CHILEAN JOURNAL of AGRICULTURAL RESEARCH

RESEARCH ARTICLE

Evaluation of European buckwheat genotypes at different elevations and seasons in Montenegro under organic farming conditions

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Received: 21 November 2024; Accepted: 3 March 2025, doi:10.4067/S0718-58392025000400504

ABSTRACT

Organic farming and the introduction of underutilized crops such as buckwheat (*Fagopyrum esculentum* Moench) play an important role in enhancing agricultural biodiversity and promoting sustainable farming practices. This study examines the variability and stability of 11 European buckwheat genotypes at two different elevations (610 and 830 m) under organic farming principles over two growing seasons. Genotype, elevation, and year factors significantly contributed to the phenotypic variation of traits. Environmental factors had the greatest impact on the variation in biomass yield (32.6%) and grain yield (28.4%), while the least influence was observed on the variation in thousand grain weight (6.3%). Higher plant height (120.11 cm) and biomass yield (14.2 t ha^{-1}) were recorded at the lower elevation (610 m), while higher grain yield (662.2 kg ha^{-1}) and thousand grain weight (22.9 g) were observed at the higher elevation (830 m). In 2015, plant height, biomass yield, thousand grain weight, and grain yield were 3.87%, 14.87%, 2.70%, and 15.95% higher, respectively, compared to 2016. Excessive rainfall in 2016 led to plant lodging, negatively affecting vegetative growth and yield. The additive main effect multiplicative interaction (AMMI) analysis showed that genotypes 'La Harpe' and 'Heljda 2' were stable and high-yielding across different environments, while 'Novosadska', 'Darja' and 'Prekumurska' exhibited positive interactions with the higher elevation environment (830 m) during the dry 2015 season. Heatmap cluster analysis indicated that genotypes 'Darja' and 'La Harpe' demonstrated broad adaptability for the analyzed traits, while 'Heljda 1' and 'Heljda 2' were particularly suited to high-rainfall conditions, achieving high grain yield or grain quality.

Key words: Adaptability, AMMI, buckwheat, heatmap cluster analysis, stability.

INTRODUCTION

The negative consequences of climate change on food production highlight the need for sustainable agricultural practices such as agroforestry, organic farming, and sustainable land management (Kumar et al., 2022). Introducing alternative crops into agroecosystems enhances crop diversification, increasing agricultural system resilience and their sustainability (Kumar et al., 2022; Rangappa et al., 2023a). Buckwheat (*Fagopyrum* spp.) is an alternative and underutilized crop that contributes to increasing agricultural diversity, offering valuable genetic resources and adaptability to marginal environments (Singh et al., 2020).

Buckwheat is a member of the Polygonaceae family, but it is often classified as a pseudocereal due to its nutritional profile, which is comparable to that of cereals like rice and wheat (Ohnishi et al., 2009; Christensen et al., 2010). It originated in the Yunnan Province of China (Ohnishi, 1990; Zhang et al., 2012) and has since been cultivated in various regions across Asia, Europe, and the Americas (Kreft and Germ, 2008; Jacquemart et al., 2012; Hunt et al., 2018). Due to its nutritional value and adaptability to different environmental conditions, buckwheat is an important crop, which is used both for human and animal nutrition (Popović et al., 2017; Kolarić et al., 2021). Buckwheat is a rich source of nutrients, including protein, vitamins, minerals, and phenolic compounds. The grain protein of buckwheat is high in arginine and lysine, which are generally limited in other cereals (Christensen et al., 2010; Chrungoo and Chetty, 2021). Phenolic compounds, such as rutin, quercetin, and flavonoids, are present in significant amounts, which contribute to their antioxidant properties (Chrungoo and Chetty 2021; Vieites-Álvarez et al., 2024).

Buckwheat is highly adaptable to a wide range of agroecological conditions, thriving in various soil types and climates. It can tolerate moisture stress, grow in sandy and rocky substrates, and perform well even in low-fertility, acidic, or poorly tilled soils (Chrungoo and Chetty, 2021; Hajong et al., 2022; Rangappa et al., 2023a; 2023b). Its short growth cycle of 70-90 d makes it particularly suited for regions with limited growing seasons (Chrungoo and Chetty, 2021). Additionally, buckwheat's allelopathic properties, due to its root residues, help naturally control weeds, thereby reducing the need for synthetic herbicides (Gfeller et al., 2018; Szwed et al., 2019).

Despite its nutritional value and ecological benefits, buckwheat production has faced difficulties in recent decades due to consistently low yields (Popović et al., 2014; Farooq et al., 2016). To overcome these challenges, organic farming has become a practical solution, making use of buckwheat's natural resilience to grow without the need for excessive fertilizers or pesticides (Popović et al., 2014; Kolarić et al., 2021; Romanovskaja et al., 2022). Organic farming not only supports environmental sustainability but also improves the nutritional content of the grains, meeting the growing demand for healthier food options (Kolarić et al., 2021; Romanovskaja et al., 2022). In mountainous regions, where conventional agriculture often faces limitations, organic farming offers a sustainable alternative (Kreft and Germ, 2008). By using organic practices, farmers can take advantage of their environmental strengths while reducing negative impacts on soil, water, and ecosystems. Adopting organic buckwheat farming presents considerable economic opportunities, particularly in areas like Montenegro, where suitable climate conditions and organic farming practices support long-term sustainability (Vreva et al., 2012).

Given the considerations mentioned earlier, it is essential to conduct research that tests the variability and stability of different buckwheat genotypes in areas with different elevations, especially when grown under organic farming principles. For this reason, the focus should be on studying the interaction between genotype and environment (GEI) to evaluate the stability of crop yields across different conditions. By growing various genotypes in diverse environments, researchers can assess their adaptive potential; however, it is important to note that different genotypes may respond differently to varying conditions, which can lead to changes in their ranking or even a crossover interaction (Li et al., 2021; Banjac et al., 2022; Priyanto et al., 2024). Many researchers have utilized additive main effects and multiplicative interaction (AMMI) analysis to examine stability of genotypes across different environments (Kandel and Srestha, 2019; Li et al., 2021; Banjac et al., 2022). This approach combines ANOVA with principal component analysis (PCA) to differentiate the effects of genotype and environment, as well as their interactions (GEI) (Gauch and Zobel, 1996). Insights gained from studying performance stability can help improve crop productivity and sustainability across various environments. A thorough understanding of GEI interactions is crucial for identifying genotypes that adapt well to specific conditions (Banjac et al., 2022). Also, identifying stable, high-yielding genotype over a wide range of environments remains a significant challenge for crop breeders (Begna, 2021).

