

## WHITE PAPER

# Innovations in Broccoli Genomics: An Introduction to Molbreeding's GenoBaits® Panels

## Summary

This white paper presents the SNP selection, design, and application of MolBreeding's **Broccoli (*Brassica oleracea*) GBTS Panel Series**, a comprehensive genomic resource developed to support oleracea breeding programs. We highlight the key traits that have been extensively studied in global *B. oleracea* research, and have compiled validated robust genome-wide SNP markers into a single, comprehensive array. A subset of these markers is annotated with their associated traits and underlying control genes, providing clear guidance for targeted selection and genetic analysis.

Using this panel, breeders can efficiently identify favorable alleles, explore the genetic architecture of their germplasm, and accelerate the development of improved oleracea varieties. This resource enables faster and more precise breeding decisions, could be incorporated into programs to meet both market demands and environmental challenges.

## Introduction

*Brassica oleracea*, a diverse species in the *Brassicaceae* family, is widely grown in temperate regions and valued for its versatility in culinary use and nutritional benefits worldwide.

Breeding *Brassica oleracea* crops remains challenging due to their genetic complexity, including whole-genome triplication, gene duplications, and repetitive sequences that create paralogs and functional redundancy. This complexity underlies the remarkable morphological diversity across morphotypes—cabbage heads, broccoli florets, kale leaves, and Brussels sprouts buds (Cheng et al., 2014). Long generation times, multiple trait targets, and variable growing conditions further slow breeding, while advances in high-throughput phenotyping and genotyping are accelerating the discovery of trait-associated genes and alleles.

Leveraging public genomic resources, MolBreeding collaborate with Zhejiang Academy of Agricultural Sciences (ZAAS) to develop the MolBreeding Broccoli GBTS Panel Series, a ready-to-use, high-density SNP marker tool that provides affordable and efficient genotyping. This panel enables breeders and researchers to track favorable alleles, uncover trait-associated genes, and accelerate the development of improved oleracea varieties with enhanced performance, resilience, and adaptability to diverse environments.

## SNP Selection & Probe Design

### SNP Calling & Filtration

A total of **102** resequencing datasets from **nine *Brassica oleracea* varieties** are used for SNP calling, including 67 datasets provided by ZAAS and 35 datasets retrieved from NCBI BioProject PRJNA312457 (Table 1). These datasets cover major cultivated forms: cabbage, broccoli, Brussels



sprouts, cauliflower, kale, landraces, and wild relatives.

A first-round SNP selection is conducted on the population gVCF (initially comprising 35,111,310 SNPs) using the following criteria:

- Minor allele frequency (MAF) > 0.35;
- SNP missing rate (NA) < 10% across the 102 accession VCF files;
- Heterozygosity < 10%;
- Prioritization of SNPs in gene-coding regions in the following order: stop-gain > non-synonymous > promoter > synonymous > 5'/3' UTR > intron > intergenic regions.

A total of 53,708 SNPs pass the filters and are selected as candidates. To fill sparse regions, selection criteria are relaxed to MAF > 0.05, heterozygosity ≤ 50%, and SNP call rate > 50%, allowing an additional 7K~8K SNPs to be included in underrepresented genomic regions.

Finally, 62,533 SNP loci are selected, and 62,533 probes are preliminarily designed using GenoBaits probe design software with the following parameters: probe length of 110 bp, GC content 30–70%, and homologous regions < 5 bp. Probe design is optimized to avoid SSRs and N regions wherever possible.

| Common Name           | Taxonomy                                  | Nr. Acc    |
|-----------------------|-------------------------------------------|------------|
| Wild                  | <i>B. oleracea</i> var. <i>oleracea</i>   | 8          |
| Broccoli              | <i>B. oleracea</i> var. <i>italica</i>    | 28         |
| Kohlrabi              | <i>B. oleracea</i> var. <i>gongylodes</i> | 8          |
| Brussels sprouts      | <i>B. oleracea</i> var. <i>gemmifera</i>  | 2          |
| (Pointed) Cabbage     | <i>B. oleracea</i> var. <i>capitata</i>   | 19         |
| Cauliflower           | <i>B. oleracea</i> var. <i>botrytis</i>   | 20         |
| Romanesco cauliflower | <i>B. oleracea</i> var. <i>botrytis</i>   | 3          |
| Chinese kale          | <i>B. oleracea</i> var. <i>alboglabra</i> | 8          |
| (Curly) Kale          | <i>B. oleracea</i> var. <i>acephala</i>   | 5          |
| Landrace              | /                                         | 1          |
| <b>Total</b>          |                                           | <b>102</b> |

Table 1 Overview of *B. oleracea* lines used for the development of broccoli panels.

### Probe Further Selection and Verification

The preliminary panel of **62,533** probes is synthesized and evaluated using **12 *Brassica oleracea* test samples** (10 cultivated varieties— purple broccoli, cauliflower, orange cauliflower, cabbage, Romanesco, Chinese kale, kale, kohlrabi, 2 broccolis and 2 wild accessions). For 62,533 SNP targets, SNP calling rate ranges from 94.51% ~98.8% among these samples, with the 99.3% accuracy on genotypes. Target regions with high genotyping uniformity and strong polymorphism are prioritized, while ensuring even distribution across all chromosomes. Following this optimization, a total of highly polymorphic **40,399 target regions** with low missing rate and low heterozygosity, are finally selected for liquid-phase GenoBaits panel production (Figure 1).

