밀 유전자원의 농업적 특성 및 적응성 분석을 통한 국내 재배환경에 적합한 유전자원 활용성 분석

이용진^{1†} · 윤진석^{1†} · 강천식² · 최유미³ · 정경희⁴ · 서용원^{1*}

¹고려대학교 생명과학대학, ²국립식량과학원, ³국립농업과학원 유전자원센터, ⁴경상남도 농업기술원

Selection of Wheat Germplasm in Korean Production Environments employing Agronomic Characteristics and Adaptation Analysis

Yong Jin Lee^{1†}, Jin Seok Yoon^{1†}, Chon-Sik Kang², Yu-Mi Choi³, Kyong Hee Joung⁴, and Yong Weon Seo^{1*}

¹Department of Biotechnology, Korea University, Seoul 02841, Republic of Korea

²National Institute of Crop Science, RDA, Wanju 55365, Republic of Korea

³National Agrobiodiversity Center, National Institute of Agricultural Science, RDA, Jeonju 54874, Republic of Korea

⁴Agricultural Product Research Department, Gyeongsangnam-do Agricultural Research and Extension Services, Jinju 52733, Republic of Korea

Abstract The National Agrobiodiversity Center of the RDA, Korea, has more than 22,700 accessions of global wheat genetic resources, including Korean wheat cultivars and landraces. Despite the numerous efforts to develop high-quality, hard winter wheat, the employment of new genetic resources into Korean wheat breeding programs is still hampered by the different growing environments. To overcome this limitation, 200 germplasms that were screened using the Genebank Management System (GMS) were evaluated in three different regions in Korea. In the 2018-2019 trial, 55 lines that showed superior field performance and high protein content were selected from among the 200 germplasms. These lines were re-evaluated in the 2019-2020 trial, and 24 lines that had suitable traits for growth, grain yield, and grain protein content in three locations were finally selected. These winter wheat germplasms also showed high yield stability throughout the three different environments in Korea. Preliminary screening using GMS information, consecutive regional tests, and quality tests could be effective procedures for the development of hard winter wheat in Korea. Therefore, introduction breeding could be a favorable breeding method aiming to improve quality, where useful genetic resources are limited.

Keywords hard winter wheat, genetic resources, adaptation, introduction breeding

Received on February 1, 2021. Revised on February 1, 2021. Accepted on February 16, 2021.

* Corresponding Author (E-mail: seoag@korea.ac.kr, Tel: +82-2-3290-3005, Fax: +82-504-393-3117)

† Author Contributions: Yong Jin Lee and Jin Seok Yoon have equal contribution, and considered as first authors.

Introduction

Wheat (*Triticum aestivum* L.) has been one of the most cultivated and important cereal crops for human dietary in the world. As the increasing population, the adaptability and yield stability of wheat are essential prerequisites to meet wheat demand. Although wheat consumption is 32.4 kg per capita per year, self-sufficiency is only 1 to 2% in Korea (KOSIS 2019). Imported wheat flour is mainly consumed as noodles (fried, steamed, Chinese noodle), followed by bread, and cookies (MAFRA 2020). This situation highlights the importance of wheat breeding programs with the aim of increasing current

production in Korea.

Wheat germplasms are valuable genetic resources to determine their potential impact on improving efficiency. Recently, the global climate change cause adverse effect on wheat development and production, and it emphasizes the need to use better adapted varieties of major crops and terrestrial crops as potential donors of useful genes (Gepts 2006, Khoury et al. 2014). However, the most cultivated crops have limited genetic diversity pools to continuous selective breeding (National Research Council 1993).

Introduction breeding leads to the development of superior wheat variety through crossing between elite variety and exotic

(non-adaptive) germplasm (Rasmusson & Phillips 1997). This breeding strategy has resulted in high productivity, quality improvement as well as increased genetic diversities in the breeding populations. This cycle of introduction breeding can increase genetic vulnerability, such as the loss of abiotic- and biotic-resistance genes due to depletion of genetic diversity (Bonnin et al. 2014). Thus, monitoring of genetic diversity at the exact growing environment is necessary for the development of wheat cultivars.

Early maturity is one of the important agronomic traits in wheat breeding programs. The effects of early maturing wheat have led to advance such as early harvesting and protection of biotic and abiotic stresses (Poehlman & Sleper 1995). In Korea, the development of early maturity winter wheat cultivars has advantages of avoiding rainfall season and efficient double cropping system. As one of the multi-cropping methods, double cropping gives economic benefits which combined by the possibility of additional profits from a second crop on the same field within a single growing season (Borchers et al. 2014). Advances in technology such as early maturity variety development and no-till equipment can promote the adoption of dual crops and make them economically viable (Shapiro et al. 1992).

There are not many cultivars that meet the satisfactory quality criteria in Korea. Therefore, wheat growers have not enough opportunity for chose of variety. To overcome these problems, it is necessary to develop wheat varieties with improved end-use quality parameters. Introducing exotic wheat germplasms into our breeding program would be an alternative way of crossing breeding.

The grain quality of wheat is determined by a complex combination of various parameters. End-use quality is determined by hardness, protein quantity & quality, and dough characteristics. Wheat grain hardness is one of the major parameters to determine the type of wheat, and it is important for end-product quality in the flour industry (Bettge & Morris 2000). Wheat is classified as soft, hard, and very hard by the basis of grain hardness (Morris 2002). The puroindolines such as PINA and PINB represent the molecular genetic control of grain hardness reside at the Hardness (*Ha*) locus on chromosome 5D (Bhave

& Morris 2008). The types of hardness depend on the presence of both puroindoline genes or the presence of a null or mutated puroindoline genes, which are called the soft wheat and hard wheat, respectively (Martin et al. 2006, Morris 2002, Xia et al. 2008).

