

Review

Systems-Level Integration of Stress Signaling, Multi-Omics, and Predictive Breeding for Abiotic Stress Tolerance in *Brassica* Crops

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Highlights

What are the main findings?

- Abiotic stress tolerance in *Brassica* crops is governed by multilayered regulatory networks integrating stress perception, osmotic adjustment, ROS homeostasis, and photosynthetic adaptation.
- Allopolyploidy drives regulatory innovation through gene dosage effects, subgenome specialization, homoeologous gene divergence, and alternative splicing.

What are the implications of the main findings?

- Integration of multi-omics data with genomic selection and genome editing enables predictive breeding for complex stress-resilience traits.
- Combined-stress models, microbiome-assisted strategies, and wild allele mining are essential for developing climate-resilient *Brassica* cultivars.

Abstract

Climate change is increasing the frequency and severity of abiotic stresses, including drought, salinity, waterlogging, and temperature extremes, thereby threatening the productivity and quality of *Brassica* crops. This review synthesizes recent progress in abiotic stress tolerance from physiological, genetic, epigenetic, and multi-omics perspectives, with an emphasis on how mechanistic discoveries can be translated into breeding decisions. We first outline the signaling hierarchy that links stress perception at the plasma membrane and cell wall interface to Ca²⁺ signaling, MAPK cascades, hormone crosstalk, osmotic adjustment, ROS homeostasis, and metabolic reprogramming. We then examine the genetic architecture of stress tolerance through QTL mapping, GWAS, and functional genomics, highlighting how allopolyploidy, subgenome specialization, homoeologous gene divergence, and alternative splicing create both opportunities and complications for *Brassica* improvement. We further evaluate how transcriptomic, epigenomic, metabolomic, and microbiome-related data are revealing regulatory complexity but remain underused for prediction and causal inference. Major bottlenecks include the inefficient conversion of association signals into validated functional markers, the descriptive rather than predictive use of multi-omics datasets, limited mechanistic understanding of combined stresses, and insufficient field validation across genetic backgrounds. Finally, we discuss integrated breeding strategies, including marker-assisted selection, genomic selection, genome editing, wild germplasm utilization, microbiome-assisted approaches, and synthetic biology. By



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connecting stress biology with translational breeding, this review provides a framework for developing climate-resilient *Brassica* cultivars.

Keywords: *Brassica* crops; abiotic stress tolerance; stress regulatory networks; allopolyploidy; multi-omics; predictive breeding

1. Introduction

Climate change is intensifying abiotic stresses such as drought, salinity, waterlogging, and extreme temperatures, creating major constraints on agricultural productivity [1–4]. In *Brassica* crops, stress timing is particularly important because exposure before or during flowering causes the largest losses in both yield and quality [5]. Stress can also reduce germination, impair seedling establishment, disrupt vegetative growth, and compromise final productivity and market quality [6–8]. Historical analyses indicate that abiotic stresses explain a large proportion of variation in productivity, and future heat-related damage is expected to exceed historical levels [9]. These observations underscore the need to move beyond descriptive stress physiology toward predictive and breeding-oriented frameworks.

Brassica crops are generally stress-sensitive, and conventional breeding is constrained by long selection cycles, complex genotype-by-environment ($G \times E$) interactions, and limited use of diverse genetic resources [2,10,11]. Recent advances have begun to overcome these limitations. Genome-wide association study (GWAS) has identified loci such as *BnaA8.MYB60* for cold tolerance in *Brassica napus* L. [12] and candidate genes associated with drought tolerance in *Brassica juncea* (L.) Czern. [13], while genome-wide analyses of stress-responsive gene families, including *CAT*, *POD*, *SnRK*, *CHYR*, and *R2R3-MYB*, have expanded the molecular toolbox for stress improvement [14–18]. Multi-omics studies have also provided mechanistic insights into heat tolerance in pakchoi [19] and salt tolerance in rapeseed [11]. Nevertheless, three bottlenecks remain unresolved. First, GWAS signals are still inefficiently converted into functional breeding markers because many significant SNPs are non-coding and their biological relevance is often inferred from linkage disequilibrium rather than validated causality. Second, transcriptomic, proteomic, and metabolomic datasets are frequently used as descriptive lists of differentially expressed genes, proteins, or metabolites rather than as inputs for predictive models [10]. Third, the literature remains biased toward drought and salinity, whereas temperature extremes, waterlogging, heavy metal toxicity, and multifactorial stress combinations are less systematically studied. A critical synthesis that links physiological traits, molecular mechanisms, statistical associations, and breeding decisions is therefore needed.

Previous reviews have often emphasized individual stress types, single omics layers, or specific breeding tools. In contrast, this review integrates physiological mechanisms, genetic architecture, epigenetic regulation, multi-omics interpretation, and breeding applications within a unified systems-level framework, with particular attention to the allopolyploid nature of *Brassica* crops. The review covers stress perception, signal transduction, osmotic regulation, ROS control, photosynthetic protection, and metabolic adaptation, while also highlighting emerging regulatory layers such as DNA methylation, histone modification, RNA methylation, alternative splicing (AS), and small RNAs. It further discusses genome editing and functional validation as translational tools. Overall, the review aims to bridge mechanistic stress biology with precision breeding strategies for stress-resilient *Brassica* improvement.

2. From Stress Perception to Metabolic Adaptation: Regulatory Networks in Brassica Crops

2.1. Multilayered Signaling Networks in Stress Perception and Transduction

Stress perception in *Brassica* crops begins at the plasma membrane–cell wall interface, where receptor-like kinases and proteins (RLKs/RLPs) contribute to the early recognition of osmotic, ionic, and mechanical perturbations (Figure 1) [20–22]. Although *LYK4* overexpression in *B. juncea* enhances tolerance to *Alternaria brassicicola* through MAPK activation [20], its direct role in abiotic stress tolerance remains unresolved and should be interpreted mainly as evidence for potential biotic-abiotic signaling crosstalk. Genome-wide surveys have identified 96 wall-associated kinase (WAK) genes in Chinese cabbage and 493 *RLK* and 228 *RLP* genes in *B. juncea*, providing valuable resources for dissecting membrane-based stress perception [21,22]. WAKs are likely to contribute to cell wall integrity sensing under osmotic stress, whereas *LYK*-family members may participate in crosstalk between pathogen-associated and abiotic signals. However, most *Brassica* *RLK*/*RLP* candidates still lack direct functional validation under abiotic stress. Therefore, expression responsiveness, although useful for candidate prioritization, should not be equated with causal involvement.

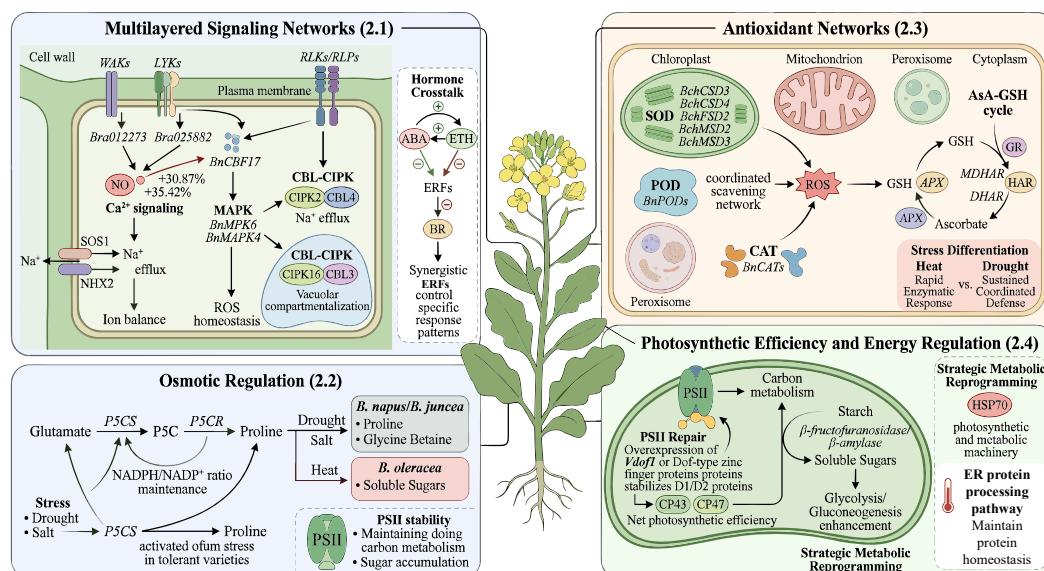


Figure 1. Multilayered regulatory networks of abiotic stress responses in *Brassica* crops. Section 2.1 summarizes stress perception and signal transduction at the plasma membrane–cell wall interface, including RLKs/RLPs, Ca²⁺ signaling, MAPK cascades, ion transport, and hormone crosstalk. Section 2.2 highlights osmotic regulation through proline, soluble sugars, betaine, and other osmoprotective metabolites. Section 2.3 presents enzymatic and non-enzymatic antioxidant systems, including SOD, POD, CAT, and the AsA-GSH cycle, which coordinate ROS scavenging across subcellular compartments. Section 2.4 illustrates photosynthetic protection, carbon metabolism reprogramming, and protein homeostasis as integrated components of energy allocation under stress.

Calcium signaling is a central second-messenger system in stress transduction. In winter rapeseed, melatonin-induced cold tolerance involves NO- and Ca²⁺-dependent activation of MAPK cascades, with melatonin pretreatment increasing NO content and intracellular Ca²⁺ concentration [23]. These data support a model in which Ca²⁺ functions upstream of antioxidant activation and osmotic adjustment during melatonin-mediated cold acclimation. Under low temperature, the ICE1-CBF pathway regulates *COR* gene expression and MAPK phosphorylation, thereby improving membrane stability and osmotic regulation in *B. napus*; overexpression of *BnCBFs* further enhances freezing tolerance [24].

Under salinity, plasma membrane SOS1 promotes Na⁺ efflux, whereas CBL-CIPK modules regulate ion transport and vacuolar sequestration through targets such as NHX2 [25,26]. The BnaCIPK2-BnaCBL4 and BnaCIPK16-BnaCBL3 interactions support a modular model of ion signaling in *B. napus* [26]. Many core signaling components have been functionally validated, but complete perception-to-response cascades remain largely proposed models. However, translating these modules into field-ready breeding targets requires evaluation of pleiotropic effects, tissue specificity, and performance across genetic backgrounds.

MAPK cascades provide an additional layer of specificity and signal amplification. Under salt stress, mutation of BnMPK6 alters the expression of *SOS1* and *NHX1*, impairing K⁺/Na⁺ balance and disrupting ROS homeostasis in *B. napus* [27], whereas MAPK activation also participates in melatonin-induced cold tolerance downstream of NO and Ca²⁺ signaling [23]. Overexpression of *NtHSP17.6* in *B. napus* enhances tolerance to heat, drought, and salinity, and overexpression of *BrPP5.2* confers heat tolerance through chaperone-like activity and regulation of stress-responsive genes [28,29]. These observations indicate functional divergence among MAPK components: some modules primarily control ion and redox balance, whereas others coordinate broader protective responses. Future work should therefore distinguish pathway specificity from redundancy using loss-of-function, gain-of-function, and multiplex editing approaches.

Hormone crosstalk further increases the complexity of *Brassica* stress signaling. ABA is a central regulator of drought and salinity responses, but its effects are shaped by interactions with ethylene (ETH), salicylic acid (SA), jasmonic acid (JA), and brassinosteroids (BRs). ETH can activate ERF transcription factors (TFs) and modulate ion transport, potentially antagonizing ABA-mediated ion homeostasis under salinity [30,31]. SA influences ABA accumulation and stomatal behavior under drought, and exogenous SA can improve gas exchange and mineral ion balance under drought and salinity [32–34]. JA interacts with ABA and other hormones to enhance stress tolerance [32], while BR and JA treatments can improve osmotic adjustment and antioxidant capacity under drought in *B. rapa* [35]. Importantly, BR-ABA interactions are context-dependent: BR signaling may antagonize ABA to sustain growth under mild stress but cooperate with ABA-dependent defense programs under severe stress [35]. Because many hormone studies rely on exogenous applications, their breeding relevance should be validated through genetic, physiological, and field-based experiments in *Brassica* backgrounds. Most supporting evidence for these signaling cascades comes from controlled laboratory experiments; extrapolation to field conditions requires careful validation given more complex and variable environmental contexts.

