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ABSTRACT

Common buckwheat (Fagopyrum esculentum), a pseudo-cereal crop initially grown in Southern China, belongs to the Polygonaceae family. It has been cultivated extensively in Asia, America, and Europe, exhibiting traits like out-crossing and self-incompatibility. This review aims to consolidate studies on buckwheat's genetic diversity and population structure, utilizing a range of morphological and genetic traits for analysis. Genotyping is pivotal for pinpointing and assessing genes that offer agronomic benefits, and for comprehending population structures and allele frequency variations. Linkage models were first established in the 1980s using allozyme and morphological markers. Common buckwheat displays variations in its morphological traits, potentially attributable to its out-crossing behavior, also referred to as self-incompatibility. Allozyme markers were widely employed in population genetic research until the early 2000s. Conversely, RAPD analysis utilizes short 11 bp DNA fragments, amplified by PCR using RAPD primers at low annealing temperatures to facilitate DNA binding. The evolution of PCR technology spurred the development of diverse DNA marker schemes for linkage mapping in the 2000s. Nonetheless, these PCR-based markers failed to cover the entire genome, posing challenges for buckwheat genetic analysis. The emergence of next-generation sequencing has enabled genome-wide assessments across various species, buckwheat included. Recently, approximately 8,885 markers, representing 757 loci, were mapped to eight linkage groups in buckwheat, proving effective for genomic selection aimed at enhancing yield.

Keywords: Genomic insights, Genetic diversity, Morphological markers, RAPD, and Self-incompatibility.

INTRODUCTION:

Common buckwheat (F. esculentum Moench) is a pseudo-grain primarily found in southwest China, believed to be its place of origin, distribution, and diversification (Konishi et al., 2005). Buckwheat sprouts are rich in antioxidants, particularly rutin, vitexin, and flavonoids, compared to other cereals (Nam et al., 2018). These glycosides are present in buckwheat flowers and green leaves (Nešović et al., 2021). Buckwheat flowers are valuable sources of nectar and medicinal properties, with the honey produced potentially serving as a supplement to combat pathogens resistant to arsenals (Abedin et al., 2020; Islam et al., 2016; Sayed et al., 2015; Begum et al., 2018; Kreft et al., 2006; Abedin et al., 2021).
Two methods are commonly used to study buckwheat genetic diversity: morphological markers and genetic markers. In the morphological study, grains from cultivated common buckwheat collected from various regions were examined for seed size, width, thickness, and weight, as well as seed value and the ratio of seed width to length. In the molecular study, allozyme markers were employed to analyze genetic resources from cultivars. Self-incompatibility poses a significant challenge to *F. esculentum* cultivation, hindering pure line production and promoting outcrossing (Matsui and Yasui, 2020). Polymerase chain reaction (PCR) was extensively used for genetic polymorphism analysis of common buckwheat in the 2000s (Balážová et al., 2018). Random amplification of polymorphic DNA (RAPD) markers has been used to study the initial cultivation of common buckwheat. Amplified Fragment Length Polymorphism (AFLP) markers have been used to investigate genetic linkages between cultivated populations and the main cultivation of common buckwheat (Konishi et al., 2005). RAPD markers are associated with a self-pollinating gene, while AFLP markers are linked to a shattering gene. Simple Sequence Repeat (SSRs), also known as microsatellite markers, is commonly used to study genetic variability across various crop species. Due to their codominant inheritance and high variability, microsatellite markers offer diverse applications. Several recent studies have explored microsatellite markers to assess the genetic diversity of cultivated *F. esculentum*. "Next Generation Sequencing" has been employed for improving and breeding agriculturally important buckwheat varieties. DNA microarrays, constructed from gene sequences obtained through NGS, are used for quantitative trait locus (QTL) analysis and genetic selection in common buckwheat cultivation (Yasui, 2020). The primary focus of this review is to summarize studies on the genetic diversity and population structure of buckwheat, utilizing various morphological and genetic traits for analysis.