The gluten content makes up 85% of total protein content in the endosperm of wheat grain and affects the rheological and pasting properties. Wheat grain quality depends on its gluten properties, including the viscoelastic of wheat dough. Gluten components are polymeric glutenins and monomeric gliadins, and they are closely associated with bread-making quality (Dhaka & Khatkar 2015). Glutenins consist of high molecular weight-glutenin subunit (HMW-GS) and low molecular weight-glutenin subunit (LMW-GS) glutenin subunits (Rasheed et al. 2014). The HMW-GSs and LMW-GSs are located at *Glu-1* loci and *Glu-3* loci, respectively, which are crucial genetically fixed parameters in the process of bread making. For example, 5 + 10 subunit encoded at *Glu-D1* have a positive effect, and 2 + 12 subunit encoded at *Glu-D1* have a negative effect on bread making quality. Previous studies on the relationships between HMW-GS and LMW-GS have led to successful wheat breeding development (Anjum et al. 2007, Dhaka & Khatkar 2015, Gupta et al. 1994, Peña et al. 1994).

In this study, we selected 200 wheat germplasms with superior agronomic characteristics *via* the GMS (Germplasm Managing System) database. The wheat germplasms were investigated for the extent of genetic variability and correlations among agronomic traits, quality parameters and adaptability in three different locations of Korean from 2018 to 2020. Among 200 wheat genetic resources, we selected 55 wheat germplasm with superior agronomic traits, qualities, and adaptability. We evaluated the selected 55 germplasms in the same locations of Korea from 2019 to 2020, and 24 germplasms with superior field performances were selected. The obtained results would be useful for the wheat breeding programs aiming to introduce alien genetic resources in the breeding populations.

Materials and Methods

Plant materials

Total 200 wheat germplasms that were screened for optimum heading dates as well as HMW-GS score of 6-10 *via* GMS (Genebank Management System) were incorporated in this study (Table 1).

Each line was planted (12 kg/10 a) at three locations; mid-north part (Namyangju-si, Gyeonggi-do), middle part (Wanju-gun, Jeollabuk-do), and south part (Jinju-si, Gyeongsangnam-do) of Korea with 2 replications on October 4, October 25, and October 29, respectively, in 2018. The test locations are at

Table 1. Origins and developmental status of the wheat germplasms used in this study.

Origin ^z	Line developmental status				Total
	Landrace	Breeding line	Cultivar	Unknown	
AFG	2				2
ARG				1	1
AUS				1	1
AUT				1	1
BGR			2	1	3
CAN				2	2
CHN	17		30	18	65
COL				1	1
ETH	1				1
HUN				1	1
IND	3			3	6
ISR				1	1
ITA				1	1
JPN				3	3
MEX	1	35		32	68
MNG				1	1
PRK				5	5
RUS				1	1
UKR				1	1
USA			1	1	2
UZB				1	1
Unknown				32	32
Total	24	35	33	108	200

^zCountry code. AFG, Afghanistan; ARG, Argentina; AUS, Australia; AUT, Austria; BGR, Bulgaria; CAN, Canada; CHN, China; COL, Colombia; ETH, Ethiopia; HUN, Hungary; IND, India; ISR, Israel; ITA, Italy; JPN, Japan; MEX, Mexico; MNG, Mongolia; PRK, North Korea; RUS, Russia; UKR, Ukraine; USA, United States of America; UZB, Uzbekistan.

latitude 37° 35' north and longitude 127° 14' east (for Namyangju-si), latitude 35° 50' north and longitude 127° 2' east (for Wanju-gun), latitude 35° 12' north and longitude 128° 7' east (for Jinju-si).

Evaluation of agronomic traits, grain protein content, and PCR markers

Numerous agronomic traits such as days to heading, days to maturing, plant height, spike length, and yield were measured according to the instruction of National Agrobiodiversity Center of Korea. Grain protein content was measured with non-destructive NIR (near-infrared) method (Kim et al. 2016). Genetic compositions of high-molecular weight glutenin subunit seed storage proteins in each line such as *Glu1-Bx* (Butow et al. 2004, Ma et al. 2003), *Glu1-Dx* and *Glu1-Dy* (Liu et al. 2008), and *Glu-B3b* and *Glu-B3g* (Wang et al. 2009) were analyzed. Furthermore, either presence or absence of genes for grain properties such as grain hardness [*Pina*, *Pinb-D1b*, *Pinb-D1c*, and *Pinb-D1d* (Huang & Brûlé-Babel 2011)], polyphenol oxidase [*PPO-A1a*, *PPO-A1b*, *PPO-D1a*, and *PPO-D1b* (He et al. 2007)], and waxy [*Wx-A1*, *Wx-B1*, and *Wx-D1* (Nakamura et al. 2002)] of each line were determined. Genotypes for vernalization genes *Vrn* [*Vrn-A1*, *Vrn-B1*, and *Vrn-D1* (Yan et al. 2004)] in each line was determined. The resistance genes for stem rust [*Sr22* (Periyannan et al. 2011)] and leaf rust [*Lr34* and *Lr46* (Skowrońska et al. 2019)] were analyzed. The PCR information are listed in Supplementary Table 1.

Statistical analysis

All data analysis used in this study including correlation and analysis of variance (ANOVA) were carried out using R program in R studio (Version 1.2.5033 - © 2009-2019 RStudio, Inc.).

Results and Discussions

Agronomic traits of selected 200 wheat germplasms at three locations in 2018–2019

National Agrobiodiversity Center maintained 22,709 wheat

germplasms and investigated agronomic traits and HMW-GS (high molecular weight-glutenin subunit) compositions of 4,200 germplasms from 2015 to 2017. Among 4,200 germplasms, 200 germplasms with average heading dates earlier than May 16 and *Glu-1* score from 6 to 10 were selected. Those 200 germplasms were planted in mid-north part (Namyangju-si, Gyeonggi-do), middle part (Wanju-gun, Jeollabuk-do), and south part (Jinju-si, Gyeongsangnam-do) of Korea.

