Research Article

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Influence of genotype and environment on field pea composition and milling traits

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Abstract

BACKGROUND: The rise in popularity of field peas (*Pisum sativum*) can be linked to their advantageous health and nutritional properties. Field pea seeds, yellow or green, are often consumed as an ingredient after being dehulled, split, and ground into flour. This study investigated the effects of genotype, growing location, and their interaction on milling of peas and on the chemical and physical characteristics of pea seeds by testing eight genotypes of yellow peas grown in four different locations.

RESULTS: The growing location influenced the contents of ash, fat, and protein in the seeds, measured by near-infrared reflectance spectroscopy. A positive correlation was observed between seed weight and surface area, evaluated by image analysis. Seeds were milled with an ultracentrifugal mill for measurement of dehulling and splitting efficiency (DSE), and quantification of coarse flour and fine flour yield. Positive correlations were observed between both DSE and coarse fraction and DSE and flour yield. Genotype and location affected DSE and coarse fraction, with a greater influence from the growing location. Fine flour yield was impacted by pea genotype. The milling traits had significant genotype × location interaction.

CONCLUSION: This study demonstrated that genotype and growing location influenced the milling of yellow peas and the fine flour yield. This information can assist breeding programs to select cultivars to achieve a more efficient milling and improve quality and use of yellow peas.

Keywords: pulse milling; pea; genotype by environment interaction; pulse flour

INTRODUCTION

The benefits of cultivating field peas (*Pisum sativum*), also known as dry peas, are present in the field and on the table. Pulses, such as field peas, can form a symbiotic relationship with *Rhizobium* bacteria and fix atmospheric nitrogen in the soil, thus improving soil fertility and reducing the need for fertilizers.[1] A fraction of legume production is used for animal feed;[2] and although an inexpensive and nutritious source of protein, starch, fiber, vitamins, and minerals in human diet,[3] field peas need to be processed before human consumption.

Milling is a way to process pulse seeds and allows for the creation of food products with value-added pulse flour.[4] The estimated food supply for pulse flour in the USA grew exponentially over the past few years, going from 4000 t in 2017 to 291 000 t in 2022.[5] Pulse flours have proven to effectively improve functionality and enhance the nutritional content of foods when used for partial replacement of wheat or in gluten-free formulations.[6] Therefore, the use of pulse flour as an ingredient is being driven by the interest of the food industry to develop nutritiously improved products for the consumers.[7] Pulses are milled into flour by removing the seed coat (dehulling), dividing the two cotyledons (splitting), and reducing to flour (milling), with dehulling and splitting usually done simultaneously.[8] The need for efficient processing technologies is an evident challenge for a broader utilization of pulses,[1] added to the negative economic impact of inefficient milling. A 'minimal' loss of 1% of the 2023 dry edible pea production in the USA would equate to more than $2.7 million.[9]

An understanding of whether genetic factors influence crop quality is essential for directing breeding efforts. Good understanding of the interaction between genotype (G) and growing location (i.e. environment, E) is necessary for breeders and growers to choose superior genotypes for specific locations.[10] Identifying which characteristics are modulated by the genetic components can aid in the development of varieties with improved performance and for specific applications. To improve the efficiency of pea milling, it is necessary to understand if G and E can impact the dehulling, splitting, and milling of the seeds.

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Identifying these G × E interactions is essential for accurate estimation of milling quality of pulses,[11] yet the role of genetics in regulating milling properties of pulses is still largely unknown.[12] Many G × E studies have been conducted with field peas, but with a focus on dry matter and seed yield,[13] chemical composition and physical properties,[14-19] nutrients and antinutrients content,[20] and flour and bread properties.[4] A study by Black *et al*[21] showed that a large variation in the dehulling quality of peas was observed across different cultivars and pretreatment solutions. Similarly, an investigation of the effect of G on the milling of peas concluded that wrinkled peas required more energy to mill than the smooth pea cultivars, and that texture analysis could be used to predict milling efficiency.[22] Milling settings were also concluded to impact the damaged starch content of yellow pea flour.[23]

An improved understanding of the milling performance of pulses allows breeding programs to make more informed decisions on cultivar development.[24] The impact of G and E on milling of pulses was previously reported for lentils[12,25,26] and chickpeas.[11] However, the same influences were not previously examined for field peas. Hence, the objective of this study is to evaluate the genetic and environmental effects and G × E interaction (GEI) on the milling of peas, as well as the effect of G, E, and GEI on pea physical characteristics and composition.

MATERIALS AND METHODS

Pea samples and plot management

Eight genotypes of field pea with yellow cotyledons were used in this study: cultivars 'AAC Carver', 'CDC Inca', 'Delta', 'DS Admiral', 'Early Star', 'Jetset', and advanced breeding lines 'PS17100008', 'PS17100022'. All genotypes were grown in four different locations in the USA: Dayton and Pullman (Washington), and Richland and Sidney (Montana). Average temperature and total precipitation for the growing months for the Washington locations were obtained from WSU AgWeatherNet (https://weather.wsu.edu). Weather data for Sidney were obtained from North Dakota Agricultural Weather Network (https://ndawn.ndsu.nodak.edu), and data for Richland were obtained from the National Oceanic & Atmospheric Administration (https://www.ncei.noaa.gov) for the closest reporting site. A randomized complete block was used as the experimental design, with four replications for the Montana locations and three replications for the Washington locations.