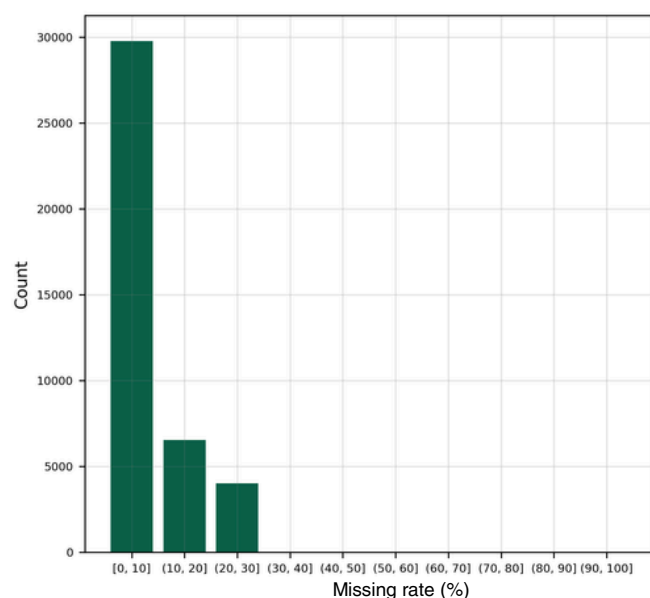
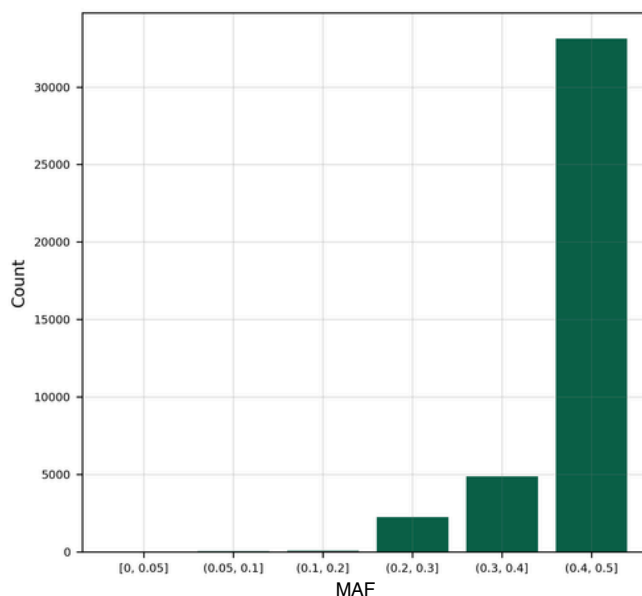
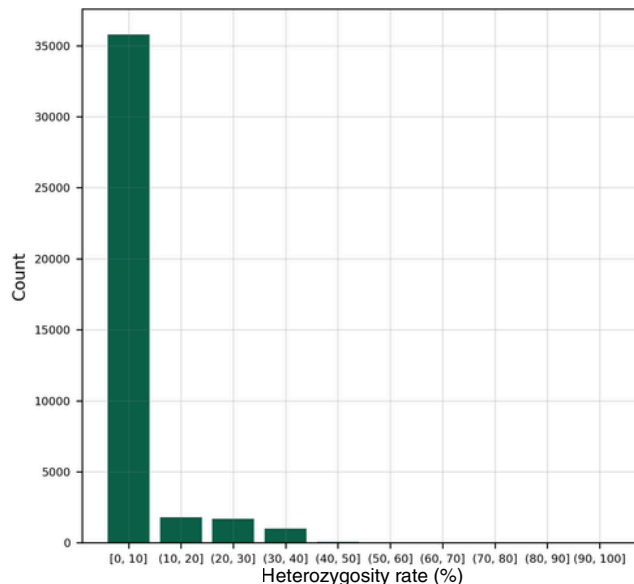


Figure 1. Overview of SNP quality Metrics: SNP Missing Rate, Heterozygosity, and MAF



“ Harnessing SNPs in and around key genes, the panel empowers insight for precise trait breeding in *B. oleracea* ”

### Key Traits in *Brassica oleracea*.

*Brassica oleracea* is one of the most economically important vegetable crops worldwide. Through domestication and selection, this single species has diversified into approximately ten widely cultivated morphotypes, including head cabbage, broccoli, cauliflower, kale, kohlrabi, Brussels sprouts, etc. These morphotypes exhibit remarkable variation in harvested organs, developmental timing, and quality attributes (Quiros & Farnham, 2011).

Breeding objectives vary substantially among morphotypes and production regions worldwide, with a strong emphasis on both morphology and quality. For leafy head types such as cabbage, breeders prioritize stable head formation, delayed bolting, and winter hardiness, whereas inflorescence crops such as broccoli and cauliflower require synchronized floral transition, curd development, and optimized flowering time. In contrast, kale breeding focuses more on leaf texture, pigmentation, nutritional content, rather than reproductive traits. These divergent breeding targets highlight the complex trait-specific genetic regulation underlying morphotype differentiation within *B. oleracea*.

To dissect the genetic basis of this phenotypic diversity, extensive inter-variety and interspecific crossing experiments, together with molecular genetics researches, have been conducted. Enabling the identification of favorable alleles help introgression of desirable traits from donor *oleracea* morphotypes or related Brassica species into elite breeding lines.

