Role of Endogenous Hormonal System in the Realization of the Antistress Action of Plant Growth Regulators on Plants

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ABSTRACT

The endogenous hormonal system plays a leading role in the regulation of growth and development of plants. This regulatory system responds sensitively to even slight changes in the plant environment, which is manifested in reorganization of the hormonal status. Detrimental factors lead to an imbalance in the content of different groups of phytohormones, as a rule reflected in the accumulation of abscisic acid (ABA) and in a parallel decline in the content of metabolism-stimulating hormones indole-3-acetic acid (IAA) and cytokinins. In total, these changes in hormone content in plants are related to inducing an effective stress response of plants accompanied however with inhibition of growth processes and plant productivity. This makes actual application of natural growth regulators characterized by growth-stimulating and anti-stress activity to increase both plant resistance and productivity as well. The present mini review mainly concentrates on the shifts in endogenous phytohormones (ABA, IAA and cytokinins) level caused by application of hormones such as, brassinosteroids, cytokinins and salicylic acid (SA). The main attention is focused on the role of these changes in endogenous hormones in the realization of growth-stimulating and protective function of applied plant growth regulators (PGRs). It is necessary to underline that simultaneous analysis of different groups of phytohomones allowed us to reveal a complex pattern of changes in plant hormonal system in response to treatments with exogenous growth regulators and to evaluate their contribution to the control of resistance to stress factors.

CONTENTS

INTRODUCTION

Terrestrial plants are sessile and can not move away from environmental stress conditions that can negatively affect their growth and development. As a result, they have to develop and/or strengthen various biochemical and physiological mechanisms to respond and adapt to these stress conditions and thus, acquire stress tolerance. These mechanisms are in fact, based on the effective integration system regulating cell metabolic activity involved in mediating the switch from normal to stress genetic programs and enabling adequate protection at whole organism level. Hormonal system is the essential link in regulation of these processes, which also cross-talk with other signalling pathways in coordination of the defence reaction start, which enables adaptation of plants to stressful environments (Wu *et al.* 2007; Ashraf *et al.* 2008).

Hormonal system is very sensitive to the slightest chan-

ges in conditions for plant growth (Davies *et al.* 2005). Stress-adaptation is determined by the pattern of changes in the concentration ratio of several interacting hormones as well as by the ability of each of hormones to influence endogenous levels of the others (Reski 2006; Malladi and Burns 2007; Goda *et al.* 2008; Schachtman and Goodger 2008; Zhao 2008). Plants control the endogenous level of phytohormones through their synthesis, conjugation with different compounds, transport in plants as well as through their degradation, based on different expression of genes, encoding enzymes of hormone metabolism (Chen *et al.* 2005; Woodward and Bartel 2005; Grennan 2006; Wasternack 2007; Catinot *et al.* 2008; Stirk *et al.* 2008; Symons *et al.* 2008; Wasilewska *et al.* 2008).

The mutual effect of hormones on concentration of each other is frequently due to ability of almost each hormone to influence synthesis, degradation or transport of other hormones (Goda *et al.* 2008). Thus, auxin mediates a very

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rapid negative control of the cytokinin pool by mainly suppressing the biosynthesis via the isopentenyladenosine-5-monophosphate-independent pathway (Nordstrom *et al.* 2004). Ethylene production has been shown to be regulated by several hormones due to their influence on transcript or protein levels of ethylene biosynthesis genes (Vandenbussche and Van Der Straeten 2007). Thus, auxin is a strong stimulator of ethylene production, resulting from auxin-induced accumulation of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase transcript. On the contrary ethylene may influence not only synthesis of auxin, but also its transport. Thus, the ethylene-induced inhibition of lateral roots formation is dependent on auxin influx mediated by the *Arabidopsis* AUX1 protein. Effects of cytokinins on ethylene production are believed to be important components of the mechanism of action of these hormones in plants. Elevated ethylene production was observed in cytokinin-treated plants, which is likely to be due to stabilization of ACC synthase compared to non-treated plants. Opposite effect on ethylene production has been demonstrated for ABA. Recent studies show that increased concentrations of ABA are required to prevent excess ethylene production from tissues under water deficit condition (LeNoble *et al.* 2004). Hormonal effects on the degradation of other hormones have also been reported. Thus application of ABA to maize (*Zea mays*) and wheat (*Triticum aestivum*) plants induced expression of cytokinin oxidase gene (CKX) and activity of corresponding enzyme catalyzing oxidative decay of cytokinins (Brugiere *et al.* 2003; Vysotskaya *et al.* 2009).

