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ISSN 1927-6478



Biological Evidence

Vol.16 No.3 2026



2026
03

Publisher

Sophia Publishing Group

Edited by

Editorial Team of Biological Evidence

Email: edit@be.bioscipublisher.com

Website: <http://bioscipublisher.com/index.php/be>

Address:

11388 Stevenston Hwy,

PO Box 96016,

Richmond, V7A 5J5, British Columbia

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Biological Evidence (ISSN 1927-6478) is an open access, peer reviewed journal published online by BioSci Publisher.

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Review Paper

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Multiple Utilization Pathways of Buckwheat: Food, Feed, Medicine and Eco-Tourism

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Received: 25 Mar., 2026

Accepted: 20 Apr., 2026

Published: 06 May, 2026

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Preferred citation for this article:Zhu X.L., 2026, Multiple utilization pathways of buckwheat: food, feed, medicine and eco-tourism, Bioscience Evidence, 16(3): 126-139 (doi: [10.5376/be.2026.16.0011](https://doi.org/10.5376/be.2026.16.0011))

Abstract Buckwheat is increasingly recognized as a highly multifunctional pseudocereal whose value extends far beyond conventional grain production. This review places particular emphasis on the distinctions between common buckwheat (*Fagopyrum esculentum*) and Tartary buckwheat (*Fagopyrum tataricum*), especially regarding reproductive biology, rutin accumulation, environmental adaptability, and product quality characteristics. Buckwheat possesses a combination of relatively uncommon traits within a single crop species, including adaptability to low-input cultivation systems, broad ecological plasticity, a well-balanced amino acid composition, high concentrations of flavonoids and phenolic compounds, and deep cultural integration within regional food traditions. These characteristics collectively support the extensive utilization of buckwheat in gluten-free and functional foods, livestock feed ingredients and feed additives, phytochemical-based health applications, and flower landscape-oriented agro-tourism systems, while simultaneously contributing to local cultural identity and rural revitalization. At the same time, the development of the buckwheat industry continues to face several important constraints related to breeding systems, production stability, processing technologies, and evidence-based functional evaluation. The present study argues that buckwheat should not be regarded as an isolated niche crop with limited applications, but rather as a strategically integrative crop whose food, feed, medicinal, ecological, and cultural functions can be developed synergistically. Such an integrated utilization model is particularly significant for mountain agriculture, marginal land use, circular bioeconomy development, and regionally differentiated rural development strategies.

Keywords *Fagopyrum esculentum*; *Fagopyrum tataricum*; Functional food; Rutin; Flavonoids; Feed utilization; Pharmacological activity; Agro-tourism; Rural revitalization

1 Introduction

Buckwheat has increasingly been described as a “smart crop” or a “climate-resilient pseudocereal” because of its value in nutritional security, ecological adaptation, and regional economic development. Compared with conventional cereal crops, common buckwheat (*Fagopyrum esculentum*) and Tartary buckwheat (*Fagopyrum tataricum*) belong to the same genus within the Polygonaceae family, but they differ considerably in genetic background, environmental adaptation, and the accumulation of functional compounds (He and Zhou, 2022).

Both common buckwheat and Tartary buckwheat generally show better protein quality and higher mineral contents than many traditional cereal grains. Buckwheat grains are rich in lysine, arginine, and soluble dietary fiber, while common cereals such as wheat and maize are usually deficient in lysine. For this reason, buckwheat has long been considered an important plant resource for improving amino acid balance in human diets (Ahmed et al., 2014). In particular, Tartary buckwheat contains much higher levels of flavonoids, especially rutin, than common buckwheat. Because of this characteristic, Tartary buckwheat has gradually become an important raw material for functional foods and natural antioxidant products.

Compared with major grain crops that often depend heavily on a single commodity market, buckwheat has more diverse utilization pathways. The grains can be consumed directly as food or further processed into functional starch products, flavonoid extracts, and plant-based feed ingredients. Bran and hulls can be used in dietary fiber products, pillow filling materials, and biomass utilization. Buckwheat straw can also serve as roughage for ruminants or as ecological mulching material. Therefore, buckwheat is not only regarded as a crop suitable for

future low-carbon agriculture, but also as an important link connecting nutritional health, ecological farming, and rural revitalization.

The present review includes both common buckwheat and Tartary buckwheat, with particular attention given to their similarities and differences in morphology, physiology, bioactive compounds, and end-use properties. This study also attempts to connect several research areas that are often discussed separately, including plant science, food chemistry, animal nutrition, pharmacology, and rural development studies. Through this integrated perspective, it becomes easier to understand how buckwheat can simultaneously function as a low-input crop, a functional food resource, a feed ingredient, a medicinal raw material, and a landscape-cultural asset. This review aims to provide theoretical support and practical references for promoting the transition of buckwheat from a traditional minor crop into a modern specialty crop with nutritional, ecological, and economic value.

2 Botanical Characteristics and Genetic Resources of Buckwheat

2.1 Taxonomy and classification of buckwheat species

Buckwheat belongs to the genus *Fagopyrum* in the family Polygonaceae. Its taxonomic position is relatively clear in crop science, but it still has important research value in phylogeny, utilization of wild relatives, and the study of breeding relationships. Modern studies generally recognize common buckwheat (*Fagopyrum esculentum* Moench) and Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) as the two most important cultivated buckwheat species. Among them, common buckwheat is widely distributed in temperate regions and is the main raw material used in buckwheat food processing worldwide. Tartary buckwheat is mainly cultivated in the mountainous regions of southwestern China, the margins of the Qinghai-Tibet Plateau, the Himalayan region, Japan, Korea, and some parts of Europe. Because of its high rutin content and stronger cold tolerance, Tartary buckwheat has received more attention in functional food and medicinal research.

The differences between common buckwheat and Tartary buckwheat are not limited to morphology. More importantly, they differ in reproductive biology and genetic structure. Common buckwheat usually shows heterostylous flowers and self-incompatibility. Populations contain both pin flowers and thrum flowers, and seed production mainly depends on insect pollination and cross-fertilization. In contrast, Tartary buckwheat is generally self-compatible, making it easier to maintain homozygosity and genetic stability in breeding populations.

2.2 Morphological and physiological characteristics

The basic morphology of buckwheat is easy to recognize, but its agricultural value largely depends on the interaction between morphological and physiological traits. Both common buckwheat and Tartary buckwheat are dicotyledonous herbaceous plants with erect or semi-erect stems, heart-shaped or arrow-shaped leaves, raceme or corymb inflorescences, and typical triangular achenes. Flowers of common buckwheat are usually white or pale pink. The plants produce abundant flowers over a relatively long flowering period and possess well-developed nectaries. Because of this, common buckwheat also has value as a nectar source crop and as a landscape plant in ecological tourism systems. Tartary buckwheat plants are generally shorter and more robust, with smaller seeds, thicker hulls, and a stronger bitter taste. However, Tartary buckwheat usually accumulates much higher levels of flavonoids than common buckwheat.

The heterostylous flower structure of common buckwheat is one of its most representative morphological and reproductive characteristics. Pin flowers and thrum flowers differ in style length, stamen height, and pollen morphology. Effective fertilization usually occurs only between different flower morphs. Fawcett et al. (2023) used high-quality genome analysis to reveal the genetic basis of heterostyly and domestication history in common buckwheat. Their study showed that the S-locus region has a complex structure closely associated with self-incompatibility, floral differentiation, and population reproductive behavior.

Buckwheat is characterized by a short growth period, rapid seedling emergence, fast early growth, and relatively good adaptation to low-input environments. Many cultivars require only about 70~90 days from sowing to maturity, making buckwheat suitable for high-altitude regions, multiple-cropping systems, post-disaster replanting, and short-season agriculture. However, buckwheat should not be considered a completely stress-resistant crop.

Seedling growth and flowering stages are sensitive to low temperature, frost, heat stress, drought, and strong wind. In particular, common buckwheat often shows increased empty grain rates and yield reduction when high temperatures, continuous rainfall, or insufficient pollinator activity occur during flowering (Penin et al., 2021). The physiological advantages of Tartary buckwheat are mainly reflected in its adaptation to high-altitude, cool, and nutrient-poor environments. Its relatively stable grain production under these conditions is associated with its shorter growth cycle, stronger root nutrient uptake ability, and higher secondary metabolic activity.

2.3 Genetic diversity and germplasm resources

Because common buckwheat is cross-pollinated and self-incompatible, natural populations usually maintain high heterozygosity and abundant genetic variation. Although Tartary buckwheat is mainly self-pollinating, long-term cultivation in mountainous regions, ethnic communities, and different altitudinal environments has also resulted in substantial ecological differentiation and the formation of diverse local landraces.

The wild ancestral form *F. esculentum* ssp. *ancestrale* is closely related to cultivated common buckwheat and provides valuable material for studying domestication traits such as larger seed size, reduced seed shattering, altered flowering behavior, and adaptation expansion. Similarly, the wild ancestral type *F. tataricum* ssp. *potanini* is important for understanding high rutin accumulation, bitter taste formation, and mountain adaptation in Tartary buckwheat. Compared with cultivated forms, wild buckwheat relatives usually show stronger seed shattering and seed dormancy. Although these traits are unfavorable for direct cultivation, they may contain genes related to stress tolerance and ecological adaptation.

2.4 Adaptation to marginal environments and climate resilience

Buckwheat is often considered suitable for marginal land cultivation, but this conclusion depends on environmental conditions and species differences. Both common buckwheat and Tartary buckwheat have relatively short growth periods and low nutrient requirements. They can therefore play important roles in areas with poor soil fertility, short frost-free seasons, or high risks for major cereal production. In mountain agricultural systems, buckwheat can function as a supplementary grain crop, a rotation crop, a nectar source plant, and an ecological landscape crop. Compared with high-input crops such as maize, wheat, and rice, buckwheat depends less on chemical fertilizers and irrigation, making it suitable for resource-limited agricultural regions.

However, the climate resilience of buckwheat should not be oversimplified. Although it shows relatively strong tolerance to poor soils and cool climates, it is still sensitive to salt stress, heat stress, drought, and frost. For example, Zhang et al. (2023) investigated the effects of salt stress on root morphology, carbon and nitrogen metabolism, and yield formation in Tartary buckwheat. Their study showed that increasing salt concentration inhibited root growth, disrupted carbon-nitrogen metabolic balance, and ultimately reduced grain yield.

Tartary buckwheat is generally better adapted to high-altitude and cool environments than common buckwheat, which corresponds closely with its long-term geographical distribution. Tartary buckwheat is widely cultivated in Liangshan Yi Autonomous Prefecture, the Yunnan Plateau, mountainous regions of Guizhou, and parts of the Himalayan region. It can complete reproductive development within a short growing season while accumulating high levels of flavonoids in the grain. This may be related to adaptation to strong ultraviolet radiation, large day-night temperature differences, and environmental stress conditions. In comparison, common buckwheat is more suitable for temperate and cool regions, but its flowering and seed setting are strongly influenced by weather conditions and pollinator activity. Under the increasing frequency of extreme climate events, breeding programs for common buckwheat need to pay greater attention to seed-setting stability, heat tolerance, and flowering regulation.

3 Nutritional Composition

3.1 Carbohydrates, proteins, lipids, and dietary fiber

Buckwheat grains are mainly composed of carbohydrates, but their nutritional profile differs from many traditional cereals because the starch matrix is accompanied by relatively high-quality proteins and dietary fiber components. Studies comparing common buckwheat (*Fagopyrum esculentum*) and Tartary buckwheat

(*Fagopyrum tataricum*) have shown that different milling fractions, including flour, semolina, bran, and husk layers, vary considerably in nutrient composition. Protein, lipid, mineral, and antioxidant-related compounds are unevenly distributed among these fractions, with bran and coarse fractions generally containing higher concentrations of nutritionally valuable compounds (Sinkovič et al., 2022) (Figure 1).

Buckwheat protein is especially important because it is naturally gluten-free and contains a relatively balanced essential amino acid profile compared with many cereal grains. However, its digestibility and functional properties may change depending on processing conditions and the surrounding food matrix. Dietary fiber further strengthens the value of buckwheat as a functional food ingredient. In both common buckwheat and Tartary buckwheat, fiber-rich fractions such as bran often contain higher levels of bioactive compounds than refined flour fractions. As a result, less refined buckwheat products are usually considered nutritionally superior to highly processed forms.



Figure 1 Representative samples of common buckwheat (*Fagopyrum esculentum* Moench) and Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) whole grain and seven grain fractions obtained by traditional stone-milling (Adapted from Sinkovič et al., 2022)

3.2 Vitamins, minerals, and essential amino acids

Buckwheat is considered a good source of minerals such as magnesium, phosphorus, iron, zinc, and potassium. It also contains vitamins and has a more balanced amino acid composition than many refined cereal products. These nutritional advantages are especially important in gluten-free food systems, where products based mainly on rice flour or purified starches often show nutritional limitations.

Research on bread fortification demonstrated that the addition of buckwheat flour increased protein content, improved amino acid scores relative to wheat bread, and enhanced antioxidant properties as well as inositol phosphate levels (Kowalski et al., 2022). Both common buckwheat and Tartary buckwheat contribute nutritional benefits, although Tartary buckwheat is generally associated with higher levels of bioactive compounds. The amino acid quality of buckwheat proteins makes them valuable in the development of nutritionally improved gluten-free foods, where protein quality is often a major concern.

3.3 Bioactive compounds: rutin, quercetin, flavonoids, and polyphenols

The biological value of buckwheat is closely related to its flavonoid composition. Rutin is the best-known compound, but the phytochemical profile of buckwheat is much more complex. In addition to rutin, buckwheat contains quercetin, orientin, isoorientin, vitexin, isovitexin, phenolic acids, fagopyritols, D-chiro-inositol derivatives, bioactive peptides, and other specialized metabolites. Tartary buckwheat usually contains much higher rutin concentrations than common buckwheat, which is one reason why Tartary buckwheat is more frequently studied for medicinal and functional food applications. However, processing conditions and the activity of endogenous rutinase can strongly influence rutin retention in final products (Wang et al., 2024).

The distribution of these compounds is highly tissue-specific. Flowers, leaves, bran, and some coarse milling fractions generally contain higher concentrations of flavonoids and polyphenols than refined flour. This uneven distribution means that nutritional quality is closely connected with processing methods and fraction selection. In recent years, rutin content has become not only a nutritional trait but also an important target in breeding and metabolic regulation research, especially in Tartary buckwheat improvement programs focused on functional food development.

4 Processing Technologies and Food Applications

4.1 Traditional buckwheat foods in different cultures

Traditional buckwheat foods are not simply “coarse grain foods.” They developed through the interaction of climate, religion, ethnic dietary habits, and local processing techniques in different regions. In Japan, the most representative buckwheat food is soba noodles, which are mainly made from common buckwheat (*Fagopyrum esculentum*). Traditional soba usually contains a certain proportion of wheat flour because pure buckwheat dough lacks gluten and breaks easily during processing. According to the Japanese Ministry of Agriculture, Forestry and Fisheries, soba is regarded as a traditional regional food closely linked with local identity. In Nagano, Yamagata, and Hokkaido, soba is not only consumed as a daily staple food, but is also connected with tourism, hand-made noodle experiences, and seasonal festivals.

In Europe, especially in the Brittany region of France, buckwheat has long been known as “blé noir” or “sarrasin.” Common buckwheat flour is traditionally used to prepare savory galettes. Today, galettes are still promoted by regional tourism authorities as a typical local specialty food. In Eastern Europe and Slavic regions, roasted buckwheat groats are commonly processed into kasha, a porridge-like or rice-like dish made from whole buckwheat kernels. Compared with Japanese soba, these foods are relatively simple in processing, but they are highly filling and suitable for long-term storage, which matched the dietary needs of traditional agricultural societies in cold regions.

In South Asia, buckwheat has a different cultural role. During religious fasting festivals such as Navaratri and Shivratri, people in northern India commonly consume buckwheat flour known as kuttu ka atta. Since buckwheat is not classified as a conventional cereal grain in Hindu dietary practice, it can be consumed during fasting periods when grains are prohibited. Therefore, the cultural importance of buckwheat in this context comes not only from nutrition, but also from its acceptance within religious dietary systems. In Nepal, Bhutan, and Himalayan mountain regions, both common buckwheat and Tartary buckwheat (*Fagopyrum tataricum*) are used to prepare flatbreads, noodle soups, and local traditional foods. These products are closely associated with high-altitude agriculture, short growing seasons, and local mountain lifestyles.

4.2 Buckwheat flour and noodle processing technologies

The main challenge in buckwheat flour processing is that buckwheat proteins cannot form the three-dimensional elastic gluten network found in wheat dough. In wheat flour, glutenins and gliadins interact after water addition and kneading, producing a viscoelastic structure that gives dough strength and extensibility. Buckwheat proteins have relatively high nutritional quality, but they cannot provide the same structural support. As the proportion of buckwheat flour increases in noodles, pasta, and bread products, problems such as strand breakage, higher cooking loss, rough texture, and reduced elasticity become more common.

De Arcangelis et al. (2020) studied gluten-free buckwheat pasta and found that proper control of starch pre-gelatinization improved product structure and cooking quality. In the absence of a gluten network, buckwheat products rely more heavily on starch gelatinization, protein gel formation, or external structuring agents to maintain processing stability.

The particle size, dehulling degree, and milling method of buckwheat flour also strongly influence final product quality. Whole buckwheat flour retains more dietary fiber, minerals, and phenolic compounds, but it usually produces darker color and rougher texture, and may weaken dough-forming properties. Refined flour has better sensory acceptance, but some nutritional compounds are lost during processing. Sinkovič et al. (2021) analyzed

different stone-milled fractions from common buckwheat and Tartary buckwheat and showed clear differences in mineral composition, nutritional value, and bioactive compound distribution among milling fractions. Their study demonstrated that flour fractionation can significantly affect the nutritional and functional quality of buckwheat foods.

4.3 Fermented buckwheat products and beverages

Buckwheat naturally has a strong cereal-like, nutty, and sometimes slightly bitter flavor. Some consumers consider the taste too heavy or coarse. Fermentation with lactic acid bacteria, yeasts, or mixed microbial cultures can modify sugars, proteins, and phenolic compounds in buckwheat substrates, improving acidity, aroma, and texture. Fermentation can also reduce the effects of certain antinutritional factors and promote the release of proteins and phenolic substances.

Matejčková et al. (2017) developed a fermented probiotic product using buckwheat substrate and *Lactobacillus rhamnosus*. Their results showed that buckwheat could support probiotic growth and maintain product stability during fermentation. Compared with traditional dairy-based probiotic foods, fermented buckwheat products have advantages such as being gluten-free, plant-based, and nutritionally complex. These characteristics make them suitable for lactose-intolerant individuals, vegetarian consumers, and people interested in gut health. Therefore, the significance of fermented buckwheat foods is not limited to flavor improvement or shelf-life extension; fermentation also increases the value of buckwheat as a functional food carrier.

Xiao et al. (2024) investigated the addition of Tartary buckwheat to kombucha fermentation systems. The study showed that Tartary buckwheat significantly increased total phenolics, total flavonoids, and rutin content in the beverage, while also improving DPPH and ABTS radical scavenging activity. This suggests that Tartary buckwheat not only contributes more phytochemicals to the fermentation system, but may also promote the release of bound phenolic compounds during microbial fermentation. After proper formulation adjustment, the addition of Tartary buckwheat did not significantly reduce sensory acceptance. Instead, its cereal and mild nutty aroma complemented the sweet-sour flavor of fruit kombucha, and some treatment groups achieved higher sensory scores than traditional kombucha products.

4.4 Gluten-free and functional food development

The value of buckwheat in gluten-free food development is not only related to the absence of gluten. Buckwheat also has higher nutritional density than many gluten-free products based mainly on corn starch, potato starch, or refined rice flour. Many commercial gluten-free foods meet the basic dietary needs of people with celiac disease or gluten sensitivity, but they are often low in protein, dietary fiber, and minerals, while producing relatively high glycemic responses.

The incorporation of buckwheat flour can significantly improve total phenolic content and antioxidant activity in gluten-free bread, while also enhancing protein, dietary fiber, and functional phytochemical composition compared with conventional starch-based gluten-free formulations (Brites et al., 2022). Both common buckwheat and Tartary buckwheat contain rutin, phenolic compounds, and antioxidant substances that help compensate for the “high starch and low nutritional density” problem common in many commercial gluten-free products. However, increasing the proportion of buckwheat flour also affects bread volume, crumb structure, and texture stability. Since buckwheat lacks a gluten network, dough gas retention becomes weaker. Industrial application therefore requires the use of hydrocolloids, composite starch systems, and optimized processing conditions to balance nutritional quality and processing performance.

Buckwheat has even greater potential in biscuits, cookies, and snack products. Compared with bread, these products depend less on gluten structure and can tolerate higher levels of buckwheat flour. Buckwheat flour can be blended with almond flour, oat flour, quinoa flour, or rice flour to improve flavor, texture, and nutritional composition. In many cases, the darker color and nutty flavor of buckwheat become sensory advantages rather than limitations, especially in low-sugar, high-fiber, plant-based, or functional snack products.

5 Nutritional Value and Feed Utilization

5.1 Nutritional composition of buckwheat straw, grain, and by-products

Buckwheat grains contain starch, protein, lipids, minerals, and moderate amounts of dietary fiber, making them suitable as both energy and protein feed resources. Common buckwheat (*Fagopyrum esculentum*) is more frequently used in grain-based feed formulations because of its relatively mild flavor and broader cultivation scale, whereas Tartary buckwheat (*Fagopyrum tataricum*) has attracted more attention for functional feed applications due to its high flavonoid content, especially rutin. In contrast, buckwheat bran, hulls, and processing by-products contain higher levels of cellulose, hemicellulose, lignin, and phenolic compounds. Although their digestibility is lower than that of grains, these by-products still have value in ruminant roughage systems and in the development of functional feed additives. Tartary buckwheat by-products are particularly rich in rutin, quercetin, and other polyphenols, which may help regulate oxidative stress, intestinal microbiota, and inflammatory responses in animals.

The use of buckwheat straw as roughage is mainly limited by its relatively high degree of lignification and fiber accumulation. Untreated straw is hard in texture, has poor palatability, and is not easily degraded by rumen microorganisms. This is one reason why buckwheat straw has not been widely utilized in animal production systems for a long time. Cao et al. (2023) investigated the effects of extrusion processing on the physical structure, chemical composition, and in vitro ruminal digestibility of buckwheat straw (Figure 2). Their results showed that extrusion treatment changed the structural characteristics of the straw, reduced hardness and chewiness, and improved water-holding capacity and ruminal degradation performance. The treatment also increased the accessibility of structural carbohydrates to rumen microorganisms, suggesting that suitable physical processing can improve the feeding value of buckwheat straw.

Different buckwheat components therefore have different feeding roles. Grains are more suitable as energy and protein sources in compound feed and may partially replace conventional cereal ingredients. Bran and hulls are more appropriate as sources of dietary fiber, antioxidant compounds, and phytochemicals. Straw should preferably enter roughage systems after extrusion, fermentation, alkalization, or microbial treatment. Compared with major feed crops such as maize, wheat, and soybean, buckwheat does not have a clear advantage for large-scale substitution. However, it has more specific value in specialty feeds, functional feed additives, and regional circular agriculture systems, especially in mountainous areas where cultivated buckwheat species are already part of traditional farming systems.

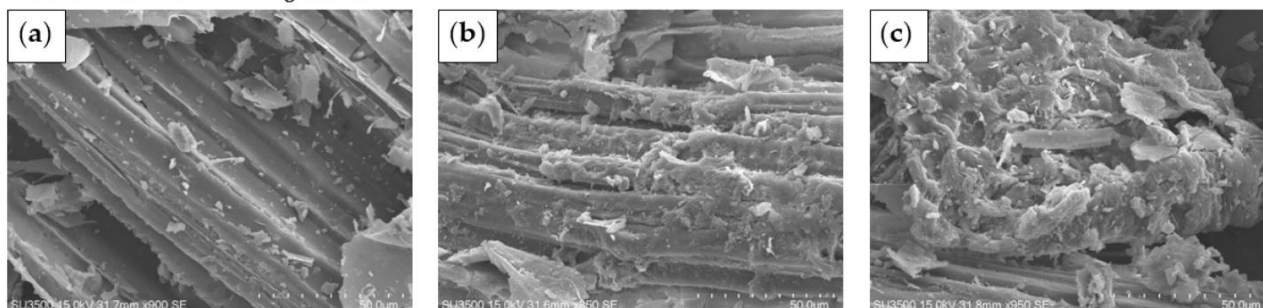
5.2 Digestibility and feed efficiency in livestock

Buckwheat grains contain relatively high-quality proteins and minerals, but the higher levels of fiber and phenolic compounds in hulls, bran, and some by-products may influence nutrient digestion and absorption. In studies involving weaned piglets, Cui et al. (2019) evaluated the combined effects of Tartary buckwheat flavonoids and *Lactobacillus plantarum*. The combination improved growth performance, nutrient digestibility, antioxidant status, and fecal microbial composition in weaned piglets. Their findings suggested that Tartary buckwheat flavonoids do not function simply as nutrient sources. Instead, they may support animal performance through antioxidant activity and regulation of intestinal microecology. This is especially important in weaned piglets because intestinal barrier function is still unstable during the post-weaning stage, and oxidative stress together with microbial imbalance can negatively affect growth and health.

Buckwheat has also been studied in poultry nutrition. Chowdhury and Koh (2018) examined the effects of buckwheat-based diets on phytase activity and nutrient digestibility in broiler chickens. Birds fed buckwheat-containing diets showed significantly higher natural phytase activity in the digestive tract, particularly in the crop and gizzard. The increased phytase activity promoted phytate degradation and released bound phosphorus, calcium, and other minerals, thereby improving phosphorus bioavailability and ileal nutrient digestibility. Compared with conventional corn-soybean meal diets, the buckwheat diets increased phosphorus digestibility and also improved the apparent digestibility of crude protein and several amino acids. The study indicated that buckwheat can function as a natural phytase source under low-phosphorus feeding conditions and

may partly reduce the need for supplemental inorganic phosphorus and exogenous phytase enzymes. This is important because excessive phosphorus supplementation in livestock production contributes to environmental pollution through phosphorus excretion.

Before in vitro rumen digestion:



After in vitro rumen digestion:

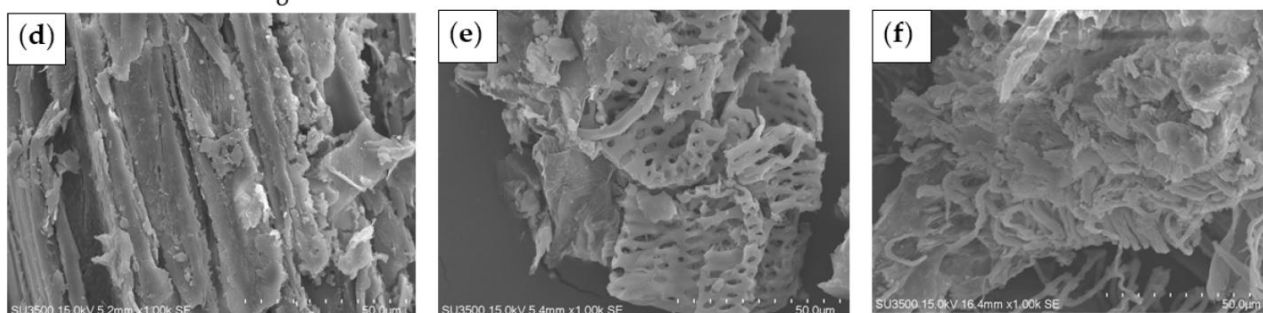


Figure 2 Scanning electron microscopy (SEM) of buckwheat straw before and after in vitro rumen digestion. (a-c) Untreated, once-expanded, and secondary-expanded buckwheat straw before digestion; (d-f) Untreated, once-expanded, and secondary-expanded buckwheat straw after digestion (Adopted from Cao et al., 2023)

6 Medicinal Components and Pharmacological Activities of Tartary Buckwheat

6.1 Bioactive phytochemicals in Tartary buckwheat

Tartary buckwheat (*Fagopyrum tataricum*) contains many functional compounds, including flavonoids, phenolic acids, polysaccharides, proteins and bioactive peptides, as well as D-chiro-inositol-related compounds. Among these substances, rutin is the most representative flavonoid glycoside, while quercetin is commonly regarded as the major active form produced after rutin hydrolysis. Buckwheat protein hydrolysates and peptides have shown potential antioxidant, antidiabetic, antihypertensive, antimicrobial, and anticancer activities. Their structural diversity also provides a basis for the development of functional foods and food-medicine homologous products (Zhu, 2021). Although buckwheat proteins are naturally gluten-free, they may still cause allergic reactions in some individuals. Therefore, medicinal utilization of buckwheat should not focus only on efficacy, but also include safety evaluation.

The concentration of bioactive compounds differs greatly among different parts of the plant. Tartary buckwheat sprouts, flowers, leaves, seed coats, and bran usually contain much higher levels of flavonoids and polyphenols than refined grain flour (Figure 3). Experiments using methanol extracts of Tartary buckwheat sprouts showed that the total flavonoid content reached 98.6 mg/g. Among the six major flavonoids detected, rutin and quercetin were the dominant compounds. Rutin content reached 89.81 mg/g in the crude extract and 31.50 mg/g in sprouts, while quercetin contents were 23.34 mg/g and 8.17 mg/g, respectively (Zhong et al., 2022).

It is important to note that the medicinal composition of buckwheat is highly dynamic rather than chemically fixed. Variety, cultivation environment, germination treatment, thermal processing, fermentation, milling methods, and storage conditions can all influence its chemical profile. In Tartary buckwheat, rutin can be hydrolyzed into quercetin by endogenous rutinoidase during processing. Although quercetin has strong biological activity, its

bitterness and lower stability may reduce consumer acceptance. In other words, the medicinal value of buckwheat is not automatically guaranteed by the plant itself. It depends heavily on compound standardization, processing control, and bioavailability studies.

