, etc. The plant can be gradually osmotically adapting itself and redistributing water in its organs, unless the wa-

ter stress is too strong and the integrity of the plant's physiological systems is not affected. Thus, the plant retains a high relative water content for a longer period of time (C l o s e, 1996; B r e s t i č, O l š o v s k á, 2001; S a n c h e z - U r d a n e t a et al., 2005; H a y, P o r t e r, 2006). Osmotic adaptation (adjustment) of the tissues to the water deficit maintains, during low water potentials, the gradients of water flow and the intake of water from soil. It also allows for stomatal adaptation and continuation of transpiration and photosynthesis (B r e s t i č, O l š o v s k á, 2001; G o n z a l e z et al., 2008).

The openness of stomata is usually in a closer correlation with the soil water content than with the leaf water content. The role of the chemical signal between the roots and the leaves is probably played by abscisic acid (ABA). This is synthesised mainly in mature leaves and roots and it can control the opening and closing of stomata independently on the hydraulic effect (P o s p í š i l o v á, 2003; W i t t e n m y e r, M e r b a c h, 2005; J i a n g, H a r t u n g, 2008).

Exogenously applied ABA also induces the closing of stomata. It thus influences the photosynthetic and transpiration rates. During soil drought, it improves the plants' water management and maintains their water status stable for a longer period of time. Thus, ABA has a favourable effect on the resistance of plants to drought (P o s p í š i l o v á, 2003). Participation of ABA in control of stomata

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has been accepted mainly in isohydric plants, as confirmed, for example, by the works of Š a f r á n k o v á et al. (2007) with barleys, or by the works of S t u c h l í -k o v á et al. (2007) and Č e s k á et al. (2008) with maize. These are plants with a balanced water status, without large fluctuations of their water potential throughout the day. As P o s p í š i l o v á (2003) also writes, the more recent data prove the participation of ABA in the closing of stomata even in anisohydric plants (such as sunflower, sugar beet). In them, there are stronger fluctuations in water content, concentration of cell solution and water potential during the day. This fact has been confirmed, for example, by the works of P o s p í š i l o v á and B a ť k o v á (2004) or H e j n á k et al. (2008).

Other phytohormones can also play a role in the control of the openness of stomata, especially in interaction with ABA. Cytokinins or ethylene can limit or postpone the closing of stomata induced by abscisic acid or inhibit the accumulation of ABA induced by water stress (P o s p í š i l o v á et al., 2000, 2005; T a n a k a et al., 2005; S t u c h l í k o v á et al., 2007). On the contrary, jasmonates can stimulate this action (S t o l l et al., 2000). Cytokinins are probably involved in the communication between roots and stems during water stress. Their constant supply from roots is necessary to maintain the maximum openness of stomata. Reduction of this supply is a sensitive signal that roots are under stress (I t a i , 1999).

Some studies mentioned insensitivity of stomata to the supplied cytokinins. The reason was probably the fact that there already was the optimum concentration of endogenous cytokinins for the opening of stomata. Another explanation is that cytokinins support the opening of stomata most effectively in the photosynthetically mature leaves and most scientists used the youngest fully developed leaf. The objective of this work was to obtain new knowledge about the effect of ABA and cytokinin benzylaminopurine (BAP) applied during a water deficit on the exchange of gases in the leaves of stressed plants *Beta vulgaris* L.

MATERIAL AND METHOD

Young plants of sugar beet, the Takt variety, were used for the experiment (*Beta vulgaris* L., ssp. *vulgaris* var. *altissima* Döll). The experiments were taking place in a physiological greenhouse under controlled conditions: with a 14-h photo-period (14 h of light / 10 h of darkness), in temperatures of between 20 and 25 °C during the day and between 16 and 20 °C at night, and the relative air humidity was between 50 and 60%.