2.2. Osmotic Regulation and Osmolyte Metabolism

Osmotic adjustment is a major component of abiotic stress tolerance in *Brassica* crops, with proline, glycine betaine, soluble sugars, and related metabolites contributing to cellular water retention and redox stability [2]. Proline is synthesized mainly through the glutamate pathway, in which Δ^1 -pyrroline-5-carboxylate synthetase (P5CS) converts glutamate to P5C and P5C reductase (P5CR) subsequently produces proline [36]. Beyond its osmotic role, proline can help stabilize the NADPH:NADP⁺ ratio during stress, thereby supporting chloroplast redox balance [36]. Exogenous proline application can enhance stress tolerance in *Brassica* crops [37], but many studies remain correlative or treatment-based. Direct genetic evidence, such as *Brassica*-specific knockout or allelic analyses of *P5CS* genes, is still limited compared with model species. Similarly, the majority of *BnaDofs* in *B. napus* were differentially regulated upon exposure to stress, which were associated with the functional terms ‘oxidation–reduction process’ and ‘response to oxidative stress’, suggesting a potential role in ROS-mediated signalling [38]. Glucosinolates and erucic acid have also been linked to drought resistance during seed germination through effects on sugar

metabolism, hormonal balance, and antioxidant responses [39]. These findings suggest that osmolytes should be viewed not only as compatible solutes but also as regulatory metabolites with potential signaling functions.

Osmolyte accumulation differs among *Brassica* species, developmental stages, and stress types, but current evidence should be interpreted cautiously because it is often based on limited genotype panels. Salt-tolerant *B. napus* genotypes tend to accumulate more proline under stress, with *P5CS* expression being upregulated [40]. Tolerant cauliflower genotypes also show higher proline accumulation and antioxidant enzyme activity than sensitive genotypes [37]. In *B. napus*, salt-tolerant varieties may maintain more efficient sucrose-starch conversion under salinity [40], and drought-tolerant *B. juncea* genotypes show increased proline levels together with enhanced SOD and POD activities [41]. Although vegetable *Brassica* crops are often reported to rely strongly on soluble sugars under heat stress, whereas oilseed *Brassica* species frequently accumulate proline and glycine betaine under drought or salinity, this pattern should not be generalized without broader comparative studies. The molecular basis of these differences, including promoter variation, TF specificity, feedback regulation, and source-sink carbon partitioning, remains an important knowledge gap.

Root system architecture represents a core belowground adaptive component of abiotic stress tolerance. Under drought and salinity stress, *Brassica* crops remodel root morphology, including changes in primary root length, lateral root density, root hair development, and root-to-shoot ratio, to optimize water and nutrient foraging and reduce ion uptake. Under waterlogging stress, aerenchyma formation in roots improves internal oxygen diffusion and mitigates hypoxic damage [42]. Root architectural traits are genetically controlled and co-localize with multiple stress tolerance QTLs [42], making them promising targets for stress-resilience breeding; however, their phenotyping difficulty and strong environmental plasticity have limited their widespread use in selection programs.

2.3. Enzymatic and Non-Enzymatic Antioxidant Networks

The antioxidant system is central to limiting stress-induced reactive oxygen species (ROS) accumulation. Superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) constitute the first enzymatic defense layer by converting superoxide and hydrogen peroxide into less damaging products. In pakchoi, *BchCSD3*, *BchCSD4*, and chloroplast-localized *BchFSD2* are upregulated under drought and salt stress, while mitochondrial *BchMSD2* and *BchMSD3* also participate in stress responses; genome-wide analysis identified 13 *BchSOD* genes [43]. Functional characterization showed that *BchFSD2* overexpression reduces superoxide and MDA accumulation while increasing CAT, POD, and SOD activities under salinity, supporting its role in salt tolerance [43]. PODs consume excess H_2O_2 through phenolic oxidation, and 109 *BnPOD* genes have been identified in *B. napus*, many of which respond to heat and salt stress [15]. CAT completes the enzymatic detoxification of H_2O_2 by converting it into water and oxygen; genome-wide analyses have identified 16 *CAT* genes in *B. napus*, 8 in *Brassica rapa* L., and 7 in *Brassica oleracea* L. [14], as well as 9 in *B. juncea* [44]. The *CAT* family in *B. juncea* and *B. rapa* has been comprehensively characterized, revealing strong evolutionary conservation together with isozyme-specific differences in heme-binding affinity among CAT1, CAT2, and CAT3 [44]. In *B. napus*, the expanded *CAT* family and its homoeologous expression patterns under stress provide useful insight into how *CAT*-mediated ROS detoxification has diversified after allopolyploidization [14]. The predicted peroxisomal localization of *BnCAT2* further highlights its central role in limiting H_2O_2 toxicity generated during photorespiration and stress induced oxidative metabolism [45]. Together with SOD and POD, *CAT* therefore contributes to a compartmentalized antioxidant system in which chloroplasts, mitochon-

dria, peroxisomes, and the cytosol are protected by partially specialized but functionally coordinated ROS-scavenging enzymes.

Non-enzymatic antioxidants, including flavonoids, glutathione (GSH), ascorbic acid (AsA), and tocopherols, complement the enzymatic defense system by directly scavenging ROS, buffering cellular redox status, and modulating stress-responsive signaling pathways. Flavonoids are particularly important because they function not only as radical scavengers but also as stress-regulated secondary metabolites linked to transcriptional reprogramming. Under drought stress, GWAS and multi-omics analysis in *B. napus* identified 60 candidate genes associated with phenolic acid and flavonoid biosynthesis, with several flavonoid biosynthetic genes and TFs being upregulated under drought conditions [46]. GSH is a core component of the AsA-GSH cycle and cooperates with AsA to reduce H_2O_2 and maintain redox homeostasis [47]. Its regeneration depends largely on glutathione reductase (GR). In *B. juncea* and *B. rapa*, 6 and 4 GR family members were identified, respectively; these cytoplasmic GR genes showed differential expression under drought and heat stress, suggesting that GSH recycling is actively adjusted according to stress intensity and duration [48]. H_2O_2 detoxification is further sustained through the AsA-GSH cycle, in which APX uses ascorbate as an electron donor to reduce H_2O_2 , while oxidized ascorbate is regenerated by MDHAR and DHAR using GSH as a reducing substrate [32]. Exogenous α -tocopherol and ascorbic acid have been shown to enhance APX, MDHAR, DHAR, and GR activities, thereby lowering H_2O_2 and MDA accumulation in salt-stressed rapeseed [47]. These findings indicate that osmoprotectants and antioxidants do not operate as separate defense layers but instead form an integrated osmotic-adjustment–antioxidant-defense network that maintains both cellular water status and redox balance. In this network, proline, soluble sugars, flavonoids, GSH, AsA, and tocopherols act cooperatively to stabilize membranes, protect proteins and photosynthetic structures, and fine-tune stress signaling.

The antioxidant response also differs markedly between heat and drought stress. High-temperature stress generally induces a rapid enzymatic response, reflected by the upregulation of antioxidant enzyme gene families in pakchoi, with POD genes showing clear temporal expression dynamics [19]. By contrast, drought stress usually requires a more sustained coordination between enzymatic ROS detoxification and non-enzymatic osmoprotection. During early drought responses, increased SOD and CAT activities help restrict ROS accumulation, whereas proline, glycine betaine, soluble sugars, and phenolic compounds contribute to osmotic adjustment, maintenance of cell turgor, and protection of membrane integrity. At the same time, elevated activities of POD, APX, and GR reinforce H_2O_2 removal and reduce oxidative damage under prolonged stress [2,49]. This coordinated response is evident in *B. napus*, where the combined accumulation of proline and soluble sugars together with increased SOD, POD, and CAT activities protects membranes from lipid peroxidation [50]. Thus, heat stress appears to rely more strongly on rapid enzymatic ROS clearance, whereas drought tolerance depends on the longer-term integration of osmotic homeostasis, antioxidant recycling, and membrane stabilization. This distinction is important for breeding because antioxidant capacity should not be evaluated as a single static trait; instead, stress type, exposure duration, tissue specificity, and the balance between enzymatic and non-enzymatic defenses should all be considered when selecting stress-resilient *Brassica* genotypes.

2.4. Photosynthetic Efficiency and Metabolic Energy Regulation Under Stress

Photosynthetic protection and metabolic energy allocation are tightly interconnected components of abiotic stress adaptation. Among the photosynthetic complexes, photosystem II (PSII) is particularly vulnerable because it is the primary site of light energy conversion and is highly sensitive to oxidative, thermal, and dehydration-induced dam-

age. Therefore, the maintenance of PSII repair and turnover is essential for sustaining photosynthetic performance under adverse conditions. Transgenic studies confirm that *Vdof1* enhances PSII stability under drought by protecting core subunits D1/D2 to maintain photosynthetic efficiency, while genome-wide predictions indicate that Dof family members are widely implicated in stress-regulated photosynthesis [51]. In this context, TF-based regulatory modules, including Dof-type zinc finger proteins, represent promising targets for reconstructing stress-responsive networks that stabilize photosynthetic machinery under adverse environments [10]. These PSII-protective mechanisms are not merely defensive responses; they also preserve the energy supply required for osmotic adjustment, antioxidant metabolism, protein repair, and other stress-adaptive processes.

Stress-induced reprogramming of carbon metabolism provides an additional layer of metabolic flexibility. Dynamic adjustment of sucrose–starch conversion allows plants to redistribute carbon between immediate energy production, osmotic protection, and storage reserves. In rapeseed under drought stress, sulfur-containing compounds, including glucosinolates and erucic acid, were shown to regulate sugar metabolism and hormone levels, thereby enhancing osmotic adjustment and antioxidant systems [39]. This response supports cellular osmotic balance while also providing metabolic substrates for stress defense. Integrated transcriptomic and proteomic analysis of non-heading Chinese cabbage under heat stress revealed that up-regulated DEGs and DAPs were significantly enriched in starch and sucrose metabolism, glycolysis/gluconeogenesis, and protein processing in the endoplasmic reticulum, whereas down-regulated genes were mainly associated with plant hormone signaling and secondary metabolic processes, indicating strategic metabolic reallocation under stress [19]. Similarly, in *B. napus* under soil-amendment treatment with brackish-water drip irrigation, DEGs and DAMs related to photosynthesis, energy metabolism, and carbohydrate metabolism were enriched, with genes such as *BanC02G0540200ZS* being up-regulated [52]. Under high-temperature stress, studies in non-heading Chinese cabbage further showed that heat shock proteins (HSPs), particularly HSP70, play a central role in protecting photosynthetic and metabolic machinery through the reprogramming of carbon and energy metabolism [19]. Proteomic evidence also indicated that β -fructofuranosidase and β -amylase were consistently up-regulated at the protein level, while HSP70s, including BAA01g40860.1, enriched in the ER protein-processing pathway were linked to key enzymes involved in photosynthesis, supporting the maintenance of essential metabolic pathways under heat stress [19].

Different abiotic stresses therefore impose distinct demands on photosynthesis and carbon allocation. Drought stress mainly favors starch degradation and soluble sugar accumulation to support osmotic adjustment and maintain cellular hydration, whereas heat stress appears to prioritize glycolysis, ER protein processing, and chaperone-mediated protection to maintain protein homeostasis. Thus, PSII repair, carbon remobilization, sugar metabolism, and protein quality control should be viewed as an integrated energy-management system rather than as independent stress-response modules. From a breeding perspective, this implies that photosynthetic tolerance should not be evaluated solely by chlorophyll content or photosynthetic rate; instead, PSII stability, carbon partitioning, soluble sugar accumulation, and stress-induced protein protection should be assessed together to identify *Brassica* genotypes capable of maintaining both energy capture and metabolic flexibility under stress.

