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Exogenous methyl jasmonate regulates cytokinin content by modulating cytokinin oxidase activity in wheat seedlings under salinity

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ABSTRACT

The treatment of 4-days-old wheat seedlings with methyl jasmonate (MeJA) in concentration optimal for their growth (0.1 μM) resulted in a rapid transient almost two-fold increase in the level of cytokinins (CKs). MeJA-induced accumulation of CKs was due to inhibition of both cytokinin oxidase (CKX) (cytokinin oxidase/dehydrogenase, EC 1.5.99.12) gene expression and activity of this enzyme. Pretreatment of wheat seedlings with MeJA decreased the growth-retarding effect of sodium chloride salinity and accelerated growth recovery after withdrawal of NaCl from the incubation medium. We speculate that this protective effect of the hormone might be due to MeJA's ability to prevent the salinity-induced decline in CK concentration that was caused by inhibition of gene expression and activity of CKX in wheat seedlings. The data might indicate an important role for endogenous cytokinins in the implementation of growth-promoting and protective effects of exogenous MeJA application on wheat plants.

1. Introduction

Jasmonic acid (JA) and its active derivatives, in particular methyl jasmonate (MeJA), are important signaling molecules involved in the regulation of plant growth and developmental processes, such as seed germination, root growth, formation of generative organs, flowering, transport of assimilates, nutrient storage, tuber formation, fruit ripening, and senescence (Creelman and Mullet, 1995; Balbi and Devoto, 2008; Wasternack and Hause, 2013). It is now well known that jasmonates (JAs) act, together with ethylene, to establish induced systemic resistance in response to insect wounding and attack by various pathogens, associated with expression of *PR*-genes and other protective proteins (Bari and Jones, 2009; Wasternack and Hause, 2013; Mithöfer et al., 2014). There is, however, a lot of information on the involvement of jasmonates in the regulation of plant resistance to abiotic stresses as indicated by evidence of increased biosynthesis of jasmonates in different cultures in response to drought, salinity, extreme temperatures, heavy metals, ozone, UV-radiance, as well as the decline of their damaging effects on plant growth exerted by exogenous jasmonates (Walia et al., 2007; Santino et al., 2013). Thus, it is evident that jasmonates, which have multiple functions in plants, are one of the main classes of plant hormones (Santino et al., 2013; Wasternack and Hause, 2013).

It has long been established that high levels of endogenous JAs, which are induced in response to environmental stresses, or exogenous application of JAs lead to growth retardation of plants (Noir et al., 2013). In addition, JAs inhibit mitosis, block both the G1/S and G2/M transitions in the cell cycle, and delay the onset of endoreduplication (Swiatek et al., 2002; Noir et al., 2013). Furthermore, a number of studies have shown that activation of JA signaling leads to repression of photosynthesis and photosynthesis-related gene expression (Attaran et al., 2014; Shyu and Brutnell, 2015). Thus, jasmonates are believed to play a key role in the transition from growth- to defense-oriented metabolism in plants (Shyu and Brutnell, 2015). However, in addition to their well known defense function, an increasing number of studies have provided evidence for an involvement of JAs together with other plant hormones in regulation of such integral physiological processes as growth, development, and differentiation of plants (Liu et al., 2015).

Plant growth and development are tightly coordinated temporally and spatially during ontogenesis by the action of plant

Abbreviations: MeJA, methyl jasmonate; CKs, cytokinins; CKX, cytokinin oxidase; JA, jasmonic acid; JAs, jasmonates; IPA, isopentenyladenosine.

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hormonal system both under optimal conditions and environmental changes. Regulation of developmental processes and defense responses is determined by the pattern of changes in the concentration ratio of several interacting hormones as well as by the ability of each hormone to influence endogenous content of the others (Reski, 2006; Shakirova et al., 2010). This suggests that the multiple physiological effects of jasmonates are might be due to their influence on the content of different plant hormones. Indeed, there are reports on the effect of jasmonates on the content of individual hormones in plants, and the diversity of regulatory action of jasmonates is due to their positive and negative interaction with other phytohormones, such as ethylene and SA (Bari and Jones, 2009; Mur et al., 2013), ABA (Hossain et al., 2011), auxins (Hentrich et al., 2013), gibberellins (Song et al., 2014), and brassinosteroids (Peng et al., 2011). However, little is known about the effect of jasmonates on the content of cytokinin hormones, and available data concerning changes in the content of cytokinins in response to JA treatment are rather contradictory (Dermastia et al., 1994; Bandurska et al., 2003; Ananieva et al., 2004; Battal et al., 2008; Schäfer et al., 2015), which may be due to the use of different plant species and organs as well as application of rather broad range of JAs concentrations.

In the present study, we found that incubation of wheat seedlings on the medium with MeJA resulted in rapid almost twofold transient accumulation of cytokinins (CKs) but had no effect on the contents of ABA and IAA. These data suggest an important role of CKs in the implementation of physiological effects of exogenously applied MeJA on wheat plants, including its protective effect, since CKs are known to be involved in the control of a broad spectrum of metabolic processes under normal conditions some of which contribute significantly to plant stress adaptation (Chernyad'ev, 2009; Wu et al., 2014).