### Morphological Markers

Common buckwheat exhibits variations in its morphological characteristics, possibly due to its outcrossing nature, also known as self-incompatibility. Variations are evident in several traits, including seed size, shape, leaf size, leaf shape, flower color, leaf lobes, initiation of flowering, plant branching, raceme length, maturity time, and seed coat color (Table 1). A notable variation is observed in flower morphology: common buckwheat flowers are perfect but incomplete, lacking petals but featuring a calyx composed of pink, white, and pink-white colored sepals measuring 6-7 mm. The tepals are connected by a 2-3 mm long pedicle (Cawoy et al., 2009). These flowers produce yellow-colored nec- tarines attached to stamens, and the petals are bundled dimly at the branch tips with short pedicels appearing at the leaf axils. Common buckwheat employs a distylous self-incompatibility (SI) breeding system. Within this system, two distinct flower types exist: long-styled flowers, or "pin flowers," characterized by long styles and short stamens, and short-styled flowers, commonly known as “thrum flowers,” which have short styles and long stamens. Pollen grains from thrum flowers are larger than those from pin flowers.

Intra-morph incompatibility occurs in the style during crosses between thrum plants, where thrum pollen tube growth is inhibited in the upper part of the style, accompanied by hypertrophy at the tips of the pollen tubes. Conversely, when pollinating between pin flowers, pollen grain growth is hindered at the midpoint of the female genital part of the common buckwheat flowers, without any signs of hypertrophy (Matsui and Yasui, 2020). The S locus governs both self-incompatibility (SI) and flower style (long or short). Additionally, thrum plants exhibit heterozygosity at this locus (Ss), while pin flowers are characterized by homozygosity (ss) (Matsui and Yasui, 2020). Notably, the SS genotype is absent in this system (Yasui et al., 2012). Ohnishi and colleagues identified an inactive morphological variant of cultivated common buckwheat through sib-crosses and utilized them as genetic traits. Ohnishi identified 37 morphological markers governed by a single gene and revealed linkage relationships among 22 common buckwheat genes (Wang et al., 2017). Although morphological traits may be considered outdated, they can still provide valuable insights for buckwheat improvement.

### Allozyme Markers

Codominant or allozyme markers, which are forms of isozymes, have been widely used as reliable indicators of genetic variation among populations of crop species at different levels. Ohnishi and her colleagues identified various allozyme markers that have been instrumental in the development of
genetic resources for common buckwheat (Yasui, 2020). In 1987, Ohnishi and Ohta developed an initial linkage map for cultivated common buckwheat, incorporating several allozyme markers (Table 1) (Ohnishi and Ohta, 1987). Subsequent studies elucidated the global population structure of cultivated common buckwheat using 64 populations predominantly collected from Asian countries such as China, Japan, Korea, and Nepal (Ohnishi, 1993). Another role of allozyme markers is to aid in tracing the origin of common buckwheat. Ohnishi identified the first natural progenitor species of common buckwheat through allozyme marker analysis (Konishi et al., 2006). Additionally, a study utilized 18 allozyme and ISSR markers to assess polymorphisms and establish the genetic structure of the common buckwheat genome (Table 1) (Zhou et al., 2012).

**Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) Markers**

Random Amplified Polymorphic DNA (RAPD) is a crucial marker used for identifying genotypes and varieties across a wide range of plants (Korir et al., 2013). RAPD analysis amplifies small DNA fragments of about 11 bp using PCR with RAPD primers at low annealing temperatures, which promote DNA binding. Tables 2 lists various RAPD and SCAR markers along with their respective primers. While RAPD is a cost-effective method, it may be less effective when using small primers and short annealing temperatures. Balážová et al. (2018) employed seven RAPD markers to evaluate 17 buckwheat varieties, identifying 52 genomic segments, of which 38 were polymorphic. Another study utilized three RAPD primers to detect polymorphisms between populations and cultivars, identifying three cultivar-specific RAPD markers. Sharma and Jana (2002) used eight primers to assess genetic diversity, identifying 240 fragments, of which 63.75% were monomorphic and 37.25% were polymorphic. Murai and Ohnishi (1996) analyzed 42 buckwheat landraces using 32 RAPD primers and found 45.9% polymorphic genes. Sequence Characterized Amplified Regions (SCAR) markers are another approach for common buckwheat development, enhancing the robustness of PCR products. SCAR markers amplify DNA fragments using specific 14-29 bp primers (Bhagyawant, 2016), often designed based on RAPD primer sequences. Both RAPD and SCAR markers have been integrated into buckwheat linkage maps. Despite their simplicity and affordability, RAPD markers can lack repeatability, which led to the development of Amplified Fragment Length Polymorphism (AFLP) technology (Vuylsteke et al., 2007). AFLP was designed to address the repeatability issues of RAPD markers and has been used to create genome-wide chromosomal maps for common buckwheat, identifying 222 AFLP genetic markers (Talukdar and Sinjushin, 2015). Table 3 provides a collection of AFLP markers with their respective primers. Nagano et al. (2001) used 500 polymorphic loci from hybrid genes, obtained from crosses between common buckwheat and wild buckwheat (F2 progeny), to identify a marker (N7) with length polymorphisms across parent lines. Yasui et al. (2008) utilized AFLP libraries to identify markers associated with the dwarf E locus and to study gene cloning processes. AFLP genome analysis has significantly advanced buckwheat genetics and crop development research by enabling high-accuracy genotype analysis.

**Simple Sequence Repeat (SSR) and Expressed Sequence Tag (EST) Markers**

Simple sequence repeats (SSR) markers, also known as microsatellite markers, consist of tandem repeats of short nucleotide sequences and are widely distributed throughout the genomes of eukaryotic organisms. Due to their co-dominant nature, multifactorial inheritance, and high mutability, SSR markers are valuable tools for studying broad genetic variations within and between populations. They offer an extensive range of applications because of their co-dominant mode of inheritance. Several studies have utilized SSR markers to characterize genetic resources, establish genetic relationships, and investigate population structures of various crops. The uses of SSR markers are detailed in Table 4. In 2011, Hara et al. developed SSR genetic tools for buckwheat’s genome, revealing significant genetic heterogeneity among common buckwheat cultivars. Microsatellite markers have also been employed to understand the limited gene flow characteristics of common buckwheat (Konishi et al., 2006). In 2009, Ma et al. constructed 136 microsatellite markers primarily used to assess genetic diversity in Korean buckwheat. The applications of these EST markers are listed in Table 5. To enhance common buckwheat cultivation in 2011, Hara et al. developed 170 sets of...
PCR primers based on EST sequences. They successfully identified linkage relationships among 63 co-dominant markers by analyzing restriction endonuclease site polymorphisms and length polymorphisms of PCR products. Bashir et al. (2021) analyzed genetic polymorphisms in 52 buckwheat landraces using 7 SSR markers, revealing substantial genetic variations within populations. Overall, the development and utilization of microsatellite and EST markers have significantly advanced techniques in population genetics studies and breeding research of buckwheat.

NGS-Based DNA Array and Genotyping by Sequencing (GBS)