This study explores the potential for organic buckwheat production in Montenegro's mountainous regions by evaluating how genotype, elevation, and seasonal variations affect agro-morphological traits. The objectives were to: (i) Assess the impact of genotype, elevation, and season on yield and related traits; (ii) evaluate the stability of buckwheat genotypes across different agroecological conditions; (iii) analyze the relationships between the traits and genotypes in various environments; and (iv) determine which trait serves as the best phenotypic marker for selecting the most suitable genotypes across different agroecological conditions.

MATERIALS AND METHODS

Plant material and experimental design

In the municipality of Bijelo Polje, Montenegro, we conducted an experiment in 2015 and 2016 at two different elevations, 610 m a.s.l. (locality Pašića Polje) and 830 m a.s.l. (locality Laholo), to evaluate the agronomic performance of 11 European buckwheat (*Fagopyrum esculentum* Moench) varieties. The tested genotypes included: Godijevo (G1; Montenegro), Novosadska (G2; Serbia), Heljda 1 (G3) and Heljda 2 (G4; Bosnia and Herzegovina), Darja (G5), Prekumurska (G6), and Čebelica (G7; Slovenia), Bamby (G8; Austria), Češka (G9; Czech Republic), La Harpe (G10; France), and Spačinska (G11; Slovakia). The trial was organized using a randomized complete block design (RCBD) with four replicates, under an organic farming system, and each elementary plot measured 10 m^2. The distance between plots was 0.5 m. Each variety was sown with the same number of seeds, 75 g per plot, at a depth of 3 to 4 cm. The following analyses were performed: Plant height (cm), biomass yield (t ha^{-1}), grain yield (kg ha^{-1}) and thousand grain weight (g) of the tested buckwheat varieties. Plant height was measured at the physiological maturity stage (end of fruit ripening), with 10 plants selected per elementary plot. A random sample of plants was taken from each plot, after the removal of the border rows (0.5 m) to avoid edge effects.

In both elevations, the pre-grassland was a natural meadow, managed under an organic farming system. Deep plowing was done in mid-April, up to 30 cm (plow). Pre-sowing preparation was carried out a few days before sowing, with a tiller to prepare the soil in order to achieve favorable conditions for uniform sowing and uniform germination and crop emergence. With this procedure, a finely crumbly structure of the soil was achieved. Sowing was done in the last decade of May in both examined seasons and in both localities. There was no crop care during the growing season, no fertilizers were applied, and weeds were removed mechanically. Harvesting was done by hand after the marginal rows of 0.5 m width were removed from the plots, which were not covered by the protective belt. The harvesting was carried out in the first decade of September in both growing seasons and at both elevations. After harvesting, plants were carefully tied into bundles, which, after being measured and marked, were left in a drafty place to dry. The threshing was done manually, and the chaff was separated using a sieve.

Before starting the experiment, a soil analysis was performed (sample from a depth of 0-30 cm) (Table 1). The results of the agrochemical analysis of the soil showed that the experiments were based on poorly fertile, loam soil (USDA, 2017). The results show that it is an acidic soil (on 610 m) and a very acidic soil (on 830 m). The soil in both elevations was quite humus, but the humus is of poor quality due to the low pH value, its acidity comes from the parent substrate and the influence of the humid climate. In both elevations, the soil is weakly calcareous with a low content of accessible P and a medium content of accessible K.

Table 1. Agrochemical analysis of soil at both examined elevations.

Elevation	pH in H_2O	pH in KCl	$CaCO_3$	Humus	P_2O_5	K_2O
m			%	%	mg 100 g^{-1}	mg 100 g^{-1}
610	5.41	4.83	3.2	4.82	1.2	12.1
830	4.65	4.08	3.5	4.31	0.9	16.7

Meteorological conditions

The municipality of Bijelo Polje is located in the mountainous part of Montenegro. Meteorological data were retrieved from the website of the Institute of Hydrometeorology and Seismology (http://www.meteo.co.me) (Figure 1).

In both growing seasons, April had slightly lower precipitation than the long-term average. In April 2015, temperatures were close to the multi-year average (9.7 °C), while in 2016 they were higher (13.6 °C). May 2015 was warmer (16.9 °C) but drier (35.5 mm precipitation), while May 2016 was cooler (13.9 °C) with significantly more rainfall (120 mm). These conditions were suitable for buckwheat germination, which occurs at temperatures as low as 5 °C. Buckwheat was sown in late May under favorable conditions for early growth. June temperatures were close to the average (18.9 °C in 2015 and 20.3 °C in 2016), with slightly above-average rainfall (90.4 mm in 2015 and 86 mm in 2016), providing optimal conditions for plant development.

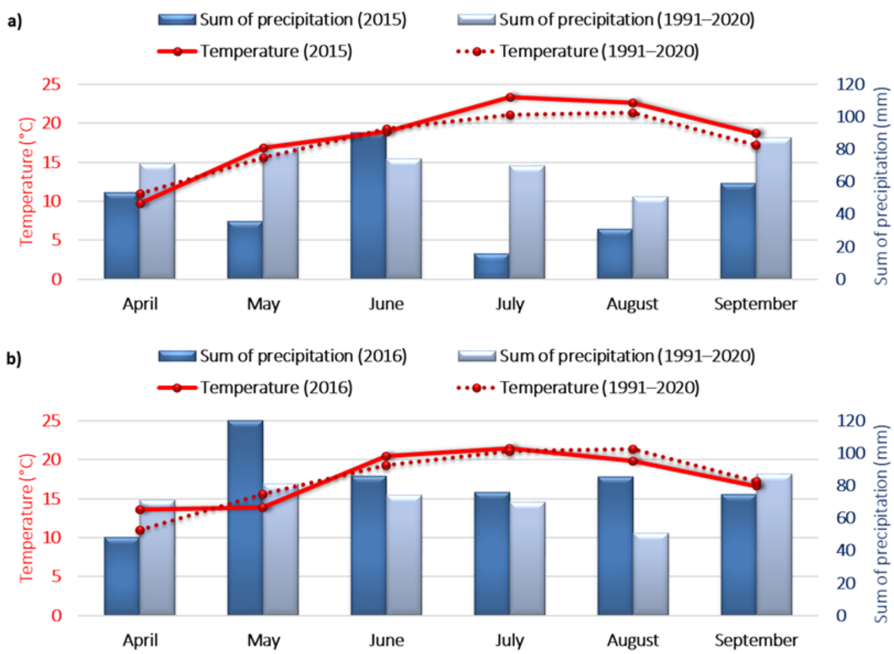

Figure 1. Mean monthly temperatures and sum of precipitation in 2015 (a) and 2016 (b) growing seasons compared to multi-year averages.

