Full Length Article

A study of the morphological and geographical diversity of Korean indigenous buckwheat landraces for breeding

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A B S T R A C T

This study was carried out to explore the significance of morphology-based selection for breeding, using a case study of indigenous buckwheat landrace from Korea. Although many studies have utilized molecular methods to investigate germplasm diversity, this research underscores the often-overlooked importance of morphology-based selection in breeding strategies. Furthermore, it highlights the necessity of considering geographical information only when it accurately reflects the actual environment. The study collected and analyzed five traits from 96 samples across 27 varieties. The hypothesis was that these 27 samples might have been grouped by region due to similar environmental conditions. This hypothesis was tested using a dendrogram, clustering, correlation analysis of each trait, and PCA analysis. The results showed that morphological traits are a crucial factor in germplasm or landrace selection and that they should be considered the basic information for breeding purposes. Morphological traits were found to be random regardless of the locations where they were collected, indicating that they should be used to determine breeding targets rather than geographic locations. The study suggested that understanding the physiological traits of specific germplasm groups can accelerate breeding efforts. Overall, this study provided important insights into the selection of germplasm or landrace for breeding purposes, highlighting the importance of morphology-based selection and the need to consider environmental factors.

1. Introduction

Numerous studies have examined germplasm diversity using molecular approaches, especially next-generation sequencing technologies (Dar et al., 2018; Yasui, 2020). Indeed, they revealed important differences and even the evolutionary lineages to facilitate better breeding strategies (Dar et al., 2018; Yabe & Iwata, 2020). However, the breeding is based on the traits, not the DNA itself (Varshney & Dubey, 2009; Singh et al., 2020). Although genotype by the environment is essential for breeding targeting a wide area, it could be studied better using molecular information but the phenotype in the given local is crucial for local breeding (Karunathilake et al., 2023; Sheikh et al., 2024; Mansoor and Chung, 2024a; Mansoor et al., 2024b). The importance of geographical information is repeatedly emphasized for collecting germplasm or landraces (Engels et al., 1995). It is because geographical information is believed to provide the environmental information responsible for the genetic diversity associated with morphological diversity. However, it is not always true since the earth's surface is dynamically different, generating numerous kinds of environments. Thus, the selection for germplasm or landrace should be based on morphological information rather than geographical one.

Buckwheat is pseudocereal which is cultivated and consumed in limited regions of the world, such as Eastern Europe and eastern Asia (Ikeda, 2002; Suzuki et al., 2020). However, buckwheat has many health-

beneficial ingredients, for instance, flavonoids, dietary fiber, resistant starch, various minerals, and vitamins (Cui et al., 2019; Luthar et al., 2021b; Ruan et al., 2022). In addition, because of the recent rapid climate change and instability of the international situation, discovering new grain cultivars which are growing fast and stable is becoming essential. Because buckwheat is resistant to environmental changes and can be harvested quickly, there are historical examples of it being used as a substitute crop when crop failures occur (Park & Chang, 2002). Because of these reasons, buckwheat has many reasons to breed new cultivars for the future. However, the effort for breeding buckwheat is lacking, and collecting various genetic resources is insufficient. To add the effort for buckwheat breeding, the current study collects and analyzes five traits from a total of 96 samples of 27 varieties. Our hypothesis is 27 varieties might be grouped by region because the close region might have similar environments. We are going to test the hypothesis by a dendrogram, clustering, correlation of each trait, and PCA analysis. This research is a case study that demonstrates the importance of morphology-based selection using buckwheat (*Fagopyrum esculentum)* landrace indigenous to Korea. Also, the perspectives from the current study might be possibly applied to other crop genetic resource collections.

2. Materials and methods

2.1. Plant materials

Twenty-seven landraces of buckwheat (*F. esculentum*) from various provinces in Korea were randomly selected (Table 1). The collection of the germplasms was used in this experiment reflected the passport information released by the germplasm management system (GMS) held by the National Agrobiodiversity Center (NAC). Each landrace consisted of 4 plants in each pot (inner diameter 16 cm, height 12 cm), in which 80 % of the pot space was filled with 430 g of artificial soil (Dduksim-e, Nongwoo Bio Co., Ltd) at the glasshouse of Jeju National University from 5 May 2019, to 9 July 2019. Those pots were completely randomized. The average temperature of the glasshouse was kept from 28 °C to 32 °C, and all plants were evenly watered in the amount of 200 ml at 09:00 AM and 4:00 PM twice. The height was measured using a tape measure from the ground to the terminal bud, and the number of stem nodes was obtained immediately after the flowering date. The flowering date of each buckwheat (*F. esculentum*) variety was determined by the date that half of the flower buds bloomed on individual plants. However, 12 landraces (IT101120, IT105699, IT108752, IT111123, IT113084, IT113582, IT185685, IT185687, IT191108, IT208826, IT210198, and IT250616) survived three replicates during the growth period.

