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Inhibition of α-amylase acting in hexaploid triticale lines by exogenous abscisic acid

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Abstract

Hexaploid triticale introgressive lines developed after recombination of A-genome with A^m-genome of diploid wheat (*Triticum monococcum*) were analysed in respect of grains responsiveness to exogenous ABA treatment. This was assessed by *in vivo* bioassay as grain germination indices, and by α-amylase assay as quantity of synthesised α-amylase measured with the technique of radial diffusion in agarose gel. The results showed an important diminishing of seedling length caused by ABA (variable in different lines) as well as genotype dependant variability of α-amylase synthesis inhibition. The differences of ABA responsiveness were seen both in whole grains and in embryoless half-grains as a direct reaction of the aleurone layer. Variation of grain sensitivity to ABA treatment compared with two sprouting resistance indices showed a significant correlation with Falling Number values in grains, but not with a dormant grains germination in spikes. This is an evidence that in triticale precocious starch decompose in unripened and ungerminated grains is dependent on genotype ABA-responsiveness of the aleurone layer.

Additional key words: ABA-responsiveness, dormancy, enzyme radial diffusion, Falling Number, germination indices, introgressive lines.

Introduction

Attempts to obtain a biochemical marker for grain dormancy have resulted in an indication that the phytohormone ABA is involved in different germination ability of non-dormant and dormant grains (Ried and Walker-Simmons 1990, Basra *et al.* 1993, Schuurink *et al.* 1993). ABA contents were similar in both non-dormant and dormant wheat grains, but the effects of ABA on germination of isolated embryos were directly proportional to the degree of dormancy in whole grains (Walker-Simmons 1987).

Based on these results the responsiveness of wheat grains to ABA treatment evaluated under laboratory conditions as an inhibition of germination was found to be an effective criterion for assessing sprouting-resistant germplasm (Basra *et al.* 1993). Also suppression of α-amylase synthesis was used to characterise the genotype responsiveness to exogenous ABA and selection of sprouting resistant stocks of rye (Masojć *et al.* 1995, 1997).

Both aforementioned cereals (wheat and rye) are known to have strong correlation of two commonly used sprouting indices: harvest-ripe grains germination in spikes (illustrating level of dormancy) and Falling Number values in whole grains mill. In opposite to this triticale stocks have shown relatively weak or no correlations between this two sprouting indices. This was found as a characteristic feature of triticale seed physiology (Sodkiewicz 1999, Zych 2000).

In the present study, the genetically determined sensitivity to exogenous ABA treatment was assessed in introgressive triticale recombinants (Tc/Tm lines) variable in spikes sprouting resistance. ABA-responsiveness measured as a grain germination indices and quantity of synthesised α-amylase was correlated with both sprouting resistance indices to analyse their ABA-dependence.

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Abbreviations: ABA - abscisic acid; FN - falling number; PI - promptness index; SR - sprouting resistance.

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Materials and methods

The material consisted of 57 hexaploid triticale introgressive lines (Tc/Tm) developed as a result of A-genome recombination with A^m-genome of diploid wheat *Triticum monococcum* var. *macedonicum* Papag. In a three-year experiment, grain dormancy in mature spikes was assessed as the average number of grains sprouting in response to laboratory artificial wetting (SR) and falling number (FN) value of whole grain mill was evaluated in seconds, as well as correlation between these two indices of sprouting resistance was estimated (see Sodkiewicz 1999). Since the SR/FN correlation was not statistically significant (r = -0.247, $P > 0.05$), Tc/Tm lines with maximum and minimum values of these indices in a matching and aninverse system were selected and used for particular experiments on ABA responsiveness.

***In vivo* bioassay:** It was carried out with 13 lines after the decay of postharvest dormancy. For every line two repetitions with 30 grains each were imbibed at 23 °C in water (1W and 2W) and 10^{-5}M ABA solution (1A and 2A) on Petri dishes covered with plastic cups. The numbers of germinated grains were scored daily until all grains germinated in some plates. Ten days after the beginning of imbibition the length of every seedling was measured. Six different indices of germination were evaluated (see Table 1) to search for the best one characterising ABA responsiveness. The promptness index (PI) was calculated as: $PI = 3(D_1) + 2(D_2) + D_3$, where D_1, D_2, D_3 were the numbers of grains germinating on days 1, 2, and 3, respectively (Mc Crate *et al.* 1982). The corrected promptness index (CPI) was calculated using the formula: $CPI = (90 - PI_{H_2O}) + PI_{ABA}$, where PI_{H_2O} was the PI of a given Tc/Tm line in a water (control), and PI_{ABA} was the PI of the cultivar in the ABA solution (Mc Crate *et al.* 1982).

