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RESEARCH ARTICLE

Characterization of yellow pea (*Pisum sativum* L.) genotypes for performance (agronomic and quality) and stability across environments

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Abstract

Background and Objectives: Yellow peas (*Pisum sativum* L.) are preferred for protein isolation. We characterized 21 yellow pea genotypes grown at Fairfield and Pullman, Washington in 2020 and 2021. To evaluate yellow pea genotypes for agronomic and quality traits across environments, data were collected on seed, flour, protein isolate, and starch-rich byproducts.

Findings: Seed yield and weight dropped by over 61% and 11%, respectively, in 2021 when compared to 2020. About 17%–20% of the flour was recovered as protein isolated with a protein recovery rate of 66%–69% and about 61%–64% was converted to starch-rich byproduct. Heritability was fairly high for seed weight (TSW) and protein isolate yield but low for seed yield and the functional properties. Some genotypes combined merits for multiple traits based on the degree of relationship between those traits. It was possible to identify genotypes with favorable combinations of seed yield with either seed weight or flour protein as these two traits exhibited weak correlation with seed yield. Owing to a positive correlation between flour protein concentration and PIYLD, two genotypes combined the best of the two traits.

Significance and Novelty: Overall, the findings of this study can be used to develop a tailored pea breeding program focused on plant-based protein. As starch-rich byproduct is the largest portion produced due to protein extraction, it would be beneficial to conduct more research on this component to facilitate product development.

KEYWORDS

functional properties, mean performance, protein isolate yield, protein isolation, purity, stability

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1 | INTRODUCTION

Pulses such as peas, chickpea, and lentils are important in human diets as they are sources of protein (Pradhan et al., 2019). Pea (*Pisum sativum* L.) is one of the first crops to be domesticated in the fertile crescent around 11,000 years ago (Abbo et al., 2017), and its uses have since expanded to the production of plant-based proteins with a growing market share (Boukid, 2021). Because of shifts in consumer choices based on health, ethical, religious, and environmental issues, food industries are focusing on pea proteins to replace proteins from animals, wheat, and soybean (Lam et al., 2018). Because the market dictates the target products of a breeding program, tailored pea breeding for plant-based protein production may be required. Productivity and quality are the key aspects required in pea cultivars when the end use is as plant-based proteins. The functional properties of proteins are also important in determining the application of plant proteins in the food industry. For example, protein solubility (PS) is important for beverages made of plant proteins (Lu et al., 2020). As a result, multiple traits must be evaluated in pea lines grown across multiple environments (location–year combinations) in what are known as multienvironment trials.

Yield and related traits are heavily influenced by plant development processes such as germination, vegetative growth, tillering or branching, flowering, and seed filling (Pradhan et al., 2019). Weather conditions that affect these processes can also have an impact on yield and quality. Plant breeders must address several breeding-related issues, such as genotype performance and adaptation to target environments, as well as the relationship between measured traits. Different statistical tools, such as GGE biplot (Yan et al., 2000), AMMI (Gauch, 1988), and WAASBY (Olivoto et al., 2019), can be used to assess the mean performance and stability of genotypes. The GGE biplot procedure is useful to address several breeding issues, including ranking of genotypes for performance and stability, mega-environment delineation, and identifying environments that are representative with good discriminative ability (Yan, 2002; Yan et al., 2000). Genotype-by-trait analysis (Yan & Rajcan, 2002), analogous to GGE biplot analysis, or a Pearson correlations coefficient analysis can be used to understand the relationships among traits. The USDA's grain legume breeding program at Pullman, Washington targets three market classes of pea (spring green, spring yellow, and wintergreen and yellow). As the color of the protein isolates is predominantly determined by the color of the cotyledons, yellow peas are preferred to produce protein isolates with yellow color. The yellow pea breeding program currently focuses on end-uses such as flour and split peas. Improvement for plant-based protein has yet to be done, so evaluating the performance of available yellow pea genotypes for protein isolate yield (PIYLD), protein purity, and functional properties is important. Proteins are typically assessed for a variety of technofunctional properties that affect their food applications, including PS, water and oil-holding capacity (OHC), emulsification, foaming, and gelation properties (Lam et al., 2018). For this study, we considered data from the advanced yield trials (AYTs) as well as data generated on seed, flour, protein isolates, and starch-rich byproducts. The objectives of this study were to: (1) evaluate the performance of yellow pea genotypes for agronomic and quality parameters, (2) assess the performance stability of genotypes across target environments in terms of yield, seed weight, and quality parameters, and (3) assess the interrelationship among the agronomic and quality traits.

2 | MATERIALS AND METHODS

2.1 | Genotypes, field trials, and agronomic traits

We used 21 yellow pea genotypes (Table 1) that were tested in AYTs in two locations in the US state of Washington, namely, Fairfield (47° 23′ 7′′ N/117° 10′ 18′′ W) and Pullman (46° 43′ 47′′ N/117° 10′ 54′′ W) during the 2020 and 2021 cropping seasons. Six of the entries (Carousel, DS Admiral, Delta, Universal, Kite, and Peregrine) were commercial cultivars and serve as checks. The field trials were laid out in randomized completed block design with three replications. Weather

TABLE 1 List of genotypes with their code and name as used in this study.

Code	Name	Code	Name
G041	Carousel	G124	PS16NZ0004
G042	Ds Admiral	G153	PS16100107
G043	Delta	G155	PS16NZ0005
G044	Kite	G156	PS17100008
G046	Peregrine	G157	PS17100022
G054	PS14100068	G158	PS17100046
G058	PS1410B0003	G160	PS17100137
G061	Universal	G162	PS17100236
G119	PS16100094	G163	PS17100239
G122	PS16100111	G164	PS17100240
G123	PS16NZ0003		

data for the test locations over the two seasons were accessed from AgWeatherNet (https://weather.wsu.edu).

