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## **Research Article**

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# Identification and evaluation of important agronomic traits in 44 Polish wheat varieties (*Triticum polonicum* L.) grown on the Qinghai Plateau, China

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### Abstract

The lack of excellent wheat germplasm resources on the Qinghai-Tibet Plateau has led to a gradual decrease in genetic diversity and an increasingly narrow genetic background in wheat grown in this region. Rational use of excellent genes from wheat relatives is important to increase genetic diversity, broaden the genetic base and achieve high yield and quality in common wheat. The objective of this study was to use principal component and cluster analyses of 13 important agronomic traits of 44 Polish wheat varieties over 3 years and comprehensively evaluate them to screen for excellent germplasm resources, thus providing the basic material for broadening the genetic base of Qinghai-Tibet Plateau wheat germplasm resources.

#### Introduction

Polish wheat (*Triticum polonicum* L.), a tetraploid naked-grain cultivated species (2n = 4x = 28, AABB), is mainly distributed along the Mediterranean coast and in Ethiopia. It is grown sporadically in Tibet, China, and has been mixed with common wheat in Henan Province and the Xinjiang Uygur Autonomous Region. Polish wheat has many advantages due to its large spike size, full grain size, excellent quality and strong tillering power (Chen *et al.*, 1985; Yang *et al.*, 2000). Studies have shown that Polish wheat has a thousand grain weight (TGW) of <60 g (Wiwart *et al.*, 2013) and a seed crude protein content (SCPC) of <18% (Dong and Zheng, 2000), while exhibiting abundant genetic diversity in all types of storage proteins (Liu and Shepherd, 1996; Sissons and Batey, 2003; Gálová *et al.*, 2011). In addition, the grain protein, essential trace micronutrients and straight-chain starch contents are higher than those in common wheat (Rodríguez-Quijano *et al.*, 2003; Bieńkowska *et al.*, 2018; Bienkowska *et al.*, 2020). Due to these excellent properties, Polish wheat varieties have become important genetic resources for improving common wheat.

A number of studies have been performed on Polish wheat, including the identification of quantitative trait loci for grain characteristics (Li et al., 2012; Yang et al., 2012; Shen et al., 2014), the analysis of karyotype by fluorescence in situ hybridization (Kwiatek et al., 2016), the cloning of transporter proteins (Peng et al., 2018a, 2018b; Jiang et al., 2021) and the discovery and localization of the long glume gene P (Watanabe et al., 1996; Wang et al., 2002). Recently, the long glume trait control gene P1 was cloned (Liu et al., 2021; Chai et al., 2022). In terms of utilizing Polish wheat for breeding, studies have focused on the super-parental phenomenon of some traits in the progeny of crosses between Polish and common wheat (Shen et al., 2014), whereby researchers have introduced the excellent characteristics of high TGW, higher tiller number, good water use efficiency, drought tolerance and disease resistance into the common wheat background (Germanov, 1979; Hakimi et al., 1996; Yuan and Li, 1998; Liu et al., 2012; Wiwart et al., 2013). However, previous research has been focused mainly on the identification and evaluation of particular traits in a few materials (Wang et al., 2017; Cheng et al., 2020; Jiang et al., 2021). Moreover the phenotypic studies that have been carried out on large Polish wheat populations have been only preliminarily evaluated based on just 1 year of trait data (Dong et al., 2007; Shen et al., 2021), and extensive long-term systematic evaluation of the varieties for specific areas of application is lacking, which is not conducive to the continuous and extensive breeding utilization of Polish wheat.

In this study, we conducted long-term systematic phenotypic identification and evaluation of Polish wheat populations collected worldwide and grown on the Qinghai Plateau. The aim was to identify breeding materials with excellent traits that are suited to the unique climate of the Qinghai Plateau, to promote the utilization of Polish wheat in breeding programmes and to further lay the material foundation for the expansion of the genetic base of wheat grown on the Qinghai Plateau for the purpose of improving the quality of this crop.

### **Material and methods**

The 44 Polish wheat materials used in this study were obtained from the United States Department of Agriculture (http://www. ars-grin.gov/). All were kept in Qinghai Provincial Key Laboratory of Crop Molecular Breeding. The material originated from 15 countries, with that from Ethiopia accounting for 27.27%, followed by Portugal and the USA, which accounted for 13.64 and 11.36%, respectively. The test materials were grown for 3 years and showed stable performance in the field, with a germination rate of  $\geq$ 90% (further information is detailed in online Supplementary Tables S1 and S2).

#### **Experimental design**

This study was conducted over three consecutive years from 2020 to 2022 at the Haidong Eco-Agriculture Experimental station (102°19′32″E, 36°28′60″N) of the Northwest Institute of Plateau Biology, Chinese Academy of Sciences. The area is a typical transition mosaic from the Loess Plateau to the Qinghai-Tibet Plateau, with an elevation of 2016 m, average annual temperature of 3.2–8.6°C, average annual rainfall of 319.2–531.9 mm, evaporation rate of 1275.6–1861 mm, average duration of annual sunshine of 2708–3636 h and frost-free period of about 90 d.

