Can ABA signaling be used to develop drought tolerant wheat?

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ABSTRACT

Wheat yield and quality can be compromised by drought stress and preharvest sprouting (PHS) on the mother plant. It is believed that problems with PHS are due to lack of grain dormancy partly resulting from lack of ABA sensitivity. The long term goal is to use wheat mutants with increased sensitivity to ABA to increase grain dormancy and drought tolerance. ABA is required to set up grain dormancy and embryo desiccation tolerance during embryo maturation, and stimulate storage of nutrients. ABA also inhibits germination of mature seeds and stimulates stomatal closure in response to drought stress. A screen for wheat mutants with altered response to ABA in seed germination has been used as a first step to isolate wheat plants with increased ABA sensitivity. Mutants have been characterized for changes in ABA dose-response. In addition, ABA hypersensitive grain germination appears to correspond to reduced vegetative transpiration under drought stress and decreased carbon isotope discrimination.

INTRODUCTION

The plant hormone abscisic acid (ABA) regulates the establishment of seed dormancy and adaption to environmental stresses including drought, cold, and salt (reviewed by 1). It is known that increased ABA sensitivity results in increased seed dormancy and drought tolerance in Arabidopsis and canola (2-4). Interestingly, increased ABA sensitivity appears to give increased cold tolerance in wheat (5). This study has identified wheat mutants with increased sensitivity to ABA with a long term view towards evaluating their efficacy in increasing resistance to drought stress and preventing preharvest sprouting through deepening grain dormancy. These mutants will be referred to as wheat *ABA responsive mutants* (W*arm*).

ABA induces seed dormancy and desiccation tolerance during embryo maturation (6). Seeds are said to be dormant when they fail to germinate even when environmental conditions favour germination. Seed dormancy can alleviated by cold imbibition or by a period of dry storage termed after-ripening. The role of ABA in establishing seed dormancy and in drought tolerance has been established through the study of ABA biosynthesis and signalling mutants in Arabidopsis, maize, tobacco, wheat and tomato (7). Reduced ABA biosynthesis and ABA-insensitivity result in reduced seed dormancy in many species, and in maize result in vivipary, a tendency to germinate on the mother plant (68, 69, 78). The maize *VIVIPAROUS1* (*VP1*) gene encodes a transcription factor required for ABA response (8). It has been shown the *VP1* homolog of wheat is misspliced and that transformation of wheat with the oat *VP1* gives increased dormancy and prevents preharvest sprouting (9). Moreover, dormancy-releasing treatments are associated with a decline in ABA content in cereals, and resistance to PHS can be associated with higher sensitivity to ABA (10, reviewed by 11). PHS tolerance is also associated with red caryopsis colour in wheat, and negatively regulated by gibberellin (GA) in sorghum (12-15).

ABA biosynthesis and response mutants also exhibit altered water relations (reviewed by 16). ABA is synthesized in response to drying soil and induces drought adaptive responses including stomatal closure and induction of osmoprotectants such proline, glycine-betaine, and dehydrins. ABA hypersensitive mutants are expected to close stomata at lower concentrations of ABA resulting in decreased transpiration under drought stress (4, 17). This study investigates whether this can result in increased transpiration efficiency (TE, carbon fixed/water transpired).

Although both ABA-dependent and ABA-independent components contribute to drought tolerance and to drought-regulated gene expression (18, 19), it seemed likely that ABA hypersensitivity would lead to higher TE by ABA regulation of stomatal closure. Transpiration was examined using a gravimetric estimation of whole plant transpiration (17). TE was examined using carbon isotope discrimination. It has been demonstrated that wheat plants with increased transpiration efficiency under dry conditions can be bred by selecting plants that show reduced discrimination against $^{13}CO_2$ versus $^{12}CO_2$ in carbon fixation under moist growing conditions (20, 21). Rebetzke et al 2002 demonstrated that such plants can also show higher yield under drought conditions in Australia (22).

