A Study of the Morphological and Geographical Diversity of Korean Indigenous Buckwheat Landraces for Breeding

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Abstract

This study examines the importance of morphology-based selection for breeding using a case study of indigenous buckwheat landrace from Korea. While numerous studies have used molecular approaches to examine germplasm diversity, this study highlights the crucial role of morphology-based selection, which is often overlooked in breeding strategies. Additionally, the study emphasizes the need to consider geographical information only when it reflects the actual environment. The results show 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 suggests that understanding the physiological traits of specific germplasm groups can accelerate breeding efforts. Overall, this study provides 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.

Keywords: buckwheat breeding; cluster analysis; landrace; phenotypic variation; principal component analysis;

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 (Singh et al., 2020; Varshney & Dubey, 2009). Although genotype by the environment is essential for breeding targeting a wide area, it could be studied better using molecular information. The phenotype in the given local is crucial for local breeding. The importance of geographical information is repeatedly emphasized for collecting germplasm or landraces (Engels, 1955). 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 geological 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, Golob, et al., 2021; 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 samples might be grouped by region because the close region might have similar environments. We are going to confirm 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.

Materials and Methods

Plant materials

Twenty-seven landraces of buckwheat (*F. esculentum*) from various provinces in Korea were randomly selected (Table 1). The collection of the germplasms 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 replications in each pot (inner diameter 16 cm, height 12 cm), in which 80 % of the pot space was filled with 430 grams 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.

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 data (Plant height, the number of totals, main, side node, and flowering date) by using the "Hmisc" package.

Results

The total number of nodes is comprised of the number of main and side nodes. More nodes mean that more flowering could occur, which is associated with the yield. The main nodes are attached to the main stem, which is related to plant height, although the length between nodes is also associated with plant height. They characterize vertical traits. On the other hand, the side nodes are from diverged branches of the main nodes. Thus, they determine the horizontal shapes. Nbclust revealed that twenty-seven landrace with five agronomic traits such as height, number of total, main, side nodes, and flowering date is grouped into three clusters (Figure 1). The cluster dendrogram based on Nbclust presents clusters 1, 2, and 3 in green, red, and blue color, respectively (Figure 2). It is confirmed by the Kruskal-Wallis rank sum test showing that every trait observed in the current study does not have a difference within each cluster except a minor difference in flowering date in cluster 3, unlike the results without clustering (Table 2). The results of Principal component analysis (PCA) indicate that the variance sources and the factors responsible for this clustering can be grouped into two; one is the number of side nodes and total nodes, and the other is the number of main nodes, flowering date, and plant height (Figure 3). The direction of vectors of all traits is toward the right side, indicating they are indeed the factors to differentiate those landraces, although the number of total nodes and side nodes is almost overlapped. The magnitude of PC1 responsible for the variances of all traits is 80.56 %, while they are split into two directions, upward and downward. However, this split explains a relatively small magnitude, 13.30 %. Overall, the results of PCA elucidate that plant height, number of nodes, and flowering date can explain the variances of those landraces used in the current study.

The correlations among five traits in all-cluster; clusters 1, 2, and 3 have distinctive characters, which is important because different characters determine the architecture and harvest time (Table 3). For all clusters, the correlations among all traits are significant, ranging from 0.47 to 1.00. In cluster 1, the correlations between plant height and the number of main nodes; the correlations between the number of total nodes and side nodes were high (0.80 and 1.00, respectively). In cluster 2, the correlations between plant height and the number of total nodes, main nodes, and side nodes (0.61, 0.76, and 0.60, respectively), between the number of total nodes and side nodes (0.69 and 1.00, respectively), and between the number of main nodes and side nodes (0.66) were medium to high. In cluster 3, the correlations between the number of total nodes and side nodes and flowering date (0.99 and 0.66, respectively), between the number of main nodes and flowering date (0.61), and between the number of side nodes and flowering date (0.64) was high. Based on the results of correlations in clusters, the morphological characters could be described as followings. For all-cluster, taller landrace highly tends to have more main nodes and late flowering. Interestingly, those landraces that have more number of main nodes highly tend to delay flowering. Overall, when these tall landraces have more main nodes with a medium number of side nodes with late flowering. In addition, they may or may not have a more number of side nodes meaning the architecture could be narrow. However, the morphological characters are changed when those clusters are grouped. For cluster 1, the taller plants tend to have more number of main nodes as all-cluster, while the number of side nodes and flowering date are not related.

Moreover, the relationship between the number of main nodes and side nodes suggests that the architectural structure of the landraces cannot be easily determined. It is possible that some landraces have a narrow architecture with fewer side nodes, while others exhibit a bush-like architecture with a higher number of side nodes.

In Cluster 2, the landraces exhibit similar patterns to Cluster 1 in terms of plant height and four other traits. However, they tend to have more total nodes and side nodes when they have a higher number of main nodes, indicating a greater number of branches both vertically and horizontally. This suggests that these landraces are more likely to have a bush-like architecture.

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

Across all clusters (Cluster 1, 2, and 3), landraces with a higher total number of nodes tend to have more side nodes. This highlights the importance of side nodes for yield, considering that flowering occurs at the nodes. Therefore, the number of side nodes becomes a crucial factor to consider for maximizing yield. (Figure 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 (Chauhan et al., 2010; Park & Chang, 2002; Zhang et al., 2017; Zhou et al., 2018).

There are lots of phenotypes to consider for buckwheat breeding (Alekseyeva, 2002; Chauhan et al., 2010; Sun et al., 2020; Zhang et al., 2017; Zhou et al., 2018). 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, Fabjan, et al., 2021). 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 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 (Clark & Bullock, 2007; Kalaitzoglou et al., 2019; S. Lee & Ham, 1992; Treadwell & Huang, 2008). Plant architecture is one of those various morphological traits. Many factors could also affect it (Luthar, Fabjan, et al., 2021). 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 (E. M. 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, researchers 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, researchers can gain a deeper understanding of its cultural significance and contribute to its sustainable production and utilization, benefiting local communities and economies.

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.

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