

## Evaluation of genetic characteristics and physicochemical property of Korean wheat landraces (*Triticum aestivum* L.)

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**Abstract:** This study has evaluated the genetic characteristics and wheat processing-related properties of four Korean wheat landraces (KWLs). The KWLs were found to possess the vernalization alleles *vrn-A1*, *vrn-B1*, and *Vrn-D1* and the photoperiod alleles *Ppd-A1b*, *Ppd-B1b*, and *Ppd-D1a*. The Korean cultivated variety Keumgang also shared these alleles with the exception of *vrn-D1*. With regard to grain hardness, KWL 2 was shown to possess *Pina-D1a* and *Pinb-D1b* like Keumgang, while other KWLs were classified as carrying *Pina-D1a* and *Pinb-D1a*. All KWLs were found to be non-waxy, carrying the alleles *Wx-A1a*, *Wx-B1a*, and *Wx-D1a*. With regard to the *polyphenol oxidase* (PPO) genes, all four KWLs carried low-activity alleles, in contrast to the Keumgang sample. The assessment of physicochemical properties revealed that KWL 1, 3, and 4 had a higher amylose content but a lower protein content than KWL 2 and Keumgang. In tests of solvent retention capacity KWL 1 and KWL 2 exhibited the lowest and highest values, respectively, for all four solvents used in the tests. With regard to the dough properties, the results of Mixolab analysis indicated a faster starch gelatinisation in KWL1, while in KWL 2 a high water absorption and the longest dough development and stability times were found. KWL 3 and 4 exhibited similar dough behaviours. Principal component analysis of the four KWL lines revealed distinct clustering based on their physicochemical and dough-related traits.

**Keywords:** flour quality; genetic resources; marker-assisted selection; Mixolab; solvent retention capacity

Crops have been cultivated through collection, domestication, and selective breeding (Bradshaw 2016). With the advancement of cultivation, plant breeding techniques have been developed to enhance yield and ensure stable production (Ranabhatt & Kapor 2017). This led to the improvement of genetic characteristics associated with important traits,

such as growth period, flowering time, and product quality (Boote et al. 2001; Slafer et al. 2009; Nogué et al. 2019). Research at the molecular level has been conducted to optimise breeding techniques, leading to the development of marker-assisted selection (Pandurangan et al. 2021). This method enables the genetic evaluation of new varieties and has proven

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useful for identifying genes associated with both agronomic and quality-related traits (Song et al. 2023).

The evaluation of crop traits involves both genetic characteristics and processing quality (McGuire et al. 1988; McLauchlan et al. 2001; Sheng et al. 2022). In wheat, in particular, its grain is consumed into flour which serves as wide range of products (bread, pasta, noodles, cakes biscuits et.). Therefore, physicochemical properties and processing traits are evaluated, as they are considered important determinants of end-use quality (Soboka et al. 2017; Shang et al. 2021; Best et al. 2023). The starch and protein composition of flour significantly influences dough characteristics and quality (Magnus et al. 2000; Miyazaki et al. 2006; Schuster et al. 2022).

Solvent retention capacity tests are one of the most widely used methods for evaluating the physicochemical characteristics of flour. In this method, four solvents, i.e., water, lactic acid, sodium carbonate, and sucrose, are used to quantify the capacity of flour to absorb and retain water, gluten, damaged starch, and pentosan, respectively (Kweon et al. 2011). This analysis enables a thorough assessment of flour quality and processing performance (Van Steertegem et al. 2013). Moreover, to comprehensively understand dough behaviour under realistic processing conditions, rheological analysis is essential (Sun et al. 2023). The dough testing instrument Mixolab is commonly used for this purpose, as it can assess the physical properties of dough under both mechanical and thermal conditions, even when the sample size is small (Stoenescu et al. 2010; Bressiani et al. 2019; Singh et al. 2019). The dough characteristics measured via Mixolab, including water absorption, development, and stability, have a significant impact on the quality of final products (Cato & Mills 2008; Dhaka & Khatkar 2013).

A comprehensive understanding of both genetic characteristics and processing-related traits is fundamental to evaluating wheat quality and its potential for industrial utilisation (Hoque & Islam 2024). Therefore, in this study, we assessed these traits in four Korean wheat landraces (KWLs) cultivated in the Gyeongsangnam-do region (South Korea) and analysed the properties of the obtained dough to determine their quality and explore industrial applicability.

## MATERIAL AND METHODS

**Plant materials.** Four Korean wheat landrace lines, previously classified based on agronomic traits and

phylogenetic relationships, were selected for the evaluation of their potential as breeding resources (Lee et al. 2024). In addition, the wheat variety Keumgang, which is widely cultivated in Korea, was included as a comparison group to compare genetic traits as well as flour and dough characteristics. These were sown in 2023 and harvested in 2024 from a field in Gyeongsangnam-do Agricultural Research and Extension Services. The field followed a randomised complete block design with three replications and plots were composed each 10 m in length and spaced 0.18 m apart. Fertilisers were applied at standard rates of 91 kg/ha for N<sub>2</sub>, 74 kg/ha for P<sub>2</sub>O<sub>5</sub>, and 39 kg/ha for K<sub>2</sub>O according to the Rural Development Administration Standard Cultivation Method (RDA 2012). Different types of commercially available wheat flours (bread flour, all-purpose flour, cake flour) were also included to comprehensively compare flour and dough characteristics.