2.2. Statistical analysis

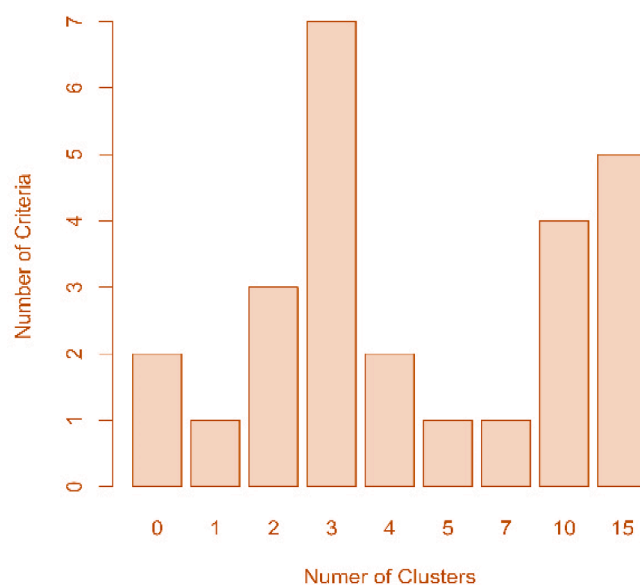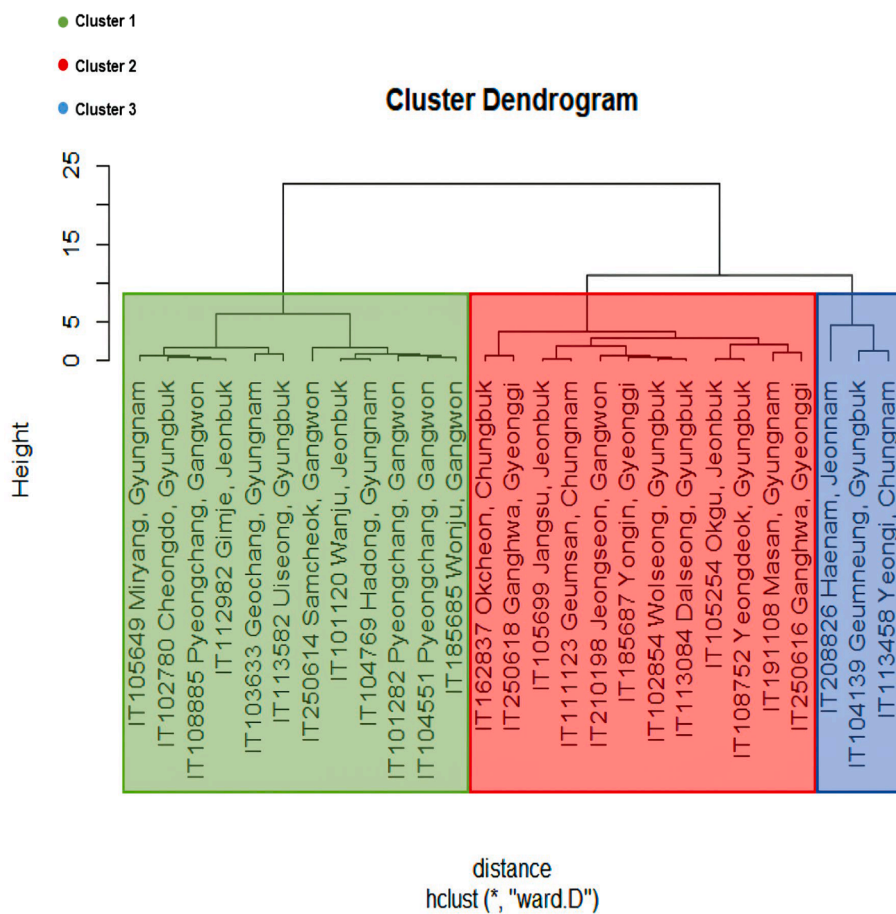Plant height, the number of totals, main, side node, and flowering date data of the buckwheat (*F. esculentum*) were used in statistical analysis. The statistical analysis was analyzed by R software (Ver. 4.1.2, the R foundation for statistical computing, Vienna, Austria). The data were standardized before the cluster analysis using the "caret" package in R software, with Euclidean distance used for clustering. The analysis was performed step by step, beginning with the determination of the best number of clusters and the application of k-means clustering by "NbClust" packages. A dendrogram was then created, and the results were merged for easier understanding. Before the comparison, each buckwheat (*F. esculentum*) variety's traits among and within the clusters were confirmed normality by the Shapiro-Wilk normality test (Shapiro & Wilk, 1965). Some of the data sets failed to satisfy normality. Therefore, The Kruskal-Wallis test was used for data analysis (Kruskal et al., 2014). Spearman's rank correlation coefficient test (Spearman, 2010) was conducted between measured variables (Plant height, the number of totals, main, side node, and flowering date) by using the "Hmisc" package. Additionally, principal component analysis (PCA) was performed with the same package to summarize the relationships among plant height, side node, total node, main node, and flowering date.

