



RELATIONSHIP BETWEEN PRE-HARVEST SPROUTING AND FUNCTIONAL MARKERS ASSOCIATED WITH GRAIN WEIGHT, *TaSus2-2B*, *TaGW2-6A*, AND *TaCwi-A1*, IN KOREAN WHEAT CULTIVARS

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SUMMARY

Pre-harvest sprouting (PHS) resistance, measured by germination index, and grain traits, including grain length, width, thickness, roundness, test weight, and 1000-kernel weight, were evaluated for four years in this study. Functional markers associated with grain weight, *TaSus2-2B*, *TaGW2-6A*, and *TaCwi-A1*, were also evaluated because grain weight is related to PHS resistance in Korean wheat cultivars. The objective of the study was to determine the relationships among PHS and grain traits, and functional markers to improve PHS resistance in Korean wheat breeding programs. Grain traits, test weight, 1000-kernel weight, and germination index were influenced significantly by genotype, year, and the interaction between year and genotype. Germination index values correlated positively with 1000-kernel weight ($r = 0.568$, $P < 0.001$) and grain traits, except grain roundness. White-grained wheat cultivars showed wider length, width, and thickness of grain and 1000-kernel weight than red-grained wheat cultivars. Wheat cultivars that carried *TaSus2-2B* with the Hap-L haplotype showed a higher germination index (0.61) than *TaSus2-2B* with the Hap-H haplotype (0.18). Wheats that carried the *TaGW2-6A-G* haplotype showed higher 1000-kernel weight (44.62 g) than the *TaGW2-6A-A* haplotype (39.75 g), although there was no significant difference in germination index between Korean wheat cultivars. Red-grained wheat cultivars that carried the *TaCwi-A1a* allele showed higher test weights than with the *TaCwi-A1b* allele and the white-grained wheats that carried the *TaCwi-A1a* allele exhibited wider length and thickness of grain and higher 1000-kernel weight than those with the *TaCwi-A1b* allele.

Keywords: pre-harvest sprouting, germination index, *TaSus2-2B*, *TaGW2-6A*, *TaCwi-A1*

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INTRODUCTION

Pre-harvest sprouting (PHS), which refers to the germination of physiologically mature grains in spikes before harvesting under rainfall and high humidity conditions, leads to significant economic losses (30% decreased yield) due to a reduction both the yield and quality of grain in

wheat (Groos *et al.*, 2002). PHS has frequently occurred in the southern part of Korea because the rainy season overlaps with the harvest time for wheat, as in China and Japan (Xiao *et al.*, 2002; Yanagisawa *et al.*, 2005; Kim *et al.*, 2012).

PHS resistance has been one of the most important traits in Korean wheat breeding

programs. Seed dormancy has been a major consideration for the selection of PHS-resistant lines because dormant wheat lines do not germinate well under wet weather conditions although many environmental factors, quantitative traits, loci, and functional markers have been related to PHS and seed dormancy (Groos *et al.*, 2002; Nyachiro *et al.*, 2002; Biddulph *et al.*, 2005; Kulwal *et al.*, 2005; Mares *et al.*, 2005; Mori *et al.*, 2005; Kumar *et al.*, 2006; Imtiaz *et al.*, 2008; Liu *et al.*, 2008; Munkvold *et al.*, 2009; Liu *et al.*, 2011; Kulwal *et al.*, 2012). Seed dormancy has generally been assessed with the germination index, which is the most reliable predictor of PHS resistance (Shorter *et al.*, 2005). Germination index is consistent across years and correlates positively with PHS index measured with artificial wetting in Korean wheat cultivars (Kim *et al.*, 2012).

PHS resistance is influenced by the morphological structure of spikes, such as the structure and erectness of wheat spikes, the openness of florets, and the tenacity of glumes (King and Richards, 1984). Red-grained cultivars are generally more resistant to PHS than white-grains because the red color (R) genes are associated with seed dormancy (Flintham, 2000; Groos *et al.*, 2002; Himi *et al.*, 2002; Mares *et al.*, 2005). Protein content, hardness, and diameter of grains correlated with PHS resistance in a greenhouse study (Barnard *et al.*, 2005). Germination index correlates positively with grain traits although no significant relationship between agronomic traits and germination index was found in Korean wheat cultivars (Shin *et al.*, 2013). White-grained Korean wheat cultivars showed higher germination indices than red-grained wheats and also showed wider length, width, and thickness of grain than red-grains (Shin *et al.*, 2013).

Sucrose synthase 2 (Sus2), grain width (GW2) and cell-wall invertase (CWI) genes have been associated with yield traits, especially 1000-kernel weight, and developed as functional markers to evaluate 1000-kernel weight in Chinese wheat cultivars (Liu *et al.*, 2012). The Hap-H haplotype at the TaSus2-2B locus positively correlates positively with increased 1000-kernel weight in Chinese wheat cultivars (Jiang *et al.*, 2011). The Hap-6A-A haplotype at the TaGW2-6A locus was significantly

associated with increased grain width and 1000-kernel weight in Chinese wheats cultivated during two crop seasons (Su *et al.*, 2011). Wheat cultivars carrying the TaCwi-A1a allele showed higher 1000-kernel weight than the TaCwi-A1b allele in two sets of Chinese landraces and two sets of commercial wheat cultivars (Ma *et al.*, 2012). The grain weight of Korean wheat cultivars was related to PHS resistance in our previous study (Shin *et al.*, 2013). Thus, in this study, we sought to determine the relationship between pre-harvest sprouting and functional markers associated with grain weight, TaSus2 2B, TaGW2-6A, and TaCwi-A1, to provide useful information for improving PHS resistance in Korean wheat breeding programs.

