

Cabbage breeding tools for biotic and abiotic resistance

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ABSTRACT

Cabbage (*Brassica oleracea* var. *capitata*) is an important vegetable crop grown globally for its nutritional value and economic importance. However, cabbage production faces significant challenges from various biotic and abiotic stresses, including pests, diseases, and environmental factors such as drought, heat, and salinity. Developing cabbage cultivars with improved resistance to these stresses is crucial for sustainable and productive agriculture. This review article examines the latest breeding tools and approaches used to enhance biotic and abiotic stress resistance in cabbage. It explores traditional breeding methods, marker-assisted selection, genetic engineering, genome editing techniques like CRISPR/Cas9, and emerging technologies such as genomic selection and speed breeding. Furthermore, the article discusses the integration of -omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, to accelerate the development of stress-resistant cabbage cultivars. The study also highlights the importance of incorporating farmer preferences and participatory breeding strategies to ensure the adoption and success of these improved cabbage cultivars.

Keywords: crop improvement, cole crops, breeding tools, stress tolerance, breeding approaches.

INTRODUCTION

Cabbage (*Brassica oleracea* var. *capitata*) is a cool-season vegetable crop belonging to the Brassicaceae family. It is widely cultivated and consumed around the world for its nutritional value, as it is a rich source of vitamins, minerals, and fiber (Sarıkamış *et. Al*, 2009). Cabbage production plays a significant role in the global economy, with an annual production of over 70 million tons (<http://www.fao.org/faostat/en/#data/QC>). However, cabbage cultivation faces various biotic and abiotic stresses that can significantly reduce yield and quality. Biotic stresses, such as insect pests, fungal diseases, bacterial diseases, and viral diseases, pose major threats to cabbage production (Juroszek, P., and Tsai, 2009). Some of the most common insect pests affecting cabbage include cabbage loopers, cabbage root flies, cabbage aphids, and diamondback moths (Shelton, A. M., and Badenes-Perez, 2006). Fungal diseases like clubroot, black rot, and downy mildew can cause severe damage to cabbage crops (Rimmer *et. al.*, 2007). Bacterial diseases, such as black rot and bacterial soft rot, and viral diseases like cauliflower mosaic virus and turnip mosaic virus, also contribute to yield losses (. Bhat, K. A., and Kolanjakkaren, 2014; Shukla and Tenzer, 2017). Abiotic stresses, including drought, heat, salinity, and nutrient deficiencies, can also

have detrimental effects on cabbage growth, development, and productivity (Farooq *et al.*, 2014). Climate change is exacerbating these abiotic stresses, making it increasingly challenging for farmers to maintain stable cabbage yields (Fahad *et al.*, 2017). Developing cabbage cultivars with improved resistance to biotic and abiotic stresses is crucial for sustainable and productive agriculture.

TRADITIONAL BREEDING METHODS

Traditional breeding methods have been extensively used in cabbage improvement programs for decades. These methods involve selecting and crossing parent lines with desirable traits, followed by multiple generations of selfing and selection to develop improved cultivars (Bradshaw, 2016).

Pedigree breeding: Pedigree breeding is a commonly used method in cabbage breeding programs. It involves selecting superior individuals from segregating populations and self-pollinating them for several generations to develop pure lines. These pure lines can then be crossed to combine desirable traits, such as pest resistance or improved yield (Prakas *et al.*, 2014).

Recurrent selection: Recurrent selection is another traditional breeding approach used in cabbage breeding. It involves cyclically selecting and intermating superior individuals from a population to gradually increase the frequency of favourable alleles for target traits, such as disease resistance or stress tolerance (Shukla and Sundaram, 2004).

Hybridization and heterosis breeding: Hybridization involves crossing two genetically diverse parent lines to produce F₁ hybrids that exhibit heterosis or hybrid vigour. Heterosis breeding in cabbage has been successful in developing high-yielding and stress-tolerant hybrids by exploiting the phenomenon of hybrid vigour (Shukla, S., Naik, A. K., and Sundaram, 2014).