**Evaluation of genetic characteristics.** Genomic DNA was extracted from young leaf tissues of wheat plants using the Genomic DNA Prep Kit (BIOFACT™, South Korea) according to the manufacturer's instructions. DNA concentration and quality were measured using a Nano spectrophotometer (Nabi UV/Vis MicroDigital, Korea). PCR amplification was performed in a 25 µL reaction volume containing 2 µL of DNA template (20 ng/µL), 2.5 µL of forward and reverse primer (10 pmol/ µL), 8 µL of 2X Prime Taq Premix (Genet Bio, Korea), and 12.5 µL of distilled water using T100™ Thermal Cycler (Bio–Rad, USA). Genetic markers were selected to identify *vernalization* (*Vrn*) genes and *photoperiod response* (*Ppd*) genes (Fu et al. 2005; Beales et al. 2007; Bentley et al. 2011; Seki et al. 2011). Moreover, the *Puroindoline* (*Pin*) genes related to seed hardness, and *Waxy* genes associated with amylose content were used (Qamar et al. 2014; Nakamura et al. 2002; Saito et al. 2009), also *Polyphenol Oxidase* (*PPO*) genes indicating the polyphenol oxidase activity associated with dough colour were employed (He et al. 2007; Si et al. 2012; Hystad et al. 2015). PCR conditions were optimised for each molecular marker.

**Analysis of the physicochemical properties of seeds.** Starch extraction was performed according to the method of the total starch assay kit (Megazyme, USA). Amylose content was determined based on the method described in Williams et al. (1970). In brief, a starch sample (20 mg) was thoroughly mixed with 0.5 N KOH solution, then the dispersed samples were transferred to volumetric flasks and diluted with dis-

tilled water up to a volume of 100 mL. 10 mL of this solution was transferred to a volumetric flask and added with 0.1 N HCl and iodine reagent. The final volume was adjusted to 50 mL. After an incubation period of 5 min, absorbance was measured at 625 nm. Protein contents in wheat grains were determined using the AACCI method 46-30.01 (2010) and Primacs™ SNC-100 (SKALAR, Netherlands), respectively. Grain samples (0.1 g) were combusted at 1 000 °C, and water vapour and interfering substances were removed using a scrubber. The total carbon content was measured using a non-dispersive infrared (NDIR) detector. Subsequently, nitrogen oxides were converted into nitrogen gas via a copper reduction column and quantified using a thermal conductivity detector (TCD). The crude protein content was estimated to multiply the nitrogen content by 5.7, which is the nitrogen conversion factor for wheat.

#### Measurement of flour and dough properties.

Solvent retention capacity (SRC) was measured using the AACCI Method No. 56-11 (2000). Four different solvents were used: distilled water, 5% lactic acid (*w/w*), 5% sodium carbonate (*w/w*), and 50% sucrose (*w/w*). A total of 25 mL of each solvent was mixed with 5 g of each flour in 50-mL tubes. The mixtures were stirred for 20 min, followed by centrifugation at 1 500 × *g* for 15 min. Removing the supernatant, inverting the tubes and placed on tissue paper for 10 min to dry excess liquid. The weight of the precipitation was then measured, and the SRC (%) was calculated using the formula by Haynes et al. (2009) (see below on this page).

The Mixolab analysis (Mixolab Chopin, France) was performed using the AACCI Approved Method 54-60.01 (2010) and Dubat (2010). In brief, a sample of flour (50 g) with a moisture content normalised to 14% and distilled water was mixed into Mixolab to achieve a total weight of 75 g. During dough development, the maximum torque was maintained at 1.1 ± 0.05 Nm, and the dough mixing speed was set to 80 rpm. Dough temperature was controlled throughout the mixing process using the Chopin+ protocol. The results obtained were analysed using Chopin Mixolab Ver. 3.14 (Chopin, France). This software measures the characteristics of dough mixtures based on five torque-related features, referred to as C1 to C5. Specifically, C1 represents the maximum

torque during the initial mixing phase, C2 reflects protein strength, C3 indicates the degree of starch gelatinisation, C4 represents the stability of gelatinised starch, and C5 is an indicator of starch retrogradation. Additionally, Chopin Mixolab calculates the values of slopes α, β, and γ, which represent the rate of protein weakening, starch gelatinisation, and enzymatic starch degradation, respectively (Tripette & Chopin 2005; Koksel et al. 2009; Hoang et al. 2023).

**Statistical analysis.** All measurements were performed in triplicate, and the average values were calculated. The obtained data were statistically analysed using Analyse-it (Ver. 6.15, Analyse-it Software, Ltd., United Kingdom) and R (Ver. 4.4.1, R Foundation for Statistical Computing, Austria). Duncan's multiple range test was conducted using the duncan.test function in the R package agricolae. Correlation analysis and principal component analysis (PCA) were performed using the “scatter plot matrix with a line graph” and “correlation monoplplot of biplot/monoplplot” functions in Analyse-it.