MATERIALS AND METHODS

Plant materials

In total, 26 Korean wheat cultivars were sown in a randomized complete blocks block design with two replicates in the Upland Crop Experimental Farm of National Institute of Crop Science, Rural Development Administration (Korea) during four years, from 2010 to 2013, on 50% clay loam soil. Seeds were sown on October 25; each plot consisted of three 4 -m rows, spaced 25 cm apart and cultivated with the standard method for wheat at the National Institute of Crop Science. Fertilizer was applied at 5:7:5 kg/10 a (N:P:K) before sowing and weeds, insects, and disease were controlled stringently. No supplemental irrigation was used. Spikes of each cultivar in the field were harvested at maturity, when all the green color had disappeared from the leaves and stems. Spikes were dried under room conditions for 7 days until the moisture content of the grain was ~14% and they were then gently threshed by hand and kept at -20°C to preserve dormancy (Mares, 1983).

Grain traits

Test weight and 1000-kernel weight were measured using a Grain Scale (Seedburo Equipment Co., USA) and a Seed Counter (Pfeuffer GmbH, Germany), respectively. Grain

length, grain width, grain thickness, and grain roundness were measured using SeedCount image analysis (SeedCount Australasia Pty Ltd., Australia). Grain color was determined by soaking 20 sound kernels in 1 M NaOH solution for 2 h; red-grained cultivars showed a dark red color and white-grains exhibited a straw-yellow color.

Germination index

Germination index was measured according to the procedure of Walker-Simmons (1988). Sound grains were incubated on a single layer of filter paper, wetted with distilled water, in the dark for 7 days at 20°C. Germinated grains were counted daily and removed. Germination index was calculated from the following formula: Germination index = $(7 \times n_1 + 6 \times n_2 + \dots + 1 \times n_7) / (7 \text{ days} \times \text{total grains})$, where $n_1, n_2, n_3, \dots,$ and n_7 are the number of grains that had germinated on Day 1, Day 2, ..., and Day 7. The maximum index is 1.0 if all grains germinate by Day 1, with lower indices indicating increasing levels of grain dormancy or reduced germination (Mares and Mrva, 2001).

DNA extraction and PCR analysis

Five seeds of each line were grown in a temperature-controlled greenhouse to analyze the allelic variations of TaSus2-2B, TaGW2-6A, and TaCwi-A1. At 2 weeks after germination, single leaves from each of five plants of each cultivar were collected, bulked, snap-frozen in liquid nitrogen, and stored at -80°C until needed. Genomic DNA was extracted from young leaf tissue (100 mg) using a genomic DNA prep kit (Solgent Co., Korea) according to the manufacturer's instructions. Allelic variations at the TaSus2-2B, TaGW2-6A, and TaCwi-A1 loci were evaluated using the procedures described by Jiang *et al.* (2011), Su *et al.* (2011), and Ma *et al.* (2012), respectively.

Statistical analysis

Statistical analyses were performed using the SAS 9.0 for windows (SAS Institute, NC, USA) using Fisher's least significant difference test (LSD), analysis of variance (ANOVA) and pair-

wise t-test. The genotype \times year component was used as the error term and sources of variation in the model were considered to be fixed effects. Pearson's correlation coefficients were also determined and statistical significance levels were set at $P < 0.05$ unless otherwise specified. All analyses were determined at least in triplicate, and all were averaged.

RESULTS AND DISCUSSION

Effect of genotype and environment on grain traits and germination index

Year, genotype, location, and their interactions significantly influenced the grain traits, including length, width, thickness, roundness of grain, test weight, and 1000-kernel weight, of Korean wheat cultivars (Table 1). Grain traits generally are affected by genotypes and environmental conditions, such as climate, soil traits or nitrogen status (Halverson and Zeleny, 1988). Several molecular markers associated with specific loci for grain traits were reported in previous studies (Sun *et al.*, 2009; Gegas *et al.*, 2010; Tsilo *et al.*, 2010; Prashant *et al.*, 2012). Genotypes have accounted for the largest proportion of the variation among length, thickness, and roundness of grain, 1000-kernel weight in Korean wheat cultivars (89.9, 83.3, 95.0, and 93.6%, respectively). Grain width and test weight were affected by both genotype (48.7 and 45.6%, respectively) and environment (48.7 and 50.0%, respectively). These results indicate that grain traits were primarily influenced by genotype and/or year rather than the interaction between year and genotype.