At the genomic level, *B. oleracea* differs from its close relative and model plant *Arabidopsis thaliana*. Its genome is characterized by high gene redundancy, extensive subgenome fractionation, frequent chromosomal rearrangements, and a high proportion of repetitive elements. These features are a consequence of a lineage-specific whole-genome triplication event followed by extensive gene loss and chromosomal reshuffling. Although many duplicated genes have lost during diploidization, gene families involved in metabolism, photosynthesis, development, and growth regulation are preferentially retained, with subsequent divergence in gene function or expression patterns (S. Liu et al., 2014). As a result, many traits in *B. oleracea* are governed by multiple paralogous loci rather than single gene. Functional divergence, dosage effects, and differential expression among retained gene copies collectively contribute to quantitative trait variation.

To maximize the practical utility of MolBreeding Broccoli Panels for breeding and genetic analysis, we systematically review genes associated with agronomic traits, morphotype, quality, abiotic stress tolerance, and biotic resistance. Below, we provide a brief overview of genes with overlapping SNPs included in the 40K broccoli panel (Table 2, Table 3).

## Agronomic Traits

### Fertility Utilization in *Brassica oleracea* Breeding and Cultivation

**Fertility control** is a cornerstone of hybrid seed production and breeding in *Brassica oleracea*. Male sterility systems enable efficient exploitation of heterosis by eliminating the need for manual emasculation and ensuring high purity of hybrid seed, while flowering time regulation is critical for adaptation to production environments and crop scheduling.

At the nuclear level, genic male sterility (**GMS**) arises from mutations that impair anther or pollen development and has been effectively applied in breeding. The recessive *ms3* locus has been fine-mapped, with **BoTPD1** proposed as the causal gene; loss of function disrupts tapetum development and pollen formation, and an insertion-based molecular marker enables precise identification of male-sterile plants (Han et al., 2018). Spontaneous male-sterile lines such as 83121A harbor an LTR retrotransposon insertion in **BoCYP704B1**, leading to defective sporopollenin synthesis and abnormal pollen walls, with linked markers facilitating efficient selection (Ji et al., 2017).

At the cytoplasmic level, cytoplasmic male sterility (**CMS**) is governed by mitochondrial genome variation rather than nuclear SNPs. The Ogura CMS system, originally derived from radish, confers stable male sterility in *B. oleracea* and is restored by the nuclear *Rfo* gene introgressed through interspecific hybridization (W. Ren et al., 2022; Shu et al., 2016; Yu et al., 2020). The targeted cytoplasm-specific markers or organellar genome sequencing are could be designed for CMS identification and breeding applications.

| Agronomic Traits                              | Genes               | # Associated Markers |
|-----------------------------------------------|---------------------|----------------------|
| Fertility                                     | <i>C09_53909499</i> | 1                    |
| Genic male sterility                          | <i>BoCYP704B1</i>   | 2                    |
|                                               | <i>ms3 (BoTPD1)</i> | 1                    |
| Chloroplast development                       | <i>BoAP1</i>        | 3                    |
| Flowering<br>Fruit width<br>Helix fruit shape | <i>BoCAL</i>        | 2                    |
|                                               | <i>BoFLC1</i>       | 3                    |
|                                               | <i>BoFLC2</i>       | 1                    |
|                                               | <i>BoFLC3</i>       | 3                    |
|                                               | <i>BoFUL-d</i>      | 6                    |
|                                               | <i>BoLFY</i>        | 1                    |
|                                               | <i>BoREM1</i>       | 1                    |
|                                               | <i>BoSEP2-1</i>     | 2                    |
|                                               | <i>BoSEP2-2</i>     | 3                    |
|                                               | <i>BoSOC1.2</i>     | 1                    |
|                                               | <i>BoTFL1</i>       | 2                    |
|                                               | <i>BoVRN1</i>       | 2                    |
|                                               | <i>BoVRN2</i>       | 1                    |
|                                               | <i>FRI (BoFRIa)</i> | 2                    |
|                                               | <i>FRI (BoFRIb)</i> | 11                   |
|                                               | <i>FT</i>           | 10                   |
|                                               | <i>SOC1</i>         | 2                    |

Table 2 Key agronomic traits (Reproductive & Flowering) in *Brassica oleracea*.

Together, CMS provides stable male sterility for large-scale F<sub>1</sub> hybrid production, while GMS are more preferred for flexible, gene-based sterility during line development and early-stage breeding, and together they complement each other to maximize hybrid purity and breeding efficiency.



### Flowering Time Regulation

Flowering time is a vital agronomic trait in *B. oleracea*, directly affecting seasonal adaptation, yield stability, and crop scheduling across production systems. The transition from vegetative growth to flowering in *Brassica oleracea* is controlled by a conserved regulatory network that integrates environmental and developmental signals. Central to this network are **FLC homologs**, floral repressors (Maple et al., 2024).