The experiment was conducted in a two-factor randomized group design. Sixty seeds of each material were planted in three rows on medium-fertile land. The rows were 2 m long and 0.2 m apart. Field management strategies such as fertilization, weeding, irrigation and pest control were the same as in conventional breeding fields. When the test material reached maturity, it was

Table 1. Changes in agronomic traits of plants in 2020-2022

No.	PH (cm)	SNL (cm)	SPL (cm)	Tiller (number)	No.	PH (cm)	SNL (cm)	SPL (cm)	Tiller (number)
B1	113.47 <sup>h</sup>	51.28 <sup>ab</sup>	52.80 <sup>ab</sup>	14.13 <sup>ab</sup>	B32	142.11 <sup>abcdef</sup>	57.32 <sup>ab</sup>	15.23 <sup>abcde</sup>	20.56 <sup>ab</sup>
B3	121.67 <sup>fgh</sup>	50.60 <sup>ab</sup>	61.33 <sup>ab</sup>	26.73 <sup>ab</sup>	B33	143.77 <sup>abcdefg</sup>	65.48 <sup>ab</sup>	11.95 <sup>defghijkl</sup>	16.43 <sup>ab</sup>
B4	118.60 <sup>gh</sup>	54.60 <sup>ab</sup>	59.87 <sup>ab</sup>	31.70 <sup>ab</sup>	B34	149.43 <sup>ab</sup>	67.93 <sup>a</sup>	12.33 <sup>defghijk</sup>	22.70 <sup>ab</sup>
B5	137.13 <sup>abcdefg</sup>	60.77 <sup>ab</sup>	57.32 <sup>ab</sup>	17.13 <sup>ab</sup>	B35	159.79 <sup>a</sup>	62.60 <sup>ab</sup>	15.06 <sup>abcdef</sup>	18.27 <sup>ab</sup>
B6	139.03 <sup>abcdefg</sup>	57.50 <sup>ab</sup>	65.48 <sup>ab</sup>	15.80 <sup>ab</sup>	B36	145.47 <sup>abcdefg</sup>	60.67 <sup>ab</sup>	16.43 <sup>abc</sup>	18.07 <sup>ab</sup>
B7	150.37 <sup>abcde</sup>	61.67 <sup>ab</sup>	67.93 <sup>a</sup>	19.23 <sup>ab</sup>	B37	155.70 <sup>abc</sup>	60.77 <sup>ab</sup>	17.86 <sup>a</sup>	22.89 <sup>ab</sup>
B8	140.70 <sup>abcdefgh</sup>	56.43 <sup>ab</sup>	62.60 <sup>ab</sup>	18.63 <sup>ab</sup>	B38	150.97 <sup>abcde</sup>	56.82 <sup>ab</sup>	14.17 <sup>bcdefgh</sup>	17.52 <sup>ab</sup>
B9	121.90 <sup>fgh</sup>	49.93 <sup>ab</sup>	60.67 <sup>ab</sup>	22.17 <sup>ab</sup>	B39	120.19 <sup>fgh</sup>	51.28 <sup>ab</sup>	11.60 <sup>fghijklm</sup>	33.12 <sup>ab</sup>
B10	138.97 <sup>abcdefgh</sup>	64.70 <sup>ab</sup>	60.77 <sup>ab</sup>	21.17 <sup>ab</sup>	B40	114.36 <sup>h</sup>	50.99 <sup>ab</sup>	11.82 <sup>defghijklm</sup>	35.18 <sup>ab</sup>
B11	139.50 <sup>abcdefgh</sup>	56.53 <sup>ab</sup>	56.82 <sup>ab</sup>	31.17 <sup>ab</sup>	B41	154.21 <sup>abcd</sup>	65.58 <sup>ab</sup>	12.81 <sup>defghij</sup>	18.50 <sup>ab</sup>
B12	151.03 <sup>abcde</sup>	58.03 <sup>ab</sup>	51.28 <sup>ab</sup>	22.80 <sup>ab</sup>	B42	137.23 <sup>abcdefgh</sup>	60.70 <sup>ab</sup>	9.50 <sup>jklmnop</sup>	21.43 <sup>ab</sup>
B13	132.00 <sup>bcdefgh</sup>	52.40 <sup>ab</sup>	50.99 <sup>ab</sup>	26.00 <sup>ab</sup>	B43	151.17 <sup>abcde</sup>	63.03 <sup>ab</sup>	12.17 <sup>defghijk</sup>	21.00 <sup>ab</sup>
B14	119.00 <sup>gh</sup>	50.33 <sup>ab</sup>	65.58 <sup>ab</sup>	28.13 <sup>ab</sup>	B44	157.77 <sup>ab</sup>	68.47 <sup>a</sup>	12.03 <sup>defghijk</sup>	19.77 <sup>ab</sup>
B15	127.97 <sup>defgh</sup>	62.07 <sup>ab</sup>	60.70 <sup>ab</sup>	19.03 <sup>ab</sup>	B45	119.23 <sup>gh</sup>	52.32 <sup>ab</sup>	7.00 <sup>p</sup>	35.64 <sup>a</sup>
B16	115.88 <sup>h</sup>	49.84 <sup>ab</sup>	63.03 <sup>ab</sup>	31.77 <sup>ab</sup>	B46	119.79 <sup>gh</sup>	52.23 <sup>ab</sup>	7.76 <sup>op</sup>	32.39 <sup>ab</sup>
B17	139.07 <sup>abcdefgh</sup>	57.16 <sup>ab</sup>	68.47 <sup>a</sup>	13.13 <sup>ab</sup>	B49	134.93 <sup>abcdefgh</sup>	63.17 <sup>ab</sup>	8.47 <sup>mnop</sup>	18.28 <sup>ab</sup>
B18	145.70 <sup>abcdefg</sup>	61.31 <sup>ab</sup>	52.32 <sup>ab</sup>	15.15 <sup>ab</sup>	B50	131.54 <sup>bcdefgh</sup>	55.08 <sup>ab</sup>	12.63 <sup>defghijk</sup>	21.15 <sup>ab</sup>
B19	139.87 <sup>abcdefgh</sup>	62.91 <sup>ab</sup>	52.23 <sup>ab</sup>	9.41 <sup>ab</sup>	B51	129.03 <sup>cdefgh</sup>	57.85 <sup>ab</sup>	12.50 <sup>defghijk</sup>	22.53 <sup>ab</sup>
B21	144.00 <sup>abcdefgh</sup>	64.20 <sup>ab</sup>	63.17 <sup>ab</sup>	8.37 <sup>b</sup>	B52	126.10 <sup>efgh</sup>	52.07 <sup>ab</sup>	12.33 <sup>defghijk</sup>	28.15 <sup>ab</sup>
B25	99.42 <sup>efgh</sup>	43.67 <sup>b</sup>	55.08 <sup>ab</sup>	17.32 <sup>ab</sup>	Ave	138.04	59.02	12.28	23.27
B27	136.37 <sup>abcdefgh</sup>	62.13 <sup>ab</sup>	57.85 <sup>ab</sup>	10.67 <sup>ab</sup>	Мах	159.79	68.47	17.86	35.64
B28	128.23 <sup>cdefgh</sup>	55.27 <sup>ab</sup>	52.07 <sup>ab</sup>	32.57 <sup>ab</sup>	Min	114.36	50.99	7.00	16.43
B29	114.63 <sup>h</sup>	52.80 <sup>ab</sup>	13.13 <sup>cdefghi</sup>	29.00 <sup>ab</sup>	CV(%)	10.97	9.36	21.28	26.15
B30	154.13 <sup>abcd</sup>	61.33 <sup>ab</sup>	12.67 <sup>defghij</sup>	17.80 <sup>ab</sup>	H'	3.09	3.09	3.07	3.06
B31	125.43 <sup>abcdefgh</sup>	59.87 <sup>ab</sup>	10.73 <sup>hijklmno</sup>	21.57 <sup>ab</sup>	-	-	-	-	-