Days to heading (DH) was taken at the period between date of sowing and date when 50% spikes were emerged in the population. The mean days to heading were 216, 189, and 179 in Namyangju-si (Gyeonggi-do), Wanju-gun (Jeollabuk-do), and Jinju-si (Gyeongsangnam-do), respectively. Days to maturity (DM) was counted from sowing to the date when more than 50% spikes turn to total loss of green color (physiological maturity). The mean DM were 254, 224, and 217, respectively, in three locations. The plant height (PH) was measured from

ground to the top of stem, and the mean PH were 79.6 cm, 78 cm, and 88.6 cm in Namyangju-si, Wanju-gun, and Jinju-si, respectively. The spike length (SL) was measured from the base to the tip of the spike excluding awn. The mean SL were 10.3 cm, 9 cm, and 8.8 cm in each location. The mean grain yield (GY) were recorded about 3961 kg/ha, 3622 kg/ha, and 5822 kg/ha in three locations. The grain protein contents (GP) of germplasms were measured using NIR spectroscopy and the mean GP were 16.1%, 11.5%, and 12% in three locations, respectively. Distributions of all the studied traits of 200 germplasms are illustrated in Fig. 1 and descriptive statistics of traits are shown in Table 2.

The correlation matrix (Fig. 2) among the traits showed high significant correlation (with $p<0.001$) among all traits except between plant height (PH) and grain protein content (GP) (not significant). The days to heading (DH) and maturing (DM) were positively correlated each other ($r=0.98$), and with SL ($r=0.49$ and $r=0.50$ for DH and DM, respectively) and GP ($r=0.75$ and $r=0.78$ for DH and DM, respectively), while negatively correlated with PH and GY. The analysis of variance indicated that the differences among three locations were significant (with $p<0.001$) for all measured traits (Table 3). Despite we selected 55 germplasms with superior agronomic traits and high grain protein contents (in following sections) across all three locations, these results indicated that some germplasms can be used as location-specific breeding materials in each location.

Selection of germplasm for 2019–2020 trial

We excluded germplasms having low average grain protein contents (<11%) in all three locations in 2019–2020 trial. Germplasms with late average heading date (later than May 3rd in 2018–2019 trial) except superior yielding performance were also excluded in the selection.

Since this study is conducted for employment of exotic wheat germplasms as a part of introduction breeding, the most important selection criterion was their adaptability in Korean growing environment. We selected 55 germplasms which showed yield stability, high grain protein content (GP) with superior agronomic traits, especially days to heading (DH) and

Fig. 1. The distribution of evaluated traits of 200 germplasms in three locations (2018–2019). Days to heading (A), days to maturing (B), plant height (C), spike length (D), grain yield (E), grain protein content (F).

Table 2. Descriptive statistics of agronomic traits and grain protein content for 200 germplasms in 2018-2019.

	DH ^z			DM ^y			PH ^x		
	Namyangju-si	Wanju-gun	Jinju-si	Namyangju-si	Wanju-gun	Jinju-si	Namyangju-si	Wanju-gun	Jinju-si
Mean	216.41	189.42	180.04	253.99	224.45	217.08	79.58	77.99	88.57
SD ^t	1.38	1.82	4.15	2.67	2.23	2.72	16.27	10.13	12.27
Variance	1.91	3.32	17.22	7.11	4.98	7.41	264.60	102.62	150.60
Minimum	210.00	186.00	156.00	249.00	219.00	209.00	39.67	51.00	56.00
Maximum	219.00	195.00	188.00	262.00	230.00	222.00	122.67	106.00	123.00
	SL ^w			GY ^v			GP ^u		
	Namyangju-si	Wanju-gun	Jinju-si	Namyangju-si	Wanju-gun	Jinju-si	Namyangju-si	Wanju-gun	Jinju-si
Mean	10.24	9.01	8.75	3960.78	3622.08	5821.90	16.07	11.54	11.99
SD	1.46	1.02	1.04	1687.10	1046.97	976.84	1.40	1.26	1.22
Variance	2.12	1.04	1.08	2846291.01	1096149.32	954224.51	1.95	1.58	1.50
Minimum	6.25	5.90	5.50	1187.50	750.00	3530.00	13.40	8.98	9.33
Maximum	14.33	12.00	11.80	8229.17	6520.83	9440.00	20.45	15.25	15.73

^zDH: Days to heading, ^yDM: Days to maturity, ^xPH: Plant height (cm), ^wSL: Spike length (cm), ^vGY: Grain yield (kg/ha), ^uGP: Grain protein content (%)
^tSD: Standard deviation

Fig. 2. The correlation matrix of evaluated traits of 200 germplasms in three locations (2018-2019). The scatter plots with a fitted line are displayed on the bottom of the diagonal. The value of the correlation and the significance level as stars are displayed on the top of the diagonal. *** Significant at 0.001 probability level.

grain yield (GY) in the most cultivated regions, Wanju-gun (Jeollabuk-do) and Jinju-si (Gyeongsangnam-do). The origin

of countries and improvement status of 55 selected germplasm are shown in Table 4.

Table 3. Analysis of variance (ANOVA) of studied traits among three locations in 2018-2019.

	DH ^z			DM ^y			PH ^x		
	df	MS ^t	F	df	MS	F	df	MS	F
Locations	2	63545***	8138	2	65250***	10107	2	6402***	38
Residuals	564	8		555	6		564	167	
	SL ^w			GY ^v			GP ^u		
	df	MS	F	df	MS	F	df	MS	F
Locations	2	112.5***	82	2	274022527***	179	2	1040***	629
Residuals	565	1.38		550	1528474		551	2	

^zDH: Days to heading, ^yDM: Days to maturity, ^xPH: Plant height (cm), ^wSL: Spike length (cm), ^vGY: Grain yield (kg/ha), ^uGP: Grain protein content (%)

^tMS: Mean square

***Significant at 0.001 probability level.

Table 4. Origin and developmental status of the wheat germplasms selected in 2019-2020 trial.