## RESULTS AND DISCUSSION

**Genetic analysis of vernalization and photoperiod.** Flowering time is a critical factor influencing yield potential and environmental adaptation in wheat, and it is affected by vernalization and the photoperiod (Snape et al. 2001; Dubcovsky et al. 2006; Plotnikov et al. 2024). Based on these genetic traits, wheat is classified as spring or winter wheat (Wu et al. 2017; Amo et al. 2022). In this study, the *vernalization* and *photoperiod* allelic genes of four KWLs and Keumgang were comparatively analysed (Table 1, Figure 1). The results showed *Vrn-A1*, *Vrn-B1* in all KWLs and Keumgang exhibited *vrn-A1* and *vrn-B1* as recessive alleles. Within the *Vrn-D1*, all KWLs showed dominant alleles with *Vrn-D1*, while Keumgang harboured a recessive allele with *vrn-D1*. In 25 Korean wheat cultivars, *vrn-A1* and *vrn-B1* were found to be recessive alleles, and *Vrn-D1* showed both recessive and dominant alleles (Cho et al. 2015). The frequency of the dominant *Vrn-D1* allele was shown to be higher in Asia than in Europe, Africa, and Australia (Kiss et al. 2014).

In photoperiod allelic genes, all KWLs and Keumgang were found to have the same alleles i.e., *Ppd-A1b*,

$$\text{SRC}(\%) = \left[ \frac{(\text{Tube + Gel weight}) - \text{Empty tube weight}}{\text{Flour weight}} - 1 \right] \times \left[ \frac{86}{100 - \text{Flour moisture}(\%)} \right] \times 100$$

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Table 1. Summary of genetic alleles of Korean wheat landraces

Variety	Vernalization			Photoperiod			Puroindolines		Waxy			Polyphenol oxidase		
	A	B	D	A	B	D	<i>Pina</i> D	<i>Pinb</i> D	A	B	D	A	B	D
Keumgang	<i>vrn</i>	<i>vrn</i>	<i>vrn</i>	<i>b</i>	<i>b</i>	<i>a</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>b</i>	<i>a</i>
KWL 1	<i>vrn</i>	<i>vrn</i>	<i>VRN</i>	<i>b</i>	<i>b</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>a</i>
KWL 2	<i>vrn</i>	<i>vrn</i>	<i>VRN</i>	<i>b</i>	<i>b</i>	<i>a</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>
KWL 3	<i>vrn</i>	<i>vrn</i>	<i>VRN</i>	<i>b</i>	<i>b</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>a</i>
KWL 4	<i>vrn</i>	<i>vrn</i>	<i>VRN</i>	<i>b</i>	<i>b</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>a</i>

A, B, and D indicate each genome of wheat, and each small alphabet indicates an allele; KWL – Korean wheat landrace; *VRN*, *vrn* – alleles for vernalization

*Ppd-B1b*, and *Ppd-D1a* (Figure 1, Table 1). Korean wheat cultivars have *Ppd-A1b* and *Ppd-D1a*. *Ppd-B1* show two different alleles. Same as KWLs, most of the Korean wheat cultivars were shown to have *Ppd-B1b* alleles (Cho et al. 2015). Overall, KWLs vernalization and photoperiod alleles are also the same as those found in cultivar Jokyoung, which is commonly used in speed breeding systems (Cha et al. 2025).

**Genetic analysis of puroindoline and waxy.** Wheat is classified into soft and hard wheat based on grain hardness, which is the most important trait to determine the end-use quality of products (Dubreil et al. 1998; Nadolska-Orczyk et al. 2009; Pasha et al. 2010). And grain hardness is determined by two puroindoline genes, *Pina* and *Pinb* (Morris 2002). The *Pina* and *Pinb* genes were confirmed to exhibit alleles *Pina-D1a* and *Pinb-D1b* in Keumgang. (Table 1,

Figure 2). Based on the genotype of Keumgang, all four KWLs were shown to carry *Pina-D1a* alleles. At the *Pinb-D1* locus, KWL 1, 3, and 4 carried *Pinb-D1a*, while KWL 2 carried *Pinb-D1b*.

Wheat is classified into waxy and non-waxy types depending on starch synthase activity (Dai 2010). Amylose synthesis is regulated by granule-bound starch synthase (GBSS) genes, the key enzyme responsible for amylose synthesis and known to control Waxy (*Wx*) protein (Shure et al. 1983). In this study, the primer sets for genes *Wx-A1*, *Wx-B1*, and *Wx-D1* were identified (Figure 2, Table 1). All four KWL lines and Keumgang exhibited the *Wx-A1a*, *Wx-B1a*, and *Wx-D1a* alleles. These genotypes possess the functional *Wx* gene that result in non-waxy wheat (Yamamori & Quynh 2000; Nakamura et al. 2002).