Seed yield (YLD) was recorded using harvested seed on a plot-basis and converted to tons per hectare (t/ha). The 1000-seed weight (TSW, g) of each plot was determined using a Vibe QM3i Grain Analyzer (Vibe Imaging Analytics). A second set of seed weight data was generated as part of yield trial data by weighing 100 seeds (HSW, g).

2.2 | Protein extraction procedure

With a few minor modifications, the alkaline extraction and isoelectric precipitation (AE-IP) method (Boye et al. (2010) was used to prepare protein isolates. Initially, 33.33 g of pea flour was thoroughly dispersed in 500 mL of water, and the pH was adjusted to 8.5 using NaOH while stirring for 30 min. The supernatant was then recovered after the solution was centrifuged at 5000g for 20 min at 4°C. The precipitate was also recovered as a starch-rich byproduct. The pH of the supernatant was adjusted to 4.5 using HCl and left for protein to precipitate at room temperature for 30 min without stirring. Using a Labconco Freezone 4.5 L freeze drier (LECO Corporation), the protein isolates and the starch-rich byproducts were freeze-dried for 48 h and stored at 4°C until needed for further analysis.

2.3 | Measured quality parameters

The quality parameters recorded in duplicates on flour, protein isolate, and starch-rich byproduct samples were flour protein concentration (FLPC, %), PIYLD (%), protein isolate purity (PIPC, %), PS (%), water-holding capacity (WHC, g/g), OHC (g/g), foaming capacity (FC, %), foaming stability (FS, %), byproduct yield (%), and byproduct protein concentration (%). Samples from one replication for the advance breeding lines and from all three replications for the checks were used for quality analyses.

An FP828p protein analyzer (LECO Corporation) was used to measure the protein concentrations ($N \times 6.25$, %) in the flour, protein isolate, and byproduct samples. Weights of initial flour sample (FLWt) and protein isolate produced (PIWt) were recorded and used to calculate protein recovery rate (PRR, %) using the following formula.

$$\mathrm{PRR} = \left(\frac{\mathrm{PIWt} \times \mathrm{PIPC}}{\mathrm{FLWt} \times \mathrm{FLPC}}\right) \times 100.$$

PS was quantified using the procedure described by Cui et al. (2020). Initially, 0.2% (wt/vol) protein solution was prepared in water at pH of 7.0. The Bradford protein assay was applied to determine protein concentration in the supernatant. PS of the samples was calculated as the ratio of protein concentration in the supernatant to total protein concentration in the protein isolates multiplied by 100.

Water and OHC (g/g) of the protein isolates were determined according to the procedure described by Stone et al. (2015). These parameters measure the amount of water or oil retained in the protein isolate after dispersing 0.25 g isolate in 10 mL water or oil for 30 min while vortexing every 5 min, and then centrifuging at 1000g for 15 min.

The foaming capacity and stability was determined according to the procedure described by Cui et al. (2020). Initially, a 2.5% (wt/vol) was prepared using 0.01 M NaOH solution and homogenized using multi-prep homogenizer (PRO Scientific Inc.) with 10 mm saw-tooth probe head. Volumes were recorded before homogenizing (V_0), just after homogenizing (V_1), and 30 min after homogenizing (V_2). FC and FS were calculated using the following formulae.

$$\mathrm{FC} = (V_1 - V_0)/V_0.$$

$$\mathrm{FS} = (V_1 - V_2)/(V_1 - V_0).$$

2.4 | Data analysis

Means for the genotypes were generated for each environment using the "metan" package (Olivoto & Lúcio, 2020). Boxplots were created environment-wise for seed yield, seed weight, and flour protein content using ggplot2 (Wickham, 2016) and rstatix (Kassambara, 2021). Pearson correlation coefficients were calculated among the traits using the mean values for the four environments separately. Spearman rank correlation coefficients were estimated between ranks based on GGE biplot and mean performance per se at each environment. Broad-sense heritability estimates (H^2) were calculated as a ratio of genotypic variance (V_G) to total variance ($V_G + V_E + V_{GE}$).

The "metan" package was used to perform the GGE biplot analysis, which considers both genotypic (G) main and genotype-by-environment interaction (GEI) effects. Singular value decomposition was performed on the GGE matrix, yielding the model outlined by Yan (2002).

$$Y_{ij} = \mu + \beta_i + \sum_{i-1}^{k} \lambda_l \rho_i \eta_j + \varepsilon_{ij},$$

where Y_{ij} is the mean trait value of genotype i at environment j; μ is the overall mean of the genotypes across all the environments; β_i is the mean trait value at environment j; λ_l is the singular decomposition values for the lth principal component; ρ_i is eigenvector of genotype i in the lth principal component; η_j is eigenvector of environment j in the lth principal component; and ε_{ij} is the residual associated with the ith genotype at the jth environment.

GGE biplot enables visualization of the relationship between genotypes, environments, and their interaction using various types of biplots created from the first two principal components. The position of the test genotypes relative to the ideal genotype is shown in one of the GGE biplots called the genotype ranking biplot. This GGE biplot depicts the ideal genotype, which is considered to have the highest yields in all environments, as a small circle in the center with an arrow pointing to it. Based on their distance from the ideal genotype, genotypes are ranked for both performance and stability, with the closest ones being the best genotypes across environments and the further ones being the worst.