PH, plant height(cm); SPL, spike length(cm); SNL, spike neck node length(cm); Ave, average; CV, coefficient of variation; H', genetic diversity index; different small letters indicate significant differences among different varieties at 0.05 level.

<b>Table 2.</b> Ché	inges in agronomic trait	s of seed in 2020-2022							
No.	SL (mm)	SW (mm)	SSA (mm²)	TGW (g)	No.	SL (mm)	SW (mm)	SSA (mm²)	TGW (g)
B1	7.54 <sup>klm</sup>	2.98 <sup>abcdefgh</sup>	23.58 <sup>cdefghijk</sup>	40.16 <sup>cdefghi</sup>	B32	9.97 <sup>abc</sup>	2.83 <sup>defgh</sup>	25.39 <sup>abcde</sup>	47.31 <sup>abcdefgh</sup>
B3	8.46 <sup>ghijk</sup>	3.33 <sup>abcdef</sup>	23.45 <sup>cdefghijk</sup>	50.59 <sup>abcdef</sup>	B33	7.94 <sup>ijkl</sup>	2.93 <sup>bcdefgh</sup>	18.59 <sup>qr</sup>	32.23 <sup>ghi</sup>
B4	8.09 <sup>hijkl</sup>	3.46 <sup>ab</sup>	22.35 <sup>ghijklmno</sup>	48.37 <sup>abcdefg</sup>	B34	7.72 <sup>jklm</sup>	2.94 <sup>bcdefgh</sup>	19.08 <sup>pqr</sup>	36.17 <sup>efghi</sup>
B5	9.59 <sup>abcde</sup>	3.24 <sup>abcdefg</sup>	27.02 <sup>ab</sup>	53.92 <sup>abcd</sup>	B35	10.07 <sup>a</sup>	3.11 <sup>abcdefgh</sup>	27.04 <sup>ab</sup>	58.10 <sup>ab</sup>
B6	9.68 <sup>abcde</sup>	2.99 <sup>abcdefgh</sup>	25.81 <sup>abcd</sup>	53.93 <sup>abcd</sup>	B36	9.50 <sup>abcdef</sup>	2.93 <sup>bcdefgh</sup>	24.26 <sup>bcdefghi</sup>	51.36 <sup>abcdef</sup>
B7	9.96 <sup>abc</sup>	3.12 <sup>abcdefgh</sup>	26.90 <sup>ab</sup>	57.40 <sup>ab</sup>	B37	9.50 <sup>abcdef</sup>	2.80 <sup>fgh</sup>	23.48 <sup>cdefghijk</sup>	47.85 <sup>abcdefgh</sup>
B8	9.54 <sup>abcdef</sup>	3.09 <sup>abcdefgh</sup>	24.90 <sup>abcdefg</sup>	55.05 <sup>abcd</sup>	B38	9.34 <sup>abcdefg</sup>	3.01 <sup>abcdefgh</sup>	23.44 <sup>cdefghijk</sup>	52.46 <sup>abcde</sup>
B9	8.46 <sup>ghijk</sup>	3.28 <sup>abcdefg</sup>	22.54 <sup>efghijklmn</sup>	46.60 <sup>abcdef</sup> ghi	B39	8.53 <sup>ghijk</sup>	3.08 <sup>abcdefgh</sup>	21.02 <sup>jklmnopq</sup>	42.11 <sup>bcdefghi</sup>
B10	9.78 <sup>abcd</sup>	3.47 <sup>a</sup>	27.51 <sup>a</sup>	63.32 <sup>a</sup>	B40	8.36 <sup>hijk</sup>	3.20 <sup>abcdefgh</sup>	23.61 <sup>cdefghijk</sup>	44.14 <sup>bcdefghi</sup>
B11	9.91 <sup>abc</sup>	3.11 <sup>abcdefgh</sup>	24.58 <sup>bcdefgh</sup>	55.89 <sup>abc</sup>	B41	8.38 <sup>ghijk</sup>	2.92 <sup>cdefgh</sup>	19.61 <sup>opq</sup>	41.96 <sup>bcdefghi</sup>
B12	9.80 <sup>abcd</sup>	3.06 <sup>abcdefgh</sup>	25.87 <sup>abcd</sup>	56.64 <sup>abc</sup>	B42	8.87 <sup>defghi</sup>	3.00 <sup>abcdefgh</sup>	21.08 <sup>jklmnopq</sup>	47.76 <sup>abcdefgh</sup>
B13	8.59 <sup>fghij</sup>	2.96 <sup>abcdefgh</sup>	21.46 <sup>ijklmnop</sup>	43.10 <sup>bcdefghi</sup>	B43	8.30 <sup>hijkl</sup>	2.91 <sup>defgh</sup>	19.78 <sup>nopq</sup>	42.19 <sup>bcdefghi</sup>
B14	8.33 <sup>hijkl</sup>	3.36 <sup>abcd</sup>	22.83 <sup>efghijklm</sup>	48.74 <sup>abcdefg</sup>	B44	8.14 <sup>hijkl</sup>	2.84 <sup>defgh</sup>	19.70 <sup>nopq</sup>	38.70 <sup>defghi</sup>
B15	8.79 <sup>efghi</sup>	3.05 <sup>abcdefgh</sup>	22.52 <sup>efghijklmn</sup>	47.15 <sup>abcdefghi</sup>	B45	7.70 <sup>jklm</sup>	3.36 <sup>abcde</sup>	20.74 <sup>klmnopq</sup>	45.67 <sup>abcdefghi</sup>
B16	8.47 <sup>ghijk</sup>	3.33 <sup>abcdef</sup>	23.13 <sup>defghijkl</sup>	50.55 <sup>abcdef</sup>	B46	7.75 <sup>jklm</sup>	3.37 <sup>abc</sup>	21.69 <sup>hijklmnop</sup>	45.02 <sup>bcdefghi</sup>