Prior to planting, seeds were treated with a slurry of fludioxonil (0.24 mL kg^{-1}), metalaxyl-M and -*S* isomer (0.13 mL kg^{-1}), thiabendazole (0.59 mL kg^{-1}), molybdenum (as sodium molybdate; 0.35 g kg^{-1}), and thiamethoxam (0.31 mL kg^{-1}). A seeding rate of 86 seeds/m^2 was used in the plots, with dimensions of 152.4 cm × 609.6 cm. Post-planting, pre-emergence herbicides metribuzin (210.5 g ha^{-1}) and linuron (3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea; 70.1 g ha^{-1}) were applied. After emergence, plots were trimmed to a dimension of 152.4 cm × 487.7 cm and maintained using conventional local techniques. At physiological maturity, each plot was harvested using a Zürn Plot Combine (Zürn Harvesting GmbH, Schöntal, Germany), and the seeds were further cleaned by hand. Seeds were maintained at 0 °C until processed.

Seed measurements and composition

A sample of 10 g of pea seeds was used for determination of 1000-seed weight (g), surface area (mm^2), and color components of L^*, a^*, and b^* with a Vibe QM3i Grain Analyzer (Vibe Imaging Analytics, Bnei-Brak, Israel). A Perten DA7250 NIR (PerkinElmer Inc., Shelton, CT, USA) was used to quantify moisture (g kg^{-1}, as is), protein, ash, and fat (g kg^{-1} of dry matter) of the seeds using near-infrared reflectance spectroscopy (NIRS). The measurements were obtained using the calibration for whole peas.

Dehulling, splitting, and milling

Pea samples were dehulled and split with a grain test mill (TM05C (2)-T; Satake Engineering Co., Hiroshima, Japan). The weight of the pea seeds was recorded before and after to quantify dehulling and splitting efficiency (DSE, %). Split and dehulled samples were then ground in a ZM 300 ultracentrifugal mill, equipped with a 24-teeth rotor with speed of 5534 × *g* and a 500 μm sieve (Retsch GmbH, Haan, Germany). The feed rate was approximately 7.5 g s^{-1}, and mill temperature was kept below 24 °C. The flour was then sifted through a 500 μm and a 210 μm sieve using a vibratory shaker (Great Western Mfg. Co., Leavenworth, KS, USA). Weight before and after sifting was used to determine milling loss and sifting loss. The milled sample with particle size larger than 210 μm was quantified as the coarse fraction (%). The fine flour yield (%) was determined as the ratio of the initial pea sample weight to the flour with particle size smaller than 210 μm. All the fractions were expressed as a percentage of the weight of the initial whole pea sample.

Data analysis

The pea samples were grown in four replicates in Sidney and Richland and three replicates in Pullman and Dayton, totaling 112 samples. Owing to crop loss or insufficient seed available, $n = 112$ for seed composition and physical measurements, but $n = 111$ for milling measurements.

The data points were analyzed with the PROC GLM procedure for three-way analysis of variance (ANOVA) using SAS Studio (SAS Institute, Cary, NC, USA), with main effects of replicate, G, and E. Fisher's least significant difference (LSD) was used for mean separation, with a significance level of 0.05. Pearson correlation was computed using R software.[27] The correlation between the variables studied and the design of the correlation heatmap were performed using Hmisc and corrplot packages respectively.[28,29]

RESULTS AND DISCUSSION

Seed physical attributes

The physical properties of pulses are generally used to assess conformity to standards and as quality attributes in market trading.[30] Seed characteristics can influence milling,[8] hence the importance of investigating weight and size of peas. Understanding the physical properties of the seeds is also required for designing equipment and facilities to process peas.[31]

The physical characteristics of the pea seeds obtained by image analysis had significant model *F* values for all traits according to the ANOVA (Table 1). The variation was well captured by the main effects of replication (Rep), G, and E, with R^2 values between 0.88 and 0.97. Rep was not a significant effect, and the absence of interactive effects between Rep, G, and E confirmed that the physical attributes of the field replicates were consistent.

Seed weight and surface area

The weight of the pea seeds can be used to predict key properties such as seed density, size, and milling yield.[32] G and E impacted seed weight (Table 1), in accordance with previously reported for field peas,[33] lentils,[25] and chickpeas.[11] The higher *F* value for

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Table 1. *F* values of analysis of variance on the effects of genotype and environment on the composition and physical characteristics of yellow peas

	Physical characteristics					Composition			
Source[a]	1000-seed weight	Area	L^*	a^*	b^*	Protein	Fat	Ash	Moisture
Rep	1.8	1.2	1.6	1.8	0.9	6.7**	0.9	0.7	3.1*
G	32.1***	31.3***	99.0***	11.9***	28.7***	42.3***	17.9*	2.6*	7.7***
E	319.6***	198.4***	0.4	21.0***	8.2**	139.0***	94.4***	16.2***	5.1*
G × E	2.3*	1.2	2.7*	7.0***	2.1*	4.9***	2.2	1.2	2.5*
Model R^2	0.97***	0.95***	0.95***	0.88***	0.88***	0.95***	0.91*	0.72*	0.81***

Note: No symbol represents not significant at $P < 0.05$.
[a] Source of variation: Rep, replication; G, genotype; E, environment.
*** $P < 0.0001$.
** $P > 0.0001$ but <0.001.
*$P > 0.001$ but <0.05.