3 | RESULTS

3.1 | Heritability estimates for agronomic and quality traits

Heritability estimates indicate the relative contribution of genotypic variation to the total variation for a trait of interest. Traits such as TSW and PIYLD were found to have high heritability ($H^2 \geq 0.60$; Table 2). FLPCs and PIPC had moderate heritability. Seed yield (YLD) had a heritability estimate of 0.23. Relatively low heritability values (<0.25) were estimated for functional properties of protein (PS, OHC, FC, FS, and WHC), with the lowest recorded for OHC and PS ($H^2 = 0.06$).

TABLE 2 Borad-sense heritability estimates for the traits measured in field yield trials and laboratory quality analyses.

Trait	Heritability
Seed yield (YLD)	0.23
1000-seed weight (TSW)	0.61
Flour protein concentration (FLPC)	0.42
Protein isolate yield (PIYLD)	0.60
Protein isolate purity (PIPC)	0.45
Water-holding capacity (WHC)	0.09
Oil-holding capacity (OHC)	0.06
Protein solubility (PS)	0.06
Foaming capacity (FC)	0.22
Foaming stability (FS)	0.31

3.2 | Seed yield and weight, and protein concentration of yellow pea genotypes

The 2020 season was favorable, with high precipitation and relative humidity but relatively low maximum temperature and evapotranspiration (Figure 1). On the other hand, the 2021 season was unfavorable, with very high temperature and extremely low precipitation. The difference in weather conditions between seasons was reflected in differences in seed yield and weight (Figure 2 and Supporting Information: Table S1). Pullman and Fairfield had mean seed yields of 5.6 and 5.0 t/ha in 2020 and 2.2 and 1.5 t/ha in 2021, respectively. Overall, there was a 61%–69% seed yield decrease in 2021 compared to 2020. In terms of seed weight, there was a decrease in 2021 compared to 2020 by 11%–15% (Supporting Information: Table S1). Average seed weight was 224.8 and 229.7 g in 2020 at Fairfield and Pullman, respectively; whereas it was 200.6 and 195.4 g in 2021 at Fairfield and Pullman, respectively.

Interestingly, pea samples from 2021 (adverse season) had higher FLPC than samples from 2020 (favorable season), particularly at Pullman (Supporting Information: Table S1). Fairfield outperformed Pullman in seed yield and FLPC in both years. Relatively higher variability was found among genotypes for TSW (CV = 5.4%–7.2%) and YLD (CV = 5.5%–7.0%) as compared to PIYLD (CV = 3.3%–4.0%) and FLPC (CV = 2.6%–5.9%) (Supporting Information: Table S1).

Two measurements of seed weight were made: one with a Vibe QM3i Grain Analyzer and the other manually, by weighing 100 seeds, and understanding the relationship between these two datasets is crucial. The correlation coefficient between the two sets of seed weight data was 0.86*** (data not shown), indicating that either method can be used to generate seed weight data. In the subsequent sections, however, seed weight refers to TSW predicted with the Vibe QM3i Grain Analyzer. The Vibe QM3i also predicts several seed-related measurements which we did not present in this paper. In all four environments, significant positive correlation coefficients (0.54*–0.58**) were found between FLPC and PIYLD (Table 3). Both FLPC and PIYLD were found to be negatively correlated with TSW, even if the correlation coefficients were nonsignificant in most of the cases. The correlation coefficients for seed yield (YLD) with TSW, FLPC, and PIYLD were also nonsignificant.