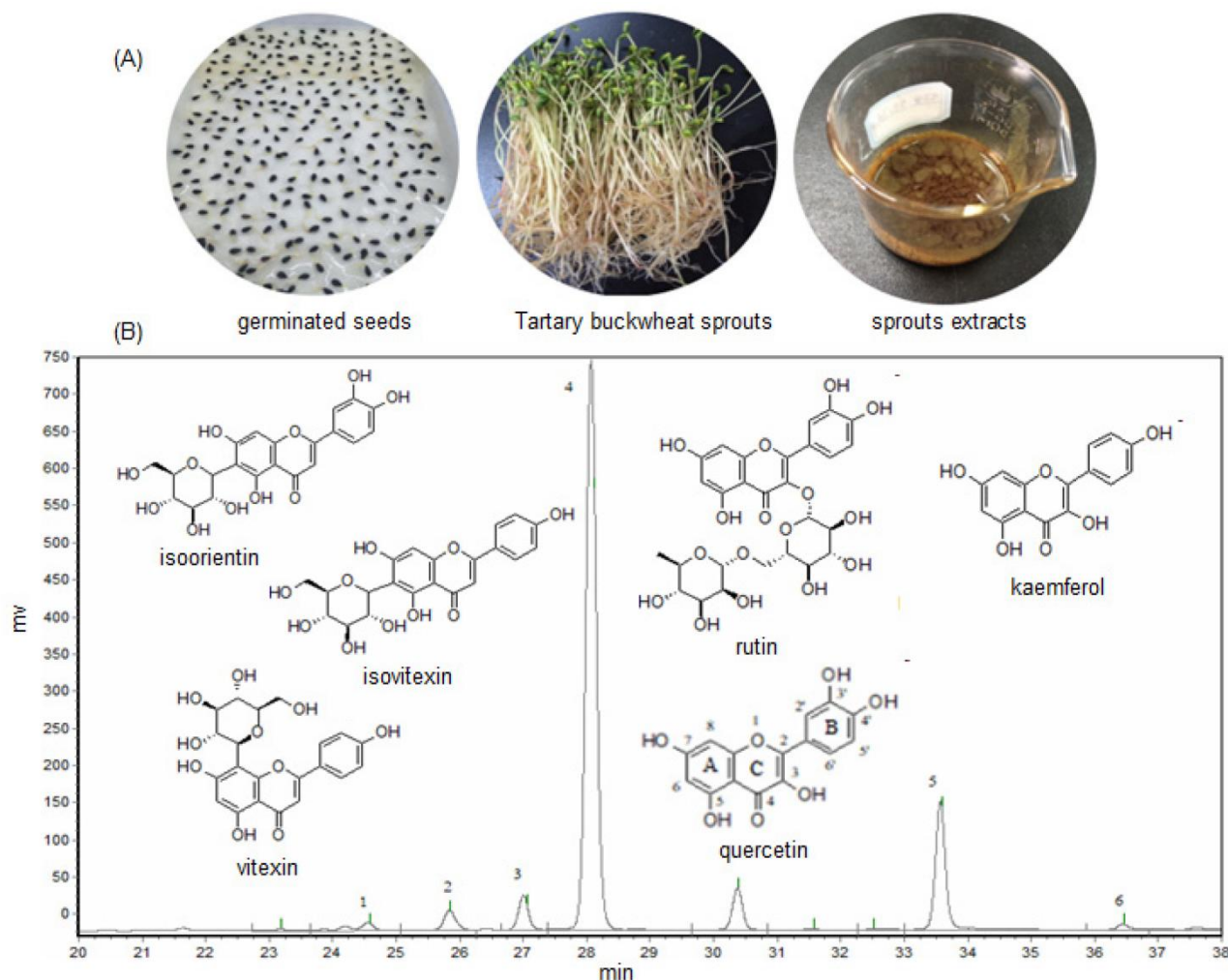


Figure 3 The germinated seeds, sprouts, and methanol extract (A), and the typical HPLC chromatogram of methanol extract (B) of Tartary buckwheat sprout cultures. Here, 1—isorientin, 2—vitexin, 3—isoovitexin, 4—rutin, 5—quercetin, 6—kaemferol (Adopted from Zhong et al., 2022)

6.2 Antidiabetic and cardiovascular protective effects

The health effects of Tartary buckwheat as a functional food are mainly related to the combined actions of dietary fiber, resistant starch, D-chiro-inositol, rutin, quercetin, and other polyphenols. Buckwheat may regulate metabolism by slowing carbohydrate digestion and absorption, improving insulin sensitivity, and reducing oxidative stress caused by hyperglycemia. Buckwheat flavonoids, especially rutin and quercetin, may also contribute to cardiovascular protection through antioxidant activity, inhibition of lipid peroxidation, improvement of lipid metabolism, and maintenance of vascular endothelial function (Giménez-Bastida and Zielinski, 2015).

Zou et al. (2023) reviewed the bioactive compounds, health effects, and industrial applications of Tartary buckwheat and concluded that its antidiabetic and cardiovascular protective functions are associated with the combined effects of flavonoids, polyphenols, dietary fiber, proteins, polysaccharides, and D-chiro-inositol. Tartary buckwheat flavonoids and phenolic compounds may delay carbohydrate digestion by inhibiting α -amylase and α -glucosidase activities. They may also participate in blood glucose regulation through improved insulin sensitivity, modulation of gut microbiota, and reduction of oxidative stress.

Compared with common buckwheat (*Fagopyrum esculentum*), Tartary buckwheat generally contains much higher levels of rutin and other flavonoids. Because of this difference, Tartary buckwheat has attracted greater attention in studies related to metabolic syndrome, diabetes, and cardiovascular diseases. However, most current evidence still comes from cell experiments, animal studies, and functional food evaluations. More long-term human intervention studies are still needed before these effects can be translated into clinical nutritional recommendations.

6.3 Anticancer and antimicrobial properties

As an underutilized crop, Tartary buckwheat has attracted increasing attention because of its potential anticancer properties. Its anticancer effects are believed to result from a complex phytochemical system rather than from one single therapeutic compound (Hassan and Ganai, 2025). Rutin, quercetin, phenolic acids, polysaccharides, and buckwheat-derived bioactive peptides may contribute to anticancer activity through antioxidant effects, anti-inflammatory responses, induction of tumor cell apoptosis, and inhibition of abnormal cell proliferation.

Tartary buckwheat is usually considered more valuable than common buckwheat in anticancer-related research because its flavonoid content is generally much higher. This makes it an important candidate for the screening of functional anticancer compounds and the development of health-oriented foods. Nevertheless, current evidence remains largely limited to in vitro experiments and animal models. At present, Tartary buckwheat should be regarded mainly as a source of preventive functional foods and natural bioactive compounds rather than as a direct substitute for clinical anticancer drugs.

In addition to anticancer activity, Tartary buckwheat also shows certain antimicrobial potential. Some studies have reported that buckwheat phenolics and peptides can inhibit the growth of several bacterial strains and may interfere with microbial metabolism or membrane integrity. However, antimicrobial activity is strongly influenced by extraction methods, processing conditions, and compound concentration. Therefore, more standardized studies are needed before buckwheat-derived antimicrobial compounds can be widely applied in medicine or food preservation systems.

6.4 Anti-obesity and metabolic regulation functions

Obesity is usually associated with lipid metabolism disorders, chronic low-grade inflammation, insulin resistance, and gut microbiota imbalance. Because these metabolic disturbances involve multiple physiological pathways, single compounds often have limited effects. The advantage of buckwheat lies in its multi-target component system. Dietary fiber can influence satiety and intestinal fermentation, proteins and peptides may regulate lipid metabolism, flavonoids and polyphenols can reduce oxidative stress and inflammation, and some compounds may further affect bile acid metabolism through interactions with gut microbiota.

Bae and Kim (2022) did not only measure flavonoids or antioxidant compounds in germinated buckwheat materials. Instead, they used an in vitro gastrointestinal digestion model to investigate the antioxidant and anti-obesity activities of digested products obtained after simulated digestion. This approach better reflects the physiological state after human consumption. The digested products of germinated buckwheat still maintained strong free radical scavenging activity and reducing power. In lipid metabolism-related experiments, they also showed the potential to inhibit lipid accumulation and alleviate obesity-related oxidative stress.

The germination process may activate endogenous enzyme systems in buckwheat grains and promote the release of bound phenolics and flavonoids, thereby improving the bioaccessibility of small active molecules. Therefore, the anti-obesity effects of buckwheat are not simply determined by its original nutritional composition. They are closely associated with the continuous process of germination, gastrointestinal digestion, release of active compounds, and regulation of lipid metabolism.

7 Buckwheat Landscape Utilization and Eco-Tourism

7.1 Flowering buckwheat landscapes and rural aesthetics

During the flowering period, large areas of buckwheat fields form white, pink, or light purple landscapes with strong visual appeal and obvious seasonal characteristics. The attraction of buckwheat landscapes is not only

related to their visual beauty. More importantly, buckwheat flowers can combine agricultural seasons, local food culture, and ethnic or regional identity into a short but emotionally recognizable rural experience.

In recent years, the Hahaheba tobacco-growing area in Mianning County, Liangshan Yi Autonomous Prefecture, Sichuan Province, has developed a representative “buckwheat flower sea + agro-cultural tourism” model. After tobacco harvesting, local farmers use winter fallow land to rotate with buckwheat, creating large white flowering fields in autumn. During the flowering season, the buckwheat flowers appear “like snow covering the fields,” forming impressive rural scenery together with mountains, villages, and tobacco fields. The local government even organized the first “Buckwheat Flower Festival,” which attracted more than 30,000 visitors for sightseeing, photography, and rural tourism experiences. Unlike traditional agricultural tourism focused only on crop viewing, Mianning integrated buckwheat flower landscapes with grain–tobacco rotation systems, Yi ethnic culture, local food experiences, and rural leisure activities. Visitors can not only enjoy the flower fields, but also participate in buckwheat food preparation, farming activities, stargazing camping, and local barbecue consumption. Local households have also benefited from flower tourism through farmhouse businesses, sales of local agricultural products, and livestream e-commerce activities.

7.2 Integration with rural revitalization strategies

Weining Yi, Hui and Miao Autonomous County in Guizhou Province is one of the three major Tartary buckwheat production regions in China and has long been known as the “Buckwheat Plateau.” In recent years, the county has gradually formed an integrated development model combining Tartary buckwheat cultivation, deep processing industries, and ecological tourism. Supported by its cold high-altitude environment above 2,300 m, Weining maintains about 150,000 mu of stable buckwheat cultivation each year, with annual production exceeding 260,000 tons. Several regional brands, including “Mingqiaoxiang,” “Mabaidashan,” and “Qianhe,” have also been developed.

Through policy support for leading enterprises such as Guizhou Weining Qiaoyuan Agricultural Co., Ltd., the county has promoted the development of a complete Tartary buckwheat industrial chain. More than twenty processed products have been developed, including buckwheat rice, buckwheat tea, buckwheat noodles, buckwheat crisps, and flower cakes. These products are sold to many provinces, including Hunan, Guangdong, and Jiangsu. The annual industrial output value exceeds 30 million yuan and has increased the income of thousands of farming households. At the same time, several thousand mu of flowering buckwheat fields have been combined with rural tourism development. In areas such as Bandi Township, Yi ethnic cultural patterns are incorporated into artistic buckwheat field designs. During the flowering season, the plateau buckwheat landscapes appear “snow-like and cloud-like,” attracting large numbers of tourists for photography, sightseeing, and ethnic cultural activities.

8 Current Challenges and Future Development Directions

8.1 Limitations in breeding and production systems

Although common buckwheat (*Fagopyrum esculentum*) and Tartary buckwheat (*Fagopyrum tataricum*) are both important cultivated buckwheat species, the major breeding problems are different between them. The biggest limitation in common buckwheat is its heterostylous flower structure and self-incompatibility system. This biological characteristic naturally promotes cross-pollination, making it difficult to achieve stable homozygous lines and consistent genetic improvement. Therefore, breeding of common buckwheat cannot rely only on traditional population selection. Factors such as S-locus regulation, flower-type ratio, pollination efficiency, and population genetic structure need to be considered together as an integrated breeding problem.

In the future, breeding of common buckwheat should focus more on the utilization of self-compatible materials, pollination control, resistance to seed shattering, and uniform maturity. In contrast, breeding of Tartary buckwheat should pay more attention to stable yield, reduced bitterness, stress resistance, and adaptability to mechanized cultivation. Only by combining genomic breeding, physiological regulation, mechanized farming systems, and region-specific production models can the buckwheat industry move from small-scale specialty cultivation toward stable, standardized, and sustainable production.

8.2 Processing technology bottlenecks

Both common buckwheat and Tartary buckwheat are gluten-free crops. Because they lack gluten proteins, their dough-forming ability is relatively weak. As a result, buckwheat-based noodles, bread, biscuits, and extruded foods often show poor elasticity, high breakage rates, rough texture, and unstable storage quality. Tartary buckwheat faces an additional challenge. Although its high rutin content is considered one of its most valuable functional traits, it is also associated with strong rutinase activity and the formation of bitterness.

Suzuki et al. (2021) reported that the *in vitro* rutinase activity of the Tartary buckwheat variety “Manten-Kirari” was two orders of magnitude lower than that of the conventional rutinase variety “Hokkai T8.” In dough prepared from “Hokkai T8,” rutin was almost completely hydrolyzed within 10 minutes after water addition, whereas only partial hydrolysis occurred in “Manten-Kirari” dough even after 6 hours. Among 29 evaluators, 27 identified clear bitterness in flour made from “Hokkai T8,” while no bitterness was reported for “Manten-Kirari” flour. More importantly, noodles produced with “Manten-Kirari” retained about 90% of their rutin content and showed only slight or no bitterness. However, high-moisture systems such as some pancake-type products still cannot completely prevent rutin hydrolysis. In addition, contamination of the processing chain with seeds or flour from normal rutinase varieties may reduce rutin retention. Future processing of Tartary buckwheat therefore requires a complete quality-control system covering seed purity, flour grading, moisture control, low-temperature storage, pH regulation, and final product formulation, rather than relying only on low-rutinase cultivars as a single technological solution.

8.3 Future trends in sustainable buckwheat utilization

The future development of buckwheat should not focus on only one specialized function. Instead, a comprehensive system based on the differentiated utilization of common buckwheat and Tartary buckwheat should be established. Future breeding programs should move beyond simple yield-oriented goals and focus on multiple traits at the same time, including self-compatibility, resistance to seed shattering, uniform maturity, suitability for mechanized harvesting, low bitterness, rutin retention, and food-processing quality. The future market potential of buckwheat lies more in high-value-added products than in low-value raw grain sales.

Sustainable utilization of buckwheat will increasingly depend on low-input agriculture, mountain agriculture, and circular bioeconomy systems. The future value of buckwheat is also reflected in the integration of agricultural landscapes, local culture, and regional branding. One promising pathway is to establish closed-loop systems in mountainous and cool-climate regions that combine buckwheat cultivation, food processing experiences, local cuisine, cultural tourism, and by-product utilization. In this way, buckwheat can simultaneously provide food, ecological, cultural, and economic functions. Under such a model, buckwheat will no longer remain only a “potential crop” frequently mentioned in review papers, but may become a practical and sustainable specialty crop supporting low-input agriculture and rural revitalization.

Author Contributions

The author conducted this study, including literature review, data analysis, and the drafting and revision of the manuscript. The author has read and approved the final version of the manuscript.

Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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Research Insights

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Rhizosphere Microbiome Remodeling Enhances Salt-Alkali Tolerance in Proso Millet (*Panicum miliaceum* L.)

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Received: 02 Apr., 2026

Accepted: 10 May, 2026

Published: 17 May, 2026

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Preferred citation for this article:

Huang W.Z., and Liang K.W., 2026, Rhizosphere microbiome remodeling enhances salt-alkali tolerance in proso millet (*Panicum miliaceum* L.), Bioscience Evidence, 16(3): 140-156 (doi: [10.5376/be.2026.16.0012](https://doi.org/10.5376/be.2026.16.0012))

Abstract Saline-alkaline stress is gradually becoming a major factor limiting agricultural productivity because it simultaneously causes osmotic stress, ion toxicity, nutrient precipitation, and high-pH damage within the same soil environment. Under this background, proso millet (*Panicum miliaceum* L.) has gained renewed attention. This study integrates current evidence on how proso millet achieves tolerance under saline-alkaline conditions through coordinated strategies at the morphological, physiological, biochemical, and molecular levels, and further discusses how rhizosphere microbiome reshaping strengthens these response mechanisms. The saline-alkaline tolerance of proso millet depends on the integrated coordination of root structural plasticity, osmotic adjustment, Na⁺/K⁺ homeostasis, antioxidant defense, and cell wall remodeling mechanisms.

The study also analyzes grain metabolic reprogramming in proso millet under saline-alkaline stress, including changes in the composition of amino acids, phenolic acids, flavonoids, organic acids, and antioxidant compounds, and discusses their potential significance for nutritional quality and functional food development. Finally, the study evaluates the application potential of proso millet in saline-alkaline land utilization, ecological restoration, low-input agriculture, and diversified food and feed systems. Rhizosphere microbiome reshaping is not a secondary result of saline-alkaline tolerance in proso millet, but rather a key component of the tolerance formation process itself, and it represents an important future research direction for breeding, microbiome engineering, and the design of climate-adaptive cropping systems.

Keywords Proso millet (*Panicum miliaceum* L.); Saline-alkali stress; Rhizosphere microbiome; Ion homeostasis; Antioxidant defense; Functional metabolites; Climate-resilient agriculture

1 Introduction

Soil salinization and alkalization have become major limiting factors in global agricultural production, especially in arid, semi-arid, and irrigated agricultural regions. The global area of saline soils has reached about 17 million km² and continues to expand under the influence of climate change, improper irrigation practices, and poor drainage conditions (Hassani et al., 2021). The main problem of saline-alkaline soils is the combined stress of salt and high pH. Salt stress reduces soil water potential and causes ion toxicity, while alkaline conditions decrease nutrient availability, disturb membrane transport, and inhibit root function. These effects finally lead to lower seed germination, unstable crop yield, and reduced land-use efficiency (Li and Yang, 2023). Saline-alkaline agriculture is not only an issue of soil management, but also an important topic related to crop structure adjustment and agricultural resilience.

Under this background, minor cereals with strong stress resistance have received increasing attention again. Compared with major staple crops that highly depend on resource input, minor cereals usually show better drought resistance, tolerance to poor soil conditions, and stronger adaptability to environmental stress. They can also maintain relatively stable yields on marginal land (Mudnakudu-Nagaraju et al., 2025). Proso millet is a typical example. It has a short growth period and low water requirement, and it also contains high nutritional value, including rich bioactive compounds, a low glycemic index, and gluten-free characteristics. Therefore, it is considered a potential crop for adapting to climate change and promoting agricultural diversification.

Proso millet originated in the dry farming regions of northern China, and its long domestication history has given it strong environmental adaptability. It is a self-pollinated C4 crop that can mature within 60~90 d and maintain relatively stable productivity under drought and saline-alkaline conditions (Baltensperger, 2002). Significant differences exist among genotypes in salt-alkaline tolerance, nutritional quality, and environmental adaptability.

The rhizosphere is not simply the soil surrounding roots, but an important interface where plants interact with microorganisms. Plant-associated microorganisms can improve plant salt tolerance through different mechanisms, including promoting nutrient uptake, regulating Na⁺/K⁺ balance, enhancing antioxidant capacity, and improving the soil environment (Zhao et al., 2020). Recent studies on proso millet have started to combine plant physiology, transcriptomics, and rhizosphere microbial community analysis, showing that the microbiome has become an essential component for understanding its saline-alkaline tolerance mechanisms (Yuan et al., 2023).

This study explains the characteristics of saline-alkaline stress and its effects on plant growth performance, summarizes the ecophysiological adaptation mechanisms that help proso millet survive and recover under saline-alkaline conditions, discusses how the rhizosphere microbiome is reshaped during stress and how microbial recruitment enhances plant tolerance, evaluates the effects of saline-alkaline stress on millet metabolism and nutritional quality, and analyzes the potential role of this crop in soil improvement, marginal land agriculture, feed security, and climate-resilient food systems. The aim is to provide theoretical references for the agricultural development of saline-alkaline land and the utilization of stress-resistant crops.

2 Saline-Alkali Stress and Ecophysiological Adaptation

2.1 Physicochemical characteristics of saline-alkali soils

Saline-alkali soil is not defined by a single threshold. Instead, it is characterized by a series of interconnected physicochemical imbalances. The continuous accumulation of soluble salts lowers soil osmotic potential, while sodium ions usually dominate both soil exchange sites and soil solution composition. In alkaline saline soils, bicarbonates and carbonates increase soil pH, sometimes above 8.5, which further promotes nutrient precipitation and weakens transport processes on the root surface. Therefore, crops growing in this type of soil are not simply facing “reduced water uptake,” but are exposed to a rhizosphere environment whose chemical properties have already been altered (Mukhopadhyay et al., 2021).

From the plant perspective, high concentrations of Na⁺ are especially damaging because they compete with K⁺ at ion transport and enzyme activation sites. Excess external sodium also disrupts cell membrane stability and induces secondary injuries, including oxidative stress. In alkaline soils, high pH further intensifies these problems by reducing the solubility of micronutrients and interfering with proton-driven transport systems. As a result, plants experience a combined stress environment in which water absorption, ion selectivity, and nutrient assimilation are all restricted at the same time.

2.2 Physiological damage caused by saline-alkali stress

Seed germination is one of the earliest life processes affected by saline-alkali stress. Germinating seeds must quickly mobilize stored reserves and establish root-soil contact, but high salinity reduces seed imbibition efficiency and alters the activity of enzymes required for reserve degradation. Elevated pH also suppresses root emergence and affects the maintenance of membrane integrity. In broomcorn millet, evaluations of different genotypes under alkaline stress showed clear differences in seed germination and seedling growth. This not only demonstrates the high sensitivity of the germination stage to saline-alkali conditions, but also indicates the existence of exploitable genetic variation (Ma et al., 2021).

Photosynthesis is then inhibited through several interconnected mechanisms. Salt stress reduces stomatal conductance, suppresses chlorophyll metabolism, damages chloroplast ultrastructure, and limits carbon assimilation. Since ion toxicity and osmotic stress often occur simultaneously, canopy symptoms usually show both dehydration and metabolic inhibition. Alkaline conditions further aggravate the problem by disrupting nutrient supply to the photosynthetic system. In proso millet and related systems, salt-tolerant genotypes are

generally better able to maintain leaf structure and photosynthetic activity than sensitive genotypes. This suggests that maintaining photosynthetic continuity is itself an important tolerance trait rather than only a secondary result of stress resistance.

Oxidative damage is another typical feature of saline-alkali stress. Reactive oxygen species (ROS) normally function as important signaling molecules, but under severe stress they accumulate excessively, causing lipid peroxidation, membrane leakage, protein damage, and cellular dysfunction. Many recent reviews on plant systems have identified ROS regulation as a central component of stress-resistance biology rather than just a secondary symptom. Salt-tolerant millet materials also show lower oxidative damage and stronger inducible antioxidant capacity, further indicating that controlled ROS scavenging plays an important role in plant tolerance under real stress conditions (Mittler et al., 2022).

Roots suffer from both direct and indirect damage. High salinity inhibits root elongation, while high pH suppresses root tip growth, root hair development, and nutrient uptake. Because roots are also responsible for maintaining ion selectivity and supporting beneficial microorganisms, root injury can trigger cascading effects on whole-plant adaptation. In millet, tolerant and sensitive genotypes differ significantly in root structure, surface integrity, and transport-related transcriptional responses, suggesting that root resilience forms the basis of shoot resilience (Yuan et al., 2022).

2.3 Agricultural challenges under saline-alkali conditions

At the field scale, saline-alkali stress not only reduces land quality but also limits the flexibility of agricultural management. It lowers seedling emergence, restricts cultivar selection, and decreases fertilizer use efficiency because plants cannot effectively absorb and assimilate nutrients applied to the soil. In areas with unstable rainfall, poor drainage, or secondary salinization, saline-alkali conditions also increase yield fluctuations between years. Therefore, saline-alkali agriculture is not only a productivity issue, but also a challenge for agricultural sustainability (Negacz et al., 2022).

The ecological costs are also serious. Salinization alters microbial diversity, biogeochemical cycling, and soil structural stability, while alkaline conditions further restrict nutrient cycling processes. In other words, degraded saline-alkali land is not simply soil with “too much salt,” but a land environment whose biological functions have been partially reshaped and are no longer suitable for conventional agricultural production. This is also why crop selection must be considered together with rhizosphere management.

3 Ecophysiological Adaptation Mechanisms of Broomcorn Millet under Saline-Alkali Stress

3.1 Morphological adaptation mechanism

The morphological adaptation of broomcorn millet under saline-alkali stress is not simply “slow growth,” but a structural adjustment aimed at survival and reproductive completion. As a typical drought-tolerant and barren-soil-tolerant minor cereal crop, broomcorn millet has characteristics such as a short growth period, strong root adaptability, and relatively moderate aboveground biomass investment. These features allow it to improve reproductive stability under stress by reducing the cost of vegetative growth in saline-alkali environments.

Root structure is the most crop-specific morphological basis for saline-alkali adaptation in broomcorn millet. An alkali stress evaluation of 296 millet germplasm resources showed that Ma et al. (2021) used mixed alkali concentrations of 80 mmol/L and 40 mmol/L to evaluate alkali tolerance at the germination and seedling stages, respectively. At the seedling stage, plant height, green leaf area, biomass, and root structure were further measured. Green leaf area can serve as a direct indicator of alkali tolerance during the seedling stage, while changes in root structure reflect the ability of the plant to maintain absorption functions under the combined effects of high pH and ion stress. Finally, 12 alkali-tolerant resources and 41 sensitive resources were identified.

The morphological adaptation mechanism of broomcorn millet can be summarized as the coordination of “low-consumption canopy-functional root system-rapid recovery.” Moderate control of leaf area expansion can reduce transpiration and ion transport burden, while maintaining the root absorption interface helps acquire water,

K⁺, and essential mineral nutrients. Compared with major cereal crops such as maize and wheat, the value of broomcorn millet in saline-alkali land is not in forming a large vegetative body, but in achieving stable production on marginal soils through a short growth cycle and strong root plasticity.

3.2 Osmotic adjustment mechanism

Saline-alkali stress first causes a decrease in external water potential, leading to inhibited water uptake, reduced cell turgor pressure, and restricted leaf expansion in broomcorn millet seedlings. To maintain cellular water status, broomcorn millet accumulates compatible solutes such as proline, soluble sugars, and soluble proteins. The role of these substances is not simply to “increase content,” but to improve osmotic adjustment capacity without significantly interfering with enzyme activity, while also stabilizing membrane structure and protein conformation.

An early study on mixed salt tolerance in 16 broomcorn millet genotypes found that under salt concentrations of 160 and 200 mmol/L, the proline content in shoots of materials such as Zhongwei Dahuangmi and Ningmi No.4 increased significantly, while the relative Na⁺/K⁺ ratio remained low. This indicates that osmotic adjustment and ion homeostasis together determine salt tolerance performance (Liu et al., 2012).

Octoploid broomcorn millet showed stronger antioxidant capacity under salt stress, with lower MDA content in leaves and significantly increased soluble sugar and proline content (Li et al., 2025). MDA is an important indicator of membrane lipid peroxidation damage. Its reduction suggests that the accumulation of osmotic adjustment substances is not an isolated response, but part of an integrated salt tolerance system involving membrane protection and antioxidant defense.

The core significance of the osmotic adjustment mechanism in broomcorn millet lies in maintaining cellular water status and metabolic continuity through the accumulation of small organic molecules, thereby avoiding excessive dependence on inorganic ions such as Na⁺ and Cl⁻ for osmotic compensation and reducing the risk of ion toxicity. This is also an important reason why some tolerant materials can still recover growth after rehydration or stress relief, even though growth inhibition occurs during saline-alkali stress.

3.3 Ion homeostasis and transport regulation mechanism

For broomcorn millet, the major damage caused by saline-alkali stress is not only “water deficiency,” but also excessive Na⁺ accumulation and difficulty in maintaining K⁺ levels. After entering cells, Na⁺ interferes with K⁺-dependent enzyme activity, disrupts membrane potential, and affects protein synthesis. Therefore, the key to salt tolerance in broomcorn millet is to limit Na⁺ uptake, promote Na⁺ efflux or vacuolar sequestration, and maintain a high K⁺/Na⁺ ratio. Studies on the molecular mechanisms of plant salt stress generally suggest that ion exclusion, compartmentalization, long-distance transport, and signal transduction together form the core regulatory network of crop salt tolerance (Ma et al., 2022).

A comparative study between broomcorn millet lines ST47 and SS212 provided direct evidence for this mechanism. Yuan et al. (2021) found that under 1% NaCl stress, the salt-tolerant variety ST47 maintained a better Na⁺/K⁺ balance and reduced toxicity by limiting Na⁺ uptake, promoting vacuolar Na⁺ sequestration, and enhancing Na⁺ efflux capacity. KEGG pathway analysis showed that pathways related to Na⁺ regulation, ion transport, and cell wall biosynthesis were significantly regulated in ST47. This indicates that salt tolerance in broomcorn millet is not simply a change in ion content, but a dynamic regulatory process involving transport proteins, signaling pathways, and tissue structure.

The adaptation of broomcorn millet to saline-alkali stress cannot simply be understood as “salt tolerance + alkali tolerance.” Neutral salt stress mainly highlights Na⁺ toxicity and K⁺ retention, while alkali stress additionally involves the effects of high pH on root absorption, cell wall stability, and proton pump activity. As a stress-tolerant minor crop, broomcorn millet has important research value because it can reveal the special strategies used by cereal crops to maintain ion homeostasis under complex saline-alkali conditions.