The climatic differences between 2015 and 2016 were most notable in July and August, key months for flowering and grain filling. In 2015, temperatures were slightly higher than the multi-year average (23.4 °C in July and 22.6 °C in August), while in 2016, temperatures were closer to the average (21.5 °C in July and 19.8 °C in August). However, precipitation was much lower in 2015 (46.2 mm) compared to 2016 (161.3 mm). The lower-elevation plot near the Lim River likely benefited from higher humidity during the drier 2015 season, reducing the impact of limited rainfall. In contrast, the heavy rainfall in 2016 caused lodging of the plants, especially at the lower elevation, which limited vegetative growth and reduced pollination and grain yield.

In September, when the buckwheat harvest occurred, less precipitation was observed in 2015 (59 mm) compared to the long-term average (87 mm), along with higher temperatures (18.7 °C compared to 17.2 °C). In 2016, temperatures (16.6 °C) were within the multi-year average, while precipitation (74.6 mm) was slightly below average. The reduced rainfall in September of 2015 was beneficial for harvesting, as excess rain could have disrupted fieldwork and affected grain quality.

Statistical analysis

The normality of the data distribution was tested using the Shapiro-Wilk test, and the homogeneity of variances was tested using Levene's test. The results indicated that the data followed a normal distribution and confirmed the homogeneity of variances. The analysis of phenotypic variability of the analyzed traits was conducted using ANOVA in R version 4.3.2 (R Project for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, https://www.R-project.org/). Multiple comparisons of variants of analyzed factors (genotype, locality, and season) in relation to analyzed traits were carried out using Tukey's HSD test at two levels of statistical significance (1% and 5%). In order to analyze the influence of the analyzed factors and their interaction on the variation of grain yield, an additive main effects and multiplicative interaction (AMMI) analysis was carried out.

The AMMI analysis combines elements of both ANOVA and principal component analysis (PCA) to decompose the genotype (G) and environment (E) effects and their interaction (GEI) into additive main and multiplicative interaction effects (Gauch and Zobel, 1996). The analysis was performed using the GenStat 18th edition for Windows, trial version (VSN International, 2018, https://www.vsni.co.uk). The obtained PCA scores (IPCA1 and IPCA2) were used to generate AMMI1 (IPCA1 *vs*. mean value of analyzed trait) and AMMI2 (IPCA1 *vs*. IPCA2) biplots, created in Microsoft Excel.

A hierarchical cluster analysis was conducted using Ward's method to illustrate the interrelationships among the analyzed traits and genotypes across different seasons and at both elevations. The data were scaled for standardization, and a distance matrix was calculated using Euclidean distance. Clusters were formed for rows (genotypes) and columns (traits) and were visualized through heatmaps, with clearly marked cluster groups for the rows (genotypes). The determination of the number of clusters into four groups was based on an analysis of the hierarchical clustering results, including dendrogram visualization and consideration of within-group variation. This approach facilitates a deeper understanding of the interdependencies between traits and genotypes, aiding in the identification of patterns that may play a significant role in further research and selection of genotypes. This analysis was performed using the R version 4.3.2.

RESULTS AND DISCUSSION

Influence of genotype, elevation and season on buckwheat grain yield components

Buckwheat is characterized by its exceptional resistance to various abiotic and biotic factors, which is why it is suitable for growing according to the principles of organic production, even in adverse environmental conditions (Chrungoo and Chetty, 2021; Hajong et al., 2022; Rangappa et al., 2023a). However, yield components and buckwheat yield are significantly influenced by a number of factors: Genotype (Li et al., 2021; Janovská et al., 2021), season, i.e., climatic factors (Vreva et al., 2012; Ghiselli et al., 2016; Kolarić et al., 2021; Janovská et al., 2021), cultivation system (Popović et al., 2013; Romanovskaja et al., 2022), sowing methods (Sobhani et al., 2014); altitude (Ghiselli et al., 2016), applied fertilizer (Kolarić et al., 2021). Therefore, it is very important to examine the influence of genotype and environmental factors on yield and yield components of buckwheat. In addition, organically produced buckwheat can provide additional value on the market and in regions where agricultural production is more difficult, so it is very important that such research is carried out according to the principles of organic production, as well as in conditions of higher altitudes.

The results of this research indicate that the factors genotype, elevation and year have a significant influence on yield components and buckwheat yield, while the interaction of the examined factors has a different effect on the phenotypic expression of the analyzed traits (Table 2). In order to select suitable buckwheat genotypes for organic production or for different climatic conditions, characterization and evaluation of genetic resources are essential (Janovská et al., 2021). The share of the influence of the genotype factor in the total variation ranged from 39.3% for biomass yield to as much as 90.6% for the thousand grain weight. The observed significant genetic variability within the studied germplasm suggests that the population has the potential to adapt to environmental challenges, thereby facilitating the development of improved varieties with enhanced productivity and yield stability (Janovská et al., 2021). These results indicate that the thousand grain weight is a genotype-specific trait, predominantly influenced by genetic factors. Similar findings were reported by Zečević et al. (2014) and Urošević et al. (2023), who studied the effects of genotype and environment on the thousand grain weight in wheat. Additionally, Janovská et al. (2021) highlighted significant diversity in the thousand grain weight among different buckwheat genotypes and years in a broader germplasm study. This diversity underscores the value of this trait for selecting suitable materials for varying climatic conditions.

When considering only the influence of environmental factors (elevation and year), the greatest contribution to variation was observed in biomass yield (32.6%), followed by grain yield (28.4%), plant height (21.27%), and thousand grain weight (6.3%). These results suggest that biomass yield and grain yield are complex quantitative polygenic traits, heavily influenced by environmental factors and their interactions.