In the pots filled with soil, 5 plants were grown in the area of 0.05 m$^{2.}$ Before the experiment was started, the plants were divided into 6 variants with 4 repetitions:

1. the control variant: plants irrigated throughout the experiment
2. plants stressed by drought: complete interruption of irrigation at the beginning of the experiment when the plants were 8 weeks old

3. plants stressed by drought + 3 days after interruption of irrigation, 100 µM solution of ABA was applied
4. plants stressed by drought + 3 days after interruption of irrigation, 1 µM solution of BAP was applied
5. plants stressed by drought + 3 days after interruption of irrigation, 10 µM solution of BAP was applied
6. plants stressed by drought + 3 days after interruption of irrigation, 10 µM solution of BAP and 100 µM solution of ABA were applied.

With its nature, the experiment builds on the experimental works published by P o s p í š i l o v á et al. (2001), P o s p í š i l o v á , B a ť k o v á (2004) and P o s p í š i l o -v á et al. (2005). In their experiments, ABA and cytokinins had been applied on the plants before the water stress was induced, and therefore the plants in the control variants were treated with water. In the experiment, which is presented in this article, ABA and BAP were applied to the plants until during the water stress. For this reason and for the purpose of assessment of the practical impacts of the application of these growth regulators, the plants irrigated throughout the experiment (variant 1) and plants with interrupted irrigation, which were not treated with water during the stress (variant 2), were used as the control groups. In the control variant (variant 1), the soil moisture was maintained at 60–70% of the maximum capillary water capacity throughout the experiment. The amount of irrigation water necessary for ensuring this level was identified gravimetrically by continual weighting of the pots, in which plants were grown. In the groups 2 through 6, irrigation was interrupted when the plants were 8 weeks old (with 5 to 6 leaves). The growth regulators (benzylaminopurine and abscisic acid, including their combination) were applied by a sprinkler on the plants' leaves 3 days after interruption of irrigation. 1 µM and 10 µM concentration levels of benzylaminopurine (BAP) and 100 µM concentration level of abscisic acid (ABA) were used in an application dose, which according to conversion, corresponded to 100 cm^3 m^{-2}. Citovett was used as the soaking agent.

The responses of plants to the changes of moisture content in the soil were continuously monitored in all variants, at the level of physiology of leaves by gravimetrical identification of the relative water content in the leaves by means of the leaf-disk method according to Č a t s k ý (1960) and by gasometrical measurement of the photosynthetic rate (P_N) and the transpiration rate (E) (F l e x a s et al., 2004).

The relative water content (RWC) was identified by weighing the leaf disks in fresh state, after saturation with water and after drying them up, using the following formula:

RWC = [(fresh weight – weight of dried matter) / (weight in full saturation – weight of dried matter)] × 100 %. Eq. (1)

The photosynthetic and transpiration rates were identified in intact leaves by means of a commercial portable gasometrical infrared analyser LCA-4 (*ADC Bio Scien-*

tific Ltd., Hoddesdon, UK) with a leaf chamber *LC4/PLC4BT-1/E*.

To calculate the photosynthetic rate (P_N), the following formula was used:

$$P_N = u_s \, \Delta c \; (\mu mol \; CO_2 \; m^{-2} \; s^{-1}), \; Eq. \; (2)$$

where Δc is the difference between the CO_2 concentration levels and the inlet and outlet of the chamber ($\mu mol \; mol^{-1}$) and where u_s is the amount of air flow per m^2 of leaf area ($mol \; m^{-2} \; s^{-1}$).

To calculate the transpiration rate (E), the following formula was used:

$$E = u_s \, \Delta W \; (mmol \; H_2O \; m^{-2} \; s^{-1}), \; Eq. \; (3)$$

where ΔW is the difference between the concentration levels of water vapour at the inlet and outlet of the chamber ($mol \; mol^{-1}$) and where u_s is the amount of air flow per m^2 of leaf area ($mol \; s^{-1} \; m^{-2}$).