3.4 Antioxidant defense system

Saline-alkali stress disturbs chloroplasts, mitochondria, and plasma membrane systems in broomcorn millet, leading to increased accumulation of reactive oxygen species (ROS). ROS are not completely harmful because they also participate in stress signal transduction. However, if they are not removed in time, they can cause membrane lipid peroxidation, protein oxidation, and cellular structural damage. Broomcorn millet maintains a dynamic balance between ROS production and scavenging by increasing the activities of antioxidant enzymes such as SOD, POD, CAT, and APX, together with non-enzymatic antioxidant components including proline, soluble sugars, flavonoids, and phenolic compounds.

Research on alkali stress showed that alkali-tolerant broomcorn millet materials possess stronger antioxidant defense capacity. Under alkali stress, the activities of antioxidant enzymes and the contents of osmotic adjustment substances in millet leaves increased simultaneously. Tolerant materials showed lower MDA content and lower electrolyte leakage rates, indicating less oxidative damage to membrane systems (Ma et al., 2021). Antioxidant defense is not simply a passive repair process after saline-alkali stress, but an active adaptation mechanism that helps broomcorn millet maintain leaf function, stomatal structure, and the integrity of photosynthetic tissues.

Studies on exogenous 24-epibrassinolide showed that hormone regulation can alleviate alkali stress injury in broomcorn millet by improving photosynthesis and antioxidant capacity (Ma et al., 2023a) (Figure 1). Exogenous treatment can regulate photosynthesis-related genes, antioxidant-related pathways, and the accumulation of effective metabolites. This suggests that the antioxidant defense system of broomcorn millet is influenced not only by stress intensity, but also by plant hormone signaling regulation.

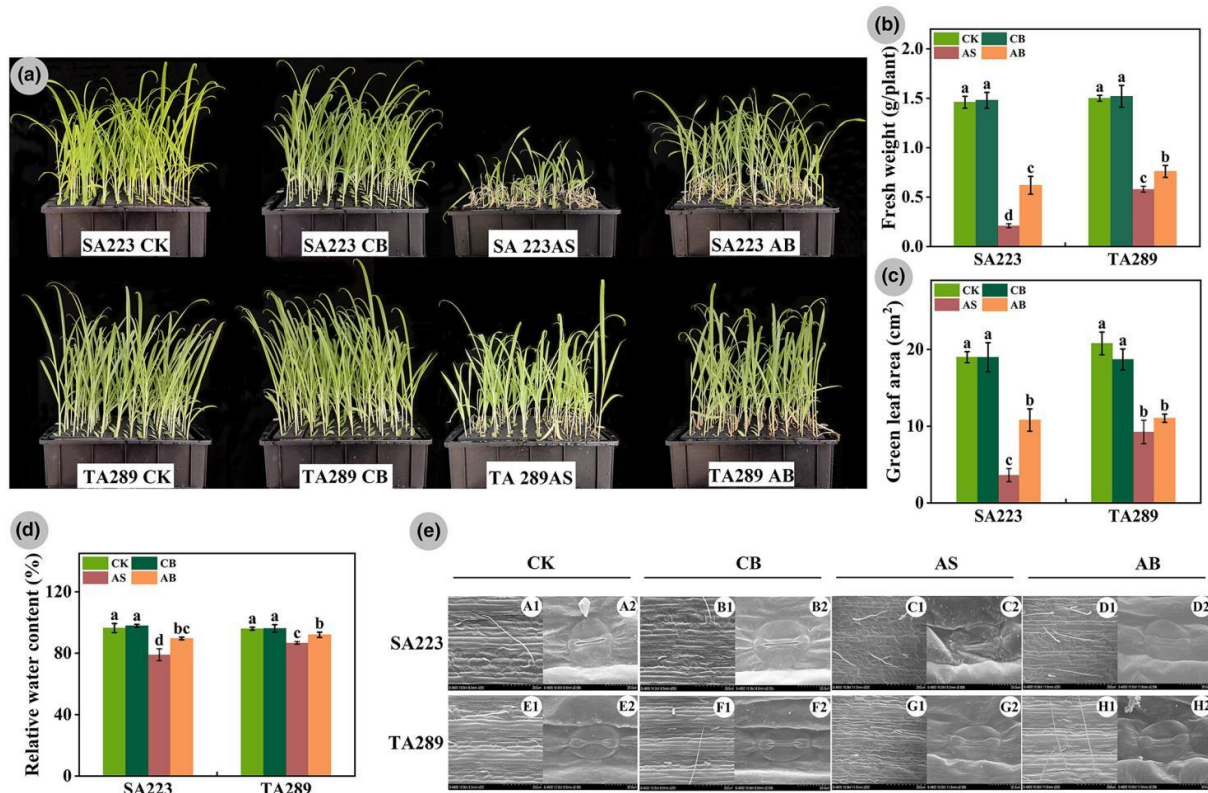


Figure 1 The growth status and leaf characteristics of SA223 and TA289 under different treatments. (a) The phenotypic differences of broomcorn millet plants grown in hydroponic culture for 7 days under different conditions. (b) The fresh weight per plant. (c) Green leaf area per plant. (d) Relative water content. (e) Scanning electron microscope of leaf surface. CK, CE, AS, and AE denote control (nutrient solution only), nutrient solution + 0.5 mg/L 24-Epicastasterone, 40 mM mixed alkali (molar ratio $\text{NaHCO}_3:\text{Na}_2\text{CO}_3 = 9:1$), and 40 mM mixed alkali + 0.5 mg/L 24-Epicastasterone. Data are mean \pm standard error (n = 5). Different alphabetical letters show significant variations among the treatments (p < 0.05, least significant difference test). The error bars indicate the standard errors of the means (Adopted from Ma et al., 2023a)

4 Reshaping of the Rhizosphere Microbiome under Saline-Alkali Stress

4.1 Composition and ecological functions of the rhizosphere microbiome

The rhizosphere microbiome of broomcorn millet is not just a group of “associated organisms” around the root surface. It is an important ecological unit involved in adaptation to saline-alkali soils. Bacteria, fungi, archaea, and micro-eukaryotes in the rhizosphere jointly participate in organic matter decomposition, nitrogen/phosphorus/sulfur cycling, mineral activation, pathogen suppression, and root signal transmission. These functions become especially important in saline-alkali soils because high salinity, high pH, and ion imbalance reduce the availability of nutrients such as phosphorus, iron, and zinc, while also inhibiting the activity of common microorganisms. Under such conditions, microbial communities that can maintain nutrient transformation and stabilize rhizosphere metabolism largely determine whether broomcorn millet can continuously absorb nutrients and maintain growth on marginal land.

The rhizosphere microbiome is directly linked with salt-stress adaptation. Yuan et al. (2023) used the salt-tolerant variety ST47 and the salt-sensitive variety SS212 under a three-month salt-stress pot experiment. They simultaneously analyzed plant phenotype, physiological traits, microstructure, Na⁺ homeostasis-related genes, and rhizosphere bacterial and fungal communities. The salt-tolerant millet not only showed better Na⁺ balance and more stable tissue structure, but also reshaped the rhizosphere microbial community toward a direction beneficial for salt-stress buffering and soil nutrient cycling.

4.2 Changes in microbial communities under saline-alkali stress

The influence of saline-alkali stress on the rhizosphere microbial community of broomcorn millet is not simply a matter of “suppressing all microorganisms.” Instead, it shows clear selectivity. High-salt conditions eliminate some sensitive microbial groups while enriching functional microorganisms that can tolerate high osmotic pressure, high Na⁺ concentration, and high pH conditions.

More specifically, under high salinity, bacterial groups such as Nocardioideae, Saccharimonadales, and Nitrospiraceae become enriched in the millet rhizosphere. These groups are commonly associated with organic matter decomposition, nutrient transformation, and stress-resistant ecological niches. At the same time, fungi such as *Opiculomyces*, *Alternaria*, and *Cryptococcus* are also enriched in the rhizosphere and may participate in organic matter degradation and nutrient absorption. Under high-salt treatment, the rhizosphere recruits these specific bacteria and fungi, which promotes soil nutrient cycling and is associated with improved nutrient uptake capacity in broomcorn millet (Chen et al., 2025).

The adaptation of broomcorn millet to saline-alkali soils does not rely only on the roots “tolerating” unfavorable conditions. Instead, the plant reorganizes the microbial community through rhizosphere selection mechanisms into a structure more favorable for nutrient supply and stress buffering. In other words, the changes in the rhizosphere microbiome under saline-alkali stress have clear ecological functions: on one hand, they reduce nutrient limitations caused by salinity; on the other hand, they improve the resilience of the rhizosphere system under unstable environmental conditions.

4.3 Beneficial rhizosphere microorganisms in broomcorn millet

4.3.1 Plant Growth-Promoting Rhizobacteria (PGPR)

Plant growth-promoting rhizobacteria are one of the most valuable microbial resources for improving saline-alkali adaptation in broomcorn millet. PGPR can promote plant growth through nitrogen fixation, phosphate solubilization, IAA production, extracellular polysaccharide secretion, siderophore synthesis, and regulation of ACC deaminase activity. Under saline-alkali stress, these functions are converted into more direct stress-resistance effects. Extracellular polysaccharides can form protective biofilms on the root surface and reduce direct Na⁺ damage. ACC deaminase can lower stress-induced ethylene levels and alleviate root growth inhibition. Phosphate solubilization and siderophore production help improve the low availability of phosphorus and iron under high-pH conditions.

Salt stress can induce plants to recruit specific root-associated bacterial communities, but long-term salt tolerance is usually provided not by a single strain, but by bacterial consortia with complementary functions (Li et al., 2021). For broomcorn millet, this means future studies should not only search for a single “universal salt-tolerant bacterium.” Instead, synthetic microbial communities should be constructed around highly enriched rhizosphere bacteria of millet. For example, strains with different abilities such as phosphate solubilization, IAA production, antioxidant induction, and extracellular polysaccharide formation can be combined to form compound microbial agents suitable for millet production in saline-alkali soils.

4.3.2 Arbuscular Mycorrhizal Fungi (AMF)

Arbuscular mycorrhizal fungi have special importance in crop adaptation to saline-alkali soils. AMF can expand the root absorption area through fungal hyphae, improve phosphorus uptake efficiency, enhance water utilization, and influence Na^+/K^+ balance and antioxidant enzyme activity. This mechanism is especially important for broomcorn millet because saline-alkali soils are often characterized by low phosphorus availability, poor soil structure, and restricted root absorption. These limitations are difficult to overcome through root function alone.

Liu et al. (2024) analyzed the rhizosphere microbial community structure and metabolic characteristics under broomcorn millet/mung bean intercropping and further examined the relationship between microbial communities and nutrient limitation. Intercropping altered the rhizosphere microbial structures of both crops and affected nutrient utilization status. This indicates that the rhizosphere of broomcorn millet is not fixed, but can be reshaped by planting systems and rhizosphere interactions. Under saline-alkali conditions, coordinated regulation of the rhizosphere using AMF together with nitrogen-fixing microorganisms and phosphate-solubilizing bacteria may become an effective way to improve nutrient uptake and yield stability in broomcorn millet.

In saline-alkali cultivation systems, AMF should not simply be regarded as external additives. Instead, they should be incorporated into a broader “rhizosphere ecological management” framework. Future studies could focus on AMF colonization rates, hyphal density, changes in soil available phosphorus, Na^+/K^+ ratios, and yield performance among different millet varieties in order to identify which genotypes are more suitable for stable symbiosis with AMF.

4.3.3 Endophytic and salt-tolerant microorganisms

Endophytic and salt-tolerant microorganisms are another important potential resource for saline-alkali adaptation in broomcorn millet. Compared with ordinary rhizosphere microorganisms, endophytes can enter internal plant tissues such as roots, stems, and leaves, forming relatively stable colonization relationships within the host. Therefore, they are more likely to provide long-term effects under continuous salt stress. Salt-tolerant endophytes can help plants resist saline-alkali damage by regulating plant hormones, improving antioxidant capacity, promoting osmotic adjustment substance accumulation, enhancing membrane stability, and stimulating root growth.

Salt-tolerant bacteria isolated from saline environments have shown the ability to produce plant growth-promoting substances. Radhakrishnan and Krishnasamy (2024) screened four salt-tolerant bacterial strains and suggested that these microorganisms could be used to promote plant growth and improve soil conditions. Since broomcorn millet is often cultivated on dry, barren, and saline marginal lands, its rhizosphere and endophytic environments are likely to contain microbial resources already adapted to combined stresses.

The saline-alkali tolerance of broomcorn millet should therefore be evaluated not only from the perspective of “plant material,” but also from the perspective of “associated microbial material.” Salt-tolerant millet varieties may possess not only stronger ion homeostasis and antioxidant capacity, but also a greater ability to recruit or maintain salt-tolerant endophytes and rhizosphere microorganisms. In the future, functional strains could be isolated from the rhizosphere and tissues of salt-tolerant materials such as ST47 and tested for their growth-promoting effects on sensitive varieties. This would support the establishment of a “salt-tolerant variety + functional microbial agent” production model for saline-alkali soils.

4.4 Root exudates and microbial recruitment

Root exudates are the key medium through which broomcorn millet regulates the rhizosphere microbiome. Sugars, amino acids, organic acids, phenolic acids, flavonoids, lipids, and purine compounds not only provide carbon and nitrogen sources for microorganisms, but also play roles in chemotaxis, signal recognition, and selective screening. Under saline-alkali stress, the composition of root exudates usually changes, thereby influencing which microorganisms can migrate to the rhizosphere, colonize roots, and form stable communities. Root exudates can drive microbial recruitment and community assembly and promote plant health and stress resistance by influencing microbial chemotaxis, community diversity, and functional complementarity (Yang et al., 2025).

The reshaping of the rhizosphere microbiome in broomcorn millet is probably not a passive outcome, but an actively driven process induced by stress-related metabolic changes in roots. Under saline-alkali stress, millet roots may release organic acids to regulate rhizosphere microdomain pH, provide sugars and amino acids as substrates for salt-tolerant growth-promoting bacteria, and use phenolic or flavonoid compounds as selective signaling molecules. The final microbial recruitment pattern is determined not by a single exudate, but by the overall “metabolite combination” formed by roots under saline-alkali conditions. Future research on broomcorn millet should combine rhizosphere metabolomics with 16S/ITS sequencing to clarify which exudates are associated with enriched groups such as Nocardioideae, Nitriliruptoraceae, and Cryptococcus.

4.5 Plant-microbe synergistic mechanisms under saline-alkali stress

The plant-microbe synergistic mechanisms of broomcorn millet under saline-alkali stress are mainly reflected in four aspects. First, microorganisms alleviate nutrient limitations in saline-alkali soils by improving nutrient availability, such as promoting organic matter decomposition, phosphorus activation, and nitrogen cycling. Second, microorganisms reduce direct Na⁺ damage to roots through extracellular polysaccharides, biofilms, and ion adsorption. Third, PGPR and endophytes can regulate root elongation, lateral root formation, and stomatal behavior through IAA, ACC deaminase, and ABA-related pathways. Fourth, microorganisms can induce antioxidant enzyme systems and osmotic adjustment processes in the host, helping plants maintain higher cellular stability under ROS accumulation and membrane lipid peroxidation stress.

Salt-tolerant broomcorn millet under salt stress does not rely only on its own gene expression regulation. It also shows simultaneous reshaping of rhizosphere bacterial and fungal communities. Under saline-alkali conditions, nutrient accumulation in broomcorn millet is closely related to changes in rhizosphere microorganisms. Specific bacteria and fungi enriched under high-salt treatment are associated with soil nutrient cycling, nutrient absorption, and organic matter decomposition.

Cultivation of broomcorn millet on saline-alkali land should therefore not rely only on evaluating varietal salt tolerance, but should also pay attention to the recovery of soil microbial functions. In the future, salt-tolerant variety screening, rhizosphere functional microorganism isolation, AMF inoculation, organic fertilizer improvement, and intercropping systems can be integrated into a comprehensive rhizosphere regulation strategy. Only in this way can the stress-resistance potential of broomcorn millet be effectively translated into stable yields on saline-alkali soils.

5 Metabolic Reprogramming Induced by Saline-Alkali Stress and Agricultural Applications

5.1 Protein accumulation and nitrogen metabolism

The study of protein accumulation in broomcorn millet grains under saline-alkali stress should not simply follow the generalized conclusion that “stress increases protein content.” Instead, it should be understood within the combined framework of nitrogen redistribution, amino acid synthesis, and restricted grain filling. Saline-alkali conditions simultaneously affect root nitrogen uptake, nitrogen assimilation, amino acid transport, and grain protein deposition. Different proportions of neutral salts and alkaline salts produce different effects on millet germination and seedling growth, and they can distinguish the relative contributions of osmotic stress, ion toxicity, and high-pH damage. This indicates that the metabolic changes of broomcorn millet under saline-alkali stress are not caused by a single salt effect, but by the combined influence of multiple stresses altering growth and material allocation.

From the perspective of nitrogen metabolism, the accumulation of proline, soluble proteins, and related amino acids in millet under stress is not simply an increase in “protective substances.” It also reflects the shift of nitrogen allocation from structural growth to osmotic adjustment and stress defense. Ravichandran et al. (2025), through comparative transcriptomic and metabolic pathway analyses of foxtail millet and broomcorn millet under salt stress, showed that both millet crops activate complex metabolic interactions, enzyme activity changes, and transcription factor regulation under stress conditions. Amino acid metabolism, antioxidant defense, and energy metabolism jointly participate in stress responses. Therefore, nitrogen metabolic reprogramming in broomcorn millet should be regarded as a “growth-defense trade-off”: part of the nitrogen is used to synthesize proline, soluble proteins, and stress-related enzymes, rather than being fully deposited into grain storage proteins.

As a result, grain protein changes in saline-alkali environments may occur in two directions. First, yield reduction may lead to a relative concentration of protein in the grains. Second, severe stress may suppress grain filling and nitrogen transport, thereby limiting protein deposition. In agricultural applications, evaluation under saline-alkali conditions should not focus only on yield. Grain crude protein, free amino acids, proline, glutamine synthetase activity, and nitrogen harvest index should also be measured simultaneously. Only by combining “yield stability” with “nitrogen metabolic quality” can truly suitable broomcorn millet materials for saline-alkali land utilization be identified.

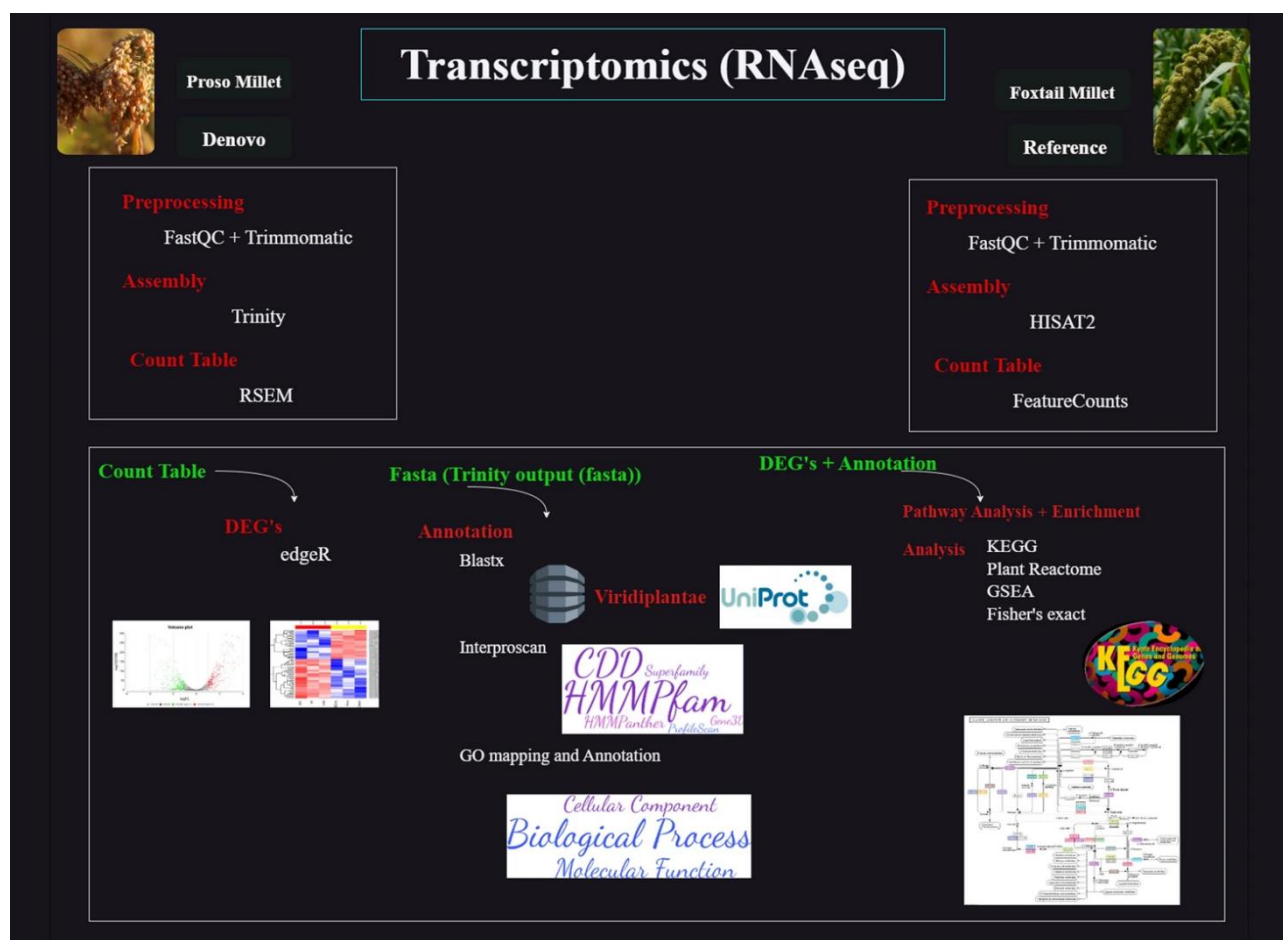


Figure 2 A detailed workflow of data collection, processing, analysis, and visualization used in this study for identifying and evaluating important transcription factors and pathways in foxtail and proso millets under salt stress (Adopted from Ravichandran et al., 2025)

5.2 Accumulation of bioactive compounds

Alkaline stress not only changes yield-related traits, but also affects the metabolite composition in broomcorn millet grains. Using two millet varieties, S223 and T289, Ma et al. (2023b) analyzed the effects of alkaline stress on non-volatile and volatile metabolites in mature grains. A total of 933 non-volatile metabolites and 313 volatile

metabolites were identified. Alkaline stress caused differential accumulation of 114 and 89 non-volatile metabolites in the alkali-sensitive and alkali-tolerant varieties, respectively, while 16 and 20 volatile metabolites also showed significant changes.

More importantly, alkaline stress altered pathways related to phenylpropanoid biosynthesis, flavonoids, flavone and flavonol biosynthesis, valine/leucine/isoleucine biosynthesis, arginine and proline metabolism, tryptophan metabolism, and ascorbate metabolism. This means that metabolic reprogramming in millet grains involves both primary and secondary metabolism. Branched-chain amino acids, arginine, and proline reflect changes in nitrogen metabolism and osmotic regulation, while phenylpropanoid and flavonoid pathways are closely associated with antioxidant activity and the accumulation of functional compounds.

From an agricultural perspective, these findings have dual significance. On one hand, alkaline stress may reduce yield or alter grain filling processes. On the other hand, moderate stress may increase the content of certain phenolic acids, flavonoids, amino acid derivatives, and organic acids, making broomcorn millet a promising raw material for functional foods produced on saline-alkali land. Therefore, broomcorn millet production in saline-alkali areas should not only pursue “stress resistance and yield maintenance,” but also explore the combined goal of “stable yield plus improved functional quality.” For example, in mildly to moderately saline-alkali soils, varieties that can maintain yield while accumulating higher levels of phenolic acids and flavonoids could provide valuable raw materials for functional millet rice, millet flour, and fermented foods.

5.3 Antioxidant and functional metabolites

The accumulation of antioxidant metabolites in broomcorn millet is an important link connecting stress resistance with its development as a functional food ingredient. Under stress conditions, metabolites such as phenolic acids, flavonoids, amino acids and their derivatives, and organic acids increase in millet grains, and the metabolic changes are more obvious in alkali-sensitive materials. This suggests that alkaline stress induces a more active antioxidant metabolic network in millet grains, although the response intensity and direction differ among varieties.

Xiang et al. (2023) investigated changes in phenolic composition and antioxidant activity during the germination of three millet varieties. The study found that total phenolic content and total flavonoid content increased significantly as germination progressed. After six days of germination, free total phenolic content increased by 6.30–8.66 times, while bound total phenolic content increased by 77.65%–116.18%. At the same time, compounds such as feruloyl quinic acid and N, N'-bis-(p-coumaroyl)-putrescine were reported for the first time during millet germination.

The functional metabolites of broomcorn millet show strong plasticity. Saline-alkali stress in the field can reshape grain metabolism, while postharvest processes such as germination and fermentation can further release or generate antioxidant active compounds. In agricultural applications, a complete chain of “saline-alkali cultivation-variety selection-germination processing-functional food development” can be established. For example, germination treatment of millet harvested from saline-alkali soils may further increase phenolic acid and flavonoid contents, supporting the development of antioxidant cereal powders, germinated millet beverages, and low-GI composite foods.

5.4 Nutritional and health value of broomcorn millet

The nutritional value of broomcorn millet should not only be understood from the traditional perspective of “coarse grains.” It should also be analyzed within the dual framework of climate-resilient food crops and functional food ingredients. Broomcorn millet is rich in protein and dietary fiber, and some nutritional indicators are superior to those of common cereals. Its gluten-free characteristics and relatively low glycemic index make it suitable for people with gluten intolerance, type 2 diabetes, and cardiovascular metabolic risks (Pavithra and Rawat, 2024).

Broomcorn millet is not only a traditional grain for cooking, but also has potential applications in the modern food industry. Research on millet nutritional biscuits evaluated biscuit quality from the perspectives of protein networks, tribology, and *in vitro* digestion. Biscuits made from different millet raw materials showed differences in protein network connectivity and porosity. In some samples, the protein network area reached 45.12%, while the lowest porosity was 10.33%, indicating that varietal differences directly affect the structure and digestive properties of baked products (Hu et al., 2025).

Research on cakes fortified with fermented millet bran dietary fiber also demonstrates practical value. After adding fermented millet dietary fiber, the total phenolic content of the product reached 0.46 mg GAE/g, the DPPH radical scavenging rate reached 66.84%, and the ABTS⁺ scavenging rate reached 87.01%, while predicted glucose release was reduced (Xiao et al., 2023). Millet and its by-products can therefore serve not only as basic raw materials for gluten-free foods, but also as ingredients with enhanced antioxidant and low-glycemic potential through fermentation and dietary fiber enrichment.

Metabolic reprogramming induced by saline-alkali stress has clear agricultural application directions. First, saline-alkali tolerant millet can be used to develop specialty grain crops for saline-alkali land. Second, high phenolic acid, high flavonoid, and high protein resources can be screened to establish functional quality breeding indicators. Third, processing technologies such as germination, fermentation, baking, and extrusion can be combined to improve the added value of millet products. In this way, broomcorn millet is no longer just a “survival crop” grown on saline-alkali land, but can become a characteristic crop connecting saline-alkali land management, nutritional health, and the functional food industry.

6 Agricultural and Ecological Applications of Proso Millet

6.1 Improvement of saline-alkali soils

The value of proso millet in saline-alkali soils is not only reflected in its own tolerance to saline environments, but also in its ability to improve the soil ecological environment. Salt-tolerant cultivation helps promote root-mediated soil stabilization, organic matter input, and rhizosphere microecological activation. In broomcorn millet, salt stress induces the enrichment of specific beneficial microorganisms and changes the structure of microbial communities related to nutrient cycling, indicating that this crop can promote the biological functions of saline-alkali soils toward a more productive state (Yuan et al., 2023).

This does not mean that proso millet alone can “restore” all saline-alkali soils. Instead, it shows that the crop is suitable as a biological synergistic factor within integrated land improvement strategies. These strategies usually also include drainage systems, water management, organic amendments, and microbial inoculants. Compared with highly salt-sensitive crops, proso millet can maintain root activity for a longer time, thereby creating favorable conditions for rhizosphere-driven soil improvement.

6.2 Sustainable crop production on marginal lands

The key to agricultural production on marginal lands is not achieving the highest yield in a single season, but maintaining stable returns under conditions of low water, low fertilizer input, climate fluctuations, and poor soil quality. Proso millet fits well within this production logic. It can generally grow under marginal soil and low-input conditions, with a growth period of about 65-75 days or 70-90 days. It requires relatively low amounts of water and fertilizer and shows strong adaptability to drought, poor soils, and climate variability (Nandini et al., 2025).

A long-term crop rotation experiment conducted in northeastern Colorado, USA, from 1995 to 2016 analyzed the relationship between water use and yield in proso millet. Nielsen and Vigil (2017) used multi-year dryland farming data to establish a water-limited yield model and identified environmental factors causing deviations between actual millet yield and theoretical water-based yield. Proso millet should not be regarded as a “low-yield substitute crop,” but rather as a short-season crop suitable for dryland rotation systems, improving water use efficiency and reducing production risks.

A more practical example comes from Karnataka, India. In 2025, it was reported that nearly 3 000 farmers in the drought-prone regions of Dharwad and Haveri introduced millet crops into degraded lands after long-term drought with support from the CROPS4HD project. The cultivation area expanded from about 50 acres to more than 2 000 acres across 23 villages, and a farmer producer company with around 5 000 members was established. The project promoted several millet crops, including proso millet, and reduced farmers' risks through seed supply, technical training, and market access support.

Agriculture on marginal lands is not only about crop stress resistance, but also about seed supply, technical services, farmer organizations, and market outlets. If proso millet is to be widely promoted in saline, drought-prone, and low-fertility areas, it should be integrated with crop rotation systems, cooperative organizations, contract purchasing, and functional food development, rather than being planted only as a scattered minor grain crop.