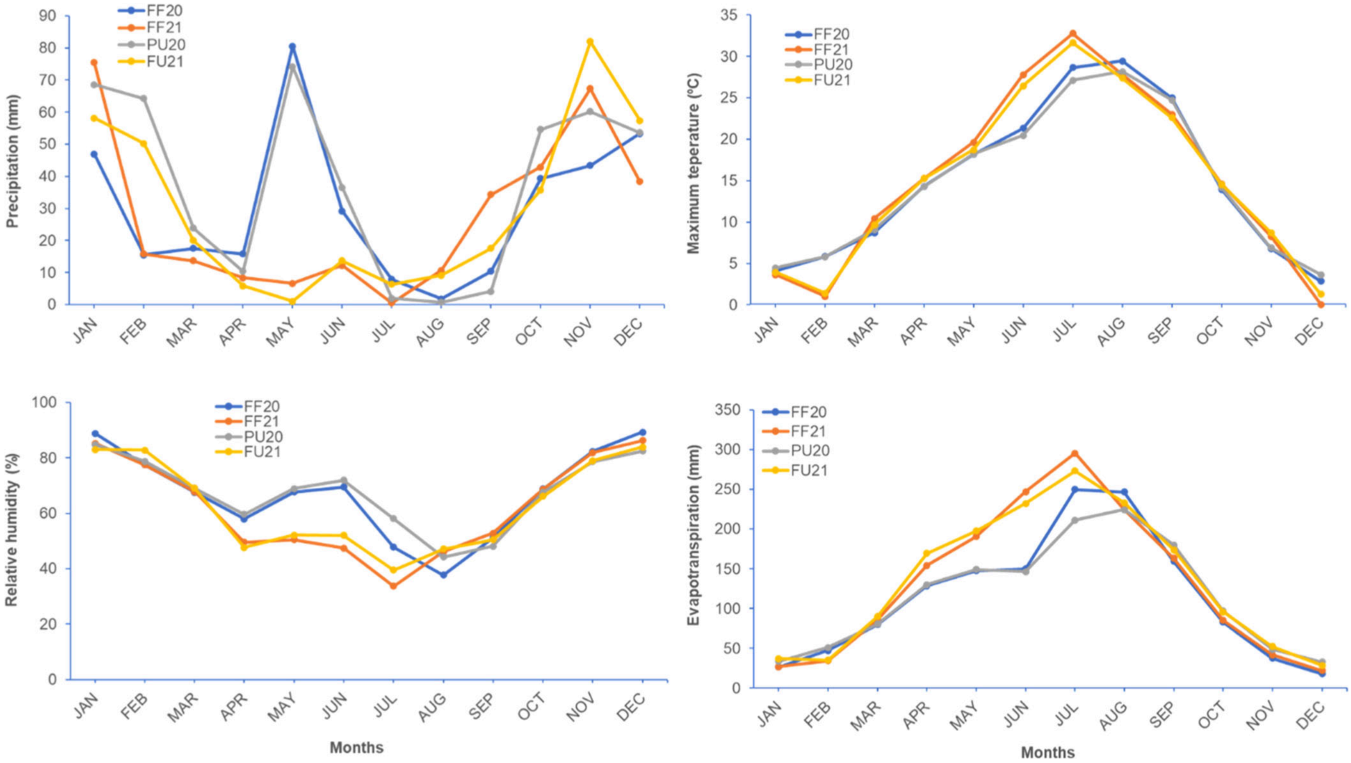

FIGURE 1 Monthly distribution of four weather parameters for the environments (location–year combinations: FF20 = Fairfield in 2020, FF21 = Fairfield in 2021, PU20 = Pullman in 2020, PU21 = Pullman in 2021). [Color figure can be viewed at wileyonlinelibrary.com]

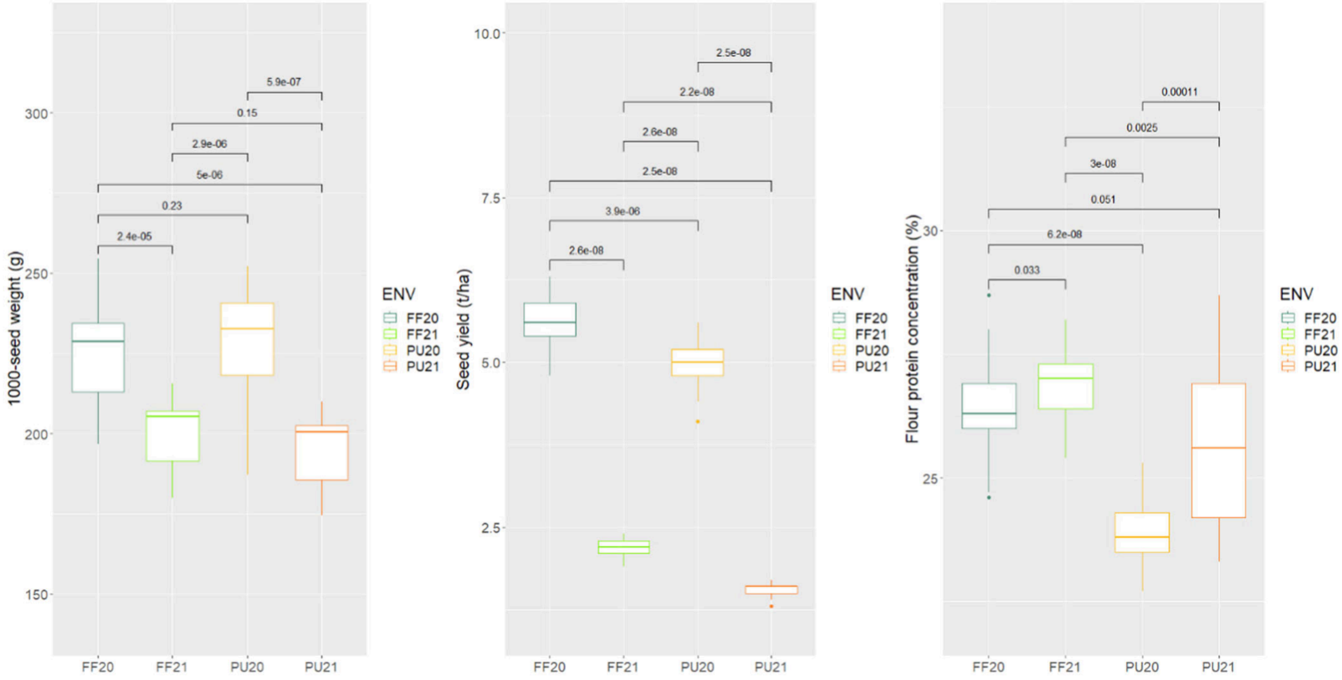

FIGURE 2 Boxplots depicting variability in seed weight, seed yield, and flour protein concentration for yellow pea genotypes across the four environments (FF20: Fairfield, FF21: Fairfield in 2021, PU20: Pullman in 2020, and PU21: Pullman in 2021). p-Values for the difference between the environments was shown for each pair-wise comparison. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Correlation coefficients among YLD, TSW, PIYLD, BPYLD, PIPC, BPPC, FLPC, PRR, WHC, OHC, PS, FC, and FS in the four environments.