The water use efficiency was calculated as a relation between the photosynthetic rate and the transpiration rate:

$$WUE = P_N/E \; (mmol \; CO_2 \; mol^{-1} \; H_2O). \; Eq. \; (4)$$

The physiological parameters were always measured in 3 fully developed leaves in each experimental pot. The first determination of P_N and E was carried out 3 days after the plants were divided into variants and after irrigation was interrupted, just before the application of the growth regulators (3 sd), then after another 2 hours (3 sd + 2 h) and then 1, 3, 5, 7 and 9 days after the application of the growth regulators (4 sd, 6 sd, 8 sd, 10 sd and 12 sd). This was always measured in the morning between 10.00 and 12.00. In the assimilation chamber, the temperature was 25°C, radiation was 750 $\mu mol \; m^{-2} \; s^{-1}$, air humidity was 50% and CO_2 concentration was 350 $\mu mol \; mol^{-1}$ during the measurements. The levels were always measured after 1 minute. From the whole series of measurements, a set of 8 measurements was selected, during which the conditions inside the chamber stabilised (i.e. after 10–14 minutes), that is, when the measured photosynthetic rates were steady. These 8 figures were used to calculate the mean, and the individual means were then included in the assessed results.

The statistical assessment was carried out by means of a variance analysis with $\alpha = 0.05$ in the computer software Statistica, version 6.1 CZ, module ANOVA. The tables show the mean levels obtained from measurements in three series of experiments, including the statement of standard errors.

RESULTS

The total water output through evapo-transpiration in the control variant 1 was at the level of 800–1,000 g H_2O per pot during the 12 assessed days in the 3 series of experiments. In order to maintain the soil moisture at the level of 70% of the maximum capillary water capacity during this period, this water was continually replenished by irrigation. During the 3 series of experiments, in the variants with interrupted irrigation (variants 2–6), evapo-transpiration reduced soil moisture on the average down to 50–60% of the maximum capillary water capacity at the end of the monitored period. As Table 1 shows, this reductiondid not result in a statistically significant decrease of the RWC level in the young plants of sugar beet, the Takt variety.

In the irrigated control variant (variant 1), the mean levels of RWC from 85.5% to 89.8% were measured at individual points in time. In the variant with interrupted irrigation (variant 2), levels of RWC between 85.1–88.9% were identified and in the variants with interrupted irrigation and applied growth regulators ABA and BAP (variants 3–6), levels between 82.1 and 89.1% were identified. At the individual moments of measurement, no statistically significant differences between the various versions of treatment were identified. This means that the young plants of sugar beet retained high RWC during the experiment and neither the interrupted irrigation, nor the application of ABA nor the application of BAP had a statistically significant effect on the levels identified by measurement.

Tables 2 and 3 show the levels of photosynthetic rate P_N and the transpiration E rate measured on the leaves of the young plants of sugar beet at the dates of 3–12 sd. In the irrigated control variant (variant 1), the mean levels of P_N at 13.40 ± 0.65 $\mu mol \; CO_2 \; m^{-2} \; s^{-1}$ and E at 3.16 ± 0.29 $mmol \; H_2O \; m^{-2} \; s^{-1}$ were measured. Although RWC in the leaves remained at a very high level, the water stress resulting from interruption of irrigation, was manifested in variant 2 by partial closing of stomata and by statistically significant reduction of P_N and E. In the plants in this

Table 1. The relative water content (RWC) in % in the leaves of sugar beet plants, the Takt variety, at individual points of time of the measurement – the mean levels measured in 3 series of experiments

