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Registration of Zak *ERA8* Soft White Spring Wheat Germplasm with Enhanced Response to ABA and Increased Seed Dormancy

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ABSTRACT

Zak *ERA8* (ENHANCED RESPONSE to ABA8) (Reg. No. GP-966, PI 669443) is a unique line derived from soft white spring wheat (*Triticum aestivum* L.) cultivar Zak that has increased seed dormancy but after-ripens within 10 to 16 wk. The goal in developing this germplasm was to use increased seed dormancy to improve tolerance to preharvest sprouting, a problem that can cause severe economic losses. This germplasm was developed by USDA–ARS, Pullman, WA, in collaboration with Washington State University. Zak *ERA8* was tested under experimental number 60.1.27.10. The *ERA8* mutation was generated by chemical mutagenesis followed by selection for the inability to germinate on abscisic acid (ABA) concentrations too low to inhibit wild-type Zak seed germination. The semidominant Zak *ERA8* line has been backcrossed twice to wild-type Zak. Following the first backcross, Zak *ERA8* showed similar morphological and grain quality traits to the original Zak cultivar.

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Preharvest sprouting (PHS) refers to the germination of mature grain on the mother plant when rain or moist conditions occur before harvest. It is a problem in cereals such as wheat (*Triticum aestivum* L.) because selection for rapid seedling emergence has led to inadequate seed dormancy at maturity to resist germination during rain events (DePauw and McCaig, 1991; Gerjets et al., 2010). Dormant seeds fail to germinate under moist conditions (Finkelstein et al., 2008). Wheat seeds have the highest dormancy and PHS resistance at physiological maturity and then gradually lose dormancy during dry storage through the process of dry after-ripening (Paterson et al., 1989). The duration of dry after-ripening required to germinate efficiently is genetically determined. Because wheat with white kernels has less seed dormancy than wheat with red kernels, PHS susceptibility limits the geographic area for white wheat production and also causes serious economic losses when major rainfall events strike areas that grow white wheat (Flintham, 2000; Himi et al., 2002). Kernel color is not the sole determining factor of seed dormancy. The plant hormone abscisic acid (ABA) also induces and maintains seed dormancy. Higher ABA accumulation and sensitivity are associated with higher seed dormancy and PHS tolerance in barley (*Hordeum vulgare* L.) and wheat (Walker-Simmons, 1987; Barrero et al., 2009; Schramm et al., 2010; Schramm et al., 2012). The objective of this research was to develop a soft white wheat with increased seed dormancy by selecting a mutation resulting in increased sensitivity to ABA during seed germination. Zak *ERA8* (ENHANCED RESPONSE to ABA8) (Reg. No. GP-966, PI 669443) was developed by the USDA–ARS, Pullman, WA, with assistance from Washington State University and tested under experimental number 60.1.27.10. Zak *ERA8* fails to germinate on low ABA concentrations that did not strongly inhibit wild-type Zak germination, has increased seed dormancy at maturity, and loses dormancy more slowly through after-ripening (Schramm et al., 2013).

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Abbreviations: ABA, abscisic acid; EMS, ethyl methanesulfonate; PHS, preharvest sprouting; SKCS, single-kernel characterization system.

Materials and Methods

The soft white spring cultivar Zak (PI 607839) has superior end-use quality but has fairly low seed dormancy and high PHS susceptibility (Kidwell et al., 2002; Schramm et al., 2013; S.A. Martinez, unpublished data). Previous work described the isolation of the *ERA8* mutation from an ethyl methanesulfonate (EMS)-mutagenized M_2 Zak population and the first backcross (BC_1, Cross 60) of Zak *ERA8* to wild-type Zak (Schramm et al., 2013). Briefly, partly after-ripened grain was imbibed on germination disks moistened with 5 μM of ABA, and mutants that failed to germinate in the presence of ABA were selected. Caryopses (referred to as seeds) used for germination studies (Tables 1 and 2) were harvested at physiological maturity from plants grown side-by-side in the greenhouse as previously described (Schramm et al., 2013). Seeds were dry after-ripened at room temperature in open containers for the indicated number of weeks following harvest. Germination phenotypes were characterized by placing whole seeds on germination disks (Anchor Paper) moistened with 6 mL of the indicated concentrations of (±)-ABA (Phytotechnology, Inc.) in an MES-buffered, pH 5.5 (2-[N-morpholino] ethane sulfonic acid, Sigma-Aldrich) solution in a Petri dish. Imbibing seeds were incubated at 30°C in the dark for 5 d, and germination was scored daily (Table 1). Seeds were also imbibed on MES-buffer alone as a mock control and to observe degree of seed dormancy in the absence of ABA. The Zak *ERA8* germplasm being released was derived from a bulked F_4 increase of the BC_1F_3 line 60.1.27.10, where BC_1F_3 was a cross of the Zak *ERA8* M_6 to wild-type Zak (Cross 60; Schramm et al., 2013). The M_6 parent was derived by single plant descent from the M_2 with selection for ABA hypersensitive seed germination. The second backcross population (BC_2, Cross 95) was a cross of BC_1F_3 line 60.9.26.5 (60.1.27.10 sibling) to wild-type Zak. The BC_2F_2 seeds were allowed to dry after-ripen in an open container for 5 wk at room temperature before plating on 2 μM ABA (Table 2). Analysis of goodness-of-fit to Mendelian segregation models was performed using the Chi-square (χ^2) test as previously described for the BC_1F_2 population (Schramm et al., 2013).