	YLD	PIYLD	BPYLD	PIPC	BPPC	FLPC	PRR	WHC	OHC	PS	FC	FS
Fairfield in 2020												
TSW	−0.09	−0.08	0.01	0.11	0.09	−0.01	0.05	0.11	0.52*	−0.12	−0.24	−0.24
YLD		0.37	0.07	0.12	0.29	0.09	0.26	−0.31	0.34	0.16	0.00	−0.34
PIYLD			−0.34	0.06	0.44*	0.58**	0.44*	−0.24	−0.08	−0.17	0.56**	−0.22
BPYLD				−0.07	−0.72***	−0.48*	0.13	−0.08	0.06	0.31	−0.23	−0.02
PIPC					0.09	0.05	0.33	0.33	0.06	−0.17	0.36	−0.23
BPPC						0.40	0.06	−0.01	0.35	−0.26	0.40	−0.45*
FLPC							−0.38	0.27	−0.24	−0.26	0.14	−0.17
PRR								−0.34	0.14	0.08	0.51*	−0.09
WHC									−0.10	−0.32	0.08	−0.11
OHC										0.15	−0.11	−0.41
PS											−0.27	−0.07
FC												−0.17
Fairfield in 2021												
TSW	−0.05	−0.24	0.22	−0.11	0.05	−0.14	−0.17	0.00	0.31	0.34	−0.11	−0.07
YLD		0.18	0.06	−0.44*	0.40	−0.13	0.33	0.35	0.26	−0.05	−0.02	−0.01
PIYLD			−0.53*	0.06	0.40	0.58**	0.56*	0.48	−0.34	−0.28	−0.14	0.02
BPYLD				−0.14	−0.53*	−0.69**	−0.09	−0.03	0.30	0.18	0.00	−0.21
PIPC					−0.24	0.31	−0.02	−0.47*	−0.45*	−0.18	0.05	−0.33
BPPC						0.36	0.14	0.19	0.26	0.05	−0.14	−0.03
FLPC							−0.18	−0.02	−0.33	−0.24	−0.09	−0.12
PRR								0.30	−0.14	−0.34	−0.13	−0.03
WHC									−0.16	0.03	0.11	0.31
OHC										0.14	−0.06	−0.10
PS											−0.17	0.22
FC												0.60**
Pullman in 2020												
TSW	−0.11	−0.43*	0.32	−0.03	−0.16	−0.23	−0.28	0.02	−0.11	0.13	−0.41	0.16
YLD		0.39	−0.01	0.02	0.61**	0.11	0.35	−0.48*	0.20	−0.12	−0.07	0.45*
PIYLD			−0.59**	0.08	0.37	0.58**	0.64**	−0.28	−0.13	−0.35	0.59**	0.26
BPYLD				−0.07	−0.31	−0.61**	−0.08	0.04	0.20	−0.05	−0.34	−0.23
PIPC					0.13	0.24	−0.37	0.56**	0.30	0.02	0.17	0.14
BPPC						0.36	0.12	−0.12	0.49*	0.08	0.17	0.63**
FLPC							−0.15	0.03	−0.28	−0.17	0.29	−0.02
PRR								−0.46*	0.04	−0.38	0.35	0.24
WHC									0.18	−0.02	0.21	−0.05
OHC										0.14	0.05	0.39
PS											−0.13	−0.01
FC												0.09

(Continues)

TABLE 3 (Continued)

	YLD	PIYLD	BPYLD	PIPC	BPPC	FLPC	PRR	WHC	OHC	PS	FC	FS
Pullman in 2021												
TSW	−0.07	−0.14	−0.14	−0.21	0.07	−0.10	−0.17	0.13	0.27	0.32	−0.21	−0.02
YLD		0.04	−0.07	−0.36	−0.21	−0.40	0.42	−0.16	0.03	−0.17	−0.07	0.21
PIYLD			−0.53*	0.23	0.53*	0.54*	0.45*	0.10	0.30	−0.15	0.17	0.07
BPYLD				−0.03	−0.55*	−0.41	−0.05	−0.39	−0.19	−0.19	0.12	−0.30
PIPC					0.38	0.76***	−0.34	−0.07	0.22	−0.17	0.24	−0.34
BPPC						0.62**	−0.13	0.29	0.54*	−0.07	0.44*	0.02
FLPC							−0.45*	0.03	0.27	−0.02	0.24	−0.06
PRR								0.07	0.07	−0.20	−0.01	0.08
WHC									−0.05	−0.33	−0.01	−0.15
OHC										0.12	0.50*	0.08
PS											−0.26	0.07
FC												−0.01

Abbreviations: BPPC, byproduct protein concentration; BPYLD, byproduct yield; FC, foaming capacity; FLPC, flour protein concentration; FS, foaming stability; OHC, oil-holding capacity; PIPC, protein isolate purity; PIYLD, protein isolate yield; PRR, protein recovery rate; PS, protein solubility; TSW, seed weight; WHC, water-holding capacity; YLD, seed yield.

*Statistical significance at 0.05 probability levels.

**Statistical significance at 0.01 probability levels.

***Statistical significance at 0.001 probability levels.

3.3 | Purity and functional properties of pea protein isolates

PIYLD and purity, as well as the functional properties, are crucial characteristics for commercial pea protein production. We evaluated a total of 21 yellow pea genotypes grown over two locations and years for these important traits. An AE-IP method yielded an average of 17.2%–19.5% protein isolate (Supporting Information: Table S1) and 61.4%–64.3% starch-rich byproduct (Supporting Information: Table S2). The protein isolates had an average protein purity of 90%–92% (Supporting Information: Table S2). Approximately 66%–69% of the protein in the flour was recovered in the protein isolates (Supporting Information: Table S2). Isolates produced from Fairfield samples had slightly higher protein purity when compared to isolates produced from Pullman samples. The purity of protein isolates was slightly higher in 2021 in samples from both locations. The low level of variability for PIPC in all four environments (Supporting Information: Table S2) suggests that the isolates for all genotypes had comparable protein concentrations.

The mean WHC was 2.5–2.9 g/g (Supporting Information: Table S3), which was within the range (1.9–4.8 g/g) reported by Lam et al. (2018). The mean OHC of protein isolates in the current study was 4.0 g/g in all the environments. Mean PS ranged from 56.4% at Fairfield in 2020 to 68.3% at Pullman in 2021 (Supporting Information: Table S4). In both years, samples from Fairfield were associated with lower PS compared to samples from Pullman. PS is generally dependent on the pH used to prepare protein solutions for the Bradford assay. Even though higher pH (>9.0) can result in higher solubility, we performed a solubility test at pH 7.0 in the current study because most protein beverages are served at this pH. The protein isolates had an average FC ranging from 53.7% to 60.4% in the four environments, and an average of 78.0%–82.1% of the foam remained intact after 30 min.