Origin ^z	Line developmental status				Total
	Landrace	Breeding line	Cultivar	Unknown	
ARG				1	1
BGR			1		1
CHN	4		9		13
IND				1	1
MEX		17		9	26
MNG				1	1
UKR				1	1
Unknown				11	11
Total	4	17	10	24	55

^zCountry code. ARG, Argentina; BGR, Bulgaria; CHN, China; IND, India; MEX, Mexico; MNG, Mongolia; UKR, Ukraine.

Agronomic traits of selected 55 wheat germplasms at three locations in 2019-2020

A total of 55 selected germplasms were planted on Oct. 19, 25, and 30 in 2019 at three locations, Namyangju-si, Wanju-gun, and Jinju-si, respectively. Distributions of all the traits measurements of selected 55 germplasms are illustrated in Fig. 3 and their descriptive statistics are shown in Table 5. The mean DH and DM of selected 55 germplasms were reduced in 2019-2020 trial of Namyangju-si and Wanju-gun, compared to that of 2018-2019 trial because one of the major selection criteria was DH. The mean grain yield (GY) and grain protein content (GP) were increased in Namyangju-si and Wanju-gun.

The correlation matrix among the traits of 55 selected germplasms in 2019-2020 trial is shown in Fig. 4. The DH

Fig. 3. The distribution of evaluated traits of 55 selected germplasms in three locations (2019-2020). Days to heading (A), days to maturing (B), plant height (C), spike length (D), grain yield (E), grain protein content (F).

was significantly correlated with all other traits and DM was also significantly correlated with all other traits except SL (not significant). The grain protein content (GP) was positively

Table 5. Descriptive statistics of agronomic traits and grain protein content for 55 selected germplasms in 2019-2020.

	DH ^z			DM ^y			PH ^x		
	Namyangju-si	Wanju-gun	Jinju-si	Namyangju-si	Wanju-gun	Jinju-si	Namyangju-si	Wanju-gun	Jinju-si
Mean	200.04	186.31	182.24	238.42	223.60	217.02	103.23	86.40	96.13
SD ^t	3.85	6.27	7.60	1.91	4.54	5.43	18.24	17.95	14.69
Variance	14.81	39.37	57.78	3.66	20.58	29.54	332.79	322.05	215.89
Minimum	193.00	176.00	168.00	235.00	218.00	208.00	76.33	63.00	64.00
Maximum	208.00	198.00	200.00	242.00	235.00	231.00	149.17	124.00	129.00
	SL ^w			GY ^v			GP ^u		
	Namyangju-si	Wanju-gun	Jinju-si	Namyangju-si	Wanju-gun	Jinju-si	Namyangju-si	Wanju-gun	Jinju-si
Mean	8.43	8.22	8.83	4316.1	3619.51	5716.70	17.10	12.03	10.76
SD	1.38	1.28	1.22	1195.82	961.40	1814.42	1.21	1.57	1.27
Variance	1.90	1.64	1.48	1429977	924293.40	3292128.00	1.47	2.47	1.62
Minimum	5.00	5.13	5.20	1218.75	1739.58	1813.54	14.16	9.37	8.69
Maximum	12.00	12.80	11.90	6458.33	6156.25	11728.12	20.51	17.03	13.69

^zDH: Days to heading, ^yDM: Days to maturity, ^xPH: Plant height (cm), ^wSL: Spike length (cm), ^vGY: Grain yield (kg/ha), ^uGP: Grain protein content (%)
^tSD: Standard deviation

Fig. 4. The correlation matrix of evaluated traits of 55 selected germplasms in three locations (2019-2020). The scatter plots with a fitted line are displayed on the bottom of the diagonal. The value of the correlation and the significance level as stars are displayed on the top of the diagonal. *** Significant at 0.001 probability level. **Significant at 0.01 probability level.

correlated with DH and DM for two years. These results indicate that the grain protein content is higher in the germplasms with late maturity than the germplasms with early maturity, and the germplasms that grown in the locations with late DH and DM accumulated more GP than in the germplasms that grown

in the locations with early DH and DM. The analysis of variance for two years of selected 55 germplasms (Table 6) indicated that all the traits are significantly different (with $p < 0.001$) among three locations and two years, except GY. The grain yield was significantly different among locations, while years and interaction between locations and years were not significant. The results indicate that the yield of selected 55 germplasms is mainly affected by locations and is stable through year trials.

Selection of germplasm for 2020–2021 trial

The selected 55 germplasms in 2019-2020 trial were the lines ranked from 1 to 55 based on the agronomic and quality performances such as DH, GY, and GP as well as field phenotyping. We selected top 70% among 55 germplasms in each location (Namyangju-si, Wanju-gun, and Jinju-si) that showed favorite agronomic performances in 2019-2020 and previous trial of 2018-2019 results. The germplasms that overlapped among locations of selected top 70% were selected as candidate germplasms for the 2020-2021 trial. The germplasms exhibited better scores in Wanju-gun and Jinju-si were given more weight values than Namyangju-si for selection.

Finally, a total of 24 germplasms were selected for 2020-2021 trial from the 3 regional tests (5 from Wanju-gun, 4 from

Table 6. Analysis of variance (ANOVA) for traits of germplasms on three locations in two years (2018-2019 and 2019-2020).