Table 1. Germination phenotype of soft white spring wheat Zak *ERA8* and Zak wild type in the presence and absence of abscisic acid (ABA) over multiple generations, after-ripening time points, and ABA concentrations.

Genotype	Gen.†	% Germination‡		ABA§	After-ripened
		No hormone	ABA		
		——— % ———		μM	wk
Zak	na	96.7	73.3	5	6
Zak *ERA8*	BC_1F_3	23.3	0	5	6
Zak	na	96.7	33.3	5	6
Zak *ERA8*	BC_1F_5	23.3	6.7	5	6
Zak	na	–	90.0	2	6
Zak *ERA8*	BC_1F_6	–	3.3	2	6
Zak	na	100	100	5	16
Zak *ERA8*	BC_1F_5	93.3	83.3	5	16
Zak	na	100	100	5	28
Zak *ERA8*	BC_1F_6	100	100	5	28

† Generation of seeds tested. na = not applicable.

‡ n = 30; germination at Day 5 of imbibition.

§ Concentration of ABA used in germination assays.

The χ^2 statistic is calculated by $\Sigma[(O-E)^2/(E)]$, where O is the observed number of seeds germinated or ungerminated, and E is the expected number of seeds germinated/ungerminated based on the Mendelian segregation model. A χ^2 distribution table was used to determine the p values based on the χ^2 statistic and the degrees of freedom = 1. The model fits the observed values when $p > 0.05$ and does not fit when $p < 0.05$.

Zak *ERA8* (60.1.27.10) and wild-type Zak were compared in field trials conducted in 2011 and 2012 at the Washington State University Spillman Research Farm, Pullman, WA. Plots of the dimensions 167 cm by 238 cm (5.5 by 8 ft) were sown using a custom-designed Wintersteiger Classic small plot combine in a randomized complete block design with five replications. Fertility and herbicide treatments were applied according to Washington State University Extension Guides for Eastern Washington (https://pubs.wsu.edu). Propiconazole (TILT-Syngenta) was applied in two applications according to the labeled rates to control stripe rust (*Puccinia striiformis* Westend f. sp. *tritici*). Plant development was compared 50 d after planting by rating Zadoks' growth stage in 2012 (Table 3; Zadoks et al., 1974). Plant height was determined after senescence and based on the average distance from the soil surface to the top of the canopy on a plot basis. Grain yield and test weight were measured with a Wintersteiger Classic small plot combine

Table 2. Segregation analysis of BC_2F_2 seed germination on abscisic acid (ABA).

Genotype	n†	Gen.‡	Not germ.§	Germ.§	χ^2			p value		
					3:1	1:3	1:2:1	3:1	1:3	1:2:1
95.3 segregating	100	BC_2F_2	61	39	7.59	50.35	0.166	0.006	<0.001	0.68
95.6 segregating	100	BC_2F_2	63	37	5.30	56.81	0.661	0.021	<0.001	0.42
+/+	30	parent#	2	28						
ERA8/*ERA8*	150	parent#	143	7						
+/*ERA8*	24	BC_2F_1	16	8						
F_2 expected¶	100				27	71	41			

† Number of seeds tested for germination, after-ripened for 5 wk past physiological maturity; df = 1.

‡ Generation of seeds tested.

§ Number of seeds that had germinated (Germ.) and not germinated (Not germ.) after 5 d of imbibition on 2 μM (±) ABA.

¶ Number of seeds expected to germinate after 5 d of imbibition for each single gene segregation ratio.