PHYSIOLOGICAL IMPORTANCE OF PHYTOHORMONES

Each phytohormone is known to be involved in regulation of such integral physiological processes as growth, development and differentiation of plants under normal conditions and under environmental changes indicating an active interaction of hormones with each other (Chow and McCourt 2004; Arteca and Arteca 2008; Goda *et al.* 2008; Kuppusamy *et al.* 2008; Bari and Jones 2009). There are convincing evidences of multi-functionality of hormones and overlap between each set of hormone-inducible effects. They were obtained in experiments using mutants defective in hormonal synthesis or response as well as in experiments with plants treated with exogenous hormones or inhibitors of their biosynthesis (Chow and McCourt 2004; Reski 2006; Goda *et al.* 2008; Wang and Zhang 2008; Urano *et al.* 2009).

Indole-3-acetic acid (IAA), the main representative of auxins in plants, plays a special role in modulating tropic responses to light and gravitation, formation of roots, shoots and reproductive organs, development and differentiation of vessels, apical dominance. All these effects are achieved through regulation of expression of the family of IAA-responsive genes (Woodward and Bartel 2005). A key role in the regulation of growth, morphogenesis, inhibition of apical dominance in shoots, biogenesis of chloroplasts, processes of photosynthesis and nitrogen metabolism, transpiration, senescence and others belongs to cytokinins (Hirose *et al.* 2007, 2008), which is also due to induction of cytokinin-responsive genes (Boonman *et al.* 2007; Zubo *et al.* 2008). Moreover, cytokinins are involved in the control of adaptation of plants to changing environment (Kudoyarova *et al.* 2007; Srivastava *et al.* 2007). Gibberellins are important in regulation of the transition from dormancy to germination, elongation of hypocotyls and stem, extension of leaves, formation of flowers and fruit set as well as in plant response to environmental factors (such as, light and temperature) realized by means of induction of the gibberellin-sensitive genes (Huerta *et al.* 2008). Ethylene is involved in regulation of such important processes as cell extension growth, senescence, formation of reproductive organs, leaf abscission, fruit ripening and graviptropism. The hormone is also implicated in triggering defence reactions in respon-

ses to biotic and abiotic stresses, which is achieved through expression of ethylene-sensitive genes (Chen *et al.* 2005). Salicylic acid (SA) and methylsalicylate participate in regulation of various physiological processes including flowering, thermogenesis, stomatal closure and ion transport as well as in initiation of protective responses of plants against biotic and abiotic stresses, which is also due to induction of a wide range of genes by SA (Rajjou *et al.* 2006; Horvath *et al.* 2007). Jasmonic acid (JA) and its derivative methyljasmonate, which are nowadays classified as phytohormones, induce a wide range of physiological and developmental responses, namely flowering, tuberization, fruit ripening, storage processes, senescence, transport of photoassimilates, induction of defence reactions in response to wounding, attack of pathogens and pests, action of abiotic stress factors such as drought and salinity, which is due to activation of transcription of a wide spectrum of jasmonate sensitive genes (Walia *et al.* 2007; Wasternack 2007). Brassinosteroids (BR), steroid phytohormones belong to a unique group of hormones that manifest their physiological activity in extremely low concentrations. They play a key role in induction of germination and cell growth by division and extension, stem elongation, pollen tube growth, differentiation of xylem, leaf epinasty, photosynthesis, fruit ripening and adaptation to different stresses (Mussig 2005; Bajguz and Hayat 2009). Adaptation of various physiological processes in plants is also realized through induction of genes sensitive to BR (Haubrick and Assmann 2006; Kagale *et al.* 2007). Abscisic acid (ABA) is the phytohormone of vital importance implicated in regulation of plant growth and development within the whole period of ontogenesis including ripening, senescence, seed dormancy and germination, development of lateral roots, functioning of stomata, transition from the vegetative to the reproductive phase (Christmann *et al.* 2006; Wang and Zhang 2008; Wasilewska *et al.* 2008).