3. Results

The total number of nodes was comprised of the number of main and side nodes. More nodes meant that more flowering could occur, which was associated with the yield. The main nodes were attached to the main stem, which was related to plant height, although the length between nodes was also associated with plant height. They characterized vertical traits. On the other hand, the side nodes came from diverged branches of the main nodes. Thus, they determined the horizontal shapes. Nbclust revealed that twenty-seven landraces with five agronomic traits such as height, number of total, main, side nodes, and flowering date were grouped into three clusters. (Fig. 1). The cluster dendrogram based on Nbclust presented clusters 1, 2, and 3 in green, red, and blue color, respectively (Fig. 2). It was confirmed by the Kruskal-Wallis rank sum test, which showed that every trait observed in the current study did not have a difference within each cluster except for a minor difference in flowering date in cluster 3, unlike the results without clustering (Table 2). The results of Principal Component Analysis (PCA) indicated that the variance sources and the factors responsible for this clustering could be grouped into two: one was the number of side nodes and total nodes, and the other was the number of main nodes, flowering date, and

Table 1
Buckwheat sampled region and IT number.

IT Number	Origin	IT Number	Origin
IT250614	Samcheok, Gangwon	IT102854	Wolseong, Gyungbuk
IT185685	Wonju, Gangwon	IT113582	Uiseong, Gyungbuk
IT210198	Jeongseon, Gangwon	IT102780	Cheongdo, Gyungbuk
IT101282	Pyeongchang, Gangwon	IT250616	Ganghwa, Gyeonggi
IT104551	Pyeongchang, Gangwon	IT250618	Ganghwa, Gyeonggi
IT108885	Pyeongchang, Gangwon	IT208826	Haenam, Jeonnam
IT185687	Yongin, Gyeonggi	IT112982	Gimje, Jeonbuk
IT103633	Geochang, Gyungnam	IT105254	Okgu, Jeonbuk
IT191108	Masan, Gyungnam	IT101120	Wanju, Jeonbuk
IT105649	Miryang, Gyungnam	IT105699	Jangsu, Jeonbuk
IT104769	Hadong, Gyungnam	IT111123	Geumsan, Chungnam
IT104139	Geumneung, Gyungbuk	IT113458	Yeongi, Chungnam
IT113084	Dalseong, Gyungbuk	IT162837	Okcheon, Chungbuk
IT108752	Yeongdeok, Gyungbuk		

Fig. 1. Nbclust result. Determining the best number of clusters in a buckwheat data set with 27 varieties with five traits (height, number of total, main, side nodes, and flowering date).

Fig. 2. The result of Cluster Dendrogram and K-means clustering results. Cluster dendrogram of 27 varieties of buckwheat's five traits (plant height, number of totals, main, side node, and flowering date) by ward.D method. The green, red, and blue background represents clusters 1, 2, 3 by K-means clustering, respectively.

Table 2
Kruskal-Wallis rank sum test *P*-values at buckwheat five traits among and within clusters.

