Influence of exogenous abscisic acid on alterations in protein expression in the proteome of *Triticosecale* seedlings

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Abstract The aim of this study was to investigate molecular basis of germination inhibition of *Triticosecale* under the influence of exogenous ABA. Seedlings were isolated from seeds after 48 h of germination in water (control sample) and 100 μ M ABA solution. It was observed that the applied concentration of phytohormone caused a significant inhibition of germination and a suppressed contribution of polysomes in the total ribosomal fraction. To identify proteins whose expression was affected by ABA, a proteomic approach employing 2D electrophoresis was used. Proteome maps for both control and ABA-treated samples displayed over 2,200 Coomassie Brilliant Blue-stained spots each. Protein spots showing either increase or decrease in spot intensity under the ABA influence were identified by mass spectrometry. Many of the identified proteins whose expression was stimulated by ABA proved to be stress-related, involved in nucleotide metabolism or playing a regulatory and signal transduction role, whereas proteins whose expression decreased are known to be involved in numerous metabolic pathways (including energy, carbohydrate or nucleotide metabolism) as well as related to genetic information processing and protein synthesis. Our results show that the germination inhibition caused by ABA is a result of multigene, diversified actions leading together to triticale growth suppression.

Keywords Triticale (*Triticosecale*) · Germination · Abscisic acid · Polysomes · Proteome · 2D PAGE

Abbreviations

2D PAGE	Two dimensional electrophoresis in polyacrylamide gel
ABA	Abscisic acid
CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
DOC	Sodium deoxycholate
DTT	Dithiothreitol
EGTA	Ethylenoglycol- <i>O</i> – <i>O'</i> -bis(2-aminoethyl)- <i>N,N,N',N'</i> tetraacetic acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
IEF	Isoelectrofocusing
M	Monosomes
pI	Isoelectric point
PTE	Polyoxyethylene-10-tridecyl ether
P	Polysomes
S	Ribosome subunits

Introduction

Annual survival of plant species depends on its ability to withstand unfavorable environmental conditions and on its efficiency of reproduction. Seeds do not only play an extremely important role as dispersal units in the plant life cycle but are also one of the most vital components of the world's diet. Triticale has a high crop potential and is readily used in animal breeding as a fodder of high alimentary values, good digestibility and yield efficiency (Boulter and Parthier 1982).

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Physiological and biochemical processes in plant organisms are under the continuous control of phytohormone regulation. Absciscic acid (ABA) is a universal plant growth regulator implicated in the control of many developmental and physiological processes during the whole plant life. It is commonly known as a growth inhibitor and plays an important role in embryo development, stress response, senescence, etc. By the ability to induce and, most likely, maintain dormancy, ABA is known to circumvent seed germination and seedling growth in the early stages of its growth (Kucera et al. 2005; Bewley 1997; Koornneef et al. 2002; Pawłowski 2007), which naturally prevents precocious germination when still on the mother plant. Pre-harvest sprouting may cause severe economic problems for agriculture by yield drop as well as diminishing grain functionality and viability.

It was observed before that by incubating mature seeds in exogenous solution of absciscic acid, the prevention of radicle extension and therefore the completion of germination may be strongly delayed as long as the inhibitor is present (Schopfer and Plachy 1985). Although many studies have been performed to gain the understanding of ABA involvement in seed germination and dormancy (Garciarubrio et al. 1997; Ananieva and Ananiev 1997; Pawłowski 2009), the process is still not fully understood.

During the whole plant life, ABA causes desiccation tolerance acquisition, which is essential during seed maturation. It was observed that stress conditions such as dehydration or excessive salinity may also cause similar proteins accumulation (Close 1997; Borovskii et al. 2002). The level of protein synthesis at the time is determined by the mRNA synthesis and the efficiency of translation. The dynamics of this process may be correlated with the formation of polysomes. Investigation of polyribosome contribution gives some valuable information concerning the overall capacity of protein synthesis in a tissue.

