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IAA-induced electrophysiological changes in wheat coleoptile cell plasmalemma during gravitropic reaction

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The fluorescent probe dis-C_3-(5) (3,3'-dipropyl-2,2'-thiodicarbocyanine iodide) was applied to investigate what passive and active cation transport changes occur in the plasmalemma during the different stages of gravitropic reaction. The coleoptiles were oriented horizontally for 30 s, 1 min, 3 min, 30 min. A membrane fraction enriched with the plasmalemma was isolated from such gravitropically irritated coleoptiles. The cation permeability of the plasmalemma was shown to be intensified under 30 s, *i. e.* the potassium diffusive potential (KDP) was decreased, but $1 \cdot 10^{-5}$ M IAA *in vivo* reduced the passive cation transport, and the KDP was on the increase. To characterize the gravitropic reaction, the following sequence of its processes can be suggested: gravitation force (g) effect → shift of cell structures → change of passive cation transport or H^+ potential through the plasmalemma → Ca^{2+} concentration changes → formation of IAA complexes and IAA transport.

Key words: wheat coleoptile, fluorescent probe dis-C_3-(5), potassium diffusive potential, gravitropic reaction

INTRODUCTION

Stems, coleoptiles and other organs are characterized by a negative gravitropic reaction curve because of a more intensive growth by elongation of the lower part of the organ, while in roots, which exhibit a positive gravitropic reaction, the growth is intensified in the upper part [1]. Following irritation, auxin (indole-3-acetic acid, IAA) concentration in the lower part of the axial organ of a horizontally positioned plant increases [2, 3] and the character of auxin action is determined by the hormone receptory system, which during the polarized growth is also already asymmetrical [4]. Ca^{2+} ion permeability of the plasmalemma is shown to increase as soon as within the first 1–2 minutes of gravitropic response, resulting in a suppressed formation of IAA–protein complexes [5]. However, so far it is not clear whether analogous changes can take place in the functioning of the monovalent cation transport systems and what kind of physiological and biochemical changes occur after irritation at the phase of polarized growth. The aim of the present work was to evaluate the character of changes in the electrophysiological properties of the plasmalemma during gravitropic reaction.

MATERIALS AND METHODS

Wheat (*Triticum aestivum* L., cultivars 'Arcus' and 'Selpek') 4-d etiolated shoot coleoptiles and their cell plasmalemma fragment were chosen as the study object. The wheat coleoptile consists of uniform cells which have insignificant differences, all in the phase of growth by elongation.

While studying the effect of IAA *in vivo*, wheat coleoptiles were grown with regard to the model of gravitropic response phase differentiation [6]. The coleoptile fragments (upon removing the first leaf) were laterally irritated with 12 g (g is free fall acceleration) for 3 min in a laboratory-made special centrifuge and then immediatelly decapitated and grown for 2 hours in an inverted position in special vessels – stands: in one case in water, in the other in a solution containing IAA $1 \cdot 10^{-5}$ M. Control variant coleoptiles (not exposed to graviinduction) were decapitated and grown in an inverted position for 2 hours in H_2O. When studying the effect of the initial stages of gravitropic response on the electrophysiological properties of the plasmalemma, the coleoptiles were horizontally oriented for 30 s, 1 min, 3 min, 30 min. From such gravitropically irritated coleoptiles a membrane fraction enriched with plasmalemma was isolated.

The plasmalemma fraction was isolated according to Leonard and Hotchkiss [7] and modifications [8, 9] by the method of differential centrifugation in sucrose density gradient. The whole homogenization and isolation procedure was carried out at a temperature of +4 °C.