Source	Df	Height	Total node number	Side node number	Main node number	Flowering date
All [1]	26	0.00 ***, [3]	0.00 ***	0.00 ***	0.00 ***	0.00 ***
Cluster 1 [2]	11	0.09 NS	0.47 NS	0.12 NS	0.47 NS	0.16 NS
Cluster 2	11	0.81 NS	0.91 NS	0.55 NS	0.85 NS	0.21 NS
Cluster 3	2	0.07 NS	0.34 NS	0.31 NS	0.31 NS	0.04 *

[1] Among buckwheat clusters.
[2] Within each cluster.
[3] *Significant at 0.05, ** Significant at 0.01, *** Significant at 0.001 probability level, and NS, nonsignificant at $P<0.05$.

plant height (Fig. 3). The direction of vectors of all traits was toward the right side, indicating they were indeed the factors that differentiated those landraces, although the number of total nodes and side nodes was almost overlapped. The magnitude of PC1 responsible for the variances of all traits was 80.56 %, while they were split into two directions, upward and downward. However, this split explained a relatively small magnitude of 13.30 %. Overall, the results of PCA elucidated that plant height, number of nodes, and flowering date could explain the variances of those landraces used in the current study.

The correlations among five traits across all clusters, specifically clusters 1, 2, and 3, exhibited distinctive characteristics, which were crucial because these differences determined plant architecture and harvest time (Table 3). In all clusters, the correlations among all traits were significant, ranging from 0.47 to 1.00, and were statistically significant. All observed correlations were positive.

In cluster 1, the positive correlations between plant height and the number of main nodes, and between the number of total nodes and side nodes, were 0.80 and 1.00, respectively; both were statistically

significant. In cluster 2, the positive correlations between plant height and the number of total nodes, main nodes, and side nodes were 0.61, 0.76, and 0.60, respectively; between the number of total nodes and side nodes were 0.69 and 1.00, respectively; and between the number of main nodes and side nodes was 0.66, all of which were statistically significant. In cluster 3, the positive correlations between the number of total nodes and side nodes, and flowering date were 0.99 and 0.66, respectively; between the number of main nodes and flowering date was 0.61; and between the number of side nodes and flowering date was 0.64; all were statistically significant.

Based on the results of the correlations in clusters, the morphological characters could be described as follows. For all clusters, taller landraces highly tended to have more main nodes and delayed flowering. Interestingly, those landraces that had a greater number of main nodes were highly inclined to delay flowering. For cluster 1, the taller plants tended to have more main nodes as with all clusters, while the number of side nodes and flowering date were not related.

(a)

(b)
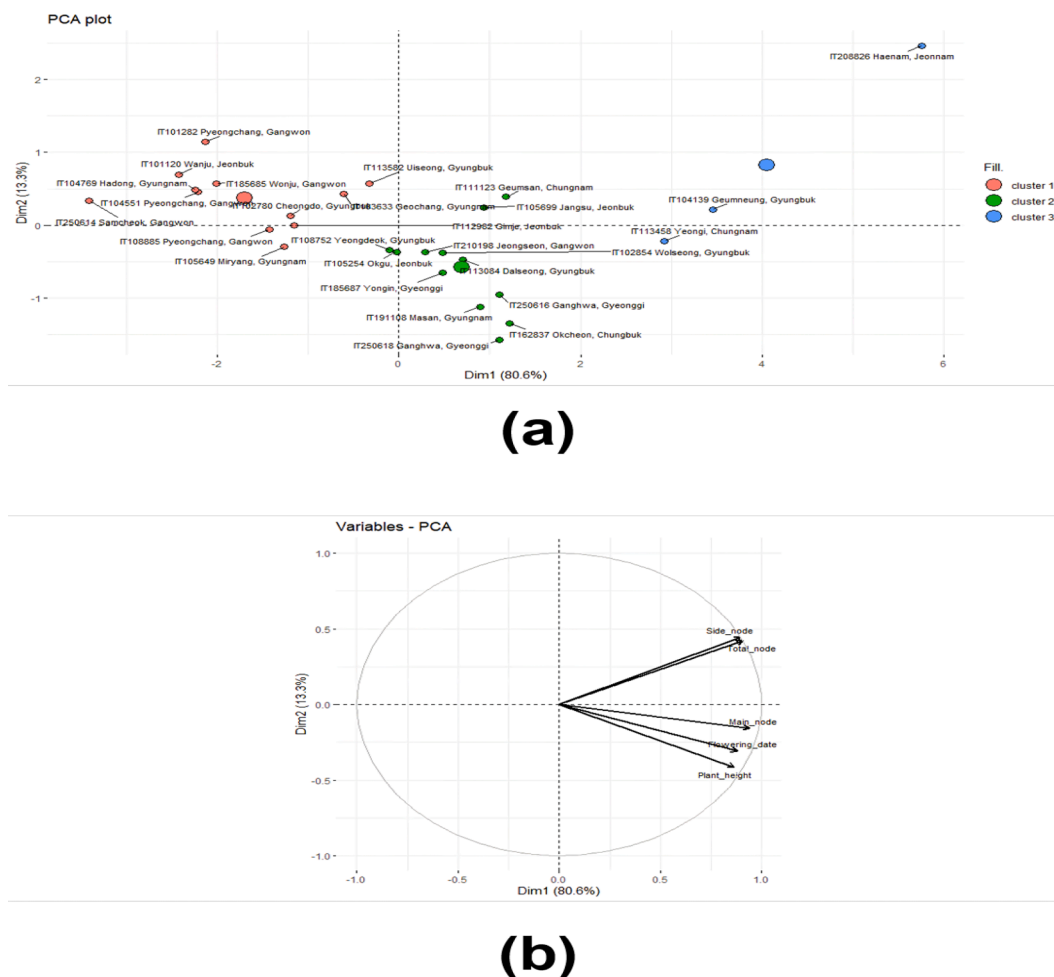
Fig. 3. Result of PCA analysis with data set with 27 varieties with five traits (height, number of total, main, side nodes, and flowering date). (a) PCA plot with each sample and cluster. The big dot in the figure means the cluster region. (b) PCA plat with variable *ar*-rows.

