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Next-generation biotechnology and breeding strategies for the development of biotic stress-resistant sugar beet

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Abstract: Sugar beet is a high-sucrose crop primarily used for sugar production, and it is now gaining attention as a promising source for bioethanol production and cattle feed. This crop is also utilized for the extraction of pectin and the production of molasses. Despite its utilization across a wide range of industries, abiotic and biotic stress factors greatly reduce the productivity of the cash crop sugar beet. In this paper, we focus on the development of biotic stress-resistant sugar beet plants through various classical and biotechnological applications. In recent decades, omics technologies, next-generation sequencing, gene editing/silencing, and overexpression techniques have been utilized to explore the molecular mechanisms underlying biotic stress responses in sugar beet. Although conventional breeding is a time-consuming and lengthy procedure, it still maintains its relevance in the sugar beet sector. Türkiye's first disease-resistant local and national sugar beet varieties, Türkşeker 2023 and Türkşeker 2053, are products of classical breeding and have begun to be cultivated in the country. Rhizomania, Cercospora leaf spot, cyst nematode, Rhizoctonia root rot, and sugar beet root maggot are the main biotic stress factors that negatively affect plant health and agricultural productivity in sugar beet. Currently, the development of new traits for resistance to fungi, viruses, pests, and nematodes is most commonly expected in sugar beet production. The integration of molecular markers in conventional breeding procedures, as well as next-generation biotechnological approaches including transgenic and CRISPR/Cas9 technologies, has provided a reliable means of improving biotic stress resistance in sugar beet. Regulatory frameworks regarding genetically modified plants, including sugar beet, are crucial for food safety and environmental impact and require discussion. The present review summarizes the applications of next-generation biotechnological techniques, omics technologies, and traditional breeding strategies to improve biotic stress resistance in sugar beet.

Key words: *Beta vulgaris*, biotic stress, biotechnological techniques, traditional breeding, sugar beet disease

1. Introduction

After sugarcane, sugar beet is widely recognized for its sugar production across many countries, including temperate regions of the world. It supplies approximately 21% of the global sugar demand (Bulut et al., 2023). In addition to being one of the main components of human nutrition, beet crop wastes are widely used for bioethanol production, as fossil fuel substitutes, as biofertilizers, and as food additives. Biotic and abiotic stresses affect all plants, including sugar beet, causing a decrease in yield. In sugar beet, viruses, nematodes, bacteria, and fungi are responsible for major diseases such as rhizomania, Cercospora leaf spot, cyst nematode, Rhizoctonia root rot, and sugar beet root maggot. These diseases negatively affect sugar production by altering the phenotypes, physiology, biochemical composition, and molecular structures of the sugar beet plant. Global sugar consumption is expected to grow at an annual rate of approximately 1.4%, reaching 203 million tons by 2029, driven by population and income growth. Therefore, global sugar production is expected to grow by 15%, rising from 176 million tons in the base period to 203 million tons by 2029 (OECD/FAO, 2020). As