3. Genetic Dissection and Functional Genomics of Stress Tolerance

3.1. QTL Mapping: From Population Design to Gene Discovery

Classical QTL mapping, primarily based on bi-parental populations such as recombinant inbred lines (RILs) and near-isogenic lines (NILs), has been widely used to dissect the

genetic architecture of abiotic stress tolerance in *Brassica* crops (Table 1) [1,53]. In *B. napus*, candidate drought-tolerant genes were identified using F_{2:6} and F_{2:8} RIL populations combined with transcriptome sequencing; these candidates mainly encoded *FLC*, *bHLH105*, and *TGA4* and were associated with TF activity, ETH signaling, and chlorophyll biosynthesis pathways [54]. Under salt stress during germination, QTL mapping in *B. napus* detected 17 QTLs, including four major QTLs on chromosome C3, and identified five candidate genes associated with membrane damage repair and ion transport [55]. Further analysis with an F_{2:3} population in the same study identified 27 QTLs for salt tolerance at germination, with a major cluster on chromosome C3 explaining up to 17.57% of the phenotypic variance, further supporting this genomic region as a recurrent hotspot for salt tolerance during early development [55].

Table 1. Representative QTL and GWAS studies identifying genetic loci and candidate genes associated with abiotic stress tolerance in *Brassica* crops.

Species	Stress Type/Condition	Mapping Approach and Population	Developmental Stage/Trait Focus	Major Loci, Markers, or Candidate Genes	Main Breeding Relevance	Reference
<i>Brassica napus</i> L.	Cold stress	GWAS using 217 rapeseed accessions	Seedling-stage cold tolerance and MDA content	<i>BnaA8.MYB60</i> haplotypes associated with cold tolerance	Provides a candidate transcription-factor locus for cold-tolerance marker development and haplotype-based selection	[12]
<i>Brassica juncea</i> (L.) Czern.	Drought stress	GWAS integrated with WGCNA using 193 accessions	Germination and seedling-stage drought tolerance	77 SNPs integrated into 27 QTLs; 11 candidate genes, including SAUR, LEA, and AP2/ERF-related genes; key B06 region containing <i>BjuB022264</i> , <i>BjuB022292</i> , <i>BjuB022282</i> , and <i>BjuB022235</i>	Links association signals with co-expression modules and drought-tolerant haplotypes useful for candidate-gene prioritization	[13]
<i>Brassica napus</i> L.	Drought stress	QTL mapping combined with transcriptome sequencing using F _{2:6} and F _{2:8} RIL populations	Drought-tolerance-related agronomic and physiological traits	Candidate genes mainly related to <i>FLC</i> , <i>bHLH105</i> , and <i>TGA4</i> , involving transcriptional regulation, ETH signaling, and chlorophyll biosynthesis	Demonstrates the value of combining linkage mapping with transcriptomics to refine drought-tolerance candidates	[54]
<i>Brassica napus</i> L.	Salt stress	QTL mapping and salt-tolerant germplasm identification	Germination-stage salt tolerance	17 QTLs, including four major QTLs on chromosome C3; candidate genes associated with membrane damage repair and ion transport	Identifies chromosome C3 as an important genomic region for salt-tolerance improvement during early development	[55]

Table 1. Cont.

Species	Stress Type/ Condition	Mapping Approach and Population	Developmental Stage/Trait Focus	Major Loci, Markers, or Candidate Genes	Main Breeding Relevance	Reference
<i>Brassica juncea</i> (L.) Czern.	Salt stress	SSR-based linkage mapping using an F _{2:3} population	Eight morpho-physiological traits under salinity	15 QTLs; major QTLs on linkage group C4 explaining up to 59.34% of phenotypic variance for siliquae per plant	Provides marker-linked loci for salinity-tolerance breeding in Indian mustard	[56]
<i>Brassica juncea</i> (L.) Czern. introgression lines derived from <i>B. carinata</i>	Drought stress under rainfed conditions	QTL dissection in introgression lines	Yield-related traits under drought, including silique length and 1000-seed weight	24 QTLs, including <i>qSL.2B.1</i> and <i>qTSW.3B.2</i> ; co-localized regions containing stress-related candidates such as <i>SOS2</i> , <i>BRI1</i> , <i>MAPK3</i> , and <i>WRKY33</i>	Highlights the value of wild/allied-species introgression for introducing favorable drought-tolerance and yield-related alleles	[57]
<i>Brassica rapa</i> L.	Summer high-temperature stress	GWAS using 52 accessions and 200,255 GBS-derived SNPs	Field and controlled-environment traits, including stability score, bolting, axillary bud formation, calcium deficiency, and boron deficiency	Candidate genes included GATA TF 21, auxin efflux carrier component 2, bHLH35-like TF, and VQ motif-containing protein 10	Provides heat-stress-associated loci relevant to vegetable Brassica breeding under summer cultivation conditions	[58]

Beyond *B. napus*, QTL mapping has provided useful genetic information in other *Brassica* species, although the level of validation varies among studies. In *B. juncea*, QTL mapping under salt stress using an F_{2:3} population and an SSR-based linkage map identified 15 QTLs for eight morpho-physiological traits, with several major QTLs on linkage group C4 explaining up to 59.34% of the phenotypic variance for siliquae per plant, providing potentially useful markers for salt tolerance breeding [56]. For drought tolerance, QTL dissection in *B. juncea* introgression lines carrying *B. carinata* segments detected 24 QTLs under rainfed conditions, including *qSL.2B.1* for silique length and *qTSW.3B.2* for 1000-seed weight, with several favorable alleles contributed by the *B. carinata* parent [57]. Within the same introgression-line population, co-localized QTLs encompassed candidate genes such as *SOS2*, *BRI1*, *MAPK3*, and *WRKY33*, which are known to participate in abiotic stress responses [57]. Such pleiotropic or co-localized QTL regions are particularly valuable because they may enable simultaneous improvement of stress tolerance and yield-related traits through marker-assisted selection (MAS), although their effects must be confirmed across environments and genetic backgrounds before deployment in breeding programs [57]. Strong G × E interactions are a major reason for the limited stability of QTL effects: many QTLs detected under one stress intensity or trial site cannot be replicated under other conditions, which restricts the direct application of linked markers in broad-range breeding [57]. In *B. rapa*, a GWAS under summer high-temperature stress identified significant SNPs and candidate genes associated with heat tolerance, including TFs and stress-associated proteins, highlighting the value of association mapping for dissecting heat tolerance in vegetable *Brassica* crops [58]. Furthermore, a preliminary QTL analysis based on thesis research identified 58 loci associated with root system architecture and seedling stage

waterlogging tolerance in *B. napus*, offering candidate targets for waterlogging tolerance improvement [42]. Collectively, these examples show that QTL mapping continues to reveal both conserved and species-specific genetic components of abiotic stress tolerance across *Brassica* crops.

Different mapping populations provide complementary advantages for QTL discovery. F_{2:8} RIL populations offer genetic stability and reusability, making them suitable for multi-environment analysis of complex traits [54]; however, their mapping resolution is limited by the number of recombination events captured during population development. NILs, chromosome segment substitution lines (CSSLs), and introgression lines are powerful tools for QTL fine mapping and candidate gene validation in *Brassica* crops, as they reduce background genetic noise and narrow down target intervals [56,59]. In *B. juncea*, both F_{2:3} families and introgression lines have proven useful for initial QTL detection and validation across environments [56,57]. Overall, bi-parental QTL mapping is effective for detecting rare or large-effect alleles but generally has limited resolution, whereas GWAS offers higher mapping resolution but is less efficient for rare alleles and is more sensitive to population structure. A practical strategy for *Brassica* stress-tolerance research is therefore to combine RIL-based discovery, NIL-based fine mapping, and GWAS-based validation in diverse germplasm panels. Such integration would improve confidence in candidate loci, accelerate causal gene identification, and increase the utility of QTL-linked markers for MAS.

3.2. GWAS Applications for Abiotic Stress Tolerance in Brassica Crops

GWAS has become a powerful approach for dissecting the genetic basis of abiotic stress tolerance in *Brassica* crops, particularly when diverse germplasm panels and high-density SNP datasets are available (Table 1). A GWAS of 217 rapeseed accessions for cold tolerance at the seedling stage identified *BnaA8.MYB60* as a major locus, with haplotypes of this gene significantly associated with cold tolerance and malondialdehyde (MDA) content, illustrating the value of GWAS for identifying stress-responsive TFs involved in low-temperature adaptation [12]. By integrating GWAS with metabolome data, SNPs associated with phenolic acid and flavonoid synthesis during the flowering period of *B. napus* were identified, including a candidate SNP in the intron of *PAL1* associated with ABA accumulation under drought treatment [46]. Compared with single-omics GWAS, multi-omics-integrated GWAS strategies, including mGWAS and eGWAS, can connect genetic variants with intermediate molecular phenotypes such as metabolites and transcripts, thereby improving candidate gene prioritization and biological interpretation. In addition to single-stress studies, GWAS-based multi-omics integration provides a useful framework for dissecting the genetic basis of metabolite-associated stress tolerance [46].

GWAS has also been extended to other *Brassica* species and stress contexts. In *B. juncea*, GWAS combined with WGCNA across 193 accessions under drought stress at germination and seedling stages identified 77 SNPs integrated into 27 QTLs and 11 candidate genes, including *SAUR*, *LEA*, and AP2/ERF TFs [13]. A key region on chromosome B06 (23.955–24.089 Mb) contained four drought-related genes, *BjuB022264*, *BjuB022292*, *BjuB022282*, and *BjuB022235*, with haplotype Hap1 specifically detected in tolerant materials. For terminal heat stress in *B. juncea*, GWAS identified significant SNP associations on chromosomes A01, A06, and B04 related to seed oil content, with candidate genes including fatty acyl-CoA reductase (*FAR3*) and MYB TFs [60]. Similarly, a comprehensive GWAS of salt tolerance in *B. napus* has identified abundant significant loci, with a subset of SNPs co-localized across multiple tolerance traits, highlighting the highly polygenic genetic architecture of salt tolerance [61]. Candidate genes included GATA TF 21, auxin efflux carrier component 2, TF bHLH35 like, and VQ motif containing protein 10, which are linked to heat and drought stress responses [58]. Another QTL mapping study in *B. rapa* under

heat stress identified 57 SNPs associated with yield related traits, explaining 6.52–23.43% of the phenotypic variance, and provided additional candidate genes for heat tolerance [62]. Moreover, a drought GWAS in *B. napus* using stress susceptibility index (SSI) and stress tolerance index (STI) identified 314 SNPs across all chromosomes, offering candidate markers for drought tolerance at the seedling stage [63]. Despite these advances, GWAS in *Brassica* crops still faces important limitations, including population structure confounding, limited power to detect rare alleles, environment-specific associations, and the frequent difficulty of distinguishing causal variants from linked markers. $G \times E$ interactions further reduce the reproducibility of association signals: significant loci identified in a single environment often fail to be validated across multi location trials, because stress intensity, timing, and co-occurring environmental factors jointly shape the phenotypic expression of tolerance traits. These limitations can be reduced by using multi-environment phenotyping, mixed linear models, haplotype analysis, transcriptomic and metabolomic validation, and cross-species comparative genomics. Therefore, the greatest value of GWAS for *Brassica* improvement lies not simply in listing significant SNPs, but in prioritizing robust, biologically interpretable loci that can be converted into functional markers or experimentally validated targets for stress-resilience breeding. Pangenomic approaches further expand usable genetic variation in *Brassica* crops: they capture structural and presence-absence variations missed by single reference genomes, enabling the discovery of novel stress resistance alleles and the optimization of breeding tools to harness underutilized germplasm for enhanced species resilience [64,65].