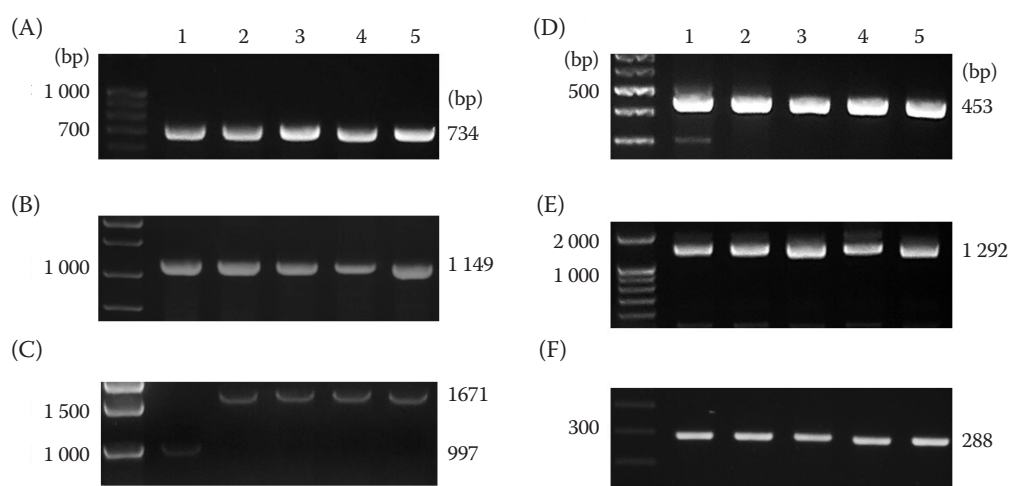


Figure 1. Identification of vernalization (*vrn*) and photoperiod (*PPD*) genes of Korean wheat landraces: *vrn-A1*, 734 bp (A); *vrn-B1*, 1149 bp (B); *vrn-D1*, 997 bp and *Vrn-D1*, 1671 bp (C); *PPD-A1b*, 453 bp (D); *PPD-B1b*, 1292 bp (E); *PPD-D1a*, 288 bp (F)

Each number indicates that 1 is Keumgang, a Korean wheat cultivar and 2–5 are Korean wheat landraces, respectively



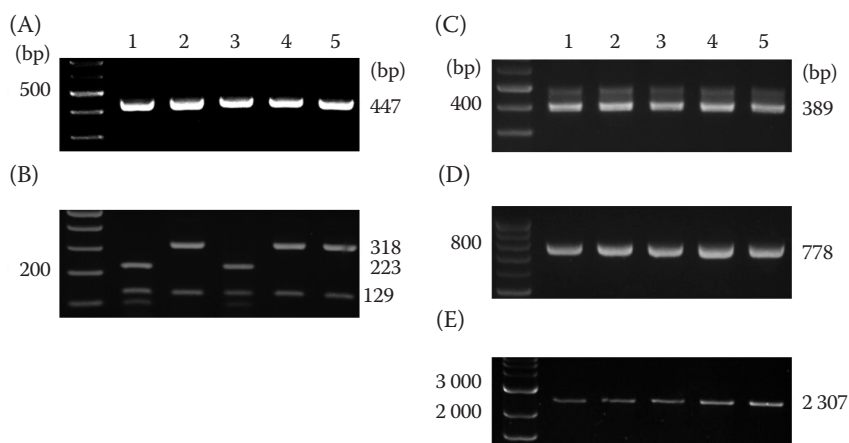


Figure 2. Identification of puroindoline (*Pin*) and waxy (*Wx*) genes of Korean wheat landraces: *Pina-D1a*, 447 bp (A); *Pinb-D1a*, 320 bp and *Pinb-D1b*, 200 bp (B); *Wx-A1*, 389 bp (C); *Wx-B1*, 778 bp (D); *Wx-D1*, 2 307 bp (E). Each number indicates that 1 is Keumgang, a Korean wheat cultivar and 2–5 are Korean wheat landraces, respectively.

**Genetic analysis of polyphenol oxidase.** Wheat products vary widely depending on flour and dough properties. Dough has darkening during preparation and cooking over time, especially frozen doughs and broken fermentations, and that is regulated by the polyphenol oxidase (PPO) (Fuerst et al. 2010; Xue et al. 2021; Yu et al. 2024). In this study, *PPO-A1*, *PPO-B1*, and *PPO-D1* were used as molecular markers to identify *PPO* allelic variants in the KWL lines (Table 1, Figure 3). At the *PPO-A1* allele, KWL 2 and Keumgang carried *PPO-A1a*, whereas KWL 1, 3,

and 4 carried *PPO-A1b*. At the *PPO-B1* allele, Keumgang exhibited the *PPO-B1a* allele, while all four KWLs possessed the *PPO-B1b* allele. Lastly, *PPO-D1* allele, all four KWLs exhibited the same genotype as Keumgang, *PPO-D1a*. In Korean cultivars, the *PPO-A1a* allele had a higher PPO activity that dough is more darkening, whereas those carrying the *PPO-A1b* allele exhibited a significantly higher brightness (Kim et al. 2012). A previous study on 50 Korean wheat cultivars shows that 45 of them exhibited *PPO-D1a* (Choi et al. 2022), and the same was observed in all four KWLs.

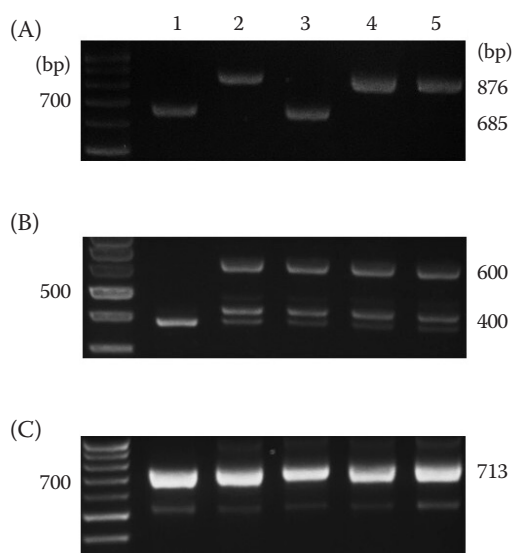


Figure 3. Identification of polyphenol oxidase (*PPO*) genes of Korean wheat landraces: *PPO-A1a*, 685 bp and *PPO-A1b*, 876 bp (A); *PPO-B1a*, 400 bp and *PPO-B1b*, 400 + 600 bp (B); *PPO-D1a*, 713 bp (C).