The correlation coefficients for functional property traits with PIPC were mostly nonsignificant (Table 3). However, PIPC exhibited inconsistent correlations in the four environments with WHC and OHC. For example, the correlation coefficients between PIPC and WHC were positive in 2020 at both Pullman (0.56**) and Fairfield (0.33) but negative for Fairfield in 2021 (−0.47*). Correlation coefficients for PS with foaming properties (FC and FS), WHC, and OHC were generally nonsignificant.

3.4 | GEI and stability of genotypes

GGE biplot can be used to assess the mean performance and stability of genotypes across environments. We considered four traits (TSW, YLD, FLPC, and PIYLD) for GGE biplot analysis. The ideal genotype, which is positioned in the center of concentric circles with an arrow pointing to it, is the highest-yielding genotype across all environments (Yan et al., 2007). The ideal genotype is used as a reference to rank genotypes for mean performance and stability. In general, genotypes closer to the ideal genotype are more desirable than those far from it (Yan & Tinker, 2006).

For TSW, G123 (PS16NZ0003), G058 (PS1410B0003), G158 (PS17100046), G046 (Peregrine), and G157 (PS17100022) were the top five genotypes (Figure 3a). The mean seed weights for these five genotypes were at least 10 g over the overall means in all the four environments (Supporting Information: Table S1). On the contrary, G043 (Delta), G153 (PS16100107), G061 (Universal), G119 (PS16100094), and G164 (PS17100240) were at the bottom with respect to mean seed weight and stability (Figure 3a), with below average seed weight in one or more of the environments (Supporting Information: Table S1).

In the case of YLD, the top five entries were G155 (PS16NZ0005), G061 (Universal), G164 (PS17100240), G157 (PS17100022), and G122 (PS16100111) (Figure 3b). These five genotypes had 0.1–0.5 t/ha more yield compared to the mean of all the genotypes in each environment (Supporting Information: Table S1). The five genotypes ranked at the bottom using the GGE biplot for YLD were G153 (PS16100107), G043 (Delta), G119 (PS16100094), G046 (Peregrine), and G042 (Ds Admiral) (Figure 3b), which were associated with below-average yields at each environment (Supporting Information: Table S1). G157 (PS17100022), one of the highest-yielding genotypes, also had a high seed weight. Some genotypes like G046 (Peregrine) and G123 (PS16NZ0003) had high seed weight but low seed yield. On the other hand, G164 (PS17100240) and G061 (Universal) were among the high yielders but with low seed weight.

The GGE biplot for FLPC indicated that G153 (PS16100107), G054 (PS14100068), G162 (PS17100236), G061 (Universal), and G043 (Delta) were the top five high protein genotypes across environments (Figure 4). All these genotypes had above-average FLPC in all the four

