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STRESS MESSENGERS JASMONIC ACID AND ABSCISIC ACID NEGATIVELY REGULATE PLANT CELL CYCLE

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Summary. Environmental stress affects plant growth and development. Several plant hormones, such as salicylic acid, abscisic acid, jasmonic acid and ethylene play a crucial role in altering plant morphology in response to stress. Developmental regulation often has the cell cycle machinery among its targets. We analyzed the effect of jasmonic acid (JA) and abscisic acid (ABA) on cell cycle progression in synchronized tobacco BY-2 cells. Both compounds were found to prevent DNA replication, keeping the cells in the G1 stage, when applied just before the G1/S transition. However, ABA did not have any effect on subsequent phases of the cell cycle when applied at a later stage, whereas JA effectively prevented mitosis on application during DNA synthesis. This demonstrates that JA treatment can freeze synchronized BY-2 cells in both the G1 and G2 stages of the cell cycle. Jasmonate administered after the S-phase was less effective in decreasing the mitotic index, suggesting that cell sensitivity towards JA is dependent on the cell cycle phase. In parallel, we employed a more complex system: *Arabidopsis thaliana* roots, where JA induced root growth inhibition was well documented. We used detailed image analysis of the root in order to describe the effects of jasmonic acid on the function of the root apical meristem.

Key words: Jasmonic acid, Abscisic acid, cell cycle, BY-2, *Arabidopsis thaliana*, mitotic index

Abbreviations: JA – jasmonic acid, ABA – abscisic acid, G1, G2 – resting phases of the cell cycle, S – phase of DNA synthesis, M-mitosis, DAPI – 4',6-Diamidino-2-phenylindole dihydrochloride.

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Introduction

Exposure to stress in plants is often followed by growth retardation and reduced fresh weight and seed or fruit production. This is crucial and troublesome in agriculture, hence a deeper understanding of the mechanisms occurring during various stress responses is essential. Jasmonic acid (JA) and abscisic acid (ABA) are among those plant hormones, which mediate in certain types of stress responses and their action results also in a negative regulation of plant growth. ABA is involved in many aspects of water-limiting stresses such as drought, salt stress and cold (Xiong et al., 2002), whereas JA function is mainly attributed to wounding and pathogen response (Creelman and Mullet, 1995). Nevertheless, the action of the two hormones seems to be closely related as the JA-insensitive mutant jin4 is hypersensitive to ABA during germination (Berger et al., 1996). The work of Birkenmeier and Ryan (1998) demonstrated another level of interaction between ABA and JA-dependent signaling pathways, by showing that the mechanical damage, usually associated with activation of jasmonate response, causes also a local desiccation and accumulation of ABA at the site of injury. According to those data the wound response can be seen as combined action of two hormones: ABA is a local factor and JA acts both on local and systemic level. The action of ABA involves mobilization of calcium stores, activation of MAPK and phosphoinositol cascade which leads to regulation of ion channels and changes in protein phosphorylation and gene expression such as the late embryogenesis abundant (LEA) family (Xiong et al., 2002). On the level of plant morphology water stress and (or) exogenous ABA inhibit root growth in sunflower (Robertson, 1990), maize (Sacks et al., 1997) and *Arabidopsis* (Leung et al., 1994) and decrease leaf blade elongation in wheat (Shuppler, et al., 1998). All those events were associated with a decrease in mitotic activity in corresponding tissue. The work of Wang et al. (1998) demonstrated that ABA and cold stress induce expression of ICK1 a cell cycle regulator, from KRP family, which interacts with CDKA and cyclin D3 proteins and inhibits in vitro the activity of the CDK complexes.

In contrast with ABA, much less is known about the mechanism of jasmonate signaling. While the list of JA-up and down regulated genes is constantly growing (Creelman and Mullet 1995, Schenk et al., 2000), the primary factors which control their expression are unknown. Some authors indicate calcium fluxes, phosphatase and kinase activities as essential elements (Rojo et al., 1998; Leon et al., 1998). Similarly to ABA, exogenous JA inhibits root growth; however it remained unclear whether meristematic activity was affected. In our experiments we demonstrated that, as predicted from the literature data, in synchronized tobacco BY-2 cells ABA inhibits the cell cycle entry at the G1/S transition and does not interfere with further stages. Analysis of the *Arabidopsis* roots grown in the presence of JA indicates that JA affects both cell size and the frequency of the cell divisions; hence it has an effect similar to ABA. However, unlike ABA, it also inhibits G2/M transition in synchronized tobacco BY-cells.

Materials and methods:

Tissue culture and synchronisation.