6.3 Proso millet as a functional feed resource

Proso millet also has strong agricultural potential as a feed resource. Unlike crops used only for grain production, proso millet can be used as silage or harvested forage and is suitable for dry regions, short growing seasons, and low-input crop-livestock farming systems. Research on forage production, feeding value, and silage suitability compared proso millet with whole-crop maize and Sudan grass hybrids (Wei et al., 2022). Although the fresh forage yield of proso millet was lower than that of maize and Sudan grass hybrids, its relative feed value was higher than that of Sudan grass hybrids. In addition, proso millet has a short growth cycle and rapid growth rate, making it an alternative forage resource in low-input regions.

The study also dynamically monitored the silage fermentation process by measuring fermentation quality on days 1, 2, 3, 5, 10, 15, 20, 30, and 45 after ensiling. During the fermentation process, lactic acid and acetic acid contents increased, while pH rapidly declined in the early stage and then became stable. However, the dry matter loss of proso millet was relatively high, indicating that harvesting time, moisture content, and silage additives still need optimization when using proso millet as a silage crop.

Therefore, the feed application of proso millet should not only emphasize that it “can be used as feed,” but should further distinguish four utilization pathways: grain feed, fresh forage, hay, and silage. In saline and drought-prone areas, proso millet can be rotated or intercropped with legume forages or feed legumes to improve protein supply and soil nitrogen cycling. In integrated crop-livestock farming systems, millet grain, straw, and processing by-products can be utilized together to form a “grain-feed-livestock-fertilizer” recycling model. This dual-purpose utilization capacity can provide farmers with greater flexibility when facing grain price fluctuations, feed costs, and climate risks.

6.4 Contribution to food security and sustainable development

The contribution of proso millet to food security is mainly reflected in three aspects. First, it expands the boundaries of food production on marginal lands. Second, it increases cereal diversity and dietary diversity. Third, it provides a crop foundation for low-input, low-water-consumption, and climate-adaptive agriculture. Millet crops possess strong climate resilience, high nutritional value, and significant rural economic potential, and they are expected to play a greater role in global food security and sustainable food systems in the future (Sharma et al., 2025).

After the “International Year of Millets” in 2023, millet crops were further incorporated into discussions on nutritional security, climate adaptation, and sustainable agriculture. Millets can help reduce dependence on high-input agricultural systems and are closely related to the United Nations Sustainable Development Goals, including zero hunger, climate action, and poverty reduction. In addition, the industrialization of millet cultivation and processing may create income opportunities for rural communities (Mohan et al., 2025).

For proso millet, this value is especially important. It is neither simply a famine-relief crop nor just a traditional minor grain for local diets. Instead, it is a multifunctional crop that can connect saline-alkali land utilization,

dryland farming, functional foods, feed resources, and farmer income growth. In the future, if coordinated development can be achieved among breeding salt-tolerant varieties, saline-land cultivation systems, forage utilization, functional food development, and regional brand building, proso millet may become not only an alternative crop for marginal lands, but also a strategic minor cereal for improving agricultural system resilience under climate change.

7 Current Challenges, Future Perspectives, and Conclusions

7.1 Limitations in understanding rhizosphere microbial networks

In recent years, research on salt-alkali stress in proso millet (*Panicum miliaceum* L.) has gradually expanded from simple physiological measurements to studies of the rhizosphere microbiome. However, this field is still transitioning from descriptive studies to mechanism-based research. Salt-tolerant millet materials can alter rhizosphere bacterial and fungal communities under salt stress and enrich microbial groups related to nutrient cycling, salt buffering, and organic matter transformation. Nevertheless, there is still limited understanding of how these microorganisms form stable networks, how they influence the host through metabolic interactions, and whether different microbial functions can replace one another.

One major difficulty in rhizosphere microbial network research is that community behavior is not equal to the behavior of a single strain. Salt stress can induce plants to recruit specific rhizosphere bacteria, but long-term salt tolerance is usually supported by bacterial communities with complementary functions rather than by one “universal strain” (Li et al., 2021). Even if some bacterial genera are enriched in the rhizosphere of salt-tolerant millet, it cannot be directly concluded that inoculation with a single strain will stably improve salt tolerance. The actual effect may come from a functional rhizosphere network jointly formed by bacteria, fungi, and their metabolites.

In addition, field saline-alkali environments are highly complex. Salinity level, Na^+/Cl^- ratio, carbonate and bicarbonate content, soil pH, texture, organic matter level, irrigation system, and previous crops can all influence rhizosphere microbial assembly. Therefore, microbial communities that perform well in one saline soil may fail to colonize effectively in another soil type, or may not show growth-promoting effects at all. For millet, future research should not only answer “which microorganisms are enriched,” but also clarify “under which soil conditions,” “recruited by which millet genotype,” “through which metabolic pathways,” and “whether the effect can remain stable across continuous growing seasons in the field.” These are the key issues that must be solved before rhizosphere microbiome research can move from basic science to agricultural application.

7.2 Multi-omics approaches in rhizosphere research

An important future direction for millet salt-alkali adaptation research is the establishment of an integrated multi-omics framework linking “host genotype–root exudates–rhizosphere microorganisms–ion homeostasis–yield and quality.” Previous studies usually measured physiological traits, transcriptomes, metabolites, or microbial communities separately. Although such studies can reveal changes at a specific level, they often fail to explain the causal relationships among different biological layers. For example, a salt-tolerant material showing a high K^+/Na^+ ratio may be related to transporter gene expression, regulation of microbial communities by root exudates, or interactions among root architecture and soil nutrient status. Without integrated analysis, it is difficult to identify the real limiting factor.

The improvement of millet genomic resources has provided the basis for this type of research. Zou et al. (2019) completed a high-quality chromosome-level genome assembly of millet, identifying 55,930 protein-coding genes and 339 miRNAs. They suggested that this genomic resource could support studies on stress resistance, C4 photosynthesis, and breeding improvement. This means that traits such as salt tolerance, alkali tolerance, root development, and rhizosphere recruitment ability can now be connected with genomic variation, promoting millet breeding from traditional experience-based selection to genome-assisted and microbiome-assisted breeding.

Methodologically, an ideal experimental design should simultaneously conduct phenotypic analysis, ionomics, root exudate metabolomics, rhizosphere metagenomics, metatranscriptomics, and host transcriptomics using the

same salt-tolerant and salt-sensitive millet materials. Research on wild soybean under salt stress has already provided a useful example. Researchers found that salt stress induced roots to secrete xanthine, which then recruited beneficial *Pseudomonas* species and improved host salt tolerance. Such studies indicate that only by placing “root exudates-microorganisms-host tolerance” within the same experimental system can the active recruitment mechanism of plants truly be explained. For millet, future work should further identify which organic acids, phenolic acids, amino acids, or sugars are associated with the enrichment of salt-tolerant rhizosphere microbial communities, and verify whether these metabolites can consistently induce beneficial microbial colonization.

7.3 Microbiome-assisted breeding strategies

Microbiome-assisted breeding should not be considered an additional technique outside traditional salt-tolerance breeding. Instead, it should become an important part of improving millet adaptation to saline-alkali soils. The basic concept is that host genotype not only determines root structure, ion transport, and antioxidant capacity, but also affects root exudate composition and rhizosphere microbial assembly. Therefore, two millet materials with similar phenotypes may show completely different field stability under continuous saline-alkali cultivation because of differences in their ability to recruit beneficial microorganisms.

In recent years, plant microbiome breeding studies have proposed that plant genetic backgrounds should be matched with compatible microbial inoculants, rather than treating microbial products as universal inputs independent of crop genotype. Future breeding should simultaneously consider plant genetics, microbial functions, and environmental adaptability, and enhance stress resistance through matching host genotypes with microbial inoculants (Shi et al., 2026). This idea is especially suitable for millet because it is often cultivated on marginal lands affected by drought, nutrient deficiency, and salinity, where environmental heterogeneity is high and simple selection for high-yield materials cannot guarantee stable field performance.

Breeding indicators with greater practical value for millet should expand from yield alone to comprehensive adaptability traits, including stable K^+/Na^+ selectivity, root recovery ability, biomass regeneration after salt stress, root exudate plasticity, enrichment capacity of beneficial rhizosphere microbes, and stability of microbial community networks. The rhizosphere microbiome is regulated by both environment and host genotype. Using the plant's natural ability to recruit beneficial microorganisms is becoming an important direction for precision microbial engineering and stress-resistant agriculture in the future (Shen et al., 2024). Therefore, salt-alkali breeding in millet should not only select materials that are “salt tolerant themselves,” but also materials that can “stably establish beneficial rhizosphere systems.”

7.4 Synthetic microbial communities and biofertilizers

In future millet production on saline-alkali land, microbial intervention technologies will likely shift from single-strain inoculation to synthetic microbial communities (SynComs). Although single strains are easier to screen and produce, they often face weak colonization ability, competition from native microorganisms, and unstable performance in the field. In contrast, synthetic communities can be designed according to functional complementarity, allowing different members to perform functions such as phosphate solubilization, nitrogen fixation, IAA production, extracellular polysaccharide secretion, antioxidant induction, and ion homeostasis regulation. In this way, they can provide more stable growth-promoting and stress-resistant effects.

SynComs are not only modular tools for studying plant-microbe interactions, but can also be used to promote plant growth and improve stress adaptation. Their construction strategies include bottom-up screening of functional strains and top-down simplification and reconstruction of native microbial communities (Xu et al., 2025). This provides a clear path for millet cultivation on saline-alkali land: first isolate locally adapted strains from the rhizosphere of millet grown long-term in saline soils, then construct functional microbial consortia based on complementary traits, and finally test their compatibility with different millet genotypes.

However, commercialization of synthetic microbial communities still faces major challenges. Plant microbiome research requires more unified standards for community construction, causal verification, data reporting, and field

evaluation. Otherwise, results from different studies are difficult to compare, and application outcomes are difficult to reproduce consistently (Northen et al., 2024). For millet, future biofertilizer development should avoid the concept of “universal microbial products.” Instead, it should emphasize regionalized and genotype-specific formulations. In other words, localized microbial consortia should be developed according to different saline-alkali soil types, soil textures, and millet cultivars, and their effects on yield, quality, and soil improvement should be validated through long-term and multi-location field trials.

7.5 Future role of millet in climate-resilient agriculture

The value of millet in climate-resilient agriculture comes from its multiple adaptive traits. It has a short growth cycle, high water-use efficiency, and strong adaptability to marginal lands. At the same time, it also has nutritional value, gluten-free characteristics, and potential for functional food development. Unlike major crops that depend on high inputs for high yields, millet is more suitable for low-input, low-water-consumption, risk-resistant, and diversified agricultural systems. Its role is not to replace rice, wheat, or maize, but to provide more flexible options for future food systems.

From the perspective of global agricultural trends, climate change, soil degradation, and water shortages are driving agricultural systems away from a single high-yield target toward a balance among stable production, nutritional security, and ecological sustainability. As an ancient and stress-tolerant minor cereal crop, millet deserves renewed attention in this context. Its short growth period makes it suitable for filling crop rotation gaps; its drought and poor-soil tolerance make it suitable for marginal lands; its nutritional quality supports functional food development; and the plasticity of its rhizosphere microbiome gives it potential as an important crop platform for biologically regulated agriculture.

In conclusion, the salt-alkali adaptation mechanism of millet should no longer be understood as simple physiological tolerance. Instead, it should be viewed as the combined result of plant genotype, root structure, ion homeostasis, metabolic regulation, and rhizosphere microbiome interactions. Future research should advance in three directions. First, multi-omics technologies should be used to reveal the causal mechanisms of salt-alkali tolerance in millet. Second, microbiome recruitment ability should be incorporated into breeding evaluation systems. Third, localized synthetic microbial communities and ecological cultivation technologies suitable for millet production on saline-alkali soils should be developed. Only in this way can millet evolve from a traditional minor grain crop into a strategic crop resource serving saline-alkali land utilization, food security, healthy food production, and climate-resilient agriculture.

Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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Research Perspective

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Advances in Water-Saving and High-Yield Cultivation Technologies for Winter Wheat under Climate Change

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Received: 11 Apr., 2026

Accepted: 20 May, 2026

Published: 07 Jun., 2026

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Preferred citation for this article:

Ma Z.Q., Liu Z.Y., and Wang W., 2026, Advances in water-saving and high-yield cultivation technologies for winter wheat under climate change, Bioscience Evidence, 16(3): 157-170 (doi: [10.5376/be.2026.16.0013](https://doi.org/10.5376/be.2026.16.0013))

Abstract Winter wheat is one of the major food crops in the world and plays an important role in ensuring global food security. However, extreme weather events and water shortages caused by climate change have created serious challenges for winter wheat production. The water demand of winter wheat changes greatly at different growth stages. Water demand is highest during the jointing-booting stage and grain filling stage, while the demand is relatively low during winter dormancy. Water deficit can cause stomatal closure and reduced transpiration, which further suppresses photosynthesis and accelerates leaf senescence. Combined high-temperature and drought stress can shorten the grain filling period and reduce thousand-grain weight. Improving water use efficiency (WUE) is the key to achieving both high yield and water saving. The rational use of deep soil water, efficient regulation of photosynthesis, and stress “memory” mechanisms can enhance the drought resistance of wheat. This study systematically reviews the physiological basis, cultivation technologies, and breeding progress of water-saving and high-yield cultivation of winter wheat under climate change conditions. It also discusses future development directions by combining digital agriculture and regional practices, providing references for coping with climate change and water resource crises.

Keywords Winter wheat; Water-saving cultivation; High yield; Climate change; Water use efficiency

1 Introduction

Winter wheat is one of the most important crops in the global food system and an important source of dietary energy and plant protein for people living in temperate agricultural regions. Compared with rice and maize, wheat has a wider adaptation range, more processing and utilization methods, and more active international trade. It plays an irreplaceable role in ensuring food security, stabilizing agricultural product supply, and maintaining the resilience of agricultural economies. Shiferaw et al. (2013) pointed out that wheat is not only an important staple food for people in developing countries, but also a key crop supporting agricultural livelihoods in many arid and semi-arid regions.

Rising temperatures, changes in the spatial and temporal distribution of precipitation, and the increasing occurrence of heat waves and drought events are changing the water–heat matching pattern in traditional winter wheat production areas (Jägermeyr et al., 2021). Especially in arid and semi-arid regions such as the North China Plain, northwest dryland areas, South Asia, and the Middle East, winter wheat often faces the contradiction between increased water demand during rapid spring growth and insufficient precipitation combined with enhanced evapotranspiration. Traditional irrigation methods have supported high wheat yields for a certain period, but they have also caused problems such as groundwater overexploitation, low irrigation efficiency, and intensified competition for agricultural water use. Improving water use efficiency has become an urgent goal in winter wheat production. The development of technologies such as remote sensing, unmanned aerial vehicles, and multi-angle spectral monitoring has also made dynamic monitoring of winter wheat water status and water use efficiency possible.

In actual production, high yield of winter wheat usually depends on sufficient water and nutrient supply, while water saving requires reducing irrigation frequency and total irrigation amount. This creates an internal contradiction between yield goals and resource limitations. Water-saving and high-yield production of winter wheat does not simply mean “less irrigation.” Instead, based on the water demand characteristics at different

growth stages, limited water resources should be preferentially allocated to key periods such as jointing, booting, flowering, and grain filling. At the same time, comprehensive measures are also needed, including soil moisture conservation, straw mulching, conservation tillage, coordinated water and fertilizer management, breeding of drought-resistant varieties, and precision irrigation decision-making (Liu et al., 2020).

Based on this background, this study mainly reviews the production problems, theoretical basis, and technical approaches of winter wheat under conditions of water resource limitation and increasing climate risk. The study analyzes the global importance of winter wheat production and the water stress problems caused by climate change, and explains the central role of improving water use efficiency in modern wheat production. In the future, winter wheat production should shift from traditional experience-based irrigation to precise diagnosis and intelligent decision-making, and from simply pursuing yield per unit area to the coordinated improvement of yield, water use efficiency, and ecological sustainability. This transition will help relieve pressure from water shortages and also provide references for stable yield improvement and efficiency enhancement of food crops under climate change.

2 Water Requirement Characteristics of Winter Wheat under Climate Change

2.1 Seasonal water requirement pattern

The water demand of winter wheat shows a clear seasonal pattern. From the seedling stage to the spring regreening stage, water consumption is relatively low because of low temperature and slow plant growth. During the winter dormancy period, water demand decreases further. After entering the jointing and booting stages in spring, rising temperatures and rapid vegetative growth cause water demand to increase quickly, usually reaching the highest level during the whole growth period. During the grain filling stage and late maturity stage, water demand decreases slightly but still remains relatively high to ensure grain filling and dry matter accumulation. Under strong light conditions and warm climates, the water requirement of winter wheat significantly increases around the heading stage. In late spring and early summer, especially during the late grain filling stage, plant transpiration gradually weakens. Water supply during key growth stages, particularly from jointing to flowering, is extremely important because water shortage during this period can significantly reduce yield. In regions such as the North China Plain, the average water requirement during the wheat growing season is about 400-500 mm, and more than 50% of the total water consumption occurs during the booting stage (Sun et al., 2024).

2.2 Physiological responses to water deficit

Under drought stress, wheat undergoes a series of physiological adjustments to adapt to water deficiency. In terms of stomatal regulation, drought stress increases the concentration of abscisic acid (ABA) in plants, which induces stomatal closure and reduces water loss through transpiration. However, stomatal closure also limits the entry of carbon dioxide, thereby suppressing photosynthesis. During short-term drought, stomatal closure is the main reason for reduced photosynthetic activity, while long-term and severe drought further downregulates genes related to photosynthetic metabolic pathways, causing additional damage to photosynthetic capacity (Li et al., 2023). Regarding transpiration, water shortage decreases leaf water potential and stomatal conductance, leading to a significant reduction in transpiration rate. Although this helps reduce water consumption, it also restricts nutrient transport within the plant. In addition, drought stress often causes oxidative stress because overload of the photosynthetic electron transport chain leads to the production of reactive oxygen species. Wheat improves cellular protection ability by increasing the activity of antioxidant enzymes such as peroxidase and superoxide dismutase, as well as accumulating osmotic adjustment substances including proline and betaine.

2.3 Effects of combined heat and drought stress

Climate change not only causes drought but also frequently leads to combined heat and drought stress. When high temperature occurs during the grain filling stage, the grain filling process is often accelerated and shortened, resulting in insufficient transfer of assimilates into grains and ultimately reducing thousand-grain weight. Under additional high-temperature conditions above 30 °C, the single-grain weight of spring wheat may decrease by 5%~12% (Wang et al., 2025). The combined effects of heat and drought can also intensify metabolic disorders in plants. Under water-deficient conditions aggravated by high temperature, stomata in wheat may close prematurely,

further affecting photosynthesis and accelerating leaf senescence, which eventually causes major yield reduction (Ye et al., 2024).

2.4 Water use efficiency under changing environmental conditions

Water Use Efficiency (WUE) is an important indicator for evaluating the water-saving production capacity of crops, and it can be assessed at both physiological and field levels. “Physiological WUE” usually refers to the ratio between CO₂ absorbed through photosynthesis and water lost through transpiration per unit leaf area (PN/Tr), reflecting the balance between photosynthetic efficiency and water loss under stomatal regulation. “Field WUE” refers to the dry matter yield or grain yield obtained per unit of crop water consumption (total evapotranspiration), commonly expressed as kg·ha⁻¹·mm⁻¹. Improvement of WUE can be achieved either by increasing yield or reducing water consumption. The use of modern water-saving irrigation methods and efficient cultivation technologies can significantly improve field WUE (Wu et al., 2025). Studies on winter wheat in northern China have shown that moderately reducing irrigation water combined with high-yield cultivation practices can increase WUE by more than 10% (Yang et al., 2025).

3 Physiological Basis of Water-Saving and High-Yield Cultivation

3.1 Root structure and utilization of deep soil water

The basis of water-saving and high-yield cultivation in winter wheat is not simply reducing water consumption above ground. More importantly, the root system must be able to convert limited soil water into effective grain yield under uneven spatial and temporal distribution of water. The growth period of winter wheat spans autumn, winter, spring, and summer. From regreening to grain filling, surface soil drought often occurs, while deeper soil layers may still retain water stored before winter or from earlier rainfall events.

Li et al. (2022) conducted two seasons of winter wheat pot experiments at the Luancheng Agro-Ecosystem Experimental Station on the North China Plain. Different soil depths of 0.5, 1.0, 1.5, and 2.0 m and water supply levels of 90~500 mm were established to simulate different rooting depths and soil water availability conditions. Under similar seasonal evapotranspiration, shallower root systems produced lower grain yield. Deep root systems not only increased the amount of available soil water, but also changed the distribution of water consumption during different growth stages, allowing more water to be used during reproductive growth. Deep roots improved “root efficiency,” meaning that higher grain production was achieved with lower root growth cost.

Odone et al. (2024) studied deep rooting traits of 14 winter wheat genotypes using the RadiMax semi-field root phenotyping platform in Denmark. Minirhizotrons reaching depths of 2.5 m were used to observe root growth, and water labeled with ¹⁵N and ²H was injected into soil layers at 1.6~1.8 m to determine whether deep roots actually functioned in absorption. Differences in deep root development were observed among winter wheat genotypes. Deep roots were associated with deep water uptake, nitrogen absorption, grain yield, and drought resistance. Genotypes with deeper roots showed larger reductions in water stress and greater yield increases when they could access deep soil layers. Therefore, drought resistance in winter wheat should not only be evaluated by plant height, leaf area, or spike number above ground, but also by deep rooting ability as an important trait for climate-adaptive breeding.

Sun et al. (2020) compared eight representative winter wheat cultivars grown in dryland areas of Shaanxi Province from the 1940s to the 2010s. Modern cultivars showed larger root surface areas under drought conditions, especially in the 0~40 cm soil layer. At the same time, grain yield significantly increased with cultivar improvement over decades, and water use efficiency increased by an average of 47.07% from early cultivars to modern cultivars. Long-term dryland breeding and cultivation selection did not simply produce “larger root systems,” but gradually achieved a more balanced coordination among root size, water uptake efficiency, and aboveground yield formation.

3.2 Regulation of photosynthesis under drought conditions

The effect of drought on winter wheat yield is ultimately reflected in reduced photosynthetic carbon assimilation and insufficient grain filling. Closure of leaf stomata can reduce water loss, but it also limits CO₂ entry into

mesophyll cells, leading to lower net photosynthetic rate. Under prolonged drought stress, chloroplast structure may be damaged, photosystem II electron transport may be inhibited, and reactive oxygen species may accumulate, causing premature leaf senescence.

Li et al. (2023) studied the effects of warming and drought stress on the coupling relationship between photosynthesis and transpiration in winter wheat on the North China Plain. Drought first reduced water loss by decreasing stomatal conductance. However, with increasing stress intensity, the decline in photosynthesis was no longer caused only by stomatal limitation, but also by downregulation of photosynthetic metabolism. In other words, mild water deficit may temporarily improve water use efficiency, but long-term or severe drought disrupts carbon-water coupling, turning water saving into yield reduction. Therefore, severe water deficit should be avoided during jointing, flowering, and early grain filling stages because photosynthetic capacity during these periods directly affects grain number and thousand-kernel weight.

During grain filling, the flag leaf is the major source of assimilates in late growth stages, and the duration of its photosynthetic activity directly affects grain fullness. Naseer et al. (2024) used the winter wheat cultivar “Xinong 979” in the dry farming region of the Loess Plateau to study the combined effects of drought and weak light during grain filling. Treatments included irrigation levels of 100%, 75%, 50%, and 25%, along with different shading durations. As irrigation decreased and shading time increased, net photosynthetic rate, transpiration rate, stomatal conductance, and intercellular CO₂ concentration all declined significantly. Under the treatment of 12 days of shading combined with the lowest irrigation level, photosynthetic gas exchange parameters showed the greatest decline. At the same time, chlorophyll fluorescence parameters Fv/Fm, qP, and quantum yield also decreased, indicating damage to PSII reaction centers (Figure 1). Drought and shading together reduced spike number, thousand-kernel weight, and grain yield, indicating that the grain filling stage is not simply a period where “less water is acceptable,” but rather a critical window when the photosynthetic system of winter wheat requires strong protection.

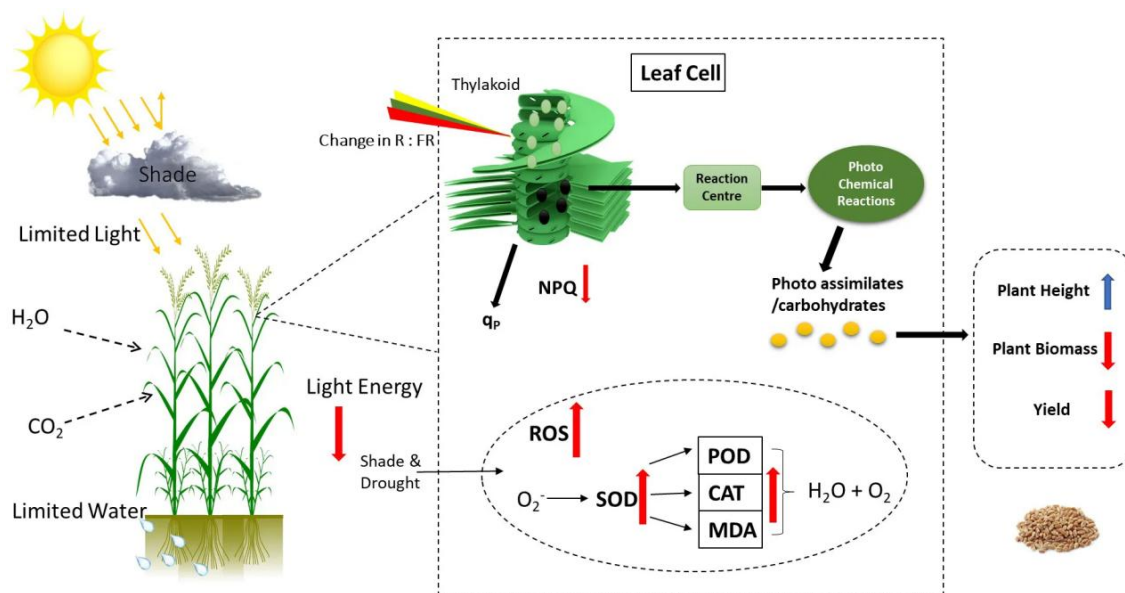


Figure 1 The comprehensive model of physiological metabolism regulation in winter wheat plants under drought and shading stress. Changing the light environment and drought conditions regulate the photosynthetic activity, photochemical efficacy, and antioxidant enzyme activities to adapt the environmental stress. The distribution and regulation of photo-assimilates affect the agronomic characteristics, and yield of winter wheat plants (Adopted from Naseer et al., 2024)

3.3 Water stress memory and adaptation mechanisms

Winter wheat has a certain capacity to adapt to water stress. Because the growth period is relatively long, winter wheat often experiences multiple stress events, including mild drought at the seedling stage, water fluctuations during regreening, spring drought after jointing, and hot dry wind during grain filling. If mild stress at early stages activates adaptive mechanisms, drought tolerance at later stages may be improved.

Amini et al. (2023) investigated drought stress memory in common wheat and synthetic wheat germplasm. Treatments included normal irrigation, secondary drought stress after seed priming, drought at jointing followed by severe drought at flowering, and single drought stress at flowering. A total of 27 wheat genotypes were evaluated. Plants exposed to moderate drought during jointing and then severe drought during flowering showed a more effective enzymatic antioxidant system, which reduced later yield loss. Clear differences in stress memory responses were found among genotypes, and synthetic wheat generally performed better than common wheat in grain yield, yield components, and drought resistance index. Changes in proline, soluble sugars, peroxidase, catalase, and ascorbate peroxidase under secondary stress indicated that osmotic adjustment and antioxidant defense are important physiological bases for stress memory formation in winter wheat.

3.4 Source-sink coordination during grain filling

The grain filling stage is the final critical period for yield formation in winter wheat and also the stage most vulnerable to yield risk under water-saving cultivation. The “source” mainly refers to assimilate-producing organs such as the flag leaf, the second leaf below the flag leaf, stems, and spikes, while the “sink” mainly refers to the grains.

Fang et al. (2024) quantitatively analyzed source-sink relationships using 13 years of semi-controlled field experiment data involving six bread wheat genotypes under combinations of high temperature, water deficit, and nitrogen deficiency. Across different environments and genotypes, grain biomass was on average about 10% higher than newly produced aboveground biomass after flowering. This indicates that grain filling does not rely entirely on newly formed photosynthates after anthesis, but also depends on remobilization of assimilates stored before flowering. More importantly, as stress intensity increased, the relative contribution of pre-anthesis assimilates to grain biomass increased from nearly 0 to 100%. This contribution was more strongly affected by water and nitrogen conditions than by temperature. Under drought or nitrogen deficiency, the ability of winter wheat to effectively transport previously accumulated carbohydrates into grains becomes the key factor determining grain filling stability.

On the Huang-Huai-Hai Plain of China, optimized irrigation can also improve source-sink relationships by delaying flag leaf senescence. Yan et al. (2023) reported in a two-year experiment that under traditional border irrigation, a border length of 40 m achieved a good balance between water saving and high yield. Compared with other border lengths, the L40 treatment enhanced antioxidant enzyme activity and sucrose metabolism in the flag leaf after flowering, delayed declines in SPAD value and chlorophyll fluorescence parameters, and promoted grain filling rate and thousand-kernel weight. In contrast, excessively short borders reduced yield, while excessively long borders lowered water productivity.