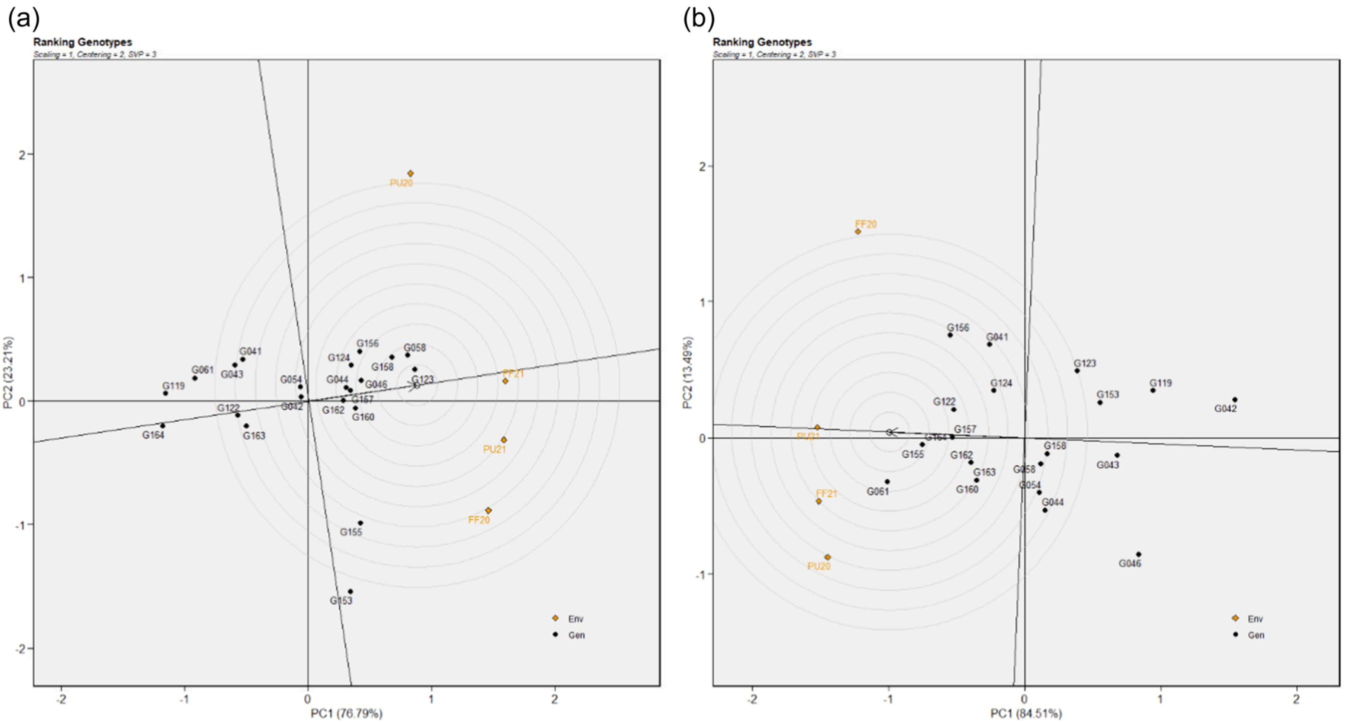

FIGURE 3 Ranking of the 21 yellow pea genotypes based on performance and stability for two traits: (a) 1000-seed weight (TSW) and (b) seed yield (YLD). Test genotype comparisons were made as compared to the ideal genotype (represented by the small circle and an arrow pointing to it and having the highest mean with absolute stability). *T* test genotypes within the central concentric circle are considered the best, while genotypes further away from the central concentric circle are considered the poor with respect to the trait. [Color figure can be viewed at wileyonlinelibrary.com]

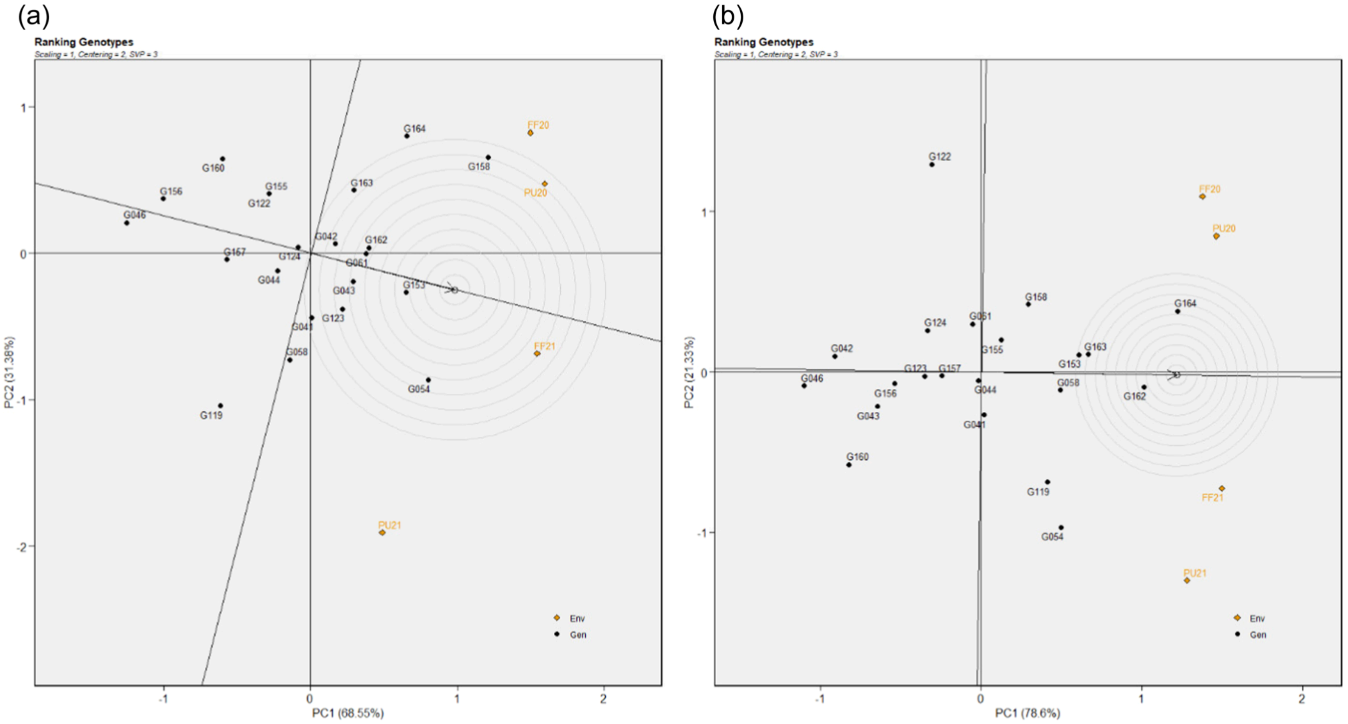

FIGURE 4 Ranking of the 21 yellow pea genotypes based on performance and stability for two traits: (a) flour protein concentration (FLPC) and (b) protein isolate yield (PIYLD). Test genotype comparisons were made as compared to the ideal genotype (represented by the small circle and an arrow pointing to it and having the highest mean with absolute stability). Test genotypes within the central concentric circle are considered the best, while genotypes further away from the central concentric circle are considered poor with respect to the trait. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 4 Rank correlations coefficients of genotype ranking by GGE biplot and by mean per se at each environment.

Traits	Fairfield in 2020	Fairfield in 2021	Pullman in 2020	Pullman in 2021
1000-seed weight (TSW)	0.67	0.93	0.88	0.77
Seed yield (YLD)	0.79	0.93	0.94	0.93
Flour protein concentration (FLPC)	0.79	0.92	0.86	0.56
Protein isolate yield (PIYLD)	0.78	0.94	0.86	0.85

environments (Supporting Information: Table S1). According to the ranking of genotypes relative to the ideal genotype, the genotypes with consistently high PIYLD across environments were G162 (PS17100236), G164 (PS17100240), G163 (PS17100239), G153 (PS16100107), and G058 (PS1410B0003) (Figure 4). These five genotypes had above-average PIYLD in each of the four environments, with mean PIYLD of 18%–20.3% (Supporting Information: Table S1). The two entries (G153 and G162) picked by both FLPC and PIYLD also had above-average seed weight in all environments with mostly average seed yield (Supporting Information: Table S1). G058 that was picked by FLPC also had above average seed weight, average or above average seed yield and PIYLD. In addition to a high yield and seed weight, G157 also had an average PIYLD.