E in comparison with G indicated that the greater variation in seed weight means occurred due to changes across the locations. Considering that weight is used for seed commercialization, breeders and growers can reckon this impact of environmental conditions on pea seed weight to adequately select growing locations. In this study, Sidney growing conditions produced the highest weight seeds (267 g per 1000 seeds), whereas the lowest weight seeds were produced in Pullman (195 g per 1000 seeds), with mean weight consistent with yellow pea seeds grown previously in the same location.[19] Overall, seeds from the Montana locations were denser than the peas from the Washington locations, which could be attributed to the higher precipitation in Sidney and Richland during the growing season (Table 4).

The size and uniformity of the seeds affect the dehulling of pulses.[34] Moreover, the use of seed size as a proxy for cooking quality was previously demonstrated for lentils.[35] In this study, the average surface area of the pea seeds was estimated by image analysis. G and E significantly affected the surface area of the yellow pea seeds, as previously reported,[33] with no significant interaction between the two effects (Table 1). Similar to seed weight, the higher *F* value for E indicates a more expressive effect of the growing conditions on the variation of seed area, meaning that breeders should take into consideration the environmental aspects when breeding for pea seed size. 'Early Star' (38.5 mm^2) and 'PS17100022' (45.1 mm^2) had the lowest and highest surface area (Table 2), with the irregular shape of 'PS17100022' explaining its large surface area (Fig. 1). Seed surface area varied significantly across all locations (Table 3); and similar to seed weight, seeds grown in the Montana locations had greater surface area than seeds from the Washington locations, potentially due to precipitation differences (Table 4). For all the growing months that data are available (Table 4), the total precipitation was higher in Sidney than in Pullman, likely explaining the 71.8 g and 8.4 mm^2 differences in mean seed weight and surface area between the two locations. Pea surface area and seed weight need to be considered by processors to design and/or identify equipment for pulse processing, and the impact of the growing conditions on seed physical characteristics is relevant for breeders when making selections for pea milling quality.

Color

The analysis of color, reported as values of L^*, a^*, and b^*, provides valuable information on the pulse seed color and its stability and is an important quality parameter for pulse consumers.[32,36]

Positive measurements of L^* indicate lightness, whereas measurements of a^* indicate redness if positive and greenness if negative. For b^*, positive measurements indicate yellowness and negative values indicate blueness. 'CDC Inca' had the darkest, most yellow seeds; at the same time, most of 'CDC Inca' seeds tested had low redness, evidenced by the lowest mean a^* among

Table 2. Mean values of physical characteristics and composition of yellow pea varieties

	Physical characteristics					Composition (g kg^{-1} dry matter)	
Genotype	1000-seed weight (g)	Surface area (mm^2)	L^*	a^*	b^*	Protein	Fat
AAC Carver	225.0 ± 22.8[d]	39.8 ± 2.7[ef]	72.1 ± 0.8[cd]	5.5 ± 0.7[bc]	19.3 ± 0.9[c]	214.4 ± 12.4[e]	17.3 ± 1.9[a]
CDC Inca	212.9 ± 28.8[e]	39.4 ± 3.1[fg]	68.5 ± 1.1[f]	4.6 ± 1.3[d]	21.8 ± 0.8[a]	236.2 ± 12.1[ab]	14.5 ± 2.1[d]
Delta	224.2 ± 31.3[e]	40.4 ± 3.9[de]	71.8 ± 0.8[d]	5.7 ± 0.5[ab]	19.2 ± 0.8[c]	231.6 ± 15.9[c]	15.8 ± 1.7[bc]
DS Admiral	233.8 ± 25.8[c]	41.0 ± 2.9[cd]	72.3 ± 0.9[c]	5.7 ± 0.8[bc]	17.9 ± 1.1[d]	225.6 ± 12.6[d]	15.3 ± 1.8[c]
Early Star	208.8 ± 23.3[d]	38.5 ± 3.0[g]	73.9 ± 0.8[b]	5.4 ± 0.3[c]	18.1 ± 0.6[d]	216.2 ± 12.0[e]	17.4 ± 2.2[a]
Jetset	242.3 ± 33.6[b]	41.9 ± 3.6[c]	70.8 ± 0.8[e]	5.6 ± 1.3[bc]	20.7 ± 1.1[b]	239.9 ± 8.3[a]	14.4 ± 1.7[d]
PS17100008	239.8 ± 34.3[bc]	43.0 ± 3.8[b]	73.8 ± 0.6[b]	6.1 ± 0.4[a]	18.2 ± 1.5[d]	223.6 ± 15.6[d]	16.4 ± 1.3[b]
PS17100022	253.8 ± 35.2[a]	45.1 ± 4.1[a]	75.2 ± 1.0[a]	5.6 ± 0.6[bc]	19.7 ± 1.2[c]	233.0 ± 14.3[bc]	15.8 ± 1.7[bc]
LSD	6.8	1.0	0.6	0.3	0.7	3.9	0.7

Note: Values express mean plus/minus standard deviation. Means with same letter within a column are not significantly different by the least significant difference (LSD) method ($P < 0.05$).