	DH ^z			DM ^y			PH ^x		
	df	MS ^t	F	df	MS	F	df	MS	F
Location	2	18904***	879	2	22107***	1809	2	2976***	13
Year	1	1562***	73	1	1751***	143	1	15468***	66
location : year	2	2522***	117	2	2076***	170	2	2847***	12
Residuals	310	8		310	12		314	235	
	SL ^w			GY ^v			GP ^u		
	df	MS	F	df	MS	F	df	MS	F
Location	2	13***	9	2	117508807***	72	2	765***	516
Year	1	58***	37	1	5593964 ^{ns}	3	1	55***	37
location : year	2	26***	17	2	3136344 ^{ns}	2	2	43***	29
Residuals	314	2		309	1625689		309	2	

^zDH: Days to heading, ^yDM: Days to maturity, ^xPH: Plant height (cm), ^wSL: Spike length (cm), ^vGY: Grain yield (kg/ha), ^uGP: Grain protein content (%)

^tMS: Mean square

***Significant at 0.001 probability level.

^{ns}not significant.

Jinju-si, 5 from Wanju-gun & Jinju-si, 1 from Namyangju-si & Wanju-gun, 4 from Namyangju-si & Jinju-si, and 5 from all three locations) and were sown in three locations, on October in 2020 for consecutive trial. The origin of country and improvement status of selected germplasm are shown in Table 7.

Among the selected 24 germplasms, some lines exhibited superior performance than others.

Three accessions (IT283227, IT293593, and IT16857) showed higher GY (from 28% to 89%) than mean GY of 24 selected germplasms among all three locations in the 2019-2020 trial. The mean DH of four accessions (IT291181, IT293624, IT166303, and IT166351) among all three locations in the 2019-2020 trial were at least one week earlier than the mean DH of 24 germplasms. Five accessions (IT291181, IT291203, IT297197, IT15804, and IT166282) had higher GP (from 12% to 19%) than mean GP of 24 selected germplasms among all three locations in the 2019-2020 trial.

The genotyping for the grain protein compositions, grain properties, disease resistance, and growth habit was carried out and the results of selected 24 germplasms are shown in Table 7. The germplasms of *Glu-1* score of 6 and 7, which were investigated in the preliminary study by National Agrobiodiversity Center were discarded during selections (2018-2020) because of a heavy selection pressure for the hard

wheat. Among the 24 selected germplasms, 25% (6 germplasms) were scored 8 and 75% (18 germplasms) were scored 10. Five lines (IT15804, IT166282, IT278241, IT293593, and IT303293) possess *Glu-b3g* allele which has been known to be associated with strong dough (Ito et al. 2011, Martínez-Cruz et al. 2011). These extra-hardy lines should be useful for making favorable quality by blending with soft/mid-hard four in the bread making industry.

As the goal of this study is to select hard wheat germplasms for adaptation in Korean production environment, we evaluated field performances and grain properties of germplasms for three consecutive years in three locations in Korea. We have experienced unusual chilling temperature during early stage of grain filling period in 2019-2020, which resulted in severe yield loss as well as reduced grain quality especially in Namyangju-si and Wanju-gun. Nevertheless, relative late or early maturity lines escaped the chilling temperature that was detrimental at the beginning of grain filling period, showed superior agronomic performances. Our results indicate that the yearly regional tests and selection based on GMS information could be an effective breeding strategy for using exotic germplasms in the breeding programs where genetic resources are limited for special quality traits.

Table 7. Genotyping for the targeted storage protein compositions, grain properties, and disease resistance of 24 germplasms.

Accession No.	Origin	Developmental Status	<i>Glu-Bx</i>	<i>Glu-Dx</i>	<i>Glu-Dy</i>	<i>Glu-B3b</i>	<i>Glu-B3g</i>	<i>PinA</i>	<i>PinB-D1b</i>	<i>PinB-D1c</i>	<i>PinB-D1d</i>
IT275964	MEX	Breeding line	7	5	10	-	-	D1a	o	-	o
IT278241	MEX	Breeding line	7	5	10	-	o	D1b	-	-	o
IT283227	MEX	Unknown	17	5	10	-	-	D1b	-	-	o
IT291175	MEX	Breeding line	7	5	10	-	-	D1b	-	-	o
IT291179	MEX	Breeding line	17	5	10	-	-	D1b	-	-	o
IT291181	MEX	Breeding line	7	5	10	-	-	D1b	-	-	o
IT291202	MEX	Breeding line	17	5	10	-	-	D1b	-	-	o
IT291203	MEX	Breeding line	7	5	10	-	-	-	-	-	o
IT291205	MEX	Breeding line	17	5	10	-	-	D1b	-	-	o
IT291270	MEX	Breeding line	7	5	10	-	-	D1b	-	-	o
IT293490	CHN	Cultivar	7	5	10	-	-	D1b	-	-	o
IT293492	CHN	Cultivar	7	5	10	-	-	-	o	-	o
IT293593	CHN	Landrace	7	5	10	-	o	-	-	-	o
IT293624	CHN	Landrace	17	5	10	-	-	D1b	-	-	o
IT297179	CHN	Cultivar	-	5	10	-	-	-	-	-	o
IT297197	CHN	Cultivar	7	5	10	-	-	D1a	-	-	o
IT303293	IND	Unknown	17	5	10	-	o	D1b	-	-	o
IT15804	Unknown	Unknown	7	5	10	-	o	D1a	o	-	o
IT16857	Unknown	Unknown	-	5	10	-	-	-	-	-	o
IT166282	Unknown	Unknown	-	5	10	-	o	D1a	o	-	o
IT166303	Unknown	Unknown	-	5	10	-	-	-	-	-	-
IT166350	Unknown	Unknown	7	5	10	-	-	D1a	-	-	-
IT166351	Unknown	Unknown	7	5	10	-	-	D1a	-	-	o
IT166364	Unknown	Unknown	7	5	10	-	-	D1b	-	-	o