4 | DISCUSSION

The GEE biplot simplifies and visually presents complex GEI in two principal components (Yan & Tinker, 2006). It helps identify genotypes that consistently perform well across environments. According to rank correlation for all the four traits, the genotype ranks based on GGE biplot closely match the genotype ranks based on mean performance in each environment (Table 4). This implies that selection using the visual GGE biplot tool was just as effective as selection based on mean performance. The GGE biplot, though, might be appealing when a large number of environments are considered.

Estimates of heritability are frequently used to assess the precision of an experiment or measurement procedures

(Piepho & Möhring, 2007). Depending on the technique used and the trait being considered, the heritability estimates have varying values. We found fairly high heritability for seed weight and PIYLD, moderate heritability for PIPC and FLPC, and low heritability for seed yield and all functional property traits. The low heritability estimates for seed yield and functional properties may indicate that the genetic variance is low or the error variance is large. Different studies reported moderate to high heritability estimates for seed yield and weight in pea (Afreen et al., 2017; Barcchiya et al., 2018; Georgieva et al., 2016; Meena et al., 2017; Singh & Dhall, 2018; Uhlarik et al., 2022; Yimam et al., 2020). Barcchiya et al. (2018) and Uhlarik et al. (2022) reported high heritability estimates for total protein in pea. Indeed, different studies estimated heritability using different methods, making direct comparison difficult. It is hard to find research reports on heritability estimates for PIYLD and purity, as well as protein functional properties, so our heritability estimates for these traits can serve as a baseline and more research may be needed to further understand genotypic contribution in determining these traits.

Weather plays a critical role in the productivity and quality of pea. Our findings indicated that unfavorable weather conditions in 2021 resulted in the reduction of seed yield and weight by more than 61% and 11%, respectively. A yield reduction of 40%–87% reported in various legume crops due to water deficit (Pradhan et al., 2019; and references therein). High-temperature stress is particularly important when combined with water deficits. For example, Bueckert et al. (2015) reported that heat stress (frequency of hot days) contributed significantly to yield loss in pea at the dryland site, but not at the irrigated site. FLPC increased slightly in 2021 (unfavorable season) compared to 2020 (favorable season). Lam et al. (2018) reported that high temperatures and low rainfall led to higher protein concentrations in peas, which was consistent with our finding that protein concentration was high in stressed environments. Better starch accumulation anticipated during favorable seasons may lower the percentage contribution of protein in seeds as protein and starch correlated negatively (Yuan et al., 2021).

Positive or nonsignificant (or weak) correlations between traits is important for simultaneously improving them. This is especially true when the goal is to improve the traits in the same direction (increasing or decreasing simultaneously). While weak correlation offers the chance to identify some genotypes combining the best of the traits of importance, strong positive correlation enables improving both traits simultaneously. We found positive relationship between FLPC and PIYLD in the current study, implying that improving one trait leads to improvements in the other. FLPC can therefore be used as an indirect selection criterion for PIYLD. Given the low throughput of the protein isolation process, using FLPC as an indirect selection criterion in pea breeding can have a significant impact, particularly in the early breeding stage when several hundred entries are evaluated. When selected genotypes based on FLPC and PIYLD were compared, two genotypes combined high values for both traits. Three of the traits we studied (TSW, FLPC, and PIYLD) had weak correlations with seed yield, implying that genotypes combining the best of these traits can be identified. For example, four genotypes (G058, G157, G153, and G162) were found to have good combination of at least three of these traits.

Protein isolation (wet, dry, or mild processes) help to partition flour samples into protein-rich and starch-rich fractions (Boukid et al., 2021). The economic feasibility of pea protein isolation can be dependent on the PIYLD and purity, as well as the functional properties of the protein produced. The AE-IP method yielded 17%–20% protein isolate, with about 66%–69% of the protein in flour sample recovered into the protein isolate. Because a considerable amount of starch-rich byproduct (61%–64%) is generally produced, it is vital to explore how starch-rich byproducts can be applied to high-value products so as to make the protein isolation process economically viable.

Protein purity and functional properties are crucial to apply pea protein isolates in food products. For example, water and OHCs are among the important functional properties, with the former affecting food texture and the latter being important for meat-binding applications (Lam et al., 2018). Our protein isolates had protein purity of over 90%, which was within the range reported (81%–95%) by Daba et al. (2022). Solubility of pea protein depends on the pH, with a “u-shaped” graph (Cui et al., 2020; Lam et al., 2018). PS hits the lowest at the isoelectric point (pH 4.0–5.0) and increasing below and above the isoelectric point. Even though inconsistencies in relationships between PIPC and PS were discovered in our study, Cui et al. (2020) reported high PS for cultivars (Trapeze and ND trial) with high FLPC and PIPC. Overall, direct comparison among studies for some of the measurements (e.g., foaming properties) can be difficult owing to differences in cultivar types and methods used (Lam et al., 2018). In conclusion, the findings from the current study can be useful to design tailored pea breeding program for protein extraction.

AUTHOR CONTRIBUTIONS

Sintayehu D. Daba contributed to planning the study, analyzing the data, writing the manuscript, and editing the paper. Rebecca J. McGee contributed to providing the data used in this study, and reviewing and editing the paper. Alecia M. Kiszonas contributed to reviewing and editing the paper.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are in the Supporting Information Tables.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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