3.3. Hierarchical Regulatory Networks: From TFs to Functional Proteins

Functional characterization of stress-responsive genes in *Brassica* crops indicates that abiotic stress tolerance is governed by a hierarchical regulatory network in which upstream TFs and chromatin regulators modulate signaling components, transporters, and downstream protective proteins [1]. Individual stress-response genes have been widely validated, but integrated hierarchical regulatory networks remain largely hypothetical frameworks. This organization is important because stress tolerance is rarely determined by single genes; instead, it depends on coordinated regulation across perception, signal amplification, ion and osmotic adjustment, ROS detoxification, and cellular repair. At the upstream regulatory level, TFs integrate stress signals and redirect gene-expression programs toward protection or acclimation. The cold-induced TF *BcWRKY53* positively regulates cold tolerance by activating the chlorophyll biosynthesis genes *BcCHLH* and *BcGUN4* [66]. However, this response is fine-tuned by the histone deacetylase *BcHDA9*, which interacts with *BcWRKY53* and represses its transcriptional activation, thereby forming a chromatin-associated negative regulatory loop in non-heading Chinese cabbage [66]. Similarly, *BrHATs* are induced by low temperature and regulate cold-responsive genes through histone acetylation, highlighting the importance of epigenetic control in stress-responsive transcriptional regulation [67]. In *B. napus*, *BnWRKY149* and *BnWRKY217* participate in ABA-associated stress signaling and regulate drought-responsive genes that may influence stomatal behavior; their induction by ABA and NaCl, together with repression under PEG treatment, suggests that WRKY-mediated responses are stress-specific rather than uniformly activated under all dehydration-related conditions [68]. Members of the MYB TF family, such as *BnaMYB111L*, have been implicated in the regulation of ROS-generating genes (e.g., *RbohB*) and cell-death-related genes (e.g., *MC4*), indicating that MYB factors can function upstream of oxidative stress signaling and stress-induced cellular remodeling [18].

Downstream of transcriptional and hormonal regulation, transporters and signaling-related proteins convert regulatory signals into physiological responses. *BnaCERK*, a sphingolipid kinase, regulates ABA biosynthesis-related gene expression and enhances

drought tolerance in *B. napus* by maintaining the balance between ceramide (Cer) and ceramide-1-phosphate (C1P); mutation of this gene impairs seed germination, indicating that sphingolipid homeostasis is closely linked to early stress adaptation [69]. More specifically, BnaCERK targets *BnaA02.SBH1* and *BnaCnn.SBH1*, both of which are highly induced by dehydration stress, supporting a functional connection between sphingolipid signaling and drought resistance [69]. Ion transport also represents a major downstream output of stress signaling. The high-affinity potassium transporter BnaA7.HAK5 is regulated by upstream MYB TFs under low-potassium stress and contributes to osmotic adjustment by enhancing K⁺ uptake [70]. In parallel, protective functional proteins execute stress defense at the cellular level. In pakchoi, *BchFSD2* has been identified as an important SOD gene for salt tolerance, functioning through ROS scavenging and maintenance of cellular redox homeostasis [43]. In *B. oleracea*, members of the phenylalanine ammonia-lyase (*PAL*) gene family are highly expressed under MeJA, low-temperature, and high-temperature treatments, suggesting that phenylpropanoid metabolism contributes to abiotic stress adaptation [71]. In *B. juncea*, *APX* and *APX-R* gene-family members are differentially expressed under drought, heat, and salt stresses, further emphasizing the importance of ROS-scavenging systems as downstream protective modules [72]. Collectively, these examples show that stress tolerance in *Brassica* crops emerges from coordinated regulation across TFs, epigenetic modifiers, transporters, signaling proteins, and antioxidant enzymes, rather than from isolated gene effects. For breeding, the most useful targets are therefore likely to be genes or modules that connect upstream regulation with stable physiological outputs, such as ion homeostasis, ROS control, photosynthetic protection, and osmotic adjustment. However, the phenotypic effects of these candidate genes are often context-dependent, and inconsistent results across genetic backgrounds and experimental conditions limit direct generalization across breeding materials.

3.4. Polyploidy-Driven Regulatory Innovations in Stress Responses

The allopolyploid nature of *B. napus* adds an additional layer of regulatory complexity to abiotic stress tolerance because many stress-responsive pathways are shaped by gene dosage effects, homoeologous gene retention, and functional divergence between the A and C subgenomes [1,10]. Polyploidization increases gene copy number, which can enhance pathway robustness through redundancy, but it can also enable regulatory specialization when duplicated genes acquire stress-, tissue-, or subgenome-specific expression patterns. For example, 114 *SnRK* genes have been identified in the *B. napus* genome and classified into three subfamilies; expression analysis showed that some members, including *BnaSnRK2.4* and *BnaSnRK2.10*, are induced by abiotic stresses, providing a foundation for functional dissection of stress-responsive *SnRK* signaling modules [16]. The *SRO* gene family also exhibits polyploid expansion in *B. napus*; 32 members have been identified across the A and C subgenomes, and *BnaSROs* show broad responsiveness to salt, drought, freezing, cold, heat, and osmotic stresses [73]. These examples indicate that gene-family expansion can increase the regulatory capacity of allopolyploid *Brassica* crops, although functional validation is still required to distinguish true adaptive innovation from retained redundancy.

Homoeologous gene differentiation is another important feature of stress regulation in polyploid *Brassica* crops. Rather than functioning as identical copies, A- and C-subgenome homoeologs may show biased expression, differential stress responsiveness, or partial functional specialization. For example, comparative genomics of antioxidant gene families reveals subgenome-biased distribution and expression patterns, with C-subgenome members enriched in ROS-scavenging pathways [14,44]. In the sphingolipid metabolism pathway, *BnaCERK* from the C subgenome regulates drought response by maintaining the Cer/C1P balance [69]. Such subgenome-associated specialization may create com-

plementary regulatory networks that allow polyploid plants to distribute stress-response functions across retained homoeologs. However, this model should be interpreted cautiously, because subgenome bias can vary by tissue, developmental stage, stress intensity, and genotype. The cold-responsive *BcWRKY53* module in non-heading Chinese cabbage, which activates the chlorophyll synthesis genes *BcCHLH* and *BcGUN4*, further illustrates how transcriptional regulators can connect stress signaling with photosynthetic maintenance [66]. Likewise, subgenome-biased expression of *BnaSnRK* genes has been observed, with a higher proportion of stress-responsive members located on the C subgenome, suggesting that subgenome-specific retention may contribute to stress-response capacity in the allotetraploid genome [16]. Therefore, subgenome specialization may represent an evolutionary advantage of polyploidy, but its breeding value depends on identifying which homoeologs produce stable and beneficial phenotypes across environments.

Polyploid-specific AS further expands regulatory plasticity in *B. napus* by generating multiple transcript isoforms from retained homoeologous genes. These AS events may help coordinate stress responses between subgenomes by modulating hormone signaling pathways, including ABA and JA signaling, as well as metabolic pathways such as glycolysis and the TCA cycle [1,10]. By increasing transcript and proteome diversity, AS provides a post-transcriptional mechanism for fine-tuning stress responses without requiring changes in gene copy number or DNA sequence. In allopolyploid crops, this mechanism may help integrate stress-adaptive features inherited from ancestral diploids, including *B. rapa* with the AA genome and *B. oleracea* with the CC genome, thereby enhancing environmental adaptability through the combined effects of gene dosage, homoeolog retention, and functional differentiation [10,74]. At the same time, extensive redundancy among polyploid paralogs complicates functional interpretation and crop improvement. For instance, 104 *BnWRKY* genes in *B. napus* exhibit variable correlation coefficients between paralogous gene pairs under stress, indicating diverse patterns of retention, subfunctionalization, and possible neofunctionalization [68]. Although redundancy can buffer plants against deleterious mutations and environmental fluctuation, it also makes targeted genetic improvement more difficult because editing a single copy may not produce a measurable phenotype if other homoeologs compensate. Consequently, future genome-editing and molecular-breeding strategies in *Brassica* should prioritize homoeolog-aware target selection, expression-guided candidate ranking, and multiplex editing where functional redundancy is likely to mask single-gene effects.

4. Transcriptional, Epigenetic, and Multi-Omics Regulation of Stress Tolerance

4.1. Transcriptomic Profiling of Stress Responses

Transcriptome analysis provides a genome-wide view of stress-induced gene-expression changes and captures the dynamic regulatory responses of *Brassica* crops to diverse abiotic stresses (Figure 2). It therefore provides a fundamental entry point for dissecting complex stress-response networks, particularly in polyploid crops where gene redundancy, homoeologous expression bias, and stress-specific transcriptional plasticity complicate functional interpretation [25,54]. Advances in high-throughput sequencing have enabled stress-responsive transcriptomes to be characterized at increasingly high resolution, establishing transcriptomics as a core platform for stress biology research in *Brassica* and other polyploid crop systems [10]. Transcriptome profiling of *B. napus* under drought stress has revealed extensive reprogramming of genes involved in water transport, osmotic adjustment, hormone signaling, and stress-responsive transcriptional regulation [54]. Similarly, transcriptomic studies in *B. napus* under salt stress have identified thousands of differentially expressed genes associated with ion homeostasis, membrane protection,

and physiological acclimation [25]. In pakchoi, integrated transcriptomic and proteomic profiling under heat stress revealed upregulation of HSPs and downregulation of phenylpropanoid/flavonoid biosynthesis, suggesting a stress-induced shift in resource allocation toward protein protection and survival-related metabolism [19]. Furthermore, integrative methylome and transcriptome analysis in non-heading Chinese cabbage demonstrated that DNA methylation changes contribute to heat stress responses by modulating genes involved in photosynthesis, stress signaling, and adaptive metabolism [75]. In *B. juncea*, transcriptomic analysis of crop wild relatives (CWRs) has been proposed as a promising strategy for identifying stress-responsive genes that may have been weakened or lost during domestication, thereby expanding the genetic reservoir available for stress-tolerance improvement [74]. In *B. napus*, transcriptome profiling of TF families, including the R2R3-MYB family, across multiple abiotic stresses has identified numerous stress-responsive members, supporting their roles as integrators of different stress-signaling pathways [18,54]. Similarly, transcriptome-based expression analysis of the *BnRH* gene family in *B. napus* under drought, salt, heat, cold, and cadmium stresses revealed that differentially expressed *BnRH* members exhibit time- and stress-specific expression patterns, further demonstrating the value of transcriptomics for dissecting gene-family-level stress responses [76].