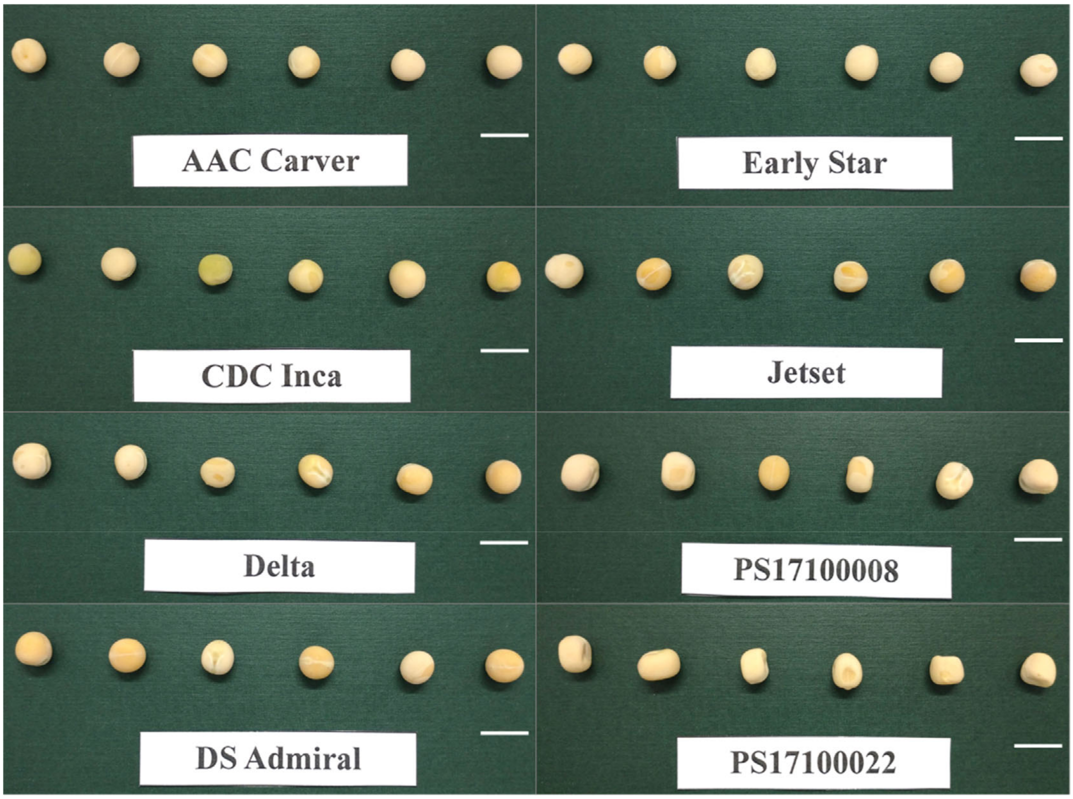

Figure 1. Yellow pea seeds from genotypes tested grown in the Richland location. Bar: 1 cm.

Table 3. Mean values of physical characteristics and composition of yellow pea across locations

	Physical characteristics					Composition (g kg^{-1} dry matter)		
Location	1000-seed weight (g)	Surface area (mm^2)	*L**	*a**	*b**	Protein	Fat	Ash
Dayton	211.7 ± 18.0^c	39.8 ± 2.7^c	72.2 ± 2.3^a	5.0 ± 1.3^c	19.7 ± 1.6^a	212.5 ± 10.8^d	16.9 ± 1.5^b	29.5 ± 0.8^c
Pullman	195.0 ± 14.1^d	37.0 ± 2.1^d	72.4 ± 2.3^a	5.3 ± 0.6^b	18.7 ± 1.2^b	223.4 ± 13.2^c	15.4 ± 1.4^c	30.0 ± 0.8^b
Richland	233.4 ± 14.3^b	40.9 ± 2.1^b	72.3 ± 2.1^a	6.0 ± 0.7^a	19.7 ± 2.0^a	241.1 ± 9.7^a	13.8 ± 1.4^d	30.7 ± 0.6^a
Sidney	266.8 ± 22.1^a	45.4 ± 2.7^a	72.3 ± 1.9^a	5.5 ± 0.6^b	19.2 ± 1.5^b	228.1 ± 12.4^b	17.4 ± 1.6^a	31.0 ± 0.8^a
LSD	4.9	0.7	0.4	0.2	0.5	2.8	0.5	0.4

Note: Values express mean plus/minus standard deviation. Means with same letter within a column are not significantly different by the least significant difference (LSD) method ($P < 0.05$).

Table 4. Environmental conditions at four experimental locations during the 2023 growing season for yellow peas

		Month				
Variable	Location	April	May	June	July	August
Average temperature (°C)	Dayton	8.2	16	18.2	23.3	22.4
	Pullman	6.8	15.3	16.7	20.7	20.3
	Richland	0.5	14.9	16.4	17.5	17.8
	Sidney	9.0	18.8	23.2	23.9	24.9
Total precipitation (mm)	Dayton	4.5	2.1	0.4	0.2	1.8
	Pullman	3.7	1.1	0.4	0.1	1.7
	Richland	1.8	4.0	5.7	6.0	2.2
	Sidney	0.8	8.8	3.5	3.0	7.0

the varieties (Fig. 1, Table 2). 'PS17100022' had the most bright seeds, with intermediate levels of yellow color. 'DS Admiral', 'PS17100008', and 'Delta' had the least yellow seeds. Overall, the color scores observed in this study were in agreement with 5- and 10-year mean values,[32] with slightly higher values of lightness and yellowness than the historical data.