The relative diminishing [%] of seedling length caused by ABA was calculated from the formula: $[(W - ABA)/W)] \times 100$, where W and ABA were the average lengths of seedlings on plates with water and ABA, respectively (Masojć *et al.* 1995).

Enzyme assay: The α-amylase activity in grains imbibed in water and ABA solution were quantified with the technique of radial diffusion in a 1.2 % (m/v) agarose gel containing 1 % (m/v) β-limit dextrin. In this method there is a linear relationship between the size of the reaction circle diameter and the logarithm of α-amylase activity expressed as a percentage of the activity in the undiluted extract measured spectrophotometrically (Daussant *et al.* 1980, Masojć and Larsson-Raźnikiewicz 1991). Dextrin was prepared by extraction of *Zea mays* starch with β-amylase.

The activity of α-amylase was assessed in whole grains imbibed in ABA solution and in water (control), as well as in embryoless half-grains of 12 Tc/Tm lines. Thirty grains (or distal halves) of every line were sterilized in 7 % sodium hypochlorite and imbibed in distilled water or 5×10^{-5} M solution of ABA (*Sigma*, St. Louis, USA) for 3 d at 25 °C. After this time grains were homogenized in 3 cm^3 of 0.02 M extraction buffer (20 mM sodium acetate containing 1 mM $CaCl_2$, pH 5.5). This was followed by centrifugation at 8 000 *g* for 10 min. The supernatant (0.02 cm^3) was poured into individual holes of 5 mm in diameter made in the gel. The diffusion of α-amylase was allowed to continue for 23 h in a moisture chamber at 7 °C. The agarose plate was stained with 5 % solution of I_2 in KI. For each triticale line diameters of diffusion circles in five replications were measured with an accuracy of 0.5 mm, and averaged as an α-amylase activity (arbitrary units).

Coefficients of linear correlation between ABA-affected germination indices and both SR and FN, as well as between α-amylase activity and both SR and FN were calculated, and linearity was verified by variation analysis in linear regression.

Results and discussion

Examinations of embryo response to exogenous ABA treatment using *in vitro* bioassay made it possible to determine the values of six different indices characterising the dynamics of grain germination or seedling development and calculation of 24 correlation coefficients between two sprouting resistance indices: either SR in a given year and in all three study years, or FN in a given year and in all three study years (Table 1).

Comparing values of germination indices of grains in H_2O with those affected by ABA, we found that the influence of exogenous ABA is reflected most clearly in diminution of the length of one-leaved seedlings (Fig. 1). Statistical analysis revealed a significant negative correlation between the average length of seedlings grown under the influence of an active concentration of ABA and FN values of individual introgressive triticale lines. The correlations were highly significant both for values from a given year and for three-year means (Table 1). In contrast, there was no correlation between ABA-affected seedling length and the average number of grains sprouted after artificial wetting of intact spikes with dormant seeds (SR). It is noteworthy that other indices characterising the dynamics of grains germination under the influence of exogenous ABA also have no

correlation with SR, but with FN as well. Since germination indices were not related to FN, the obtained results support the hypothesis that the correlation between seedling length and FN is not determined by differences in plantlet age, starting from germination and resulting from embryo response, but by the influence of ABA on later steps of development, probably as a response of the α-amylase synthesis region in endosperm.

Table 1. Linear correlation coefficients (r) of different germination indices of triticale grains obtained in condition with exogenous ABA treatment estimated with dormant grain germination in spikes (SR) and falling number values (FN) (** - statistically significant at $P < 0.01$).

Germination indices	SR - 1 year	SR - 3 years	FN - 1 year	FN - 3 years
Number of grain germinated in ABA after 2 d	-0.250	-0.154	-0.074	0.280
PI in ABA	-0.249	-0.153	-0.107	0.206
CPI (water-ABA)	-0.241	-0.298	0.332	0.281
Average length of seedlings in ABA	0.051	0.107	-0.699**	-0.727**
Relative length of seedlings in ABA	0.322	0.400	0.212	0.435
Diminution of seedlings length	0.419	0.471	0.239	0.312