2D PAGE analysis allows a global analysis of the changes in proteins expression. Some papers have already been published on the subject of plant proteomics concerning seed germination, dormancy breaking (Gallardo et al. 2001; Cangahuala Inocente et al. 2009; Yang et al. 2007; Pawłowski 2007, 2009), or the influence of various growth regulators on proteome alterations (Pawłowski 2007). However, the complicated mechanisms of germination and dormancy breaking are still not fully understood. Proteomic studies give a powerful tool for identifying alterations in protein expression in response to a variety of factors, including hormonal stimuli. In combination with mass spectrometry and accessible databases, it gives enormous possibilities in the attempt to understand many physiological processes occurring in cells (Pawłowski 2007).

The main aim of this experiment was to investigate the molecular basis of ABA involvement in germination

suppression. In order to achieve this aim, the dynamics of protein translation and alterations in proteome profiles of triticale embryos were examined.

Materials and methods

Plant material and germination conditions

In this study, caryopses of cv Ugo triticale (*Triticosecale Witt.*) isolated from germinating seeds were used. Seeds were surface sterilized in 0.5 % sodium hypochlorite for 20 min, washed thoroughly with tap water and afterwards rinsed with distilled water. 50 grains were placed on a filter paper layer in 15 cm Petri dishes containing 20 ml of distilled water (control conditions) or 100 μ M aqueous solution of absciscic acid (ABA). Seeds were germinating in the dark at 21 °C for 24, 48 and 72 h. After these time periods, embryos were isolated from seeds. All the experiments were performed in triplicates.

Polyribosomes isolation

After 24, 48 or 72 h of germination, caryopses of 2 g fresh weight were ground using mortar and pestle in 16 ml of buffer U (2 % PTE, 1 % DOC, 200 mM Tris–HCl pH 8.5, 50 mM potassium acetate, 25 mM magnesium acetate, 2 mM EGTA and 100 mg/ml Heparin), according to the method of Abe et al. (1992), and pelleted at 12,000 $\times g$ for 20 min.

The extract was purified and enriched by pelleting through 50 % sucrose pads at 330,000 $\times g$ for 90 min in a Beckman SW 55 Ti rotor. The pellet containing the total extractable fraction of polyribosomes was resuspended in buffer U. 200 μ l of aliquot (about 10 OD units) was fractionated on a gradient of 15–60 % (w/v) solution of sucrose in buffer B (50 mM Tris–HCl pH 8.5, 20 mM potassium acetate, 10 mM magnesium acetate) at 330,000 $\times g$ for 45 min. The ribosome profile was monitored by measuring the absorbance at 254 nm using a UA-5 flow recorder (ISCO, Lincoln, NE, USA) according to Davies and Abe (1995). All steps were carried at 0–4 °C. The level of polysomes in the overall ribosomal fraction was determined by excising the area under the polyribosome profile from the total fraction of polysomes, together with monosomes and ribosomal subunits. All chemicals, if not indicated otherwise, were acquired from Sigma-Aldrich.

Proteome extraction

Proteome was extracted from embryonic tissue after 48 h of germination, as recommended by Gallardo et al. (2002a, b). 300 mg of tissue was powdered in liquid nitrogen and

suspended in chilled lysate buffer containing 7.5 M urea, 2.2 M thiourea, 63 mM CHAPS, 0.25 % Triton X-100 (Bio-Rad), 5 mM Trizma base, 21 mM Trizma hydrochloride, 1.1 % ampholytes (pH 3–10), 60 U/ml DNase I, 72 µg/ml RNase A (5.8 Kunitz units/ml), 14 % protease inhibitors (Cocktail complete mini, Roche) and 8 mM DTT (Bio-Rad). Each sample was mixed, shaken on ice for 45 min and centrifuged several times at $12,000\times g$ for 10 min in order to obtain clear supernatant. The protein concentration was measured at 280 nm using a NanoDrop 1000 spectrophotometer (Thermo Scientific).