A significant G effect for the lightness (L^*), greenness–redness (a^*), and blueness–yellowness (b^*) of pea seeds was observed (Table 1), meaning that breeders need to consider color when developing yellow pea genotypes. A significant effect of G on the lightness and greenness of yellow pea seeds was previously reported.[37] The growing conditions influenced the green color of the seeds, as observed by the significant GEI value, similar to a previous report.[37] Although smaller than the influence of G and E, breeders will need to be aware that there are interactive effects contributing to the greenness of yellow peas, and genotypes will have to be trialed in environments where they would ultimately be grown to determine if they will be of sufficient quality to processors and/or consumers.

Composition of the pea seeds

The quality of pulses is closely linked to their composition. Seed quality attributes can impact pulse milling, hence the importance of also investigating these parameters.[8] The levels of ash, fat, moisture, and protein of yellow pea seeds, as quantified by NIRS, had significant model F values and the variation was captured by the effects, with R^2 between 0.72 and 0.95 (Table 1). According to the ANOVA, G and E significantly affected all the composition variables investigated in this study, with a higher impact on the protein, fat, and ash content of the seeds arising from the growing conditions rather than the G effect. No Rep × G or Rep × E interactions were observed, except for Rep × E for protein content ($P < 0.05$), which suggests that variations in the field impacted the protein content of the pea seeds within locations. Similar significance of effects was reported previously by Wang *et al.*[33] for a study of six pea cultivars across five locations over 2 years.

Protein

The high F value reinforces that the location had a relevant contribution in the variation of pea seed protein (Table 1). Similar significant effects of G and E in the protein content of peas were previously reported.[17,18,33] A similar significant influence of G with higher effect of E on the protein content of wheat is known.[38] The pea genotypes presented different levels of protein under different growing conditions, evidenced by the highly significant GEI (Table 1), also similar to previous studies.[4,18,33] 'Jetset' (240 g kg^{-1}) and 'AAC Carver' (214 g kg^{-1}) were found to have the highest and lowest protein content. Seeds from plants grown in Richland contained more protein (241 g kg^{-1}) than seeds grown in the remaining locations (Table 3). This difference could be attributed to Richland having the lowest temperature and the highest total precipitation across the locations for most of the growing months (Table 4), combined with the high impact of environmental conditions in the protein concentration of the seeds. Additionally, the nitrogen present in the soil has an impact on the accumulation of nitrogen in the seeds,[39] which could lead to variations in protein levels. Although slightly higher than the mean protein levels observed in this study, a previous study also reported that the protein content of seeds harvested at Sidney was less than at Richland.[18]

Along with genetic factors, environmental elements can affect the protein content of pea seeds. Temperature, drought, soil characteristics, and type of cultivation (conventional *vs* organic) can influence the protein content of peas.[40] Mixed results were found in previous studies on the association between legume protein content and the exogenous factors of temperature and precipitation in different locations. Elevated temperatures and low rainfall were associated with high protein by Walter *et al.*,[40] whereas Tao *et al.*[41] found that the protein content of peas was positively affected by wetter conditions throughout the crop growth stages. Climate conditions, as well as growing region and year, had no effect on the protein content of chickpeas.[42] In this study, the small variation between the highest and lowest mean protein content across locations was considered insufficiently expressive to indicate a trend. Further investigations can be done in the future, with a deeper consideration of environmental factors, including but not limited to soil composition and previous crops.

Pulses are known as a significant source of protein,[43] hence the importance of protein content for pulse commercialization. Breeders should be aware of interactive effects between genotypes and environments causing variation in the protein content of yellow peas and direct breeding efforts to obtain the expected protein quantity required by processors and consumers.

Fat

Although both G and E had significant effects, the growing location had a higher impact on the fat content of the peas, evidenced by the higher F value, than genotype did (Table 1). Similar effects were previously reported.[20] Similar to a previous study, we observed no significant interaction between G × E for the variation of fat levels in the yellow peas.[17] However, a significant GEI affected the fat content of pea cultivars, measured gravimetrically by Wang and Daun.[20] A different combination of genotypes, locations, and number of replicates could probably be the source of the different effect of the interaction than the one found in this study. An average fat content of 16 g kg^{-1} was observed across all the yellow pea samples evaluated, intermediate between that reported for yellow peas in 2022 (13 g kg^{-1}) and the 5-year mean from 2018 to 2022 (21 g kg^{-1}).[32] The mean fat content of the yellow seeds differed across all locations, with Richland seeds having the lowest (Table 3).