4 Advances in Water-Saving Cultivation Technology

4.1 Deficit irrigation strategies

Deficit irrigation (DI) is not simply reducing irrigation water input. Instead, it is a targeted regulation of irrigation timing, irrigation amount, and the lower limit of soil moisture according to the sensitivity of winter wheat to water deficit at different growth stages. From the regreening stage to jointing, winter wheat enters a period of rapid vegetative growth, with rapid expansion of leaf area and increased transpiration water consumption. The booting stage, flowering stage, and early grain-filling stage are directly related to spikelet number, grain formation, and dry matter transport to grains, so these stages require the greatest water guarantee in water-saving irrigation. In contrast, moderate water deficit during the overwintering stage, early regreening stage, or late maturity stage has relatively limited effects on final yield. Based on this, the concept of deficit irrigation has gradually formed: “moderate water control during non-critical periods and precise water supply during critical periods.”

From the perspective of regional irrigation systems, water-saving irrigation for winter wheat in the North China Plain emphasizes coordination with interannual precipitation differences. Optimal irrigation strategies differ among years with different rainfall conditions. In wet years, irrigation frequency can be reduced, and sometimes only one supplemental irrigation at flowering is enough. In normal rainfall years and dry years, two irrigations are generally required to balance water supply before and after flowering. The irrigation demand of winter wheat

varies greatly over multiple years, and irrigation water requirements may change significantly under different precipitation patterns. If irrigation is fixed without considering annual rainfall differences, water waste may occur in wet years, while water shortage may occur in dry years (Zhao et al., 2020).

The meta-analysis of irrigation-soil-climate interactions conducted by Yang et al. (2025) further explained the quantitative relationship between water saving and high yield. Many wheat irrigation experiments showed that irrigation significantly increased grain yield, spike number, grains per spike, and thousand-grain weight. However, the increase in water productivity was relatively limited because higher yield was accompanied by increased evapotranspiration. Irrigation amounts between 75 and 150 mm were more suitable for balancing grain yield and water productivity. When irrigation exceeded this range, the marginal yield increase gradually declined.

4.2 Precision irrigation technology

Traditional flood irrigation and furrow irrigation often have problems such as excessive irrigation amount, strong surface evaporation, deep percolation losses, and uneven soil water distribution. In contrast, drip irrigation, sprinkler irrigation, micro-sprinkler irrigation, and intelligent irrigation systems can improve the efficiency of water delivery to the root zone and reduce ineffective water consumption.

Yang et al. (2020) established five irrigation levels of 0.25, 0.40, 0.60, 0.80, and 1.00 ET_c in a subsurface drip irrigation experiment on winter wheat. Although the 0.25 and 0.40 ET_c treatments achieved obvious water savings, tiller number, leaf water content, leaf area index, net photosynthetic rate, and grain yield all decreased significantly. Compared with full irrigation, the 0.80 ET_c treatment showed only small differences in plant height, leaf area index, spike number, and photosynthetic-transpiration parameters, and could maintain yield relatively well. The 0.60 ET_c treatment showed a relatively high harvest index and water use efficiency. Deficit subsurface drip irrigation also promoted water extraction from the 40~140 cm soil layer, allowing crops to utilize more deep soil water storage.

Zheng et al. (2025) carried out a micro-sprinkler irrigation experiment in the North China Plain to compare the effects of different wetting depths and irrigation regimes on winter wheat yield, water productivity, and carbon emission efficiency. In years with normal rainfall, maintaining the wetting depth at 0~30 cm could sustain yield while reducing water consumption. In wet years, a wetting depth of 0~40 cm was more favorable for achieving high yield potential. Compared with drip irrigation, micro-sprinkler irrigation improved surface soil moisture distribution and, under certain conditions, reduced farmland CO₂ emission flux and improved carbon emission efficiency.

The value of intelligent irrigation systems lies in transforming “when to irrigate,” “how much to irrigate,” and “how deep to irrigate” into a calculable, monitorable, and feedback-based decision-making process. Soil moisture sensors, weather stations, UAV remote sensing, satellite remote sensing, and crop models have been integrated into irrigation management (Abdelhamid et al., 2025). Sun et al. (2024) used UAV hyperspectral technology to estimate leaf water content of winter wheat during the grain-filling stage and established the relationship between leaf water content and soil moisture to determine suitable irrigation amounts during grain filling. Since grain filling is a critical stage for grain formation, accurate evaluation of canopy water status helps improve both grain yield and water productivity. Compared with traditional soil sampling or experience-based observation, UAV monitoring has advantages such as high resolution, rapid coverage, and non-destructive measurement, making it suitable for field-scale water diagnosis (Figure 2).

4.3 Soil water conservation measures

For winter wheat, whether irrigation water can be effectively stored in soil, absorbed by roots, and protected from ineffective evaporation largely depends on tillage layer structure, straw mulching, soil organic matter, and soil porosity. If soil compaction is severe, the plow pan is thick, and infiltration capacity is poor, increasing irrigation may still result in surface runoff, deep leakage, or restricted root penetration.

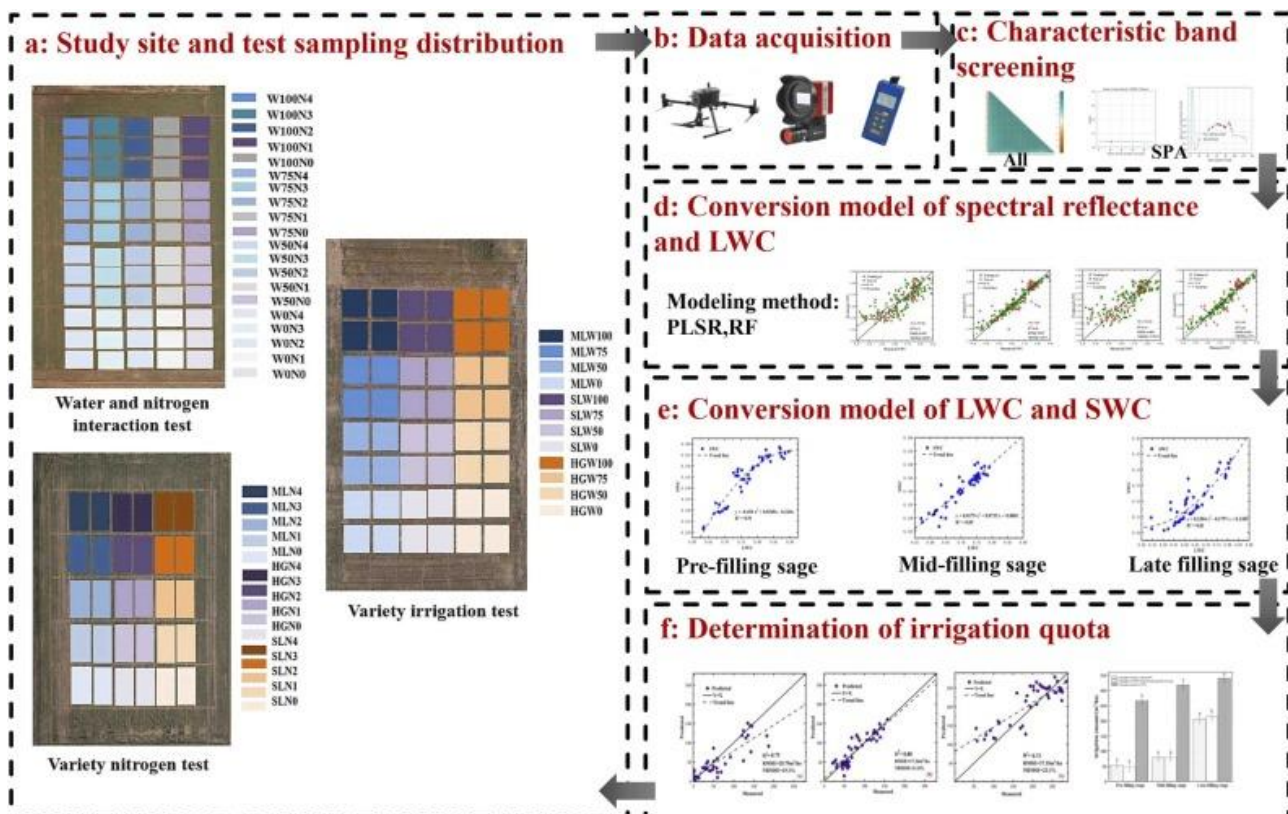


Figure 2 Test distribution and technical roadmap. Note: LWC, leaf water content; SWC, soil water content; PLSR, partial least squares regression; RF, random forest; ALL, hyperspectral band; SPA, successive projections algorithm (Adopted from Sun et al., 2024)

In dryland and semi-arid wheat systems, straw mulching can help retain more rainfall or irrigation water in the tillage layer and reduce evaporation losses from bare soil. Dong et al. (2025) studied the effects of long-term rotary tillage and straw mulching in a rainfed wheat-soybean rotation system. The results showed that appropriate tillage combined with straw management promoted dry matter accumulation, increased grain yield, and improved water use efficiency. Under unstable rainfall conditions, the role of straw mulching is not only to conserve soil moisture but also to influence final yield through improving crop growth and dry matter production.

Qiang et al. (2022) conducted field experiments in Xinxiang, Henan Province, comparing no-tillage, rotary tillage, and subsoiling combined with straw return in terms of tillage layer structure, grain yield, and WUE. Compared with no-tillage, subsoiling significantly reduced soil bulk density in the 20~40 cm layer, increased soil porosity, and reduced soil compaction in the 0~40 cm layer. Subsoiling combined with straw return also increased soil organic carbon in the tillage layer. More importantly, subsoiling promoted the downward movement of irrigation water and rainfall, creating a better soil environment for root growth. Compared with no-tillage, subsoiling increased winter wheat yield by 34.48%~38.10% and improved WUE by 19.57%~21.96%.

4.4 Integrated water and fertilizer management

Water and nutrients do not function independently. Water shortage limits nitrogen transport to the root zone and root absorption, while insufficient nitrogen supply weakens leaf area development, photosynthate accumulation, and root activity. Under water-saving conditions, reducing irrigation without adjusting fertilization can easily result in either “less water but excessive fertilizer” or “insufficient fertilizer efficiency.” On the other hand, simply increasing nitrogen fertilizer without improving water supply may increase the risk of nitrogen residue and leaching.

Wu et al. (2025) studied the effects of regulated deficit irrigation on winter wheat yield formation, water use efficiency, and nitrogen use efficiency under different soil fertility conditions. Regulated deficit irrigation was not

equally effective under all fertility levels. Under medium and low fertility conditions, moderate deficit irrigation improved WUE and partial factor productivity of nitrogen while maintaining or increasing grain yield. However, under high fertility conditions, regulated deficit irrigation could reduce grain yield or weaken nitrogen use efficiency.

5 Breeding and Genetic Improvement of Water-Saving Wheat

5.1 Drought-resistant germplasm resources

Drought resistance in wheat is not controlled by a single trait. It is determined by multiple characteristics together, including root water uptake ability, leaf water retention, stomatal regulation, osmotic adjustment, antioxidant capacity, early maturity for drought escape, and grain filling stability. In recent years, more attention has been paid to landraces, wild relatives, synthetic hexaploid wheat, and core germplasm resources preserved by international breeding institutions. Many countries and international gene banks have conserved a large number of wheat landraces and wild materials. During long-term natural selection and farmer selection, these materials gradually developed adaptation to drought, poor soils, heat stress, and other adverse environments (Khadka et al., 2020).

Common wheat experienced a genetic bottleneck during domestication and modern breeding. Some allelic variations related to drought resistance, deep rooting, and stress adaptation may have been weakened or lost. Synthetic hexaploid wheat is usually developed by crossing tetraploid durum wheat with D-genome donors such as *Aegilops tauschii*, followed by chromosome doubling. This approach allows favorable alleles from wild relatives to be reintroduced into the genetic background of common wheat. Rosyara et al. (2019) analyzed the genetic contribution of synthetic hexaploid wheat to CIMMYT spring bread wheat breeding materials and found that synthetic-derived lines had already entered the international wheat improvement system.

Mokhtari et al. (2022) evaluated 184 synthetic hexaploid wheat-derived lines under both normal irrigation and water stress conditions over two years. Large genetic variation was observed in agronomic traits such as plant height, heading date, spike traits, thousand-kernel weight, and grain yield. Under water stress, grain yield significantly decreased, but the degree of reduction differed greatly among genotypes, indicating that synthetic hexaploid derivatives contain valuable materials for drought improvement. Mokhtari et al. (2024) further screened drought-tolerant types from 91 synthetic hexaploid wheat lines. Under water stress, significant differences were observed in relative water content, leaf area index, photosynthetic pigments, proline accumulation, antioxidant enzyme activity, and malondialdehyde content. Some synthetic hexaploid materials maintained relatively high grain yield, higher leaf relative water content, and lower membrane lipid peroxidation under drought conditions.

5.2 Key genes related to drought resistance

Drought resistance in wheat involves many biological processes, including root development, water absorption, stomatal regulation, ABA signaling, reactive oxygen species scavenging, osmotic adjustment, and photosynthetic stability. In arid and semi-arid wheat-growing regions, water in the topsoil evaporates quickly, while deeper soil layers often still contain available moisture. Therefore, wheat lines with deeper root systems and higher deep-root density usually show more stable performance from stem elongation to grain filling stages.

When soil moisture decreases, ABA levels in roots and leaves increase. This induces stomatal closure to reduce transpiration and water loss, while also activating antioxidant systems, osmotic adjustment, and stress-response gene expression. Mega et al. (2019) showed that enhancing ABA receptor function could regulate water-use efficiency and drought resistance in wheat. They developed wheat materials overexpressing ABA receptors and found that total water consumption was reduced, while biomass production and grain yield per unit water increased. Mao et al. (2022) reported that wheat lines overexpressing TaPYL1-1B exhibited higher ABA sensitivity, stronger photosynthetic capacity, and higher water-use efficiency. Under water-deficit conditions, these lines showed improved drought tolerance and maintained grain yield. More importantly, the study identified a favorable allelic variation, TaPYL1-1Bn-442, in the promoter region of TaPYL1-1B. This variation contains a MYB recognition site that can be regulated by TaMYB70, thereby enhancing TaPYL1-1B expression in drought-tolerant genotypes (Figure 3).

In addition to ABA receptors, transcription factor genes are also important regulators of drought resistance in wheat. Chen et al. (2021) studied the wheat NAC transcription factor TaNAC48 and found that its expression was induced by drought, PEG treatment, hydrogen peroxide, and ABA. The gene was localized in the nucleus. Wheat lines overexpressing TaNAC48 showed higher proline content, lower water-loss rate, and reduced levels of malondialdehyde, hydrogen peroxide, and superoxide anions under drought stress. The study also demonstrated that TaAREB3 could bind to the ABRE element in the TaNAC48 promoter and activate its expression, revealing a regulatory relationship between ABA signaling and NAC transcription factors.

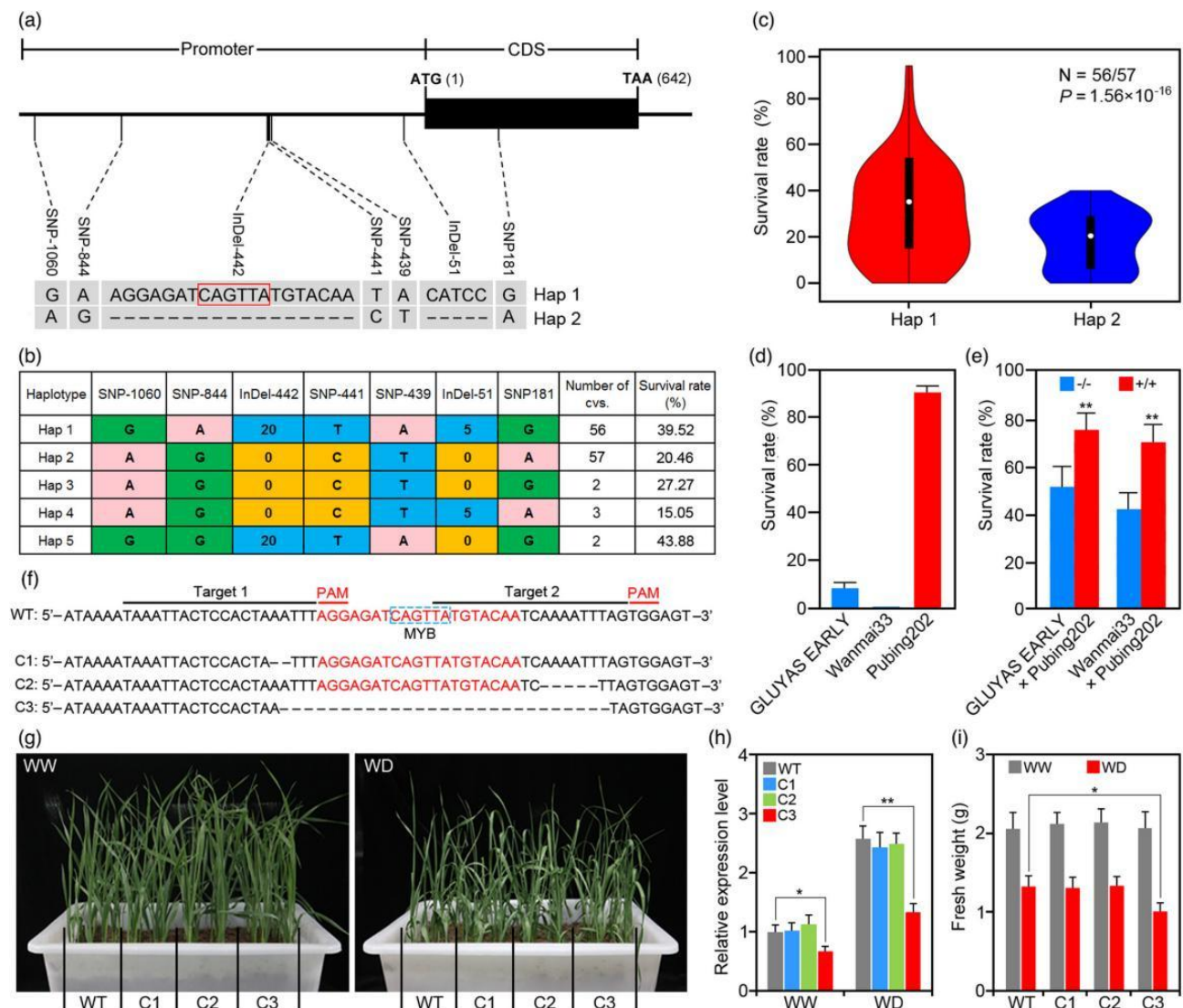


Figure 3 Genetic variations in TaPYL1-1B and their association with wheat drought tolerance (a) Distribution of DNA polymorphisms within the TaPYL1-1B promoter and the coding sequence region. The red frame indicates an MYB-binding sequence. (b) Haplotype analysis of TaPYL1-1B genotypes among 120 wheat varieties based on seven SNPs/indels. (c) Comparison of drought tolerance between wheat varieties carrying Hap 1 and Hap 2 genotypes. (d) Survival rates of wheat cv. Pubing202, Wanmai33 and GLUYAS EARLY plants under severe drought stress. (e) The survival rates of the F4 individuals carrying either the homozygous tolerant (+/+) or sensitive (-/-) allele of TaPYL1-1B in response to drought conditions. (f) Targeted mutagenesis of the 20-bp insertion via CRISPR-Cas9. Red labels indicate protospacer adjacent motif (PAM) sequences. Three independent lines were obtained harbouring deletions of the 20-bp insertion or its flanking sequence. (g) Phenotypic analysis of drought tolerance and (h) TaPYL1-1B relative expression levels in deletion mutants and WT plants under well-watered (WW) and water-deficit (WD) conditions. (i) Fresh weight of mutant and WT plants under WW and WD conditions. Data represent the mean ± SD of three replicates. Statistical significance was determined by a Student's t test, * P < 0.05; ** P < 0.01 (Adopted from Mao et al., 2022)

5.3 Marker-assisted selection and genomic breeding

Since drought resistance in wheat is controlled by multiple genes and is strongly influenced by environmental conditions, field phenotypic selection alone is easily affected by differences in year, soil, and management practices. The advantage of marker-assisted selection is that early-generation selection can be carried out around identified QTLs or candidate genes, reducing uncertainty in breeding. Genomic selection uses high-density genome-wide markers to predict breeding values and is more suitable for complex quantitative traits such as grain yield, water-use efficiency, canopy temperature, heading date, and grain filling stability. Nouraei et al. (2024) conducted a genome-wide association study on drought resistance in wheat and identified loci and candidate genes associated with drought tolerance using SNP markers.

The CIMMYT wheat breeding system adopted genomic selection relatively early for grain yield and adaptation improvement. Juliana et al. (2020) systematically analyzed the application of genomic selection for grain yield in the CIMMYT wheat breeding program. They pointed out that genomic selection can use marker information to predict yield potential in early-generation materials, shorten the breeding cycle, and identify stable high-yield lines across multiple environments. This is particularly important for drought resistance and water-saving breeding because water-stress experiments are costly, environmental variation is large, and repeated evaluations require long periods. Genomic prediction can therefore improve screening efficiency.

5.4 Gene editing and future climate-smart wheat

Traditional breeding requires hybridization, backcrossing, and many years of selection to combine favorable alleles, resulting in a relatively long breeding cycle. In contrast, the CRISPR/Cas system can achieve targeted mutation, knockout, base substitution, or expression regulation in specific genes or regulatory regions, making it suitable for precise improvement of genes with known functions. For hexaploid common wheat, gene editing is especially valuable because many genes have three homologous copies in the A, B, and D genomes. Traditional mutant screening makes it difficult to obtain functional changes in all copies simultaneously, whereas CRISPR can edit multiple homologous genes at the same time, improving both functional validation and trait improvement efficiency.

At present, drought-related gene editing in wheat is still moving from functional verification toward breeding application. Zhao et al. (2024), in their study on CRISPR/CasΦ2-mediated gene editing in wheat and rye, showed that new Cas systems are expanding the wheat gene-editing toolbox. The significance of these tools is not only that they can perform editing, but also that they may allow combined regulation of multiple drought-resistance pathways in the future.

6 Digital Agriculture and Smart Water Resource Management

6.1 Remote sensing and soil moisture monitoring

Remote sensing technology uses satellites or drones to collect land cover, vegetation index, and soil information, providing an effective way to evaluate crop growth and monitor water conditions over large agricultural areas (Zhang et al., 2021). Multispectral data from Sentinel-2, Landsat, and similar platforms can be used to estimate crop chlorophyll content and evapotranspiration through Land Surface Temperature analysis, which helps indirectly estimate soil moisture conditions. At the same time, more small soil moisture sensors are being installed in farmland, and these sensors can upload underground moisture data in real time through wireless networks. By combining these data with machine learning models, researchers and farmers can predict field water demand and carry out precise irrigation scheduling. Drone remote sensing has high spatial resolution and strong flexibility, so it is widely used in stress testing and drought diagnosis for local wheat fields.

6.2 Artificial intelligence-based irrigation decision systems

Deep learning models can integrate meteorological data, remote sensing images, and soil sensor information to predict crop water demand trends. Water resource management frameworks that combine satellite remote sensing and deep learning can improve the prediction accuracy of soil moisture and irrigation demand to more than 90%. In wheat irrigation research, algorithms such as Convolutional Neural Networks (CNN) and Long Short-Term Memory networks (LSTM) have already been tested for water demand prediction and water-saving irrigation

planning. In addition, reinforcement learning and intelligent optimization algorithms are also used to automatically regulate valves and pumping stations in response to changing field water conditions in real time. Some smart irrigation systems continuously improve their performance through reinforcement learning trial-and-error processes, making it possible to reduce water use while maintaining crop health.

6.3 Application of the internet of things in wheat production

By deploying sensor networks, it is possible to monitor temperature, humidity, soil moisture, and soil nutrients in real time, and the collected data can be transmitted wirelessly to control centers (Jawad et al., 2017). Sensor arrays combined with satellite communication technology allow real-time monitoring of soil moisture in remote farms. Automatic irrigation control systems can operate without direct human supervision. When sensors detect that soil moisture falls below a set threshold, pumps automatically start irrigation, and the pumps shut down automatically after irrigation is completed. Compared with traditional manual irrigation, automated systems reduce human error and improve irrigation accuracy. Some smart irrigation gateways can even use edge computing to run simple decision-making models directly in the field, which further reduces response time.

6.4 Big data and predictive agriculture

Short-term and long-term weather forecasts, drought indexes, and related information provided by meteorological agencies and research institutions can serve as the basis for preparing irrigation plans in advance. By mining historical yield, soil, and climate data, prediction models for crop yield and water demand can be established to support farmer decision-making. For example, long-term experimental data show that the occurrence probability of heat stress in wheat is closely related to crop yield, which can help estimate harvest risks and guide timely adjustments in planting density or irrigation strategies. In terms of water resource allocation, decision-makers can combine hydrological forecasts with crop water demand models to distribute limited water resources, such as groundwater and river water, more scientifically for regional water conservation. Climate warning systems, including drought early warning systems, can also provide guidance for spring sowing plans and water-saving irrigation measures. Big data-based decision support systems are gradually becoming more common, making wheat production more forward-looking and adaptable.

7 Challenges and Future Prospects

7.1 Balancing yield stability and water saving

Under conditions of limited resources, balancing grain yield and water use efficiency remains a major challenge. Excessive pursuit of maximum yield usually depends on large amounts of irrigation, while overly strict irrigation restriction may lead to yield reduction. Future studies should seek a compromise between physiological mechanisms and system optimization. Breeding wheat genotypes with high photosynthetic efficiency and high WUE, combined with moderate deficit irrigation strategies (such as 75% ET_c), may help achieve the goal of “moderate stress and efficiency priority” (Wu et al., 2025). At the same time, regional differences should also be considered. For example, winter wheat in southern regions generally requires more water and can rely on rainfall supplementation, while northern arid areas should focus more on soil moisture conservation and the use of drought-tolerant cultivars.

7.2 Climate uncertainty and extreme weather risks

Global warming has increased uncertainty in agricultural production. High-temperature heatwaves and long-term drought events are becoming more common and are now major factors affecting wheat yield. To cope with climate risks, it is necessary to improve the resilience of agricultural systems. This includes establishing drought early-warning systems, adjusting sowing dates and cultivar selection more flexibly, and promoting stress-resistant wheat varieties. For example, planting plans can be developed with the support of multi-model climate simulations. If a heatwave is predicted during flowering, early-maturing and heat-tolerant cultivars can be used to reduce production risks. In addition, “insurance-based agriculture” and regulation mechanisms, such as water storage reservoirs and subsidy policies, can help reduce risks for farmers. Agricultural production systems should also become more flexible. In double-cropping systems, adjusting planting density according to climate conditions can partly offset losses caused by extreme weather.

7.3 Limitations of current irrigation technologies

Although many water-saving technologies have been developed, there are still several difficulties in practical application. First, advanced irrigation equipment, such as intelligent irrigation systems, is expensive and difficult for small-scale farmers to adopt without policy support and financial subsidies. Second, natural resource conditions vary greatly among regions, and the same technology may produce different effects in different environments. Therefore, localized technical evaluation is necessary. In addition, agricultural information services and technology extension systems still need improvement. Limited farmer training and low acceptance of new technologies have slowed down the adoption process. For example, the deployment rate of intelligent irrigation systems remains low in some areas of North China, while traditional farming habits in arid regions of Northwest China also limit the promotion of improved cultivation practices. Finally, more accurate decision-support tools are still needed. Many existing AI models cannot fully simulate the complete growth process of wheat, and more practical and adaptable agronomic models should be further developed.

7.4 Future development directions of climate-smart wheat systems

In the future, the trends of “smart agriculture” and “low-carbon agriculture” will strongly influence winter wheat production systems. In smart agriculture, technologies such as 5G communication, edge computing, and artificial intelligence will be more widely applied, allowing the whole crop production process to become digitalized. Every stage, from sowing to harvesting, can be monitored through cloud-based systems. Drone pesticide spraying and automated agricultural robots may become common practices. At the same time, new sensors, such as miniature UAV multispectral sensors, will enable real-time monitoring of microclimate conditions and vegetation indicators.

Biotechnology will also contribute to water management. For example, engineered microorganisms may regulate rhizosphere environments and improve water absorption, while symbiotic microbes may enhance soil water retention capacity. Regenerative agricultural systems, including cover crop strip farming and mixed cropping, can improve soil structure and regional water cycles, thereby indirectly increasing WUE. In addition, under the background of greenhouse gas reduction, low-carbon agricultural practices will gradually be introduced into wheat production systems. Energy-saving irrigation methods, such as solar-powered pumps and drip or micro-sprinkler irrigation systems, can reduce fossil fuel consumption. Overall, future wheat production systems will integrate information technology, mechanization, and biotechnology, gradually developing toward climate-smart agriculture to achieve sustainable high yield and water-saving goals.

Author Contributions

The authors appreciate Dr Cai for the assistance in references collection and discussion for this work completion.

Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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Case study

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Cold-Resistant Radish Varieties for Winter Production in Southern China

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Received: 01 May, 2026

Accepted: 03 Jun., 2026

Published: 15 Jun., 2026

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Preferred citation for this article:Huang D.D., and Li M.H., 2026, Cold-resistant radish varieties for winter production in southern China, Bioscience Evidence, 16(3): 171-185 (doi: [10.5376/be.2026.16.0014](https://doi.org/10.5376/be.2026.16.0014))

Abstract With the development of the winter vegetable industry in southern China, climatic factors such as low temperature, cold waves, and frost have become major environmental stresses limiting stable and high-yield radish production. This study focuses on the winter agricultural climate characteristics of southern China and their effects on radish cultivation. It explains the morphological, physiological, and molecular biological basis of cold tolerance in radish and summarizes the mechanisms of osmotic adjustment, antioxidant defense systems, plant hormone regulation, and low-temperature-responsive gene networks involved in cold tolerance formation. At the same time, the study reviews the diversity of radish germplasm resources in China, local cold-tolerant varieties, advances in conventional breeding techniques, and progress in molecular breeding research. The advantages of representative cold-tolerant varieties in terms of yield, quality, and environmental adaptability are also analyzed. Combined with key cultivation practices, including optimization of sowing time, water and fertilizer management, protected cultivation, and integrated pest and disease control, this paper discusses efficient winter radish production systems and typical regional application cases. The coordinated application of cold-tolerant variety breeding and regional cultivation technologies is an important approach to improving the stability and economic benefits of winter radish production in southern China. In the future, greater efforts should be made to integrate genomics with precision breeding technologies, improve cold-tolerance evaluation systems, and promote the application of smart agriculture technologies, providing both theoretical support and technical guidance for the development of efficient, environmentally friendly, and sustainable winter vegetable production systems.