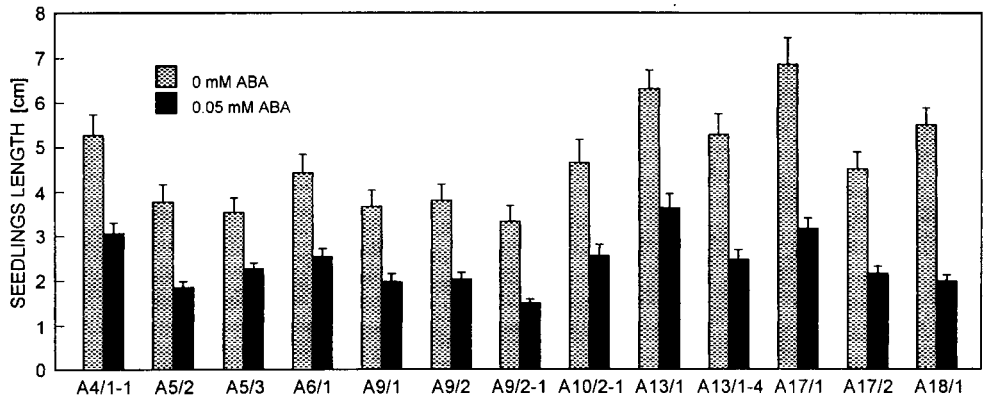

Fig. 1. The average length of seedlings of introgressive Tc/Tm lines grown in water or ABA solution.

Such a meaning is strongly supported by a statistically significant negative correlation (r = -0.938, $P < 0.01$) which was observed between FN and α-amylase activity assessed in grains after exogenous ABA treatment. In contrast, such a correlation calculated between ABA-affected α-amylase activity and SR was very weak (r = -0.089) and statistically non-significant. In control grains imbibed in water the correlations between α-amylase activity and FN was non-significant (r = -0.673). This is an evidence that genotype sensitivity to ABA is a factor which generates observed correlation between α-amylase synthesis in laboratory testing and FN values characterising analysed triticale lines in field conditions.

The separate experiment on α-amylase activity in embryoless distal halves of grains imbibed in ABA solution (Fig. 2) gave similar results as experiment based on whole grains: a statistically significant correlation of α-amylase activity with FN and a non-significant correlation with SR. Strong correlation of α-amylase synthesis with FN made possible to use this character to

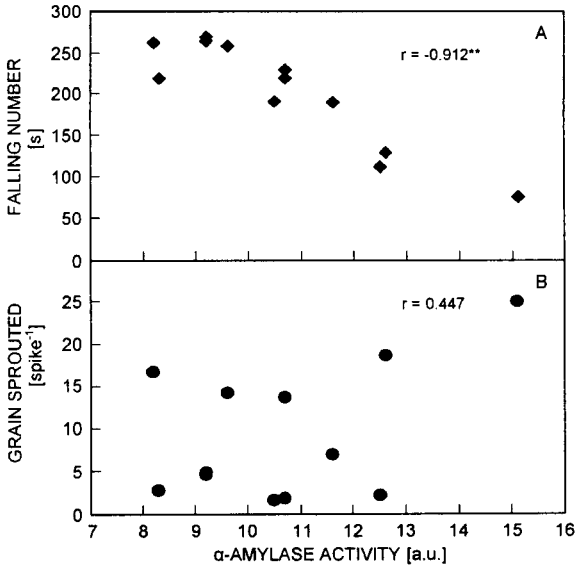

Fig. 2. Relationship of falling number (A) and grain sprouting in spikes (B) with α-amylase activity (arbitrary units) in grains treated ABA in investigations of introgressive Tc/Tm lines.

laboratory screening of triticale genotypes in respect to FN. The compatibility of results for whole grains and for half-grains lacking embryos indicated that the differences in responsiveness to ABA derived from the direct response of the aleurone layer of endosperm, which was not visible modified from a signal induced by the embryo.

Observations of seedlings shortening and α-amylase synthesis are consistent showing that FN illustrating the general level of starch decompose in field condition is influenced by genotype responsiveness to ABA. Because of correlation significance our results also shows that this ABA-sensitivity of endosperm aleurone assessed after decay of grain dormancy paralleled that of unriped caryopses. Grains dormancy assessed after harvest (SR index) showed to be independent on ABA-responsiveness during afterripening. That is in accordance with other evidences that embryo responsiveness to ABA decreases with loss of grain dormancy during after ripening (Walker-Simmons 1987, Noda *et al.* 1993).

FN assessed in grains mill samples provides information what is general (summarized) level of starch decompose by α-amylase acting in endosperm. From the point of view of caryopses development and ripening the last but not least in importance moment of starch hydrolysis is α-amylase synthesis in germination of harvest-ripen grains in spikes. However, in hexaploid triticale lines FN shoved to be uncorrelated with SR thus implying that starch hydrolysis measured as FN in great extend is done earlier (Sodkiewicz 1999). This suggests that in triticale grains the starch decompose can be done by late maturity α-amylase production which is controlled by endosperm tissue as it was observed in some wheats (Mares and Mrva 1993). Our results showed that this precocious starch hydrolysis is strongly dependant on genotype ABA-responsiveness of the aleurone layer of triticale endosperm.

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