Thus, hormones are strongly involved in regulation of plant development at normal and stress environment. They initiate and control adaptation process.

INVOLVEMENT OF ABA IN RESPONSES TO ABIOTIC STRESSES

Drought, disturbance of temperature regime, salinity and toxic ions are most important environmental factors, which are critical for survival of plants and lead to significant losses of crop productivity. Responses to these stresses are known to be interconnected, employing common signaling pathways, which allow cell adaptation and lead to similar changes in plants on morphological, physiological, biochemical and molecular/or genetic levels (Verslues *et al.* 2006; Zengin 2006; Huang *et al.* 2008; Vasquez-Robinet *et al.* 2008; Potters *et al.* 2009). Increased synthesis and accumulation of ABA having frequently a transitory pattern may be characterized as the universal response to these stressful impacts leading to water regime imbalance (Xiong *et al.* 2002; Vasquez-Robinet *et al.* 2008; Urano *et al.* 2009). Thus, ABA plays a key role in the movement of stomata and induction of a wide spectrum of genes coding for protective proteins (Chinnusamy *et al.* 2004; Christmann *et al.* 2006; Wang and Zhang 2008).

In fact, stomatal closure belongs to early plant responses to stress caused by ABA-induced increase in cytosolic Ca^{2+} concentration followed by activation of ion channels in cell membrane, loss of turgor by guard cells and decrease in stomatal aperture, ABA-induced production of H_2O_2, serving as an intermediate signal to promote stomatal closure (Reski 2006; Schachtman and Goodger 2008). It is necessary to underline that alongside with inducing the production of reactive oxygen species (ROS) ABA is involved in up-regulation of genes and activities of corresponding antioxidant enzymes (Bari and Jones 2009) providing a protection against oxidative stress caused by conditions unfavourable for plant growth. Stress-induced endogenous ABA accumulation regulates the increase in saccharopine, most

amino acids and organic acids levels (Urano *et al*. 2009). ABA is of pivotal importance for the induction of biosynthesis and accumulation of proline known as osmoprotectant which is involved in stabilization of biopolymers and cell membranes and protection against injurious action of ROS (Yu *et al*. 2008).

Induction of ABA-sensitive genes is the basis of regulatory effect of ABA rendering to plants higher stress resistant. Recently, about 2000 drought-inducible genes have been identified using whole genome oligonucleotide microarray in *Arabidopsis thaliana* during progressive drought stress. About two thirds of these genes were regulated by ABA or its analogue (PB1425) (Huang *et al*. 2008) thus, confirming the significance of ABA in protection of plants against stress. Among ABA-induced genes, important role belongs to those encoding dehydrins. These proteins are peculiar to all taxonomic groups of the plant kingdom. Their massive accumulation is observed in plant seed embryos during their dehydration. However, sharp increase in expression of dehydrin genes and accumulation of their protein products is registered in vegetative plant tissues subjected to dehydration. Dehydrins are the most abundant among stress proteins induced under these conditions (Allagulova *et al*. 2003). Physico-chemical properties of dehydrins are determined by peculiarities of their structure, including the obligatory presence of 15-aminoacid K-segment capable to form the secondary amphiphyll α-spiral. These properties enable involvement of dehydrins in protecting against denaturation, preserving integrity of cellular structures and stabilizing membranes under conditions of dehydration. Induction of expression of dehydrin genes and accumulation of their protein products also take place under normal conditions in ABA-treated plants (Allagulova *et al*. 2003; Shakirova *et al*. 2005). Sensitivity of the genes of dehydrins to ABA and different stress factors is determined by the presence in their promoters of different combinations of *cis*-regulated elements interacting with each other and with various ABA-induced *trans*-factors (Shinozaki and Yamaguchi-Shinozaki 2007; Chung and Parish 2008).