Zak (+/+) and Zak *ERA8* (−/−) parental lines used to generate cross 95; grown at the same time as the F_1 plants.

equipped with a Harvest Master Grain Gage. Seed samples (n = 100) from each plot were assayed using the Perten Single Kernel Characterization System 4100 (SKCS). Traits obtained included grain hardness and grain weight. The average single-kernel weight generated by the SKCS was used to estimate 1000 kernel weight. Grain protein concentrations were determined from a sample of each plot using the DA 7200 NIR Analyzer (Perten). Analysis of variance was performed using the MIXED procedure in SAS/STAT software (version 9.3, SAS Institute).

Characteristics

Compared with wild-type Zak, Zak *ERA8* lines showed both increased seed dormancy in the absence of ABA and decreased ability to germinate when plated on ABA across multiple generations (Table 1). Without hormone, Zak *ERA8* showed reduced germination compared with wild-type Zak, indicating that the mutation resulted in increased seed dormancy at 6 wk of after-ripening. Zak *ERA8* showed reduced germination on 5 μM ABA and on 2 μM ABA compared with wild-type Zak (Table 1). This indicates that Zak *ERA8* is hypersensitive to ABA's inhibition of seed germination at 6 wk of after-ripening. The Zak *ERA8* ABA hypersensitive germination phenotype was also apparent at 16 wk of after-ripening on 5 μM ABA. However, Zak *ERA8* showed more efficient germination in the absence of hormone with 16 wk of after-ripening (Table 1), and with 10 wk of after-ripening in a previous study (Schramm et al., 2013). After 3 yr of after-ripening, the Zak *ERA8* ABA hypersensitive phenotype could be detected only at high, 25 to 50 μM, ABA concentrations (Schramm et al., 2013).

F_2 segregation analysis following the first backcross (Cross 60) was consistent with partial dominance (Schramm et al., 2013). F_2 segregation analysis of the second backcross (Cross 95) of the Zak *ERA8* sibling to wild-type Zak was also found to be consistent with a semidominant trait (Table 2). Because it is difficult to find a condition where Zak *ERA8* shows 0% and wild-type Zak shows 100% seed germination on ABA, the germination phenotype of parents grown at the same time as the BC_2F_1 plants was used to generate the expected number of germinated seeds for a 3:1 (dominant) and 1:3 (recessive) segregation ratio for use in calculating the χ^2 statistic (as in Schramm et al., 2013). A χ^2 test indicated that neither the recessive nor dominant segregation ratio appeared to fit the observed data ($p < 0.05$). The fact that wild-type showed 93.3% (n = 30) and Zak *ERA8* 4.7% germination (n = 150) on 2 μM ABA, while the BC_2F_1 heterozygote showed an intermediate phenotype of 33.3% germination (n = 24) suggests that the *ERA8* mutation is semidominant. The germination phenotype of the heterozygous BC_2F_1 seeds was used to predict the expected number of heterozygous seeds that would germinate if *ERA8* were semidominant. If 100 F_2 seeds show segregation as a semidominant trait (1 *ERA8*/*ERA8* : 2 +/*ERA8* : 1 +/+), 25 *ERA8*/*ERA8* seeds should show 4.7% germination (1 germinated seeds), 50 +/*ERA8* seeds should show 33.3% germination (17 germinated seeds), and 25 +/+ seeds should show 93.3% germination (23 germinated seeds). Thus, for a semidominant trait, we expect 1 + 17 + 23 = 41 seeds out of 100 to germinate. As shown by a χ^2 test, the observed germination phenotypes of the 95.3 and 95.6 F_2 populations both fit the semidominant model ($p > 0.05$, 1:2:1 model in Table 2).

Analysis of agronomic traits and grain quality suggested that Zak *ERA8* has similar, but not identical, characteristics to the original Zak premutagenesis parent (Table 3). In 2011, Zak *ERA8* showed a significant increase in test weight ($p < 0.05$) and a small but significant decrease in grain yield ($p < 0.07$). Although Zak *ERA8* did not show a statistically significant decrease in yield in 2012, it showed a statistically significant decrease in yield when the 2 yr were combined (Table 4). Previous work indicated that BC_1F_3 Zak *ERA8* did not show a significant decrease in yield when grown in the greenhouse (Schramm et al., 2013). In 2012, Zak *ERA8* plots reached an average Zadoks' stage of 46.2 (boot swollen) on the same day that Zak reached a Zadoks' stage of 45.8. However, this difference in flowering time was not statistically significant. Based on SKCS analysis, Zak *ERA8* grain quality traits resembled Zak. No significant difference was observed in kernel hardness or 1000 kernel weight. However, Zak *ERA8* had significantly higher protein compared with Zak in 2012 ($p = 0.014$), but not in 2011 (Table 3). The quality traits of Zak *ERA8* were not significantly different from Zak when the data from 2011 and 2012 were

Table 3. Comparisons of agronomic and quality traits for soft white spring wheat Zak *ERA8* and wild-type Zak from field experiments conducted at Pullman, WA.