Germination index was also significantly influenced by year, genotype, and their interactions and the variation in germination index was largely accounted for by genotype (97.15%) rather than year (2.56%) or the interaction between year and genotype (0.29%). Germination index, which associated with seed dormancy, is generally used to predict PHS resistance because germination index is more stable with regard to growing conditions, including high temperature and drought during the grain filling stage, rather than other artificial wetting methods to determine the degree of PHS

(Trethowan *et al.*, 1996; Xiao *et al.*, 2002; Gubler *et al.*, 2005; Shorter *et al.*, 2005). The germination index of Korean wheat cultivars also appeared to be more stable across the year than the evaluation of PHS, which is typically evaluated by mist spraying in a humid chamber and artificial rain fall in the greenhouse, as in our previous report (Kim *et al.*, 2012).

Relationship between grain traits and germination index

Mean values for grain traits and color of Korean wheat cultivars over the four crop years are summarized in Tables 2 and 4. Cultivars grown in 2013 showed significantly wider grain width and lower test weight than those grown in other years. Grain width and thickness of grain grown in 2011 and 2012 was lower than those of other years. No significant difference was found in length or roundness of grain, 1000-kernel weight, or germination index. Grain traits ranged from 5.86 to 7.20 mm in length, from 3.12 to 3.65 mm in width, from 2.77 to 3.12 mm in thickness, from 1.65 to 1.91 in roundness, from 777.50 to 839.08 g in test weight, and from 33.00 to 50.39 g in 1000-kernel weight. Twenty Korean wheat cultivars showed red grain color and six were white. The white-grained wheat cultivars showed wider length, width, and thickness of grain (6.87, 3.51, and 3.08 mm, respectively), and higher 1000- kernel weight and germination index (46.46 g and 0.61, respectively) than red-grained wheat cultivars (6.32, 3.33, 2.89 mm, 39.44 g, and 0.18, respectively; Table 5). The relationship between grain color and PHS resistance was reported in

previous studies (Flintham *et al.*, 1999; Mori *et al.*, 2005; Mares *et al.*, 2005). Both germination index and grain traits, except grain roundness and test weight, were related to grain color in Korean wheat cultivars. White-grained Korean wheat cultivars have been developed recently because white-grained wheats have higher flour yields and lightness of flour than red grains (Shin *et al.*, 2013). Thus, improvement of PHS resistance in white-grained wheats is important in Korean wheat breeding programs. The relationships among grain traits and the germination index of Korean wheat cultivars are summarized in Table 3. There was a positive correlation among grain traits, except grain roundness and test weight. Germination index correlated positively with length ($r = 0.478, P < 0.05$), width ($r = 0.523, P < 0.01$), and thickness ($r = 0.631, P < 0.001$) of grain and 1000-kernel weight ($r = 0.586, P < 0.01$). There was no significant relationship between germination index and grain roundness or test weight. These results indicate that increasing the size or weight of the grain could increase the risk of PHS in Korean wheat cultivars. Falling number has been evaluated in connection with PHS in wheat cultivars because sprouted wheat grains show abruptly low Falling falling numbers (Trethowan *et al.*, 1996). Grain size of wheat cultivars correlated positively with alpha-amylase and negatively with falling number (Evers *et al.*, 1995). Recently developed Korean wheat cultivars showed higher grain size and weight than older cultivars (Tables 2, 4). Red-grained and small grain size wheat cultivars should be considered to improve PHS resistance in Korean wheat breeding programs.

Table 1. Effect of year, genotype and their interactions on grain traits and germination index of 26 Korean wheat cultivars.

Source of variation	df	Grain traits						Germination index
		Length	Width	Thickness	Roundness	Test weight	1000-kernel weight	
Year (Y)	3	398.89*** ^a	192.51***	109.57***	23.08***	1327.45***	92.54***	23.04***
Genotypes (G)	25	3914.08***	192.73***	574.37***	673.70***	1209.71***	2130.28***	874.45***
Y × G	75	40.82***	10.31***	5.31***	12.43***	117.24***	54.21***	2.57***

^a *** significant at $P < 0.001$.

Table 2. Means for grain traits and germination index of 26 Korean wheat cultivars grown in four crop years.