RESULTS AND DISCUSSION

W*arm* ABA sensitivity during embryo germination. Wheat caryopses (grains) are unique in that whole grains and embryos become highly insensitive to ABA once afterripened. Since dormant grains fail to germinate in the absence of ABA, grains are cut in half to stimulate germination and assess the sensitivity of the embryo to ABA-inhibition of germination. Wheat ABA-responsive mutants (*arm*) were isolated from fast-neutron mutagenized Chinese spring wheat based on the fact that their germination was inhibited by 5 μM ABA once

afterripened for 6 month (23). Five mutants that showed a reproducible increase in ABA sensitivity were further characterized in this study. The sensitivity of cut grains to ABA in germination was examined by measuring percent germination following a 120 h incubation on germination discs wetted with increasing concentration of (+)-ABA (Figure 1, data not shown). Five mutants, *arm2*, *arm3*, *arm4*, *arm5*, and *arm6* showed efficient germination in the absence of ABA (80% or higher) and decreasing germination with increasing ABA concentration. One mutant, *arm1*, showed inefficient (0-20%) germination even in the absence of ABA. The ABA-independent decrease in germination efficiency may be the result of an increase in embryo dormancy suggesting that *arm1* is not a true ABA hypersensitive mutant. Figure 1 shows an example of the ABA dose-response of wheat embryos after 48 h incubation for ABA hypersensitive *arm6* compared to embryo dormant *arm1* and wild-type (WT) Chinese spring. These results indicate that it is possible to recover mutants with increased sensitivity to ABA in grain germination.

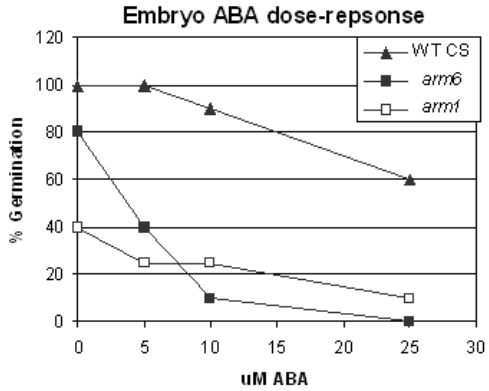

Figure 1. ABA dose-response in embryo germination. Half-grains were incubated in the dark for 48h at 30°C. W*arm6* shows increased ABA sensitivity, whereas W*arm1* shows increased embryo dormancy.

Gravimetric estimation of transpiration in drying soils. Next, we examined the hypothesis that ABA hypersensitive mutants may also show increased ABA sensitivity in vegetative tissues resulting in decreased transpiration in drying soils. Plants were grown to the 5-leaf stage in 4-inch pots. Then watering ceased, and the soil and pots were covered with plastic to prevent soil moisture loss through evaporation. The plants were weighed every 24 h for 14 days to measure loss of soil moisture through plant transpiration. The *arm1* mutant which appeared to have increased embryo dormancy showed no change in transpiration compared to wild-type Chinese spring (Figure 2). The *arm2*, *arm3*, *arm5*, and *arm6* mutants which appeared to have an ABA hypersensitive dose-response in embryo germination, on the other hand, showed slower transpiration of soil moisture compared to wild-type (Figure 2, data not shown). The leaves of ABA-hypersensitive plants appeared more green and turgid at the conclusion of the experiment. These results suggest that increased sensitivity to ABA in embryo germination correlates with decreased transpiration rate suggesting that increased ABA sensitivity may cause earlier stomatal closure in response to ABA and drying soils.

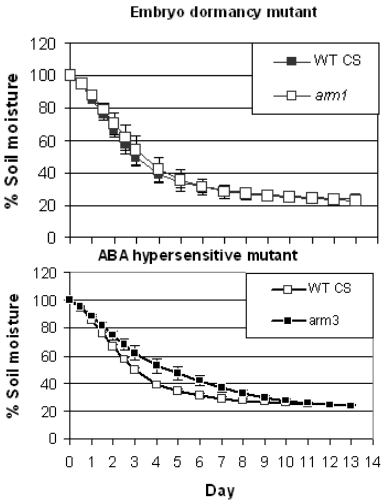

Figure 2. Gravimetic measurement of soil moisture loss due to transpiration over a time course of 14 d without watering. WT CS, wild-type Chinese spring.

The effect of W*arm* mutants on TE. We examined the hypothesis that increased ABA sensitivity could result in increased transpiration efficiency. Reduced carbon isotope discrimination under well-watered conditions has been shown to correlate with increased transpiration efficiency (22). Wheat genotypes were grown at Central Ferry WA in randomized triplicate small plots in 2007. Leaf tissue was collected from plants grown under well-watered or dry conditions. Two ABA-hypersensitive mutants, *arm3* and *arm4* show a significant decreased in carbon-isotope discrimination compared to wild-type Chinese spring under wet growth conditions. This suggests that an increase in ABA sensitivity sometimes correlates with an improvement in TE.