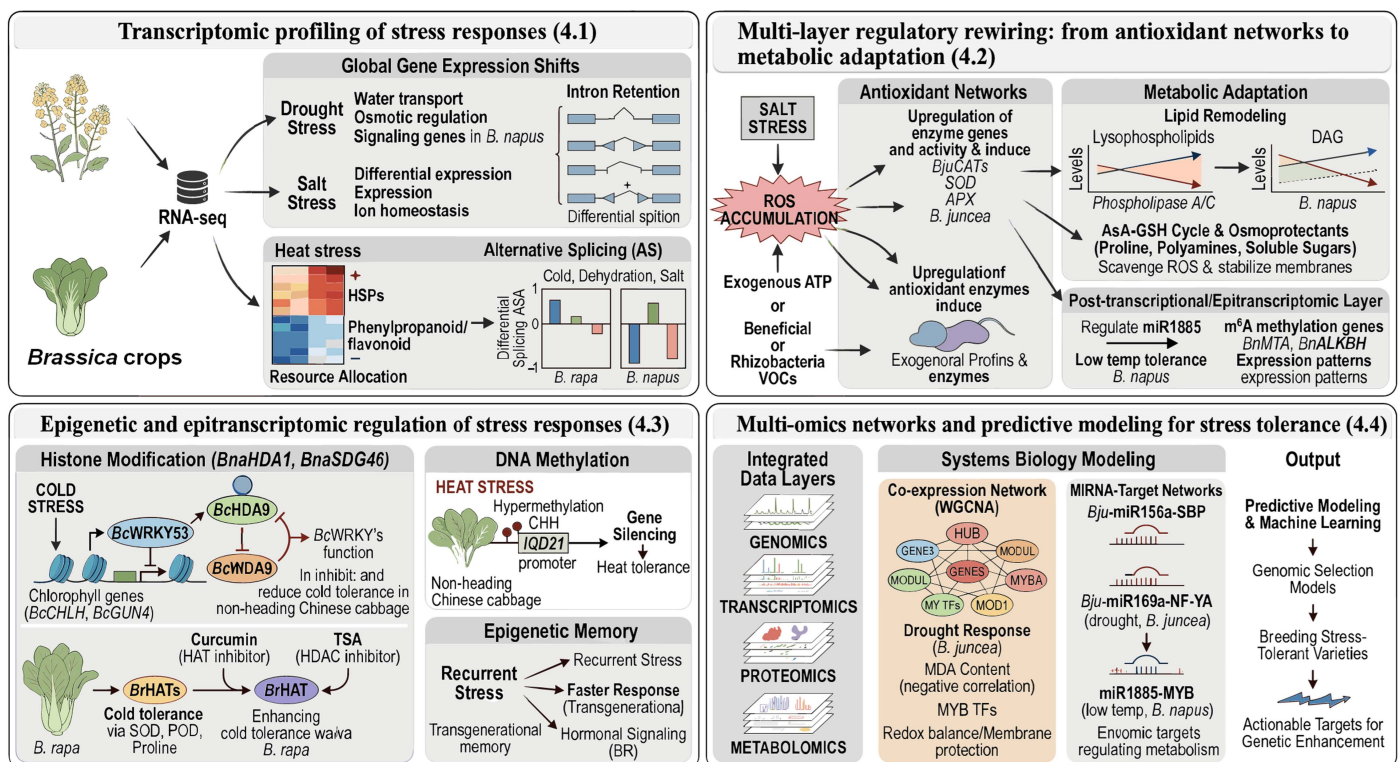


Figure 2. Transcriptional, epigenetic, and multi-omics regulation of stress tolerance in *Brassica* crops. Section 4.1: Global gene expression shifts under drought, salt, and heat stresses, together with alternative splicing events that add post-transcriptional regulatory complexity. Section 4.2: Antioxidant network activation and metabolic adaptation, including lipid remodeling, the AsA-GSH cycle, osmoprotectant accumulation, ROS scavenging, membrane stabilization, and epitranscriptomic regulation. Section 4.3: Epigenetic regulation through histone modification, HAT/HDAC-associated modulation of cold tolerance, DNA methylation, RNA methylation, and stress-memory formation. Section 4.4: Integration of genomics, transcriptomics, proteomics, and metabolomics through systems-biology approaches, including WGCNA co-expression networks, miRNA-target regulatory networks, and machine-learning-based predictive models for genomic selection of stress-tolerant varieties.

Beyond differential gene expression, AS adds an important post-transcriptional layer to stress adaptation. AS enables a single gene to generate multiple transcript isoforms,

thereby expanding regulatory and proteomic diversity without requiring additional gene copies. Transcriptome-wide analyses in *B. rapa* and *B. napus* have identified numerous genes undergoing stress-responsive differential splicing, with intron retention being the most common AS mode under cold, dehydration, salt, and ABA treatments [77]. This is particularly relevant in polyploid *Brassica* crops, where retained homoeologs may differ not only in expression level but also in transcript isoform usage. The complexity of AS regulation is further underscored by the observation that more than 70% of intron-containing plant genes undergo AS; however, the complex genome structure of polyploids remains a major challenge for accurately resolving isoform-specific expression and assigning biological function to individual splice variants [4,77]. Overall, transcriptomic profiling has substantially advanced understanding of abiotic stress responses in *Brassica* crops, but its greatest value will come from integration with functional validation, epigenomic information, proteomics, metabolomics, and physiological phenotyping. Such integration is necessary to move beyond lists of differentially expressed genes toward mechanistic models and breeding-relevant markers for stress resilience.

4.2. Multi-Layer Regulatory Rewiring: From Antioxidant Networks to Metabolic Adaptation

Salt stress promotes ROS accumulation and triggers coordinated regulation of antioxidant enzyme isoforms, redox-related metabolites, and stress-responsive signaling pathways. The *BjuCAT* gene family in *B. juncea* contributes to redox balance by supporting antioxidant enzyme activities, including SOD and APX, thereby helping to restrict oxidative damage under stress [44,72]. This fine-tuning of antioxidant capacity is also influenced by post-transcriptional regulation; for example, *BrCMF* has been reported to respond to salt stress through regulation of antioxidant-related pathways [78]. However, the precise role of *BrCMF* and its possible involvement in GSH metabolism require further experimental validation, because current evidence remains largely correlative. Beyond gene-level regulation, the interaction between enzymatic antioxidants and non-enzymatic metabolites forms a multi-layered defense system. Volatile organic compounds (VOCs) released by beneficial rhizobacteria have been shown to upregulate *BrSOD* and *BrCAT* expression and increase SOD and CAT enzyme activities under salt stress in cabbage, suggesting that microbiome-derived signals may enhance host antioxidant capacity [79]. Exogenous ATP has also been reported to induce the expression of SOD, CAT, APX, and GR genes under NaCl stress in *Brassica campestris*, indicating that chemical priming can strengthen antioxidant preparedness before or during stress exposure [80].

At the metabolite level, lipid remodeling represents a central component of stress-induced metabolic rewiring. Lipid profiling in *B. napus* showed that lysophospholipids and diacylglycerol (DAG) display stress-dependent changes that correlate with transcriptional regulation of specific phospholipase A and C family members [81]. These lipid-derived signals may contribute simultaneously to membrane remodeling and stress-signal amplification. In parallel, the AsA-GSH cycle and osmoprotectants such as polyamines act together with antioxidant enzymes to stabilize membranes, scavenge ROS, and maintain redox homeostasis [25,47,72]. Epitranscriptomic regulation further adds to this multilayered response. Transcriptome-wide analysis of the m⁶A methylation-related gene family in *B. napus* identified 33 candidate genes, including methyltransferases such as *BnMTA* and *BnMTB* and demethylases such as *BnALKBH*, whose stress-responsive expression patterns suggest a role for RNA methylation in post-transcriptional stress adaptation [82]. Small non-coding RNAs also contribute to this regulatory architecture; for example, *miR1885* has been implicated in low-temperature tolerance in *B. napus* through multi-omics analysis, linking *Brassica*-specific microRNAs (miRNAs) with stress-responsive gene networks [83]. Together, these findings indicate that stress adaptation in *Brassica* crops depends on coor-

minated regulatory rewiring across transcriptional, post-transcriptional, epigenetic, and metabolic layers rather than on isolated antioxidant responses alone.

4.3. Epigenetic and Epitranscriptomic Regulation of Stress Responses

Epigenetic and epitranscriptomic modifications, including histone acetylation/deacetylation, DNA methylation, and RNA methylation, provide *Brassica* plants with flexible regulatory mechanisms for adjusting gene expression under stress and, in some cases, retaining stress memory for faster responses to recurrent stress [1,10]. The dynamic balance between histone acetylation and deacetylation is especially important because it directly affects chromatin accessibility and the transcriptional activation or repression of stress-responsive genes. Histone modification genes have been systematically cataloged in Brassicaceae; genome-wide analysis identified 461 histone modification genes in allotetraploid *B. napus*, illustrating the extensive epigenetic regulatory capacity of polyploid *Brassica* genomes [84].

Under low-temperature stress, the histone acetyltransferase (HAT) gene family, including *BrHAT* genes in *B. rapa*, contributes to cold tolerance by regulating histone acetylation levels and activating genes involved in antioxidant defense and osmotic adjustment, including those related to SOD, POD, and proline accumulation [67]. Specifically, 29 *BrHAT* genes were identified in *B. rapa*, and both curcumin, a HAT inhibitor, and trichostatin A (TSA), an HDAC inhibitor, influenced cold resistance through modulation of histone acetylation and deacetylation [67]. These inhibitor-based experiments demonstrate that altered histone acetylation status can change the expression of low-temperature-responsive genes, supporting a direct role for chromatin regulation in cold adaptation. In non-heading Chinese cabbage, the low-temperature-induced TF *BcWRKY53* promotes cold tolerance by activating the chlorophyll biosynthesis genes *BcCHLH* and *BcGUN4*; however, the *BcHDA9* interacts with *BcWRKY53* and inhibits its activity, leading to increased histone deacetylation and reduced cold tolerance [66]. Genome-wide analyses also show that histone modification genes such as *BnaSDG46* and *BnaHDA1* are differentially expressed under salt, drought, and cold stresses, while H3K4me3 is enriched in the promoter regions of stress-responsive genes and promotes their transcription [84]. Weighted gene co-expression network analysis (WGCNA) identified a ‘green’ module significantly correlated with SPAD under nitrogen shortage, with hub genes including *BnaSDG53*, *BnaHDA1*, and *BnaSDG46* interacting with stress-adaptation genes, further supporting the central role of histone modification in environmental response regulation [84].

DNA methylation regulates stress-responsive gene expression by altering promoter accessibility and chromatin state. In non-heading Chinese cabbage, integrated methylome and transcriptome analysis demonstrated that DNA methylation changes contribute to heat stress adaptation by modulating genes associated with photosynthesis and stress responses [75]. Heat stress specifically induced CHH hypermethylation in promoters of negative heat-stress regulators such as *IQD21*, leading to transcriptional silencing and improved heat adaptation; by contrast, CG and CHG methylation were relatively stable under heat stress, suggesting that different methylation contexts have distinct regulatory roles [75]. Some stress-induced DNA methylation changes may be maintained through mitotic divisions and, in certain cases, transmitted to offspring, potentially forming transgenerational stress memory. However, the stability, specificity, and breeding utility of such stress memory in *Brassica* crops still require careful validation. RNA methylation, particularly m⁶A modification, adds another layer of regulation; 33 candidate m⁶A methylation-related genes have been identified in *B. napus*, with methyltransferases and demethylases showing stress-responsive expression patterns [82].

Epigenetic regulation is also closely connected with hormone signaling. BR signaling interacts with histone modification pathways and other hormonal signals to modulate stress responses, thereby contributing to the balance between growth and defense [32,85]. These mechanisms may provide opportunities for chemical priming, in which small-molecule epigenetic modifiers or hormone-related treatments are used to enhance stress preparedness without permanent genetic modification. Nevertheless, such approaches must be evaluated carefully because broad epigenetic modifiers can have pleiotropic effects on growth, development, and yield. Thus, epigenetic and epitranscriptomic regulation should be viewed not only as mechanistic layers of stress adaptation but also as potential, yet still developing, targets for stress-resilient *Brassica* breeding.

4.4. Multi-Omics Networks and Predictive Modeling for Stress Tolerance

Multi-omics integration, combining genomics, transcriptomics, proteomics, and metabolomics, has become essential for decoding the complex regulatory networks underlying abiotic stress tolerance in *Brassica* crops. Because stress tolerance is controlled by multiple interacting biological layers, systems biology approaches are needed to connect genetic variation, gene expression, protein abundance, metabolite accumulation, and physiological traits into interpretable models.

Co-expression network analysis is one of the most widely used tools for this type of integration. In *B. juncea*, WGCNA identified core modules associated with drought response. The expression dynamics of these modules were significantly negatively correlated with MDA content, linking transcriptional regulation with membrane stability and oxidative damage indicators [13]. miRNA-target regulatory networks further refine this multi-omics framework. Several miRNA-target pairs are experimentally confirmed, but global multi-omics regulatory networks are largely computationally inferred models. As core post-transcriptional regulators, miRNAs fine-tune stress responses via mRNA cleavage, translational repression, or epigenetic silencing, coordinating hormone crosstalk, ROS homeostasis, and growth-defense tradeoffs to complement transcriptional and epigenetic control [86–88]. miRNA cascades in allopolyploid *Brassica* show stress-type, tissue-specific, and subgenome-biased expression, comprising conserved and *Brassica*-specific miRNAs with distinct stress functions. For example, the *Bju-miR156a*-SBP and *Bju-miR169a*-NF-YA modules have been implicated in drought stress responses in *B. juncea*, adding a post-transcriptional regulatory dimension to co-expression networks [13,86]. Specifically, *miR156* targets SPL TFs to redirect resources from vegetative growth to stress defense, while the *Bna-miR169n*-NF-YA8 module has been functionally validated to modulate ABA signaling and drought tolerance in *B. napus* [89]. Integrated miRNA–mRNA transcriptome analysis in winter *B. rapa* under drought stress identified 32 differential miRNAs and multiple target genes mainly enriched in starch/sucrose metabolism, amino acid biosynthesis, and carbon metabolism pathways, playing indispensable roles in drought tolerance [90]. Additionally, integrated miRNA–mRNA analysis in *B. napus* under salt stress identified three regulatory pairs (*bna-miR160a*/*BnaA03.BAG1*, *novel-miR-126*/*BnaA08.TPS9*, and *novel-miR-70*/*BnaA07.AHA1*) involved in salt tolerance, with WGCNA further revealing five candidate genes (*WRKY6*, *WRKY70*, *NHX1*, *AVP1*, and *NAC072*) as key regulators [91]. Only a small fraction of these omics-identified candidates have been functionally validated, and heterogeneous experimental designs across studies reduce the consistency and comparability of reported findings.