Each number indicates that 1 is Keumgang, a Korean wheat cultivar and 2–5 are Korean wheat landraces, respectively.

**Physicochemical properties and Solvent retention capacity averages of flour.** The KWLs were evaluated in terms of their physicochemical properties to assess flour quality, which is a key factor affecting processing traits (Guzman et al. 2022). KWL 1, 3, and 4 exhibited higher amylose contents (28.31%, 27.98%, and 27.55%, respectively) than Keumgang (26.97%), while a lower content was observed for KWL 2 (26.20%) (Table 2). Nitrogen contents were lower in all four KWLs (1.49%, 1.64%, 1.67%, and 1.67%, respectively) than in Keumgang (1.85%). Protein contents were lower in all the KWLs (7.90%, 9.56%, 7.44%, and 8.73%) compared to Keumgang (13.05%).

The SRC test is a method used to assess the physicochemical properties of flour. Here, it was used to evaluate four characteristics: water absorption, gluten strength, damaged starch content, and pentosan content (Duyvejonck et al. 2012). With regard to water absorption by water SRC (referred to as WSRC), the results showed lower values for all KWLs (48.55%, 58.96%, 52.70% and 54.91%, respectively) than for Keumgang (62.80%). Gluten strength was evaluated based on the capacity to retain lactic acid (LASRC), and all KWLs (93.40%, 113.17 %, 97.31% and

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Table 2. Evaluation of seed physicochemical properties of Korean wheat landraces

Variety	AC	NC	PC	Crude protein	WSRC	LASRC	SCSRC	SSRC
		(%)		(N 5.7)*			(%)	
Keumgang	26.97 <sup>bc</sup>	1.85 <sup>a</sup>	13.05 <sup>a</sup>	10.55 <sup>a</sup>	62.80 <sup>a</sup>	121.68 <sup>a</sup>	78.11 <sup>a</sup>	86.40 <sup>b</sup>
KWL 1	28.31 <sup>a</sup>	1.49 <sup>c</sup>	7.90 <sup>d</sup>	8.49 <sup>c</sup>	48.55 <sup>e</sup>	93.40 <sup>e</sup>	60.66 <sup>d</sup>	69.09 <sup>d</sup>
KWL 2	26.20 <sup>c</sup>	1.64 <sup>b</sup>	9.56 <sup>b</sup>	9.35 <sup>b</sup>	58.96 <sup>b</sup>	113.17 <sup>b</sup>	75.45 <sup>b</sup>	88.19 <sup>a</sup>
KWL 3	27.98 <sup>a</sup>	1.67 <sup>b</sup>	7.44 <sup>e</sup>	9.52 <sup>b</sup>	52.70 <sup>d</sup>	97.31 <sup>d</sup>	72.54 <sup>c</sup>	82.02 <sup>c</sup>
KWL 4	27.55 <sup>a</sup>	1.67 <sup>b</sup>	8.73 <sup>c</sup>	9.52 <sup>b</sup>	54.91 <sup>c</sup>	109.01 <sup>c</sup>	73.00 <sup>c</sup>	87.66 <sup>a</sup>
Bread	24.94	2.36	13.00	13.43	69.50	136.23	82.68	96.61
All-purpose	25.11	1.78	10.00	10.12	63.44	114.97	80.14	87.33
Cake	25.79	1.46	7.00	8.32	52.61	81.88	65.10	75.94

AC – amylose content; NC – nitrogen content; PC – protein content; SRC – solvent retention capacity; WSRC – water solvent retention capacity; LASRC – lactic acid solvent retention capacity; SCSRC – sodium carbonate solvent retention capacity; SSRC – sucrose solvent retention capacity; KWL – Korean wheat landrace; bread – bread flour; all-purpose – all-purpose flour; cake – cake flour; \*N indicates nitrogen content; the alphabets were Duncan's multiple range test at a significance level of  $P < 0.05$

109.01%, respectively) were observed to have lower values than Keumgang (121.68%). Starch damage was determined based on the capacity to retain sodium carbonate (SCSRC), and the results showed lower values for all four KWLs (60.66%, 75.45%, 72.54% and 73.00%, respectively) than for Keumgang (83.14%). Finally, pentosan content was assessed based on the capacity to retain sucrose (SSRC), with results showing the highest and lowest values in KWL 2 (88.19%) and KWL 1 (69.09%), respectively.

**Characteristics of the dough obtained using flour derived from the KWLs.** Mixolab analysis was used to evaluate the dough characteristics made using flour derived from the KWLs. This tool analyses the quality of flour and the rheological properties of dough under different mechanical and thermal conditions (Jia et al. 2011). The results showed water absorption values of 50.3–54.5% for all four KWL flours, while a higher value was observed for the Keumgang flour (59.3%) (Table 3). These values are in line with