Variants of trial		The relative water content (%) in the period from 3 to 12 days after interruption of irrigation (3–12 sd); the growth regulator were applied on 3 sd						
No.	The name of variant	3 sd	3 sd + 2 h	4 sd	6 sd	8 sd	10 sd	12 sd
1	Control	89.4	89.8	89.4	88.5	86.7	85.5	86.1
2	Stress by drought	88.9	85.4	87.3	86.3	85.1	85.3	86.9
3	Stress by drought + 100 µM ABA	85.5	84.3	86.5	85.9	82.8	83.4	82.9
4	Stress by drought + 1 µM BAP	87.7	85.9	87.6	87.2	82.6	86.1	84.9
5	Stress by drought + 10 µM BAP	87.3	85.2	86.4	89.1	84.4	83.8	82.1
6	Stress by drought + 10 µM BAP + 100 µM ABA	86.6	87.2	85.5	85.7	85.4	87.3	87.4

variant, the mean levels of P_N at 5.51 ± 1.39 μmol CO_2 m^{-2} s^{-1} and E at 0.81 ± 0.18 mmol H_2O m^{-2} s^{-1} were identified during the period between 3 sd and 12 sd.

Tables 2 and 3 also show that the effect of abscisic acid (variant 3) on the parameters of gas exchange (P_N and E) changes in relation to the time elapsed from their application. At first, 2 hours after the application of ABA (3 sd + 2 h), the plants responded with a significant decrease of levels of P_N and E. In this variant, the levels of P_N at 1.83 ± 1.18 μmol CO_2 m^{-2} s^{-1} and E at 0.52 ± 0.15 mmol H_2O m^{-2} s^{-1} were measured. While in the untreated variant 2, also stressed by drought, at this point in time, the levels of P_N at 5.95 ± 1.83 μmol CO_2 m^{-2} s^{-1} and E at 0.80 ± 0.40 mmol H_2O m^{-2} s^{-1} were measured. It is apparent, the P_N was more sensitive to the application of ABA because its decrease was greater than the decrease of E. ABA had a similar effect on the reduction of the stomatal conductivity and the reduction of the photosynthetic and transpiration rates even 24 hours after its application (4 sd).

At later points in time at which measurements were carried out (6–12 sd), the effect of ABA was the opposite. After 3 days from its application (6sd), higher levels of P_N (7.65 ± 0.95 μmol CO_2 m^{-2} s^{-1}) as well as E (1.21 ± 0.17 mmol H_2O m^{-2} s^{-1}) were measured than in the plants grown under water stress without the application of growth regulators (P_N 5.74 ± 1.17 μmol CO_2 m^{-2} s^{-1} and E 0.77 ± 0.06 mmol H_2O m^{-2} s^{-1}). A similar trend was also identified at other points in time. The photosynthetic and transpiration rates of the plants stressed by lack of water were higher after the application of ABA than in the plants not treated by ABA. However, they did not reach the levels of P_N and E measured in the control non-stressed variant.

The application of BAP on sugar beet plants stressed by drought (variants 4 and 5) resulted in a significant increase of P_N and E throughout the monitored period. In the stressed plants treated with this cytokinin, specifically after application of the 1 μM solution of BAP, the mean levels of P_N at 12.95 ± 0.89 μmol CO_2 m^{-2} s^{-1} and E at 1.93 ± 0.25 mmol H_2O m^{-2} s^{-1} were measured, and after the application of the 10 μM solution of BAP, the mean level of P_N was 12.96 ± 0.96 μmol CO_2 m^{-2} s^{-1} and the mean level of E was 2.45 ± 0.31 mmol H_2O m^{-2} s^{-1}. These levels are statistically significantly higher than in the plants not treated with benzylaminopurine (variant 2); concerning P_N, the levels were virtually the same as in the irrigated control group (variant 1).

After the combined application of the 10 μM solution of BAP and the 100 μM solution of ABA (variant 6) the mean level of P_N at 11.45 ± 1.29 μmol CO_2 m^{-2} s^{-1} and the mean level of E at 2.33 ± 0.35 mmol H_2O m^{-2} s^{-1} were measured in the plants stressed by drought. It follows from the presented results that these levels of gas exchange are statistically provably higher than in the case, in which ABA alone was applied; these levels are virtually the same as the levels measured after the application of BAP alone.