The spectrofluorimetric method [10] was employed to assess the Na^+ and K^+ permeability of the membrane and the functioning of the cationic electrogenic pumps with the aid of a fluorescent probe, dis-3-(5) (3,3'-dipropyl-2,2'-thiodicarbocyanine iodide) $8.3 \cdot 10^{-9}$ M, sensitive to the potential of cyanine order at $\lambda_{excitation}$ = 570 nm, $\lambda_{fluorescence}$ = 670 nm. The fluorescent probe was applied to study the capacity of the membrane to maintain artificially created ion (concentration) and electrochemical gradients. Such ion gradients in the course of the experiment were created by saturating the vesicles obtained after purification in sucrose density gradient with a potasium medium (K^+ medium: 150 mM K_2SO_4, 150 mM sucrose, 1 mM Tris-Mes, pH 7.2). The vesicles were saturated up by osmotic shock. The membrane and shock medium (K^+ medium) ratio was 1:7. Following the shock, the suspense was concentrated by centrifugation 153000 g for 60 min. Such a preparation, called plasmalemma vesicle fraction, was used in the subsequent work. The vesicles were transferred into the incubation medium (1–2 µg protein in 600 µl Na^+ medium: 150 mM Na_2SO_4; 150 mM sucrose; 1 mM Tris-Mes pH 7.2). The K^+ premeability was induced by the ionophore valinomycine ($8.3 \cdot 10^{-9}$ M). Under these conditions K^+ ions start flowing from the inside to the outside of the vesicles and generate the K^+ diffusion potential (KDP). On adding monensin ($1 \cdot 10^{-5}$ M), a Na^+/H^+ exchange agent, Na^+ from the incubation medium flows into the vesicles. A decrease of the K^+ diffusion potential takes place (NaDP). The ATP-dependent H^+ transport was induced by adding ATP and Mg^{2+} into the medium (pH 6 both inside and outside the vesicles). Protein concentration was determined according to [11]. The test results were statistically evaluated. Only differences reliable at CF no less than 95% were considered.

RESULTS AND DISCUSSION

We studied changes that occurred in passive and active cation transport in the plasmalemma at different stages of gravitropic response development. The NaDP of non-graviinduced (control) (Figure) and graviinduced for different lenghts of time coleoptile plasmalemma fractions differed greatly. Even a 30-s horizontal position of the coleoptiles reduced the NaDP potential from 76 to 30%, implying a much higher conductivity of plasmalemma vesicles. One can see that at the same time the ATP-dependent H^+ potential increased nearly as much – from 75 to 114%. After 3 min and 30 min of gravitropic irritation the membrane cationic permeability of the membranes approached that of the control variant, *i.e.* the NaDP after a 3-min presentation in horizontal position increased up to 55%, and after 30 min it reached that of control. At the same time the functioning of the protonic pumps, which was intensified immediatelly following a 30-s irritation, as soon as within 2.5 min attained the initial level.

Thus, at the initial stages of gravitropic irritation (0.5–3 min) the cation permeability of the plasmalemma is intensified, while the proton transport through the plasmalemma becomes more intensive only in the case of a 30-s irritation. To study changes in plasmalemma electrophysiological properties during the IAA-dependent laterally polarized growth, the phase separation model was applied [6].

The effect of gravitropic irritation on the processes taking place in cell membranes of wheat coleoptile segments during polarized growth was investigated. The NaDP of the plasmalemma fractions of non-graviinduced (control) (Table) and graviinduced and with no IAA incubated coleoptiles exhibit nearly no differences and reach 55 and 56%, respectively (of KDP at a constant ATP-dependent H^+ potential). The NaDP of graviinduced coleoptiles incubated with $1 \cdot 10^{-5}$ M IAA (83%) was higher as compared to control (56%), implying that IAA *in vivo* reduced the Na^+ ion permeability of the plasmalemma. The active inward ion repumping,

Table. **Electrophysiological properties of laterally polarized wheat coleoptile cell plasmalemma during IAA–dependent gravitropically polarized growth**

Electrophysiological properties	Incubation		
	Vertical +2 h inverted without exogenous IAA	Horizontal 12 g 3 min +2 h inverted without exogenous IAA	Horizontal 12 g 3 min +2 h inverted with exogenous $1 \cdot 10^{-5}$ M IAA
NaDP (mV)	36	40	59
NaDP (% of KDP)	55	56	83
H^+ potential (% of KDP)	67	68	52