Multiple *BoFLC* paralogs have been retained following whole genome triplication, and variation at these loci contributes substantially to flowering time diversity. QTL and association studies repeatedly identify ***BoFLC.C2*** as a major locus influencing vernalization response and flowering time variation in broccoli, and allelic differences at ***BoFLC2*** have been linked to differences in vernalization sensitivity among morphotypes (Q. Li et al., 2022; Okazaki et al., 2007; Woodhouse et al., 2021). Variation in ***BoFLC3*** expression and sequence also contributes to flowering differences in certain genetic backgrounds, such as in cauliflower and subtropical broccoli germplasm, where non vernalization pathways may also operate (Guo et al., 2024; Lin et al., 2018). Like in *Arabidopsis*, vernalization promotes flowering by epigenetically silencing *BoFLC* through chromatin modification involving regulators homologous to ***VRN1*** and ***VRN2***, which establish persistent repression after cold exposure, thereby enabling the activation of downstream integrator genes such as ***SOC1*** and ***FT*** (Hong et al., 2021; Ridge et al., 2015; Vos et al., 2022). Beyond temperature cues, flowering time in *B. oleracea* is also influenced by photoperiod and plant age pathways, with circadian regulators and integrators modulating developmental timing in response to day length and growth stage (N. Li et al., 2024). The integration of these multiple pathways underlies the broad flowering variation observed among cultivars and provides multiple targets for breeding adaptation across environments.

Manipulating flowering regulators help breeders to tailor cultivars for seasonal production, shorten generation cycles, and expand environmental adaptation. Combined with male sterility systems for efficient hybrid seed production, precise flowering control enhances heterosis utilization and improves yield, quality, and stress resilience in commercial *B. oleracea* breeding programs.

### Quality and appearance in *B. oleracea*

**Leafy heading formation** is a defining central selection target of cabbages and other heading *B. oleracea* morphotypes, directly influencing market value, yield, and consumer preference. The process involves tightly rolled or layered leaves forming a compact head, a trait shaped by complex interactions of plant architecture, leaf morphology, and growth regulators. that heading is controlled by multiple quantitative trait loci (QTLs) and key regulatory genes. For example, ***BoKAN1*** gene as instrumental in shaping the leafy heads of cabbage and Brussels sprouts (Guo et al., 2024). ***BoBRX***, ***BoCLAVATA3 (BoCLV3)***, ***BoAP2-like transcription factors***, and auxin response factor (***ARF***) genes are one of central components influence meristem activity and leaf curvature, and epigenetic regulation and gene expression timing also play critical roles in head initiation and development.

**Leaf margin shape** is a highly valued ornamental trait in kale. ***BoFeL (BoLI)*** is the first candidate gene on C09 identified to confer the feathered-leaved trait (J. Ren et al., 2019). ***BoALG10*** has been later identified as responsible for maintaining the smooth leaf margin, and loss of its function disrupts the N-glycan biosynthesis pathway, resulting in an irregular feathered leaf shape (Feng et al., 2022)

In addition, other key appearance traits in *B. oleracea* have been actively studied for their impact on yield and marketability.

| Agronomic Traits                       | Genes                                        | # Associated Markers |
|----------------------------------------|----------------------------------------------|----------------------|
| Leafy heading formation                | <i>ARF3</i>                                  | 2                    |
|                                        | <i>ARF4</i>                                  | 2                    |
|                                        | <i>BoBRX</i>                                 | 2                    |
|                                        | <i>BoKAN1</i>                                | 4                    |
| Head whiteness                         | <i>C09_62541915</i>                          | 1                    |
| Flower head color (orange cauliflower) | <i>Or</i>                                    | 7                    |
| Organ size                             | <i>BoCDAG1</i>                               | 4                    |
| Leaf lobe formation                    | <i>BoALG10</i>                               | 1                    |
|                                        | <i>BoFeL</i>                                 | 2                    |
| Stem hollowness                        | <i>C09_61034396</i> ,<br><i>C09_61036417</i> | 2                    |
| Petal color                            | <i>BoCCD4</i>                                | 58                   |
| Leaf color (purple)                    | <i>BoMYBL2-1 (cabbage)</i>                   | 1                    |
|                                        | <i>BoPAP1 (chinese kale)</i>                 | 40                   |
|                                        | <i>BoPr (BoMYB2) (kale)</i>                  | 18                   |
| Hypocotyl color (purple)               | <i>BoTT8</i>                                 | 11                   |

Table 3 Key agronomic traits (Morphology & Quality) in *Brassica oleracea*.

For example, **CDAG1** regulates organ size (e.g. size of flower head) and biomass by controlling cell number and size, likely via ethylene signaling, making it a promising target to be modified for yield improvement (H. Li et al., 2017). Another interesting trait is **stem hollowness** in broccoli, which is genetically complex and sensitive to environmental conditions. Multiple loci contribute to this phenotype, with *QHS.C09-2* identified as a major QTL explaining ~15.6% of variation and presumed to be the primary determinant of hollow stems (Yu et al., 2019).

**Pigmentation** breeding in *Brassica oleracea* is driven by consumer preferences on attracting colors and nutritional values, with carotenoid- and anthocyanin-based coloration controlled by key genes. The **BoOr (Orange)** gene in cauliflower is a gain-of-function allele that promotes chloroplast differentiation and  $\beta$ -carotene accumulation, enhancing orange pigmentation without altering upstream biosynthesis (Lu et al., 2006). Anthocyanin pigmentation is regulated by the MBW complex composed of R2R3-MYB, bHLH, and WD40 proteins, which activate key structural genes in the flavonoid-based pigment (anthocyanin) biosynthesis pathway in most plants. In *B. oleracea*, variations in these regulators drive purple pigmentation, with **MYBL2-1** reducing **MYBL2** expression and enhancing anthocyanin accumulation in purple cabbage (Khusnutdinov et al., 2022). Similarly, **BoTT8** contributes to purple hypocotyls, while **BoPAP1** and **BoPr (BoMYB2)** influence purple leaf color in kale and Chinese kale (X. Liu et al., 2017; Rahim et al., 2019; B. Zhang et al., 2012).