Protein 2-D PAGE electrophoresis

Two dimensional electrophoresis in polyacrylamide gel (2D PAGE) can separate a complex protein mixture according to their isoelectric point (pI) in the first dimension and molecular mass in the second. First dimension of 2D PAGE was performed in a 24 cm Immobiline DryStrip Gel strip with a linear pH gradient from 3 to 10 (GE Healthcare) in an Ettan IPGphor 3 system (GE Healthcare), following the manufacturer's instructions. The protein extract was purified using 2D Clean-Up Kit (GE Healthcare). Prior to isoelectrofocusing, 2 mg of proteins were solubilized in rehydration buffer (7 M urea, 2 M thiourea, 2 % CHAPS, 0.5 % ampholytes (pH 3–10) (Amersham), 10 mM DTT and 0.002 % bromophenol blue) to a total volume 450 µl. Directly before the second dimension, the strip (containing separated proteins) was equilibrated in a glass tube in equilibration buffer containing 6 M urea, 75 mM Tris-HCl (pH 6.8), 30 % glycerol and 26 mM DTT (in the first step) or 220 mM iodoacetamide instead of DTT (in the second step) for 15 min each. In order to separate the proteins of the same pI according to their molecular mass, the strip was placed on top of a Precast DaltGel 12.5 (GE Healthcare) and sealed with 10 % agarose solubilized in electrode buffer containing 25 mM Trizma base, 195 mM glycine and 0.1 % SDS (Laemmli 1970). Electrophoresis was carried in an EttanDalt Six electrophoresis unit (GE Healthcare) using Laemmli buffer system. To estimate molecular masses of proteins, Low Molecular Mass Standards (Bio-Rad) were used.

Analysis of 2DE gels

After 2D PAGE, the gel was stained with Coomassie Brilliant Blue G-250 according to Neuhoff et al. (1988) and scanned on an Image Scanner using Labscan 3.0 software (GE Healthcare). The protein pattern was analyzed using ImageMaster 2D Platinum 6.0 programme. After spot detection the quantitative determination of spot intensity, including a normalization following the method of principal component analysis (Wibig 2007), was performed,

taking into consideration the average values of background optical density (OD) and spots intensity on each gel. The obtained data were subjected to a *t* test statistical analysis. 12 of up- and 12 of down-regulated proteins, who showed at least twofold difference in spot intensity in response to ABA, were cut from the gel and subjected to a mass spectrometry identification.

Mass spectrometry

Spots of interest were analyzed by liquid chromatography coupled with a Hybrid-2D-Linear Quadrupole Ion Trap-Fourier Transform Ion Cyclotron Resonance (LTQ-FT-ICR) mass spectrometer (Thermo Electron Corp., San Jose, CA, USA) in the Institute of Biochemistry and Biophysics (Polish Academy of Sciences, Warsaw, Poland). Prior to the analysis, gel slices were subjected to a standard in-gel digestion procedure (Shevchenko et al. 1996) during which gel pieces were washed with 50 % (v/v) acetonitrile, 10 mM ammonium bicarbonate solution, dehydrated with 100 % acetonitrile and completely dried in a Speedvac concentrator. Proteins were digested overnight with Sequencing Grade Modified Trypsin (Promega V5111). Peptides were eluted from gel with 0.1 % TFA, 2 % acetonitrile and applied to RP-18 precolumn (LC Packings) using 0.1 % TFA as a mobile phase. Afterwards, peptide mixtures were transferred to nano-HPLC RP-18 column (Waters), using 0–60 % acetonitrile gradient in 30 min in the presence of 0.05 % formic acid (150 nl/min flowrate). Column outlet was directly coupled with ion source of LTQ-FT-MS, working in the regime of data-dependent MS to MS/MS switch. The acquired raw data were processed by Mascot Distiller followed by Mascot Search (Matrix Science, London, UK, locally installed <http://proteom.pl/mascot>) against NCBI non-redundant database. Search parameters for precursor and product ions mass tolerance were, respectively, ± 40 ppm and ± 0.8 Da (Pappin et al. 1993).