Of particular interest in this regard is to study the processes responsible for regulation of the endogenous CK content by exogenous MeJA in wheat plants. Cytokinins play a central role in the hormonal regulation of plant growth and development (Zubo et al., 2008; Zalabák et al., 2013), and therefore their homeostasis in plant tissues is extremely important. Plants have an efficient system for maintaining the endogenous level of biologically active forms of CKs. The homeostasis of CKs depends on the balance between their biosynthesis, interconversions of active and reserve forms, and cytokinin degradation (Frébort et al., 2011). Our primary interest was to investigate the effect of MeJA on the CK degradation because biosynthesis of CKs under adverse environments is well known to decrease (Nishiyama et al., 2011; Merewitz et al., 2012), and therefore the significance of the existing pool of these phytohormones is of great importance. The key enzyme of CK degradation is cytokinin oxidase (CKX) that catalyzes the degradation of CKs by cleavage of their side chain, and this is the significant mechanism that controls the CK content in plants (Frébort et al., 2011; Avalbaev et al., 2012; Zalabák et al., 2013). Thus, the purpose of this research was to determine the ability of exogenously applied methyl jasmonate to influence gene expression and activity of cytokinin oxidase both under normal and sodium chloride salinity conditions.

In this work, we found that cytokinin oxidase is involved in the regulation of CK content by exogenous MeJA in wheat plants in optimal growth conditions as well as under salinity stress.

2. Materials and methods

2.1. Plant material and experimental design

Wheat seeds (*Triticum aestivum* L.) cv. Bashkirskaya 26 were obtained from Chishminsky Breeding Station, Bashkortostan, Russia. The seeds were surface sterilized with 96% ethanol and grown for 3 days on filter paper moistened with tap water under illumination of 200 mmol m^{-2} s^{-1} at 22–24 °C and a 16-h photoperiod. After endosperm excision, some 3-days-old seedlings were transferred onto a medium with 2% sucrose for 24 h. Then 4-days-old seedlings were exposed on 0.1 μM MeJA for different time periods. The working solution of MeJA was prepared by diluting the stock solution (1 mM MeJA in 96% ethanol) in 2% sucrose. Another part of the 3-days-old seedlings were transferred onto a medium with 2% sucrose supplemented with 0.1 μM MeJA for 24 h. Thereafter, MeJA-pretreated 4-days-old seedlings were transferred to a mixture of 2% sucrose and 2% NaCl for different time periods. Plants incubated on 2% sucrose solution served as a control in all these experiments.

2.2. Growth analysis

Growth was determined as changes in the length, fresh and dry weight of the seedlings and the activity of cell division in the apical meristem of wheat seedling roots. Experiments on the assessment of growth characteristics were conducted in three biological repeats, each variant included not less than 30 seedlings. To determine the mitotic index (MI), root apical meristem of seedlings was fixed in the mixture of acetic acid and ethanol (1:3) for 4 h. After fixation, the plant material was washed with tap water and treated with the mixture of 5% pectinase and 5% cellulase at 37 °C for 1 h. Transient squash preparations were stained with 1% acetocarmine prepared in 45% acetic acid. Thereafter, preparations were examined at 600× magnification and MI was counted by the routine method as a percent of mitotic to total number of the cells (Fusconi et al., 2006) using the Amplival microscope (Carl Zeiss, Germany). For each treatment, not less than 30 seedlings were used; the MI analysis was performed for 3000 cells in each treatment.

2.3. Quantification of cytokinins, IAA and ABA

Free cytokinins, IAA and ABA were measured in wheat seedlings (0.5 g fresh weight) by competitive ELISAs using polyclonal antibodies specific for a particular hormone and antirabbit antibodies conjugated with peroxidase (Shakirova et al., 2004; Zubo et al., 2008). For hormone extraction, seedlings were homogenized in 80% ethanol and incubated overnight at 4 °C. The homogenate was centrifuged at 18,000 × *g* for 10 min, and the supernatant was concentrated to an aqueous residue in vacuo. The total content of zeatin derivatives was determined in an aliquot of the aqueous residue using rabbit antibodies raised against zeatin riboside having high immunoreactivity towards zeatin, its riboside and nucleotide (Kudoyarova et al., 1998). The antibodies showed low cross-reactivity to dihydrozeatin and isopentenyladenine and their derivatives (Kudoyarova et al., 1998; Veselov et al., 1999). Immunoassay reliability was confirmed by dilution tests, chromatographic examination of the distribution of immunoreactivity, and comparison of the results of immunoassay against physicochemical assays (liquid chromatography–mass spectrometry (LC–MS)) (Veselov et al., 1999; Arkhipova et al., 2007). In graphs showing total cytokinin levels, data are combined values for zeatin, its riboside and nucleotide.

For IAA and ABA measurements, the remaining part of the aqueous residue was acidified with HCl to pH 2.5 and partitioned twice with diethyl ether (organic to aqueous phase 1:3). Subsequently ABA and IAA were transferred from the organic phase into 1% sodium hydrocarbonate (pH 7–8; ratio of organic to aqueous phases was 1:3), re-extracted with diethyl ether, methylated with diazomethane and immunoassayed using polyclonal antibodies to ABA and IAA (Veselov et al., 1992; Shakirova et al., 2004). IAA and ABA recovery calculated in model experiments was about 80%. Reducing the amount of extractant, based on