Keywords Radish (*Raphanus sativus* L.); Cold tolerance; Winter production; Germplasm resources; Molecular breeding; Cultivation technology; Southern China

1 Introduction

Winter vegetable production is an important part of the year-round vegetable supply system in China. In regions with suitable accumulated temperature conditions, off-season production can be achieved, creating strong market competitiveness. Southern China is one of the major winter-spring vegetable production regions and has comparative advantages for winter vegetable cultivation because of its favorable climate and resource conditions. However, it still faces the challenge of low-temperature weather events, which can reduce yield stability and product quality (Hou and Wang, 2024). Under these circumstances, improving the adaptability and stress resistance of major winter crops is of great importance for ensuring regional food security and promoting sustainable and intensive agricultural development.

Radish (*Raphanus sativus* L.) is one of the most important root vegetables in China and worldwide. It is widely cultivated across different climate zones and growing seasons. In China alone, the planting area of radish reached approximately 1.2 million hectares in 2016, with a fleshy root production of 44.6 million tons, accounting for about 40%~47% of the global planting area and production of radish (Kurina et al., 2021). According to different planting seasons, radish can be classified into spring radish, summer radish, autumn radish, and winter radish. Among them, autumn and winter radishes are dominant in many regions, accounting for approximately 20%-50% of the total autumn vegetable planting area (Zhang et al., 2019a). Because of its short growth cycle, diverse root shapes and colors, and high nutritional value, radish has become a key crop in intensive vegetable production systems and an important source of income for many small-scale farmers.

Although radish has relatively strong environmental adaptability, it is essentially a cool-season crop. Its growth process, especially the fleshy root development stage, is highly sensitive to temperature changes. It is generally accepted that the highest fleshy root yield can be achieved when day/night temperatures are maintained within the range of 19/13 °C ~24/18 °C. Both excessively high and excessively low temperatures can negatively affect fleshy root development and quality (Khan et al., 2022; Oh et al., 2022). Under low-temperature stress, secondary growth of the fleshy root, cambial activity, and root enlargement are significantly inhibited, resulting in reduced yield and deteriorated quality. Low temperatures can also damage cell membrane structures, disrupt the homeostasis of reactive oxygen species (ROS), and slow seed germination and early seedling growth, thereby reducing seedling establishment rates in winter crops. These constraints are particularly serious in open-field winter production systems in southern China, where periodic cold waves often cause substantial economic losses to farmers.

Therefore, breeding and selecting cold-tolerant radish varieties has become an important strategy for stabilizing winter radish production. At the molecular level, several transcription factors involved in low-temperature stress responses, including members of the ERF, WRKY, MYB, and CDF families, such as RsERF40, RsWRKY49, RsMYB90, and RsCDF3, have been shown to enhance cold tolerance by regulating the expression of cold-responsive (COR) genes, osmotic adjustment, cell wall reinforcement, ROS balance, and root growth under low-temperature conditions. At the population level, radish exhibits extensive genetic diversity in morphological traits, growth characteristics, and biochemical properties, with significant differences among ecological types and geographical populations.

Based on this background, the present study focuses on cold-tolerant radish varieties suitable for winter production in southern China. The objectives of this study are: (i) to explain the important role of radish in winter vegetable production systems in relation to the needs of winter vegetable cultivation in southern China; (ii) to systematically summarize the physiological and molecular mechanisms through which low-temperature stress restricts radish growth and fleshy root development; (iii) to analyze genetic diversity resources and breeding approaches related to cold tolerance and winter adaptability; and (iv) to provide theoretical support and practical references for the breeding, selection, and regional promotion of radish varieties with strong cold tolerance, thereby achieving stable yield, high productivity, and improved quality in winter radish production.

2 Agricultural Climate Characteristics of Winter in Southern China

2.1 Temperature variation patterns and occurrence of low-temperature stress

Southern China is generally characterized by a mild and humid winter climate. In southeastern China, the average daily temperature during winter is usually maintained at around 7 °C~8 °C, while the annual mean temperature in many areas ranges from 14 °C to 18 °C. The frost-free period can exceed 230~340 days (Freychet et al., 2021). Historical climate reconstruction studies have shown that although a clear long-term warming trend has occurred, especially with a rapid increase in winter temperatures since the late twentieth century, winter temperatures in southern China still exhibit considerable year-to-year variability (Fu and Ding, 2021). Under the background of global warming, extreme cold events have not disappeared but instead show clear interdecadal fluctuations. In recent decades, some regions of China have even experienced an increase in regional extreme cold events. Analyses of frost days across the country indicate that although the overall frost-free period has become longer and the number of frost days has decreased, frost and cold-wave events still occur periodically in southern China, posing potential risks to crops that are sensitive to low temperatures.

2.2 Regional differences (e.g., the Yangtze River Basin and South China subtropical regions)

There are significant agroclimatic differences between the Yangtze River Basin (YZRB) and the more southern subtropical regions. The Yangtze River Basin has a typical subtropical monsoon climate, characterized by mild and rainy winters and a frost-free period of approximately 230~326 days. However, the region is also known for frequent late-spring cold events and relatively high climate risks during the transition from winter to spring (Lei et al., 2024). In contrast, South China, including provinces such as Guangdong, Guangxi, and Hainan, has a warmer climate and a longer frost-free period. Even during the coldest month, temperatures generally remain above 1 °C (Yu et al., 2022).

Even within the Yangtze River Basin, substantial differences exist among the upper, middle, and lower reaches in terms of average temperature, precipitation, and sunshine duration. These variations result in different cropping systems and different levels of sensitivity to climate anomalies (Xu et al., 2019). Such regional climatic differences directly affect the suitable sowing windows for winter vegetables and the level of climate-related risks they face.

2.3 Effects of cold waves and frost events on vegetable crops

Although winters in southern China are generally mild, cold waves and large-scale persistent extreme low-temperature events can still cause sudden temperature drops, freezing rain, and prolonged cold conditions, thereby affecting agricultural production in southern and southeastern China. For example, the severe cold-wave events that occurred during the winters of 2008 and 2016 brought historically low temperatures and freezing rain, causing not only transportation disruptions and infrastructure damage but also serious impacts on agricultural production (Liao et al., 2020).

At the national scale, winter low-temperature events vary considerably in both frequency and intensity. Strong cold-wave events in southern China generally last longer than those in northern China, and December is the period with the highest occurrence frequency and the longest duration of cold waves (Chen et al., 2022). When extreme low temperatures occur during critical growth stages, they can significantly reduce the yields of overwintering crops such as winter wheat and rapeseed, with the degree of impact varying among regions (Xiao et al., 2018; Huang et al., 2020; Zhao et al., 2024). In the double-cropping systems of the Yangtze-Huaihe Plain, extreme weather events occurring during sowing or seedling establishment of winter crops can suppress vegetation growth throughout the growing season and ultimately reduce crop yield formation (Chen et al., 2024).

3 Physiological and Molecular Basis of Cold Tolerance in Radish

3.1 Morphological traits associated with cold tolerance

Compared with cold-sensitive materials, cold-tolerant radish genotypes can better maintain secondary growth of the fleshy root, cambial activity, and root meristem size under low-temperature conditions. Overexpression of RsERF40 and RsWRKY49 promotes elongation and radial expansion of fleshy roots or hairy roots, indicating that these genes enhance cell expansion and cell division under cold stress. RsERF40 strengthens cell wall structure by upregulating the expression of cell wall-related genes (RsCESA6 and RsEXPB3), thereby maintaining root tissue stability under low-temperature conditions. Similarly, RsWRKY49 increases root meristem size and promotes cell division, allowing roots to maintain continuous growth in cold environments.

3.2 Physiological responses (osmotic regulation and antioxidant activity)

Cold-tolerant plants usually accumulate osmotic adjustment substances such as soluble sugars and proline to stabilize cell membrane structure and maintain cellular osmotic potential. In radish, sucrose synthesized by RsSPS1 is an important osmotic regulator. Higher sucrose levels help maintain cambial activity and improve cold tolerance, whereas silencing RsSPS1 results in reduced sucrose, proline, and chlorophyll contents, while increasing the accumulation of membrane damage indicators, including malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) (Chen et al., 2025b). RsERF40 enhances osmotic regulation by activating COR genes and promoting the accumulation of cryoprotectants.

Maintaining reactive oxygen species (ROS) homeostasis is another key physiological mechanism for plant adaptation to cold stress. Low temperatures often cause excessive ROS accumulation, making an efficient antioxidant system essential. Overexpression of RsERF40, RsCDF3, and RsSHRc reduces ROS and MDA accumulation while increasing proline content and overall antioxidant capacity, thereby alleviating oxidative damage. Among them, RsCDF3 directly represses the expression of the NADPH oxidase genes RsRbohA and RsRbohC, reducing ROS production and establishing a positive feedback mechanism that favors ROS homeostasis (He et al., 2023). These findings are consistent with studies in other crops, where increased activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), together with higher levels of flavonoids and ascorbic acid, are generally associated with stronger cold tolerance (Raza et al., 2021; Liu et al., 2022; Xu et al., 2023a).

3.3 Role of plant hormones in low-temperature adaptation

Plant hormone signaling is closely linked to cold-stress responses. Cross-species studies have shown that abscisic acid (ABA), auxin, gibberellins, jasmonic acid, salicylic acid, ethylene, brassinosteroids, and cytokinins all participate in plant cold acclimation by regulating gene expression, growth and development, and ROS signaling pathways.

In radish, the ethylene-responsive factor RsERF40 clearly illustrates the integration of hormone signaling and cold signaling. As a member of the AP2/ERF transcription factor family, RsERF40 not only functions in ethylene-related signaling pathways but also regulates cold-induced COR genes and cell wall remodeling processes (Zhou et al., 2025). In addition, transcription factors from the WRKY and MYB families are often regulated by hormonal signals and contribute to balancing plant growth and defense responses under low-temperature conditions (Feng et al., 2025; Qin et al., 2026). Although studies on hormone profile changes in radish under cold stress remain limited, hormone-regulated transcription factors are considered key regulatory nodes linking hormone signaling, ROS homeostasis, and sustained growth.

3.4 Key genes and molecular regulatory pathways associated with cold tolerance

Cold tolerance in radish is regulated by both CBF-dependent and CBF-independent pathways. RsWRKY40 activates RsCBF1 and RsCBF2, while also enhancing RsSPS1 expression, forming a transcriptional regulatory module that links sucrose accumulation with the classical CBF-COR signaling cascade. Similarly, RsWRKY49 transcriptionally activates RsCBF2 and RsNR2, and variations within its promoter region are associated with differences in cold tolerance among genotypes (Chen et al., 2025a).

RsERF40 represents a CBF-independent cold-tolerance pathway. This gene directly upregulates the expression of RsCOR78, RsCOR413PM1, and several cell wall-related genes, thereby promoting cryoprotectant accumulation, maintaining osmotic stability, and supporting fleshy root growth. RsMYB90 binds to the promoter of RsCOR78 and activates RsUFGT expression, enhancing anthocyanin biosynthesis and improving ROS scavenging capacity under low-temperature conditions (Li et al., 2025).

In addition to transcription factors, the GRAS family member RsSHRc is also an important regulator of cold tolerance. Its expression is induced by low temperatures, promoting ROS scavenging, reducing MDA accumulation, and increasing proline content. In this way, it links fleshy root enlargement with stress-defense mechanisms (Li et al., 2022). Radish ICE1-like factors, such as RsICE1, are connected to the widely conserved ICE1-CBF-COR regulatory module found in many plant species. Heterologous expression of RsICE1 in rice enhances cold tolerance by increasing soluble sugar and proline accumulation and reducing membrane damage (Qian, He and Li, 2024).

3.5 Advances in omics studies (transcriptomics and metabolomics)

High-throughput transcriptomic technologies have played a critical role in identifying cold tolerance-related genes in radish. Comparative transcriptome analyses between cold-tolerant and cold-sensitive genotypes revealed that genes such as RsERF40, RsSPS1, RsWRKY40, RsWRKY49, RsCDF3, and RsSHRc are significantly induced under low-temperature conditions and are closely associated with cold-tolerant phenotypes. Therefore, these genes are considered important regulators of cold tolerance (Chen et al., 2025a). In addition, genome-wide analyses of gene families such as RsCDF and RsGRAS have identified several cold-induced members and revealed their expression dynamics during different stages of fleshy root development.

Compared with transcriptomics, metabolomic research in radish is still at a relatively early stage. However, integrated transcriptomic-metabolomic studies in other crops have revealed common patterns. Cold-tolerant genotypes generally accumulate higher levels of soluble sugars, amino acids, polyamines, flavonoids, and lignin-related metabolites. At the same time, their transcriptional regulatory networks are significantly enriched in carbohydrate metabolism, phenylpropanoid/flavonoid biosynthesis pathways, hormone signaling pathways, MAPK cascades, and ROS scavenging systems (Jian et al., 2020; Wang et al., 2021; Gao et al., 2022).

With the increasing application of multi-omics technologies in radish research, researchers are gradually establishing a systems biology framework that links morphological traits, physiological indicators, and specific regulatory genes. This framework not only helps clarify the molecular mechanisms underlying cold tolerance in radish but also accelerates breeding programs based on Marker-Assisted Selection (MAS) and Genomic Selection (GS) for the development of cold-tolerant winter radish cultivars.

4 Cold Tolerance Germplasm Resources and Breeding of Radish

4.1 Diversity of Chinese radish germplasm resources

Radish germplasm resources provide the essential genetic foundation for developing new varieties with strong stress tolerance, high yield, and superior quality. China is one of the major centers of radish diversity and production worldwide, with a cultivation area of about 1.2 million hectares and an annual production exceeding 40 million tons (Xing et al., 2024). At present, more than 2 000 radish germplasm accessions have been collected and conserved in Chinese germplasm repositories, of which approximately 95% are domestic landraces. This reflects the rich in situ genetic diversity and long domestication history of radish in China (Wang et al., 2018). Population genetic studies based on single nucleotide polymorphism (SNP) and structural variation (SV) markers have shown that radish resources from China and other East Asian regions form the major genetic groups. Clear genetic differentiation exists between these groups and European or other regional types. However, relatively frequent gene flow has also been observed among some subpopulations, accompanied by a certain risk of genetic erosion (Li et al., 2023a).

4.2 Identification of cold-tolerant landrace resources

Landraces are generally defined as heterogeneous populations that have evolved through long-term farmer selection under specific ecological, geographical, and cultivation conditions. They are valuable genetic resources for obtaining adaptive traits such as tolerance to abiotic stresses. In radish research, systematic evaluations conducted across different seasons and locations have identified excellent germplasm resources with desirable agronomic traits and strong stress resistance. For example, long-term trials carried out in Jinan, Yangling, Harbin, and other regions screened multiple germplasm accessions with outstanding overall performance based on yield, stress tolerance, and disease resistance (Qu et al., 2002). The conservation of radish germplasm resources in China places particular emphasis on collecting and preserving landraces from their regions of origin as well as resources representing different ecological types. These materials exhibit abundant phenotypic and biochemical variation.

4.3 Conventional breeding methods (selection breeding and hybrid breeding)

Traditional radish breeding mainly focuses on target traits such as high yield, early maturity, late bolting, cold tolerance, drought tolerance, heat tolerance, disease resistance, and high-quality fleshy roots. Through methods including mass selection and pedigree selection within landraces and local populations, as well as intraspecific and interspecific hybridization, favorable alleles can be continuously accumulated, resulting in varieties that combine multiple desirable traits that are difficult to achieve in a single genotype.

With the development of F_1 hybrid seed production systems based on self-incompatibility, identification of S haplotypes in parental lines has become an important step for efficient hybrid seed production. This approach eliminates the need for manual emasculation, greatly improves hybrid seed production efficiency, and accelerates the incorporation of complementary favorable traits, including stress tolerance, into commercial cultivars (Kumar and Kaushik, 2021). In addition, core germplasm collections established from large-scale germplasm resources have been widely used in the development of cytoplasmic male sterility (CMS) lines and new hybrid cultivars, further improving the efficiency of conventional breeding programs.

4.4 Molecular breeding strategies (marker-assisted selection and genomic tools)

In recent years, significant progress has been made in radish genomics research. High-quality reference genomes, genetic linkage maps, SNP and SV marker systems, and molecular markers associated with important agronomic traits have been continuously developed and improved (Kim et al., 2019). Molecular fingerprint maps based on SNPs and SVs can not only be used for accurate germplasm identification, genetic structure analysis, and core germplasm collection construction, but also provide important technical support for marker-assisted selection (MAS) and genomic studies.

At present, MAS technology has been successfully applied in radish disease-resistance breeding. Using 275 core radish germplasm accessions collected from 30 provinces in China as well as Russia, Germany, South Korea, and other countries, Ma et al. (2024) conducted artificial inoculation with Race 4, the dominant physiological race of *Plasmodiophora brassicae* in China. Disease resistance levels were evaluated using the disease index (DI). Significant differences in clubroot resistance were observed among the germplasm resources. Six highly resistant accessions and 50 resistant accessions were identified. Some materials originating from Sichuan Province, Russia, and Germany showed stable and high levels of resistance (Figure 1). The study further analyzed the geographic distribution and horticultural characteristics of these resistant germplasms and found that resistant resources were relatively abundant in southern regions. In addition, the highly resistant materials exhibited considerable genetic diversity in leaf type, fleshy root shape, and root color. Previously reported clubroot-resistant SSR molecular markers were then used to evaluate the highly resistant materials. The results indicated that these accessions possessed genetic characteristics different from those of known resistant materials, suggesting that they may carry novel resistance genes. Finally, several highly resistant accessions were crossed with elite cultivated varieties, resulting in a number of hybrid progenies with breeding potential.

At the same time, genome-wide association studies (GWAS) and transcriptome analyses have identified several important genes and transcription factors involved in cold tolerance and fleshy root development, including RsERF40 and the RsWRKY40-RsSPS1-CBF regulatory module. These findings provide potential targets for improving cold tolerance through MAS and genomic selection while maintaining normal fleshy root growth (Li et al., 2023b).



Figure 1 Phenotypic comparison between high-resistance and high-sensitivity seedlings. (a-f) Highly resistant (HR); (g-i) highly susceptible (HS). Bar scale = 1 cm (Adopted from Ma et al., 2024)

4.5 Major achievements and limitations of breeding research

Substantial progress has been achieved in radish breeding with respect to yield, quality, disease resistance, and environmental adaptability. In some breeding programs, more than half of the released cultivars have become F_1 hybrids developed using CMS technology and molecularly identified core germplasm resources. Among these achievements, clubroot resistance breeding is considered a successful example. Through the integration of diverse germplasm resources, phenotypic screening, and molecular marker technologies, breeders have not only enriched the resistance gene pool but also developed several new disease-resistant cultivars.

However, some cultivated populations still face problems related to relatively narrow genetic backgrounds and ongoing genetic erosion. In terms of cold-tolerance breeding, although the molecular regulatory mechanisms and key regulatory genes are being gradually clarified, relatively few cold-tolerant winter radish cultivars have been widely adopted in production. This is mainly due to the incomplete development of molecular markers associated with cold tolerance, the complex quantitative inheritance of cold-tolerance traits, and the lack of high-quality phenotypic data collected across multiple environments (Chang-Brahim et al., 2024; Ray et al., 2025).

5 Characteristics of Major Cold-Tolerant Radish Cultivars

5.1 Criteria for cold tolerance evaluation

The evaluation of cold tolerance in radish cultivars usually considers their survival ability under low-temperature conditions, production stability, and the ability to maintain agronomic and quality traits. Key evaluation indicators include stable seed germination and plant establishment under unfavorable low-temperature environments, reduced bolting and disease incidence, and the ability to produce marketable fleshy roots during cool seasons, under protected cultivation, or in low-temperature regions. In addition, yield stability (fleshy root yield per unit area and proportion of marketable roots), as well as the maintenance of root morphology, storage performance, and sensory quality, are widely used as important indicators for assessing cultivar adaptation to winter or off-season cultivation environments.

5.2 Morphological and agronomic traits

Cold-tolerant or winter-adapted radish cultivars generally show vigorous vegetative growth and strong fleshy root development under cool environmental conditions. Studies conducted across different ecological environments have shown that plant height, leaf number, leaf area, root length, root diameter, and individual root weight are important agronomic traits for selecting superior cultivars (Singh et al., 2021; Lahari and Tripathi, 2023; Thakur et al., 2023).

For example, under cool or protected cultivation conditions, the cultivars Ivory White F1 and Okura can achieve fleshy root yields of approximately 30~54 t·ha⁻¹, while also producing longer roots and larger root diameters (Shrestha et al., 2021).

The Chinese radish cultivar Serdtse Podmoskovya, developed in Russia, is characterized by a medium growth period (65~75 d), a high marketable root rate (81%~89%), and a relatively large individual root weight (281~533 g). It has shown good production performance under both open-field and protected cultivation conditions (Stepanov, 2023). Genetic diversity analyses have further demonstrated that radish germplasm resources exhibit extensive variation in growth duration, root shape (ranging from round to cylindrical), and biomass allocation patterns.

5.3 Quality characteristics (flavor, texture, and nutritional composition)

Cold-tolerant radish cultivars intended for winter markets must not only possess strong environmental adaptability but also meet consumer expectations for quality. Important quality traits include uniform root shape and size, smooth skin, crisp and tender flesh, and a moderate pungent flavor.

Under shade-net or off-season cultivation conditions, the hybrid cultivars Ivory White F1 and Mino Early Long White F1 showed relatively high root dry matter content (approximately 7.6%), high ascorbic acid content (approximately 19.5 mg/100 g), and high total soluble solids content (approximately 6 °Brix). These cultivars also received favorable consumer ratings for pungency and overall flavor (Dahal et al., 2020).

The cultivar Serdtse Podmoskovya produces white, juicy fleshy roots with a mildly pungent taste. Under winter production conditions, its dry matter content ranges from 6.3% to 11.0%, total sugar content from 2.6% to 3.2%, and ascorbic acid content is approximately 19~20 mg% (Stepanov, 2023). Late-maturing, large-root winter cultivars generally contain higher levels of dry matter and vitamin C, whereas early-maturing, small-root cultivars tend to have lower dry matter content but still maintain desirable ascorbic acid levels (Kurina et al., 2021).

5.4 Representative cultivars suitable for southern winter conditions

Studies conducted under various cool-season and off-season cultivation environments have identified several cultivars suitable for winter or protected production systems. Under spring and off-season cultivation conditions in Nepal, Ivory White F1 consistently produced the highest fleshy root yields and exhibited excellent quality characteristics in both net-house and shaded cultivation systems.

In addition, comparative trials conducted under winter protected cultivation conditions in Wuhan identified several Korean spring radish cultivars that demonstrated good yield and quality performance within autumn–winter protected production systems (Wan, 2010).

5.5 Comparative performance under open-field and protected cultivation conditions

Superior cold-tolerant cultivars are generally able to achieve higher and more stable yields than local check cultivars during cool-season or off-season cultivation while maintaining desirable quality characteristics.

Under shade-net and off-season production environments, Ivory White F1 produced fleshy root yields of approximately 31 t·ha⁻¹ and outperformed other improved and hybrid cultivars in root length, root circumference, dry matter content, vitamin C content, and consumer acceptance (Dahal et al., 2020).

In winter open-field trials, compared with the commonly grown cultivar Mino Early, Okura increased yield by approximately 49%, while Miyasige increased yield by approximately 22%. Both cultivars maintained good root size and attractive external appearance (Shrestha et al., 2021).

6 Winter Production Cultivation Techniques

6.1 Optimal sowing time and planting density

By properly adjusting sowing time and planting density, radish can achieve stable production under low-temperature conditions. Model analyses and long-term simulation studies have shown that optimizing sowing dates together with proper irrigation and fertilization management can significantly improve radish yield and resource-use efficiency. This indicates that sowing time should be matched with local climatic conditions and should be selected to avoid severe low-temperature damage while ensuring sufficient accumulated temperature for fleshy root development (Zhang et al., 2021).

Experimental results under different seasonal conditions further demonstrated that direct seeding with a smaller plant spacing (approximately 10 cm) is favorable for aboveground vegetative growth, whereas a wider spacing (approximately 20 cm) significantly increases root diameter, root length, and individual root weight. These findings suggest a density trade-off between leaf growth and fleshy root yield (Hudu et al., 2025). Therefore, in winter production, sowing dates should be arranged according to local cold-wave patterns to avoid periods of extreme low temperatures. At the same time, an appropriate planting density should be adopted to ensure rapid canopy closure and improved ground coverage while providing sufficient space for root enlargement.

6.2 Soil preparation and nutrient management

Radish is highly responsive to soil fertility, and improper fertilization is one of the major reasons for low nutrient-use efficiency in radish production in China. The combined application of chemical fertilizers with organic and biofertilizers, such as farmyard manure, spent mushroom substrate, nitrogen-fixing bacteria (*Azotobacter*), and phosphate-solubilizing bacteria, can significantly increase the availability of nitrogen (N), phosphorus (P), and potassium (K) in the soil. This practice promotes fleshy root growth, increases yield, and improves soil health, showing much better results than the use of chemical fertilizers alone (Shilpa et al., 2023).

In recent years, the Nutrient Expert system and nutrient requirement models based on the QUEFTS framework have enabled the accurate estimation of balanced nitrogen, phosphorus, and potassium requirements according to the nutrient demand per unit yield. These tools provide scientific support for fertilization decisions under different soil and climate conditions, thereby improving yield and economic returns, enhancing nutrient-use efficiency, and reducing environmental risks associated with excess nitrogen and phosphorus accumulation (Zhang and Ullah, 2022).

Deep plowing and careful seedbed preparation before sowing are beneficial for uniform seed emergence and early root development. These practices are particularly important for successful crop establishment under winter low-temperature conditions.

6.3 Irrigation strategies under low-temperature conditions

During winter radish production, evapotranspiration is generally low. However, maintaining suitable soil moisture remains a key factor for normal plant growth and nutrient uptake. Optimized irrigation management can reduce irrigation water use by approximately one-third to one-half while maintaining or even increasing yield, and it can also significantly decrease the risk of nitrate leaching (Gan et al., 2023).

Drip irrigation experiments have shown that maintaining moderate soil water potential (generally around 7~12 kPa) under suitable mulching conditions results in the best fleshy root growth, fresh weight accumulation, and economic returns. Both water deficit and excessive moisture can reduce production performance (Santos et al., 2022). Under protected cultivation conditions, moderate deficit irrigation combined with appropriate nitrogen application can maintain yield while improving nitrogen-use efficiency and reducing environmental impacts.

During winter irrigation, prolonged soil saturation should be avoided to prevent root injury under low-temperature conditions. In addition, irrigation should preferably be carried out during the warmer periods of the day to minimize cold damage to root growth.

6.4 Application of protected cultivation measures (mulching, tunnels, and greenhouses)

Protected cultivation is an important approach for reducing low-temperature stress and ensuring safe radish production during winter. Plastic mulches of different colors can increase rhizosphere temperature and soil heat accumulation. Transparent plastic mulch usually provides the strongest warming effect and can significantly increase root length, root diameter, and root weight while effectively reducing premature bolting caused by temperatures below 10 °C during early spring (Lee and Park, 2020).

Black plastic mulch and nonwoven fabric covers can also improve plant growth and yield. Under suitable soil moisture tension conditions, black plastic mulch generally provides higher production efficiency and economic benefits than bare-soil cultivation. In addition, organic mulches and crop residues, such as straw and by-products from Brazilian palm processing, can improve the soil moisture and temperature environment, thereby enhancing radish growth and productivity under protected cultivation systems (Gomes et al., 2020).

Low tunnels, high tunnels, and combined low-tunnel plus high-tunnel systems can significantly increase vegetable yields during cool seasons and can maintain stable production even under freezing conditions (Shiwakoti et al., 2018). For radish production, polyethylene low tunnels can improve leaf nutrient status, increase dry matter accumulation, and enhance fleshy root yield. When combined with foliar silicon application, the effects on cold tolerance and yield improvement become even more pronounced (Alhasnawi and Al-Bayati, 2023; Al-Bayati and Alhasnawi, 2025).

In seed production systems, winter greenhouses are commonly equipped with heated or insulated seedbeds and plastic covering facilities for elite plant propagation, ensuring early transplanting and protection from frost damage.

6.5 Integrated pest and disease management in winter

Winter production does not completely eliminate pest and disease problems; instead, it changes the timing and types of pests and diseases that occur. In radish seed production, protected facilities such as greenhouses and plastic tunnels still require strict weed, pest, and disease management, and pesticides should be used in accordance with relevant national regulations and approved pesticide lists.

In both open-field and protected winter cultivation systems, mulches and cover crops can serve as important components of integrated management strategies. Dead mulches not only suppress weed growth but also regulate soil moisture and temperature, thereby improving crop performance. In addition, winter cover crops dominated by

radish can effectively suppress overwintering annual weeds that emerge in autumn and early spring, reducing the need for herbicide applications before planting subsequent crops.

In vegetable rotation systems and high-tunnel production systems, overwintering cover crops, including radish, can also promote soil biological activity and nitrogen cycling without reducing the yield of subsequent cash crops. These benefits contribute to long-term soil health maintenance and improve the resilience of agricultural systems to environmental stress (Perkus et al., 2022; Elhakeem et al., 2023; Wang et al., 2023).