Cultivar	Grain traits				Test weight (g)	1000-kernel weight (g)	Germination index
	Length (mm)	Width (mm)	Thickness (mm)	Roundness			
<i>Year</i>							
2010	6.46	3.36	2.94	1.75	817.56	41.37	0.30
2011	6.41	3.31	2.95	1.75	810.96	41.21	0.29
2012	6.41	3.36	2.91	1.76	814.21	40.50	0.27
2013	6.51	3.45	2.95	1.76	803.74	41.15	0.26
LSD ^a	0.12	0.05	0.04	0.02	5.09	1.52	0.07
<i>Cultivar</i>							
Alchan	5.86	3.28	2.90	1.65	798.50	35.58	0.06
Anbaek	6.44	3.65	3.00	1.71	823.25	47.23	0.10
Baekjoong	6.77	3.53	3.01	1.78	819.38	44.28	0.61
Cheonggkye	6.06	3.23	2.78	1.74	808.79	35.95	0.04
Dahong	5.90	3.12	2.78	1.71	791.42	33.00	0.07
Eunpa	5.93	3.25	2.87	1.68	823.75	37.44	0.27
Geuru	6.87	3.55	2.99	1.80	808.04	48.03	0.33
Gobun	6.40	3.25	2.88	1.77	806.33	38.68	0.16
Hanbaek	7.20	3.58	3.21	1.79	801.67	50.39	0.87
Jeokjoong	6.82	3.53	3.00	1.79	816.04	45.43	0.63
Jinpoom	6.19	3.34	2.86	1.73	815.21	39.64	0.13
Joeun	6.22	3.31	2.98	1.69	831.58	38.24	0.38
Jokyung	7.02	3.55	3.18	1.77	812.08	48.58	0.54
Jonong	6.88	3.46	3.03	1.80	819.42	43.79	0.08
Jopoom	6.90	3.23	2.81	1.91	818.04	38.03	0.15
Keumkang	6.78	3.49	3.08	1.76	839.08	47.44	0.36
Milsung	5.98	3.20	2.84	1.70	808.25	34.89	0.05
Namhae	6.13	3.37	2.93	1.69	777.50	37.84	0.14
OI	6.25	3.38	2.91	1.72	787.17	40.10	0.73
Olgeuru	6.50	3.41	2.97	1.75	812.33	43.05	0.30
Saeol	6.76	3.20	2.77	1.90	823.92	37.02	0.06
Seodun	6.10	3.29	2.85	1.72	811.31	38.30	0.45
Sukang	6.38	3.42	2.99	1.72	829.83	42.25	0.04
Tapdong	6.16	3.39	2.91	1.70	815.75	40.31	0.08
Uri	6.55	3.22	2.79	1.85	788.83	39.42	0.06
Younbaek	6.64	3.39	3.02	1.76	814.58	42.66	0.64
LSD	0.02	0.03	0.01	0.01	1.15	0.28	0.02

^aLeast significant difference ($P < 0.05$).

Table 3. Correlation coefficients of grain traits and germination index in 26 Korean wheat cultivars.

Parameter	Grain traits					
	Length	Width	Thickness	Roundness	Test weight	1000-kernel weight
Grain width	0.613*** ^a					
Grain thickness	0.626***	0.861***				
Grain roundness	0.738***	-0.001	-0.058			
Test weight	0.264	0.251	0.246	0.114		
1000-kernel weight	0.788***	0.935***	0.871***	0.231	0.298	
Germination Index	0.478*	0.523**	0.631***	0.064	-0.018	0.568**

^aIndicates significance at * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 4. Grain color and allelic variation of *TaSus2-2B*, *TaGW2* and *TaCwi-A1* identified by PCR analysis in 26 Korean wheat cultivars.

Cultivar	Released year	Grain Color	<i>TaSus2-2B</i> ^a	<i>TaGW2</i> ^b	<i>TaCwi-A1</i> ^c
Alchan	1995	Red	<i>Hap-H</i>	<i>Hap-6A-A</i>	<i>b</i>
Anbaek	2001	Red	<i>Hap-H</i>	<i>Hap-6A-A</i>	<i>a</i>
Baekjoong	2007	White	<i>Hap-L</i>	<i>Hap-6A-A</i>	<i>b</i>
Cheonggkye	1979	Red	<i>Hap-H</i>	<i>Hap-6A-A</i>	<i>b</i>
Dahong	1979	Red	<i>Hap-H</i>	<i>Hap-6A-G</i>	<i>b</i>
Eunpa	1982	Red	<i>Hap-H</i>	<i>Hap-6A-A</i>	<i>a</i>
Geuru	1980	Red	<i>Hap-H</i>	<i>Hap-6A-G</i>	<i>b</i>
Gobun	1996	Red	<i>Hap-H</i>	<i>Hap-6A-A</i>	<i>b</i>
Hanbaek	2008	White	<i>Hap-L</i>	<i>Hap-6A-G</i>	<i>a</i>
Jeokjoong	2007	White	<i>Hap-L</i>	<i>Hap-6A-A</i>	<i>b</i>
Jinpoom	1998	Red	<i>Hap-H</i>	<i>Hap-6A-A</i>	<i>b</i>
Joeun	2000	Red	<i>Hap-L</i>	<i>Hap-6A-A</i>	<i>b</i>
Jokyung	2004	White	<i>Hap-L</i>	<i>Hap-6A-G</i>	<i>a</i>
Jonong	2003	Red	<i>Hap-H</i>	<i>Hap-6A-A</i>	<i>b</i>
Jopoom	2001	Red	<i>Hap-H</i>	<i>Hap-6A-A</i>	<i>b</i>
Keumkang	1997	White	<i>Hap-H</i>	<i>Hap-6A-G</i>	<i>b</i>
Milsung	1998	Red	<i>Hap-H</i>	<i>Hap-6A-A</i>	<i>a</i>
Namhae	1988	Red	<i>Hap-H</i>	<i>Hap-6A-A</i>	<i>b</i>
Ol	1976	Red	<i>Hap-H</i>	<i>Hap-6A-A</i>	<i>b</i>
Olgeuru	1994	Red	<i>Hap-H</i>	<i>Hap-6A-A</i>	<i>b</i>
Saeol	1997	Red	<i>Hap-H</i>	<i>Hap-6A-A</i>	<i>a</i>
Seodun	1997	Red	<i>Hap-H</i>	<i>Hap-6A-A</i>	<i>b</i>
Sukang	2008	Red	<i>Hap-H</i>	<i>Hap-6A-G</i>	<i>a</i>
Tapdong	1986	Red	<i>Hap-H</i>	<i>Hap-6A-A</i>	<i>a</i>
Uri	1992	Red	<i>Hap-H</i>	<i>Hap-6A-A</i>	<i>b</i>
Younbaek	2005	White	<i>Hap-L</i>	<i>Hap-6A-G</i>	<i>b</i>