Higher values of plant height (120.11 cm) and biomass yield (14.2 t ha^{-1}) were recorded at a lower elevation (610 m), which can be explained by specific agroecological conditions. The lower elevation, located near the Lim River, allowed for a more favorable level of air humidity, even during periods of reduced rainfall. These conditions reduced the stress caused by drought and enabled optimal development of the vegetative parts of the plants, which was reflected in higher height and total biomass. These findings are consistent with Ghiselli et al. (2016), who also reported that buckwheat plants grown at lower elevations tended to be higher. The same study found that the number of leaves per plant and the number of branch stages per plant, traits that can be directly linked to biomass yield, were higher at lower elevations. This increase was attributed to enhanced plant growth under those specific agroecological conditions. Buckwheat's ability for rapid growth and high biomass production (with an average biomass yield of 13.8 t ha^{-1} in this study) makes it a suitable crop for reducing soil erosion and provides a valuable feed source for livestock (Hajong et al., 2022). Additionally, incorporating buckwheat biomass can be beneficial as green manure due to its allelopathic properties and its ability to enrich the soil with nutrients, particularly P and N (Boglaienko et al., 2014). These characteristics make buckwheat an ideal cover crop in both organic and sustainable farming systems (Vieites-Álvarez et al., 2024). In contrast to plant height and biomass yield, the values for thousand grain weight (22.9 g at 830 m *vs*. 22.1 g at 610 m) and grain yield (662.2 kg ha^{-1} *vs*. 608.4 kg ha^{-1}) were higher at the higher elevation. This difference may be due to favorable microclimatic and soil conditions at the lower elevation, which promoted greater growth in height and biomass, leading to crop lodging. Lodging had a direct impact on reducing thousand grain weight and grain yield. These findings are consistent with the results of Ghiselli et al. (2016), who also observed higher values for thousand grain weight and grain yield in buckwheat at higher elevation, compared to values at lower elevation.

Table 2. AMMI-ANOVA for analyzed traits in 11 buckwheat genotypes grown across two elevations (610 and 830 m) during two growing seasons (2015 and 2016). Treat.: Treatment; Gen.: genotype; Env.: environment; $IPCA_1$: first interaction principal component axis; $IPCA_2$: second interaction principal component axis; Res.: residual; df: degrees of freedom; SS: sum of squares; MS: mean squares; F: F-statistic (F-value); Sig.: significance level (p-value); $^{**}p < 0.01$; $^{*}p < 0.05$; ^{ns}nonsignificant.

	Treat.	Genotype (G)	Env. (E)	Block	G × E	$IPCA_1$	$IPCA_2$	Res.	Error	Total
df	43	10	3	12	30	12	10	8	120	175
					Plant height					
SS	6400	3796	1800	353	804	647	133	23	1711	8464
MS	148.8	379.6	599.9	29.5	26.8	53.9	13.3	2.9	14.3	48.4
F	10.44^{**}	26.63^{**}	20.37^{**}	2.07^{ns}	1.88^{**}	3.78^{**}	0.93^{ns}	0.2^{ns}	–	–
Sig.	0.000	0.000	0.000	0.054	0.009	0.001	0.505	0.989	–	–
					Biomass yield					
SS	461.4	231.1	191.7	30.5	38.5	19.9	13	5.6	96.4	588.2
MS	10.73	23.11	63.91	2.54	1.28	1.66	1.30	0.71	0.80	3.36
F	13.36^{**}	28.77^{**}	25.16^{**}	3.16^{*}	1.6^{*}	2.06^{*}	1.62^{ns}	0.88^{ns}	–	–
Sig.	0.000	0.000	0.000	0.041	0.039	0.024	0.107	0.536	–	–
					Thousand grain weight					
SS	851.6	789.6	55.2	2.5	6.9	3.8	2.7	0.4	17.5	871.6
MS	19.81	78.96	18.39	0.21	0.23	0.32	0.27	0.05	0.15	4.98
F	135.64^{**}	540.76^{**}	89.52^{**}	1.41^{ns}	1.56^{*}	2.17^{*}	1.83^{ns}	0.83^{ns}	–	–
Sig.	0.000	0.000	0.000	0.172	0.047	0.017	0.063	0.952	–	–
					Grain yield					
SS	1615133	964597	516539	91074	133997	81318	46398	6280	112574	1818781
MS	37561	96460	172180	7590	4467	6777	4640	785	938	10393
F	40.04^{**}	102.82^{**}	22.69^{**}	8.09^{**}	4.76^{**}	7.22^{**}	4.95^{**}	0.84^{ns}	–	–
Sig.	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.572	–	–

The interaction between genotype and elevation did not significantly ($p > 0.05$) impact the variation in buckwheat plant height, biomass yield, and thousand grain weight (Figure 2). This indicates that the ranking of genotypes in terms of these traits remained consistent across the examined elevations. Genotype 'Darja' exhibited the greatest plant height at both elevations (≈ 127.0 cm), while genotype 'Prekumurska' achieved the highest biomass yield, with similar values across elevations (≈ 15.4 t ha^{-1}). In contrast, genotype 'Heljda 1' had the lowest values for both plant height and biomass yield at both elevations (≈ 111.0 cm and ≈ 11.9 t ha^{-1}, respectively). Genotype 'Heljda 1' recorded the highest value of thousand grain weight at both elevations (26.0 g at 610 m and 26.9 g at 830 m), while 'Bamby' exhibited the lowest values (17.85 g at 610 m and 18.50 g at 830 m). The lack of a significant interaction between genotype and elevation suggests that the genotypic performance of buckwheat for traits like plant height, biomass yield, and thousand grain weight is stable across different elevations. This stability means that these genotypes show consistent phenotypic expression for these traits, regardless of elevation, meaning that changes in elevation do not affect their relative ranking or performance for these traits. This finding is particularly valuable in breeding programs, as it suggests that genotypes such as 'Heljda 1' and 'Heljda 2' can be reliably cultivated across a range of elevations, achieving low values for vegetative plant parts and high values for thousand grain weight under varying environmental conditions. At the same time, genotype 'Bamby' consistently shows very low values for all traits at both elevations, making it unsuitable for use in breeding programs aimed at achieving higher productivity and quality in variable agroecological conditions within organic production systems. In contrast, the Genotype × Elevation interaction (GEI) had a significant effect on grain yield variation among the analyzed buckwheat genotypes. Kandel and Shrestha (2019) highlighted that variations in grain yield across different locations are influenced by local climatic conditions and soil characteristics. The largest reductions in grain yield between elevations were observed in genotypes 'Novosadska', 'Prekumurska', 'Darja', and 'Heljda 2', with decreases of 14.62%, 11.76%, 11.16%, and 10.39%, respectively, at the lower elevation of 610 m. At the higher elevation of 830 m, genotype 'Heljda 2' achieved the highest grain yield (760.87 kg ha^{-1}), whereas 'La Harpe' recorded the highest grain yield at 610 m (726.11 kg ha^{-1}) and demonstrated similar yield levels across both elevations. 'Bamby' had the lowest grain yield at both elevations (541.44 kg ha^{-1} at 610 m and 512.50 kg ha^{-1} at 830 m), highlighting its limited adaptability in terms of grain yield across varying elevations. This finding is useful for breeding programs as it allows for the identification of genotypes that are better adapted to specific elevations.