Results

In this study, triticale seeds were subjected to germination in 100 µM aqueous solution of phytohormone abscisic acid (ABA). Measurements of the fresh weight content of embryonic tissues after 48 h of germination showed significant differences between control seedlings and those subjected to ABA. Their masses amounted to 23.79 and 10.45 mg per seedling, respectively. After 72 h of germination, the masses grew to 44.77 mg in control and 12.28 mg in ABA-treated seedlings (Fig. 1). Comparing the values of fresh weight content after both time periods, it is clear that ABA caused a significant suppression in seedlings fresh weight accumulation during early stages of triticale seed germination.

Fig. 1 Changes in fresh weight of *Triticosecale* embryos and seedlings during germination in water (control) and in 100 μ M abscisic acid (ABA). Statistically significant differences between examined trials and control were indicated: ** $p < 0.01$. Standard deviation ($\pm$ SD) was determined for $n = 3$

The tendency was similar in the polysome content in the total ribosomal fraction (monosomes, polysomes and ribosomal subunits) of embryonic tissues. Figure 2 presents sedimentation profiles of the total ribosomal fractions of triticale seedlings separated in a sucrose gradient 15–60 %. The analysis of the polysome population contribution in the total ribosomal fraction showed an increase from 18.6 % after 24 h of germination under the control conditions up to 65.6 % after 48 h and a slight decrease to 64.3 % in 72 h seedlings. ABA showed an inhibitory effect on the contribution of the polysome population content. The amount of polysomes in the total ribosomal fraction of caryopses treated with exogenous ABA was greatly reduced. After 24 h of germination, it constituted only 4.6 % of the total ribosomal fraction, 24 h later 22.3 % (nearly 3 times less than in the control at the same time) and after 72 h of germination it decreased to 18.1 %.

It has been observed that after 48 h of germination, caryopses displayed the first significant difference in the fresh weight content caused by the ABA application and the maximum of polysome formation. Due to this fact, the proteomes of caryopses were analyzed at that time. In order to test the protein expression alterations caused by ABA, the 2D PAGE method coupled with mass spectrometry was used. It was observed that ABA not only caused a decrease of some protein expression but also up-regulated the expression of others. The 2D proteome profiles in the pH range from 3 to 10 were established for control and ABA-treated caryopses. Figure 3 shows the achieved electrophoretic patterns with the identified proteins marked with arrows. Proteins whose expression was stimulated by ABA (spots 1–12) are presented in Table 1 and those whose expression decreased (spots 13–24) are shown in Table 2.

Discussion

Seed germination is one of the most critical processes in plant life, when quiescent embryos transform into active,

Fig. 2 The sedimentation profiles of total ribosomal fractions isolated from *Triticosecale* embryos and percentage of polysomes (grey area): before germination (0 h), after 24, 48 and 72 h of germination in water—control (a) and in 100 μ M ABA solution (b). Absorbance was measured at 254 nm

viable new organisms. It has already been noted that ABA blocks reserve mobilization by repressing α -amylase gene transcription and therefore restricts the availability of energy and metabolites for growing embryos, alters seed carbon and nitrogen metabolism (Garciaurubrio et al. 1997), inhibits proteins and RNA synthesis (Ananieva and Ananiev 1997), water uptake, endosperm rupture, restrain further embryo extension and seedling growth after radicle emergence (Schopfer and Plachy 1985; Kucera et al. 2005). The suppression of germination is then a result of changes in many various processes occurring in tissues, often initiated by expression of the ABA-responsive genes. The present results show that an exogenously applied growth regulator, namely ABA in 100 μ M concentration, significantly influences on the physiological and biochemical behavior of germinating triticale seeds.