**Glucosinolates (GSLs)**, a group of sulfur- and nitrogen-containing secondary metabolites, are key secondary metabolites in broccoli that strongly influence bitterness and overall eating quality, contributing plants own defense system against insect and pathogen. Consumption of GSLs and their breakdown products in *B. oleracea* vegetables has been associated with reduced cancer incidence, making GSL composition an important target for quality-oriented breeding (Sarikamış, 2009; N.-Q. Zhang et al., 2018).

Over 200 kinds of GSLs have been identified, classified into 1) aliphatic GSLs; 2) indole GSLs; 3) aromatic GSLs based on their structure and derived amino acid precursors (Brian Clarke, 2010). In broccoli, GSLs are synthesized through a multi-step enzymatic pathway involving amino acid side-chain elongation, core structure formation, and diverse secondary modifications regulated by multiple enzymes and transcription factors (Miao et al., 2017). Around 100 GSLs biosynthesis genes have been identified in *B. oleracea* (S. Liu et al., 2014; Yi et al., 2015), and natural variation in GSL profiles between *B. rapa* and *B. oleracea* is largely attributed to duplication of **MAM** (methylthioalkylmalate synthase) and **AOP** (2-oxoglutarate-dependent dioxygenase) genes (S. Liu et al., 2014). Two functional AOP2 copies **BoAOP2.1** (BoGSL-ALKa) and **BoAOP2.2** (BoGSL-ALKb) in broccoli promotes the accumulation of sulforaphane. over-expression of the gene AOP2 leads to a peak level of sulforaphane content in organs of broccoli (Z. Li et al., 2014).

Other key genes like, **FMOGS-OX** loci encode flavin-monooxygenases that catalyze the S-oxygenation of methylthioalkyl GSLs into methylsulfinylalkyl forms, thereby increasing beneficial aliphatic GSLs (e.g. glucoraphanin) ; **GSL-OH**, which encodes 2-oxoacid-dependent dioxygenase, modifies GSL side chains and shifts the GSL profile toward more bitter compounds (Robin et al., 2016); and higher **ST5b** (sulfotransferase) expression strongly associate with increased aliphatic glucobrassicin, particularly during pathogen-induced defense responses in *B. oleracea* (Abuyusuf et al., 2018).

**Leaf waxiness** affects both organ appearance and market quality, and also help plants cope with biotic and abiotic stresses by the protective cuticular barrier against water loss, extreme weather, and insect damage. Waxiness is primarily controlled by genes involved in cuticular wax biosynthesis, modification, transport, and regulation. **Cgl1** confers the glossy leaf

| Agronomic Traits                   | Genes                                         | # Associated Markers |
|------------------------------------|-----------------------------------------------|----------------------|
| Glucosinolate content (bitterness) | <i>BoIAP2</i> (BoGSL-ALKa)                    | 2                    |
|                                    | <i>BoIAP2</i> (BoGSL-ALKb)                    | 1                    |
|                                    | <i>FMOGS-OX5</i>                              | 5                    |
|                                    | <i>GSL-OH</i>                                 | 3                    |
|                                    | <i>ST5b</i>                                   | 1                    |
|                                    | <i>C02_3703070, C07_49784510, C09_4860469</i> | 3                    |
|                                    | <i>C03_5128702</i> ( <i>Gluconapin</i> )      | 1                    |
| Leaf waxiness                      | <i>CGL-1</i>                                  | 11                   |
|                                    | <i>BoCER2</i>                                 | 3                    |
|                                    | <i>BoGL1</i>                                  | 1                    |
|                                    | <i>Cgl2</i> ( <i>BoWax1</i> )                 | 3                    |

Table 3 Key agronomic traits (Morphology & Quality) in *Brassica oleracea*.

Other specialized pigment-related loci include **CCD4**, which influences yellow petal color through carotenoid cleavage activity (Han et al., 2019). **BoAP1**, where a recessive mutation disrupts chloroplast development, leading to a lethal albino phenotype (Zhao et al., 2024).

phenotype in cabbage by controlling epicuticular wax accumulation (Z. Liu et al., 2017). Loss of function of three very-long-chain fatty acid (VLCFA) modification gene homologs—**Cgl2** (CER4-like), **BoGL5** (BoCER2), and **BoGL1** (CER1)—leads to wax-defective, glossy phenotypes in *B. oleracea* (Han et al., 2021; D. Liu et al., 2017a, 2017b). A R2R3-MYB transcription factor BoGL-3 likely acts upstream by regulating the expression of wax-related biosynthetic genes (Dong et al., 2019).