^a*Hap-H* and *Hap-L* haplotypes at *TaSus2-2B* locus produced 423 and 381 bp PCR fragments, respectively.

^b*Hap-6A-A* and *Hap-6A-G* produced 167 and 218 bp PCR fragments, respectively, after restriction with *TaqI* of PCR products (418 bp) of *TaGW2* gene.

^c*TaCwi-A1a* and *TaCwi-A1b* alleles at *TaCwi-A1* locus produced 402 and 404 bp PCR fragments, respectively.

Table 5. Comparison of means for grain traits and germination index (GI) between grain color and allelic variation in *TaSus2-2B*, *TaGW2-6A*, and *TaCwi-A1* in 26 Korean wheat cultivars grown in four crop years.

	No. Cultivar	Grain traits						GI
		Length (mm)	Width (mm)	Thickness (mm)	Roundness	Test weight (g)	1000-kernel weight (g)	
<i>Grain color</i>								
Red	20	6.32b ^a	3.33b	2.89b	1.75a	809.96a	39.44b	0.18b
White	6	6.87a	3.51a	3.08a	1.78a	817.14a	46.46a	0.61a
<i>TaSus2-2B</i>								
<i>Hap-H</i>	20	6.35b	3.33b	2.90b	1.75a	810.34a	39.90b	0.18b
<i>Hap-L</i>	6	6.78a	3.48a	3.07a	1.76a	815.89a	44.93a	0.61a
Red-grained								
<i>Hap-H</i>	19	6.33a	3.33a	2.89a	1.75a	808.82a	39.50a	0.17a
<i>Hap-L</i>	1	6.22a	3.31a	2.98a	1.69a	831.58a	38.24a	0.38a
White-grained								
<i>Hap-H</i>	1	6.78a	3.49a	3.08a	1.76a	839.08a	47.44a	0.36a
<i>Hap-L</i>	5	6.89a	3.52a	3.08a	1.78a	812.75a	46.27a	0.66a
<i>TaGW2-6A</i>								
<i>TaGW2-6A-A</i>	19	6.36a	3.34a	2.90b	1.75a	810.81a	39.75b	0.24a
<i>TaGW2-6A-G</i>	7	6.68a	3.44a	3.04a	1.76a	813.81a	44.62a	0.41a
Red-grained								
<i>TaGW2-6A-A</i>	17	6.31a	3.32a	2.89a	1.75a	810.00a	39.15a	0.19a
<i>TaGW2-6A-G</i>	3	6.38a	3.36a	2.92a	1.74a	809.76a	41.09a	0.15a
White-grained								
<i>TaGW2-6A-A</i>	2	6.80a	3.53a	3.01a	1.79a	817.71a	44.86a	0.62a
<i>TaGW2-6A-G</i>	4	6.91a	3.50a	3.12a	1.77a	816.85a	47.27a	0.60a
<i>TaCwi-A1</i>								
<i>TaCwi-A1a</i>	8	6.49a	3.40a	2.97a	1.75a	817.31a	42.26a	0.25a
<i>TaCwi-A1b</i>	18	6.43a	3.35a	2.92a	1.76a	809.09a	40.52a	0.30a
Red-grained								
<i>TaCwi-A1a</i>	6	6.28a	3.35a	2.90a	1.74a	820.79a	39.86a	0.10a
<i>TaCwi-A1b</i>	12	6.34a	3.32a	2.89a	1.75a	805.32a	39.26a	0.22a
White-grained								
<i>TaCwi-A1a</i>	2	7.11a	3.57a	3.20a	1.78a	806.88a	49.49a	0.71a
<i>TaCwi-A1b</i>	4	6.75b	3.49a	3.03b	1.77b	822.27a	44.95b	0.56a

^aValues followed by the same letters are not significantly different at $P < 0.05$.

Allelic variations in *TaSus2-2B*, *TaGW2-6A*, and *TaCwi-A1*

Allelic composition of *TaSus2-2B*, *TaGW2-6A*, and *TaCwi-A1* in Korean wheat cultivars is shown in Table 4 and Figure 1. The differences in grain traits and germination index, based on the allelic compositions of *TaSus2-2B*, *TaGW2-6A*, and *TaCwi-A1* are also shown in Table 5. Sucrose synthase (SUS) catalyzes the first step in the conversion of sucrose to starch in the endosperm. The wheat *Sus2* orthologous gene (*TaSus2*) was located on chromosomes 2 and three single-nucleotide polymorphisms (SNPs) were detected in *TaSus2-2B* (Jiang *et al.*, 2011).

