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## Use of diploid and tetraploid hulled wheat genotypes for animal feeding

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**Abstract:** Einkorn and emmer wheat are still cultivated in the mountainous areas of Turkey. They are resistant to biotic and abiotic stress conditions and are routinely used as food stuffs due to reasonable dietary fiber rate and vitamin and micronutrient content. Therefore, the present study was conducted to determine the feed potential of einkorn and emmer wheat populations. A total of 17 hulled wheat populations collected from different provinces of Turkey were studied during the years 2012 and 2013. Experiments were carried out in randomized complete block design with 3 replications to investigate grain yield, harvest index, chemical composition, and gas and methane production of the grain. Grain yield varied between 2.477 and 4.617 t ha<sup>-1</sup>, harvest index between 27.55% and 36.03%, crude protein (CP) between 6.69% and 16.22%, neutral detergent fiber (NDF) between 25.99% and 31.99%, acid detergent fiber (ADF) between 14.35% and 19.05%, crude oil (CO) between 1.33% and 2.05%, crude ash (CA) between 4.23% and 5.22%, metabolizable energy (ME) between 2.58 and 2.25 Mcal kg<sup>-1</sup> DM, organic matter digestibility (OMD) between 65.59% and 72.73%, and gas production (GP) between 46.01 and 52.22 mL. While einkorn populations had higher ME, OMD, CP, and CO content values, emmer wheat populations had higher ADF and NDF rates. Moreover, there were significant variations in gas and methane production in all hulled wheat populations. Genotype-trait biplot analysis clearly indicated the variation limits of NDF, ADF, CP, OMD, ME, methane, GP, CO, and CA of hulled wheat populations. There were also significant linear relationships between CP and ME (0.585) and OMD (0.613); between CO and GP (0.513); and between GP and ME (0.623) and OMD (0.696). It was concluded that with regard to higher yield and digestibility, these hulled wheat populations could be used as basic germplasm for breeding of new einkorn and emmer wheat cultivars to be used as alternative feedstuff.

**Key words:** Animal feeding, chemical composition, gas and methane production, genotype-trait biplot analysis, hulled wheat

### 1. Introduction

Wheat, including *Triticum* and *Aegilops*, belongs to the family Gramineae. *Triticum* is classified into 3 groups: diploid (2n = 14), tetraploid (2n = 28), and hexaploid (2n = 42) (Feldman et al., 1988). Turkey is known as the genetic diversity center of wild relatives of wheat. While the Middle East, Mediterranean, and West Asian gene centers have 22 species, Turkey by itself has 14 different species (Van Slageren, 1994).

The cultivation of Einkorn (*Triticum monococcum* L.) and emmer wheat (*Triticum dicoccum*) significantly decreased with the widespread use of high-yield durum (*Triticum durum* L.) and bread (*Triticum aestivum* L.) wheat cultivars. However, compared to bread and durum wheat, hulled wheat can easily be cultivated under adverse climate and soil conditions (Hammer and Perrino, 1984; Buerli, 2006; Mielke and Rodemann, 2007). Moreover, hulled wheat has strong tillering ability, resistance to drought and fungi, competitiveness to weeds, and higher

protein and amino acid content (Sharma et al., 1981; Rügger et al., 1990; D'Antuono, 1994; Schmid et al., 1994; Moudry, 1999; Cubadda and Marconi, 2002; Løje et al., 2003;).

Lopez et al. (2010) reported that antimethanogenic potential of feeds can be classified as low (>11% and ≤14%), medium (>6% and <11%) and high (>0% and <6%) based on methane percentage in total gas production (GP) after fermentation.

Genotype-trait biplot analysis is commonly used for graphical presentation of the data, which greatly enhances the ability to understand the patterns among the traits (Yan and Kang, 2003). The genotype-trait biplot describes the interrelationships among all traits on the basis of overall pattern of the data, whereas correlation coefficients only describe the relationships between 2 traits (Yan and Reid, 2008).

Hulled wheat studies have mainly concentrated on nutritive values for human beings instead of feed values for

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livestock. However, they are routinely used in human and livestock feeding. Therefore, the objective of the present study was to determine the feed quality parameters and yield potential of hulled wheat as an alternative feedstuff for animal feeding.