Trait	Year	Zak *ERA8*		Zak		*p* value†
		Mean	SE	Mean	SE	
Zadoks' stage	2012	46.2	0.66	45.8	0.66	0.68
Plant height, cm	2011	85.8	1.4	85.6	1.4	0.92
	2012	67.2	1.4	71.4	1.4	**0.048**
Yield, kg ha^{-1}	2011	3988	263	4725	263	**0.065**
	2012	2952	263	3545	263	0.13
Test weight, kg m^{-3}	2011	789	6.4	770	6.4	**0.048**
	2012	756	6.4	753	6.4	0.75
Grain protein concentrations, %	2011	9.79	0.58	9.54	0.58	0.77
	2012	14.19	0.26	13.05	0.26	**0.014**
Hardness, %	2011	13.30	3.12	5.10	3.12	0.10
	2012	18.18	1.40	18.70	1.40	0.80
1000 kernel weight, g	2011	44.6	2.0	45.5	2.0	0.75
	2012	27.6	0.9	27.3	0.9	0.83

† Differences between wild-type Zak and Zak *ERA8* in bold type are statistically significant, with a *p* value of ≤ 0.07 based on analysis of variance.

Table 4. Comparisons of agronomic and quality traits for soft white spring wheat Zak *ERA8* and wild-type Zak combined over 2 yr.

Trait	Zak *ERA8*		Zak		*p* value†
	Mean	SE	Mean	SE	
Plant height, cm	76.5	0.98	78.5	0.98	0.17
Yield, kg ha^{-1}	3470	186	4135	186	**0.02**
Test weight, kg m^{-3}	772	5.1	762	5.1	0.10
Grain protein concentrations, %	11.99	0.32	11.30	0.32	0.16
Hardness, %	15.7	1.7	11.9	1.7	0.15
1000 kernel weight, g	36.1	1.1	36.4	1.1	0.84

† Differences between wild-type Zak and Zak *ERA8* in bold type are statistically significant, with a *p* value of ≤ 0.05 based on analysis of variance.

combined (Table 4). These data suggest that the *ERA8* mutation has little effect on agronomic and grain quality traits.

Discussion

Zak *ERA8* had higher seed dormancy and ABA hypersensitivity than wild-type Zak, showed no apparent change in the grain quality traits examined, but showed a small but significant decrease in field grain yield. There are several possible explanations for the observed difference in Zak and Zak *ERA8* grain yield. One possibility is that a single backcross was not sufficient to eliminate detrimental unlinked alleles generated during mutagenesis. Alternatively, the increased seed dormancy in Zak *ERA8* may lead to reduced emergence, leading to the small reduction in yield. If so, an increased sowing rate may address this problem. Finally, the *ERA8* mutation may result in vegetative ABA hypersensitivity, leading to reduced stomatal conductance similar to that seen in the Chinese Spring ABA hypersensitive mutant *Warm4* (Pei et al., 1998; Schramm et al., 2010). Stomatal closure can reduce CO_2 uptake and photosynthesis in years with ample rainfall, leading to reduced yield. In addition, ABA hypersensitivity in vegetative tissue may increase drought tolerance through reduced stomatal conductance. Future work will need to determine if the yield difference is tightly linked to the *ERA8* locus and whether the *ERA8* mutation results in changes in vegetative ABA sensitivity. Future work will also need to examine the efficacy of the *ERA8* mutation for improving preharvest sprouting through seed dormancy in other genetic backgrounds.

Seed Availability

Zak *ERA8* seed derived from the BC_1F_3 line 60.1.27.10 used in germination and field characterization is available to wheat breeders, geneticists, and other researchers on written request to the corresponding author. It is requested that appropriate recognition of the source be given when this germplasm contributes to research and to development of new breeding lines and cultivars. Seed of the Zak *ERA8* BC_1F_4 germplasm has been deposited in the National Plant Germplasm System and will be available 5 years from date of publication.

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