The biological functions of these core modules are mainly associated with redox balance, membrane protection, osmotic adjustment, and stress-responsive transcriptional regulation. In *B. juncea*, drought-responsive modules were enriched in TF families such as MYB, and their higher expression was associated with lower MDA accumulation, sug-

gesting a protective role against oxidative stress [13]. Similarly, the coordinated activity of antioxidant enzymes, including SOD, POD, and CAT, together with osmolytes such as proline and soluble sugars, is essential for maintaining membrane stability under stress [33]. The *miR1885-MYB* regulatory module, identified through small RNA and degradome sequencing in *B. napus*, modulates glucosinolate metabolism under low-temperature stress, providing another example of how multi-omics approaches can connect regulatory RNAs, TFs, metabolites, and physiological adaptation [83]. Notably, *miR1885* is a *Brassica*-specific miRNA, reflecting lineage-specific regulatory innovation [83]. Conserved miRNA families also contribute broadly to stress tolerance: *miR398* targets Cu/Zn SOD genes to adjust ROS scavenging capacity, with confirmed roles in *B. rapa* heat response and *B. napus* heavy metal stress [92,93]; and *miR393* modulates auxin signaling to enhance osmotic stress tolerance in *B. napus* [94]. Moreover, the *Bna-miR399c-PHO2* module has been functionally characterized in *B. napus* under low phosphorus stress, where overexpression of *Bna-miR399c* enhanced phosphorus uptake and translocation, increased root biomass, and improved chlorophyll content, while *BnPHO2* was confirmed as a direct target of *Bna-miR399c* [95]. Collectively, these findings establish miRNA-mediated regulation as an integral component of the multi-layered stress regulatory network in *Brassica* crops, acting in concert with—rather than in isolation from—transcriptional and epigenetic control.

Despite these advances, miRNA research in *Brassica* stress biology remains constrained by the prevalence of descriptive expression profiling, functional redundancy of homoeologous copies in allopolyploids, and insufficient tissue- and stage-specific characterization. Further bottlenecks include non-uniform miRNA phenotyping standards and scarce tissue-resolved pangenome small RNA atlases. Nonetheless, miRNAs hold promising potential as early stress biomarkers and precision breeding targets for coordinating multiple downstream stress pathways [96].

Together, genomics, transcriptomics, proteomics, and metabolomics provide evidence for interconnected gene-protein-metabolite networks rather than linear stress-response pathways. Systems-biology modeling based on integrated datasets, including co-expression network analysis, can improve the identification of molecular targets for stress-resistance improvement in *Brassica* crops. However, a major challenge remains the transition from descriptive multi-omics integration to predictive and experimentally validated models. Additional bottlenecks include: (1) batch effects and technical heterogeneity across different omics platforms that reduce data compatibility; (2) the high dimensionality of multi-omics data increasing the risk of false-positive associations; (3) the difficulty of inferring causal relationships from correlation-based integration; (4) the high cost of multi-omics profiling limiting large-scale breeding application; and (5) insufficient integration of pangenome structural variants and miRNA molecular phenotype data. Predictive frameworks that combine multi-omics data with machine-learning algorithms, including genomic selection models that incorporate transcriptomic and epigenomic predictors, offer promising routes for accelerating the breeding of stress-tolerant varieties [97,98]. For these approaches to become practically useful, future studies should prioritize multi-environment datasets, standardized phenotyping, independent validation populations, and biologically interpretable models; researchers need to unify miRNA quantification standards, construct *Brassica* pangenome small RNA atlases, and integrate structural variants into machine learning prediction models. Overall, multi-omics integration has advanced understanding of abiotic stress responses in *Brassica* crops, but its greatest breeding value will depend on transforming complex datasets into robust predictors, functional markers, and actionable targets for stress-resilience improvement.

5. Integrated Molecular Breeding Approaches for Stress Resilience

5.1. From Phenotypic Selection to Marker-Assisted Breeding

Conventional breeding for abiotic stress tolerance in *Brassica* crops has historically relied on phenotypic screening and physiological indicators, including membrane stability, osmolyte accumulation, and stress-survival traits. Although these approaches remain valuable, they are time-consuming, strongly influenced by environmental variation, and often insufficient for distinguishing true genetic tolerance from stress escape or phenotypic plasticity. For example, under low-temperature stress, several generations of field evaluation are usually required to stabilize cold-tolerance traits, and genotype differences can be difficult to separate from environmental effects when selection is based only on phenotype [10]. The high plasticity of cold-responsive traits further reduces selection efficiency, particularly for complex quantitative traits controlled by many small-effect loci [24].

Conventional destructive phenotyping has long been a bottleneck limiting genetic gain, lagging far behind rapid genomic advances. High-throughput phenomics now offers key solutions: controlled-environment imaging platforms enable non-destructive, time-resolved stress tolerance screening in vegetable *Brassica* germplasm [99], and field spectral and thermal phenotyping allows large-scale evaluation of drought and heat tolerance in rapeseed breeding populations [100]. UAV-based remote sensing further enables canopy-level trait extraction at field scale, including canopy temperature, vegetation indices, and growth dynamics [97], while automated physiological sensors enable continuous monitoring of leaf water status and gas exchange. Despite these advances, key challenges remain: accurate dissection of component traits from imaging data remains difficult, field environmental interference reduces measurement precision, and standardized phenotyping protocols are still lacking across different morphotypes of *Brassica* crops [99,100]. Taken together, high-throughput phenomics not only alleviates the traditional phenotyping bottleneck, but also provides high-quality phenotypic data for the development and validation of molecular markers and genomic selection models. Despite current constraints in trait prediction accuracy and morphotype adaptability, integrating dynamic phenomics with genomic selection is expected to substantially accelerate genetic gain for stress resilience [97,98].

In this context, SSR and SNP markers linked to *CBF* genes and other major QTLs have provided useful tools for improving selection accuracy in rapeseed cold-tolerance breeding programs.

MAS improves the efficiency of phenotype-based breeding by enabling breeders to track alleles linked to stress-tolerance loci at early developmental stages and across environments. The central role of *CBF* genes in low-temperature signal transduction has been well established: their upregulation activates downstream cold-responsive genes, such as *COR15a*, thereby improving membrane stability and ROS-scavenging capacity [10,24]. Genome-wide identification of *CBF* genes in *B. napus* revealed 11 members, all containing the conserved AP2 domain, with higher expression in roots than in aboveground tissues, suggesting tissue-specific contributions to stress signaling [24].

Compared with conventional field selection, MAS can reduce breeding time, lower resource requirements, and improve the precision of allele introgression. Its major advantages include: (1) reduced time cost, because target genes can be selected through seedling-based DNA testing rather than repeated stress screening in later generations; (2) reduced resource input, because fewer large-scale field trials are needed under variable stress environments; and (3) improved accuracy, because multiple favorable alleles can be combined through QTL pyramiding. The identification of co-localized QTL hotspots, such as the chromosome 3B cluster in *B. juncea* encompassing *qBY.3B.2*, *qSY.3B.1*, and *qWUE.3B.1*, illustrates the potential of MAS for simultaneously improving stress tolerance and yield-related traits [57]. Similarly, SNP markers associated with drought tolerance

through GWAS, including 314 SNPs linked to SSI and STI indices, provide candidate molecular tools for MAS in *B. napus* [63]. Case studies also suggest that MAS using markers closely linked to cold-tolerance QTLs can contribute to improved survival and reduced yield loss under freezing stress [24]. The availability of high-density SNP platforms, such as the *Brassica* 60K Illumina Infinium SNP array, has further strengthened MAS by enabling broader genome coverage and more efficient trait introgression [2,10].

Nevertheless, MAS has important limitations in *Brassica* stress-tolerance breeding. Many available markers are linked to QTLs with small or environment-dependent effects, which can limit selection gain. Marker polymorphism and linkage phase may also vary among breeding populations, requiring validation before deployment. Moreover, MAS is less effective for highly polygenic traits controlled by many minor-effect loci, especially when genotype-by-environment interactions are strong. Therefore, MAS is most effective when used for validated major QTLs, gene pyramiding, or pre-breeding introgression, whereas genomic selection and multi-omics-assisted prediction may be more appropriate for complex stress-resilience traits.

5.2. Genetic Transformation and Genome Editing for Stress Improvement

Genetic transformation has provided important proof-of-concept evidence that specific regulatory and protective genes can enhance abiotic stress tolerance in *Brassica* crops. Overexpression of dehydration-responsive element-binding protein 1 (*DREB1*) significantly improves drought tolerance in *B. napus*. *DREB* TFs coordinate plant responses to water deficit by activating downstream stress-responsive genes involved in osmotic adjustment, antioxidant defense, and cellular protection [1]. Transgenic *B. napus* overexpressing *Bn-DREB1* under the control of the CaMV 35S promoter exhibited higher relative water content, greater proline accumulation, and increased antioxidant enzyme activities under drought stress, supporting the effectiveness of TF-based engineering. Overexpression of stress-protective proteins, including small HSPs, has also enhanced tolerance to multiple abiotic stresses in *B. napus* [28]. For example, overexpression of *NtHSP17.6* in *B. napus* resulted in longer roots and higher proline accumulation under salt stress than in wild-type plants, with OE3 and OE4 transgenic lines showing root lengths of 1.5–1.6 cm compared with 1.0 cm in wild-type plants under NaCl treatment [28]. These examples demonstrate the value of single-gene engineering, although broader and more stable tolerance may require stacking multiple genes that target complementary pathways, such as osmotic regulation, ROS detoxification, ion homeostasis, and protein protection.

Genome editing offers a more precise strategy for improving stress tolerance, especially through targeted modification of negative regulators, stress-responsive TFs, transporters, or regulatory elements. Targeted knockout of negative regulators through CRISPR/Cas9 has shown promise in improving abiotic stress tolerance in *Brassica* crops. CRISPR-mediated editing has already been successfully applied to specific loci in *B. napus*, demonstrating the feasibility of precise genetic improvement in this allopolyploid crop [101]. MYB TFs and ubiquitination-related pathways are also emerging as promising targets for optimizing stress responses, ion balance, and stress-associated protein turnover [1].

However, genome editing in *Brassica* crops is complicated by allopolyploid genome structure. In allotetraploid rapeseed, the AACC genome contains multiple homoeologous copies of many stress-related genes, creating functional redundancy and increasing the complexity of target selection. Editing only one copy may be insufficient if other homoeologs compensate for its function [102]. At the same time, the high sequence similarity among duplicated genes can increase off-target risk, especially when sgRNAs are designed within conserved domains. Additional practical limitations include: (1) low and genotype-dependent transformation and regeneration efficiency, which restricts editing throughput

across diverse breeding materials; (2) the difficulty of achieving simultaneous homozygous mutations in all homoeologous copies in a single generation; (3) insufficient field validation of edited lines across multiple environments and genetic backgrounds; and (4) biosafety and regulatory considerations that vary across national jurisdictions and limit the commercial deployment of edited cultivars. Therefore, effective genome editing in *Brassica* requires homoeolog-aware sgRNA design, careful evaluation of copy number, expression-guided target selection, and validation of edited alleles across stress conditions. Integrating multi-omics data with haplotype analysis can improve target prioritization and reduce the risk of ineffective or pleiotropic edits. Multiplex base editing and prime editing, which enable precise nucleotide substitutions without double-strand breaks, represent promising next-generation tools for polyploid *Brassica* improvement because they may allow simultaneous, copy-specific, and lower-risk modification of stress-related alleles.