B17	9.76 <sup>abcde</sup>	2.99 <sup>abcdefgh</sup>	24.38 <sup>bcdefgh</sup>	51.60 <sup>abcde</sup>	B49	9.56 <sup>abcde</sup>	2.91 <sup>cdefgh</sup>	25.79 <sup>abcd</sup>	57.70 <sup>ab</sup>
B18	8.52 <sup>ghijkj</sup>	2.89 <sup>cdefgh</sup>	20.44 <sup>lmnopq</sup>	41.90 <sup>bcdefghi</sup>	B50	10.02 <sup>ab</sup>	3.06 <sup>abcdefgh</sup>	26.14 <sup>abc</sup>	58.26 <sup>ab</sup>
B19	9.07 <sup>bcdefgh</sup>	3.27 <sup>abcdefg</sup>	25.07 <sup>abcdefg</sup>	53.68 <sup>abcd</sup>	B51	9.96 <sup>abc</sup>	3.07 <sup>abcdefgh</sup>	25.36 <sup>abcdef</sup>	57.64 <sup>ab</sup>
B21	9.08 <sup>abcdefgh</sup>	3.13 <sup>abcdefgh</sup>	23.43 <sup>cdefghijk</sup>	51.91 <sup>abcde</sup>	B52	8.35 <sup>hijk</sup>	3.31 <sup>abcdef</sup>	22.24 <sup>ghijklmno</sup>	50.32 <sup>abcdef</sup>
B25	7.34 <sup>lm</sup>	2.77 <sup>gh</sup>	22.44 <sup>fghijklmno</sup>	31.23 <sup>hi</sup>	Average	8.70	3.02	22.20	45.94
B27	9.02 <sup>cd efgh</sup>	3.21 <sup>abcdefgh</sup>	23.73 <sup>cdefghij</sup>	50.21 <sup>abcdef</sup>	Мах	10.07	3.37	27.04	58.26
B28	8.32 <sup>hijk</sup>	2.77 <sup>gh</sup>	18.59 <sup>qr</sup>	30.37 <sup>i</sup>	Min	6.91	2.70	16.87	32.23
B29	8.29 <sup>hijkl</sup>	3.29 <sup>abcdefg</sup>	23.23 <sup>cdefghijkl</sup>	46.76 <sup>abcdefghi</sup>	CV(%)	10.37	6.30	12.50	17.61
B30	8.25 <sup>hijkl</sup>	2.70 <sup>h</sup>	20.17 <sup>mnopq</sup>	34.53 <sup>fghi</sup>	Ц	3.09	3.09	3.08	3.08
B31	6.91 <sup>m</sup>	2.82 <sup>efgh</sup>	16.87 <sup>r</sup>	32.35 <sup>ghi</sup>	-	I	I	I	I
SL, seed lengtl varieties at 0.0	1(mm); SW, seed width(mm 5 level.	; SSA, seed surface area(m	1m²); TGW, thousand grain	weight(g); Ave, average; C'	V, coefficient of variati	on; H', genetic diversity in	dex; different small letters	s indicate significant differer	ces among different

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promptly harvested by hand, and the seeds were dried in the sun and stored in a suitable, ventilated place.

#### Determination of phenotypic traits

After harvest, 10 intact plants were randomly taken from each material type, and the plant height (PH), spike length (SPL), spike neck node length (SNL) and tillers were measured with a tape measure and a scale.

After seed threshing, 200 seeds from each variety were randomly selected to measure the seed length (SL), seed width (SW) and seed surface area (SSA) using a Marvin analysis grain photoelectric analyser with five repetitions. From each sample, 200 seeds were randomly selected and weighed with an electronic balance of an accuracy of one thousandth and the measurement was converted to TGW.

#### Determination of grain quality

Intact Polish wheat seeds were crushed in a centrifugal grinder and filtered through a 0.25 mm filter, and the assay samples were mixed

thoroughly and stored in a cool place. Determination of sample moisture content (SMC) was performed using a portable rapid moisture tester with reference to GB/T 20264-2006. The SCPC was analysed using an automatic crude protein analyser (Tacator, Sweden) with reference to GB/T 5519-2008. The water absorption rate (SWAR) of the flour was determined using a flour quality meter (Brabender, Germany), and sample hardness index (SHI) was determined using a single grain property tester according to GB/T 21304-2007. Determination of the bulk density (SBD) of the sample was carried out using an HGT-100 tester according to GB/T5498-85. All indices were measured three times.