In this work we show that ABA treatment has a strong inhibitory impact on polysome participation in protein synthesis during seed germination. Very few studies on this subject exist, mainly completed by Weidner et al. (1991, 2003, 2006). Formation of a polysome population in the presence of ABA proved to be suppressed more than three-fold during the whole time of the experiment. Absciscic acid inhibits transcription but increases RNA stability (Weidner et al. 2006), therefore protein biosynthesis takes part mainly on the mRNAs preserved in desiccated embryos. Kawaguchi et al. (2004) showed that dehydration stress causes the reduction of mRNAs recruited to polysomes, what indicates that the effect of the restriction of translation mediated by ABA and dehydration may be related. The suppression of polysome formation may be a result of ABA-mediated reduction of translation initiation, diminished rate of elongation, termination or mRNA availability.

Fig. 3 Two-dimensional electrophoretic patterns of embryonic proteins obtained from *Triticosecale* seedlings germinating for 48 h in water—control (a) and in 100 μ M solution of abscisic acid (b). Isoelectrofocusing was performed in the 24 cm polyacrylamide strips with the immobilized pH gradient ranging from 3 to 10. Proteins were visualized using Coomassie Brilliant Blue G-250. Identified spots are marked with arrows. On the left side of each gel was added a marker of protein molecular weight (kDa)

The results concerning changes in the polysome content show a slight decrease in the polysome content after 72 h of germination in comparison to 48 h seedlings both in control and ABA-treated samples. Diminishing amount of the transcripts incorporated into polysomes in the seedlings may be an indicator of triggering post-germination processes such as reduction of cell division in favor of higher extension rate and higher water uptake. Long exposition to the phytohormone may be one of the causes of low water input and polysome formation reduction in ABA-treated seedlings.

2D PAGE analysis of the proteomes of ABA-treated and non-treated seedling showed differences in proteins expression. Identified proteins were classified according to their functions into categories such as stress response, metabolism, signal transduction, protein synthesis and genetic information processing, protein degradation, cell structure.

Stress response

Many of the identified proteins can be included in the group of stress-related proteins. It is known that ABA enhances stress tolerance in plants, especially desiccation tolerance in the late stages of seed development, by induction of stress-related proteins production (Yang et al. 2007; Li et al. 2010). Because the whole process of germination might be described as the reverse of seed maturation, it is not surprising that some of the proteins involved in embryo development are still present under the influence of exogenously applied ABA, although normally during the imbibition stage of germination their abundance gradually diminishes (Han and Kermod 1996; Ostergaard et al. 2004). Absciscic acid in this work caused an increase

Table 1 Identified proteins from *Triticosecale* seedlings, whose expression increased under the influence of exogenously applied abscisic acid

Up-regulated				
Spot number	Protein identification	Organism	Accession number	Exp. M_w/pI
1.	Putative late embryogenesis abundant protein	<i>Hordeum vulgare</i>	ABS85196	96/7.8
2.	Dehydrin; DHN6	<i>Hordeum vulgare</i>	AAF01694	69/6.5
3.	26S proteasome regulatory particle triple-A ATPase subunit4b	<i>Oryza sativa</i>	BAB78495	59/6.3
4.	USP family protein	<i>Triticum aestivum</i>	AAX13276	31/5.7
5.	Nucleoside diphosphate kinase	<i>Lolium perenne</i>	AAF91407	29/6.1
6.	LEA protein	<i>Bromus inermis</i>	BAD22766	49/6.7
7.	Putative late embryogenesis abundant protein	<i>Hordeum vulgare</i>	ABS85196	103/6.3
8.	Putative late embryogenesis abundant protein	<i>Hordeum vulgare</i>	ABS85196	102/6.4
9.	Putative GTP-binding protein	<i>Oryza sativa</i>	BAD03576	58/6.1
10.	NAD-dependent formate dehydrogenase		Q9ZRI8	58/6.1
11.	Alpha-SNAP	<i>Hordeum vulgare</i>	AAP79420	48/5.2
12.	Glutathione transferase	<i>Triticum aestivum</i>	CAC94003	38/6.2