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a result, sugar beet cultivation is expected to expand due to global sugar demand. Examining the effects of biotic stress on sugar beet through specific examples, Cercospora leaf spot outbreaks in 2015 caused yield decreases of almost 30% in multiple European locations (Rangel et al. 2020). Reduced root weight and sugar content are the primary causes of yield losses linked to rhizomania, which have been found to range from 69% to 96% (Tamada et al., 1999; Tamada et al., 2021). Sugar beet cultivation underwent an important shift between 2010 and 2020, and producers were able to access high-yielding, tolerant varieties. Therefore, earlier estimates of the economic impact of beet cyst nematode are probably outdated, even if in 1999 it was projected that the yearly economic damage in Europe could reach €90 million (Müller, 1999; Daub, 2021). The degree of *Rhizoctonia solani* infection–related yield loss varies from field to field and can reach 60% (Barreto et al., 2021). With an annual agricultural value of $3 billion in the United States, the sugar beet root maggot is a destructive pest of one of only two plant species in the world from which sugar is commonly produced (Acharya et al., 2024). However, these biotic stress factors that negatively affect sugar beet production have paved the way for the use of next-generation molecular biological methods together with classical breeding. Traditional breeding approaches frequently fail to successfully combat rapidly evolving pathogens because of their time-consuming nature, limited genetic diversity, and low precision. These limitations necessitate the use of modern molecular breeding techniques. In this review, we first discuss molecular biological techniques, including genome editing, transgenics, and omics technologies, which are frequently used to develop sugar beet varieties resistant to biotic stresses. Research and development carried out to date using these technologies in sugar beet have been explored. Biotechnologically advanced molecular methods combined with traditional methods have dramatically changed sugar beet cultivation by identifying novel traits related to various biotic stresses. Therefore, a wide range of information is presented on traditional and molecular marker-assisted breeding programs for sugar beet. Sugar beet production faces major threats from biotic stress factors that cause diseases, which are also examined individually. Biotic stress, caused by a complex variety of viral, bacterial, fungal, and nematode pathogens, is still one of the major limiting factors in achieving optimal crop yield and viability; this continuing challenge has triggered the need to investigate molecular and biotechnological strategies to provide targeted and efficient solutions beyond the limitations of traditional breeding. Finally, legal regulations arising from the use of next-generation biotechnological techniques in sugar beet were evaluated on a country-by-country basis. In addition, potential solutions for growing disease-resistant sugar beet in the future were examined in depth. This article was created during the breeding process of Türkiye's first disease-resistant local and national sugar beet varieties, Türkşeker 2023 and Türkşeker 2053. It is thought that this article can play an important role in the development of new sugar beet varieties resistant to biotic and abiotic stresses in Türkiye and the world.

2. Biotechnology techniques for development of biotic stress-resistant sugar beet

2.1. Gene editing technologies

Next-generation biotechnological progress, such as gene editing with clustered regularly interspaced short palindromic repeats (CRISPR), transcription activator-like effector nucleases (TALEN), and zinc finger nucleases (ZFN) methodologies, is a promising solution to problems arising from biotic and abiotic stressors in sugar beet. As of now, these advanced gene editing techniques are used, which are more precise and amenable for developing resistant varieties against different biotic stresses (Pattanayak et al., 2023). ZFN genome editing tools are among the earliest generations of genetic modification technology in sugar beet to produce targeted host resistance against biotic stresses. ZFNs are engineered zinc finger (ZF) proteins that target triplet DNA sequences. Each zinc finger is linked to the FokI nuclease domain that cleaves DNA (Mall et al., 2021; Pattanayak et al., 2023). To create two-sided double-stranded breaks (DSBs) in DNA, adjacent ZFNs must bind to each of the opposite target strands. In turn, this binding promotes dimerization of the *Fok*I nuclease domains and DNA cleavage. DSBs may then induce DNA repair pathways in the cell. ZFNs have also been applied to various plants in the literature, such as tobacco (Townsend et al. 2009), maize (Shukla et al. 2009), *Arabidopsis thaliana* (Osakabe et al. 2010), and soybean (Bonawitz et al. 2019).

Xanthomonas, the bacterium from which TALEN is derived, is highly harmful and causes major problems in a variety of crops. Using its type III secretion system, Xanthomonas translocates transcription activator-like (TAL) proteins into the cytoplasm of the host cell during infection. TALs are primarily composed of three structures: transcriptional activation domains, nuclear localization signals, and the core tandem repeat domain. DNA binding is carried out by highly conserved repeat domains, which typically have a length of 33–35 amino acids (AA). The hypervariable residues located at the 12th and 13th positions of the repeat domain determine which target DNA the TAL protein will bind. The activator domain of TALENs is replaced by FokI, making it a target-specific genome editing tool. TALENs are employed in pairs to produce double-strand breaks via FokI. A spacer domain is used to separate each pair once they have bound to the opposing strands of the target sequence

(Zhang et al., 2014). Information on the molecular functions related to biotic stress in sugar beet would be valuable for exploring critical genes targeted for genome editing technologies such as TALEN (Misra et al., 2022a). Moreover, the identification of BTB protein genes such as *BvBTB1*, *BvBTB2*, and *BvBTB3*, as well as their role in sugar beet defense against *Cercospora beticola* infection, provides valuable insights for developing biotic stress-resistant sugar beet varieties using TALEN technology (Misra et al., 2022b). Plant defense might be increased through modification of susceptibility-related genes by TALEN technology, resulting in the generation of sugar beet varieties that resist biotic stresses.