Accession No.	<i>PPO-A1a</i>	<i>PPO-A1b</i>	<i>PPO-D1a</i>	<i>PPO-D1b</i>	<i>Wx-A1</i>	<i>Wx-B1</i>	<i>Wx-D1</i>	<i>Vrn1A</i>	<i>Vrn1B</i>	<i>Vrn1D</i>	<i>Sr22</i>	<i>Lr34</i>	<i>Lr46</i>
IT275964	-	-	o	-	o	o	-	-	-	o	-	-	-
IT278241	o	-	-	o	o	o	-	-	-	-	-	-	-
IT283227	-	-	-	o	o	o	-	o	-	-	-	o	-
IT291175	o	-	-	o	o	o	-	o	-	-	-	-	o
IT291179	-	-	-	o	o	o	-	o	o	o	-	-	o
IT291181	-	-	-	-	o	o	-	o	o	o	-	-	o
IT291202	o	-	o	-	o	o	-	o	-	o	-	o	-
IT291203	-	-	-	-	o	o	-	o	o	-	-	o	-
IT291205	o	-	o	-	o	o	-	o	-	-	-	-	-
IT291270	-	-	o	-	o	o	-	o	-	o	-	o	o
IT293490	-	-	o	-	o	o	-	-	-	-	-	-	o
IT293492	-	-	-	-	o	o	-	o	-	o	-	-	o
IT293593	-	-	o	-	o	o	-	o	o	o	-	-	o
IT293624	-	-	-	-	o	o	-	-	-	-	-	-	o
IT297179	-	-	-	-	o	o	-	o	-	-	-	-	-
IT297197	-	-	o	o	o	o	-	o	-	o	-	-	o
IT303293	-	-	-	o	o	o	-	-	-	-	o	-	o
IT15804	-	-	-	-	o	o	-	o	o	-	-	o	o
IT16857	-	-	-	-	o	o	-	-	-	-	-	-	-
IT166282	-	-	o	-	o	o	-	o	-	-	-	-	-
IT166303	-	-	o	-	o	o	-	o	-	-	-	-	-
IT166350	-	-	o	-	o	o	-	-	o	o	-	-	-
IT166351	-	-	o	-	o	o	-	-	-	o	-	-	o
IT166364	-	-	o	o	o	o	-	o	-	o	-	-	-

Acknowledgement

This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through Agri-Bio Industry Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (318088)

Supplementary Data

Supplementary Table 1 is available on the journal website.

REFERENCES

1. Anjum FM, Khan MR, Din A, Saeed M, Pasha I, Arshad MU. 2007. Wheat gluten: High molecular weight glutenin subunits - Structure, genetics, and relation to dough elasticity. *J Food Sci* 72: R56-R63.
2. Bettge AD, Morris CF. 2000. Relationships among grain hardness, pentosan fractions, and end-use quality of wheat. *Cereal Chem* 77: 241-247.
3. Bhavé M, Morris CF. 2008. Molecular genetics of puroindolines and related genes: Allelic diversity in wheat and other grasses. *Plant Mol Biol* 66: 205-219.
4. Bonnin I, Bonneuil C, Goffaux R, Montalent P, Goldringer I. 2014. Explaining the decrease in the genetic diversity of wheat in France over the 20th century. *Agric Ecosyst Environ* 195: 183-192.
5. Borchers, A, Truex-Powell E, Wallander S, Nickerson C. 2014. Multi-Cropping Practices: Recent Trends in Double Cropping. EIB-125, pp 1-16. United States Department of Agriculture, Economic Research Service
6. Butow BJ, Gale KR, Ikea J, Juhász A, Bedö Z, Tamás L, Gianibelli MC. 2004. Dissemination of the highly expressed Bx7 glutenin subunit (*Glu-B1a1* allele) in wheat as revealed by novel PCR markers and RP-HPLC. *Theor Appl Genet* 109: 1525-1535.
7. Dhaka V, Khatkar BS. 2015. Effects of gliadin/glutenin and HMW-GS/LMW-GS ratio on dough rheological properties and bread-making potential of wheat varieties. *J Food Qual* 38: 71-82.
8. Gepts P. 2006. Plant genetic resources conservation and utilization: The accomplishments and future of a societal insurance policy. *Crop Sci* 46: 2278-2292.
9. Gupta RB, Paul JG, Cornish GB, Palmer GA, Bekes F, Rathjen AJ. 1994. Allelic variation at glutenin subunit and gliadin loci, *Glu-1*, *Glu-3* and *Gli-1*, of common wheats. I. Its additive and interaction effects on dough properties. *J Cereal Sci* 19: 9-17.
10. He XY, He ZH, Zhang LP, Sun DJ, Morris CF, Fuerst EP, Xia XC. 2007. Allelic variation of polyphenol oxidase (PPO) genes located on chromosomes 2A and 2D and development of functional markers for the PPO genes in common wheat. *Theor Appl Genet* 115: 47-58.
11. Huang XQ, Brûlé-Babel A. 2011. Development of simple and co-dominant PCR markers to genotype puroindoline a and b alleles for grain hardness in bread wheat (*Triticum aestivum* L.). *J Cereal Sci* 53: 277-284.
12. Ito M, Fushie S, Maruyama-Funatsuki W, Ikeda TM, Nishio Z, Nagasawa K, Tabiki T, Yamauchi H. 2011. Effect of allelic variation in three glutenin loci on dough properties and breadmaking qualities of winter wheat. *Korean J Breed Sci* 61: 281-287.
13. Khoury CK, Bjorkman AD, Dempewolf H, Ramirez-Villegas J, Guarino L, Jarvis A, Rieseberg LH, Struik PC. 2014. Increasing homogeneity in global food supplies and the implications for food security. *Proc Natl Acad Sci USA* 111: 4001-4006.
14. Kim KH, Kang CS, Choi I, Kim H, Hyun JN, Park CS. 2016. Analysis of grain characteristics in Korean wheat and screening wheat for quality using near infrared reflectance spectroscopy. *Korean J Breed Sci* 48: 442-449.
15. KOSIS. 2019. <https://kosis.kr>.
16. Liu S, Chao S, Anderson JA. 2008. New DNA markers for high molecular weight glutenin subunits in wheat. *Theor Appl Genet* 118: 177-183.
17. Ma W, Zhang W, Gale KR. 2003. Multiplex-PCR typing of high molecular weight glutenin alleles in wheat. *Euphytica* 134: 51-60.
18. MAFRA. 2020. <https://www.mafra.go.kr>.
19. Martin JM, Meyer FD, Smidansky ED, Wanjugi H, Blechl AE, Giroux MJ. 2006. Complementation of the *pina* (null) allele with the wild type *Pina* sequence restores a soft phenotype in transgenic wheat. *Theor Appl Genet* 113: 1563-1570.
20. Martínez-Cruz E, Espitia-Rangel E, Villaseñor-Mir H, Molina-Galán J, Benítez-Riquelme I, Santacruz-Varela A, Peña-Bautista R. 2011. Dough rheology of wheat recombinant