as well as the H^+ potential, in control reached 67% (of the KDP) and almost did not differ from the plasmalemma H^+ potential of the 3-min 12-g induced and H_2O-incubated coleoptiles. The H^+ plasmalemma potential of graviinduced and IAA-incubated coleoptiles was somewhat lower (52%). Thus, in this case graviinduction did not change the K^+, Na^+ and H^+ permeability of the plasmalemma, while on addition of exogenous IAA the H^+ transport was less intensive. Thus, we see that IAA *in vivo* reduces passive cation transport and considerably intensifies the NaDP. A comparison of these data with the 46% NaDP decrease presented in Figure which is thought [5, 12] to be related to the formation of IAA–protein complexes modified by calcium allows a suggestion that the modifying effect of IAA during the gravitropic reaction polarized growth as one of the modifying links is manifested through electrophysiological changes in the plasmalemma. The obtained results are compatible with the reports stating that changing the position of the axial organ with regard to the gravitation vector results in a higher Ca^{2+} concentration in the cytosol [3], and changes in Ca^{2+} concentration level influence the formation of IAA–protein complexes in the plasmalemma and thus the intensity of the realization stage of gravitropic response [5, 12]. To characterize gravitropic response, the following sequence of its processes can be suggested: gravitation force (*g*) effect → shift of cell structures → change of passive cation transport or H^+ potential through the plasmalemma → Ca^{2+} concentration changes → formation of IAA complexes and IAA transport.

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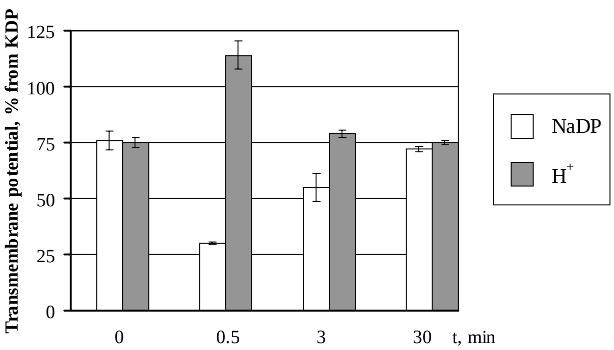

Figure. Effect of short-lasting gravitropic irritation (1 *g*) on the NaDP of horizontally oriented for 0.5–30 min wheat coleoptile cell plasmalemma vesicles and on the ATP-dependent H^+ potential. Medium inside the vesicles: 150 mM K_2SO_4, 150 mM sucrose, 1 mM Tris-Mes, pH 7.2; incubation medium for passive transport investigations: 150 mM Na_2SO_4, 150 mM sucrose, 1 mM Tris-Mes, pH 7.2, for active transport investigations – the same but pH 6.0 + 3 mM ATP and 3 mM $MgCl_2$. 100% – KDP

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KVIEÈIØ KOLEOPTILIØ LÀSTELIØ PLAZMOLEMOS ELEKTROFIZIOLOGINIAI POKYÈIAI DËL IAR POVEIKIO GRAVITROPINËS REAKCIJOS METU

S a n t r a u k a

Panaudojant cianininës eilës potencialui jautrø fluorescentiná zondà dis-C_3-(5) (3,3'-dipropil-2,2'-tiodikarbocianino jodidas) buvo tiriama, kokie katijonø pasyvaus ir aktyvaus transporto pokyèiai vyksta kvieèiø (*Triticum aestivum* L.) koleoptiliø plazmolemos fragmentuose skirtingais gravitropinës reakcijos vystymosi etapais. Koleoptilës buvo orientuotos horizontaliai 30 s, 1 min., 3 min., 30 min. Ið tokiu bûdu gravitropiðkai 1 *g* sudirgintø koleoptiliø iðskirta membraninë frakcija, prisodrinta plazmolemos. Paaiðkëjo, jog po 30 s plazmolemos laidumas katijonams yra didesnis, t. y. generuojamas maþesnis kalio difuzinis potencialas (KDP), o $1 \cdot 10^{-5}$ IAR *in vivo* poliarizuoto augimo metu maþina pasyvø katijonø transportà, generuojamas didesnis KDP. Siûloma gravitropinës reakcijos procesø seka: sunkio jëgos (*g*) poveikis → làstelës struktûrø pasislinkimas → pasyvus katijonø transporto arba H^+ potencialo per plazmolemà pasikeitimas → Ca^{2+} koncentracijos pokyèiai → IAR kompleksø susidarymas ir IAR transporto pokyèiai.