#### Genetic diversity index

The genetic diversity index was calculated as follows by referring to the method reported by Li and Nan (Li and Nan, 2019):

Genetic diversity index 
$$(H') = -\sum P_i \ln P_i$$

where  $P_i$  is the frequency of occurrence of the *i*-th level of a trait.

Table 3. Seed quality changes of different varieties in 2020-2022

No.	SMC (%)	SCPC (%)	SWAR (%)	SHI (%)	SBD (g/l)	No.	SMC (%)	SCPC (%)	SWAR (%)	SHI (%)	SBD (g/l)
B1	8.82 <sup>a</sup>	20.52 <sup>abcdef</sup>	50.78 <sup>a</sup>	62.21 <sup>ab</sup>	800.80 <sup>ab</sup>	B32	8.89 <sup>a</sup>	21.21 <sup>abcde</sup>	58.82 <sup>a</sup>	62.21 <sup>ab</sup>	786.47 <sup>abc</sup>
B3	8.42 <sup>a</sup>	21.16 <sup>abcde</sup>	59.65 <sup>a</sup>	65.73 <sup>ab</sup>	765.27 <sup>bc</sup>	B33	9.14 <sup>a</sup>	18.34 <sup>cdef</sup>	59.20 <sup>a</sup>	68.66 <sup>ab</sup>	775.30 <sup>abc</sup>
B4	8.43 <sup>a</sup>	18.88 <sup>bcdef</sup>	58.87 <sup>a</sup>	63.46 <sup>ab</sup>	754.63c	B34	9.24 <sup>a</sup>	17.17 <sup>f</sup>	57.54 <sup>a</sup>	72.30 <sup>ab</sup>	796.46 <sup>abc</sup>
B5	8.74 <sup>a</sup>	20.41 <sup>abcdef</sup>	60.51ª	65.69 <sup>ab</sup>	784.61 <sup>ab</sup>	B35	8.91 <sup>a</sup>	21.41 <sup>abcd</sup>	60.20 <sup>a</sup>	64.24 <sup>ab</sup>	786.08 <sup>abc</sup>
B6	9.05 <sup>a</sup>	20.10 <sup>abcdef</sup>	60.81 <sup>a</sup>	68.55 <sup>ab</sup>	789.32 <sup>ab</sup>	B36	8.88 <sup>a</sup>	20.22 <sup>abcdef</sup>	60.75 <sup>a</sup>	66.68 <sup>ab</sup>	790.93 <sup>abc</sup>
B7	8.74 <sup>a</sup>	21.64 <sup>abc</sup>	60.06 <sup>a</sup>	64.79 <sup>ab</sup>	794.12 <sup>abc</sup>	B37	8.91 <sup>a</sup>	21.60 <sup>abcd</sup>	60.14 <sup>a</sup>	67.40 <sup>ab</sup>	794.07 <sup>abc</sup>
B8	8.92 <sup>a</sup>	19.87 <sup>bcdef</sup>	60.75 <sup>a</sup>	86.73 <sup>a</sup>	796.62 <sup>abc</sup>	B38	9.05 <sup>a</sup>	20.38 <sup>abcdef</sup>	60.45 <sup>a</sup>	65.96 <sup>ab</sup>	793.66 <sup>abc</sup>
B9	8.46 <sup>a</sup>	21.09 <sup>abcde</sup>	56.98 <sup>a</sup>	60.0 <sup>0ab</sup>	774.62 <sup>abc</sup>	B39	8.52 <sup>a</sup>	22.15 <sup>ab</sup>	59.84 <sup>a</sup>	62.43 <sup>ab</sup>	761.77 <sup>bc</sup>
B10	8.94 <sup>a</sup>	19.92 <sup>bcdef</sup>	58.34 <sup>a</sup>	60.30 <sup>ab</sup>	796.71 <sup>abc</sup>	B40	8.99 <sup>a</sup>	22.02 <sup>abc</sup>	57.58 <sup>a</sup>	64.83 <sup>ab</sup>	777.07 <sup>abc</sup>
B11	8.83 <sup>a</sup>	19.87 <sup>bcdef</sup>	58.92a	63.83 <sup>ab</sup>	790.78 <sup>abc</sup>	B41	9.15 <sup>a</sup>	19.16 <sup>bcdef</sup>	60.85 <sup>a</sup>	65.20 <sup>ab</sup>	790.05 <sup>abc</sup>
B12	8.91 <sup>a</sup>	21.82 <sup>abc</sup>	59.20 <sup>a</sup>	62.68 <sup>ab</sup>	794.58 <sup>abc</sup>	B42	9.17 <sup>a</sup>	19.78 <sup>bcdef</sup>	59.87 <sup>a</sup>	63.09 <sup>ab</sup>	791.04 <sup>abc</sup>
B13	9.01 <sup>a</sup>	21.31 <sup>abcd</sup>	59.28 <sup>a</sup>	62.18 <sup>ab</sup>	787.21 <sup>abc</sup>	B43	9.24 <sup>a</sup>	19.04 <sup>bcdef</sup>	59.57 <sup>a</sup>	64.93 <sup>ab</sup>	789.97 <sup>abc</sup>
B14	8.54 <sup>a</sup>	21.57 <sup>abcd</sup>	59.13a	63.02 <sup>ab</sup>	764.08 <sup>bc</sup>	B44	9.08 <sup>a</sup>	19.30 <sup>bcdef</sup>	60.14 <sup>a</sup>	66.02 <sup>ab</sup>	772.77 <sup>abc</sup>
B15	8.89 <sup>a</sup>	21.15 <sup>abcde</sup>	61.39 <sup>a</sup>	63.11 <sup>ab</sup>	774.66 <sup>abc</sup>	B45	8.72 <sup>a</sup>	17.88 <sup>def</sup>	60.68 <sup>a</sup>	64.78 <sup>ab</sup>	754.38 <sup>c</sup>
B16	8.57 <sup>a</sup>	22.50 <sup>ab</sup>	58.91 <sup>a</sup>	62.89 <sup>ab</sup>	765.62 <sup>bc</sup>	B46	8.54 <sup>a</sup>	17.49 <sup>ef</sup>	60.73 <sup>a</sup>	65.53 <sup>ab</sup>	762.17 <sup>bc</sup>
B17	9.02 <sup>a</sup>	20.33 <sup>abcdef</sup>	59.17 <sup>a</sup>	64.66 <sup>ab</sup>	799.29 <sup>abc</sup>	B49	8.85 <sup>a</sup>	20.39 <sup>abcdef</sup>	59.83 <sup>a</sup>	62.41 <sup>ab</sup>	792.63a <sup>bc</sup>
B18	9.17 <sup>a</sup>	18.84 <sup>bcdef</sup>	59.51 <sup>a</sup>	64.17 <sup>ab</sup>	792.69a <sup>bc</sup>	B50	8.96 <sup>a</sup>	17.87 <sup>def</sup>	60.42 <sup>a</sup>	66.57 <sup>ab</sup>	794.09 <sup>abc</sup>
B19	8.59 <sup>a</sup>	19.50 <sup>bcdef</sup>	59.59 <sup>a</sup>	62.29 <sup>ab</sup>	775.73 <sup>abc</sup>	B51	9.01 <sup>a</sup>	17.22 <sup>f</sup>	60.26 <sup>a</sup>	66.27 <sup>ab</sup>	794.59 <sup>abc</sup>
B21	8.94 <sup>a</sup>	21.19 <sup>abcde</sup>	59.45 <sup>a</sup>	62.87 <sup>ab</sup>	789.30 <sup>abc</sup>	B52	8.79 <sup>a</sup>	20.48 <sup>abcdef</sup>	58.66 <sup>a</sup>	63.71 <sup>ab</sup>	778.10 <sup>abc</sup>
B25	9.27 <sup>a</sup>	22.31 <sup>ab</sup>	49.64 <sup>a</sup>	53.27 <sup>b</sup>	814.35 <sup>a</sup>	Ave	8.87	20.31	59.16	64.24	782.57
B27	8.84 <sup>a</sup>	21.72 <sup>abc</sup>	61.65 <sup>a</sup>	62.57 <sup>ab</sup>	768.61 <sup>abc</sup>	Max	9.27	23.72	61.83	86.73	814.35
B28	8.64 <sup>a</sup>	23.72 <sup>a</sup>	55.94 <sup>a</sup>	51.39 <sup>b</sup>	757.43 <sup>bc</sup>	Min	8.42	17.17	49.64	51.39	754.38
B29	8.48 <sup>a</sup>	21.42 <sup>abcd</sup>	59.22 <sup>a</sup>	63.26 <sup>ab</sup>	775.80 <sup>abc</sup>	CV (%)	2.67	7.37	3.91	7.66	1.80
B30	9.15 <sup>a</sup>	20.02 <sup>abcdef</sup>	57.96 <sup>a</sup>	58.40 <sup>b</sup>	774.07 <sup>abc</sup>	H′	3.78	3.78	3.78	3.78	3.78
B31	8.85 <sup>a</sup>	19.86 <sup>bcdef</sup>	61.83 <sup>a</sup>	65.08 <sup>ab</sup>	770.94 <sup>abc</sup>	-	-	-	-	-	-