Two haplotypes, *Hap-H* (423bp) and *Hap-L* (381bp), were found in *TaSus2-2B* and the *Hap-H* haplotype for high 1000-kernel weight underwent very strong positive selection in Chinese wheat breeding, but not in Europe (Jiang *et al.*, 2011). Twenty Korean wheat cultivars carried the *Hap-H* haplotype and six wheat cultivars carried the *Hap-L* haplotype at the *TaSus2-2B* locus. White-grained wheat cultivars, except Keumkang, showed the *Hap-L* haplotype. Korean wheat cultivars that carried *Hap-H* haplotype showed lower 1000-kernel weight (39.90 g) than the *Hap-L* haplotype (44.93 g), which is the opposite result to Chinese wheat cultivars (Jiang *et al.*, 2011). Wheat

cultivars that carried the Hap-H haplotype also exhibited shorter grain length (6.35 mm), width (3.33 mm), and thickness (2.90 mm) than Hap-L haplotype (6.68, 3.48, and 3.07 mm, respectively). The germination index of the Hap-H haplotype was lower (0.18) than that of the Hap-L haplotype (0.61). These results indicate that TaSus2-2B could be used to select wheat lines that carried PHS resistance in Korean wheat breeding programs, despite the opposite relationship between other grain traits and haplotypes at the TaSus2-2B locus.

TaGW2, which is the grain width (GW2) gene ortholog in wheat, influenced grain width and weight; it was located in the homologous group 6 chromosome and two SNPs have been detected in the promoter region of TaGW2-6A (Su *et al.*, 2011). A cleaved amplified polymorphic sequence (CAPS) marker was developed based on the 593 A-G polymorphism to distinguish the two haplotypes in TaGW2-6A, Hap-6A-A, and Hap-6A-G (Su *et*

al., 2011). Chinese wheat cultivars that carried Hap-6A-A were significantly associated with wider grains and higher 1000-kernel weight than Hap-6A-G in two crop seasons (Su *et al.*, 2011). Nineteen Korean wheat cultivars carried TaGW2-6A-A haplotype and seven wheat cultivars carried TaGW2-6A-G haplotype at TaGW2-6A locus. White-grained wheat cultivars, except cvs. Baekjoong and Jeokjoong, showed the TaGW2-6A-A haplotype. Korean wheat cultivars that carried the TaGW2-6A-G haplotype showed higher grain thickness and 1000-kernel weight (3.04 mm and 44.62 g, respectively) than the TaGW2-6A-A haplotype (2.90 mm and 39.75 g, respectively), which is the opposite result to Chinese wheat cultivars (Su *et al.*, 2011). However, there was no significant difference in length or width of grain, test weight, or the germination index of Korean wheat cultivars based on the TaGW2-6A alleles.

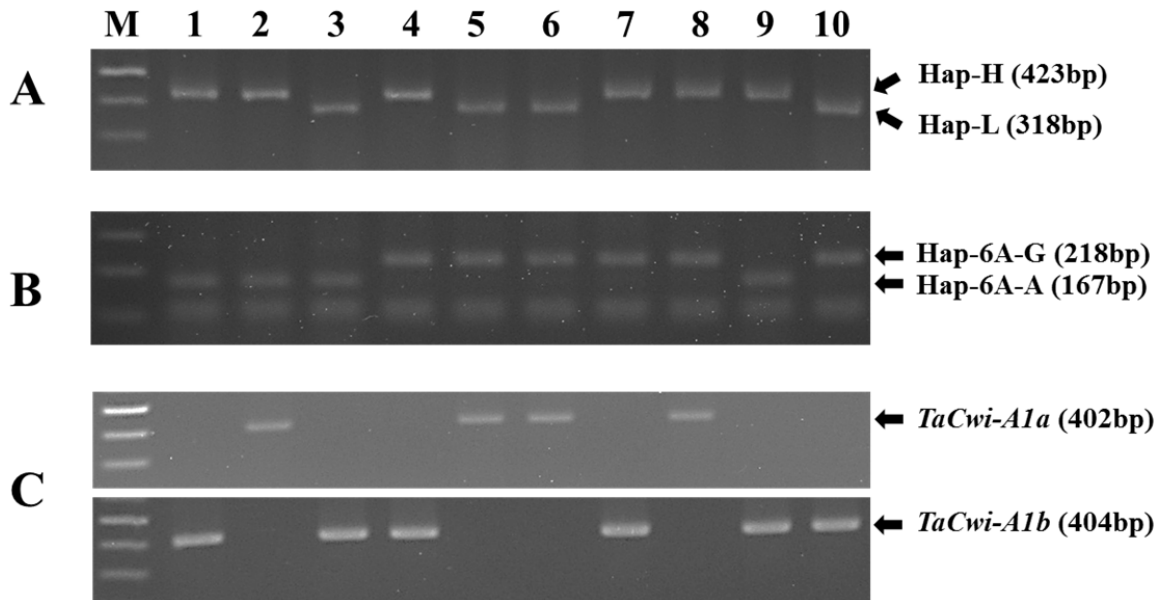


Figure 1. Gel electrophoresis of PCR-amplified *TaSus2-2B* (A), *TaGW2-6A* (B) and *TaCwi-A1* (C) in Korean wheat cultivars. M = molecular size marker, 1 = Alchan; 2 = Anbaek; 3 = Baegjoong; 4 = Dahong; 5 = Hanbaek; 6 = Jokyung; 7 = Keumkang; 8 = Sukang; 9 = Uri; 10 = Younbaek.