## Abiotic resistance

Abiotic stress tolerance in *Brassica oleracea* is controlled by diverse gene families that regulate stress perception, signaling, and protective responses. Large transcription factor families such as bHLH, CBF/DREB, WRKY, NAC, MYB, and AP2/ERF play key roles in responses to cold, heat, drought, and salinity, making them important targets for breeding and genetic improvement (Kayum et al., 2015; Raza et al., 2025; Thamilarasan et al., 2014; Wang et al., 2018). For example, over 200 *bHLH* genes in *B. oleracea* are identified and dozens of them significantly up/down-regulated under various stresses, including cold, heat, drought, and salinity (Raza et al., 2025). Freezing tolerance in cabbage is influenced by **BoCCA1**, a core circadian clock gene that enhances cold resilience via the CBF pathway; and **BoCDSP5**, whose allelic variation confers low-temperature resistance independently of CBF (Song et al., 2018, 2020).

**BoHsp70**, a heat shock protein, and the GRAS family transcription factor **BoGRAS** (*SCL13*), which is strongly upregulated in heat-tolerant lines at early developmental stages and may serve as a seedling stage selection marker (Park et al., 2013). **BoWS**, contributes to early stress perception and hormone mediated signaling to maintain water use efficiency under water stress condition (Z. Li et al., 2004).

## Biotic resistance

*B. oleracea* is vulnerable to several major diseases, including Fusarium wilt, clubroot, soft rot, and black rot. (Kayum et al., 2016). For **clubroot resistance**, no major CR genes or highly resistant lines have yet been identified in *B. oleracea* (Mehraj et al., 2020). However, two QTL regions—*qCRc7* and *Rcr7*—have been mapped from resistant cabbage cultivars, cv. GZ87 and cvs. Tekila and Kilaherb, respectively. *qCRc7* confers resistance to *Plasmodiophora brassicae* race 4, while *Rcr7* provides resistance to pathotypes 3 and 5X (Ce et al., 2021; Dakouri et al., 2018). **FOC1** gene, confers dominant **Fusarium wilt resistance** by encoding a TIR-NBS-LRR type R gene (Lv et al., 2014; Shimizu et al., 2015). **BoDMR2** has been associated with adult-stage **resistance to downy mildew** in cabbage (Wu et al., 2024). Eight additional SNPs related to **black rot resistance** on C01, C02, C04, C05 are provided by ZAAS.

## Trait-Associated Markers

SNPs located within functional gene regions, as well as within  $\pm 7$  kb flanking regions, are annotated as trait-associated markers. These markers represent genotypic variation in and near genes of interest, providing a resource for downstream phenotype–genotype association analyses (Figure 2). The **MolBreeding Broccoli Panel** acts as a genotyping tool to assess genetic diversity and support trait-informed marker selection. While the status of these SNPs may not directly indicate a trait or its resistance, they, or their combined haplotypes, are often associated with specific traits.

While these markers suggest associations, they do not guarantee causation. Functional relevance of individual markers needs to be evaluated by users in combination with their own phenotypic data. Upon validation of key loci, new molecular functional markers can be developed for routine application in marker-assisted selection and breeding programs.

Beyond SNP-level variation, over 500K structural variations (SVs) have been identified in *B. oleracea* pangenome. These SVs, including presence/absence variations (PAVs, one kind SV) affecting approximately 20% of gene, are not detectable using conventional SNP panels (Golicz et al., 2016; Guo et al., 2021; X. Li et al., 2024). Despite the presence of SNPs associated with these genes being found, the impact of SVs on gene regulation and phenotype divergence cannot be ignored (Figure 2). For example, promoter SVs introduced by transposable elements have been shown to upregulate **BoKAN1** (X. Li et al., 2024). Tandem duplication variation of **BoFLC** paralogs generate structural and expression diversity—such as duplicated *BoFLC1* copies enhancing floral repression; deletion/PAV of *BoFLC2* promoting early flowering (Kinoshita et al., 2023). Validation of individual SVs relies on targeted PCR assays; **to enable genome-scale screening**, customized SV-specific marker panels can provide an efficient solution for high-throughput characterization of structural diversity.

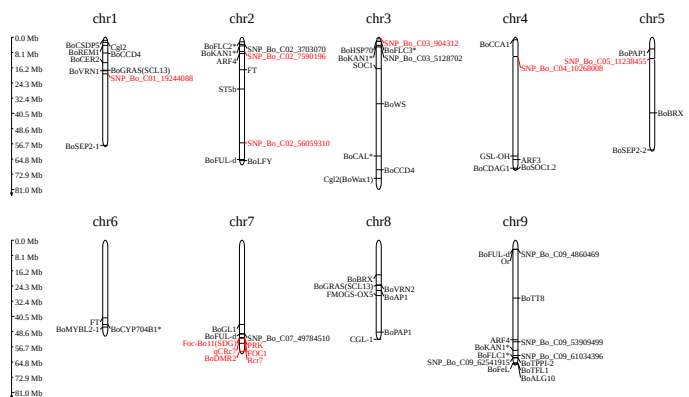


Figure 2 Genomic distribution of SNP markers associated with functional genes and QTL regions across nine *B. oleracea* chromosomes in the GenoBaits 40K Broccoli Panel. (The asterisk “\*” denotes that the gene whose expression is affected by known structural variations (Guo et al., 2021; Kinoshita et al., 2023; Thamilarasan et al., 2014).)