Table 3. Summary of Mixolab parameters

Parameters	Keumgang	KWL 1	KWL 2	KWL 3	KWL 4	Bread	All-purpose	Cake
WA (%)	59.3 <sup>a</sup>	50.3 <sup>e</sup>	54.5 <sup>b</sup>	52.8 <sup>d</sup>	53.5 <sup>c</sup>	62.2	56.0	50.5
DD (min)	7.08 <sup>a</sup>	0.78 <sup>b</sup>	2.06 <sup>b</sup>	0.95 <sup>b</sup>	0.99 <sup>b</sup>	8.85	8.33	0.90
DS (min)	9.60 <sup>a</sup>	1.35 <sup>c</sup>	8.80 <sup>a</sup>	4.40 <sup>b</sup>	4.95 <sup>b</sup>	11.40	10.90	7.00
C1 (Nm)	1.10 <sup>ab</sup>	1.14 <sup>a</sup>	1.13 <sup>ab</sup>	1.08 <sup>b</sup>	1.09 <sup>b</sup>	1.08	1.11	1.07
C2 (Nm)	0.47 <sup>a</sup>	0.37 <sup>c</sup>	0.48 <sup>a</sup>	0.41 <sup>b</sup>	0.42 <sup>b</sup>	0.62	0.61	0.44
C3 (Nm)	1.73 <sup>c</sup>	2.14 <sup>ab</sup>	2.00 <sup>b</sup>	2.28 <sup>a</sup>	2.25 <sup>a</sup>	1.69	2.01	2.36
C4 (Nm)	1.56 <sup>c</sup>	1.90 <sup>b</sup>	2.11 <sup>a</sup>	2.23 <sup>a</sup>	2.11 <sup>a</sup>	1.63	1.53	2.06
C5 (Nm)	2.53 <sup>c</sup>	2.82 <sup>b</sup>	3.25 <sup>a</sup>	3.36 <sup>a</sup>	3.52 <sup>a</sup>	2.77	3.36	3.85
$\alpha$	-0.11 <sup>d</sup>	-0.08 <sup>c</sup>	-0.07 <sup>bc</sup>	-0.07 <sup>b</sup>	-0.06 <sup>a</sup>	-0.07	-0.07	-0.08
$\beta$	0.47 <sup>b</sup>	0.68 <sup>a</sup>	0.25 <sup>c</sup>	0.38 <sup>b</sup>	0.25 <sup>c</sup>	0.22	0.31	0.44
$\gamma$	-0.03 <sup>a</sup>	-0.04 <sup>a</sup>	-0.01 <sup>a</sup>	-0.04 <sup>a</sup>	-0.03 <sup>a</sup>	-0.01	-0.13	-0.05

KWL – Korean wheat landrace; bread – bread flour; all-purpose – all-purpose flour; cake – cake flour; WA – water absorption; DD – dough development time; DS – dough stability time; C1 – the maximum point of the first mixing stage; C2 – protein strength; C3 – starch gelatinization; C4 – starch gel stability; C5 – starch retrogradation in the cooling phase;  $\alpha$  – protein network weakening range;  $\beta$  – starch gelatinization range;  $\gamma$  – cooking stability range; the alphabets were Duncan's multiple range test at a significance level of  $P < 0.05$

those normally reported in Korean wheat cultivars, which range between 50% and 60% (Kim et al. 2017). Among the four KWLs flour, those derived from KWL 2 and KWL 1 exhibited the highest (54.5%) and lowest (50.3%) values, respectively.

With regard to dough development time, no significant differences were observed among KWLs, with values ranging between 0.78 and 2.06 min. Among them, a considerably longer time was observed for KWL 2 (2.06 min). In addition, KWL 2 (8.80 min) and Keumgang (9.60 min) exhibited a longer dough stability time compared to the other KWLs (1.35–4.95 min). Protein strength (C2), which is measured in understanding the gluten network's behaviour during dough processing (Hrušková et al. 2013; Lacko-Bartošová et al. 2019). That was shown to be the highest in KWL 2 (0.48 Nm) and Keumgang (0.47 Nm), while KWL 3 (0.41 Nm) and KWL 4 (0.42 Nm) exhibited lower values close to those detected on average in Korean cultivars (0.41 Nm) (Kim et al. 2017). Protein networks influence dough development and stability time (Gao et al. 2020). These results showed that KWL 2 possesses longer

dough development and stability time than the other KWLs, because it showed a stronger gluten network.

Starch gelatinisation (C3) and the stability of gelatinised starch (C4) were higher in all four KWLs (2.00–2.28 Nm and 1.90–2.23 Nm, respectively) than in Keumgang (1.73 Nm and 1.56 Nm, respectively). Additionally, KWL 1 and KWL 2 exhibited lower  $\alpha$  values (–0.08, –0.07, respectively) compared to Keumgang (–0.11), suggesting lower protein weakening rates, while the  $\beta$  values across all four KWLs ranged between 0.25 and 0.68, indicating moderate starch gelatinisation rates. These results indicate that the KWL flours had a gelatinisation potential and stable starch networks upon heating and that their starches were showed strong mechanical properties.