Although previous research (Ghiselli et al., 2016; Janovská et al., 2021; Romanovskaja et al., 2022) showed that higher rainfall usually favors vegetative growth and increased biomass yield in buckwheat, in this research higher values of plant height (120.8 cm) and biomass yield (14.8 t ha^{-1}) were recorded during the 2015 dry season, compared to the 2016, which had a higher amount of precipitation (116.3 cm and 12.9 t ha^{-1}, respectively). This result can be explained by a combination of agroecological factors specific to the dry season, especially in lower elevation, and the impact of excessive rainfall on plants during 2016. Lower rainfall in the 2015 season reduced the risk of plant dormancy and lodging, which allowed buckwheat to fully develop vegetative parts and achieve maximum biomass yield. Also, due to the proximity of the river in lower elevations, increased air humidity helped the plants perform well even during the dry season. In the 2016 season, the higher amount of precipitation, combined with the winds in the mountainous area, led to the lodging of crops, whereby the analyzed varieties could not express their full genetic potential. Also, in 2015 season, when rainfall was lower, higher values for thousand grain weight (22.8 g) and grain yield (682.3 kg ha^{-1}) were recorded. These results align with those of Romanovskaja et al. (2022), who highlighted that while excessive moisture may be beneficial for vegetative growth, it is unfavorable for grain formation. Also, Kalinova and Vrchotova (2011) and Rangappa et al. (2023b) point out that heavy rainfall can have a negative effect on buckwheat grain formation and grain yield. Also, lodging reduces the efficiency of flowering and fertilization, negatively affecting grain quality and yield. Morishita et al. (2020) emphasize the importance of lodging resistance in buckwheat breeding to minimize the negative impact on yield.

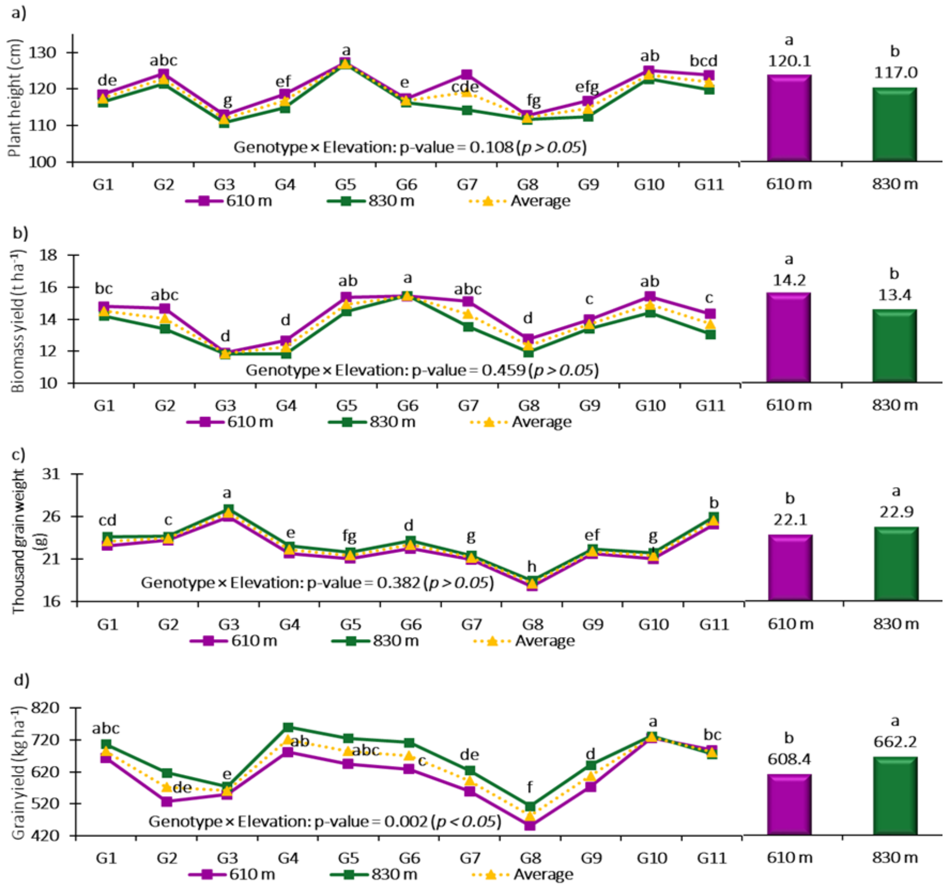

Figure 2. Phenotypic variation of buckwheat agromorphological traits in relation to genotype, elevation, and their interaction: G1 Godijevo; G2 Novosadska; G3 Heljda 1; G4 Heljda 2; G5 Darja; G6 Prekumurska; G7 Čebelica; G8 Bamby; G9 Češka; G10 La Harpe; G11 Spačinska. Different letters indicate differences (p < 0.05) according Tukey test in the average values of genotypes and elevations, respectively.

The interaction between genotype and year was found to have a significant impact on the variation in plant height, indicating that some genotypes respond differently to yearly environmental conditions (Figure 3). Notably, genotypes 'Godijevo', 'Čebelica', and 'La Harpe' contributed most to this significant Genotype × Year interaction, showing considerable shifts in their rankings for plant height across years. For instance, 'Čebelica' exhibited the greatest year-to-year variation in height, with an average difference of approximately 10 cm. On average, genotype 'Darja' recorded the highest plant height in the study (127.02 cm), whereas the shortest average height was observed in 'Heljda 1' (111.9 cm). These results align with Janovská et al. (2021), who also observed significant yearly and genotypic differences in buckwheat height. Similarly, the interaction between genotype and year significantly influenced biomass yield. Genotype 'Prekumurska' exhibited the largest variation in biomass yield between years, with the highest values of 16.97 t ha^{-1} in 2015 and 13.98 t ha^{-1} in 2016, indicating significant sensitivity to annual conditions. Additionally, 'Prekumurska' had the highest average biomass yield across seasons (15.46 t ha^{-1}). In contrast, 'Bamby' showed minimal variation in biomass yield across years (12.11 t ha^{-1} in 2015 and 12.6 t ha^{-1} in 2015), suggesting greater stability for this trait. The lowest biomass yield was recorded for genotype 'Heljda 1', which reached its lowest value in the 2016 season (11.28 t ha^{-1}). The analyzed buckwheat genotypes maintained consistent rankings in thousand grain weight across years, with higher values recorded in the 2015 season. Notably, genotype 'Novosadska' had the highest thousand grain weight, reaching 23.74 g in 2015 and 23.20 g in 2016, while the lowest values were observed in genotype 'Bamby', with 18.25 g in 2015 and 18.10 g in 2016. This consistency in rankings, along with greater variability among genotypes than between years, suggests that thousand grain weight is largely influenced by genetic factors. The differences between genotypes indicate considerable diversity within the studied population, supporting the idea that this trait can serve as an important selection criterion for breeding programs aimed at resilience in different climates. These findings are consistent with Janovská et al. (2021), who observed substantial variability in thousand grain weight in their study of 136 buckwheat accessions, underscoring its relevance in selecting genotypes for varied environmental conditions. Similarly, Rauf et al. (2020) found a high degree of heterogeneity in buckwheat germplasm, with thousand grain weight ranging from 21 to 42 g, further highlighting the trait's variability and potential for selection.