The previously mentioned molecular markers were unable to unravel the genetic mystery of self-incompatibility in common buckwheat. In 2010, Next-Generation Sequencing (NGS) was first introduced to genetics and crop development research in common buckwheat. The lists of NGS microarray and Genotyping-by-sequencing (GBS) markers are provided in Table 6. To enhance the common buckwheat breeding program, Yabe et al. (2014) developed microarray probes (50-75 bp length) using NGS based on specific genomic sequences of common buckwheat. In 2016, draft genome sequences of common buckwheat were published, along with the establishment of the Buckwheat Genome Database (BGDB) (Yasui et al., 2016). Matsui and Yasui, (2020) demonstrated that BGDB has isolated several agriculturally significant genes. Furthermore, GBS analysis has been conducted using the entire genome as a reference to identify genetic sequences unique to buckwheat crops with thrum flowers, particularly the S-allele-linked region of the S locus (Yasui et al., 2016). A significant achievement in GBS analysis was made in 2019; Mizuno and Yasui, (2019) identified 255,517 SNP locations in 46 cultivated common buckwheat genotypes. This analysis revealed significant genetic diversity, with common buckwheat separating into two genetic diversity groups: Asian and European species, showing minimal differentiation between them, consistent with previous research. The Buckwheat genome assembly was then applied to buckwheat landraces worldwide using GBS technology (Yasui et al., 2016). GBS employs genomic DNA sequences amplified by restriction enzymes and has become popular for identifying a large number of SNPs (Mizuno and Yasui, 2019).

Another study successfully detected the S-allele using GBS markers, which consisted of 333 scaffolds covering 5.4 Mb (Yasui et al., 2012). Yasui et al. (2012) demonstrated that this region contains sequences where GBS readings were found in short-styled plants but absent in long-styled plants, and it harbors two genes (SS and ss). Understanding the DNA organization in the S locus can serve as a powerful tool for elucidating genetic interactions in common buckwheat landraces (Mizuno and Yasui, 2019). In conclusion, Next-Generation Sequencing (NGS) and GBS techniques have significantly advanced genetic research and improved breeding efforts in common buckwheat.

Population Structure

In this study, we summarized research on various morphological and genetic markers used to assess the genetic and morphological diversity of cultivated common buckwheat landraces (Table 7). This information aims to guide the conservation and utilization of buckwheat genomic resources. Genomic resources are vital for developing improved plant varieties with beneficial traits, which can enhance crop management and human nutrition. Preserving the genetic resources of buckwheat is essential, given the decreasing agricultural land. As the cultivated area for buckwheat continues to decline, there is an urgent need to conserve its genetic assets. Drought and desertification, resulting from extensive deforestation, pose global threats. Additionally, many small-scale farmers cannot afford the high-cost inputs required for cultivating high-yielding grain crops like wheat and rice. Consequently, farmers are showing interest in cultivating rainfed crops like buckwheat, which can thrive in low rainfall and low fertility conditions where other local crops struggle. Buckwheat offers an affordable option for resource-poor farmers, requiring less investment while providing a reliable harvest. Furthermore, buckwheat has medicinal properties, making it a valuable crop for these farmers.

CONCLUSION:

This study aimed to review the genetic resources and population structure of buckwheat landraces using a variety of morphological and genetic markers. Initially, allozyme and morphological markers were employed as primary tools for studying the genetic resources of cultivated common buckwheat. As research progressed, RAPD and
SCAR markers became pivotal in tracing the origins of common buckwheat. In 2004, AFLP was first used to construct chromosome maps for common buckwheat, contributing to the development of cultivated common buckwheat with 54 loci containing microsatellite markers. DNA array technology plays a vital role in the characterization of cultivated buckwheat's genetic resources. The advent of NGS technology facilitated the development of BGDB, a genome database housing numerous distinct, agronomically validated genomes. Using GBS, 255,517 SNP sequences were identified in 46 cultivated buckwheat plants, suggesting the potential for gene transfer within the cultivated buckwheat genome. Genetic resources and population structure insights are essential for crop development in buckwheat. Evaluating germplasm plays a crucial role in plant germplasm management programs. The information on genetic diversity obtained from this study can aid in assessing additional germplasm collections and furthering genetic research on cultivated common buckwheat species. This, in turn, can help expand the genetic diversity of current buckwheat cultivars.

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CONFLICTS OF INTEREST:
The authors declare there is no conflict of interest.

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