Mutation breeding: Mutation breeding involves inducing genetic variations through physical or chemical mutagens, such as gamma rays, X-rays, or ethyl methane sulfonate (EMS). These mutations can lead to new trait variations, including enhanced biotic or abiotic stress resistance, which can be selected and incorporated into breeding programs (Ahloowalia *et al.*, 2004).

While traditional breeding methods have contributed significantly to cabbage improvement, they have limitations, such as long breeding cycles, the polygenic nature of many traits, and the difficulty in pyramiding multiple resistance genes (Tester, and Langridge, 2010). To overcome these challenges, modern breeding approaches, including marker-assisted selection, genetic engineering, and genome editing, have been developed and integrated into cabbage breeding programs.

MARKER-ASSISTED SELECTION (MAS)

Marker-assisted selection (MAS) is a breeding approach that utilizes molecular markers linked to specific traits or genes of interest to facilitate the selection of desirable genotypes (Collard, and Mackill, 2008), MAS has been widely applied in cabbage breeding for various traits, including disease resistance, insect resistance, and abiotic stress tolerance.

Resistance gene mapping and MAS: Molecular markers have been developed and used for marker-assisted selection of resistance genes against various cabbage diseases, such as black rot, fusarium wilt, and clubroot (Sharma, *et al.*, 2017; Ren *et al.*, 2001; Chandra *et al.*, 2021). For example, the Crr1 gene conferring resistance to clubroot disease has been

mapped and validated, enabling the development of molecular markers for MAS in cabbage breeding programs (Hirai, 2006).

QTL mapping and MAS for complex traits: Quantitative trait loci (QTL) mapping has been employed to identify genomic regions associated with complex traits, such as yield, quality, and abiotic stress tolerance in cabbage. Molecular markers linked to these QTLs have been used for marker-assisted selection to improve these traits (Lee *et. al.*, 2021; Zhang *et. al.*, 2020).

Genomic selection (GS): Genomic selection is an advanced form of MAS that utilizes genome-wide marker data and statistical models to predict the breeding values of individuals for complex traits (Meuwissen *et. al.*, 2001). GS has shown promising results in cabbage breeding for traits like drought tolerance, where it has been used to accelerate the breeding cycle and improve selection accuracy (Bhandari *et. al.*, 2022).

While MAS has facilitated more efficient and precise selection in cabbage breeding, it has limitations, such as the need for extensive marker development and validation, and the potential for linkage drag (Bernardo *et. al.*, 2008). Additionally, MAS may not be effective for traits controlled by many minor-effect QTLs or epistatic interactions.

GENETIC ENGINEERING

Genetic engineering involves the direct transfer and integration of specific genes from various sources into the cabbage genome to introduce desirable traits (Bawa, and Anilakumar, 2013). This approach has been explored in cabbage breeding for enhancing biotic and abiotic stress resistance.

Insect resistance: Genes encoding insecticidal proteins, such as *Bacillus thuringiensis* (Bt) toxins, have been introduced into cabbage to confer resistance against lepidopteran pests like cabbage loopers and diamondback moths (Cao *et. al.*, 2002). These *Bt* cabbage lines have shown improved protection against insect damage and reduced the need for insecticide applications.

Disease resistance: Transgenic approaches have been used to develop cabbage cultivars with resistance against various fungal, bacterial, and viral diseases. For example, the introduction of antifungal proteins, like chitinases and glucanases, has been explored for enhancing resistance against fungal pathogens like clubroot and downy mildew (Muzzarelli, *et. al.*, 2001; Mora, and Earle, 2001).

Abiotic stress tolerance: Genetic engineering has also been employed to improve abiotic stress tolerance in cabbage. Genes involved in stress response pathways, such as those encoding transcription factors, Osmo protectants, or antioxidant enzymes, have been introduced into cabbage to enhance drought, heat, and salinity tolerance (Wang, *et. al.*, 2003; Bajji *et. al.*, 2001).