## 2. Materials and methods

### 2.1. Materials

General information about 17 hulled wheat populations collected from Kars, Kayseri, and Kastamonu provinces is summarized in Table 1. The study was carried out during the years 2012 and 2013 in the experimental fields of the Erciyes University Faculty of Agriculture in randomized complete block design with 3 replications. Plant material was sown in April at a sowing rate of 200 kg ha<sup>-1</sup> and harvested in July during 2 successive seasons, and 120 kg ha<sup>-1</sup> N and 100 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> were applied to the soil as a base fertilizer. Each plot had 6 rows that were 3 m long, with 14-cm row spacing. The research site has a slightly alkaline

and nonsaline sandy-loam soil texture with reasonable phosphorus and poor organic matter levels (FAO, 1990; TOVEP, 1991; Van Slageren, 1994). Physicochemical soil characteristics are provided in Table 2.

Climate parameters of the experimental site are summarized in Table 3. The research site has a long-term average precipitation of 163 mm (<http://www.mgm.gov.tr/>). The second year had lower precipitation than the first year. Average temperature of the experimental years and long-term averages were close to each other but relative humidity levels of the experimental seasons were a little lower than long-term averages.

### 2.2. Chemical composition analyses

Hulled wheat grains were milled through a 1-mm sieve and used for chemical analyses. Ash content of samples was determined at 550 °C for 8 h in an ash oven while oil content of the samples was analyzed by using an ether extraction method (AOAC, 1990). The Kjeldahl method was used for N content and the protein ratio was

**Table 1.** Collection site, botanical name, chromosome number, and genome formula of hulled wheat populations.

Population	Common name	Collection sites	Botanical name	Genome formula chromosome numbers
P1		Kayseri – Epce		
P2		Kayseri – Şahmelik		
P3		Kayseri – Ayşepınar		
P4		Kayseri – Ayşepınar		
P5	Emmer wheat	Kayseri – Şahmelik	<i>Triticum dicoccum</i>	2n = 28, AABB
P6		Kayseri – Kızılören		
P7		Kayseri – Epce		
P8		Kayseri – Gümüşören		
P9		Kayseri – Yenice		
P10		Kayseri – Tomarza		
P11		Kars		
P12		Kars		
P13		Kars		
P14	Einkorn	Kastamonu	<i>Triticum monococcum</i>	2n = 14, AA
P15		Kastamonu		
P16		Kars		
P17		Kastamonu		

**Table 2.** Physical and chemical characteristics of soils of the experimental site.

Year	Texture				pH	Org. Mat. %	CaCO <sub>3</sub> %	K <sub>2</sub> O kg ha <sup>-1</sup>	P <sub>2</sub> O <sub>5</sub> kg/ha <sup>-1</sup>	EC dS/m
	Clay %	Silt %	Sand %	Class						
2012	10.23	27.14	62.63	Sand-silt	7.54	0.62	1.09	1103.6	64.2	0.28
2013	10.40	13.94	75.66	Sand-silt	7.10	0.96	0.79	1264.3	117.3	0.24

**Table 3.** Precipitation, temperature, and moisture data of experimental site.

Months	Precipitation (mm)			Temperature (°C)			Moisture (%)		
	2012	2013	Long term*	2012	2013	Long term*	2012	2013	Long term*
April	5.6	32.2	57.4	13.6	11.6	10.6	43.6	60.3	63.0
May	50.6	23.4	54.4	20.7	16.7	14.9	49.2	52.9	61.1
June	34.2	18.8	39.4	19.2	19.8	19.1	43.5	46.8	55.8
July	0.2	0.0	11.8	27.6	21.5	22.6	76.9	43.8	50.4
Total	90.6	74.4	163.0						
Mean				17.4	17.4	16.8	53.3	50.95	57.58

\*From 1970 to 2011.

calculated by multiplying the N content by 5.7 (AOAC, 1990). Neutral detergent fiber (NDF) (Van Soest and Wine, 1967) and acid detergent fiber (ADF) (Van Soest, 1963) were determined using an ANKOM 200 Fiber Analyzer (ANKOM Technology Corp., USA).