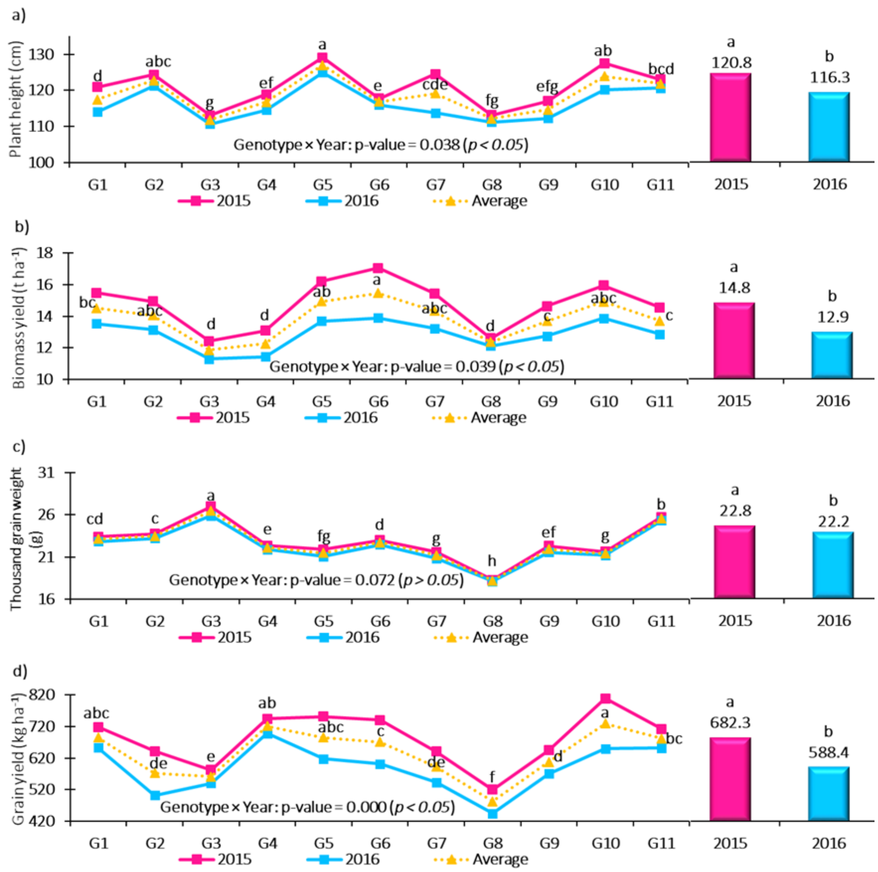

Figure 3. Phenotypic variation of buckwheat agromorphological traits in relation to genotype, year, and their interaction: G1 Godijevo; G2 Novosadska; G3 Heljda 1; G4 Heljda 2; G5 Darja; G6 Prekumurska; G7 Čebelica; G8 Bamby; G9 Češka; G10 La Harpe; G11 Spačinska. Different letters indicate differences ($p <$ 0.05) according Tukey test in the average values of genotypes and years, respectively.

In the 2016 season, genotype 'Heljda 2' achieved the highest grain yield among the tested genotypes, with 697.75 kg ha^{-1}, and exhibited minimal yield variation between the two seasons. In contrast, genotype 'Bamby' had the lowest yield in 2016, with 444.62 kg ha^{-1}. Notable reductions in grain yield between seasons were recorded for genotypes 'Novosadska', 'La Harpe', 'Prekumurska', and 'Darja', with decreases of 21.82%, 19.65%, 18.73%, and 17.85%, respectively, in 2016. Previous studies also reported substantial differences in buckwheat yield across regions and growing conditions. Kolarić et al. (2021) found significantly higher grain yields in buckwheat cultivated in Pančevo (Serbia), with yields ranging from 1.0 to 1.6 t ha^{-1} over two seasons. Similarly, Popović et al. (2014) found significantly higher average yields in conventional production for buckwheat 'Novosadska', 'Godijevo', 'Bamby', and 'Češka', grown in Bački Petrovac. Consistent with our findings, these authors observed that 'Bamby' had the lowest grain yield, while 'Češka' had the highest, falling within the average range in our study. The generally higher yields recorded in these studies can likely be attributed to the favorable agroecological conditions and higher soil fertility of the Vojvodina region, where the research was conducted. Rangappa et al. (2023a) emphasized that buckwheat performs best on lighter soils with neutral to slightly acidic pH levels, whereas heavier, wetter soils may increase the risk of lodging, negatively affecting yield. Globally, the average yield of buckwheat remains around 999.7 kg ha^{-1} (FAOSTAT, 2024). Over recent decades, buckwheat cultivation has declined worldwide, largely due to the crop's relatively low yield potential compared to other major crops (Popović et al., 2014; Farooq et al., 2016). One contributing factor to this lower yield potential is buckwheat's short growing period, as research has shown a positive correlation between longer growing periods and higher grain yields (Morishita and Tetsuka, 2001). Therefore, there is a need to select genotypes that balance yield stability and adaptability, especially in regions with diverse soil and climate conditions.

Stability performance of buckwheat grain yield

The identification of stable and high-yielding buckwheat genotypes stands as a central objective within buckwheat improvement programs (Kandel and Shrestha, 2019). The considerable Genotype-by-Environment interaction (GEI) for grain yield, along with the significance of IPCA1 and IPCA2 interaction components, suggests that a detailed examination of stability across environments is essential. This analysis is effectively visualized using the AMMI1 (mean *vs*. IPCA1) and AMMI2 (IPCA1 *vs*. IPCA2) biplots (Figure 4). Together, the first two principal interaction components account for as much as 95.31% of the total GEI, corroborating the findings of Li et al. (2021), who reported similar results with a study on 200 local populations of Tartary buckwheat.