Ash

Both G and E impacted the ash content of harvested seeds. The growing location had a greater effect on the variation of the ash levels than the genetic background did, in contrast to previous studies that reported no significant effect of environment on the ash.[17,20] Small differences in the mean ash content across the environments studied ranged from 29 g kg^{-1} (Dayton) to 31 g kg^{-1} (Sidney and Richland) (Table 3). Although statistically significant, the difference in ash content among genotypes ranged from 30.0 g kg^{-1} ('AAC Carver') to 30.8 g kg^{-1} ('CDC Inca') and was not biologically impactful (data not shown). Ash indicates the presence of minerals such as potassium and phosphorus in pea seeds.[44] However, the level of phytic acid, which is an antinutritional factor, is positively correlated with ash content in peas.[33] Therefore, pea breeders need to take into consideration the impact of the environmental factors on the ash levels of yellow peas.

Milling variables

Pulse milling is necessary to remove the seed coat and produce flour with a small particle size, allowing good miscibility with other ingredients.[24] The milling variables studied had significant model *F* values, with variation well captured by the effects of DSE, percentage of coarse fraction, and flour yield (R^2 between 0.72 and 0.83; Table 5). Replication and its interactions with G and E were not significant for the milling variables studied. The 0.62% total mean for milling loss, with $LSD_{(0.05)}$ of 0.2% across varieties and 0.1% across locations, was not substantial and hence not presented. The same was true for sifting loss, with a total mean of 1.1% and 0.1% $LSD_{(0.05)}$ for locations and varieties.

DSE

In this study, dehulling (seed coat removal) and splitting were performed simultaneously. Dehulling efficiency impacts pulse flour yield, and a high loss of mass with the pea hulls is not economically advantageous to the processors.[21,45,46] The DSE measurement was used to express this quality attribute, and was significantly affected by G and E, with a higher effect from the latter (Table 5). A similar significant effect of G on the variance of dehulling efficiency of 61 cultivars of field peas was previously reported by Black *et al*.[45] The dehulling efficiency of desi-type chickpea was also significantly affected by cultivar and location.[11] A significant GEI affected the DSE of the yellow pea seeds, but with lower effect than G and E (Table 5). For lentils, however, the interaction between G and E was not significant for dehulling efficiency.[25]

A wider range of DSE values was observed across genotypes than environments (Table 6). Similar behavior was previously reported for lentil dehulling efficiency.[25,26] Only 'DS Admiral', 'Early Star', and 'PS17100022' were significantly different from the highest DSE cultivar, 'Jetset' (Table 6). The least efficient dehulling and splitting was observed for 'PS17100022' and could have been caused by its irregular shape (Fig. 1). The total DSE mean for the entries was 76.2%, comparable to the average 79.3% dehulling efficiency reported by Black *et al*.[21] on the study of 23 field pea genotypes. Seed dehulling and splitting of the pea seeds are of major importance for pea processing. Awareness of the influence of genetic, environmental, and interaction factors on the efficiency of dehulling and splitting of peas can be useful to breeders for improvement of pea milling quality.

Coarse fraction

After dehulling, the split pea seeds were milled using an ultracentrifugal mill. The variation in the coarse fraction of milled yellow peas was well captured by the model, with R^2 of 0.87 (Table 5). G and E had highly significant effects on the variation of coarse fraction, with a higher impact from E. An average of 22.5% coarse fraction was calculated with all the samples milled. The variation of means for coarse fraction presented LSD lower than 1% among locations and varieties. 'AAC Carver', 'CDC Inca', and 'Delta' had similar coarse fraction concentrations and were the top coarse fraction entries, but they also had higher levels of DSE (Table 6). 'PS17100022' had the lowest percentage of coarse fraction, but also the lowest DSE and flour yield. Pea samples harvested from Sidney exhibited higher coarse fraction than samples grown in the other locations did (Table 6). Seeds from Pulman and Dayton had the lowest means of coarse fraction.

It is important to consider that different groups reported the use of various mill types and configurations, which poses a challenge in comparing pulse milling effects.[24] Yellow pea flour with a moderate particle size distribution and acceptable levels of starch damage was previously obtained by Gu *et al*.[23] with the use of a ultracentrifugal mill. The same 500 μm screen size was used in this study, but different rotor speeds were used. In this study, however, flour was considered to be the milled portion with particle size below <210 μm, as a reference of the definition of wheat flour.[24,47] The milled pea fraction with particle size greater than 210 μm was quantified as the coarse fraction. Using a pin mill and an impact mill, followed by air classification, Pelgrom *et al*.[48] obtained a fine and a coarse fraction of yellow pea flour. Protein, fiber, oil, and ash were more prevalent in the fine fraction and less present in the coarse fraction.[48] Unfortunately, the percentage of each fraction in relation to the initial sample weight was not reported and, therefore, cannot be compared.

Table 5. *F* values of analysis of variance on the effects of genotype and environment on the milling of yellow pea seeds

Source	DSE	Coarse fraction (>210 μm)	Fine flour yield
Rep	1.7	1.2	2.0
G	10.3***	13.0***	8.9***
E	16.6***	34.5***	1.1
G × E	3.0**	2.8*	1.9*
Model R^2	0.83***	0.87***	0.75*

Note: No symbol represents not significant at $P < 0.05$.
Abbreviations: DSE, dehulling and splitting efficiency; Rep, replication; G, genotype; E, environment.
*** $P < 0.0001$.
** $P > 0.0001$ but <0.001.
*$P > 0.001$ but <0.05.