SMC, seed moisture content (%); SCPC, seed crude protein content (%); SWAR, seed water absorption rate (%); SHI, seed hardness index (%); SBD, seed bulk density (g/l); Ave, average; CV, coefficient of variation; H', genetic diversity index; different small letters indicate significant differences among different varieties at 0.05 level.

The overall mean (*X*) and standard deviation (SD) of the study materials were calculated. Each trait was classified into 10 levels from level 1 [Xi < (X-2d)] to level 10 [Xi > (X + 2d)], with one level every 0.5 d. The relative frequency of each level was used to calculate the genetic diversity index.

#### Statistical analysis

The phenotypic data were summarized using Microsoft Excel and plotted using sigmaplot12.5. Significance analysis, correlation analysis, principal component analysis and cluster analysis (Euclidean distance class average method) of plant phenotypic morphology, grain phenotypic morphology and grain quality were performed using SPSS 21.0 software.

### **Results and analysis**

#### Plant phenotypic and grain characteristics and grain quality

Details of the plant phenotypes and morphologies are provided in Table 1. Overall, 57% of the plants exhibited a PH $\geq$ 134.93 cm, and B35 (PH of 159.79 cm) was 60.72% taller than B25. Fifty

per cent of plants had a SNL $\geq$ 57.77 cm, and compared to B25, the SNL of B44 was increased by 56.79%. The mean SPL was 12.34 cm, with 50% of the material exhibiting this SPL, but B37 showed an increase of 10.86 cm (maximum SPL of 17.86 cm) compared to B45 (minimum SPL of 7.00 cm). The mean tiller number for 40.91% of the material was 21.91, and B45 (35.64 tillers) produced 27.27 more than B21 (8.37 tillers). The effects of variety on PH, SL, SNL and tiller number were extremely significant. The year of growth affected only SNL and tiller number, and the interaction between these parameters was not significant (online Supplementary Tables S3–S6).

As shown in Table 2, 45.45% of the material exhibited a mean SL of 8.81 mm; B35 had the highest SL (10.07 mm), while B31 had the lowest (6.91 mm). The mean of seed width (SW) was 3.07 mm, with this value measured in 45.45% of the materials. Compared with B30 (2.7 mm), the B10 (3.47 mm) SW was significantly increased by 28.52%. The mean seed surface area (SSA) was 22.97 mm<sup>2</sup>, with 54.55% of the materials exhibiting this value, and compared to B31 (16.87 mm<sup>2</sup>), the SSA of B10 (27.51 mm<sup>2</sup>) was significantly increased by 63.07%. The TGW was  $\geq$ 47.57 g, as measured in 40.91% of the materials. Compared to B28 (30.37 g), the B10 (63.32 g) TGW was increased significantly by 32.95 g.