The WUE levels calculated for the young sugar beet plants as the ratio between P_N and E are shown in Table 4. The mean WUE in the irrigated control variant (variant 1) for the assessed period was 4.23. In the variant with inter-rupted irrigation (variant 2), the level of WUE was significantly higher at 6.96 for the monitored period. In the variants with interrupted irrigation and applied growth regulators (variants 3–6), WUE reached the mean levels from 4.91 to 6.71 after the application. In variants 5 and 6, the application of a higher concentration of BAP resulted in an increase of E and deterioration of WUE. However, the lowest levels of all were identified shortly after the application of ABA: 2 hours after the application, WUE was at 3.52; and 24 hours after the application WUE was at 3.32.

DISCUSSION

Lack of water in a soil, which is drying up, is sensed by roots and evokes expression of adaptation responses in plants, such as increased production of ABA, partial closing of stomata and osmotic adjustment (Brestič, Olšovská, 2001; Hay, Porter, 2006; Jiang, Hartung, 2008). The result is that RWC in the leaves of sugar beet was maintained at a high level throughout the experiment (Table 1). High levels of RWC associated with decrease of P_N and E (Tables 2 and 3) are probably a manifestation of moderate water stress, as stated by, for example, Brestič and Olšovská (2001) or Pospíšilová et al. (2005). The decrease of P_N caused by the closing of stomata and by the reduction of CO_2 content in the intercellular space is referred to as stomatal inhibition of photosynthesis (Flexas et al., 2004).

WUE expressed as the ratio of P_N/E can show whether photosynthesis is only limited stomatally during water stress or whether non-stomatal limitation is also important. According to observations of many plant species subjected to moderate water stress, the stomatal limitation of photosynthesis strongly predominates over the non-stomatal limitation (for example, Flexas et al., 2004). The results of the assessed experiment (Table 4) with the sugar beet plants, the Takt variety, are in line with this. The significantly lower levels in the stressed plants would show that photosynthesis is also limited non-stomatally. However, in the groups stressed by interruption of irrigation, higher mean levels of WUE than in the non-stressed plants were identified during the assessed period, which probably resulted from their osmotic adjustment during the water stress.

The fact that the closing of stomata and decrease of P_N and E occurred in spite of the high relative water content in the leaves of the sugar beet plants means that the parameters of gas exchange were in closer correlation with the soil water content than with the leaf water content. This fact is explained, as it has already been mentioned in the introduction, by a chemical signal about drought, which is carried by abscisic acid produced in roots and transported to leaves (Pospíšilová, 2003; Jiang and Hartung, 2008). The experimental results proved that exogenously supplied ABA also has an effect on the control of gas exchange between the sugar beet leaves and the surrounding environment.

Table 2. The effect of water stress induced by interruption of irrigation, the growth regulators ABA and BAP and the period of time elapsed from their application on the photosynthetic rate (P_N) in sugar beet plants, the Takt variety, the mean levels for 3 series of experiments. Mean ± standard error ($n = 9$)