The last 10 years have seen significant advancements in biotechnology, such as CRISPR technology, which presents a huge opportunity to create sugar beet cultivars resistant to biotic stress (Misra et al., 2023). The technology provides precise edits to the stress-resistance genes of sugar beet, making it more resilient to biotic stress conditions and reducing risks from off-target effects. In addition, the targeted use of CRISPR technology can enhance adaptability to adverse environments such as alkali and heavy metal stresses by targeting sugar beet genes related to alkalinity stress responses and cold tolerance (Misra et al., 2023; Pattanayak et al., 2023). This approach aims to enhance the tolerance of sugar beet to adverse abiotic conditions, ultimately contributing to sustainable and high-yield crop production. CRISPR technology can be employed to generate insect-resistant sugar beet varieties for biotic stress management. Such transgenes and other synthetic constructs targeting susceptibility-related genes, such as *Cry1Ab* and *Cry1C*, can be used to transform sugar beet plants for natural resistance against biotic stresses, thus modifying the plant immune system and providing protection to the crop from damage or loss (Misra et al., 2023). Genes related to sugar beet responses to abiotic and biotic stresses can be directly targeted using a combination of next-generation sequencing (NGS), gene editing, or overexpression methods. The two primary components of the CRISPR/Cas9 system are guide RNA (gRNA) and the Cas9 protein. A DNA endonuclease that is RNA-dependent, Cas9 binds to gRNA and forms a complex with it. The 20 nucleotides that make up the gRNA act as a recruiting signal for Cas9 and are complementary to the target DNA sequence. CRISPR/Cas9 differs significantly from other genome-editing technologies in that it uses RNA-DNA interactions rather than DNA-protein interactions to recognize the target DNA sequence. A particular protospacer adjacent motif (PAM), located at the 3′ end of target sequences, is the site where Cas9 and gRNA must attach for the process to work. The most commonly used PAM for Cas9 from *Streptococcus pyogenes* in genome editing is 5′-NGG-3′. Cas9 causes double-strand breaks (DSBs) in the DNA target, which triggers DNA repair. Nonhomologous end joining (NHEJ) and homology-directed repair (HDR) mechanisms are used to create gene knockouts and gene insertions or modifications, respectively. When NHEJ arbitrarily adds or removes nucleotides inside a coding region, it results in a frameshift mutation, causing a gene knockout. Hence, for all plants, including sugar beet, the NHEJ repair process is the most widely used and effective way to fix DNA damage. Conversely, precise insertion of predefined sequences derived from a donor DNA template is possible using the HDR approach. Applications of HDR in plants have been limited because of its poor editing efficiency. Our team's former review paper provides comprehensive details on all genome editing methods and their underlying molecular frameworks (Baloglu et al., 2022).

Plants have successfully improved resistance against numerous viral infections through the use of CRISPR-based gene editing. In a study conducted by Yıldırım et al. (2023), the transient expression of gRNA/Cas9 constructs targeting the expressed genes of BCTIV (Beet curly top Iran virus, genus *Becurtovirus)* in sugar beet leaves was used to evaluate the constructs' effectiveness. Using BCTIV and gRNA/Cas9 in tandem, sugar beets showed significantly reduced levels of disease symptoms, biomass loss (13%–45%), and systemic infection (10%–55%). However, following BCTIV agro-inoculation, nearly all positive control sugar beets (90%) had severe disease signs and systemic infection, along with a significant drop in biomass (68%). The gRNA/Cas9-mediated insertion or deletion (INDEL) mutations at the BCTIV genome's target loci showed significant efficiency (53%–88%), particularly for those targeting the movement gene of BCTIV and the overlapping region with genes regulating ssDNA and the capsid. This study offers the first demonstration of the ability of gRNA/Cas9 modules to prevent *Becurtovirus* infection in sugar beets using a transient expression assay. In another study, a set of BNYVV-based vectors allowing efficient expression of four recombinant proteins were developed and expressed in the model plant *Nicotiana benthamiana* and the native host, sugar beet (Jiang et al., 2019). The subcellular colocalization of proteins in the leaf, root, and stem tissues of plants with systemic infection was examined using these vectors. Furthermore, *NbPDS* guide RNAs for genome editing were delivered to transgenic plants expressing Cas9 via BNYVV-based vectors, resulting in a photobleached phenotype in systemically infected leaves. These vectors could facilitate genomic research and the expression of various proteins in sugar beet. In a recent article, Rollwage et al. (2024) reported the discovery of the *Beta vulgaris Bv-eIF(iso)4E* susceptibility factor for the beet chlorosis virus (*Polerovirus*) that carries VPg. Bv-eIF(iso)4E and the putative BChV-VPg interacted physically, while Bv-eIF4E and Bv-eIF(iso)4E isoforms, closely associated with