#### Comprehensive evaluation of the KWL lines.

The relationships among physicochemical properties, flour traits related to SRC, and dough characteristics measured by Mixolab for the KWLs were examined via correlation analysis and PCA (Figures 4 and 5). The results of correlation analysis showed that amylose content was positively correlated with the degree and rate of starch gelatinization ( $r = 0.662^*$

	AC	PC	WSRC	LASRC	SSSRC	SSRC	WA	DD	DS	C1	C2	C3	C4	C5	$\alpha$	$\beta$	$\gamma$	
AC	-	-0.885	-0.910	-0.880	-0.701	-0.715	-0.823	-0.880	-0.933	-0.079	-0.930	0.662	-0.271	-0.365	-0.119	0.683	-0.502	<0.05
PC		-	0.821	0.907	0.510	0.622	0.688	0.767	0.787	0.220	0.810	-0.670	0.003	0.236	0.164	-0.596	0.563	<0.01
WSRC			-	0.939	0.889	0.911	0.968	0.793	0.978	-0.230	0.959	-0.405	0.546	0.599	0.360	-0.872	0.597	<0.001
LASRC				-	0.791	0.878	0.898	0.680	0.894	-0.199	0.856	-0.368	0.340	0.584	0.452	-0.861	0.467	
SSSRC					-	0.966	0.972	0.579	0.875	-0.595	0.786	-0.045	0.827	0.834	0.577	-0.947	0.332	
SSRC						-	0.978	0.553	0.856	-0.583	0.771	-0.048	0.729	0.856	0.649	-0.977	0.400	
WA							-	0.767	0.787	0.220	0.810	-0.670	0.003	0.236	0.164	-0.596	0.563	
DD								-	0.808	0.278	0.844	-0.829	0.311	0.237	0.052	-0.469	0.630	
DS									-	-0.172	0.979	-0.446	0.520	0.512	0.229	-0.842	0.504	
C1										-	-0.031	-0.758	-0.741	-0.822	-0.705	0.602	0.176	
C2											-	-0.541	0.434	0.375	0.099	-0.739	0.632	
C3												-	0.247	0.294	0.357	-0.013	-0.497	
C4													-	0.812	0.578	-0.674	0.242	
C5														-	0.883	-0.812	0.114	
$\alpha$															-	-0.590	0.001	
$\beta$																-	-0.265	
$\gamma$																	-	

Figure 4. Pearson's correlation between flour physicochemical properties of Korean wheat landraces

AC – amylose content; PC – protein content; WSRC – water solvent retention capacity; LASRC – lactic acid solvent retention capacity; SCSRC – sodium carbonate solvent retention capacity; SSRC – sucrose solvent retention capacity; WA – water absorption; DD – dough development time; DS – dough stability time; C1 – the maximum point of the first mixing stage; C2 – protein strength; C3 – starch gelatinization; C4 – starch gel stability; C5 – starch retrogradation in the cooling phase;  $\alpha$  – protein network weakening range;  $\beta$  – starch gelatinization range;  $\gamma$  – cooking stability range

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and 0.683\*, respectively) and negatively correlated with protein content, water absorption, dough development, dough stability time, and protein strength ( $r = -0.885^{***}$ ,  $-0.823^{**}$ ,  $-0.880^{***}$ ,  $-0.933^{***}$ , and  $-0.930^{***}$ , respectively). In contrast, protein content was positively correlated with water absorption, dough development, and stability time ( $r = 0.688^*$ ,  $0.767^{**}$ , and  $0.787^{**}$ , respectively) and negatively correlated with the degree and rate of starch gelatinisation ( $r = 0$ ,  $-0.670^{***}$ ,  $-0.596^*$ , respectively). This result is similar to previous findings indicating negative correlations between amylose content and protein characteristics in Japanese soft wheat (Nishio et al. 2020). Starch-protein interactions play a key role in determining flour quality and dough properties. In particular, they affect the viscosity, elasticity, starch retrogradation and processability of dough (Scott & Awika 2023).

In this study, all SRC were positively correlated with water absorption, dough stability time, and protein strength ( $r = 0.968^{***}$ ,  $0.898^{***}$ ,  $0.972^{***}$ ;  $0.978^{***}$ ,  $0.978^{***}$ ,  $0.894^{***}$ ;  $0.875^{***}$ ,  $0.856^{**}$ ,  $0.959^{***}$ ,  $0.856^{***}$ ,  $0.786^*$ , and  $0.771^{**}$ , respectively), while they showed negative correlation with rate of gelatinization ( $r = -0.872^{***}$ ,  $-0.861^{***}$ ,  $-0.947^{***}$ , and  $-0.977^{***}$ , respectively). Water absorption, dough development, and dough stability times showed strong positive correlations with protein strength ( $r = 0.810^{**}$ ,  $0.844^{**}$ , and  $0.979^{***}$ , respectively). In addition, water absorption and dough stability were negatively correlated with the rate of starch gelatinisation ( $r = -0.596^{***}$  and  $-0.842^{***}$ ), while dough development showed strong negative correlations with the degree of starch gelatinisation ( $r = -0.829^{***}$ ).

PCA also showed similar results to the correlation (Figure 5). PCA explained a total variance of 87.9%, with PC1 accounting for 63.3% and PC2 accounting for 24.6%. Along the PC1 axis, amylose content, degree and rate of starch gelatinisation were associated with each other, and these parameters were found to contribute to a negative direction on this axis. Protein content, dough development time, dough stability, and protein strength, also showed strong correlations with each other, and these parameters were in the positive direction of the PC1 axis. In addition, consistent with the correlation analysis, amylose contents and protein contents exhibited negative correlations with each other.

All four KWLs were clearly separated into distinct clusters based on their different characteristics. Along the PC1 axis, KWL 2 and KWL 4 were positioned with

dough development, protein contents, all SRC, Protein network weakening range etc., whereas KWL 1 and KWL 3 were located with the range of starch gelatinisation, amylose contents, and starch gelatinisation. Furthermore, the PC2 axis contributed to further subdividing. This allowed for a clear differentiation of the characteristics between the samples. KWL 1 exhibited a range of starch gelatinisation. KWL 2 possessed a range of cooking stability, dough development time, and high protein content. KWL 3 demonstrated a degree of starch gelatinisation, which enhances the physical properties of dough. Lastly, KWL 4 showed gluten stability.