No.	The name of variant	The photosynthetic rate P_N (μmol CO_2 m^{-2} s^{-1}) in the period from 3 to 12 days after interruption of irrigation (3–12 sd), the growth regulator were applied on 3 sd						
		3 sd	3 sd + 2 h	4 sd	6 sd	8 sd	10 sd	12 sd
1	Control	12.39 ± 0.19 a	12.78 ± 0.46 a	13.29 ± 0.31 a	12.96 ± 1.01 a	14.07 ± 0.90 a	14.12 ± 0.43 a	14.17 ± 1.22 a
2	Stress by drought	4.27 ± 1.28 b	5.95 ± 1.83 b	3.62 ± 1.67 b	5.74 ± 1.17 b	5.86 ± 1.22 b	6.64 ± 1.10 b	6.47 ± 1.46 b
3	Stress by drought + 100 μM ABA	4.29 ± 1.54 b	1.83 ± 1.18 c	1.76 ± 0.92 b	7.65 ± 0.95 b	9.69 ± 1.47 c	8.11 ± 2.43 b	8.39 ± 0.70 b
4	Stress by drought + 1 μM BAP	3.22 ± 1.08 b	12.44 ± 0.82 a	12.29 ± 0.40 c	13.42 ± 0.51 a	12.68 ± 1.50 a	13.80 ± 1.02 ac	13.08 ± 1.09 a
5	Stress by drought + 10 μM BAP	4.00 ± 0.28 b	12.17 ± 0.96 a	15.47 ± 0.21 d	12.53 ± 1.52 a	12.07 ± 1.13 ac	12.27 ± 1.47 ac	13.22 ± 0.45 a
6	Stress by drought + 10 μM BAP + 100 μM ABA	3.41 ± 0.75 b	9.80 ± 1.06 d	9.32 ± 2.81 c	12.06 ± 1.33 a	12.48 ± 0.99 a	12.55 ± 0.83 c	12.46 ± 0.69 a

Note: The figures in columns labelled by the same letters for the individual times of measurement are not provably different at $\alpha < 0.05$

Table 3. The effect of water stress induced by interruption of irrigation, the growth regulators ABA and BAP and the period of time elapsed from their application on the transpiration rate (E) in sugar beet plants, the Takt variety, the mean levels for 3 series of experiments. Mean ± standard error ($n = 9$)

No.	The name of variant	The transpiration rate E (mmol H_2O m^{-2} s^{-1}) in the period from 3 to 12 days after interruption of irrigation (3–12sd), the growth regulator were applied on 3 sd						
		3 sd	3 sd + 2 h	4 sd	6 sd	8 sd	10 sd	12 sd
1	Control	2.86 ± 0.12 a	3.02 ± 0.24 a	3.16 ± 0.09 a	3.61 ± 0.14 a	3.87 ± 0.65 a	2.69 ± 0.31 a	2.89 ± 0.50 a
2	Stress by drought	0.75 ± 0.24 b	0.80 ± 0.40 b	0.76 ± 0.12 b	0.77 ± 0.06 b	0.81 ± 0.10 b	0.93 ± 0.08 b	0.87 ± 0.28 b
3	Stress by drought + 100 μM ABA	0.81 ± 0.29 b	0.52 ± 0.15 b	0.53 ± 0.16 b	1.21 ± 0.17 c	1.26 ± 0.44 bc	1.11 ± 0.38 bc	1.16 ± 0.33 bc
4	Stress by drought + 1 μM BAP	0.71 ± 0.16 b	2.62 ± 0.14 c	1.82 ± 0.13 c	1.91 ± 0.20 d	1.68 ± 0.34 cd	1.66 ± 0.27 cd	1.87 ± 0.42 c
5	Stress by drought + 10 μM BAP	0.74 ± 0.20 b	2.76 ± 0.35 ac	3.22 ± 0.17 a	2.47 ± 0.23 e	2.06 ± 0.33 d	2.01 ± 0.46 ad	2.15 ± 0.29 ac
6	Stress by drought + 10 μM BAP + 100 μM ABA	0.85 ± 0.10 b	1.65 ± 0.25 d	2.27 ± 0.72 c	2.47 ± 0.32 e	2.79 ± 0.28 e	2.50 ± 0.19 a	2.27 ± 0.33 ac

Note: The figures in columns labelled by the same letters for the individual times of measurement are not provably different at $\alpha < 0.05$

Table 4. The effect of water stress induced by interruption of irrigation, the growth regulators ABA and BAP and the period of time elapsed from their application on the water use efficiency (WUE) in sugar beet plants, the Takt variety, the mean levels for 3 series of experiments