Moreover, the relationship between the number of main nodes and side nodes suggested that the architectural structure of the landraces could not be easily determined. It was possible that some landraces had a narrow architecture with fewer side nodes, while others exhibited a bush-like architecture with a higher number of side nodes. In Cluster 2, the landraces exhibited similar patterns to Cluster 1 in terms of plant height and four other traits. However, they tended to have more total nodes and side nodes when they had a higher number of main nodes, indicating a greater number of branches both vertically and horizontally. This suggested that these landraces were more likely to have a bush-like architecture.

Cluster 3, on the other hand, showed a tendency for landraces to have more side nodes when they had more main nodes. Consequently, an increase in side nodes led to a higher total number of nodes. Interestingly, there was no correlation between plant height and the number of main nodes in this cluster, suggesting that the length of internodes in the main stem might have varied among these landraces. Additionally, the fact that the number of main nodes was not correlated with the total number of nodes but with the number of side nodes indicated a bush-like architecture for these landraces.

Across all clusters (Cluster 1, 2, and 3), landraces with a higher total number of nodes tended to have more side nodes. This highlighted the importance of side nodes for yield, considering that flowering occurred at the nodes. Therefore, the number of side nodes became a crucial factor to consider for maximizing yield (Fig. 4).

4. Discussion

Buckwheat is a crop with significant genetic diversity, and studying this diversity allows for the identification and conservation of unique and valuable genetic traits. Preserving genetic diversity is crucial for future crop improvement, as it provides a pool of genetic resources that can be tapped into to develop new varieties with improved traits, such as disease resistance, tolerance to environmental stress, and higher yields (Park & Chang, 2002). Understanding buckwheat diversity enables breeders to make informed decisions in their efforts to develop improved varieties. By studying the various landraces and their morphological characteristics, breeders can identify traits that are desirable for specific purposes, such as higher yield, better nutritional composition, or adaptability to different environmental conditions. This knowledge can then be used in breeding programs to develop new varieties that meet the needs of farmers, consumers, and the industry (Park & Chang, 2002; Chauhan et al., 2010; Zhang et al., 2017; Zhou et al., 2018).

There are lots of phenotypes to consider for buckwheat breeding (Alekseyeva, 2002; Chauhan et al., 2010; Zhang et al., 2017; Zhou et al., 2018; Sun et al., 2020). However, the current study used five of them. The flowering date is not responsible for the architectural traits; however, it is a crucial phenotype for determining early or late harvest (Luthar et al., 2021a). Those groups are admixture in the Korean peninsular. This means that morphological diversity did not come from geographical differences. Buckwheat cultivation in Korea is speculated to be started before BC 5 (Chen et al., 2018). More landraces could be introduced from abroad even after that. Thus, the current landrace could

Table 3
Spearman's rank correlation among plant height, number of total nodes, main nodes, side nodes, and flowering date.