5.3. Integrated Physiological and Molecular Breeding Strategies for Stress Tolerance

An effective breeding strategy for abiotic stress tolerance should integrate physiological screening with molecular markers, omics-based candidate selection, and functional validation. Drought and high-temperature stress directly affect plant water balance, photosynthetic performance, and membrane integrity. Relative water content is a useful indicator of water-deficit status and typically decreases under stress [103]. Photosynthetic parameters, including stomatal conductance and chlorophyll fluorescence, provide additional information on stress-induced impairment of photosystem function, particularly under heat stress [103,104]. Antioxidant enzyme activities, including SOD, CAT, and APX, reflect the capacity for ROS detoxification [43,105], whereas MDA accumulation indicates the extent of membrane lipid peroxidation [104,105]. Osmoregulatory substances such as proline and soluble sugars, together with hormone-related changes involving ABA and JA, further help quantify genotype-specific stress adaptation. For combined-stress breeding, however, it is essential to select indicators that respond specifically to stress combinations rather than simply extrapolating from individual-stress responses, because plant responses to combined stresses are often non-additive. Early maturity can also function as a stress-escape strategy by allowing plants to avoid severe heat or drought during flowering, while post-stress phenotypic plasticity, which may be influenced by plant population and developmental context, provides additional information for designing resilient breeding programs.

Molecular marker selection should be linked to pathways with clear physiological relevance. Key marker-associated pathways include: (1) TF families; (2) HSPs; (3) epigenetic markers and other epigenetic regulators associated with stress responses; and (4) hormone-pathway genes. Among heat-tolerant donor materials, six introgression lines derived from *Brassica tournefortii* × *B. juncea*, *B. juncea* × *B. carinata*, and *B. juncea* × *Erucastrum cardaminoides* have been validated as elite genetic resources for commercial breeding under heat stress [57]. These wide-hybrid introgression lines are particularly important because they introduce novel alleles that may be absent from cultivated germplasm.

A practical integrated breeding pipeline can be organized into three stages. The first stage is rapid physiological screening using RWC, photosynthetic parameters, chlorophyll fluorescence, and MDA content to identify tolerant genotypes under controlled or field stress conditions. The second stage is molecular re-screening using qPCR, RNA-seq, or marker assays to evaluate candidate genes and regulatory modules. The third stage is functional validation through genome editing, transgenic analysis, or exogenous treatments. The pre-breeding work (PBW) pipeline can complement this framework by systematically evaluating wild relatives and U-triangle species under heat and drought stress. Overall, a multi-tiered strategy that combines physiological phenotyping, molecular marker analysis,

gene-editing validation, and pre-breeding can accelerate genetic gains while reducing the risk of selecting traits that perform well only under narrow experimental conditions.

5.4. Predictive Modeling and Cost-Effective Breeding Through Genomic Selection

Genomic selection provides a promising framework for improving complex stress-tolerance traits because it uses genome-wide markers to predict breeding values rather than relying only on major QTLs or individual candidate genes. In *Brassica* breeding, GS platforms can integrate whole-genome SNP data with transcriptomic, epigenomic, and metabolomic information to predict stress tolerance in lines or hybrid combinations. Genomic selection is broadly validated in major field crops, but *Brassica*-specific multi-omics prediction models remain largely proposed strategies. Such models can support quantitative evaluation of complex traits such as drought and salt tolerance, especially when stress responses are controlled by many small-effect loci [98]. Integration of histone modification patterns and AS events may further improve model representation of abiotic stress-response pathways. Compared with MAS, which is most effective for a limited number of validated major QTLs, GS captures both major- and minor-effect loci across the genome, making it more suitable for highly polygenic stress-resilience traits. Its reliability is not uniform: prediction performance depends heavily on population relatedness, trait heritability, and environmental stability, and reported accuracy often declines when applied to unrelated breeding panels.

Despite this promise, GS in *Brassica* stress breeding faces several challenges. Prediction accuracy often declines when models are applied to genetically distant populations, and robust prediction of stress tolerance requires multi-environment training datasets because stress-related traits are strongly affected by genotype-by-environment interactions. When training data are derived from a limited number of environments, GS models tend to overfit location-specific effects and perform poorly in new target environments. Incorporating explicit $G \times E$ terms and multi-environment phenotypic records into prediction models is therefore essential for reliable field application. The high cost of multi-omics profiling also limits large-scale implementation in commercial breeding. Nevertheless, omics-informed prediction models can reduce reliance on repeated field screening, which is time-consuming and expensive under multiple stress environments. A GS platform can predict stress performance by establishing genotype-phenotype association databases and updating prediction models as new phenotypic and molecular data become available.

The practical value of GS increases when it is integrated with validated molecular targets and management-based interventions. Candidate genes validated by CRISPR/Cas9 can be incorporated into molecular design breeding pipelines. Metabolomic information related to hormone signaling, including BRs and melatonin, can help optimize exogenous regulation strategies and reduce the need for repeated field hormone-treatment trials. Likewise, nutrient-management strategies, including silicon and sulfur fertilization, have been shown to mitigate salinity and heavy-metal stress by improving photosynthetic pigment content, proline accumulation, and root elongation in *Brassica* crops, and could be integrated into model-based optimization workflows [106]. Ultimately, multi-omics-informed GS has the potential to shorten breeding cycles, reduce phenotyping costs, and support precision breeding for stress-tolerant *Brassica* varieties. However, its successful application will depend on high-quality training populations, standardized stress phenotyping, transparent model validation, and the conversion of predictive signals into biologically interpretable and breeder-usable selection criteria.

6. Challenges and Future Perspectives

6.1. Challenges of Combined Abiotic Stresses in Brassica Crops

Current research on abiotic stress tolerance in plants, including *Brassica* crops, still relies heavily on single-stress models, such as drought, salinity, or high temperature applied independently (Figure 3). Although these models are useful for dissecting individual response pathways, they do not adequately represent field conditions, where crops are commonly exposed to multiple stresses simultaneously or sequentially. For example, drought–heat combinations can intensify membrane lipid peroxidation and accelerate photosynthetic damage beyond the effects observed under either stress alone [19,107]. Combined stresses can activate regulatory responses that are absent under individual stresses, and that multifactorial exposure may produce synergistic negative effects that cannot be predicted from simplified single-stress experiments. Therefore, the continued dominance of single-stress research may lead to incomplete or misleading interpretations of stress-resistance mechanisms. This limitation is particularly important in *Brassica* crops, where most mechanistic studies remain concentrated on single stresses in oilseed rape, whereas combined-stress responses in vegetable *Brassica* species are still poorly explored. This confirms that combined-stress complexity is a universal challenge across crop families. However, *Brassica* crops face additional challenges due to their allopolyploid genome structure, which may further amplify the complexity of combined-stress regulatory networks.

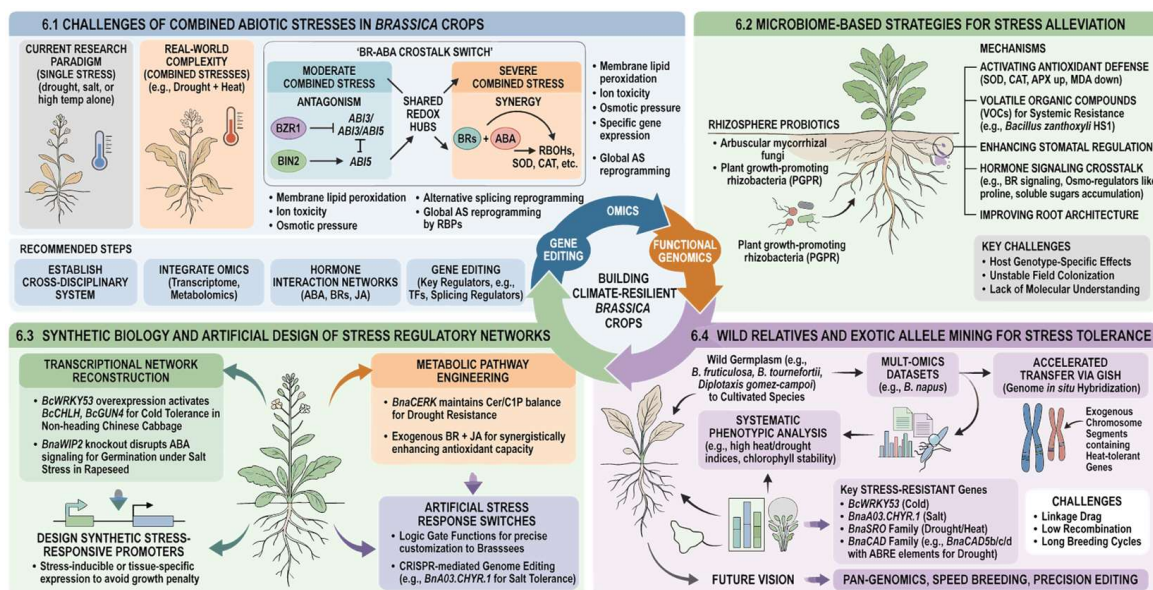


Figure 3. Challenges and emerging strategies for enhancing abiotic stress tolerance in *Brassica* crops. Section 6.1: Limitations of single-stress research paradigms and the need for field-relevant models that capture combined and sequential stress responses. Section 6.2: Microbiome-based strategies, in which rhizosphere probiotics and beneficial microorganisms alleviate stress by activating antioxidant defenses, producing volatile organic compounds (VOCs), improving stomatal regulation, modulating hormone signaling, promoting osmoprotectant accumulation, and enhancing root architecture. Key challenges include host-genotype specificity, unstable field colonization, and limited mechanistic understanding of plant–microbe interactions. Section 6.3: Synthetic biology and artificial design of stress regulatory networks, including transcriptional network reconstruction, synthetic stress-responsive promoters, metabolic pathway engineering, and inducible stress-response switches aimed at balancing stress tolerance with yield maintenance. Section 6.4: Wild relatives and exotic allele mining, emphasizing wild germplasm as reservoirs of stress-resilient alleles, genome in situ hybridization (GISH) for tracking introgressed chromosome segments, and pan-genomic and multi-omics datasets for guided allele transfer.

Under compound stress, plant responses are often nonlinear and involve reconfiguration of hormonal, redox, metabolic, and transcriptional networks. Drought and high temperature, for instance, can reshape hormone signaling by increasing ABA accumulation while simultaneously engaging BR-associated pathways [85]. Rather than acting through a fixed relationship, ABA–BR crosstalk may shift between antagonism and cooperation depending on stress intensity, developmental stage, and tissue context. Under favorable or mild-stress conditions, BR signaling may partially antagonize ABA responses to sustain growth, whereas under severe stress, BR and ABA pathways may converge to activate antioxidant defenses and promote survival [85,108]. This regulatory switch may involve shared redox hubs, including respiratory burst oxidase homologs (RBOHs), which integrate hormonal signals and modulate ROS production as both damaging molecules and stress-signaling intermediates. Salt–alkali stress adds another layer of complexity by simultaneously disturbing ion homeostasis, pH stability, osmotic adjustment, and proline metabolism. In addition, combined stresses can trigger stress-specific transcriptional and alternative-splicing responses; for example, high temperature can suppress cold-responsive genes while enhancing heat-shock protein pathway, and RNA-binding proteins may dynamically regulate AS patterns under multifactorial stress combinations [19,77,107]. However, the transcriptional, epigenetic, and metabolic reprogramming patterns that define drought–heat or salt–alkali responses in *Brassica* crops remain insufficiently characterized.