## Ready-to-Use Broccoli GBTS Panels

Three GenoBaits broccoli panels, with marker densities of 10K, 20K, and 40K, are developed based on the reference genome of *B. oleracea* var. *italica* 'B58-1' and include both trait-associated (foreground) and background SNPs. The 10K and 20K panels consist of evenly distributed subsets of the 40K panel (Tables 4; Figures 3 & 4). SNPs are generally evenly distributed across the genome, but noticeable gaps occur near centromeric regions, especially in the 10K and 20K panels, because the suppressed recombination, high repeat content, low gene density, and selective constraints of centromeres result in fewer SNPs that could be selected around centromeric regions.

| Technology | Product name                  | Core SNP | Trait | Trait Marker |
|------------|-------------------------------|----------|-------|--------------|
| GenoBaits® | GenoBaits® Broccoli 40K Panel | 38115    | 22    | 452          |
| GenoBaits® | GenoBaits® Broccoli 20K Panel | 19484    | 21    | 398          |
| GenoBaits® | GenoBaits® Broccoli 10K Panel | 10377    | 19    | 373          |

Reference Genome: *B. oleracea* var. *italica* 'B58-1'

Table 4 Overview of three ready-to-use GenoBaits broccoli panels

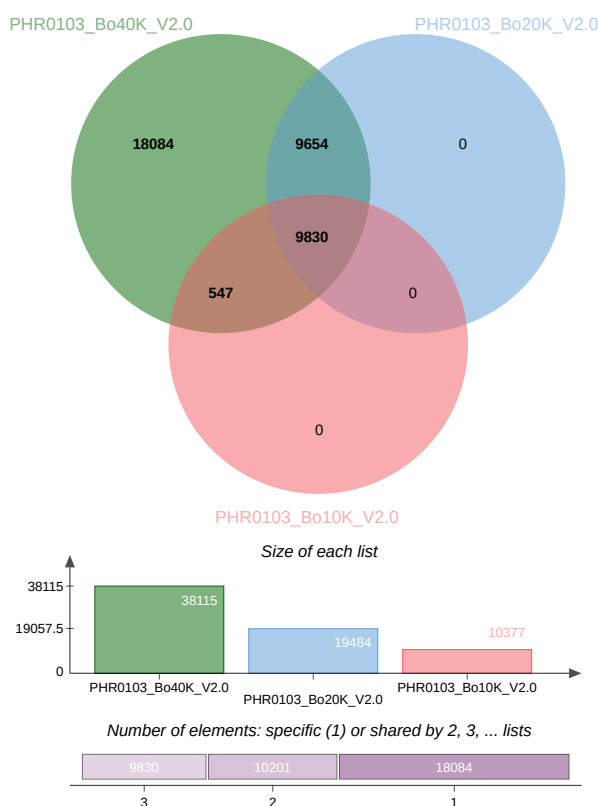


Figure 3 Venn diagram illustrating the overlap of SNP markers among three broccoli panels.



Figure 4 Distribution of SNPs in three broccoli panels: A.GenoBaits broccoli 40K, B.20K, C.10K panels)

### GenoBaits Broccoli 40K Panel

The 40K panel offers high resolution, featuring 38,115 target SNPs along with additional approx. 230K mSNPs (flanking SNPs identified on fragments captured by probes). This panel includes 452 trait-associated markers that cover 22 traits. The following metrics summarize the actual performance data from recent genotyping projects:

- Alignment Rate: 91.74%
- Coverage Uniformity (20%)\*: 94.60 %
- Mean Depth of Reads: 46.8
- Target SNP Call Rate: 97.09%

## GenoBaits Broccoli 20K Panel

The 20K panel has 19484 target SNPs and additional approx. 11K mSNPs, with 398 trait-associated markers spanning 21 traits. Project application data are being accumulated, and performance results will be reported as they become available.

## GenoBaits Broccoli 10K Panel

The 10K panel includes 10377 target SNPs and approx. 5.8K mSNPs, with 373 target markers associated with 19 traits. Recent projects utilizing this panel have shown the following average performance:

- Alignment Rate: 91.20%
- Coverage Uniformity (20%)\*: 92.92 %
- Mean Depth of Reads: 100.39
- Target SNP Call Rate: 96.95%

\***Coverage Uniformity** refers to the percentage of SNP loci whose sequencing depth is at least 20% of the average sequencing depth across all SNPs in the panel.

## Phylogenetic Trees & PCA analysis using 40K broccoli panel

The 40K Broccoli panel's robustness and accuracy are further validated through phylogenetic tree reconstruction and PCA analysis, demonstrating that the selected SNPs effectively capture the genetic relationships among *Brassica oleracea* cultivars and landraces. To assess this, forty-three publicly-available sequencing datasets representing four *B. oleracea* varieties are retrieved from NCBI (Project PRJNA1047966, Table 5),