		Total node number	Main node number	Side node number	Flowering date
Plant height	All	0.60***, [1]	0.86***	0.58***	0.73***
	Cluster 1	0.31*	0.80***	0.27*	0.42**
	Cluster 2	0.61***	0.76***	0.60***	0.56***
	Cluster 3	−0.24NS	−0.05NS	−0.25NS	−0.50NS
Total node number	All		0.65***	1.00***	0.50***
	Cluster 1		0.27*	1.00***	0.08NS
	Cluster 2		0.69***	1.00***	0.25NS
	Cluster 3		0.40NS	0.99***	0.68*
Main node number	All			0.61***	0.75***
	Cluster 1			0.22NS	0.40*
	Cluster 2			0.66***	0.57***
	Cluster 3			0.31NS	0.61*
Side node number	All				0.47***
	Cluster 1				0.06NS
	Cluster 2				0.23NS
	Cluster 3				0.64*

[1] *Significant at 0.05, ** Significant at 0.01, *** Significant at 0.001 probability level, and NS, nonsignificant at $P < 0.05$.

not be from one species. However, the current landraces are the results of the selection by natural environments and human beings to result in the maximum results yield, which can be affected by the morphological traits in the given environment. Numerous factors, such as light, temperature, soil shear resistance, wind, etc., affect the morphologies of plants (S. Lee & Ham, 1992; Clark & Bullock, 2007; Treadwell & Huang,
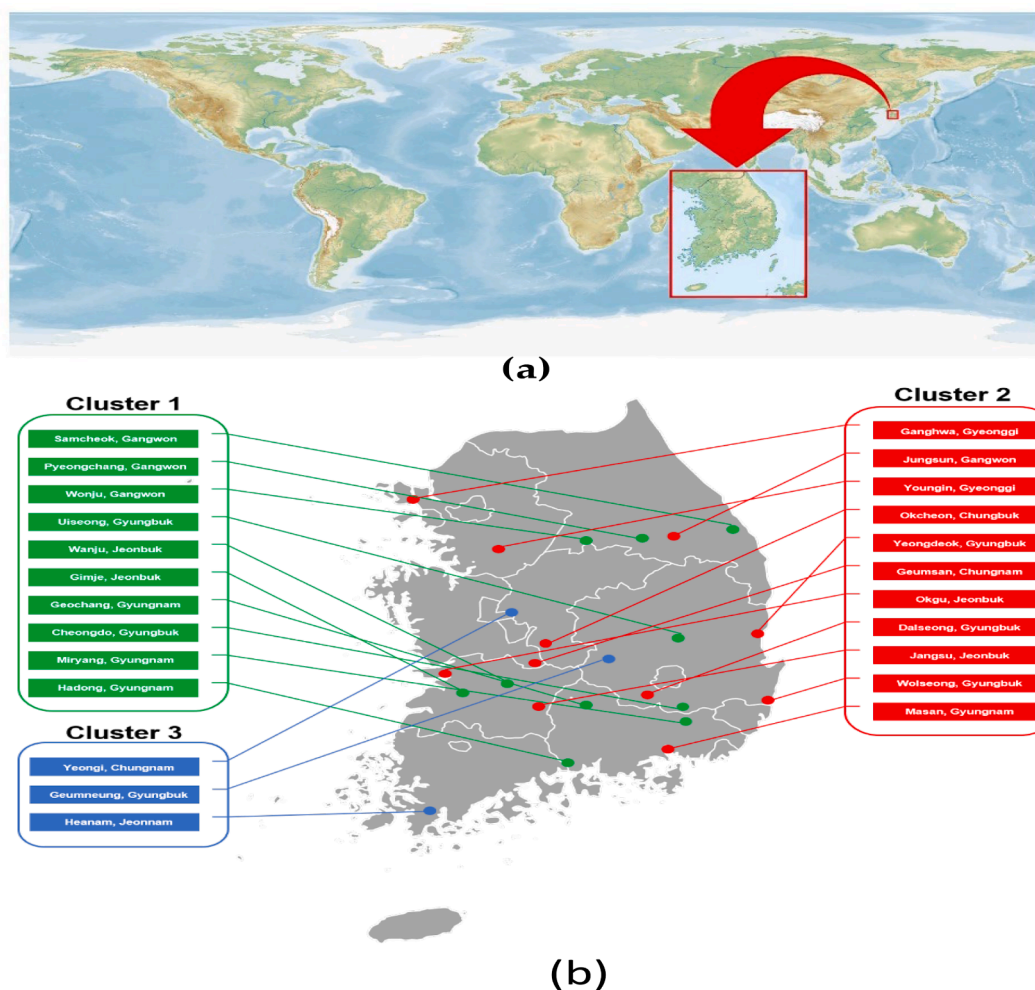
Fig. 4. Buckwheat sample location map. (a) The geographic location of Korea on the world map; (b) buckwheat sampling location in Korea.