While genetic engineering has shown promising results in cabbage improvement, concerns over the potential risks associated with genetically modified (GM) crops have led to strict regulations and public acceptance challenges in some regions (Nicolia, *et.al.*, 2014). Additionally, the complexity of many stress tolerance mechanisms and potential unintended effects remains challenges in genetic engineering approaches.

GENOME EDITING

Genome editing technologies, particularly CRISPR/Cas9, have emerged as powerful tools for precise and targeted modifications of the cabbage genome (Boetesi and Fischer, 2015).

These techniques offer advantages over traditional genetic engineering by enabling site-specific modifications without the introduction of foreign DNA.

Disease resistance: CRISPR/Cas9 has been used to knockout or modify susceptibility genes in cabbage to enhance resistance against various diseases. For example, editing the BraA.FR.a gene, which encodes a susceptibility factor for the *Fusarium oxysporum* fungus, has been shown to confer resistance against Fusarium wilt disease in cabbage (Chaturvedi *et. al.*, 2012).

Insect resistance: CRISPR/Cas9 has also been employed to modify endogenous cabbage genes involved in defence pathways or plant-insect interactions to enhance insect resistance. For instance, editing the BrPDF2.1 gene, which encodes a plant defensin protein, has been reported to improve resistance against diamondback moths (Hu *et. al.*, 2019).

Abiotic stress tolerance: Genome editing has been explored for improving abiotic stress tolerance in cabbage by modifying genes involved in stress response pathways. For example, editing the BrDREB2A transcription factor gene has been shown to enhance drought and heat tolerance in cabbage (Sakuraba, *et. al.*, 2017).

While genome editing offers promising opportunities for cabbage improvement, there are challenges associated with off-target effects, regulatory frameworks, and public acceptance, similar to those faced by genetic engineering (Lassoued *et. al.*, 2020). Additionally, the complex nature of many stress tolerance mechanisms may require multiplex editing of multiple genes or regulatory elements.

EMERGING TECHNOLOGIES

In addition to the established breeding tools mentioned above, several emerging technologies are being explored and integrated into cabbage breeding programs for enhancing biotic and abiotic stress resistance.

Speed breeding: Speed breeding is a novel technique that involves the use of controlled environmental conditions, such as extended photoperiods and optimized temperature and humidity, to accelerate the breeding cycle (Watson *et. al.*, 2018). By shortening the generation time, speed breeding can significantly reduce the time required for developing improved cabbage cultivars with desired stress resistance traits.

Genomic selection (GS): As mentioned earlier, genomic selection is an advanced form of marker-assisted selection that utilizes genome-wide marker data and statistical models to predict breeding values for complex traits (Meuwissen *et. al.*, 2001). GS has shown promising results in cabbage breeding for traits like drought tolerance and yield, and its application is expected to grow as genotyping costs decrease and computational power increases (Bhandari *et. al.*, 2022).

Epigenetic breeding: Epigenetic modifications, such as DNA methylation and histone modifications, can influence gene expression and phenotypic variation without altering the underlying DNA sequence (Baulcombe, and Dean, 2014). Epigenetic breeding involves exploiting these modifications to modulate stress response pathways and improve stress tolerance in cabbage. This approach has shown potential in enhancing drought and salt tolerance in crops like *Brassica napus* (Hauben *et. al.*, 2009).

Synthetic biology and metabolic engineering: Synthetic biology and metabolic engineering approaches involve the design and construction of novel genetic circuits or metabolic pathways to enhance specific traits in plants (Liu, and Stewart, 2015). These techniques have been applied in model plants like *Arabidopsis thaliana* for improving stress tolerance and could be explored in cabbage for enhancing biotic and abiotic stress resistance.