Table 6. Mean values of milling attributes of yellow pea across varieties and locations

	Mean value		
	DSE (%)	Coarse fraction (%)	Fine flour yield (%)
Genotype			
AAC Carver	76.6 ± 4.0ab	24.3 ± 2.8a	50.6 ± 2.1de
CDC Inca	77.6 ± 2.1a	24.2 ± 1.3a	51.6 ± 1.3cd
Delta	77.5 ± 3.1a	23.5 ± 2.1ab	52.1 ± 1.8bc
DS Admiral	74.7 ± 2.4c	21.2 ± 1.1c	51.9 ± 1.7bc
Early Star	75.9 ± 1.9bc	21.4 ± 0.7c	52.8 ± 1.5ab
Jetset	77.9 ± 2.3a	22.9 ± 1.3b	53.3 ± 1.4a
PS17100008	76.6 ± 2.2ab	21.7 ± 2.2c	53.3 ± 1.3a
PS17100022	72.5 ± 2.4d	21.1 ± 1.9c	49.8 ± 1.6e
LSD	1.4	0.9	1.1
Location			
Dayton	74.8 ± 2.1c	21.4 ± 1.5c	51.4 ± 1.4b
Pullman	74.7 ± 2.5c	21.2 ± 1.6c	51.7 ± 1.9ab
Richland	76.5 ± 2.8b	22.9 ± 1.9b	51.9 ± 1.9ab
Sidney	77.9 ± 3.4a	24.0 ± 2.2a	52.3 ± 2.3a
LSD	1.0	0.6	0.8

Note: Values express mean plus/minus standard deviation. Means with same letter within a column are not significantly different by the least significant difference (LSD) method ($P < 0.05$).
Abbreviation: DSE, dehulling and splitting efficiency (%).

Fine flour yield

Pulses can be consumed in numerous ways after adequate processing.[49] The yellow peas were milled with an ultracentrifugal mill and the flour fraction with particle size <210 μm was used to compute the yield as a percentage of the initial weight of the whole pea sample. The fine flour yield was affected significantly by G (Table 5). No significant effect of E was detected. The interaction between G and E also affected flour yield, but at a lower level of significance. The total mean flour yield of 51.9% was similar to the previously reported 55.6% flour yield of Irish-grown peas with the use of a roller mill.[50] A higher flour yield of yellow peas ranging from 82.5 to 86.9% was previously reported, but for the milling of whole pea seeds, hence the higher yield.[51] With an ultracentrifugal mill using different speed and sieve apertures, Gu *et al.*[23] reported at least 92% flour yield. However, the whole milled fraction was considered for the calculation of the yield, in contrast to the milled fine fraction <210 μm used in this study.

The range for flour yield variation across genotypes was larger than across locations, similar to the other milling attributes investigated (Table 6). 'Jetset' and 'PS17100008' had the highest mean flour yield (Table 6). Although not significantly different, the slightly lower DSE for 'PS1710008' was offset by its lower mean coarse fraction in comparison with 'Jetset', which resulted in similar mean flour yield for both entries. The lowest means of flour yield were observed for 'AAC Carver' (50.6%) and 'PS17100022' (49.8%) (Table 6). Although in the highest level of DSE, 'AAC Carver' had the highest mean coarse fraction among the varieties, which contributed to its lower yield. 'PS17100022', however, had the lowest observed mean for coarse fraction, but its irregular shape (Fig. 1) is believed to have contributed to its lower DSE and, consequently, lower flour yield.

Pulse flour is an option of utilizing pulses to meet consumer nutritional needs.[52] To use pulse flour as an ingredient in product formulation, the seeds must be milled, highlighting the relevance of flour yield. Different particle size fractions have different functionality (e.g. water absorption), and, therefore, can be end-product specific.[34] The significant effect of the environmental conditions of the growing locations on the percentage of coarse fraction and the genotypic impact on the yield of fine flour can be used to breed and grow yellow peas for specific uses based on their particle size.

Correlation between pea characteristics

The Pearson correlation coefficients between the yellow pea attributes are shown in a heatmap with corresponding significance levels (Fig. 2). A high and positive correlation was found between 1000-seed weight and surface area. As expected, the larger the peas, the greater their weight, evidenced by high weight lines 'PS1710008' and 'PS17100022', which also had large surface area (Table 2). Conversely, entries with low seed weight, such as 'Early Star', also had the smallest observed surface area. As expected, a high correlation between surface area and seed weight confirms that breeders can rely on the weight of the seeds to identify larger seeds.