The gene coding for wheat germ agglutinin (WGA), alongside with a wide range of other stress proteins, belongs to ABA-responsive genes. WGA is a typical representative of cereal lectins and a constitutive wheat protein, which concentration in plant tissues changes significantly during development. Significant reversible increase in WGA content was observed in wheat plants in response to salinity, drought, osmotic stress and heat shock, infection with fungal pathogens, preceded by accumulation of endogenous ABA (Shakirova 2001). Data showing a decline in stress-induced oxidative damage in seedlings pretreated with WGA and an accelerated renewal of growth processes during the post-stress period in these plants confirm that WGA is an active participant in ABA-induced wheat resistance (Bezrukova *et al*. 2008).

It is necessary to underline that ABA is only one although most important out of many other components of the system of hormonal regulation in stressed plants. Alongside with accumulation of ABA plant response to stress factors also involves changes in concentration of other hormones functioning in integrated regulatory system. Nowadays a lot of accumulated data shows that as a rule abiotic stresses lead to a decline in concentration of growth–promoting hormones: auxins, gibberellins, cytokinins (Alvarez *et al*. 2008; Ghanem *et al*. 2008; Wang *et al*. 2008). A number of genes encoding proteins responsive to gibberillic acid and auxin were identified as drought- and ABA-down-regulated genes, suggesting that these hormones may modulate drought stress signalling by acting antagonistically to ABA (Huang *et al*. 2008). Thus, stress-induced decline in the content of gibberellins and auxins may contribute to the development of an adequate stress response. The importance of decreased auxin level is supported by observation that mutants over-expressing GH3-gene, coding for an enzyme that catalyses conjugation of auxins, had both low IAA content and increased resistance to abiotic stresses (Park 2007).

Cross-talk between hormones controlling the same processes related to growth or defence mechanisms is achieved at many different stages of signal transduction pathways or during downstream responses (Kuppusamy *et al*. 2008; Bari and Jones 2009). These pathways frequently interact through co-regulation of stability of key transduction components. Ubiqiutin/proteosome system enabling selective protein degradation was shown to be essential for almost all of plant hormone responses. The example of hormone interaction at this level is in that gibberellins (GA) and ethylene antagonistically regulate the stability of DELLA proteins which normally function to restrain growth. GA acts to promote DELLA protein degradation, hence stimulating plant growth, whereas ethylene stabilizes DELLA proteins, thereby causing growth inhibition (Dugardeyn *et al*. 2008). Hormonal pathway interactions may also occur through the combinatorial regulation of common target genes. There are a number of evidences proving that transcriptional targets of auxin and BR significantly overlap. Consistent with this several studies found impaired auxin-responsive gene expression in BR-deficient backgrounds and synergistic enhancement of the expression levels of auxin- and BR-responsive genes by simultaneous treatment with both hormones. Ethylene and jasmonate pathways converge to induce the expression of ethylene response factor 1 (ERF1), which encodes a transcription factor required to regulate the expression of defence genes (Kuppusamy *et al*. 2008). Such induction requires the presence of both JA- and ethylene pathways because mutations blocking either of the pathways prevent the expression of ERF1.

Taking into account the multi-hormonal regulation of most of processes in plants, great attention is paid to development of modern highly sensitive and rapid methods of measuring concentration of different hormones in the same plant extract (Forcat *et al*. 2008). It is necessary to underline that simultaneous measurement of different groups of hormones in the same plants allows researchers to draw a complex picture of stress-induced reorganization of the state of hormonal system and to evaluate its importance for regulation of plant resistance.