Cell wall invertase (CWI) is involved in sink tissue development and carbon partitioning and is highly associated with grain weight (Ma *et al.*, 2012). The Cwi gene is located on wheat chromosome 2A and designated TaCwi-A1 (Ma *et al.*, 2012). Chinese wheat cultivars that carried the TaCwi-A1a allele (402bp) showed higher grain weight than those with the TaCwi-A1b allele (404bp) and these alleles have been used in Chinese wheat breeding programs for improving grain yield (Ma *et al.*, 2012). Eighteen Korean wheat cultivars carried the TaCwi-A1a allele and eight carried the TaCwi-A1b allele. White-grained wheat cultivars, except Hanbaek and Jokyoung, showed the TaCwi-A1b allele. There was no significant difference in grain traits or germination index of Korean wheat cultivars based on the TaCwi A1 alleles. However, red-grained wheat cultivars that carried the TaCwi-A1a allele showed a higher test weight (820.79 g) than those with the TaCwi-A1b allele (805.32 g). White-grained wheats that carried the TaCwi-A1a allele exhibited wider grain length (7.11 mm) and thickness (3.20 mm) and higher 1000-kernel weight (49.49 g) than the TaCwi A1b allele (6.75, 3.03 mm, and 44.96 g, respectively).

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REFERENCES

- Barnard A, van Deventer CS, Maartens H (2005). Genetic variability of preharvest sprouting – the South African situation. *Euphytica* 143: 291-296.
- Biddulph TB, Mares DJ, Plummer JA, Setter TL (2005). Drought and high temperature increases preharvest sprouting tolerance in a genotype without grain dormancy. *Euphytica* 143: 277-283.
- Evers AD, Flintham J, Kotecha K (1995). Alpha-amylase and grain size in wheat. *J. Cereal Sci.* 21: 1-3.
- Flintham J, Adlam RE, Gale MD (1999). Seedcoat and embryo dormancy in wheat. In: Weipert D (ed) *Eighth international symposium on preharvest sprouting in cereals 1998*. Association of Cereal Science Research Federal Centre for Cereal Potato and Lipid Research, Detmold, Germany. pp 77-81.
- Flintham JE (2000). Different genetic components control coat imposed and embryo-imposed dormancy in wheat. *Seed Sci. Res.* 10: 43-50.
- Gegas VC, Nazari A, Griffiths S, Simmonds J, Fish L, Orford S, Sayers L, Doonan JH, Snape JW (2010). A genetic framework for grain size and shape variation in wheat. *Plant Cell* 22: 1046-1056.
- Groos C, Gay G, Perretant MR, Gervais L, Bernard M, Dedryver F, Charmet G (2002). Study of the relationship between pre-harvest sprouting and grain color by quantitative trait loci analysis in a white \times red grain bread wheat cross. *Theor. Appl. Genet.* 104: 39-47.
- Gubler F, Millar AA, Jacobsen JV (2005) Dormancy release, ABA and pre-harvest sprouting. *Curr. Opin. Plant Biol.* 8: 183-187.
- Halverson J, Zeleny L (1988). Criteria of wheat quality. pp 15-45: in Y. Pomeranz (ed). *Wheat: chemistry and technology*. American Association of Cereal Chemists, ST. Paul, MN, USA.
- Himi E, Mars DJ, Yanagisawa A, Noda AK (2002). Effect of grain color gene (R) on grain dormancy and sensitivity of the embryo to abscisic acid (ABA) in wheat. *J. Exp. Bot.* 53: 1569-1574.
- Imtiaz M, Ogbonnaya FC, Oman J, Ginkel M (2008). Characterization of quantitative trait loci controlling genetic variation for preharvest sprouting in synthetic backcross-derived wheat lines. *Genetics* 178: 1725–1736.
- Jiang Q, Hou J, Hao C, Wang L, Ge H, Dong Y, Zhang X (2011). The wheat (*T. aestivum*) sucrose synthase 2 gene (*TaSus2*) active in endosperm development is associated with yield traits. *Funct. Integr. Genomics* 11: 49-61.
- Kim KH, Kang CS, Park JC, Shin SH, Hyun JN, Park CS (2012). Evaluation of pre-harvest sprouting in Korean wheat cultivar. *Korean J. Breed. Sci.* 44: 526-537.
- King RW, Richards RA (1984). Water uptake in relation to pre-harvest sprouting in wheat: ear characteristics. *Aust. J. Agric. Res.* 35: 327-335.