Arabidopsis thaliana seeds were sterilised with bleach and transferred to Petri dishes containing MS medium with vitamins (Duchefa), 1% sucrose and 0.8% agar. Plants were grown vertically at 23ºC, under 16 h light–8 h dark regime. BY-2 cells were maintained as described by Nagata et al. (1992). with some modifications: the culture was refreshed weekly by transfer of 0.5 ml of a 7 days old culture into 50 ml of fresh MS medium (Duchefa) pH 5.8, containing 3% (w/v) sucrose (Duchefa), 0.2 g/l KH_2PO_4 (Merck), 10 mg/l *myo*-inositol (Sigma), 1 mg/l thiamin hydrochloride (Sigma) and 0.2 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D; Serva), referred in the text as "medium". The culture was kept at 27 ºC at constant darkness and 130 rpm. For maintenance on Petri dishes medium was additionally supplied with 1% (w/v) agar (Sigma). The synchronization protocol was based on the method of Nagata (Nagata et al., 1992). More details can be found in Swiatek et al. (2002).

Root growth measurement

Position of the root tip was marked every 24 h with a scalpel at the bottom of the plastic Petri dish containing vertically grown seedlings. At the end of the experiment plates were scanned in a flatbed office scanner in transparent mode (AGFA, SnapScan 1236, SCSI). Daily increments of the root were measured in the images using Scion Image (Scion Corporation).

Microscopy

For mitotic index analysis cell were fixed in solution ethanol/acetic acid 3:1 (v/v), washed in PBS, stained with 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI) and counted (Nikon fluorescent microscope). 500 cells were counted per slide, stages from early prophase until anaphase were considered as mitosis.

Arabidopsis roots were examined under light microscope (Leitz), under 20 x magnification. Images were captured using Nikon DXM 1200 digital camera and used for cell size measurements.

Thymidine incorporation

DNA synthesis was monitored in 1 ml samples by pulse labeling with 1 µCi of [methyl-^{3}H]- thymidine (AP-Biotech) for 30 min at 28 ºC on a rotary shaker as described in Swiatek et al. (2002). Upon quench correction total DNA synthesis was expressed as Bq per µg of protein in the sample.

Results

We analyzed the architecture of the *Arabidopsis* roots upon continuous treatment with jasmonic acid for four days. The data are summarized in the Table 1. As expected, jasmonic acid caused a substantial decrease in the root growth rate, approximately 8 fold, accompanied by a decrease in the cell side in the mature part of the root. However, the cells in JA treated root are only 3 times smaller than in the untreated control;

Table 1. Comparison between untreated control, wild type *Arabidopsis thaliana* ecotype Landsberg erecta and plants treated with 20 µM JA, 7 days after germination, 4 days of treatment. Roots of 15 plants per treatment were analyzed. Data represent average values ± standard deviation. For cell production rate standard deviation was calculated by error propagation method.

Parameter:	Control	20 µM JA
Root growth rate [µm/h]	398.52±117.64	51.48±15.77
Mature cell size [µm]	121.9 ±14.02	51.84±8.63
Cell production rate [No. of new cells per file/h]	3.27±1.03	0.99±0.35

therefore the cell size alone cannot be responsible for such a dramatic difference in growth rate. Dividing the growth rate by the size of the mature cell gives a good estimate of how many cells are formed in one cortical cell file in the root per unit of time. This value represents the contribution of the cell division to the root growth. In our experimental setup JA treatment results in approximately three times less cells being produced by the meristem as compared to control. Therefore the root growth inhibition caused by jasmonic acid is caused not only by a decrease in the cell size but also by a decreased meristematic activity. In order to gain more insight into how the cell cycle progression is affected by JA we moved to the experimental system of tobacco BY-2 cells. They offer an advantage of being undifferentiated and easily synchronized, which makes them an excellent tool for studying the cell cycle separated from differentiation. When BY-2 are treated with various concentrations of JA, a dose dependent growth inhibition can be observed (Fig. 1),

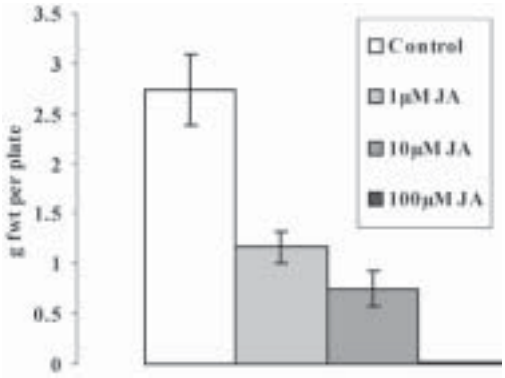

Fig. 1. The effect of various concentrations of jasmonic acid on fresh weight gain of tobacco BY-2 cells. The cells were grown in Petri dishes, on 1% agar for 3 weeks at 26 ºC in the dark.