### 2.3. In vitro gas and methane analyses

Ruminal liquids were sampled from 3 Ivesi sheep with fistulae for in vitro gas measurements. The sheep were fed a 60% alfalfa and 40% barley ration. Water and licking blocks were provided ad libitum and ruminal liquid samples were taken just before morning feeding. The liquid was rinsed through 4-layer cheese cloth and mixed with a 1:2 buffer solution. Ground samples of about 0.2 g were placed into a 100-mL syringe and then 30 mL of buffered ruminal liquid was added to the syringe. The syringes with the samples and ruminal liquids were then placed into a water bath set at 39 °C. Four additional syringes with only buffered ruminal liquid were also included in the incubation. The GP of those syringes was deducted from the GP of sample syringes to determine the net GP of each sample. Feed samples were incubated for 24 h and their GP was measured. The resultant gases were transferred to an infrared methane analyzer (Sensor Europe GmbH, Germany) with a plastic syringe and their methane productions were determined (Goel et al., 2008). The analyzer is able to measure the methane content (%) of the injected gas. The following formula was used to calculate methane production:

Methane production (mL) = total gas (mL) × methane content (%).

### 2.4. Metabolizable energy and organic matter digestibility

Metabolizable energy (ME) content and organic matter digestibility (OMD) of the feeds were calculated with the following formulae by using 24-h GP and chemical composition parameters (Menke and Steingass, 1988):

ME (Mcal kg<sup>-1</sup> DM) = (2.20 + 0.136 GP + 0.057 CP + 0.002859 CO) × 0.24,

OMD (%) = 14.88 + 0.889 GP + 0.45 CP + 0.0651 CA, where GP is 24-h gas production (mL), CP is crude protein (%), CO is crude oil (%), and CA is crude ash (%).

### 2.5. Statistical analysis

Variance and correlation analyses were performed over morphology, yield, and quality data using SAS software (SAS Institute, 1999) and then differences between mean values were tested by Duncan's multiple range tests. Additionally, in order to reveal the total variation through all traits investigated, principal component analysis was performed. Finally, all data were biplotted using Microsoft Excel to visualize the relationships among traits in all populations in the same chart (Lipkovich and Smith, 2002).

## 3. Results

Grain yield (GY) and chemical composition of hulled wheat populations are summarized in Table 4. Differences among wheat populations for all traits were significant at the  $P < 0.01$  level. GY of hulled wheat varied between 2.477 and 4.617 t ha<sup>-1</sup>, with the lowest value in P9 and the highest in P16. The lowest harvest index (HI) was observed in P7 with 27.55%, whereas the highest value was observed in P12 with 36.03%. CP content varied from 6.69% to 16.22%, with the highest value in P3 and the lowest in P5. NDF ratios varied between 5.99% in P9 and 31.99% in P17. Rates of ADF, one of the cell wall components of hulled wheat, varied from 14.35% to 19.05%, with the highest value in P15. CO content ranged from 1.33% to 2.05%, with the lowest value in P15 and the highest in P6. CA rates varied between 4.23% in P4 and 5.22% in P15.

GP, ME, and OMD parameters of hulled wheat are provided in Table 5. There was a great variation in terms of all these parameters among hulled wheat accessions. The highest GP was observed in the P2 and P12 populations, while the lowest production was observed in P17. Moreover, P11 and P17 produced the highest and

**Table 4.** Grain yield, harvest index, and chemical composition of hulled wheat populations.

Population	Grain yield (t ha <sup>-1</sup> )	Harvest index	Crude protein (%)	NDF (%)	ADF (%)	Crude oil (%)	Crude ash (%)
P1	3.048 hi	29.43 g	13.29 e	29.21 c	17.64 d	1.56 g	4.70 abc
P2	3.855 c	30.35 efg	11.62 h	26.69 ghi	16.01 h	1.87 c	4.55 bc
P3	2.973 i	31.30 cde	16.22 a	27.29 efg	16.89 e	1.81 d	4.46 bc
P4	3.187 gh	30.60 def	9.54 k	27.10 fgh	17.89 c	1.78 d	4.23 c
P5	3.015 i	28.51 h	6.69 n	29.65 c	16.91 e	1.79 d	5.23 a
P6	3.517 d	33.06 b	7.11 m	26.01 j	16.70 f	2.05 a	4.87 ab
P7	2.652 j	27.55 i	11.03 i	26.58 ij	16.40 g	1.69 e	4.79 abc
P8	2.643 j	33.29 b	7.13 m	27.63 ef	16.42 g	1.80 d	4.82 ab
P9	2.477 k	30.64 def	12.00 g	25.99 j	14.35 j	1.92 b	4.45 bc
P10	2.772 j	30.18 fg	9.72 j	27.05 fgh	16.03 h	1.81 d	4.32 bc
P11	3.395 de	29.91 fg	11.57 h	27.53 ef	16.08 h	1.79 d	4.36 bc
P12	4.068 b	36.03 a	7.30 l	27.72 e	15.53 i	1.69 e	4.67 abc
P13	3.253 fg	32.04 c	14.85 c	28.65 d	16.35 g	1.62 f	4.80 abc
P14	3.161 gh	30.49 ef	9.50 k	26.69 ghi	16.43 g	1.61 f	5.14 a
P15	3.093 hi	31.55 cd	15.99 b	30.55 b	19.05 a	1.33 i	5.22 a
P16	4.617 a	35.39 a	14.42 d	26.19 j	16.42 g	1.39 h	4.66 abc
P17	3.363 ef	31.25 cde	13.69 f	31.99 a	18.51 b	1.67 e	4.66 abc
Population	**	**	**	**	**	**	**
Year	**	**	**	**	**	**	**
pop × year	**	**	**	**	**	**	**
F values							
Population	146.10	52.44	13593.10	78.46	308.10	263.36	3.03
Year	19.39	1966.15	6903.34	110.61	4482.80	775.56	149.23
pop × year	81.02	16.49	765.46	62.41	268.05	52.71	3.99