Insights gained from the AMMI1 biplot (Figure 4a) illustrate distinct variations across environments, highlighting both additive and interaction-driven differences in grain yield. Among the environments, E1 exhibited both above-average yield and high yield stability, indicating favorable conditions for consistent performance. Conversely, environment E2 (830 m in 2015 season) although associated with the highest grain yield, presented the greatest instability among environments. In the AMMI1 biplot, both environments in 2016 season (E3 and E4) were positioned within the first quadrant, associated with below-average grain yields, thereby reflecting less favorable conditions for achieving high yields. Among the genotypes, 'La Harpe' and 'Heljda 2' emerged as high-yielding and stable, indicating their suitability across a range of conditions. This proximity to the origin and high mean values, highlights their overall adaptability, making them promising candidates for cultivation across a range of conditions within the trial. Similarly, Kandel and Shrestha (2019) also identify buckwheat genotypes that demonstrate stability and adaptability across different locations and recommend that for broader cultivation. Genotype 'Bamby', while yielding the lowest overall, demonstrated high stability. Genotypes 'Čebelica' and 'Češka' displayed average yield levels with stable performance, further affirming their adaptability across diverse environments. In contrast, 'Heljda 1', 'Spačinska', 'Novosadska', 'Prekumurska', and 'Darja' exhibited significant instability, with 'Spačinska', 'Prekumurska', and 'Darja' reaching above-average yields. The observed instability in these genotypes highlights their potential suitability for targeted environments rather than broad adaptability. Incorporating IPCA2 into the analysis has led to a refined understanding of genotype stability (Figure 4b). Genotype 'La Harpe', although marked by a low IPCA1 value, exhibits a high IPCA2 value, positioning it notably distant from the origin, yet demonstrating a favorable interaction with environment E1 (610 m in 2015 season). Similarly, genotype 'Heljda 2', also characterized by a low IPCA1 and high IPCA2 value, aligns well with environment E4 (830 m in 2016 season), suggesting its adaptability within that specific environment. The genotypes identified as unstable, 'Novosadska', 'Darja', and 'Prekumurska', present high IPCA1 but low IPCA2 values. Their placement in proximity to environment E2 (610 m in 2016 season) on the biplot suggests a positive interaction within this particular environment, which was favorable for achieving high grain yield. Genotypes 'Heljda' and 'Spačinska', which displayed considerable overall instability, showed a positive interaction with environment E3 (610 m in 2016), reflecting their specific affinity for favorable conditions. From this analysis, it can be concluded that certain genotypes display specific adaptability to particular environments. In contrast, genotypes 'Godijevo', 'Čebelica', 'Bamby', and 'Češka', with low values for both IPCA1 and IPCA2, were located near the origin, signifying a pronounced overall stability in grain yield across the different environments.

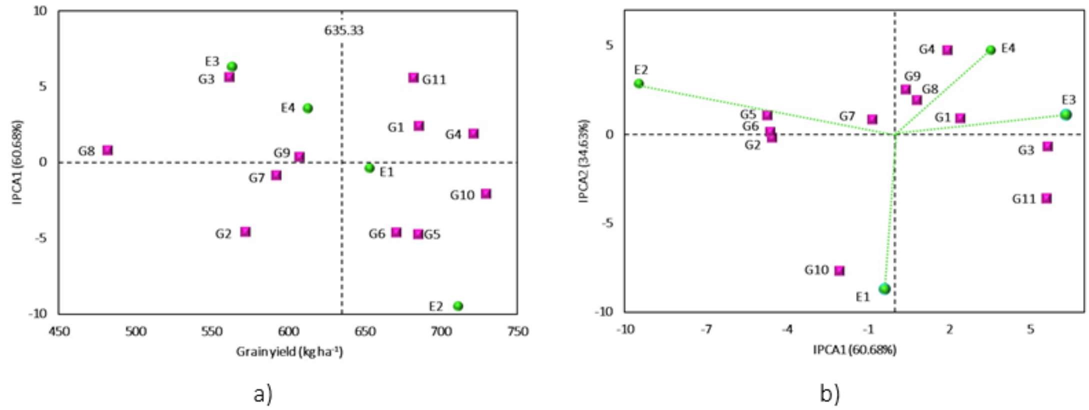

Figure 4. Additive main effect multiplicative interaction (AMMI) 1 (mean value *vs*. IPCA1) (a) and AMMI 2 (IPCA1 *vs*. IPCA2) biplot (b) for assessing the stability of 11 buckwheat genotypes, in terms of grain yield, grown across four environments. G1 Godijevo; G2 Novosadska; G3 Heljda 1; G4 Heljda 2; G5 Darja; G6 Prekumurska; G7 Čebelica; G8 Bamby; G9 Češka; G10 La Harpe; G11 Spačinska; elevation 610 m in 2015 (E1) and 2016 (E3); elevation 830 m in 2015 (E2) and 2016 (E4). $IPCA_1$: First interaction principal component axis; $IPCA_2$: second interaction principal component axis.

Interconnection among traits and genotypes

A heatmap cluster analysis was conducted, separately for each tested environment, which clusters both traits (columns) and genotypes (rows) in a same time. Different shades of pink on the heatmap graph represent above-average values for the given trait, while white indicates average values, and various intensities of green represent below-average values of the trait. Stronger shades of pink correspond to higher values, whereas stronger shades of green indicate lower values (Figure 5).

The heatmap cluster analysis conducted for both tested environments revealed consistent patterns of trait clustering across elevations, shaped by seasonal climatic conditions. In the drier 2015 season, a distinct cluster was observed for thousand grain weight at both elevations, while traits such as plant height, biomass yield, and grain yield were grouped together within the same cluster (Figures 5a and 5c). The strong positive association between plant height and grain yield can be attributed to the plants' ability to remain upright under limited rainfall, allowing for enhanced vegetative growth and more efficient resource utilization to support yield. These findings align with Morishita and Tetsuka (2001), who reported a significant positive correlation between plant height and seed yield in buckwheat. Similarly, Romanovskaja et al. (2022) highlighted strong positive correlations between buckwheat grain yield and biomass yield under organic farming conditions. Under these conditions, genotype 'Heljda 1' formed a distinct cluster, characterized by high thousand grain weight but low values for all other traits. Additionally, genotype 'Bamby' also clustered separately, exhibiting low values for most traits. In contrast, genotypes 'Darja' and 'La Harpe' clustered together, consistently showing high values for plant height, biomass yield, and grain yield, except for thousand grain weight. This suggests that 'Darja' and 'La Harpe' possess traits suitable for maximizing productivity under limited rainfall conditions.