- lines in relation to allelic variants of *Glu-1* and *Glu-3* loci. Cereal Res Commun 39: 386-393.
21. Morris CF. 2002. Puroindolines: the molecular basis of wheat grain hardness. Plant Mol Biol Puroindolines: the molecular genetic basis of wheat grain hardness. Plant Mol Biol 48: 633-647.
22. Nakamura T, Vrinten P, Saito M, Konda M. 2002. Rapid classification of partial waxy wheats using PCR-based markers. Genome 45: 1150-1156.
23. National Research Council. 1993. Genetic Vulnerability and Crop Diversity. pp 47-84. Managing Global Genetic Resources: Agricultural Crop Issues and Policies. The National Academies Press, Washington, DC.
24. Peña RJ, Zarco-Hernandez J, Amaya-Celis A, Mujeeb-Kazi A. 1994. Relationships between chromosome 1B-encoded glutenin subunit compositions and bread-making quality characteristics of some durum wheat (*Triticum turgidum*) cultivars. J Cereal Sci 19: 243-249.
25. Periyannan SK, Bansal UK, Bariana HS, Pumphrey M, Lagudah ES. 2011. A robust molecular marker for the detection of shortened introgressed segment carrying the stem rust resistance gene *Sr22* in common wheat. Theor Appl Genet 122: 1-7.
26. Poehlman JM, Sleper DA. 1995. Methods in Plant Breeding. pp 216-84. Breeding Field Crops. 4th ed. Iowa State University Press, Ames, Iowa.
27. Rasheed A, Xia X, Yan Y, Appels R, Mahmood T, He Z. 2014. Wheat seed storage proteins: Advances in molecular genetics, diversity and breeding applications. J Cereal Sci 60: 11-24.
28. Rasmusson DC, Phillips RL. 1997. Plant breeding progress and genetic diversity from de novo variation and elevated epistasis. Crop Sci 37: 303-310.
29. Shapiro BI, Brorsen BW, Doster DH. 1992. Adoption of double-cropping soybeans and wheat. J Agric Appl Econ 24: 33-40.
30. Skowrońska R. 2019. Development of multiplex PCR to detect slow rust resistance genes *Lr34* and *Lr46* in wheat. J Appl Genet 60: 301-304.
31. Wang LH, Zhao XL, He ZH, Ma W, Appels R, Peña RJ, Xia XC. 2009. Characterization of low-molecular-weight glutenin subunit *Glu-B3* genes and development of STS markers in common wheat (*Triticum aestivum* L.). Theor Appl Genet 118: 525-539.
32. Xia L, Geng H, Chen X, He Z, Lillemo M, Morris CF. 2008. Silencing of puroindoline a alters the kernel texture in transgenic bread wheat. J Cereal Sci 47: 331-338.
33. Yan L, Helguera M, Kato K, Fukuyama S, Sherman J, Dubcovsky J. 2004. Allelic variation at the *VRN-1* promoter region in polyploid wheat. Theor Appl Genet 109: 1677-1686.

Supplementary Table 1. PCR primers and conditions used in this study.