RESISTANCE-INDUCING PHYTOHORMONES

The problem of stress-resistance is most important for plant breeding and is under a steadfast attention of researchers all over the world. This is indeed the case, since information concerning the chain of reactions taking place in plants in response to extreme external conditions may really contribute to an increase in plant resistance and productivity. These goals are achieved not only by means of selection of stress tolerant cultivars, but also through the purposeful manipulation of adaptation with the help of growth regulators (Ashraf *et al*. 2008). Due to this, the interest of researchers to growth regulators is not casual. These substances combine the properties of growth activators and inductors of unspecific resistance which reveal a perspective for their practical application in plant growth. The survival of plants is due to their ability to resist extreme environment and to adapt to it. However, realization of natural protective mechanisms that take place in plants, when conditions become worse, is well known to be accompanied by a decline in their productivity (Wu *et al*. 2007). And since plant breeding demands an intensification of not only plant resistance, but in the first place of productivity, aimed application of regulators of plant growth and development capable to increase both of them are of high priority. It is obvious that not any PGR may be used, but those, which are characterized by a wide spectrum of their protective action. Effectiveness of application of PGRs depends strongly on their acting concentration (the use of excessively high concentrations may cause an opposite result). Effective concentrations are different with different cultivars, phases of plant development and means of treatment.

SALICYLIC ACID – INDUCTOR OF UNSPECIFIC PLANT RESISTANCE

Salicylic acid (SA) is an endogenous growth regulator, which exogenous application provides a perspective for increasing plant resistance and productivity due to its revealed key role in induction of systemic acquired resistance (SAR). SA-induced expression of pathogenesis-related (PR) proteins is in the basis of this phenomenon (Yang *et al.* 2004). A significant amount of experimental data demonstrates with convincing evidence participation of both endogenous and exogenous SA in establishing resistance of different plant species not only to biotic, but also to various abiotic stress factors such as oxidative stress, salinity, high and low temperature, water stress, heavy metals (Yang *et al.* 2004; Liu *et al.* 2006; Horvath *et al.* 2007). This allows the classification of this regulator as a plant stress hormone. An important impact for realization of SA-induced resistance may be exerted by the effect of SA on hormonal status of plants. Thus, seed sowing or treatment of seedlings with SA had a growth stimulating effect linked to fast transitory parallel accumulation of IAA and ABA. This growth stimulating effect manifested also in increased productivity of wheat plants (Shakirova *et al.* 2003). Stimulating effect of SA is likely to be due to SA-induced increase in concentration of IAA observed in wheat plants. Accumulation of ABA in SA-treated plants may be of pivotal importance for induction of expression of *TADHN* gene for dehydrin, accumulation of WGA and proline, as well as for activation of antioxidant enzymes (Shakirova 2007), all these effects being important components of SA-induced pre-adaptation to a forthcoming stress. Pretreatment of wheat seedlings with SA decreased significantly the level of injurious effect of salinity on their growth. Moreover, after removal of the stressor from the media, these SA-pretreated plants restored quickly their growth potential, which was likely to be due to diminished amplitude of stress-induced shifts in the content of IAA, cytokinins as well as that of ROS production and activity of antioxidant enzymes reflected in a decline in malondialdehyde content and electrolyte leakage.

Consequently, accumulation of ABA in the course of pretreatment of plant with SA is likely to play a key role in SA-induced resistance of wheat plants (Shakirova *et al.* 2003). Other data may also support the notion that SA interact with ABA in establishing plant resistance to abiotic stresses (Liu *et al.* 2006). In total, the data showing a key role of SA in induction of SAR and establishment of a complex of protective reactions in response to abiotic stresses allows to consider SA as an inducer of unspecific plant resistance. Thus, the state of hormonal and pro-/antioxidant systems in SA-treated plants makes a significant contribution to the SA-induced unspecific resistance (Shakirova 2007), opening a wide perspective for the usage of this endogenous growth regulator in agriculture.

INVOLVEMENT OF CYTOKININS IN PROTECTIVE RESPONSE OF PLANTS

Since cytokinins are known to be necessary for plant growth, attempts have been made to apply synthetic cytokinins to increase their growth rate and productivity (Liu and Huang 2002). It is important to note that to exert a beneficial effect on plant growth the changes in concentration of cytokinins inside the plants should be in a physiological range and not superoptimal. Exceedingly high levels of cytokinins may result in an opposite effect, which is frequently the case with *ipt*-transgenic plants. These plants overproduce cytokinins due to expression of genes coding for isopentenyl-transferase responsible for cytokinin synthesis in bacteria and plants. In such transgenic plants accumulation of high toxic cytokinins levels results in a disturbance of normal plant growth and development (Ainley *et al.* 1993; Hare *et al.* 1997). Necessity of cytokinins for shoot physiology was supported by the growth inhibition observed in transgenic plants deficient for cytokinins (Werner *et al.* 2008). These