NDF: neutral detergent fiber; ADF: acid detergent fiber; \*\*: P < 0.01. Different letters in the same column show significant difference.

lowest methane, respectively. Maximum ME values were observed in P3 with 2.58 while the lowest value was seen in P5 and P17 with 2.25 Mcal kg<sup>-1</sup> DM. OMD values ranged from 72.73% in P3 to 65.69% in P5, respectively.

Genotype-trait biplot graphing based on chemometric evaluation of hulled wheat populations showed that PCA1 and PCA2 accounted for 46% and 24% of variation, respectively (Figure). Totally, 70% of cumulative variation was explained by those 2 main components. Population and quantitative parameters were then biplotted on the same graph to visualize the relationships between populations

and these parameters by using 2 main components (Figure). The P15, P13, P16, and P17 populations were found to be prominent with their NDF, ADF, and methane percentages; P8, P14, and P15 with their CA values; P4, P6, P10, P9, and P12 with GP; and P1, P3, P11, and P12 with their GY, CP, ME, and OMD levels.

Linear correlations among quantitative parameters are summarized in Table 6. There were significant positive relationships between GY and HI; between GP and CO content; between GP and methane; between methane and ME and OMD; and between ME and OMD parameters.

**Table 5.** Gas production, net methane, methane percentage, ME, and OMD of hulled wheat populations.

Population	Gas production (mL)	CH <sub>4</sub> (mL)	CH <sub>4</sub> % of total gas	ME (Mcal kg <sup>-1</sup> DM)	OMD (%)
P1	51.59 ab	9.77 abc	19.11 cd	2.45 bc	71.32 ab
P2	52.01 a	9.91 abc	19.02 cd	2.46 b	71.22 ab
P3	51.57 ab	10.03 ab	19.39 bcd	2.58 a	72.73 a
P4	50.43 abc	9.86 abc	19.49 abcd	2.36 cdef	68.64 cd
P5	48.63 cd	9.57 bcd	19.66 abc	2.25 h	65.69 e
P6	50.67 abc	9.83 abc	19.37 bcd	2.32 fgh	67.87 d
P7	49.96 abc	9.50 bcd	18.98 cd	2.39 bcdef	69.24 bcd
P8	50.23 abc	9.57 bcd	19.03 cd	2.33 defgh	67.56 de
P9	50.52 abc	9.86 abc	19.48 abcd	2.46 b	71.07 ab
P10	49.38 bcd	9.65 abcd	19.48 abcd	2.33 efgh	67.63 de
P11	50.69 abc	10.12 a	19.82 ab	2.43 bcd	70.15 bc
P12	52.22 a	9.92 abc	18.88 d	2.38 bcdef	69.50 bcd
P13	49.04 cd	9.66 abcd	19.59 abcd	2.42 bcde	70.05 bc
P14	48.44 cd	9.80 abc	20.10 a	2.26 gh	67.44 de
P15	47.06 de	9.43 cd	19.20 ab	2.34 defg	69.25 bcd
P16	48.27 dc	9.68 abcd	19.96 ab	2.42 bcd	70.24 bc
P17	46.01 e	9.24 d	20.04 ab	2.25 h	65.74 e
Population	**	*	**	**	**
Year	**	**	**	**	**
Pop × year	**	**	NS	**	**
F values					
Population	5.44	1.95	3.72	9.24	8.58
Year	302.50	805.35	426.50	280.86	852.56
Pop × year	4.56	4.19	1.40	4.42	4.48