Table 1. Carbon-isotope discrimination, Δ

Treatment	Wet		Dry	
Genotype	Av	SD	Av	SD
WT	18.14	0.61	17.97	0.58
arm1	18.47	0.79	18.16	0.72
arm2	18.23	0.54	17.53	0.43
arm3	17.68	0.43	17.45	0.51
arm4	17.95	0.54	17.71	0.58
arm6	18.10	0.50	17.85	0.34

Av average, SD standard deviation, n=6

One concern with ABA-hypersensitive germplasm is that the associated decrease in stomatal aperture may result in a decrease in CO_2 uptake leading to inefficient photosynthesis and reduced yield. To investigate this we conducted a preliminary experiment measuring plant height and yield per plant in well-watered plants grown under controlled greenhouse conditions (Table 2). Only the *arm1* line showed a significant decrease in plant height. Future work will investigate whether this may be due to some change in GA signalling. Of the high TE lines, *arm3* line showed no decrease in yield whereas *arm4* showed some decrease in yield. These results suggest that it may be possible to improve TE and drought tolerance without sacrificing yield by introducing ABA hypersensitive mutations into wheat.

Table 2. Greenhouse yield per plant

	Plant height		[1]Yield	
Genotype	Av	SD	Av.	SD
WT	111.65	3.23	5.15	0.80
arm1	97.31	3.72	4.72	0.68
arm2	107.03	6.59	4.86	0.09
arm3	111.6	1.74	5.16	0.36
arm4	103.65	2.34	4.18	0.77
arm6	104.35	5.76	5.23	0.55

[1]Yield was measured as gm of grain per plant, height in cm, n=12

MATERIALS AND METHODS

ABA dose-response experiment. Plating assays were performed to compare percent germination of dormant cut half-grains (referred to as embryos), on varying concentrations of (+)-ABA (gift of S. Abrams) in 5 μM MES, pH 5.5 as described by Walker-Simmons (10). Each plate contained 30 seeds representing 3 subsamples of 10 seeds each. Germination was scored as percent germination radicle emergence every 24 hours for 5 days.

Gravimetric measurement of transpiration. Gravimetric measurement of soil moisture lost through plant transpiration was performed according to Pei et al (17) with modifications for wheat. Plants were grown in a growth chamber under 16-hour day 350-400 umol m^{-2} s^{-1} light at temperatures of 22°C day/15°C night. Plants were moved to the greenhouse at the 4-leaf stage (Zadok Scale 14), and allowed to acclimate for 7 days to a photoperiod of 16 hours and temperatures 18-22°C day/15-17°C night. Pots were saturated with water one day before the experiment, leaving pots evenly moist on day 1 (~430 g). 11 plants at approximately the same developmental stage (Zadok 15) and size were selected (4). To avoid water evaporation from soil surface, pots were covered with transparent cellophane wrap. Watering ceased and pots were weighed individually every 12 hours for the first 48 hours at 12 pm and 12 am and every 24 hours thereafter for 14 days at 12 pm. At the end of the experiment, shoots were weighed after oven-drying to 70°C for 48 hours to obtain dry weight. Dry weights were subtracted from the daily pot weights to calculate percent soil moisture loss.

Carbon-isotope discrimination measurements. Wild-type Chinese Spring and M_7 *arm* wheat lines were grown in Central Ferry, WA in 2007. Well-watered plants were irrigated using line-source irrigation. Drought-stressed ("dry") plants were grown on residual soil moisture. The fully expanded leaf below the flag leaf was harvested from six individuals per genotype at one week post-anthesis, representing two random plants from three different plots. Leaves were dried at 70ºC for 72 h, ground, weighed, and submitted to the Bioanalysis Stable Isotope Core Laboratory at Washington State University. δ values were calculated using a two-point normalization determined by fitting a regression line through values for the two standards. Carbon isotope discrimination was calculated as $\Delta = (\delta_a - \delta_p) / (1 + (\delta_p / 1000))$, where δ_a (-8‰) is the isotopic composition of air and δ_p is the plant isotopic composition (20).

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