Target		Primer set	PCR conditions	Product size
<i>Glu1-Bx</i>	<i>Bx17</i> <i>non-BX17</i>	F: CGCAACAGCCAGGACAATT R: AGAGTTCTATCACTGCCTGGT	95°C 5 m - [95°C 30 s - 58°C 30 s - 72°C 1 m] 38 cycle - 72°C 5 m	699 bp 630 bp or 766 bp
<i>Glu1-Dx</i>		F: GGGACAATACGAGCAGCAAA R: CTGTGTTCCGGTTGTGCCA	95°C 5 m - [95°C 30 s - 60°C 30 s - 72°C 1 m] 38 cycle - 72°C 5 m	<i>Dx2</i> : 299 bp <i>Dx5</i> : 281 bp
<i>Glu1-Dly</i>		F: CGCAAGACAATATGAGCAAACT R: TTGCCCTTGTCTCTGTGTC		<i>Dy10</i> : 397 bp <i>Dy12</i> : 415 bp
<i>Glu-B3b</i>		F: ATCAGGTGTAAAAGTGATAG R: TGCTACATCGACATATCCA	94°C 5 m - [94°C 35 s - 56°C 35 s - 72°C 90 s] 38 cycle - 72°C 8 m	1570 bp
<i>Glu-B3g</i>		F: CCAAGAAATACTAGTTAACAAGTAGTC R: GTTGGGGTTGGGAAACA	94°C 5 m - [94°C 35 s - 60°C 35 s - 72°C 90 s] 38 cycle - 72°C 8 m	853 bp
<i>Pinb-D1b</i>		Pinb-F1: TCCTCTAGCTCTCCTTGCTC Pinb-R1: AGCCACTAGGGAACCTGTCAG Pinb-D1b-F1a: ATCACAAAATGGTGGGAAGA	94°C 5 m - [95°C 30 s - 60°C 30 s - 72°C 30 s] 15 cycle - [95°C 10 s - 45°C 30 s - 72°C 5 s] 5 cycle - [95°C 10 s - 53°C 30 s - 72°C 5 s] 15 cycle - 72°C 8 m	<i>Pinb</i> : 423 bp <i>pinb-D1b</i> : 232 bp
<i>Pinb-D1c</i>		Pinb-F1: TCCTCTAGCTCTCCTTGCTC Pinb-R1: AGCCACTAGGGAACCTGTCAG Pinb-D1c-R1b: TAGGTGCTATCTGGCTCA	94°C 5 m - [95°C 30 s - 61°C 30 s - 72°C 30 s] 15 cycle - [95°C 10 s - 48°C 30 s - 72°C 5 s] 5 cycle - [95°C 10 s - 53°C 30 s - 72°C 5 s] 15 cycle - 72°C 8 m	<i>Pinb</i> : 423 bp <i>pinb-D1c</i> : 269 bp
<i>Pinb-D1d</i>		Pinb-F1: TCCTCTAGCTCTCCTTGCTC Pinb-R1: AGCCACTAGGGAACCTGTCAG Pinb-D1d-F1a: TAGGCCACAAAATGGT	94°C 5 m - [95°C 30 s - 60°C 30 s - 72°C 30 s] 15 cycle - [95°C 10 s - 45°C 30 s - 72°C 5 s] 5 cycle - [95°C 10 s - 53°C 30 s - 72°C 5 s] 15 cycle - 72°C 8 m	<i>Pinb</i> : 423 bp <i>pinb-D1d</i> : 236 bp
<i>Pina-D1</i>		Pina-F1a: ACTGGACGAAAAAGCAGTGG Pina-R1a: GATTGACCCCTGGATGATGT Pina-R2a: TCACCCAATGCTGAAGACAC Pina-F1a: ACTGGACGAAAAAGCAGTGG Pina-R1a: GATTGACCCCTGGATGATGT Pina-R2b: GTTTGTCGGCGAGGTAGAAAG	94°C 5 m - [95°C 30 s - 60°C 30 s - 72°C 60 s] 35 cycle - 72°C 8 m	<i>Pina-D1a</i> : 704 bp <i>Pina-D1b</i> : 922 bp <i>Pina-D1a</i> : 704 bp <i>Pina-D1b</i> : 1033 bp
<i>PPO-D1a</i>		F: TGCTGACCGACCTTGACTCC R: CTCGTCACCGTCACCCGTAT	95°C 5 m - [95°C 30 s - 59°C 30 s - 72°C 1 m] 40 cycle - 72°C 5 m	713 bp
<i>PPO-D1b</i>		F: TGAAGCTGCCGTCATCTAC R: AAGTTGCCCATGTCTCTCGCC		490 bp
<i>PPO-A1ab</i>	<i>A1a</i> <i>A1b</i>	F: AACTGTGGCTCTTCTTCCCA R: AAGAACTTGCCCATGTCCGC	95°C 5 m - [95°C 30 s - 60°C 30 s - 72°C 1 m] 40 cycle - 72°C 5 m	a: 685 bp b: 876 bp
<i>Wx-A1</i>		F: TCGTGTTCGTCGGCGCCGAGATGG R: CCGCGCTTGTAGCAGTGGAAGTACC	95°C 5 m - [95°C 30 s - 65°C 30 s - 72°C 2 m] 32 cycle - 72°C 7 m	a: 389 bp b: 370 bp
<i>Wx-B1</i>		F: CTGGCTGCTACCTCAAGAGCAACT R: CTGACGTCCATGCCGTTGACGA		425 bp
<i>Wx-D1</i>		F: CTGGCTGCTACCTCAAGAGCAACT R: CTGTTTACCATGATCGCTCCCTT		a: 2307 bp b: 1731 bp
<i>Vrn</i>	<i>Vrn-A1</i> <i>Vrn-B1</i> <i>Vrn-D1</i>	VmA1F: GAAAGGAAAAATCTGCTCG VmB1F: CAGTACCCCTGCTACCACTG VmD1F: CGACCCGGGCGGCACGAGTG Vm1R: TGCACCTTCCC(CG)CGCCCAT	94°C 5 m - [94°C 45 s - 55°C 1 m - 72°C 1 m] 40 cycles - 72°C 5 m 94°C 5 m - [94°C 45 s - 58°C 1 m - 72°C 1 m] 40 cycles - 72°C 5 m 94°C 5 m - [94°C 45 s - 60°C 1 m - 72°C 1 m] 40 cycles - 72°C 5 m	500 bp 1000 bp 750 bp
<i>Sr22</i>		F: TTCCATAAGTTCCTACAGTAC R: TAGACAAACAAGATTAGCAC	94°C 5 m - [94°C 45 s - 58°C 1 m - 72°C 1 m] 40 cycles - 72°C 5 m	200 bp
<i>Lr</i>	<i>Lr34</i> <i>Lr46</i>	F: GTTGGTTAAGACTGGTGATGG R: TGCTTGCTATTGCTGAATAGT F: GGTCTTCTGGGCTTTGATCCTG R: GTTGCTAGGGACCCGTAGTGG	94°C 5 m - [94°C 45 s - 60°C 30 s - 72°C 1 m] 40 cycles - 72°C 5 m	R: 150 bp S: 229 bp 242 bp