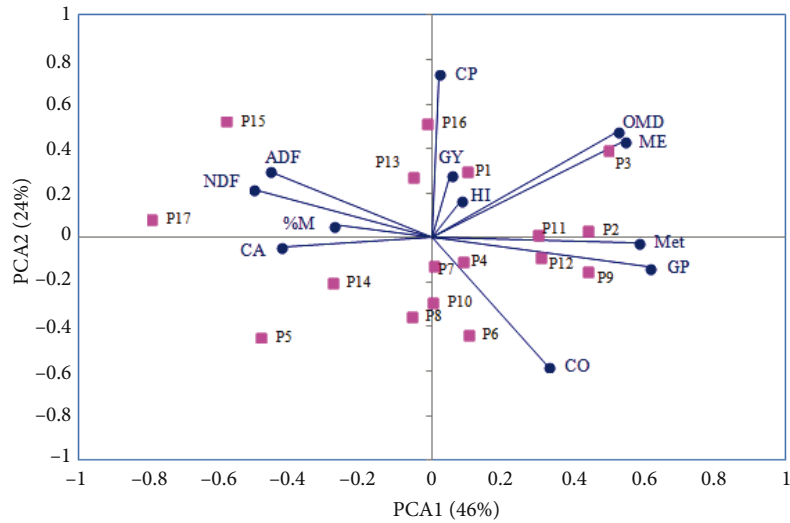
ME: Metabolizable energy (MJ/kg), OMD: organic matter digestibility (%), \*\*: P < 0.01; \*: P < 0.05; NS: nonsignificant. Different letters in the same column show significant difference.

There were also significant negative correlations between NDF and GP and methane; between ADF and CO, GP, and ME; between GP and methane; between CA and ME; and between GP and methane percentage (M%) parameters (Table 6).

#### 4. Discussion

Since precipitation of the experimental seasons significantly fluctuated, irrigations were performed to fulfill sufficient germination and emergence in both years. Moreover, the 50% decrease in precipitation (May and

June) and lower temperatures (April, May, and June) of the second year resulted in significant variations in grain yield and chemical compositions of the years. Additionally, genetic variations among the hulled wheat germplasm against heat and drought stress led to significant year × genotype interactions. Peterson (1992) showed that yield and quality of wheat were significantly affected by genotype, environment, and genotype × environment interaction. Other factors such as summer and winter sowing, amount and distribution of precipitation, plant density and tillering (Gebre-Mariam and Larter, 1979),



**Figure.** Genotype-trait biplot polygon of hulled wheat populations and quantitative traits based on 2 main principal components. GY: Grain yield; HI: harvest index; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; CO: crude oil; CA: crude ash; GP: gas production; Met: methane; %M: methane %; ME: metabolizable energy; OMD: organic matter digestibility.

**Table 6.** Relationships among quantitative parameters of hulled wheat germplasm.

	GY	HI	CP	NDF	ADF	CO	CA	GP	Met	M%	ME	OMD
GY	1											
HI	<b>0.641</b>	1										
CP	0.073	-0.041	1									
NDF	-0.085	-0.129	0.227	1								
ADF	0.014	-0.128	0.291	<b>0.714</b>	1							
CO	-0.294	-0.178	<b>-0.545</b>	-0.426	<b>-0.484</b>	1						
CA	-0.030	-0.019	-0.121	0.361	0.309	-0.377	1					
GP	0.025	0.052	-0.218	<b>-0.572</b>	<b>-0.526</b>	<b>0.513</b>	-0.451	1				
Met	0.191	0.104	-0.046	<b>-0.623</b>	<b>-0.520</b>	0.415	-0.482	<b>0.787</b>	1			
M%	0.175	-0.036	0.150	0.136	0.114	-0.162	0.035	<b>-0.622</b>	-0.099	1		
ME	0.064	0.061	<b>0.585</b>	-0.370	-0.310	0.050	<b>-0.517</b>	<b>0.623</b>	<b>0.595</b>	-0.343	1	
OMD	0.125	0.081	<b>0.613</b>	-0.383	-0.293	-0.064	-0.418	<b>0.608</b>	<b>0.616</b>	-0.345	<b>0.966</b>	1