results prompt that synthetic cytokinins should be applied to plants in optimal concentration. This may be achieved by introducing cytokinin synthesizing bacteria into the plant rhizosphere (Arkhipova *et al.* 2007). These bacteria have been shown to produce cytokinin bound to polysaccharides. Slow dissociation of hormones from this complex maintained their increased concentration in physiological range and invariably stimulated plant growth. The consequences of such treatments for ability of plants to cope with stresses cannot be easily predicted.

Cytokinin content is mostly decreased in stressed plants (Alvarez *et al.* 2008; Wang *et al.* 2008), which is believed to contribute to their survival under unfavourable conditions, since the decline in plant growth rate due to a drop in cytokinin content should help a plant to mobilize resources necessary for defence reactions. Although this response may be beneficial for survival of plants, this decline in plant growth rate and productivity is not what farmers may be happy with. It may seem rather unexpected, but quite a promising perspective that treatment of plants with synthetic cytokinins frequently increased their resistance to high temperature, drought, flooding, heavy metals and other unfavourable external factors (Hare *et al.* 1997; Liu and Huang 2002). This may be explained by up-regulation of some genes important for protection of plants against stresses (enabling increased level of proline, isoforms of PR protein, osmotin, etc.) observed in plants treated with exogenous cytokinins (Hare *et al.* 1997). Recent data confirmed involvement of cytokinins in the responses triggering protection of plants against abiotic and biotic stresses. Thus, the balance of cytokinin and auxin influenced the expression of specific peroxidases providing a defence regulating mechanism (Potters *et al.* 2007). Expression of cytokinin responsive genes was enhanced under salinity, dehydration and low temperature stress in rice (Hirose *et al.* 2007). Genome-wide analyses of *Arabidopsis* seedlings using the approximately 8,300-element Affymetrix Arabidopsis GeneChips (Affymetrix, Santa Clara, CA) to examine cytokinin-responsive gene revealed an effect of cytokinins on redox state. Thus, both a glutathione *S*-transferase and a glutaredoxin were up-regulated (Rashotte *et al.* 2003). The usage of transgenic plants adds new evidences of cytokinin implication in stress responses. Overexpression of genes that code for cytokinin degrading oxidase (CKX) enzymes was used to produce tobacco and *Arabidopsis* plants with reduced cytokinin content. These plants show a phenotype called the cytokinin deficiency syndrome. Ultrastructural changes observed in meristem and leaf cells of cytokinin deficient transgenic tobacco plants were indicative of enhanced oxidative stress suggesting an involvement of cytokinins in limiting oxidative damage (Werner *et al.* 2008). During drought transcripts encoding antioxidative enzymes were up-regulated in the transgenic plants expressing an isopentenyltransferase gene driven by a stress-induced promoter (Rivero *et al.* 2007). In these experiments transcript expression correlated well with changes in enzymatic activities for the different ROS-scavenging enzymes resulting in outstanding drought tolerance. Thus, all the data indicate involvement of cytokinins in stress signalling.

Since cytokinins maintain stomata in open state, increasing in this way the transpiration losses by plants (Davies *et al.* 2005), they are likely to make plants more susceptible to drought. However no such effect was observed in plants inoculated with cytokinin producing bacteria, which stomata closed under conditions of water deficit despite accumulation of cytokinins (Arkhipova *et al.* 2007). In this case stomatal closure was shown to be due to accumulation of ABA in parallel to cytokinins in inoculated plants. In some experiments, effect of cytokinins on stress resistance of plants was shown to be modified by cytokinin-induced increase in ethylene production (Hare *et al.* 1997). Thus, ability of cytokinins to diminish stress-induced inhibition of growth in stressed plants and simultaneously to protect them against stresses may be partially due to cytokinin-induced changes in concentration of other hormones in

plants.