Table 2 Identified proteins from *Triticosecale* seedlings, whose expression decreased under the influence of exogenously applied abscisic acid

Down-regulated				
Spot number	Protein identification	Organism	Accession number	Exp. M_w/pI
13.	Os08g0538000; similar to putative glycyl-tRNA synthetase	<i>Oryza sativa</i>	NP_001062369	93/5.9
14.	Os09g0535300	<i>Oryza sativa</i>	NP_001063780	56/5.9
15.	Glyceraldehyde-3-phosphate dehydrogenase, cytosolic		P26517	52/6.3
16.	Glutathione transferase F4	<i>Triticum aestivum</i>	CAD29477	41/5.9
17.	Glucose and ribitol dehydrogenase homolog—barley	<i>Hordeum vulgare</i>	T06212	45/6.3
18.	Dehydroascorbate reductase	<i>Triticum aestivum</i>	AAL71854	41/5.9
19.	Os05g0460000; similar to 70 kDa heat shock cognate protein 1	<i>Oryza sativa</i>	NP_001055754	45/6.3
20.	Heat shock protein cognate 70		AAA86903	27/6.4
21.	ATP synthase beta subunit	<i>Triticum aestivum</i>	CAA52636	68/5.6
22.	Ribosomal protein S7	<i>Triticum aestivum</i>	AAW50993	47/5.8
23.	HSC70-1 (heat shock cognate 70 kDa protein 1); ATP binding	<i>Arabidopsis thaliana</i>	NP_001119156	47/6.1
24.	α -Tubulin	<i>Hordeum vulgare</i>	AAB08791	35/5.7

in the expression of some LEA (late embryogenesis proteins—spots 1, 6, 7, 8), dehydrin DHN6 (spot 2), NAD-dependent formate dehydrogenase (spot 10), USP family protein (spot 4), and glutathione transferase (spot 12).

LEAs are known to be ABA responsive (Finkelstein and Rock 2002; Grelet et al. 2005) and protect developing embryos from stresses by replacing water and maintaining protein and membrane structure (Close 1997; Han and Kermode 1996). DHN6 was another stress protein up-regulated by ABA. The increase of this protein expression was especially high (more than threefold). Dehydrins are known to play protective functions, preventing protein coagulation and plasma membrane destabilization, what is important for embryo prevention during seed maturation. The up-regulation of DHN6 occurs not only after ABA treatment but also under low water availability or cold stress condition (Rinne et al. 1999; Borovskii et al. 2002) but we still lack the complete knowledge about their exact role in the cell. The up-regulation of NAD-dependent formate dehydrogenase and USP family protein gives another evidence of ABA cross-talk with stress response pathways. The former, due to the ability to oxidize accumulating under stress formate into CO_2 in the presence of NAD^+ , may complement the respiration when the efficiency of Krebs cycle diminishes. Both the transcript and the enzyme were detected under many stresses such as hypoxia, drought, cold, wounding, and osmotic stress (Ambard-Bretteville et al. 2003; Kim et al. 2007; Li et al. 2010). The latter, USP family proteins, are also often expressed in many species under the unfavorable conditions, providing the organism with the ability to protect from various stresses and therefore this proteins constitute a useful target in stress tolerance engineering. Suggested function of the proteins containing USP domain is nucleotide binding and signal transduction (Nachin et al. 2005;

Isokpehi et al. 2011). Its function under ABA conditions has not been studied yet.