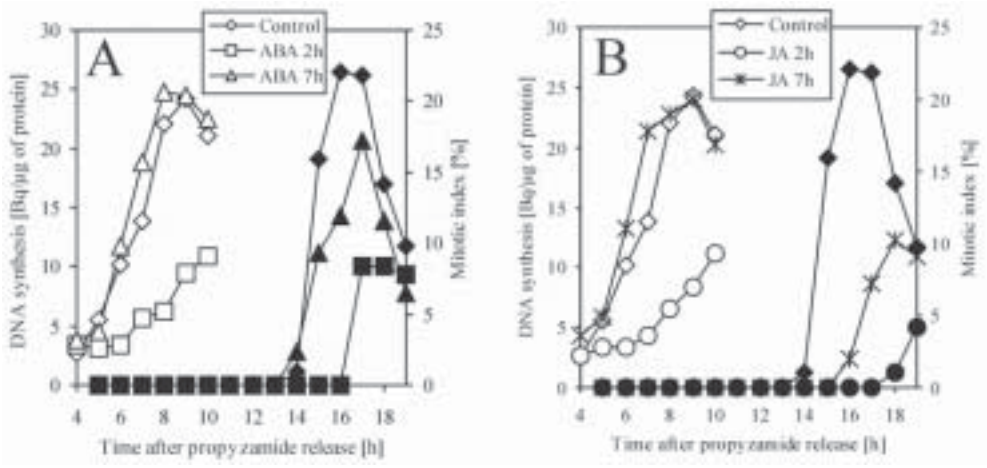

Fig. 2. The effect of 200 µM ABA and 100 µM JA on the cell cycle progression in propyzamide released tobacco BY-2 cells compared to untreated control. Hollow symbols: thymidine incorporation, full symbols mitotic index. A: cultures were supplied with ABA at two hours (G1) and seven hours (S-phase), B: cultures were supplied with JA at two hours (G1) and seven hours (S-phase).

which is clearly related to the inhibition of the cell proliferation since there was little variation in the cell size in the treated cultures. In subsequent experiment we compared the effect of JA and ABA on the cell cycle progression in the propyzamide-synchronized culture. Both hormones were applied at two time points: during G1 and during S-phase (Fig. 2). When either ABA or JA were applied during G1 a strong reduction in the DNA synthesis occurred, measured by thymidine incorporation, which was followed by a substantial reduction of mitotic index, which was more pronounced in JA treated culture. When applied during replication ABA nor JA had any effect on the rate of DNA synthesis, however the mitotic index was significantly reduced in the JA treated culture. We obtained very similar result when aphidicolin-synchronized culture was employed. When both hormones were applied to the cells synchronized at the beginning of the S-phase, only JA caused a reduction and delay of the mitotic peak (Fig. 3).

Discussion

Root growth inhibition is a typical plant reaction to exogenous jasmonate. It has been implemented in screening for JA insensitive mutants, for example coi1-1, jin1, jin4 and jar1 have been isolated this way. Although the wound and pathogen responses seem functionally distant from the root development control, nevertheless all the mutants mentioned above display defects in defense responses as well. Interestingly; little was known about the possible targets of JA action in roots. Our studies on *Arabidopsis* roots and in tobacco BY-2 indicate that JA can inhibit the cell cycle in the root meri-

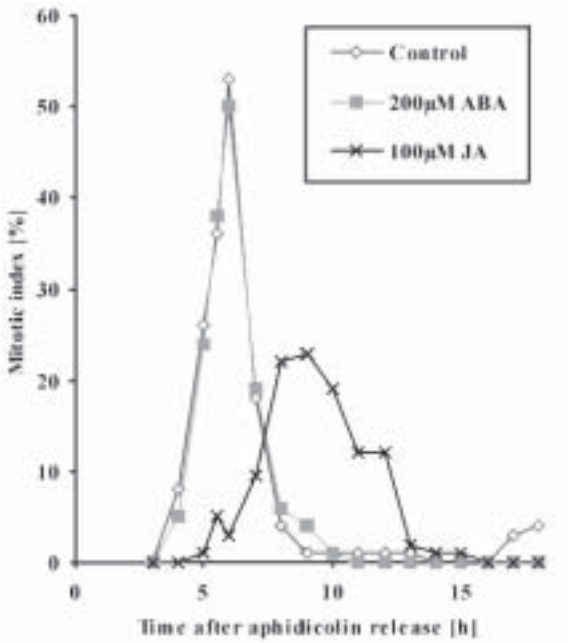

Fig. 3. The effect of 200 μM ABA and 100 μM JA on the mitotic index in aphidicolin released tobacco BY-2 cells compared to untreated control. Both JA and ABA were applied immediately after the release, which corresponds to early S-phase of the cell cycle.

stem. This indicates that systemic signals which are elevated in stress responses, like JA and ABA can directly influence vegetative growth. Possibly, because the stress reaction mobilizes plant metabolism into different pathways, so that the limited metabolite and energy supply is re-directed towards restructuring of the tissue. However there is still a remaining question of how exactly the cell cycle machinery is affected. In case of ABA, $p27^{Kip}$ related protein (KRP) seems a good candidate, because we observed that ABA effects G1/S transition but has no effect on the other stages of the cell cycle, which is in agreement with data of Wang et al. (1998), who indicated cyclinD3/CDKA complex as a target for inhibition by KRP1 while cyclin D3 is expressed mainly in late G1. Nevertheless this hypothesis would require experimental evidence. Direct targets of jasmonic acid still remain enigmatic. Since it blocks G1/S transition, it would be interesting to examine the expression of the KRP family since it seems a likely target for ABA action. G2/M arrest on the other hand seems unique for jasmonic acid and more difficult to explain, because our data indicate that BY-2 cells which are engaged in replication are most sensitive to this arrest (Swiatek et. al 2002).

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