In the 2016 season, traits clustered into two main groups at both elevations: Grain yield and thousand grain weight in one cluster, and plant height and biomass yield in another (Figures 5b and 5d). This pattern indicates a negative correlation between plant height and biomass yield with grain yield and thousand grain weight, likely caused by lodging due to excessive rainfall. Lodging, which reduces plant stability, negatively impacts the translocation of resources towards grain development, thereby lowering yield and grain quality. A similar negative correlation between plant height and thousand grain weight in buckwheat was observed by Janovská et al. (2021). At the lower elevation in 2016, genotypes 'Darja', 'La Harpe', and 'Novosadska' exhibited high values for plant height and biomass yield (Figure 5b). These genotypes also maintained above-average values for these traits at the higher elevation, demonstrating their broad adaptability (Figure 5d). Genotype 'Spačinska' formed a unique cluster at the lower elevation, grouping with genotypes 'Heljda 1' and 'Heljda 2', and was characterized by high grain yield but below-average biomass yield (Figure 5b). At the higher elevation in the

2016 season, 'Heljda 1' and 'Heljda 2' demonstrated specific clustering patterns. 'Heljda 1' showed the highest thousand grain weight but very low biomass yield, while 'Heljda 2' exhibited the highest grain yield with the lowest biomass yield (Figure 5d). These results suggest that these genotypes may be particularly suited for environments where balancing grain quality and yield is critical. Across all environments, genotype 'Bamby' consistently displayed the lowest average values for all traits.

Based on the conducted analysis, it is evident that plant responses to agroclimatic conditions shape the association between genotypes and traits. Genotype 'Heljda 1' is a promising genetic resource for enhancing thousand grain weight. This genotype, along with 'Heljda 2', demonstrated specific adaptability to high-rainfall conditions. Genotypes 'Darja' and 'La Harpe' consistently performed well across all environments, making them candidates for broader cultivation.

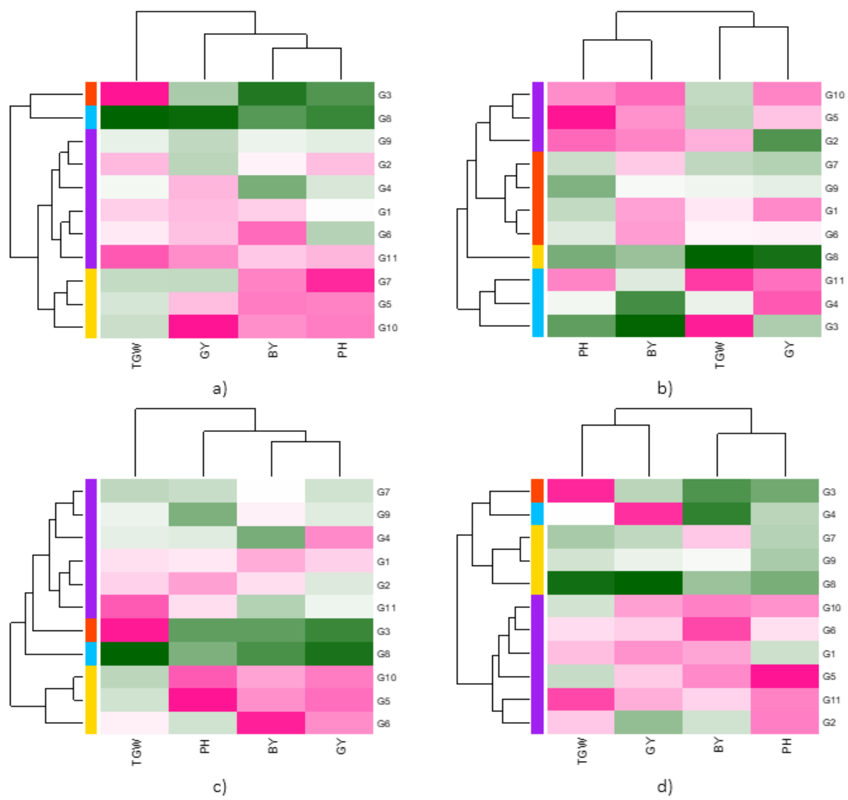

Figure 5. Heatmap cluster analysis for analyzed traits of 11 buckwheat genotypes grown in different environments. TGW: Thousand grain weight; PH: plant height; BY: biomass yield; GY: grain yield; G1 Godijevo; G2 Novosadska; G3 Heljda 1; G4 Heljda 2; G5 Darja; G6 Prekumurska; G7 Čebelica; G8 Bamby; G9 Češka; G10 La Harpe; G11 Spačinska; elevation 610 m in 2015 (a) and 2016 (b), elevation 830 m in 2015 (c) and 2016 (d).

CONCLUSIONS

This study revealed substantial phenotypic variability among 11 European buckwheat genotypes, highlighting the importance of genotype, environmental factors, such as elevation and season in shaping agromorphological traits. Environmental factors accounted for the largest share of variation in biomass and grain yield, while the thousand grain weight was the least affected by changes in environmental conditions. Lower elevation (610 m) favored vegetative growth under both dry and high-rainfall conditions but had a limiting effect on grain yield and thousand grain weight. Traits reached higher values during the drier growing season, as excessive rainfall led to lodging, which negatively impacted plant growth, grain yield, and grain quality. Grain yield is a reliable phenotypic indicator for evaluating the stability of the genotypes due to its sensitivity to genotype-environment interactions. The genotypes 'La Harpe' and 'Heljda 2' exhibited consistent performance and high yields across environments, while 'Novosadska', 'Darja', and 'Prekumurska' adapted well to the drier conditions at higher elevation (830 m). In seasons with higher rainfall, 'Heljda 1' demonstrates high grain quality, while 'Heljda 2' achieves high grain yield, with both varieties characterized by low plant height, making them well-suited for ecological farming systems in moisture-rich environments. These findings provide valuable insights for the selection of suitable buckwheat genotypes for cultivation in specific agroecological conditions, supporting the integration of this underutilized crop into sustainable agricultural production systems.

Author contribution

Conceptualization: M.V., V.Z. Methodology: M.V., V.Z., J.B., S.A.L. Software: M.V., M.M.S., M.S. Validation: V.Z., J.B., K.L. Formal analysis: M.V., J.B., K.L. Investigation: M.V., V.Z. Resources: M.V. Data curation: M.V., J.B., S.A.L. Writing-original draft: M.V., V.Z., M.M.S. Writing-review & editing: M.V., M.M.S., V.Z., M.S. Visualization: M.V., M.M.S., M.S., K.L. Supervision: V.Z. Project administration: V.Z. Funding acquisition: M.V. All co-authors reviewed the final version and approved the manuscript before submission.

Acknowledgements

We acknowledge the support of the management of the Municipality of Bijelo Polje during the implementation of this research. This research was financially supported by the Ministry of Science, Technological Development, and Innovation of the Republic of Serbia, grant numbers: 451-03-136/2025-03/200054 and 451-03-136/2025-03/200216.

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