

Marker-assisted backcrossing (MABC) is the core technical pathway for achieving gene pyramiding. Its basic framework includes foreground selection, recombinant selection, and background selection, aiming to restore the recurrent parent genome as quickly as possible while introducing target genes (Haque et al., 2021). In multiple crops, MABC can restore more than 95% of the recurrent parent genome within 3–4 backcrosses and successfully pyramid 4–10 resistance or tolerance loci, which is significantly faster than traditional backcrossing methods (Das et al., 2018; Pandit et al., 2021). With the introduction of high-density SNP chips, KASP, multiplex detection systems, and genomic selection models, breeders can now simultaneously track multiple loci, optimize population size, and improve pyramiding success rates, providing an actionable approach for modular improvement of complex target traits in soybean (Jarallah et al., 2025).

4.2 Breeding efficiency and limiting factors

Gene pyramiding through MAS improves traditional breeding efficiency. Its core advantage lies in the ability to directly identify target gene combinations at the seedling or early-generation stage, thereby reducing the number of materials carried into subsequent generations and saving field space, phenotyping costs, and breeding cycle time (Haque et al., 2021). In multiple crops, marker-assisted gene pyramiding can essentially restore elite genetic backgrounds within 2–4 backcrosses, whereas traditional backcrossing usually requires about six generations. It can also pyramid multiple resistance or tolerance loci, enhancing resistance levels and yield stability (Kumar et al., 2018; Haque et al., 2021). This means that breeders can not only simultaneously track multiple target loci through multi-marker detection, but also focus resources on the most promising recombinants using foreground and background selection, improving the accumulation efficiency of favorable genes in populations (Ramalingam et al., 2020).

However, multi-gene pyramiding is not without cost. As the number of target genes increases, the required population size rapidly expands; at the same time, gene linkage, QTL×QTL interactions, and donor fragment linkage drag increase screening difficulty and may weaken the expression of target genes in new genetic backgrounds (Das et al., 2017). Even if molecular detection confirms the target genotypes, rigorous background selection and multi-environment field trials are still needed to validate the combined performance of resistance, stress tolerance, yield, and adaptability.

4.3 Application of gene pyramiding in soybean breeding

In soybean breeding, MAS-based gene pyramiding has gradually shifted from theoretical strategy to practical application, with the most mature field still being disease-resistance breeding (Figure 3). Studies have used marker-assisted backcrossing to simultaneously introduce the *Phytophthora* root rot resistance gene *Rps2*, powdery mildew resistance gene *Rmd-c*, and effective nodulation-related genes into high-yielding variety backgrounds, resulting in new materials with multiple resistances and good agronomic performance. This demonstrates the feasibility of simultaneous multi-gene introgression in soybean (Ramalingam et al., 2020). The same concept also applies to the pyramiding of soybean cyst nematode resistance genes *Rhg1/Rhg4*, different *Rps* genes, and rust resistance genes, aiming to build broader-spectrum and more durable resistance systems within a single variety (Haque et al., 2021).

In terms of abiotic stress, although mature pyramiding cases reported in soybean are still fewer than those in crops such as rice, the relevant technical pathway has become relatively clear. As major-effect QTLs and candidate genes related to drought, salt, waterlogging, and heat tolerance continue to be validated, breeders can use MAS to integrate loci corresponding to different stress-resistance mechanisms—such as genes related to osmotic regulation, maintenance of ion homeostasis, and optimization of root architecture—into the same elite genetic background, thereby improving varietal stability and adaptability under complex environments (Dormatey et al., 2020; Pandit et al., 2021). In quality improvement, gene pyramiding also has clear potential. For example, joint selection of protein- and oil-related QTLs, fatty acid composition loci, and low-antinutritional-factor genes is expected to simultaneously improve nutritional value, processing quality, and marketability (Das et al., 2017; Zampieri et al., 2023).