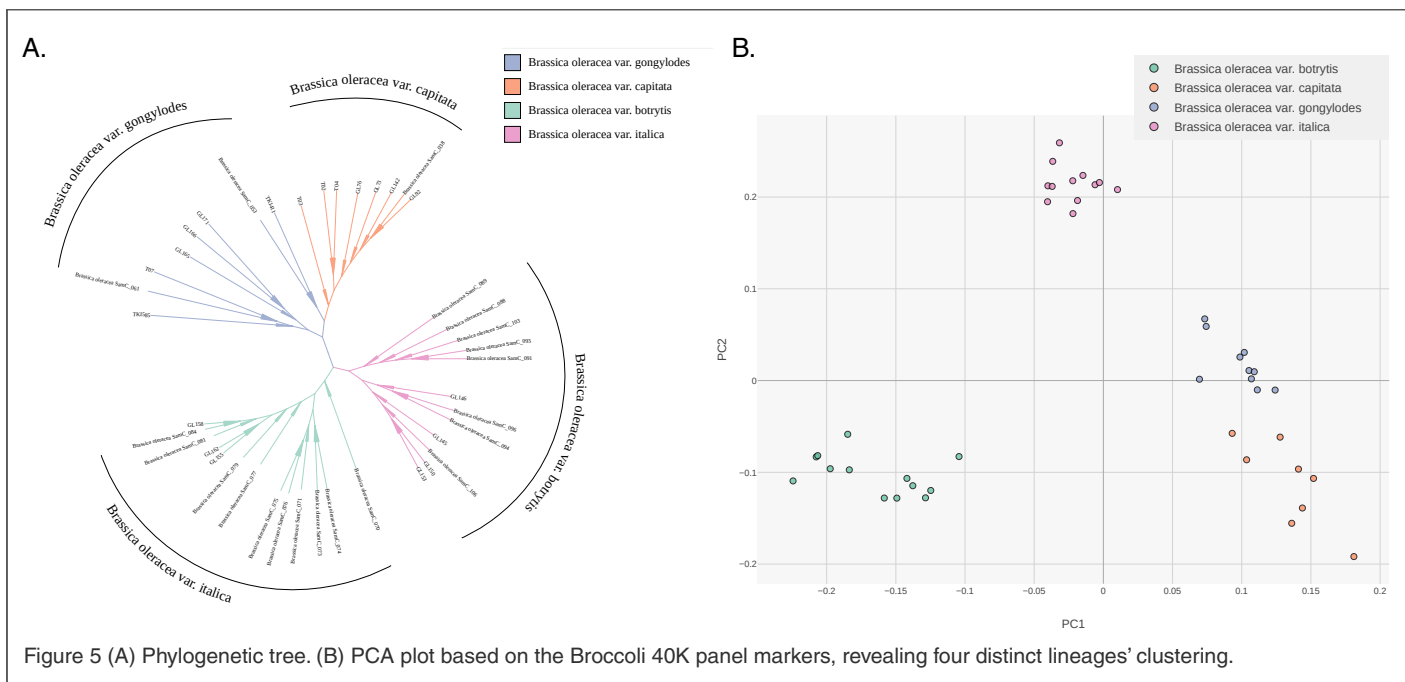
including 13 var. *botrytis*, 8 var. *capitata*, 10 var. *gongylodes*, and 12 var. *italica*.

Compared to the high SNP call rate achieved in our in-house analyses using the broccoli 40K marker panel, the public resequencing datasets contained a relatively higher proportion of SNPs that are missing or could not be directly called out. As a result, the resolution of both the phylogenetic tree and PCA plots is moderate; fine-scale evolutionary relationships among accessions are less clearly defined.

Nevertheless, both analyses still clearly reveal the four major clusters corresponding to the four *B. oleracea* varieties. PCA plots recapitulate the closer genetic relationship between var. *italica* and var. *botrytis* as their more recent divergence compare to var. *capitata* and var. *gongylodes* (Figure 5). Even under conditions of incomplete SNP data, the 40K SNP panel captures the overall population structure and relative genetic distances among varieties, highlighting its robustness for genotyping and diversity analyses.

| Varieties                                       | Number    |
|-------------------------------------------------|-----------|
| <i>Brassica oleracea</i> var. <i>botrytis</i>   | 13        |
| <i>Brassica oleracea</i> var. <i>capitata</i>   | 8         |
| <i>Brassica oleracea</i> var. <i>gongylodes</i> | 10        |
| <i>Brassica oleracea</i> var. <i>italica</i>    | 12        |
| <b>Total</b>                                    | <b>43</b> |

Table 5 Summary of *B. oleracea* accessions used for PCA analysis and phylogenetic tree reconstruction.



## Conclusion

MolBreeding's Broccoli Panels are designed for modern molecular breeding programs, featuring high-density probes that have been efficiently captured and rigorously validated across diverse *Brassica oleracea* varieties. This panel offers broad versatility, applicable not only to broccoli but also to Brussels sprouts, cauliflower, kale, kohlrabi, cabbage, and related C-genome *Brassica* vegetables. These three high-density broccoli SNP panels support a wide range of applications—including GWAS, precise variety identification, exploration of elite traits in wild accessions, and data-driven breeding decisions—thereby accelerating the development of superior cultivars.

## Call to Action

We invite the *Brassica* researchers and industry community to collaborate with us in utilizing GenoBaits Broccoli Panels to accelerate breeding and genetic discovery. Through this collaboration, we aim to unlock more potential of *brassica* genetics in modern breeding programs, enabling the development of superior cultivars with enhanced yield, disease resistance, and flavor quality, and shaping a more innovative and sustainable future for *B. oleracea* cultivation.

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## **MolBreeding Biotechnology | [cs@molbreeding.com](mailto:cs@molbreeding.com)**

**Americas** | 10 Pasteur, Suite 150, Irvine, CA 92618 United States | +1-515-508-9174

**EMEA** | Unit 10, West Point Business Park, West Road, Harlow, Essex, CM20 2BU United Kingdom | +44 (0) 1279 940 983

**Asia** | 538B, 5/F, Building 1W, 1 Science Park W Ave, Science Park, Pak Shek Kok, Hong Kong SAR, China | + (852) 5130-1856