No.	The name of variant	The water use efficiency (P_N/E) in the period from 3 to 12 days after interruption of irrigation (3–12 sd), the growth regulator were applied on 3 sd							
		3 sd	3 sd + 2 h	4 sd	6 sd	8 sd	10 sd	12 sd	Average
1	Control	4.33	4.23	4.22	3.59	3.64	5.25	4.90	4.23
2	Stress by drought	5.69	7.44	4.76	7.45	7.23	7.14	7.44	6.96
3	Stress by drought + 100 μM ABA	5.29	3.52	3.32	6.32	7.69	7.31	7.23	6.43
4	Stress by drought + 1 μM BAP	4.54	4.75	6.75	7.03	7.55	8.31	6.99	6.71
5	Stress by drought + 10 μM BAP	5.41	4.41	4.80	5.07	5.86	6.10	6.15	5.29
6	Stress by drought + 10 μM BAP + 100 μM ABA	4.01	5.94	4.11	4.88	4.47	5.02	5.49	4.91

The low levels of P_N and E (Tables 2 and 3), measured at the first measurement times after the application of ABA, can be explained as follows: the application of ABA on the leaves caused the plants to close their stomata more than the non-treated plants. Molecules of water are smaller than molecules of CO_2 and with the same concentration gradients, they diffuse faster, that is, more easily. Based on this, the stomata apparently limited the movement, that is, the intake of CO_2 more than output of water. This resulted in deterioration of WUE (Table 4). However, the partial closing of stomata probably slowed down the progression in development of water stress in the treated plants. This was why at later points in time after the application of ABA, the measured levels of P_N and E were higher and the WUE levels determined by measurement were approximately the same in comparison with the plants in variant 2, which were stressed, but were not treated with abscisic acid. The results thus support the opinion of P o s p í š i l o v á (2003) and P o s p í š i l o v á, B a ť k o v á (2004), that the improving effect of ABA on water stress is probably of a general nature.

The favourable effect of benzylaminopurine (BAP) on the parameters of exchange of gases in the sugar beet leaves was very strong during the moderate water stress. The confrontation of the results shown in Tables 2 and 3 with the findings published by other authors shows that the effect of cytokinins on reduction of the negative impact of water stress is species-specific. A favourable effect, improving the parameters of gas exchange, was identified by P o s p í š i l o v á and B a ť k o v á (2004) in experiments with sugar beet. However, the effect was less significant. In the same experiment, the application of benzyladenine on maize plants did not influence the parameters of gas exchange; and when this substance was applied on the beans, the stomatal conductivity and transpiration rate were reduced. However, in another experiment with bean plants, the effect of benzyladenine on P_N and E was positive (P o s p í š i l o v á et al., 2001). The differences in the effect are probably determined by the use of various concentration levels of cytokinins. This is also supported, for example, by the experiments of R u l c o v á (2000) who identified a negative effect of higher concentration levels of benzyladenine on P_N and E. The time of application probably also has its importance – before the inducement of water stress or during the water stress – and so does the method of application – sprinkling, watering or wetting the roots (P o s p í š i l o v á et al., 2001). Higher levels of E after treatment of stressed plants with cytokinins also might have a negative effect.

Water losses combined with a higher transpiration rate can result in faster deterioration of the water stress in plants treated in this way (faster decrease of RWC) in the next phase of dehydration. This should be the subject matter of further research.