Addressing combined abiotic stresses requires a cross-disciplinary framework that integrates realistic stress simulation, multi-omics profiling, functional genomics, and predictive breeding. Transcriptomic, epigenomic, proteomic, and metabolomic analyses should be used to characterize hormone interaction networks, particularly ABA, BR, and JA crosstalk, and to test the proposed ABA–BR regulatory switch under drought–heat combinations. Candidate regulators, including stress-responsive TFs, RNA-binding proteins, and splicing factors, should then be validated through gene editing, transgenic approaches, or allele-specific functional analysis. In parallel, experimental platforms should be developed to simulate salt–alkali stress more accurately and to dissect the coupling between ion transport, pH regulation, osmolyte accumulation, and root-system adaptation. More sophisticated stress models are also needed to incorporate growth-stage-specific temperature thresholds, especially in crops such as cabbage, where stress timing strongly influences productivity. Overall, future progress in *Brassica* stress biology will depend on moving from simplified single-stress models toward field-relevant multifactorial systems that can reveal emergent regulatory networks and support climate-resilient breeding.

6.2. Microbiome-Based Strategies for Stress Alleviation

Microbiome-based strategies offer a promising complementary route for improving abiotic stress tolerance in *Brassica* crops. Beneficial rhizosphere microorganisms, including *Arbuscular mycorrhizal* fungi and plant growth-promoting rhizobacteria (PGPR), can enhance drought resistance through multiple mechanisms, including improved nutrient and water acquisition, modulation of hormone signaling, activation of antioxidant defenses, and stimulation of root growth. PGPR can increase the activities of SOD, CAT, and APX, thereby reducing drought-induced ROS accumulation and limiting membrane lipid peroxidation, as reflected by lower MDA levels. For example, *Bacillus zanthoxyli* HS1 induces systemic resistance through the secretion of VOCs, upregulates antioxidant-related gene expression, and improves stomatal regulation, thereby contributing to better water balance under stress [79]. Beneficial microbes may also interact with hormone pathways, including BR-associated signaling, to regulate downstream stress-responsive genes and promote the accumulation of osmoprotectants such as proline and soluble sugars [79,85]. BR-enhanced tolerance may also be linked to PGPR-mediated improvements in root architecture and

symbiotic performance, indirectly supporting water and nutrient uptake under stress [109]. These hormone–microbe interactions can reduce oxidative damage and partially alleviate stress-induced growth inhibition.

Despite this potential, microbiome-mediated stress tolerance in *Brassica* crops remain at an early stage compared with model plants and some cereal systems. Several challenges limit translation to field application. First, microbial effects are often host-genotype specific, meaning that a strain effective in one *Brassica* genotype may not perform similarly in another. Second, the colonization and persistence of introduced microorganisms are unstable under complex field conditions, where soil type, native microbiota, climate, agronomic practice, and plant developmental stage all influence microbial establishment. Third, the molecular dialogue between *Brassica* roots and beneficial microbes remain insufficiently understood, especially regarding hormone crosstalk, microbial VOC perception, immune modulation, and stress-specific root exudation patterns. Future studies should therefore prioritize the identification of *Brassica*-adapted microbial strains, the use of synthetic microbial communities with complementary functions, and the integration of microbiome profiling with transcriptomics, metabolomics, and root phenotyping. Such approaches could support the development of stable microbial products that improve stress resilience without compromising yield or crop quality.

6.3. Synthetic Biology and Artificial Design of Stress Regulatory Networks

Synthetic biology provides an emerging framework for moving beyond single-gene manipulation toward the rational design of stress-responsive regulatory networks. In *Brassica* crops, this approach could be used to engineer transcriptional modules, inducible promoters, metabolic pathways, and genome-editing strategies that enhance stress tolerance while minimizing growth penalties. Several functional studies already illustrate the potential of targeted regulatory modification. For example, overexpression of the cold-induced TF *BcWRKY53* enhances low-temperature tolerance in non-heading Chinese cabbage by activating the chlorophyll biosynthesis genes *BcCHLH* and *BcGUN4* [66]. Similarly, studies on *BnaWIP2* indicate that WIP-family transcriptional regulators can influence ABA-associated germination and salt-stress responses, highlighting this regulatory family as a potential target for stress-related network engineering [110]. These examples suggest that TF families such as WRKY, MYB, and WIP could be used as components of designed stress-responsive modules. Importantly, synthetic stress-inducible or tissue-specific promoters may help avoid the yield penalties often associated with constitutive overexpression of defense genes by activating protective pathways only when and where they are needed.

Metabolic pathway engineering is another promising direction for improving stress resilience. Stress responses can be strengthened by adjusting antioxidant metabolism, lipid signaling, osmoprotectant biosynthesis, or hormone balance. For instance, *BnaC-ERK* in the sphingolipid metabolism pathway positively regulates drought resistance by maintaining the balance between ceramide (Cer) and ceramide-1-phosphate (C1P) [69]. Small-molecule-mediated regulation can also support stress adaptation; exogenous brassinolide and JA can enhance antioxidant capacity and osmolyte accumulation, thereby reducing drought-induced damage [32,85]. In polyploid *Brassica* crops, synthetic biology is particularly attractive because it may help overcome gene redundancy by designing regulatory modules that coordinate multiple homoeologous genes rather than modifying only one copy. Such multiplex regulation could be especially useful for pathways where functional compensation limits the phenotypic effects of single-gene editing.

A more advanced goal is the construction of inducible stress-response switches that integrate transcriptional, epigenetic, and post-transcriptional regulation. Non-coding RNA–TF networks provide useful design targets; for example, miRNA–MYB regulatory

modules in *B. juncea* are involved in drought responses [86], while the SRO gene family participates in stress responses in rapeseed. These multi-level regulatory strategies could be combined with CRISPR-mediated genome editing, such as editing *BnA03.CHYR.1* to enhance salt tolerance [17], to develop more precise and customizable stress-tolerance traits. In the longer term, synthetic stress-response circuits with logic-gate-like behavior could allow plants to activate defense pathways only under specific stress combinations and deactivate them after stress relief. Although this remains largely conceptual in *Brassica*, it provides a useful future direction for balancing stress tolerance with yield potential. Near-term progress will depend on validated stress-inducible promoters, predictable regulatory modules, genotype-specific transformation systems, and careful evaluation of pleiotropic effects under field conditions. Systematic orphan gene profiling in rapeseed provides a candidate resource for future synthetic biology applications [111].

6.4. Wild Relatives and Exotic Allele Mining for Stress Tolerance

Wild relatives and exotic germplasm represent valuable reservoirs of stress-resilient alleles that can broaden the genetic base of cultivated *Brassica* crops. Species such as *B. fruticulosa*, *B. tournefortii*, and members of *Diplotaxis* have been exposed to long-term natural selection under harsh environments and may therefore carry alleles associated with heat, drought, salinity, and poor-soil tolerance. Mining these alleles and transferring them into cultivated backgrounds can help overcome the limitations of narrow genetic diversity in elite breeding pools.

Systematic phenotyping is the first step toward identifying useful wild donors. Evaluation of heat- and drought-stress indices during germination and seedling stages across 25 wild and U-triangle *Brassica* species identified genotypes such as *B. tournefortii* and *Diplotaxis gomez-campoii* that maintained stronger growth under high temperature. Their higher chlorophyll stability and improved osmolyte accumulation compared with cultivated species suggest that they may provide valuable germplasm for discovering heat- and drought-tolerance loci [103]. However, the value of such donors depends not only on stress tolerance but also on cross-compatibility, fertility, linkage drag, and the ability to recover agronomically acceptable progeny.

Genome in situ hybridization (GISH) can support wild allele introgression by tracking alien chromosome fragments in cultivated genetic backgrounds. Instead of ‘infiltration’ of wild alleles, the process should be viewed as targeted introgression of wild chromosome segments carrying potentially useful stress-tolerance loci. Nevertheless, wild germplasm utilization remains challenging because introgressed fragments may carry unfavorable linked traits, recombination between divergent chromosomes may be limited, and repeated backcrossing can extend breeding cycles. These limitations highlight the need to combine cytogenetic tracking with MAS, genomic prediction, and speed breeding.

The combined use of wild alleles, GISH, pan-genomics, and genome editing can accelerate the development of stress-tolerant *Brassica* germplasm. In the future, pan-genomics can identify presence–absence variation and structural variants absent from elite cultivars, speed breeding can shorten introgression cycles, and precision genome editing can recreate favorable wild alleles without transferring large deleterious chromosomal segments. Together, these strategies could enable more efficient use of wild diversity while reducing linkage drag.

7. Conclusions

Substantial progress has been made in understanding the physiological, genetic, epigenetic, and multi-omics basis of abiotic stress tolerance in *Brassica* crops. Research has revealed key mechanisms involving stress perception, calcium and MAPK signaling,

hormone crosstalk, osmotic adjustment, ROS detoxification, photosynthetic protection, transcriptional regulation, epigenetic modification, and polyploid genome evolution. However, several bottlenecks continue to limit translation into breeding. First, the conversion of GWAS-identified loci into validated functional markers remains inefficient, particularly because many significant SNPs are located in non-coding regions and are supported mainly by linkage rather than causal evidence. Second, multi-omics datasets are still used largely for descriptive analysis, whereas predictive and mechanistic integration remains underdeveloped. Third, most studies focus on drought and salinity as single stresses, while combined stresses, which better reflect field conditions, remain insufficiently studied despite their synergistic and non-additive effects.

To overcome these limitations, future *Brassica* stress-resilience research should prioritize systems-level strategies that connect molecular mechanisms with breeding outcomes. We propose an explicit three-tier roadmap for future research priorities. In the short term, priority should be given to standardizing multi-environment phenotyping protocols and generating replicated $G \times E$ datasets to stabilize QTL/GWAS signals and improve genomic prediction reliability. In the medium term, research should focus on functional validation of key regulatory modules, multi-omics causal inference, and translational tools including precision genome editing and wild allele introgression. In the long term, the field should move toward integrated systems breeding platforms that combine multi-omics prediction, genome design, and field-validated stress-resilient cultivars to sustain productivity under changing climate conditions. In parallel, wild relatives, microbiome-based approaches, synthetic biology, and speed breeding can diversify the sources and strategies available for developing stress-resilient germplasm. The convergence of systems biology, functional genomics, predictive breeding, and targeted allele deployment will be essential for transforming mechanistic knowledge into climate-resilient *Brassica* varieties capable of sustaining productivity under increasingly unstable environmental conditions.

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Abbreviations

The following abbreviations are used in this manuscript:

ABA	Absciscic acid
AS	Alternative splicing
BR	Brassinosteroid
CAT	Catalase
CSSLs	Chromosome segment substitution lines

CWR	Crop wild relative
DAMs	Differentially accumulated metabolites
DAPs	Differentially accumulated proteins
DEGs	Differentially expressed genes
DHAR	Dehydroascorbate reductase
eGWAS	Expression genome-wide association study
ETH	Ethylene
G×E	Genotype-by-environment
GISH	Genome in situ hybridization
GR	Glutathione reductase
GSH	Glutathione
GWAS	Genome-wide association study
HAT	Histone acetyltransferase
HSP	Heat shock protein
JA	Jasmonic acid
MAS	Marker-assisted selection
MDA	Malondialdehyde
MeJA	Methyl jasmonate
miRNA	MicroRNA
mGWAS	Metabolite genome-wide association study
NILs	Near-isogenic lines
PAL	Phenylalanine ammonia-lyase
PBW	Pre-breeding work
PGPR	Plant growth-promoting rhizobacteria
POD	Peroxidase
PSII	Photosystem II
QTL	Quantitative trait locus
RILs	Recombinant inbred lines
RLKs/RLPs	Receptor-like kinases and proteins
ROS	Reactive oxygen species
SA	Salicylic acid
SOD	Superoxide dismutase
SSI	Stress susceptibility index
SSR	Simple sequence repeat
STI	Stress tolerance index
TF	Transcription factor
VOCs	Volatile organic compounds
WAK	Wall-associated kinase
WGCNA	Weighted gene co-expression network analysis

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