In order to support the above results, PCA (PC1: 59.2%, PC2: 17.5%) was conducted to validate the characteristics of KWLs, using commercial flours (hard, medium, and soft) and Keumgang as reference standards (Figure 6). The analysis revealed that the hard wheat flour was distinguished by superior sol-

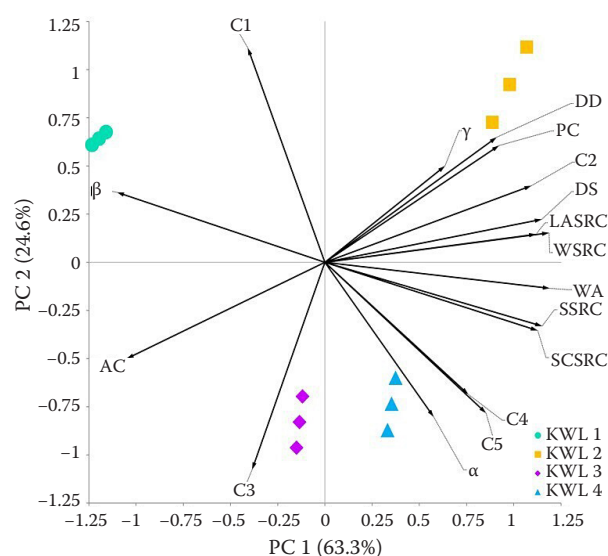


Figure 5. Principal component analysis based on flour physicochemical properties

AC – amylose content; PC – protein content; WSRC – water solvent retention capacity; LASRC – lactic acid solvent retention capacity; SCSRC – sodium carbonate solvent retention capacity; SSRC – sucrose solvent retention capacity; WA – water absorption; DD – dough development time; DS – dough stability time; C1 – the maximum point of the first mixing stage; C2 – protein strength; C3 – starch gelatinization; C4 – starch gel stability; C5 – starch retrogradation in the cooling phase;  $\alpha$  – protein network weakening range;  $\beta$  – starch gelatinization range;  $\gamma$  – cooking stability range; KWL – Korean wheat landrace



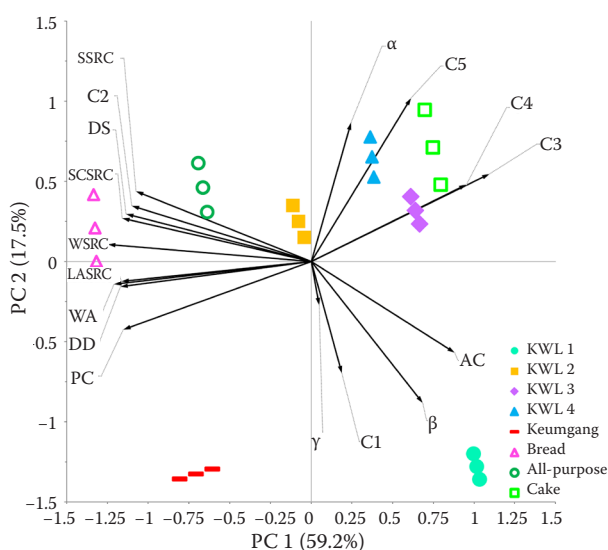


Figure 6. Principal component analysis based on seed physicochemical properties

AC – amylose content; PC – protein content; WSRC – water solvent retention capacity; LASRC – lactic acid solvent retention capacity; SCSRC – sodium carbonate solvent retention capacity; SSRC – sucrose solvent retention capacity; WA – water absorption; DD – dough development time; DS – dough stability time; C1 – the maximum point of the first mixing stage; C2 – protein strength; C3 – starch gelatinization; C4 – starch gel stability; C5 – starch retrogradation in the cooling phase;  $\alpha$  – protein network weakening range;  $\beta$  – starch gelatinization range;  $\gamma$  – cooking stability range; KWL – Korean wheat landrace

vent retention capacities, dough development time and stability. Medium wheat flour was positioned intermediately, whereas the soft flour was clustered by its lower protein contents and higher gelatinisation. Among the KWLs, KWL 2 was situated near the medium wheat flour group. KWL 3 and KWL 4 lines were clustered near the soft wheat flour group. While the KWL 1 were not clustered with reference standards and a high relation at rate of gelatinisation and amylose contents than the other tested flours.

## CONCLUSION

In this study, genetic characteristics and traits related to flour quality were comprehensively evaluated in four KWLs. Molecular marker analysis revealed allelic variation in genes related to vernalization, photoperiod sensitivity, grain hardness, starch composition, and polyphenol oxidase activity. Notably,

based on the observed *PPO* genotype, the KWLs examined were shown to exhibit a lower predicted enzymatic browning potential compared to the Korean wheat cultivar Keumgang.

Significant variations were also found in the physicochemical and dough-related properties among the KWLs, which further differentiated their processing performance. Result of PCA, the KWLs clustered into distinct groups based on protein and amylose contents, dough development time, and range of starch gelatinisation. these four KWLs present different genetic and flour quality characteristics of value. These four KWLs have different genetic characteristics and physicochemical properties.

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