7 Case Studies of Winter Radish Production Systems

7.1 Case study in the Yangtze River delta region

The Yangtze River Delta region, including Shanghai and Zhejiang, is one of the major autumn-winter radish production areas in China. It is characterized by a temperate-subtropical monsoon climate and highly intensive vegetable rotation systems. Field experiments conducted in Zhejiang and Shanghai showed that radish is commonly grown as a rotational crop after rice or leafy vegetables. Although its growing period is relatively short, the input levels of nitrogen (N), phosphorus (P), and potassium (K) are generally high (Zhang et al., 2019b). The use of scientific nutrient management tools such as the QUEFTS model and Nutrient Expert for balanced fertilization can significantly improve fleshy root yield and nutrient use efficiency compared with traditional farmer fertilization practices (Figure 2). These results indicate that science-based fertilization management plays an important role in supporting the high-input winter production systems in this region.

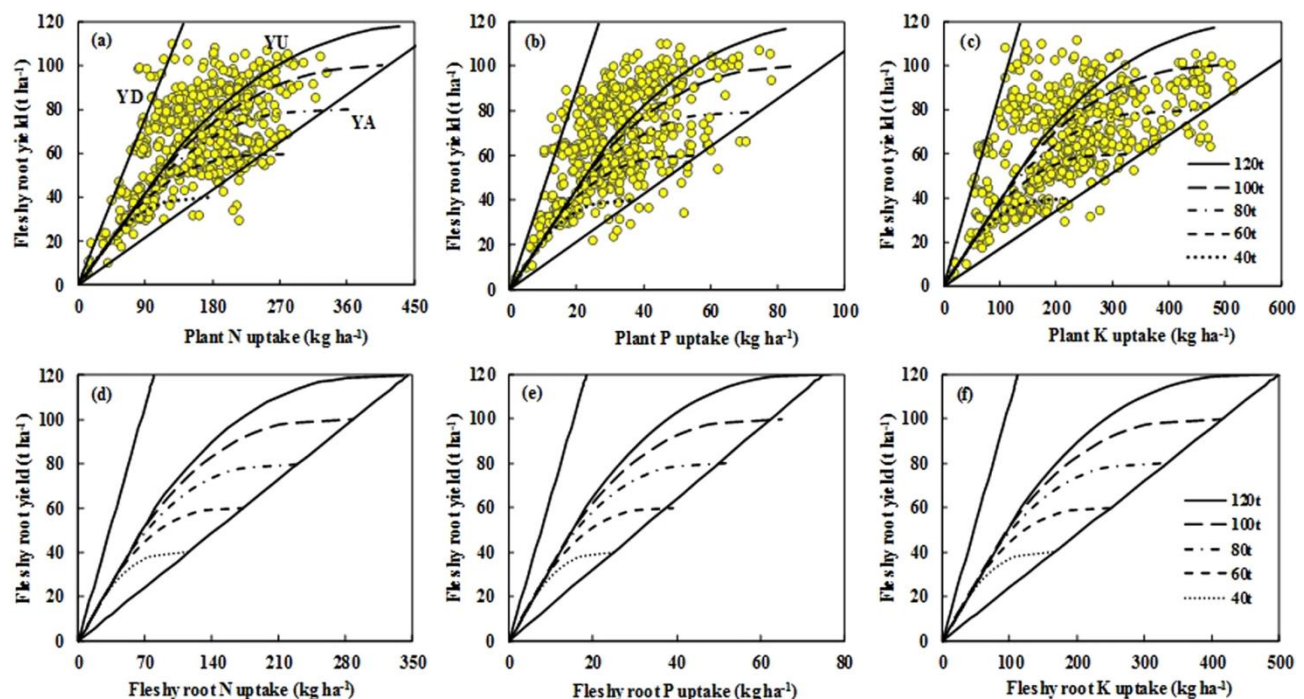


Figure 2 Relationships between fleshy root yield and N, P, and K accumulation in the total plant dry matter at maturity (a-c) and fleshy root N, P, and K removal in fleshy root dry matter (d-f) under different potential yields predicted by the QUEFTS model. YD, YA, and YU represent the maximum dilution, maximum accumulation, and balanced uptake of N, P, and K in the total plant dry matter or in the fleshy roots dry matter, respectively. These parameters were calculated by the QUEFTS model after excluding the upper and lower 2.5 percentiles of all internal efficiency data ($HI \geq 0.4$). The potential yield ranged from 40 to 120 t ha⁻¹ (Adopted from Zhang et al., 2019b)

7.2 Case study in southern China (e.g., Guangdong and Guangxi)

Subtropical regions such as Jiangxi and Chongqing, which represent the warm and humid ecological conditions of southern China, widely practice autumn-winter radish production. These areas are characterized by relatively mild temperatures and considerable variation in soil conditions. Soil organic matter content and available nitrogen, phosphorus, and potassium levels differ greatly among locations. Therefore, site-specific nutrient and water management strategies are required to fully realize yield potential according to local soil characteristics (Zhang et

al., 2019a). Under these climatic conditions, radish is commonly rotated with crops such as cabbage, tomato, or potato, forming intensive vegetable production systems. Winter production takes advantage of the long frost-free period, but growers must also deal with challenges such as high rainfall and soil acidification.

7.3 Farmer adoption and local adaptation strategies

In the major radish-growing regions of China, farmers often adjust sowing dates, planting density, and fertilization programs according to local temperature conditions and soil characteristics to improve production efficiency. Studies on off-season radish production in Nepal using insect-proof net houses or shading facilities showed that farmers commonly achieve off-season production by shifting sowing dates to late winter or early spring, using shading structures to reduce heat and strong light stress, and selecting hybrid varieties that can maintain stable fleshy root development under unsuitable temperature conditions. In China, the promotion of improved hybrid varieties, optimization of plant spacing, adoption of drip irrigation, and implementation of integrated nutrient management have become important technical approaches for maintaining uniform root size and reducing physiological disorders during cool-season production.

7.4 Yield and economic benefit analysis

Data from large-scale multi-location experiments showed that, under suitable climatic conditions and optimized water and fertilizer management, the economic yield of radish in China can reach 30–33 t·ha⁻¹ or even higher. A significant positive relationship has been found between yield and the balanced uptake of nitrogen, phosphorus, and potassium nutrients (Sharma et al., 2025). Economic analyses of nutrient management and irrigation strategies indicated that optimized fertilization programs and drip irrigation systems can significantly increase net returns and benefit–cost ratios compared with traditional farmer practices. The main reasons are higher yields and improved efficiency in the use of production inputs.

8 Future Prospects and Research Directions

8.1 Integration of genomics and precision breeding

The availability of high-quality, chromosome-level radish genomes, together with abundant SNP (single nucleotide polymorphism) and SV (structural variation) marker resources, has provided a strong foundation for genome-assisted breeding. Genome-wide association studies (GWAS) and transcriptome analyses have identified several important regulators involved in low-temperature response and plant growth, including the RsWRKY40-RsSPS1-CBF regulatory module, RsERF40, RsWRKY49, and RsCDF3 (Xu et al., 2023b). These findings provide technical support for precise molecular marker-assisted selection (MAS), genomic selection (GS), and speed breeding aimed at improving complex traits such as cold tolerance, late bolting, and yield.

8.2 Breeding climate-adapted radish varieties

Climate change is expected to alter temperature and precipitation patterns, which will further affect the geographic distribution of both wild and cultivated radish populations. Wild radish and diverse cultivated germplasm contain a large number of stress-resistant alleles that can be used to improve resistance to various biotic and abiotic stresses (Han et al., 2023). By combining these genetic resources with knowledge of low-temperature response regulatory networks, including regulators such as RsWRKY40, RsERF40, and RsWRKY49, it will be possible to develop new winter radish varieties with multiple stress-resistance traits, including tolerance to cold, drought, and heat. Such varieties will be better suited to future climate conditions that are expected to become more complex and variable.

8.3 Development of smart agriculture and digital cultivation technologies

Internet of Things (IoT)-based environmental monitoring systems and smart greenhouse technologies have shown clear advantages in radish production. Compared with conventional cultivation methods, these technologies have achieved better performance in seed germination, plant growth, and resource-use efficiency, demonstrating the value of sensor-based management systems (Lafta and Abdullah, 2024). In the future, the integration of sensor networks, automated control systems, artificial intelligence (AI), and big data analytics will further improve irrigation management, temperature regulation, and nutrient supply. These technologies can also provide accurate decision-making support for winter radish production in greenhouses and protected cultivation systems.

8.4 Strengthening regional breeding and extension systems

Studies on genetic diversity and the development of core germplasm collections indicate that greater attention should be given to the strategic use of germplasm resources from different geographic regions as well as wild relatives during breeding programs. At present, several regional breeding projects have successfully combined genomic technologies, controlled-environment screening methods (such as artificial-light cultivation), and field evaluations to develop high-performance radish lines suitable for protected cultivation and cold-region production (Sinyavina et al., 2023). In the future, stronger collaboration among research institutions, agricultural extension services, and farmers will be needed to accelerate the transfer and application of research results in winter radish production.

Author Contributions

We are grateful to Ms. Xuan for her critically reading the manuscript and providing valuable feedback.

Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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Research Report

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The Causal Inference Layer in Complex Trait Genetics: A Unified Statistical Framework from Fine-Mapping to Cross-Trait Integration

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Received: 22 May, 2026

Accepted: 22 Jun., 2026

Published: 30 Jun., 2026

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Preferred citation for this article:

Fang X.J., 2026, The causal inference layer in complex trait genetics: a unified statistical framework from fine-mapping to cross-trait integration, Bioscience Evidence, 16(3): 186-201 (doi: [10.5376/be.2026.16.0015](https://doi.org/10.5376/be.2026.16.0015))

Abstract Genome-wide association studies (GWAS) have identified a large number of loci associated with complex traits and diseases. However, most of these signals arise from linkage disequilibrium (LD) rather than directly reflecting causal variants, thereby limiting their mechanistic interpretability. Probabilistic fine-mapping addresses this limitation by introducing posterior inclusion probabilities (PIPs) and credible sets, shifting the inferential target from a single significant locus to a distribution of causal probabilities and enabling a systematic characterization of genetic uncertainty. In recent years, with the growing availability of functional annotation data, multi-ancestry studies, and multi-omics resources, fine-mapping methods have continued to expand in both model architecture and application scope. Nevertheless, a unified theoretical perspective across these methods remains lacking. In this study, we develop a unified statistical framework centered on causal configurations by systematically integrating fine-mapping and colocalization analyses within a Bayesian inferential framework. Under this framework, fastPAINTOR constructs annotation-informed priors using functional annotations, whereas CAVIAR and its extension McCAVIAR strengthen likelihood-based constraints through LD structure and cross-study information. Colocalization analysis further extends the inferential target from a single-trait setting to a multi-trait space, enabling probabilistic modeling of cross-trait causal consistency. Accordingly, research on complex traits can be organized into a continuous inferential pipeline from GWAS to fine-mapping, and further to colocalization and transcriptome-wide association studies (TWAS), thereby progressively translating statistical associations into biological mechanistic interpretation. On this basis, we further propose a method-selection strategy based on inferential hierarchy, clarifying the trade-offs among computational complexity, causal resolution, and information sources across different methods, and summarizing a practical workflow of “hierarchical inference.” This framework is applicable not only to studies of human complex diseases, but also to applied contexts such as crop genetic improvement, where it can be used to assess causal consistency across environments and populations. By unifying fine-mapping and colocalization within the same causal inference layer, this study provides statistical genetics with a consistent conceptual language and analytical paradigm, thereby facilitating the systematic transition of complex trait research from association discovery to mechanistic interpretation and causal inference.

Keywords Complex traits; Fine-mapping; Causal inference; Colocalization analysis; Credible sets; Multi-omics integration

1 Introduction

Genome-wide association studies (GWAS) have substantially advanced the dissection of the genetic architecture of complex traits and diseases over the past decade, leading to the identification of numerous genomic regions associated with phenotypic variation in both human populations and crop systems (Lapierre et al., 2020). However, the evidence provided by GWAS is primarily statistical association rather than direct causal interpretation. Because linkage disequilibrium (LD) is widespread across the genome, significantly associated loci often function only as marker SNPs correlated with the true functional variants, rather than representing the actual causal variants with biological effects. At the same time, multiple independent causal signals may coexist within a single associated region, making the assumption that the most significant SNP directly corresponds to the causal variant statistically untenable in many cases (Hutchinson et al., 2020). Accordingly, how to distinguish causal signals from correlated signals within associated regions has become an important issue linking population genetic analysis with functional genomic interpretation.

From the perspective of the development of statistical genetics, genomic analysis of complex traits has not been built upon a single statistic, but has instead progressed around a series of different inferential targets. GWAS

characterizes the evidence of association between loci and traits, SNP heritability reflects the proportion of phenotypic variance explained at the population level under a given model and marker set, and polygenic risk scores (PRS/PGS) further integrate these effects into predictive functions at the individual level (Fang and Wu, 2026; Fang, 2026a; 2026b). Nevertheless, this inferential chain remains incomplete at the level of causal interpretation. In particular, when LD structure is complex and multiple causal variants may coexist within a region, the central question is no longer merely whether a genomic region is associated with a trait, but rather which variants are more likely to be truly causal and how much uncertainty is attached to such judgments. Fine-mapping emerged precisely in response to this problem. Its objective is not to repeat the screening of significant loci, but to provide a probabilistic characterization of the plausibility of causal variants under given data and modeling assumptions, thereby introducing causal probability as a more informative inferential target in statistical genetics.

To address this objective, fine-mapping generally takes posterior inclusion probability (PIP) and credible sets as its core analytical quantities. Unlike conventional GWAS, which mainly relies on significance thresholds to make binary decisions, fine-mapping emphasizes continuous probabilistic estimation of causal configurations conditional on the observed data and model assumptions. As a result, the focus of inference shifts from whether an association exists to which candidate variants should be retained and how likely each of them is to be causal. Within this framework, a credible set is no longer treated as a subsidiary result centered on a single candidate locus, but rather as the smallest set of candidate variants constructed under a predefined coverage probability. This approach directly incorporates uncertainty into the inferential process and provides a more operational basis for subsequent functional validation and cross-omics integration (Kichaev et al., 2016; Hutchinson et al., 2020).

However, moving from association signals to causal probability distributions is far from straightforward. First, complex LD structure induces strong statistical correlations among multiple SNPs, giving rise to substantial non-identifiability and making it difficult to isolate the true causal variant from a group of correlated variants based on any single statistic alone. Second, the presence of multiple causal variants is not uncommon in complex traits, which means that traditional stepwise regression or conditional analysis cannot always identify independent effects in a stable manner and may instead lead to incorrect inference when the model is inadequately specified (Hutchinson et al., 2020). Therefore, the development of fine-mapping should not be viewed merely as a further refinement of analytical procedures. More fundamentally, it requires probabilistic models that explicitly characterize LD structure and the space of causal configurations while also allowing uncertainty to be properly represented and propagated.

In recent years, Bayesian probabilistic mapping approaches have gradually become the dominant framework in this field. These methods typically perform joint modeling of GWAS summary statistics and LD structure, while incorporating prior distributions to estimate the posterior probability that each candidate variant is causal, thereby providing a probabilistic representation of the causal architecture within local genomic regions. Although these methods all pursue the same general objective, they differ in their modeling emphasis. fastPAINTOR constructs annotation-informed priors by integrating functional annotations and improves computational efficiency through approximate Bayesian inference, making it more suitable for large-scale and multi-trait analyses. By contrast, CAVIAR and its extension MsCAVIAR do not rely on functional annotation, but instead model LD structure and multiple causal configurations directly, while improving the consistency and resolution of causal localization through the integration of cross-study or multi-ancestry data (Lapierre et al., 2020). Compared with traditional single-variant analysis, these approaches show clearer advantages in statistical power, the compactness of credible sets, and the consistency of results across cohorts (Hutchinson et al., 2020).

On this basis, colocalization analysis further establishes the connection between fine-mapping and functional genomics. When GWAS and molecular QTL signals overlap within the same genomic region, the key question is not simply whether they co-occur, but whether that overlap is driven by the same causal variant. Colocalization analysis is designed precisely to address this issue. Under a unified probabilistic framework, it evaluates the likelihood that GWAS signals and molecular QTL signals, such as eQTLs, sQTLs, pQTLs, or TWAS signals,

share the same causal variant, thereby distinguishing true causal concordance from superficial overlap generated solely by LD. With the development of multi-causal models, methods such as SuSiE have further improved the precision of causal decomposition and colocalization analysis, enabling cross-omics integration to move beyond simple signal overlap toward a more rigorous probabilistic characterization of causal consistency.

Against this background, the present study approaches fine-mapping from the perspective of the overall inferential framework of statistical genetics and interprets it as a key layer of causal inference situated between association discovery, variance explanation, and individual-level prediction. Focusing on representative methods including fastPAINTOR, CAVIAR, and MsCAVIAR, this study compares their differences in modeling assumptions, applicable boundaries, and computational characteristics, further clarifies the methodological meaning of PIP and credible sets as inferential targets for causal probability, and discusses their relationship with colocalization analysis. On this basis, the study seeks to propose a method-selection framework that balances computational efficiency, localization accuracy, and data conditions, thereby providing a more operational basis for empirical analysis in different research settings. From this perspective, fine-mapping should no longer be viewed merely as an auxiliary technique applied after GWAS, but rather as a core inferential step through which statistical genetics moves from association evidence toward causal interpretation.

2 Statistical Foundations of Fine-Mapping

2.1 Causal probabilities and credible sets: from association statistics to causal inference targets

The primary objective of fine-mapping is not merely to identify statistically significant loci, but to characterize, under complex linkage disequilibrium (LD) structures and multi-causal architectures, which variants are causal and how uncertainty is distributed across them. From this perspective, the inferential target of fine-mapping departs fundamentally from GWAS association statistics and instead becomes a new statistical object: the posterior distribution over causal configurations (i.e., a causal estimand).

In the conventional GWAS framework, single-variant tests provide evidence that a genomic region harbors an association signal but cannot distinguish true causal variants from correlated LD proxies (Spain and Barrett, 2015; Schaid et al., 2018). To address this limitation, Bayesian fine-mapping introduces the posterior inclusion probability (PIP) as a core quantity, defined as the probability that a given variant is causal conditional on the observed data and LD structure:

$$\text{PIP}_j = P(\gamma_j = 1 | \text{Data}, \text{LD})$$

where γ_j is an indicator of whether SNP j is causal. Within this framework, PIP is not a surrogate for statistical significance but a formally defined estimand that quantifies causal probability under explicit model assumptions (Hutchinson et al., 2020).

By ranking SNPs according to their posterior inclusion probabilities (PIPs) and cumulatively summing them until a predefined coverage threshold, such as 95%, is reached, one can construct the smallest candidate variant set, namely the credible set (Hutchinson et al., 2020; Shrestha et al., 2024). Under the assumption that the model is correctly specified, this set contains the true causal variant with a given probability. Unlike the traditional point-estimation logic that directly treats the most significant SNP as the causal variant, the credible set embodies a set-based inferential framework. Rather than forcing a single locus to bear the full burden of causal interpretation, it retains, under a probabilistic constraint, a candidate space capable of covering the true causal variant. In this way, uncertainty in causal inference is explicitly incorporated into the statistical analysis. From the perspective of the broader framework of statistical genetics, GWAS primarily provides evidence of association between loci and traits, SNP heritability corresponds to the explanation of variance at the population level (Fang, 2026a), and PRS serves individual-level prediction (Fang, 2026b), whereas PIP and the credible set describe the causal probability distribution at the locus level, thereby forming a key intermediate layer between association analysis and functional interpretation (Figure 1).

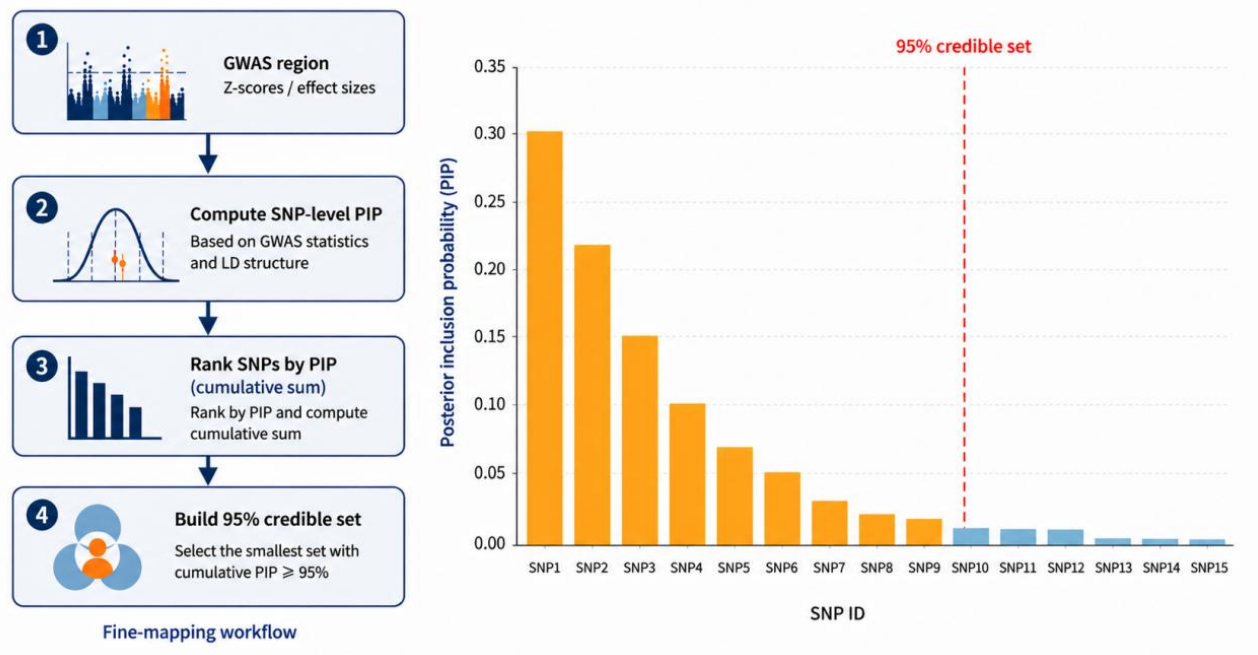


Figure 1 Workflow and illustrative example of credible set construction based on posterior inclusion probability (PIP)

Note: The left panel illustrates the core statistical workflow of fine-mapping: starting from GWAS summary statistics (e.g., Z-scores or effect sizes) within a genomic region, SNP-level posterior inclusion probabilities (PIPs) are computed, ranked, and cumulatively summed to construct a credible set under a predefined coverage threshold (e.g., 95%). The right panel presents an example PIP distribution, where each bar represents the PIP of a candidate SNP, and the dashed line indicates the cutoff at which the cumulative probability reaches 95%

In practical applications, the size of a credible set is not fixed, but is jointly determined by the complexity of local LD structure and the number of underlying causal variants. When LD relationships are relatively simple and only a single causal variant exists within a region, the credible set is usually compact. By contrast, under high-LD backgrounds or in the presence of multiple causal variants, the candidate set often expands substantially, which essentially reflects increased statistical non-identifiability (Hutchinson et al., 2019). In recent years, calibrated credible sets have further improved coverage estimation, bringing it closer to the underlying causal architecture (Shrestha et al., 2024). Therefore, this probability-set-based analytical framework not only enhances the interpretability of fine-mapping results, but also provides a relatively unified input form for functional annotation integration and cross-omics analysis.

2.2 Paradigm shift from single-variant testing to probabilistic inference

From the perspective of statistical inference, the difference between fine-mapping and traditional single-variant GWAS testing is not merely a refinement of analytical steps, but a fundamental shift in both the inferential target and the object of inference. Traditional GWAS mainly relies on single-SNP tests and determines whether a locus is associated with a trait through a significance threshold (Schaid et al., 2018). This approach is efficient for genome-wide scanning, but it rests on a strong simplifying assumption, namely, that the most significant SNP can serve as a proxy for the causal variant. This assumption often fails in the presence of complex LD structure or multiple causal signals, thereby leading to incorrect attribution or bias in the prioritization of candidate loci.

In contrast, Bayesian fine-mapping does not attempt to select a single “best” locus directly from significance results. Rather, it reformulates the problem as the estimation of the posterior distribution over all possible causal configurations, conditional on the observed data and the LD structure. Within this framework, PIP provides a continuous probabilistic characterization of the causal plausibility of an individual locus, whereas the credible set defines the candidate space under a prespecified probability constraint. For example, if three SNPs within a region have PIPs of 0.60, 0.25, and 0.10, respectively, they jointly constitute a 95% credible set, rather than only the

most significant locus being retained (Hutchinson et al., 2020). This treatment more adequately reflects the uncertainty of local signals and substantially reduces the risk of prioritizing false positives driven by LD structure.

More importantly, once statistical inference is advanced from significance testing to probabilistic characterization, the output of fine-mapping is no longer limited to the interpretation of local association signals, but can be further integrated with other statistical modules. PIP and credible sets can be used for functional annotation integration, support cross-population comparisons, and facilitate colocalization analysis, thereby maintaining a relatively consistent measurement basis across different data domains (Kichaev et al., 2014; Gerber et al., 2023). For this reason, fine-mapping should not be understood merely as a downstream filtering step following GWAS, but rather as an important inferential layer linking association analysis to mechanistic interpretation.

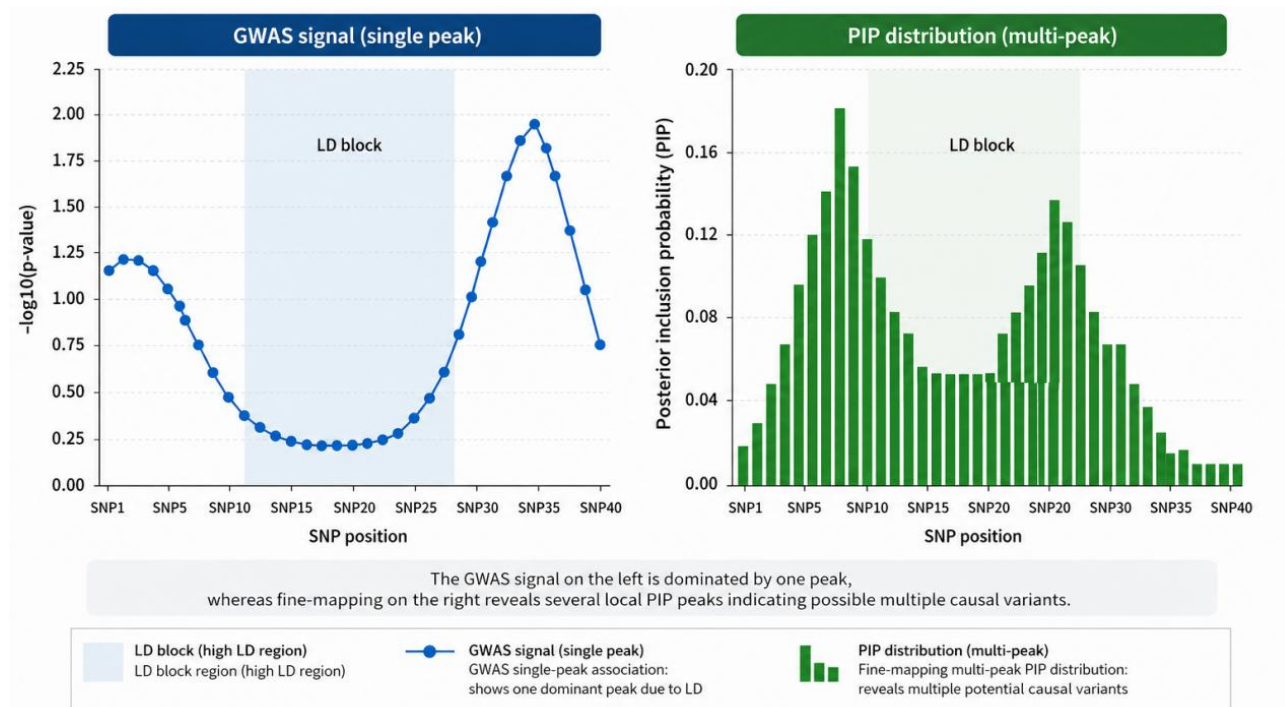


Figure 2 p-value vs PIP under an LD block: contrast between single-peak association signals and multi-peak causal probability distributions

Note: The left panel shows GWAS association signals represented by $-\log_{10}(p)$, typically characterized by a single dominant peak within an LD block. The right panel shows the corresponding posterior inclusion probability (PIP) distribution obtained from fine-mapping, revealing multiple local peaks that reflect potential causal variants. The shaded region denotes the LD block

2.3 Theoretical implications: from point estimates to causal distributions

From a more general statistical perspective, the central significance of fine-mapping lies in its transformation of causal inference from the identification of a single locus into the estimation of a posterior distribution. A credible set can be understood as the smallest set estimator constructed under a coverage probability constraint, and this logic is highly consistent with that of interval estimation and confidence set inference (Hutchinson et al., 2019; 2020). Within this framework, the effects of LD structure and the presence of multiple causal variants are no longer treated as nuisances in the analysis, but are instead directly incorporated into the model, allowing causal inference to shift from deterministic judgment to probabilistic characterization. Compared with approaches that rely on a single significant locus to draw conclusions, this strategy more faithfully captures the uncertainty inherent in the genetic architecture of complex traits and avoids excessive simplification of local association signals.

At the same time, once causal probabilities are expressed in the form of PIP and credible sets, they can be propagated into downstream analyses, thereby providing a common probabilistic basis for integrative studies at different levels. For example, in joint analyses of GWAS and eQTL data, researchers may compare the overlap of

credible sets or examine joint posterior probabilities to assess whether different signals are likely to share the same causal variant. In this sense, fine-mapping is not merely a tool for improving localization accuracy within a genomic region; it also serves to transmit uncertainty into subsequent analyses, enabling functional annotation integration, colocalization analysis, and cross-omics research to proceed on a unified probabilistic scale.