Also glutathione transferases are members of a diversified group of enzymes protecting cells from a wide range of reactive substrates. Those genes were already found to be responsive to abscisic acid as well as many abiotic stresses (Xu et al. 2002). They are involved in detoxification and became a marker of plant response to stress (Dixon et al. 2002). On the other hand, we found that one of the proteins from this group, glutathione transferase F4 (spot 16), was down-regulated in response to applied abscisic acid. Peng et al. (2009) examined two strains of wheat differing in stress tolerance and obtained the substantial up-regulation of glutathione transferase F4 under stress conditions only in the tolerant strain. This data suggest that the ability to generate detoxification reactions with this protein involvement is dependent on the stress susceptibility of the species. It could be also concluded that not all of the stress and ABA-responsive pathways are common.

ABA caused also a negative effect on HSC70 (heat shock protein cognate) expression (spots 19, 20, 23). HSC70 belong to the group of chaperones, which bind to newly synthesized proteins, preventing from aggregation and improper folding during a very active time of plant development (Sung et al. 2001). The low level of heat shock proteins seems to coincide with the low-efficient protein yield and the germination inhibition in the presence of ABA.

On the basis of the previous results as well as the results of this study, we may observe an evident cross-talk between ABA and stress response. It is not surprising, since stress-inducible genes expression in many cases is conditioned by ABA-dependent phosphorylation of transcriptional factors binding to ABRE (ABA-responsive elements) regulatory sequences (Yamaguchi-Shinozaki and Shinozaki 2006). Abscisic acid is a main player in setting a seed dormancy and

triggers the processes allowing the embryo to survive in a quiescent state. Considering the severe conditions naturally imposed on seeds during desiccation, the relationship between stress and the phytohormone is indispensable.

Metabolism

The results of our work indicate that abscisic acid regulates many aspects of cell energy, carbohydrate, and nucleotide metabolism and causes complex and diversified effects on cell functions. Our findings indicate that ABA-mediated germination inhibition rely to a large extent on metabolism suppression. Germination and subsequent steps of seedling growth require large amounts of energy released from the seed storage reserves and converted into energy mainly by glycolysis. Yang et al. (2007) identified many glycolytic enzymes up-regulated during barley germination. In our study, the applied concentration of ABA has shown an inhibitory effect on the synthesis of many proteins involved in metabolism such as glyceraldehyde-3-phosphate dehydrogenase (spot 15), glucose and ribitol dehydrogenase homolog (spot 17), dehydroascorbate reductase (spot 18), and ATP synthase beta subunit (spot 21). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is the enzyme involved not only in glycolysis but also in other functions related to plant stress. It was demonstrated that the amount of GAPDH transcripts in rice grows significantly in response to exogenous ABA treatment and drops considerably after few hours (Zhang et al. 2011). Although most of this protein functions in relation to stress remain largely unknown, it was suggested that it may take part in protection against high ROS levels and generate NADPH when other proteins undergo inactivation or degradation (Chang et al. 2000; Bustos et al. 2008).

Down-regulation of ATP synthase beta subunit suggests the contribution of ABA to regulating the energy metabolism in germinating seedlings. Glucose and ribitol dehydrogenase is involved in an alternative carbohydrate metabolism and its gene is known to be ABA responsive (Ostergaard et al. 2004). Dehydroascorbate reductase (identified as spot 18) reduces dehydroascorbate to ascorbic acid, which protects cells against oxidative stress. Deficiency of this protein regulates cell elongation and cell cycle, what was already shown to lead to suppression of leaf expansion, deceleration of root growth and delay of flowering (Chen and Gallie 2006).

The up-regulated protein in this category is nucleoside diphosphate kinase (NDPK) (spot 5). As a housekeeping enzyme, it provides nucleoside phosphates for nucleic acid synthesis and plays a role in signal transduction. Its function is not entirely known yet. The results obtained in this work are not consistent with the results of Yano et al. (1995) or Kim et al. (2008), who detected an increase of NDPK during

germination and seedling growth. However, Harris et al. (1994) detected the up-regulation of NDPK as a result of stress response, which suggests that the role of NDPK in plants is diversified and needs further investigation.