In bold, significant values (except diagonal) at the level of significance of 0.050; GY: grain yield; HI: harvest index; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; CO: crude oil; CA: crude ash; GP: gas production; Met: methane; M%: methane %; ME: metabolizable energy; OMD: organic matter digestibility.

and grain development may also affect the grain yields of wheat genotypes (Akgün et al., 2007). Grain yield values of the present study were similar to those of Troccoli and Codianni (2005), Konvalina et al. (2011), and Shewry et al. (2013). There were great variations in GYs and HIs of

hulled wheat populations; such differences mainly come from various genetic backgrounds within the germplasm (Feil, 1992). Chemical compositions of the hulled wheat germplasm also varied significantly in this study. It was reported that hulled wheat contained more CP and CA but

less CO than common wheat (D'Egidio et al., 1993; Abdel-Aal et al., 1995; Løje et al., 2003). CP contents of the hulled wheat germplasm of the present study were quite similar to those of Abdel-Aal et al. (1995), Acquistucci et al. (1995), Wieser (2000), Grausgruber et al. (2004), and Stehno et al. (2010), but lower than that of Suchowilska et al. (2009). Cubadda and Marconi (1994) dehulled the genetic material of their experiment before analysis, and therefore they had higher CP ratios than the present study. The protein ratio is significantly affected by genetics and environmental factors such as soil, climate, and fertilizer application (Plant Breeding International, 1990). CA contents of the current study were lower than that of Abdel-Aal et al. (1995) and higher than that of Grausgruber et al. (2004). CO contents, on the other hand, were quite similar to those of Abdel-Aal et al. (1995), Piergiovanni et al. (1996), and Grausgruber et al. (2004), but lower than those of Suchowilska et al. (2009) and Pelillo et al. (2010). So far, there has not been any literature found on the NDF and ADF contents of hulled wheat. These 2 parameters are the natural part of the feeds, affect digestibility, and limit the consumption of feeds by animals. ADF and NDF content of hulled wheat, compared to other cereals, are similar to those of oat but higher than those of sorghum, barley, and maize. However, the CP content of hulled wheat is usually higher than that of the other cereals (NRC, 1996, 2001). There were great variations in the GP of hulled wheat populations. Higher NDF contents indicate lower GP rates; therefore, P17 had the lowest GP. When NDF content increases, the amount of carbohydrates decreases; consequently, the feed stuff of microorganisms sharply decreases. Blümmel and Ørskov (1993) demonstrated that the amount of gas after fermentation depends on the amount of digestible feedstuff in the rumen. That is why increasing the amount of fermentable carbohydrate of feedstuff results in higher

gas emissions. None of the hulled wheat populations had antimethanogenic effects, considering the ranges (from 18.88% to 20.10%) suggested by Lopez et al. (2010). The highest ME and OMD of the P3 population can be explained by its higher CP content. There is a positive correlation between ME and OMD and CP content; therefore, higher protein content resulted in increasing ME and OMD values.

Genotype-trait biplot graphing clearly demonstrated that CP, OMD, ME, GP, and CO were the main contributors to total variation by their larger vector lengths (Akçura, 2011). Thus, hulled wheat populations of P17, P15, P16, and P3 with the largest vector lengths respectively for NDF, ADF, CP, and ME can be preferred as parental material by plant breeders (Yan and Rajcan, 2002). Generally, it is expected that the highest variation in terms of any trait matches with the related population in the biplot graph, so this can contribute to the understanding of the relationship between the traits and the population (Yan and Kang, 2003). These close relationships were especially true for NDF, ADF, and ME of P15, P17, and P3. However, P14 deviated significantly from CP, since the biplot describes the interrelationships among all traits on the basis of overall pattern of the data (Yan and Reid, 2008).

In brief, the current results revealed that hulled wheat germplasm with reasonable GY even under dry conditions, higher OMD and CP and CO contents, and lower ADF and NDF and methane production can be a new source for animal feeding. Biplot analysis contributed to easily understanding the overall relationships between the traits and the populations. Population P16 can be nominated for grain yield, P1 and P3 for CP, P6 for CO, P9 for NDF and ADF, P9 for ME and OMD, and finally P17 for lower gas and methane production. Moreover, those populations can also be used in further breeding studies for einkorn and emmer wheat.

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