**Figure 1.** Correlation analysis between important traits in polish wheat. PH, plant height (cm); SPL, spike length (cm); SSNL, spike stem node length (cm); SL, seed length (mm); SW, seed width (mm); SSA, seed surface area (mm<sup>2</sup>); TGW, thousand grain weight (g); SMC, seed moisture content (%); SCPC, seed crude protein content (%); SWAR, seed water absorption rate (%); SHI, seed hardness index; SBD, seed bulk density (g/l); SD, standard deviation; CV, coefficient of variation (%); *H*<sup>'</sup>, genetic diversity index.\*Significant differences at 5% probability level; \*\*significant differences at 1% probability level.

The effect of variety on the grain phenotypic traits and TGW was significant, while the effect of year of growth was not significant. However, the interaction between variety and year of growth on the grain phenotypic traits and TGW was significant (online Supplementary Table S6).

As shown in Table 3, the mean SMC of 56.82% of the materials was >8.87%, and compared with B3 (8.42%), the SMC of B25 (9.27%) was significantly increased by 10.10%. The mean SCPC was 20.31%, as measured in 54.55% of the material, with a significant increase of 38.15% seen in the SCPC of B28 (23.72%) compared to B34 (17.17%). Overall, 68.18% of the materials had a SWAR  $\geq$ 59.16%, with a significant increase of 24.56% seen in B31 (61.83%) compared to B25 (49.64%). The material with a seed hardness index (SHI) ≥64.24% accounted for 47.73%, although the SHI of B28 (86.73%) was 68.77% greater than that of B8 (51.39%). In total, 56.82% of the material had a SBD  $\geq$ 782.57 g/l, with an increase of 7.95% seen in B45 (814.35 g/l) compared to B25 (754.38 g/l). The effect of year and variety on grain crude protein content was significant or extremely significant, but the interaction between year and variety was not significant. The effects of variety and the interaction between variety and year with regard to grain moisture content, water absorption, hardness index and bulk density were extremely significant; however, the effect of year was not significant (online Supplementary Table S6). Following a comprehensive analysis of the above, the coefficient of variation and genetic diversity of the 44 Polish wheat varieties in terms of plant phenotypic morphology, grain phenotypic morphology and grain quality traits were high.

# Relationship between phenotypic and grain characteristics and grain quality

As shown in Fig. 1, the TGW was highly significantly positively correlated with SL  $(0.82^{**})$ , SW  $(0.51^{**})$ , SSA  $(0.87^{**})$  and SWAR  $(0.41^{**})$ . The SMC was highly positively correlated with SBD  $(0.66^{**})$ , and the SWAR was highly positively correlated

with SHI (0.47\*\*). The SCPC of the seeds was highly significantly negatively correlated with the SHI  $(-0.45^{**})$ .

# Principal component analysis between plant phenotypic morphology, grain phenotypic morphology and grain quality

The indicators in Table 1 were standardized, and the data were subjected to principal component analysis to obtain the correlation matrix for each evaluation parameter of Polish wheat. A total of four principal components were obtained (online Supplementary Table S7). It can be seen that principal component 1 combined 30.91% of all information, mainly reflecting the characteristics of Polish wheat in terms of SMC, TGW, SNL, SL and PL. Principal component 2 combined 54.19% of all information, mainly reflecting the characteristics of Polish wheat in terms of SSA, SW and TGW. Principal component 3 combined 71.05% of all information, mainly reflecting the characteristics of Polish wheat in terms of SPL and SCPC. The characteristics of Polish wheat were reflected by 71.05% of all the information in principal component 3, mainly from SPL and SCPC, and 80.55% of all the information in principal component 4, mainly from SL (online Supplementary Table S8).

As shown in Table 4, the ratio of the eigenvalues corresponding to each principal component to the sum of the total eigenvalues of the extracted principal components was used as the weight. The scores for the first four main components of each material and the sum of the product of the corresponding weights were used in our assessment (online Supplementary Table S9, Supplementary Formula I). The top 10 varieties screened using our comprehensive evaluation were B35, B7, B12, B37, B10, B38, B8, B36, B6 and B17, among which B35, B7, B12, B37 and B10 had higher comprehensive evaluation indices, with *F*-values of 70.15, 69.73, 69.59, 68.74 and 68.54, respectively.

The clustering analysis of each index of the Polish wheat test materials was performed using the Euclidean distance class average method (Fig. 2). The results showed that at the level of Euclidean genetic distance (GD) 10, the 44 Polish wheat varieties

 Table 4. Comprehensive evaluation results between different polish wheat materials

No.	Fs	Rank	No.	Fs	Rank	No.	Fs	Rank
B1	64.03	30	B17	67.85	10	B37	68.74	4
B3	63.88	31	B18	65.77	22	B38	68.37	6
B4	61.63	40	B19	66.35	18	B39	62.05	38
B5	66.95	17	B21	67.69	11	B40	62.78	37
B6	68.00	9	B25	61.69	39	B41	66.33	19
B7	69.73	2	B27	65.87	20	B42	65.46	23
B8	68.34	7	B28	61.10	43	B43	65.87	21
B9	63.82	32	B29	63.40	33	B44	65.19	24
B10	68.55	5	B30	64.59	26	B45	60.76	44
B11	67.51	12	B31	61.19	42	B46	61.43	41
B12	69.59	3	B32	67.15	15	B49	67.31	14
B13	64.45	28	B33	63.30	36	B50	67.46	13
B14	63.33	35	B34	64.98	25	B51	67.01	16
B15	64.46	27	B35	70.15	1	B52	64.45	29
B16	63.39	34	B36	68.16	8	-	-	-

S, synthesis;  $F_n$  (n = 1, 2, 3, 4, ... s).