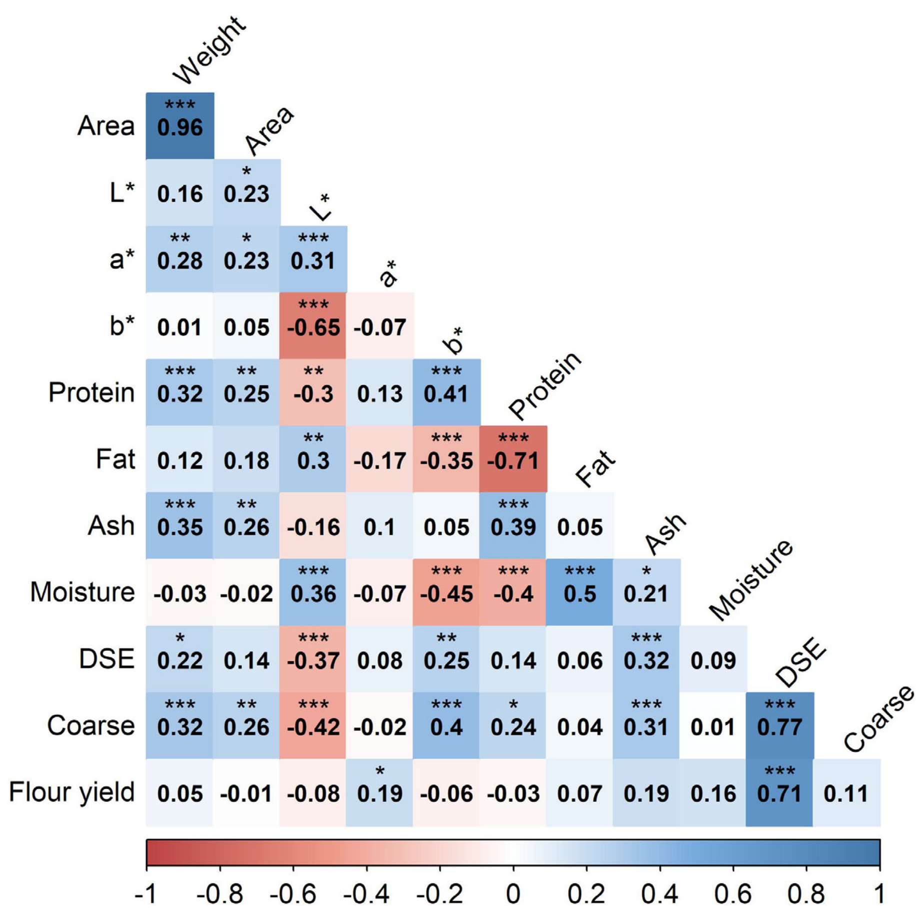

Figure 2. Pearson correlation coefficients for pea variables. Weight, 1000-seed weight; Area, seed surface area; DSE, dehulling and splitting efficiency; Coarse, coarse fraction. ***, $P < 0.0001$; **, $P > 0.0001$ but <0.001; *, $P > 0.001$ but <0.05. No symbol represents not significant at $P < 0.05$.

A positive correlation between ash content and seed weight was observed in this study. Correspondingly, a previous study found a positive correlation between the ash content and grain yield of peas.[18] With the positive correlation between weight and ash, selecting for a high seed weight could also mean selecting for high ash levels. The positive correlation between ash and phytic acid[33] could be a concern. However, most of the ash is in the seed coat and is usually removed in the dehulling step.

A strong, highly significant positive correlation was observed between DSE and flour yield (0.71, $P < 0.0001$). Intuitively, the higher the efficiency, especially in the dehulling and splitting step, the more split peas will be available to be milled into flour. High DSE was also positively correlated with coarse fractions; higher weight of dehulled samples implies that more samples will be milled and distributed to both fractions. 'Jetset' and 'PS17100008' had the highest mean flour yield, and both were in the highest mean level of DSE and middle levels of coarse percentage (Table 6).

Based on previous publications, it was expected that the surface area of the seeds would impact pea flour yield. However, no highly significant correlation was observed between fine flour yield and surface area. The lack of correlation between coarse fraction and fine flour yield and the positive strong correlation between fine flour yield and DSE indicated that selecting for good DSE is a way to achieve high fine flour yield. A strong negative correlation was observed between the fat and the protein content of the pea seeds, evidenced by 'AAC Carver' and 'Early Star', which had the highest observed levels of fat and the lowest levels of protein (Table 2). Similarly, 'CDC Inca' and 'Jetset' had high protein and low fat content. A strong negative correlation was found between protein and fat levels in chickpeas.[42] A positive, but lower correlation between fat and protein content in field peas was previously reported.[14]

Moisture was positively correlated with fat and negatively correlated with protein. Also highly significant, protein was positively correlated with ash in a moderate degree (0.39, $P < 0.0001$). In a previous study, Nikolopoulou *et al.*[14] studied three field pea cultivars across three locations over 2 years and also observed a positive correlation between protein and ash, but with a higher degree of correlation (0.77). Since our investigation found that G had a significant effect on the fat and ash content of the peas, the higher number of genotypes in our study could be the source of this difference. For color, a negative correlation (−0.65, $P < 0.0001$) was observed between L^* and b^*, indicating that samples with high levels of yellowness appeared darker. 'CDC Inca' was a good example, being the G with high yellowness mean, but also the darkest seeds (Table 2). A moderate positive correlation between the lightness (L^*) and the greenness–redness (a^*) of yellow peas was observed, whereas a previous study found no significant correlation between the two attributes.[37] Different locations and genotypes could be the cause for this disparity, considering that G influenced L^* and b^*, and the E had a greater impact on greenness than G did.

CONCLUSION

The effects of G and E on the milling of field peas and their correlation with seed characteristics were not previously reported. G significantly affected the dehulling and splitting of seeds and the flour yield of yellow peas. E influenced DSE and the coarse fraction of milled seeds, and a positive correlation between DSE and fine flour yield was observed. Significant interactions between G and E were observed for all the milling parameters, but as a lower source of variation than the main effects of G and E. These results indicate that breeding programs can select pea genotypes for better milling efficiency.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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