In addition, because PIP is fundamentally defined in probability space and does not depend on the form of a specific statistical measure, such as a p-value or effect size, it is more readily comparable across different study designs, populations, and omics data types. This property allows it to serve as a common language for expressing causal information across data domains and provides a methodological basis for cross-study integration (Shrestha et al., 2024). This is particularly valuable in multi-ancestry studies, where differences in LD structure across populations can be regarded as a naturally occurring additional constraint. Different LD patterns alter the correlations between non-causal variants and true causal loci, thereby helping to further shrink credible sets and improve causal resolution (Spain and Barrett, 2015). This feature also gives fine-mapping greater practical value in multi-ancestry research and naturally extends to the development of subsequent methods such as MsCAVIAR.

3 fastPAINTOR: An Annotation-Informed Model for Causal Probability

3.1 Model framework: joint inference of statistical evidence and functional annotation

Within the unified Bayesian framework of fine-mapping, the key distinction among methods lies not in whether probabilistic inference is performed, but in how the prior distribution over causal configurations is specified. From this perspective, fastPAINTOR can be understood as a prototypical *annotation-informed causal model*, whose central objective is to establish a probabilistic mapping between GWAS statistical evidence and functional annotations.

In general form, the fine-mapping problem can be expressed as estimating the posterior distribution over the causal configuration vector γ :

$$p(\gamma|D,R,A) \propto p(D|\gamma,R) p(\gamma|A)$$

From the perspective of statistical genetics, a basic fine-mapping model characterizes the distribution of causal probabilities under LD constraints, whereas fastPAINTOR further describes an annotation-informed causal probability distribution conditional on functional annotations, thereby giving its inferential target stronger biological interpretability. To estimate the posterior distribution within this framework, fastPAINTOR adopts approximate Bayesian methods, such as variational inference or importance sampling, in order to avoid the exponential enumeration of the full causal configuration space or the high computational cost associated with traditional Markov chain Monte Carlo (MCMC) approaches (Talukdar et al., 2023). Its optimization objective can be expressed as the evidence lower bound (ELBO):

$$\Pr_{q(\gamma)}(\gamma_j=1|a_j) = \text{logit}^{-1}(\alpha_0 + \alpha^T a_j)$$

where a_j denotes the annotation vector for SNP j (e.g., eQTL status, chromatin accessibility, transcription factor binding sites). This formulation effectively transforms biological functional potential into statistical prior probability, enabling a principled integration of functional and association evidence (Kichaev et al., 2014).

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$$\mathcal{A}(q) = \mathbb{E}_{q(\gamma)}[\log p(D|\gamma,R) + \log p(\gamma|A)] - \mathbb{E}_{q(\gamma)}[\log q(\gamma)]$$

This approximation achieves a favorable balance between computational efficiency and inferential accuracy, enabling scalable application to genome-wide datasets and multi-trait analyses.

3.2 Methodological features: information integration, scalability, and resolution

From a methodological perspective, the strengths of fastPAINTOR are first reflected in its ability to directly incorporate external functional information into the modeling of causal probabilities. Variants located in enhancer or promoter regions, or supported by eQTL evidence, are assigned higher prior weights by the model. As a result, the posterior inclusion probability (PIP) reflects not only the strength of statistical association, but also signals of functional relevance, thereby giving candidate causal variants greater biological interpretability (Kichaev et al., 2016).

At the same time, fastPAINTOR also shows strong scalability at the computational level. By adopting approximate Bayesian inference, the method avoids the computational bottleneck of MCMC in high-dimensional causal configuration spaces, allowing it to maintain relatively high efficiency under large-scale GWAS data and multiple annotation settings. This property is particularly important for current large-sample studies represented by biobank-scale datasets.

On this basis, the incorporation of functional annotations can further improve the resolution of causal localization. In particular, under multi-trait or multi-annotation settings, fastPAINTOR can often effectively reduce the size of the credible set. Previous studies have shown that, while maintaining relatively high coverage, the inclusion of functional annotations can reduce the candidate set by approximately 40%~60% (Kichaev et al., 2016). For studies in which downstream experimental validation resources are limited, such compression of the candidate space has direct practical value.

When these features are considered within a unified framework, the main advantage of fastPAINTOR can be understood as arising from the optimization of prior structure. In other words, when the likelihood model remains unchanged, the introduction of functional annotations improves the identifiability of the causal probability distribution, thereby enhancing the model's ability to distinguish true causal variants.

3.3 Model limitations and sources of bias

Although fastPAINTOR has clear advantages in information integration and computational efficiency, its inferential performance also depends heavily on the validity of the prior model itself. As a result, while this method improves interpretability, it also introduces new methodological risks.

3.3.1 Prior misspecification

When functional annotations are noisy, incomplete, or biased, the prior distribution may distort posterior inference, leading to over-prioritization of non-causal variants or underestimation of true causal variants. This issue can be viewed as a prior-induced shift in the causal estimand and may require mitigation through multi-annotation integration or sensitivity analyses.

3.3.2 Limited transferability across populations

Functional annotations are often tissue-specific or population-specific. In cross-ancestry studies or non-model organisms, annotation information may not generalize, potentially reducing model performance. Consequently, interpretation of results in such contexts should be approached with caution.

3.3.3 Approximation error

While variational inference improves scalability, it may underestimate posterior uncertainty in regions with complex LD structure or multiple causal variants. This can result in overly compact credible sets or the omission of true causal variants.

4 (Ms)CAVIAR: Likelihood-Driven Modeling of Cross-Study Causal Consistency

4.1 CAVIAR: an LD-driven model of causal configurations

Within the unified Bayesian framework of fine-mapping, CAVIAR (CAusal Variants Identification in Associated Regions) represents a class of models in which inference is driven primarily by the likelihood structure. In contrast to fastPAINTOR, which incorporates functional annotations through an explicit prior, CAVIAR relies solely on GWAS summary statistics and LD structure to infer the posterior distribution over causal configurations, without invoking external biological information (Lapierre et al., 2020).

Formally, CAVIAR models the joint distribution of GWAS Z-scores. For a genomic region containing m SNPs, let $Z=(Z_1, \dots, Z_m)$ denote the vector of marginal association statistics. Conditional on a causal configuration γ , the model assumes:

$$Z \sim N(0, \Sigma + \sigma^2 I)$$

where Σ is the covariance matrix determined by LD structure, and σ^2 captures residual variance. The key feature of this model lies in its explicit incorporation of LD structure into the likelihood function, thereby allowing correlated signals and causal signals to be distinguished within the same statistical framework.

Under this framework, CAVIAR evaluates (either exactly or approximately) the space of possible causal configurations and computes posterior inclusion probabilities (PIPs) for each SNP, from which credible sets are constructed under a specified coverage constraint (e.g., 95%). Importantly, the model allows for multiple causal variants ($K \geq 1$) within a locus, thereby addressing the “signal ambiguity” that arises in LD-rich regions.

From a unified perspective, CAVIAR characterizes a causal probability distribution that is primarily determined jointly by LD structure and association statistics, namely an LD-driven causal estimand. Compared with models that rely on annotation information, it is not directly affected by the quality of external functional data. As a result, it often shows greater robustness when functional annotations are unavailable, when the annotations themselves may be biased, or when the study is conducted in a cross-species context.

4.2 MsCAVIAR: extending the likelihood to cross-study causal consistency

Building on the single-study formulation, MsCAVIAR (Multiple Study CAVIAR) extends the inferential target from identifying causal variants within a single study to assessing causal sharing across studies or populations.

This extension fundamentally alters the causal estimand: rather than estimating the posterior probability that a variant is causal within a single dataset, MsCAVIAR estimates the posterior probability that a variant is jointly causal across multiple studies.

To achieve this, MsCAVIAR introduces a random-effects model to capture heterogeneity in effect sizes across studies. Let β_{js} denote the effect of SNP j in study s , then:

$$\beta_{js} \sim N(\mu_j, \tau_j^2)$$

Where μ_j represents the shared (mean) effect across studies and τ_j^2 captures cross-study heterogeneity. By jointly modeling summary statistics from multiple studies together with their respective LD structures, MsCAVIAR can estimate not only study-specific PIPs, that is, within-study causal probabilities, but also the shared causal probability across studies.

The importance of this modeling strategy lies in the fact that differences in LD structure across populations are no longer treated merely as background conditions of the analysis, but rather as a source of information that can be actively exploited. If the correlation structure of a given SNP differs across populations, then the association strength generated by non-causal variants will usually fluctuate with changes in LD patterns, whereas true causal variants are more likely to exhibit relatively consistent signals across studies. MsCAVIAR takes advantage of this contrast to eliminate spurious signals and further reduce the size of the credible set (Lapierre et al., 2020).

Empirical studies have shown that in cross-ancestry GWAS analyses, such as those of lipid traits and type 2 diabetes, MsCAVIAR can significantly reduce the size of the credible set relative to single-study methods, often by about 20% or more, while still maintaining high causal coverage. This indicates that cross-study integration improves not only statistical power, but also the robustness of causal inference.

4.3 Methodological trade-offs: likelihood vs prior, robustness vs resolution

From a methodological standpoint, (Ms)CAVIAR and fastPAINTOR form a natural contrast within the unified framework:

- (1) fastPAINTOR: improves identifiability by optimizing the prior
- (2) CAVIAR / MsCAVIAR: improves identifiability by refining the likelihood

For CAVIAR and MsCAVIAR, one prominent advantage is that they do not rely on functional annotations and are therefore less sensitive to bias in external information. This property is particularly important when annotation resources are limited, when annotation quality is unstable, or when the study organism is not a model species with well-developed functional information. In comparison, these methods depend more directly on the LD structure itself and the statistical constraints it provides, and thus often show greater robustness when functional information is lacking.

A further advantage of MsCAVIAR lies in its use of cross-population differences in LD structure. LD patterns are not identical across populations, and this provides additional leverage for separating causal signals from non-causal correlated signals. For this reason, MsCAVIAR can improve resolution through cross-study integration in situations where single-study analysis is insufficient. This gain in information derived from heterogeneity is essentially a cross-LD decoupling mechanism that is not available within a single-study framework.

However, the main limitation of these methods is also clear, namely their relatively high computational cost. Because they require evaluation of high-dimensional causal configuration spaces, computational complexity increases rapidly as the number of candidate SNPs grows and the number of potential causal variants rises. In MsCAVIAR, this problem is further amplified by joint modeling across multiple studies (Lapierre et al., 2020). As a result, in practical applications, different methods tend to serve distinct roles. fastPAINTOR is better suited for genome-wide scanning and high-throughput analysis, whereas (Ms)CAVIAR is more appropriate for fine-scale dissection of candidate regions and validation studies under cross-population settings.

With continued algorithmic optimization and ongoing improvements in computational resources, cross-study fine-mapping is expected to play an increasingly important role in complex trait research and may gradually become part of the standard analytical workflow.

5 Colocalization and eQTL/TWAS Interfaces: A Multi-Trait Causal Inference Layer

5.1 From signal overlap to causal sharing: reformulating the problem

Although GWAS has identified a large number of loci associated with complex traits, these statistical signals do not directly reveal their underlying molecular mechanisms. Molecular quantitative trait loci (QTLs), including expression QTLs (eQTLs), splicing QTLs (sQTLs), and protein QTLs (pQTLs), provide critical insights into how genetic variants influence phenotypes through regulatory processes at the transcriptional and translational levels (Okamoto et al., 2023). In parallel, transcriptome-wide association studies (TWAS) aggregate genetic effects at the gene level by predicting gene expression, thereby prioritizing candidate genes.

It should be noted, however, that the overlap of GWAS signals with molecular QTL or TWAS signals within the same genomic region does not necessarily imply that they are driven by the same causal variant. Because linkage disequilibrium (LD) and allelic heterogeneity may coexist, multiple highly correlated variants within a region can affect different phenotypes separately, thereby producing overlapping signals at the statistical level without reflecting true causal consistency. This phenomenon is commonly referred to as false colocalization. Colocalization analysis is designed precisely to address this question by modeling cross-trait causal sharing within a probabilistic framework (Giambartolomei et al., 2013; Hormozdiari et al., 2016).

From the perspective of this study, if fine-mapping characterizes the within-trait causal probability distribution (via PIP), then colocalization extends this to: the joint posterior distribution over shared causal configurations across traits, thereby forming a critical bridge between locus-level causal inference and mechanistic interpretation.

5.2 Bayesian foundations: from single-causal to multi-causal models

The statistical foundation of colocalization lies in evaluating competing causal hypotheses under a Bayesian framework. A canonical example is COLOC, which defines five mutually exclusive hypotheses within a genomic region:

- H_0 : neither trait is associated
- H_1 : only the GWAS trait is associated
- H_2 : only the QTL trait is associated
- H_3 : both traits are associated, but with distinct causal variants
- H_4 : both traits share the same causal variant

Posterior probabilities $P(H_i|\text{data})$ are computed for each hypothesis, with $P(H_4|\text{data})$ (commonly denoted as PP4) serving as the primary measure of colocalization evidence (Giambartolomei et al., 2013).

The strength of this framework lies in the fact that it does not rely on a single lead SNP for inference. Instead, it makes use of the overall information contained in the credible set at the regional level and explicitly incorporates uncertainty into the inferential process. Therefore, compared with approaches that rely solely on peak overlap, COLOC provides a more rigorous statistical interpretation. However, an important assumption of COLOC is that each trait has at most one causal variant within the region.

In studies of complex traits, multiple causal signals are not uncommon, and this single-causal-variant assumption is often overly restrictive. For this reason, a series of extended methods have been developed to accommodate more complex genetic architectures. eCAVIAR allows multiple causal variants within a region and combines LD structure to calculate the colocalization posterior probability (CLPP); moloc extends the colocalization framework to joint modeling of multiple traits; and HyPrColoc is further designed for high-dimensional multi-trait colocalization analysis. Although these methods differ in their specific implementations, they share a common conceptual basis: all of them extend the causal configuration space in fine-mapping into a joint space across multiple phenotypes. From a unified perspective, colocalization can therefore be understood as a further extension of fine-mapping along the phenotypic dimension.

5.3 Interface with fine-mapping: from variant-level to cross-trait inference

Colocalization analysis is naturally coupled with fine-mapping methods such as fastPAINTOR and (Ms)CAVIAR. Their relationship can be summarized as follows:

fine-mapping provides variant-level posterior probabilities (PIPs) and credible sets for each trait
colocalization evaluates whether these probabilities imply shared causality across traits

Formally, cross-trait causal sharing can be conceptualized as a function of trait-specific PIPs and LD structure:

$$P(\text{shared causal}) \approx f(\text{PIP}_{\text{GWAS}}, \text{PIP}_{\text{QTL}}, \text{LD})$$

In practical research, this relationship is usually implemented as a two-stage inferential workflow. The first stage operates at the locus level, where researchers apply fastPAINTOR or (Ms)CAVIAR to perform fine-mapping for a single phenotype, thereby obtaining high-resolution PIP distributions and credible sets. The second stage then moves to the cross-phenotype level, where methods such as COLOC, eCAVIAR, or moloc are used, based on the results of the first stage, to evaluate the probability of colocalization between GWAS signals and QTL or TWAS signals.

The practical value of this analytical workflow lies in the clear complementarity among these methods. fastPAINTOR primarily reduces the candidate space by integrating annotation information, whereas (Ms)CAVIAR improves inferential robustness through LD structure and cross-population differences.

Colocalization analysis further examines causal consistency through joint inference across phenotypes. When combined with TWAS-based integration at the gene level, this workflow ultimately forms a complete inferential chain from GWAS to fine-mapping, then to colocalization and TWAS, and finally to candidate gene identification:

GWAS→fine-mapping→colocalization→TWAS→candidate gene identification

This continuous analytical path indicates that the focus of statistical genetics has moved beyond the identification of association signals and is gradually shifting toward the systematic interpretation of molecular mechanisms (Hormozdiari et al., 2016; Okamoto et al., 2023).

6 Practical Guidelines for Method Selection: A Layer-Based Decision Framework

6.1 A unified view of trade-offs: computation, identifiability, and information sources

In fine-mapping and cross-omics analysis, method selection is not simply a matter of tool preference, but rather the result of a systematic trade-off among different inferential strategies. At its core, this trade-off usually involves three mutually constraining aspects: computational complexity, causal resolution or identifiability, and the structure of information sources, including LD, functional annotations, and cross-study data. Within a unified Bayesian framework, the differences among methods can essentially be understood as different ways of imposing constraints on the same causal configuration space by drawing on different types of information (Schaid et al., 2018; Hutchinson et al., 2020).

(1) FastPAINTOR: improving resolution through prior information

The core of fastPAINTOR lies in its use of functional annotations to construct informed priors, thereby increasing the identifiability of causal variants while keeping the likelihood structure, that is, the LD structure, unchanged (Kichaev et al., 2014, 2016). The strengths of this method are mainly reflected in two respects. On the one hand, it can operate efficiently at the genome-wide scale and is therefore suitable for large-scale analyses. On the other hand, when functional annotation information is sufficiently informative, it can substantially shrink the credible set and thus improve the ranking resolution of candidate causal variants. Previous studies have shown that integrating functional annotations not only reduces the size of the candidate set but also improves the prioritization of causal variants (Kichaev et al., 2016; Zou et al., 2021).

However, this very advantage also defines its limitation. The inferential results of fastPAINTOR are highly sensitive to the quality of annotations. Once functional annotations contain noise, missingness, or systematic bias, these defects may be propagated into the posterior inference and introduce systematic error. From a statistical perspective, fastPAINTOR is therefore essentially an annotation-informed prior model that improves causal resolution by relying on prior information.

(2) CAVIAR: robustness through likelihood modeling

Unlike fastPAINTOR, CAVIAR does not rely on functional annotations, but instead distinguishes correlated signals from causal signals by explicitly modeling LD structure at the likelihood level (Schaid et al., 2018). This characteristic makes it less dependent on external information and therefore generally more robust when functional annotations are sparse or of uncertain reliability. At the same time, CAVIAR allows multiple causal variants to exist within a region, giving it considerable flexibility in handling complex genetic architectures.

This robustness, however, comes at a cost. Because CAVIAR must evaluate high-dimensional causal configuration spaces, its computational complexity increases rapidly as the number of candidate SNPs and the number of potential causal variants rise. CAVIAR is therefore best understood as a likelihood-driven model that emphasizes robustness in causal inference.

(3) MsCAVIAR: enhancing identifiability through cross-study information

MsCAVIAR extends CAVIAR into cross-study or cross-population settings. By jointly modeling summary signals from multiple studies together with their respective LD structures, it further improves the identification of causal variants (Lapierre et al., 2020). Its most important advantage lies in its ability to exploit differences in LD patterns

across studies, thereby removing non-causal signals more effectively and improving the assessment of causal consistency across studies.

At the same time, MsCAVIAR places higher demands on the data. It depends on the completeness and comparability of multi-study datasets, and because it combines statistical information across several studies, its computational burden is also substantially increased. MsCAVIAR can therefore be regarded as a cross-study model that extends the scope of causal inference and improves identifiability through cross-study information.

(4) Colocalization methods: inference of cross-trait causal consistency

Colocalization methods, such as COLOC and eCAVIAR, extend the inferential target from a single phenotype to multiple phenotypes, focusing on whether different phenotypes share the same causal variant and thereby enabling probabilistic modeling of cross-trait causal consistency (Giambartolomei et al., 2013; Hormozdiari et al., 2016). Whereas fine-mapping primarily concerns locus-level causal probabilities, colocalization methods further expand the inferential target to shared causal probability, allowing GWAS signals to be connected more directly to molecular mechanisms.

The performance of these methods, however, is typically influenced by the quality of downstream molecular QTL data and the degree of matching across tissues and cell types. For this reason, colocalization methods are best understood as a framework for causal inference at the cross-trait level.

6.2 A layer-based decision strategy

In practical research, method selection is better organized according to inferential layers rather than based on empirical preference alone. Different stages of analysis address different questions, and the methods used should accordingly change with the inferential goal.

(1) Genome-wide scanning stage

At the genome-wide scanning stage, the main objective is usually to narrow the candidate space as quickly as possible in order to complete an initial screen in large-scale datasets. In this context, fastPAINTOR is often an appropriate choice. Its use presupposes access to high-quality functional annotation information. By leveraging annotation-informed prioritization, the method can balance efficiency with candidate-space reduction in high-throughput analysis settings (Kichaev et al., 2016; Zou et al., 2021).

(2) Regional fine-resolution stage

Once the analysis enters the stage of fine-scale dissection of candidate regions, the focus shifts from broad screening to the robust identification of sets of causal variants. At this stage, CAVIAR is often more advantageous. Especially when functional annotations are insufficient or local LD structure is complex, its independence from prior information allows its robustness to become more evident (Schaid et al., 2018).

(3) Cross-study integration stage

When the research goal further shifts to evaluating causal consistency across studies or ancestries, MsCAVIAR becomes more suitable. It can exploit LD differences across studies to enhance identification, thereby improving both causal consistency assessment and localization resolution in multi-cohort or multi-ancestry studies (Lapierre et al., 2020).

(4) Mechanistic interpretation stage

At the stage of mechanistic interpretation, the focus is no longer limited to locating causal variants, but rather to linking genetic variants with molecular functional processes. At this point, colocalization analysis combined with TWAS can more effectively evaluate cross-trait causal consistency and connect GWAS findings with potential molecular mechanisms (Giambartolomei et al., 2013; Okamoto et al., 2023).

6.3 A unified strategy: hierarchical inference pipeline

Integrating the above considerations, method selection in complex trait genetics can be summarized as a unified inferential pipeline:

GWAS→fine-mapping→colocalization→TWAS/functional validation

Within this framework, the various methods are not simple substitutes for one another, but rather different steps that progressively constrain and refine the same causal problem at different inferential layers (Schaid et al., 2018; Hutchinson et al., 2020). Among them, fastPAINTOR and CAVIAR mainly act on the causal space of a single phenotype, MsCAVIAR further addresses causal consistency across studies, and colocalization analysis extends the inference to cross-trait causal consistency.

The value of this layered inferential strategy lies in its ability to maximize the integration of information from different sources, including LD structure, functional annotations, cross-study data, and cross-omics information, while seeking a balance between computational efficiency and inferential precision and reducing, as much as possible, the systematic bias that may arise from any single method. In this sense, method selection should not be understood as a comparison among interchangeable techniques, but rather as a layered decision-making process centered on the same causal inferential objective.

7 Discussion: A Unified Framework from Association Signals to the Causal Inference Layer

7.1 From method complementarity to a unified statistical framework

Existing fine-mapping methods are often regarded as a collection of tools developed for different research scenarios. However, from the unified perspective of statistical genetics, these methods all essentially perform inference on the same causal configuration space under different informational constraints. The differences among fastPAINTOR, CAVIAR, and its extension MsCAVIAR can be understood as reflecting different emphases on distinct components of the Bayesian model: fastPAINTOR primarily strengthens the prior structure through functional annotations (Kichaev et al., 2014, 2016), CAVIAR reinforces likelihood constraints through explicit modeling of LD structure (Schaid et al., 2018), and MsCAVIAR further introduces additional data constraints through cross-study integration (Lapierre et al., 2020).

Accordingly, the complementarity among these methods should not be viewed as a simple empirical combination, but rather as the result of progressively strengthening constraints on the same causal estimand across different informational dimensions. Within this framework, method selection is no longer merely a matter of choosing among tools, but instead involves determining which path most effectively approximates the causal probability distribution under given data conditions. In this sense, fine-mapping methods should not be regarded as isolated techniques, but as different implementations within a unified statistical framework.

7.2 Dual pathways of information integration: prior enrichment and data expansion

The key to improving fine-mapping resolution lies in increasing the identifiability of causal configurations. In the current methodological landscape, this objective is pursued mainly along two paths. One path relies on strengthening prior information through the incorporation of functional annotations. Methods represented by fastPAINTOR transform biological information, such as eQTLs, ATAC-seq, and ChIP-seq data, into informed priors, thereby increasing the posterior probability of candidate causal variants (Kichaev et al., 2016; Zou et al., 2021). When annotation quality is high, this strategy can not only shrink credible sets but also enhance the biological interpretability of the results. However, this path is highly dependent on annotation quality. Once functional information is biased or incomplete, it may lead to prior misspecification and consequently affect posterior inference (Kichaev et al., 2016).

The other path takes the form of expanding the observed data, particularly through the integration of multi-population or multi-study datasets to exploit differences in LD structure and thereby enhance the identification of causal variants. Methods such as MsCAVIAR follow this logic by jointly modeling summary signals from different studies, thus improving both the robustness and the resolution of causal inference (Lapierre et al., 2020). Compared with annotation-dependent methods, this approach does not directly rely on external functional information and can more effectively eliminate spurious signals driven by LD. Its cost, however, is that it places greater demands on data quality, comparability across studies, and computational resources (Schaid et al., 2018).

From a unified perspective, these two paths correspond to improving the identifiability of causal inference either by enhancing prior information or by expanding observed data. In practice, the combination of high-quality functional annotations and multi-ancestry data integration often provides a favorable balance between efficiency and robustness.

7.3 From single-trait to multi-trait inference: extending the causal layer

Fine-mapping mainly addresses the distribution of causal probabilities under a single-trait setting, whereas colocalization analysis further extends this inferential framework into the multi-trait space by evaluating whether different traits share causal variants, thereby enabling probabilistic modeling of cross-trait causal consistency (Giambartolomei et al., 2013; Hormozdiari et al., 2016). In statistical terms, this extension corresponds to a shift from univariate posterior distributions to joint posterior distributions.

In practical workflows, this extension forms a continuous inferential chain:

GWAS→fine-mapping→colocalization→TWAS

This pipeline embeds statistical association signals into a causal pathway linking variants, genes, and phenotypes, thereby facilitating the transition from association discovery to mechanistic interpretation (Okamoto et al., 2023).

7.4 Implications for crop genetic improvement

Although probabilistic fine-mapping methods were primarily developed in human genetics, they also hold considerable promise for crop genetic improvement. Plant traits are often shaped by strong environmental dependence and complex population structure, which means that GWAS results obtained under a single environment or within a single population often have limited stability (Schaid et al., 2018).

In this context, the probabilistic framework centered on PIP and credible sets can be used to evaluate the consistency of causal signals across multiple environments and populations, thereby identifying more stable genetic factors. More specifically, MsCAVIAR can be applied to validate causal consistency across populations (Lapierre et al., 2020), colocalization analysis can help connect genetic variants with molecular mechanisms (Giambartolomei et al., 2013), and functional annotation integration can be used to prioritize candidate loci (Kichaev et al., 2016). The combination of these approaches provides a more reliable statistical basis for marker-assisted selection (MAS) and the prioritization of targets for gene editing.

7.5 Integration with downstream causal inference methods

Fine-mapping and colocalization analysis together provide an important foundation for higher-level causal inference methods. In Mendelian randomization (MR) analysis, selecting instrumental variables on the basis of credible sets can reduce false positives driven by LD and thereby improve the validity of the instruments (Broekema et al., 2020). At the same time, by combining colocalization and TWAS results, it is possible to construct multilayer causal networks linking variants, genes, pathways, and phenotypes, thus extending the analytical perspective from single-gene interpretation to systems-level biological interpretation (Okamoto et al., 2023).

Therefore, within a unified framework, the role of fine-mapping is not merely to refine association signals locally, but to transform statistical association results into candidate sets that can be used for downstream causal inference, thereby serving as a key intermediate layer in the broader causal analysis chain.

7.6 Future directions: toward an integrated causal inference framework

Future developments in statistical genetics are likely to continue advancing along the direction of a unified causal inference framework. Important directions include the deeper integration of multi-ancestry and multi-omics data (Lapierre et al., 2020), the development of multi-trait and network-level models such as Genomic SEM, and the further application of machine learning methods to high-dimensional causal inference. However, despite the expansion of methodological forms, the central challenge remains unchanged: how to maintain interpretability and reproducibility in causal inference under complex data structures.

Against this background, the probabilistic framework centered on PIP and credible sets will continue to serve as an important connecting foundation across different methods, functioning as a common language of representation and supporting the ongoing transition of statistical genetics from association analysis toward causal inference (Schaid et al., 2018).

8 Conclusion

The development of probabilistic fine-mapping marks an important shift in the research paradigm of statistical genetics. The focus of complex trait research is no longer confined to the detection of association signals, but is gradually moving toward an inferential framework centered on causal probability distributions. Within this framework, genetic variants are no longer ranked solely according to significance levels, but are instead characterized probabilistically through posterior inclusion probabilities (PIPs) and credible sets, thereby enabling a more systematic representation of uncertainty in causal inference. This shift means that research on complex traits is moving from the localization of association signals toward the analysis of causal mechanisms.

The main contribution of this study lies in its attempt to bring different fine-mapping methods into a common causal configuration framework from a unified Bayesian perspective. Although these methods differ in their implementation, they all essentially serve to constrain and characterize the same causal space. Methods that rely on functional annotations primarily improve resolution through prior information, whereas methods that rely on LD structure enhance robustness through likelihood-based modeling, and cross-study integrative methods further improve the identifiability of causal signals by expanding the data basis. Thus, the differences among these methods are better understood as arising from the way information is utilized, rather than from differences in the inferential target itself.

On this basis, the inferential framework can also be naturally extended to the multi-trait level. By modeling the probability that different phenotypes share causal variants, colocalization analysis extends causal inference from the single-trait setting to the evaluation of cross-trait causal consistency, thereby establishing a statistical connection between genetic variation and molecular mechanisms. In this way, research on complex traits gradually forms a continuous inferential path, beginning with GWAS, proceeding through fine-mapping and colocalization analysis, and ultimately moving toward functional interpretation and candidate gene identification.

From a practical perspective, method selection should be organized systematically according to inferential layers and data structure. Different methods are not simple substitutes for one another, but rather different steps designed to address different questions within the same causal inference process. Only by combining these methods appropriately in specific research contexts is it possible to achieve a balance among computational efficiency, inferential robustness, and biological interpretability, and thereby improve the overall reliability of complex trait analysis.

Author Contributions

Xuanjun Fang conducted the study, including literature review, data analysis, and drafting and revising the manuscript. The author has read and approved the final version of the manuscript.

Acknowledgments

This work was supported by the Major Program of the National Natural Science Foundation of China (Grant No. 30490254).

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