Figure 2. Cluster analysis of polish wheat materials in phenotypic morphology, grain phenotypic morphology and grain quality.

yielded three clusters: cluster I contained one variety each from Australia and Ukraine; cluster II contained 13 varieties from Ethiopia, one from Egypt and one from Portugal; cluster III contained 29 varieties, including five each from the USA and Portugal, three each from Iran and Hungary, two each from Ethiopia, Italy and Cyprus, one each from Egypt, Portugal, Jordan, UK, Germany, Argentina and Romania and two of unknown origin. The cluster analysis showed that the means of SPL, SMC, SCPC and SBD were relatively high in cluster I. The materials from Ethiopia accounted for a larger component of cluster II, and the mean values for tiller number and SW were both higher. The materials in cluster III were widely distributed, among which there was only one material from Ethiopia, and the mean values of PH, SNL, SL, SSA, TGW, SWAR and SHI were higher, but the values for the rest of the traits were relatively low. Therefore, when using Polish wheat varieties as parents in breeding, the materials should be selected from different clusters depending on the target traits (online Supplementary Table S10, Supplementary Formula II).

#### Discussion

At present, domestic and international research on Polish wheat traits is still focused mainly on the evaluation and identification of specific traits in a few materials (Peng, et al., 2018a, 2018b; Jiang et al., 2021), and there has been a lack of long-term comprehensive systematic evaluation in areas of specific application, which to some extent hinders the breeding use of Polish wheat under different ecological conditions. As a result, Polish wheat is not continuously and widely used as breeding material (Li, 2001; Cheng et al., 2020). This study was based on the statistical analysis of phenotypic data from 44 Polish wheat varieties obtained from 2020 to 2022 (3 years) on the Qinghai Plateau. We combined data analysis methods widely used in the classification of germplasm resources of various crops, such as correlation analysis, principal component analysis and cluster analysis, to comprehensively evaluate the performance of each trait and visually reflect the variation of different traits among factors according to the high and low eigenvalues. This avoided the one-sided judgement associated with the traditional use of a single indicator (Michał, et al., 2016) and thus provided a reliable basis for the screening of excellent Polish wheat materials.

Polish wheat has excellent characteristics, including TGW and SCPC, and is regarded as an ideal germplasm resource for the yield and quality improvement of common wheat (Wiwart et al., 2013; Bieńkowska et al., 2018). However, studies have shown that the performance of Polish wheat with high TGW and SCPC varies greatly under different ecological conditions. Dong et al. (2007) planted 58 Polish wheat varieties from 21 countries in Sichuan and conducted a phenotypic evaluation study for one growing season, which included eight agronomic trait phenotypes such as PH, SPL and TGW (Dong et al., 2007). The results showed that the PH in Sichuan was 101.67-171.33 cm (average: 146.39); the SPL was 8.00-20.33 cm (average: 12.33 cm); and the TGW was 12.00-52.00 g (average: 27.36 g). Compared with this study, the average PH in Sichuan was 11.46 cm higher than in Qinghai, the average SPL in Sichuan was 0.01 cm less than in Qinghai and the TGW in Sichuan was 20.21 g less than in Qinghai. The results of this study are consistent with the phenotypic variation pattern of important agronomic traits of wheat in Sichuan and Qinghai reported by Gao et al. (2013).

Related studies have also shown that Polish wheat can reach more than 60 g TGW (Wiwart *et al.*, 2013) and contain a SCPC of up to 18% (Dong and Zheng, 2000). The results of the present study showed that the average TGW of the 44 Polish wheat varieties grown in the eastern agricultural region of Qinghai was 47.57 g, and the material with the highest TGW showed a 3-year average of up to 63.32 g. The average SCPC of the seeds was 20.31%, and the material with the highest SCPC showed a 3-year average of up to 23.72%. The yield and quality levels were in general agreement with those reported previously. Thus, it seems that the special climatic conditions in Qinghai are suitable for optimizing the high TGW and SCPC traits of Polish wheat. This will certainly be beneficial to the utilization of the Polish varieties for wheat breeding on the Qinghai plateau and other regions with similar climates.

The principal component and cluster analyses in this study showed the 44 Polish wheat varieties to be grouped into three clusters. Five varieties (B35, B7, B12, B37 and B10) with the best multivariate scores and three varieties with higher TGW [B10 (63.32 g), B50 (58.26 g), B35 (58.1 g)] were included in cluster III. The four materials with higher SCPC were B28 (23.72%), B16 (22.50%), B39 (22.15%) and B40 (22.02%), from cluster II. For the improvement of yield traits such as TGW, we suggest the use of Polish wheat materials from cluster III. However, to improve qualities such as SCPC, we suggest the varieties from Ethiopia and Mediterranean coastal countries in cluster II. Therefore, clusters II and III should be regarded as suitable sources of excellent varieties of Polish wheat for use on the Qinghai plateau and areas with similar climatic conditions. It is also worth noting that different varieties of Polish wheat should be selected as breeding parents according to specific breeding objectives for the improvement of common wheat.

#### Conclusions

Clustering and principal component analyses were applied to classify and compare relevant indicators for a comprehensive evaluation of 44 Polish wheat varieties over 3 years on the Qinghai plateau. The findings revealed that five varieties with the best multivariate scores were B35 (70.15), B7 (69.73), B12 (69.59), B37 (68.74) and B10 (68.54). Different Polish wheat lines should be selected as parents for wheat breeding programmes according to the specific traits of interest for use in regions with similar climatic conditions to the Qinghai Plateau. To improve yield traits such as TGW, we suggest using Polish wheat materials from cluster III with high TGW. However, for the improvement of qualities such as SCPC, we suggest the selection of varieties from Ethiopia and Mediterranean coastal countries in cluster II.

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