One of the ways of studying the interactions between ABA and BAP is the combined application of both substances on plants. In this way, it was, for example, found out that BAP reduces or postpones the effect of ABA on the closing of stomata during water stress in maize plants (S t u c h l í k o v á et al., 2007). The results of the assessed experiment correspond to this. When these substances were applied together on the sugar beet plants of the Takt variety, influenced by a moderate water stress, BAP eliminated the effect of ABA on P_N and E (Tables 2 and 3). Similarly P o s p í š i l o v á (2003) found out in sugar beet plants under a moderate water stress that cytokinins, in combined application with abscisic acid, were reducing the closing of stomata induced by ABA. However, when the cytokinins were applied only after ABA, the already closed stomata did not open. Cytokinins can have effect not only at the level of the closing cells and can not only mitigate the effect of ABA on the closing of stomata but they can also partly inhibit the accumulation of ABA induced by water stress (P o s p í š i l o v á et al., 2005). Therefore, the effect of an applied combination of BAP and ABA on the parameters of gas exchange and water use efficiency apparently depends on the type of the experimental plant, the used concentration levels of the substances, the time of their application and the method of their application. Probably, the water status in the plant (the degree of water stress) also plays its role.

CONCLUSION

The experimental results proved the effect of exogenously applied abscisic acid and benzylaminopurine on the control of gas exchange in the leaves of sugar beet under water stress. Shortly after it is applied, ABA reduces P_N and E and slows down the progression of development of water stress in the treated plants. BAP significantly increases the parameters of gas exchange in the treated plants. However, during a prolonged lack of water, there is a risk that the higher transpiration rate will accelerate the depletion of water and will deteriorate the water stress.

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Fytohormonální regulace výměny plynů v rostlinách *Beta vulgaris* L. při vodním stresu.

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Příspěvek hodnotí vliv kyseliny abscisové (ABA) a cytokininu benzylaminopurinu (BAP) na regulaci výměny plynů v listech cukrovky při vodním stresu.

Pro nádobové pokusy v řízených podmínkách ve skleníku byly použity rostliny odrůdy Takt s 5–6 vytvořenými listy. ABA v koncentraci 100 μM a BAP v koncentraci 1 μM a 10 μM byly aplikovány na listy tři dny po rozdělení rostlin do variant a přerušení zálivky. Relativní obsah vody v listech (RWC) byl měřen terčíkovou metodou podle Č a t s k é h o (1960), rychlost fotosyntézy (P$_N$), rychlost transpirace (E) a efektivita využití vody (WUE) byly měřeny gazometricky (F l e x a s et al., 2004) těsně před a dále 2 hodiny a 1, 3, 5, 7 a 9 dnů po aplikaci růstových regulátorů. Pokus byl statisticky vyhodnocen analýzou rozptylu při α = 0.05 v počítačovém programu Statistica, verze 6.1 CZ, modul ANOVA.

Zjištěné hodnoty RWC jsou uvedeny v tab. 1, naměřené hodnoty P$_N$ v tab. 2 a E v tab. 3 a vypočítané hodnoty WUE v tab. 4. Rostliny si po celou dobu po přerušení zálivky udržely vysoký RWC, ale došlo ke statisticky významnému snížení P$_N$ a E a také ke zlepšení WUE ve srovnání s nestresovanými rostlinami. Aplikace ABA nejdříve snížila u stresovaných rostlin P$_N$ a E a došlo ke zhoršení WUE (2 a 24 hodiny po aplikaci). V pozdějších termínech byly naopak naměřeny P$_N$ a E vyšší a WUE přibližně stejná ve srovnání se stresovanými, ale ABA neošetřenými rostlinami. Nedosáhly ale hodnot P$_N$ a E naměřených u kontrolní nestresované varianty. Aplikace BAP na suchem stresované rostliny se v celém sledovaném období projevila statisticky významně vyššími hodnotami P$_N$ a E v porovnání s rostlinami neošetřenými BAP. Rozdíly v účinku použitých koncentrací BAP nebyly v průměru za celé sledované období statisticky významné. Při společné aplikaci obou látek eliminoval BAP vliv ABA. Naměřené hodnoty P$_N$ a E byly statisticky průkazně vyšší než při použití samotné ABA, prakticky na úrovni hodnot naměřených po dodání samotného BAP.

cukrovka; vodní stres; rychlost fotosyntézy; rychlost transpirace; kyselina abscisová; benzylaminopurin

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