BRASSINOSTEROIDS AND ESTABLISHMENT OF STRESS RESISTANCE

Soon after identification of brassinolide, being the first representative of a new class of phytohormones brassinosteroids (BR) in plants, laboratory and field experiments revealed not only their growth stimulating activity, but also protective action in plants of different species experiencing low or high temperature, drought, salinity, toxic effect of heavy metals, oxidative stress and attack of pathogens (Kagale *et al.* 2007; Bajguz and Hayat 2009). All these effects lead to increased productivity of BR-treated plants. Combination of these growth stimulating and protective properties of exogenous BR is due to their tight interaction with other hormones (ethylene, auxin, gibberellins, ABA, SA, JA) (Arteca and Arteca 2008; Vert *et al.* 2008; Bari and Jones 2009).

Experiments with wheat seedlings revealed that 24-epibrassinolide (EBR)-treatment resulted in a fast and lasting two-fold accumulation of cytokinins in plants on the background of the absence of any noticeable changes in concentration of IAA or ABA (Avalbaev *et al.* 2003). It is important to note that treatment of canola seedlings with brassinolide had also no effect on concentration of endogenous ABA in leaf tissue (Kurepin et al. 2008). EBR enables maintaining concentration of active forms of cytokinins immunoreactive to antiserum obtained against zeatin riboside. This effect is achieved through a decline in both expression of CKX gene and activity of corresponding enzyme (Avalbaev *et al.* 2006). These results allow us to suggest that endogenous cytokinins may serve as mediators intensifying physiological action of EBR in wheat plants. Evidence of this is also provided by prevention of a sharp decline in cytokinin content in wheat seedlings pretreated with EBR correlating with a reduced level of injurious effect of salt-stress on the seedlings growth (own unpublished results). As mentioned above, cytokinins are involved in the control of plant resistance to stresses, especially to those leading to dehydration (Davies *et al.* 2005). In connection with this, maintaining cytokinin concentration on the control level in salt-stressed BR-pre-treated plants may be important for establishment of EBR-mediated stress responses.

Moreover, EBR was shown to induce expression of *TADHN* dehydrin and *WGA* genes in wheat seedling (Shakirova *et al.* 2002; Allagulova *et al.* 2007), while these proteins, as mentioned above, are important components of plant protection against injurious stress factors of environment. It is worthwhile to underline that EBR-treatment itself does not lead to changes in ABA content in wheat plants. This indicates that BR may control the level of wheat TADHN dehydrin and lectin in an ABA-independent manner, these proteins making an important contribution to the effect of pre-adaptation of EBR-treated plants to the possible forthcoming stresses. In connection with the presented data, it is important to underline, that in *Arabidopsis* plants some genes were co-regulated by BR and both ABA and drought (Huang *et al.* 2008).

Despite the numerous data demonstrating effectiveness of application of BR to increase plant productivity, the chain of reactions underlying the BR-induced increase in plant resistance to various injurious factors of environment are still not understood. Moreover, the role of endogenous BR in establishment of stress resistance remains unclear (Jager *et al.* 2008). However, it is the practice itself that dictates the necessity of the study of mechanisms of BR-induced effects. It opens new perspectives for increasing plant defense based on the application of BR in extremely low concentrations in agriculture. This increases the importance of the study of the mechanisms of BR-induced unspecific resistance of cultivated plants.

CONCLUSION

In total, the available data and/or literature display great possibilities of application of growth regulators with the aim of increasing productivity of plants frequently subjected to changing environment. Exogenous application of hormones not only stimulates plant growth and development, but also contributes to inducing unspecific resistance to various unfavourable factors of environment. It is due to combination of the properties of growth activators and inductors of unspecific resistance, for example, revealed for cytokinins, BR and SA, that those hormones have such a great potential for practice. Their application in extremely low concentrations triggers a wide range of response reactions appearing to be due their action on endogenous system of hormonal regulation of plant metabolism in total, which prepares plants to subsequent stress situations. This reflects in a decline in the level of injurious action of stress environmental factors on intensity of the growth processes in particular and in an increase in plant productivity in total. In connection with this, the study of the pattern of changes in the state of hormonal system caused by exogenous growth regulators seems to be very important to achieve an increase in plant productivity.

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