2008; Kalaitzoglou et al., 2019). Plant architecture is one of those various morphological traits. Many factors could also affect it (Luthar et al., 2021a). One of the most important factors could be sunlight (Gardiner et al., 2016; Kumar et al., 2020). If plants need a certain number of nodes that is directly related to the number of leaves and yield to have successful photosynthesis, they have to develop a strategy to place their nodes vertically or horizontally. The side node is a horizontal trait in the plant architecture. No matter how the nodes are developed, the number of total nodes is highly associated with the number of side nodes regardless of the number of main nodes and plant height. It might be because of the adaptation to receive the light in the given environments. The Korean peninsula is about 1,100 km in length and 300 km in width. This length is long enough to affect the day length of some crops, such as soybean (Ballaré et al., 1996; Patil et al., 2001). If the day length affects the flowering date, there should be a clear pattern on the map. However, the flowering date difference was random. The reason for this could be found in the buckwheat cultivation practice. Buckwheat is not cultivated on the fertile soil in the field. Rather, it is grown in the barren area on the slopes of the mountains or in the poor soil near the field. Even within the same area, the mountain's daylight length is much shorter than the plane field. This could create a random day long regardless of the latitude in the 1,100 km long area. Indeed, the Korean peninsular has lots of mountains and rivers that create various environments, although it is not large (Lee et al., 2019; Nawaz et al., 2020). These randomly created differences could affect the evolution/adaptation of the buckwheat landraces to form the current morphology in each location. The flowering date, one of the other morphological traits in the current study, could be grouped into three clusters. However, there was no pattern geologically. studying buckwheat diversity we can identify landraces or genetic traits that are more resilient and adaptable to changing conditions. This information can be used to develop climate-smart varieties that can thrive in different regions, have increased resistance to pests and diseases, and tolerate extreme weather events, ultimately ensuring food security in the face of environmental challenges. Thus studying buckwheat diversity, we can gain a deeper understanding of its cultural significance and contribute to its sustainable production and utilization, benefiting local communities and economies.

5. Conclusion

The results in the current study imply the following. First, the landrace or germplasm should be chosen based on the given crop's important morphological traits, not geographical information. Instead, environmental diversity should be considered since there could be diverse environments with no pattern in the given area, even though small, like the Korean peninsular. Second, the utilization of certain germplasm or landrace needs to examine the groups based on morphological or other physiological traits because there could be a required combination of traits within that group to accelerate breeding speed to stack those traits into the new breeding cultivar.

Consent to participate

All authors consent to participate in the manuscript publication.

Consent for publication

All authors approved the manuscript to be published.

Ethics approval

Not applicable.

Data availability statement

The raw data is available when requested from the author.

CRediT authorship contribution statement

Gyung Deok Han: Writing – original draft, Software, Methodology, Investigation, Data curation. **Sheikh Mansoor:** Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation. **Jaeyoung Kim:** Visualization, Methodology, Data curation. **Jieun Park:** Writing – review & editing, Validation, Methodology, Data curation. **Seong Heo:** Visualization, Formal analysis, Data curation. **Ju-Kyung Yu:** Visualization, Validation, Investigation, Formal analysis, Data curation. **Seong Hoon Kim:** Visualization, Formal analysis, Data curation. **Yong Suk Chung:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contribution

GDH and JK conducted experiments and preliminary data analyses; SM, JK, JP, SH, JY, SHK and YSC analyzed the data and wrote the draft of the manuscript. SHK and YSC. conceived and supervised the project. All authors were involved in editing the manuscript

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2024.103387.

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