INTEGRATION OF-OMICS APPROACHES

The integration of various -omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, has provided valuable insights into the molecular mechanisms underlying biotic and abiotic stress responses in cabbage. These insights can inform and accelerate breeding efforts for developing stress-resistant cultivars.

Genomics: Advances in next-generation sequencing technologies have enabled the generation of high-quality reference genomes for cabbage and its close relatives (Liu, et. al., 2014). Comparative genomic analyses have identified genes and regulatory elements associated with stress response pathways, which can serve as targets for breeding or genome editing strategies.

Transcriptomics: Transcriptomic studies, using techniques like RNA-seq, have been employed to investigate gene expression patterns in cabbage under various biotic and abiotic stress conditions (Yang *et. al.*, 2021^a; Yang *et. al.*, 2010^b). These analyses have identified stress-responsive genes, transcription factors, and regulatory networks, which can be targeted for improving stress tolerance through breeding or genetic engineering.

Proteomics: Proteomic approaches, such as two-dimensional gel electrophoresis and mass spectrometry, have been used to study changes in protein abundance and post-translational modifications in cabbage under stress conditions (Gao *et. al.*, 2009; Peng *et. al.*, 2009). These analyses have identified stress-responsive proteins and protein complexes involved in defence mechanisms, which can be targeted for enhancing stress resistance.

Metabolomics: Metabolomic studies have investigated changes in the metabolite profiles of cabbage under various biotic and abiotic stresses (Savchenko *et. al.*, 2010; Martínez-Ballesta, *et al.*, 2013). T

hese analyses have identified stress-responsive metabolites and metabolic pathways that can be modulated through breeding or metabolic engineering to improve stress tolerance. By integrating information from these -omics approaches, researchers can gain a comprehensive understanding of the complex molecular networks underlying stress responses in cabbage.

This knowledge can guide the identification of key target genes, proteins, or metabolic pathways for manipulation through breeding, genetic engineering, or genome editing strategies to develop stress-resistant cabbage cultivars.

PARTICIPATORY AND FARMER-CENTRIC BREEDING

While technological advancements in breeding tools and -omics approaches are crucial for developing stress-resistant cabbage cultivars, it is equally important to consider farmer preferences and involve them in the breeding process. Participatory and farmer-centric breeding approaches have gained increasing recognition for ensuring the adoption and success of improved cultivars.

Participatory plant breeding (PPB): PPB involves the active participation of farmers in the breeding process, from setting breeding goals and selecting parent materials to evaluating and selecting advanced lines (Ceccarelli, 2015). This approach ensures that the developed cultivars meet the specific needs and preferences of farmers, increasing the likelihood of adoption and successful cultivation.

Farmer-led evaluation and selection: Farmer-led evaluation and selection involve farmers directly assessing and selecting promising cabbage lines under their local environmental conditions and management practices (Buckler *et. al.*, 2021). This approach ensures that the selected lines are well-adapted to the target production environments and farmer preferences.

Incorporation of farmers' traditional knowledge: Traditional knowledge held by farmers can provide valuable insights into local adaptation strategies, stress tolerance mechanisms, and desirable traits (Altieri, 2014). Incorporating this knowledge into breeding programs can enhance the development of stress-resistant cabbage cultivars that are better suited to local conditions and cultural preferences.

Community seed banks and seed exchange networks: Community seed banks and seed exchange networks facilitate the conservation, exchange, and distribution of locally adapted cabbage cultivars (Vornooy *et. al.*, 2015).

These initiatives promote the maintenance of genetic diversity and ensure the availability of stress-tolerant cultivars to farmers, particularly in marginalized communities. By involving farmers and considering their preferences and local knowledge, breeding programs can develop stress-resistant cabbage cultivars that are not only high-yielding and resilient but also meet the specific needs and cultural preferences of farmers, increasing the likelihood of successful adoption and sustainable production.