Signal transduction

GTP-binding proteins are important in a cell signal transduction chain initiated by phytohormones (Terry et al. 1993), therefore the especially high (more than threefold) up-regulation of triticale GTP-binding protein (spot 9) expression by ABA is not surprising.

Protein synthesis/genetic information processing

Uwer et al. (1998) suggest that an arrest of embryo growth between globular and heart stages may be caused by the loss of Glycyl-tRNA synthetase (spot 13) during embryogenesis. Abscisic acid in our study caused a decrease in the protein showing similarity to this enzyme. This may suggest that ABA is directly responsible for maintenance of dormancy and inhibition of germination by the inhibition of specific protein expression.

A responsiveness of the ribosomal protein S7 (corresponding to spot 22) to abscisic is a novel finding. The protein reveals down-regulation in relation to the phytohormone. It is known as an initiator of 30S ribosomal subunit assembly and plays important role in protein synthesis by implication in tRNA and mRNA interactions (Donotsova et al. 1991). The down-regulation of this protein may be one of the explanations of the decrease of polysome formation and growth inhibition obtained in this work in the presence of exogenously applied ABA.

Protein degradation

The main player in a protein degradation pathway of plant cells is 26S proteasome. It may regulate numerous processes, such as division or development by degradation of unwanted or regulatory proteins; it can ensure proper protein folding and assembling of complexes by controlling ubiquitin-mediated degradation (Wolf and Hilt 2004; Borghetti et al. 2002). Expression of 26S proteasome regulatory particle triple-A ATPase subunit 4b (spot 3) was positively (more than threefold) modulated by ABA, indicating that protein cleavage is an especially important control point in processes connected to germination inhibition and dormancy breaking.

Cell structure

Two of the identified proteins are involved in cell structure formation (Alpha-SNAP and α -tubulin). The up-regulated

protein identified as Alpha-SNAP (spot 11) is important for proper cellular organization, division and expansion. It is also crucial for intracellular vesicle trafficking and fusion (Bachem et al. 2000; Leyman et al. 2000). The role of alpha-SNAP in ABA-mediated germination suppression has not been reported yet, but it was found to be important in a process of stomatal closure triggered by ABA on a non-transcriptional level (Geelen et al. 2002). It is involved in starch and soluble sugars accumulation in leaves (Bachem et al. 2000). The other protein from the group, down-regulated α -tubulin, marked here as spot 24, is one of the key components in controlling seed germination (Pawlowski 2009). Giani et al. (1998) indicated that a drop in α -tubulin transcript is correlated with the inhibition of coleoptile elongation in response to ABA in rice. Olinevich et al. (2002) observed a decrease in microtubules and α -tubulin content in wheat roots in the presence of the hormone, together with an enhancement in the stability of the microtubules, what affirms ABA-related suppression of cell extensibility, leading to growth retardation.

The down-regulated hypothetical protein Os09g0535300 has not been characterized yet, either for its responsiveness to the phytohormone or for its functions under stress conditions; however, the ethylene-responsive homolog protein XAP-5 (CIRCADIAN TIMEKEEPER XCT) from *A. thaliana* is suggested to play a role in ethylene-induced growth inhibition and hypocotyl shortening by modulating the activity of some transcription factors. It may also be connected to a circadian clock and signal transduction (Ellison et al. 2011).

Conclusions

As expected, exogenously applied abscisic acid caused inhibition of germination of triticale seeds. It has also been shown to play a suppressive role in polysome formation and to influence on expression of various proteins, altogether leading to the inhibition of growth and development. On the basis of our results we may conclude that the applied phytohormone mainly decelerates metabolism, protein translation, cell elongation and influences the expression of stress-related proteins in triticale.

Author contribution Ewa Szypulska and Stanisław Weidner were responsible for experimental design, Sylwia Świgońska provided all the technical support, Anna Badowiec performed the research and prepared the manuscript under the guidance of Stanisław Weidner.

Conflict of interest The authors state that they have no conflict of interest.

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