- Kulwal PL, Kumar N, Gaur A, Khurana P, Khurana JP, Tyagi AK, Balyan HS, Gupta PK (2005). Mapping of a major QTL for pre-harvest sprouting tolerance on chromosome 3A in bread wheat. *Theor. Appl. Genet.* 111:1052-1059.
- Kulwal P, Ishikawa G, David-Benscher D, Feng Z, Yu LX, Jadhav A, Mehetre S, Sorrells ME (2012). Association mapping for pre-harvest sprouting resistance in white winter wheat. *Theor. Appl. Genet.* 125: 793-805.
- Kumar N, Kulwal PL, Gaur A, Tyagi AK, Khurana JP, Khurana P, Balyan HS, Gupta PK (2006). QTL analysis for grain weight in common wheat. *Euphytica* 151:135-144.
- Liu S, Cai S, Graybosch R, Chen C, Bai G (2008). Quantitative trait loci for resistance to pre-harvest sprouting in U.S. hard white winter wheat Rio Blanco. *Theor. Appl. Genet.* 117: 691-699.
- Liu S, Bai G, Cai S, Chen C (2011). Dissection of genetic components of preharvest sprouting resistance in white wheat. *Mol. Breed.* 27: 511-523.
- Liu Y, He Z, Appels R, Xia X (2012). Functional markers in wheat: current status and future prospects. *Theor. Appl. Genet.* 125: 1-10.
- Ma D, Yan J, He Z, Wu L, Xia X (2012). Characterization of a cell wall invertase gene TaCwi-A1 on common wheat chromosome 2A and development of functional markers. *Mol. Breeding* 29: 43-52.
- Mares DJ (1983). Preservation of dormancy in freshly harvested wheat grain. *Aust. J Agric. Res.* 34: 33-38.
- Mares DJ, Mrva K (2001). Mapping quantitative trait loci associated with variation in dormancy in Australian wheat. *Aust. J Agric. Res.* 52: 1257-1266.
- Mares D, Mrva K, Cheong J, Williams K, Watson B, Storlie E, Sutherland M, Zou Y (2005) A QTL located on chromosome 4A associated with dormancy in white- and red-grained wheats of diverse origin. *Theor. Appl. Genet.* 111: 1357-1364.
- Mori M, Uchino N, Chono M, Kato K, Miura H (2005). Mapping QTLs for grain dormancy on wheat chromosome 3A and the group 4 chromosomes, and their combined effect. *Theor. Appl. Genet.* 110: 1315-1323.
- Munkvold JD, Tanaka J, Benscher D, Sorrells ME (2009). Mapping quantitative trait loci for preharvest sprouting resistance in white wheat. *Theor. Appl. Genet.* 119: 1223-1235.
- Nyachiro JM, Clarke FR, DePauw RM, Knox RE, Armstrong KC (2002). Temperature effects on seed germination and expression of seed dormancy in wheat. *Euphytica* 126: 123-127.
- Prashant R, Kadoo N, Desale C, Kore P, Dhaliwal HS, Chhuneja P, Gupta V (2012). Kernel morphometric traits in hexaploid wheat (*Triticum aestivum* L.) are modulated by intricate QTL × QTL and genotype × environment interactions. *J. Cereal Sci.* 56: 432-439.
- Shin SH, Kim KH, Kang CS, Park JC, Hyun JN, Park CS (2013). Effects of agronomic characteristics and grain morphology on pre-harvest sprouting in Korean wheat cultivar. *Korean J. Crop Sci.* 45: 346-357.
- Shorter SC, Munro CA, Hodgkinson J (2005). Predicting pre-harvest sprouting susceptibility in New Zealand wheat cultivars. *Euphytica* 143: 309-312.
- Su Z, Hao C, Wang L, Dong Y, Zhang X (2011). Identification and development of a functional marker of *TaGW2* associated with grain weight in bread wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 122: 211-223.
- Sun XY, Wu K, Zhao Y, Kong FM, Han GZ, Jiang HM, Huang XJ, Li RJ, Wang HG, Li SS (2009). QTL analysis of kernel shape and weight using recombinant inbred lines in wheat. *Euphytica* 165: 615-624.
- Trethowan RM, Rajaram S, Ellison FW (1996). Pre-harvest sprouting tolerance of wheat in the field and under rain simulation. *Aust. J. Agric. Research* 47: 705-716.
- Tsilo TJ, Hareland GA, Simsek S, Chao S, Anderson JM (2010). Genome mapping of kernel characteristics in hard red spring wheat breeding lines. *Theor. Appl. Genet.* 121: 717-730.
- Walker-Simmons MK (1988). Enhancement of ABA responsiveness in wheat embryos at higher temperature. *Plant Cell Environ.* 11: 769-775.
- Xiao SH, Zhang XY, Yan CS, Lin H (2002). Germplasm improvement for preharvest sprouting resistance in Chinese white-grained wheat: An overview of the current strategy. *Euphytica* 126: 35-38.
- Yanagisawa A, Nishimura T, Amano Y, Torada A, Shibata S (2005). Development of winter wheat with excellent resistance to harvest sprouting and rain damage. *Euphytica* 143: 313-318.