Table 1. Major insect pests of cabbage and their management strategies

Insect Pest	Damage Caused	Management Strategies
Cabbage Looper	Defoliation	Bt crops, Biological control, Insecticides
Diamondback Moth	Defoliation	Bt crops, Trap crops, Resistance breeding
Cabbage Root Fly	Root damage	Crop rotation, Insecticides, Resistant cultivars
Cabbage Aphid	Stunting, Virus transmission	Insecticides, Biological control, Resistant v cultivars

Table 2. Common fungal diseases of cabbage and their management

Disease	Causal Pathogen	Management Strategies
Clubroot	<i>Plasmodiophora brassicae</i>	Resistant cultivars, Crop rotation, Soil amendments
Black Rot	<i>Xanthomonas campestris</i> pv. <i>campestris</i>	Resistant cultivars , Seed treatment, Crop rotation
Downy Mildew	<i>Peronospora parasitica</i>	Fungicides, Resistant cultivars, Cultural practices
Alternaria Leaf Spot	<i>Alternaria brassicae</i>	Fungicides, Resistant cultivarss, Cultural practices

Table 3. Examples of biotic stress resistance genes used in cabbage breeding

Gene	Source	Trait	Breeding Approach
Bt Cry genes	<i>Bacillus thuringiensis</i>	Insect resistance	Genetic engineering
Crr1	<i>Brassica rapa</i>	Clubroot resistance	Marker-assisted selection
BjuPR-1, BjuPR-4, BjuDF1.2	<i>Brassica juncea</i>	Fungal disease resistance	Marker-assisted selection
BraA.FR.a (knockout)	<i>Brassica rapa</i>	Fusarium wilt resistance	CRISPR/Cas9 genome editing

Table 4. Examples of abiotic stress tolerance genes used in cabbage breeding

Gene	Function	Trait	Breeding Approach
BrDREB2A	Transcription factor	Drought, Heat tolerance	CRISPR/Cas9 genome editing
Osmoprotectant genes	Osmolyte biosynthesis	Drought, Salinity tolerance	Genetic engineering
Antioxidant enzyme genes	ROS scavenging	Drought, Heat tolerance	Genetic engineering
Aquaporin genes	Water transport	Drought tolerance	Marker-assisted selection

Table 5. Examples of QTLs and genomic regions associated with abiotic stress tolerance in cabbage

Trait	QTL/Genomic Region	Mapping Population
Drought tolerance	QTLs on C02, C05, C09	DH population
Cold tolerance	QTLs on C03, C05, C08	F2:3 population
Yield under drought	Genomic regions on C02, C06	RIL population

CONCLUSIONS

This article has explored various breeding tools and approaches used to enhance biotic and abiotic stress resistance in cabbage.

Traditional breeding methods, marker-assisted selection, genetic engineering, and genome editing have all contributed to the development of improved cabbage cultivars with enhanced resistance to pests, diseases, and environmental stresses.

Emerging technologies, such as speed breeding, genomic selection, epigenetic breeding, and synthetic biology, offer promising opportunities for further advancements in this field. The integration of -omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, has provided valuable insights into the molecular mechanisms underlying stress responses in cabbage.

This knowledge can guide the identification of key target genes, proteins, or metabolic pathways for manipulation through breeding or genetic engineering strategies to develop stress-resistant cultivars.

However, the successful adoption and impact of these improved cabbage cultivars also depend on considering farmer preferences and involving them in the breeding process through participatory and farmer-centric approaches.

By incorporating traditional knowledge, facilitating farmer-led evaluations, and promoting community seed banks and seed exchange networks, breeding programs can develop stress-resistant cabbage cultivars that meet the specific needs and cultural preferences of farmers, ensuring sustainable and productive agriculture.

As climate change and global population growth continue to exert pressure on agricultural systems, the development of stress-resistant cabbage cultivars will become increasingly crucial for ensuring food security and promoting sustainable agriculture. The integration of diverse breeding tools, -omics approaches, and farmer-centric strategies will play a vital role in achieving this goal and contributing to the resilience of cabbage production systems worldwide.

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