beet mild yellowing virus (BMYV), also interacted with VPg. Since mutations within the expected cap-binding pocket of Bv-eIF(iso)4E resulted in a loss of interaction, it was shown that these VPg-eIF interactions within the *Polerovirus*–beet pathosystem were highly specialized. To examine the potential of eIFs as a resource for resistance to beet-infecting *Poleroviruses*, sugar beet plants were subjected to genome editing using CRISPR/Cas9, which led to the deletion of various eIFs. *Bv-eIF(iso)4E*^{KO} plants had significantly reduced BChV accumulation and a drop in infection rate from 100% to 28.86%, while there was no effect on BMYV accumulation. These findings bolster the notion that eIFs are good candidates for *Polerovirus* resistance in sugar beet breeding.

Sequence data of sugar beet (Dohm et al., 2014) and wild relative beet genomes, together with gene editing/silencing and overexpression technologies, have provided useful information on the molecular responses to biotic and abiotic stress in sugar beet in the last few decades. The development of sugar beet cultivars resistant to biotic stress caused by insects and pathogens may benefit from the introgression of alleles from wild ancestors and related species, genetic transformation, and in vitro methods. The use of available biotechnological tools is of vital importance in developing climate-smart sugar beet cultivars that can withstand various abiotic and biotic stresses and ensure sustainable, high-yield production under changing environmental conditions.

2.2. Genetic transformation technologies

Approximately 20% of the world's sugar is obtained from the cultivation of sugar beet, but a major portion is affected by numerous biotic and abiotic stresses that cause substantial annual yield losses (Misra et al., 2022a). Therefore, genetic transformation techniques have been developed to introduce new transgenic traits into sugar beet within a short period of time, rather than relying on conventional, time-consuming breeding methods (Misra et al., 2022a). *Agrobacterium*-mediated and PEG-mediated transformation are the most prevalent methods used in sugar beet genetic engineering, providing opportunities to produce new traits, such as fungus/virus tolerance, pest/nematode resistance, herbicide tolerance, and salt stress mitigation. Advanced biotechnological methods have the potential to identify major genes related to various stresses in sugar beet, leading to diverse, cost-effective, and sustainable cultivation practices (Misra et al., 2023). The transformation efficiencies of various biotechnological tools differ markedly, ranging from 6.2% to 30.6% for *Agrobacterium*-mediated transformation, and from 0.9% to 7% for particle bombardment. In contrast to traditional breeding, biotechnology has shifted toward methodologies such as speed breeding, which have been useful in addressing stress challenges arising from both biotic and abiotic factors in sugar beet production (Mall et al., 2021).

Two common viruses in sugar beet that seriously impair productivity are beet necrotic yellow vein virus (BNYVV) and beet severe curly top virus (BSCTV). RNA silencing was employed to establish resistance to BNYVV by introducing constructs containing the viral coat protein-encoding DNA sequence, *CP21*, in both sense and antisense orientations. Moreover, since most sugar beet-growing fields are coinfected with BNYVV and BSCTV, experiments were carried out to examine potential interactions between these viruses and their impact on transgenic plant resistance to BNYVV. It has been demonstrated that the RNA silencing-mediated tolerance to BNYVV in transgenic sugar beets is specific and does not diminish upon coinfection with a heterologous virus (Khoshnami et al., 2023). Bajestani et al. (2022) employed a successful approach to create transgenic sugar beet plants resistant to BNYVV by expressing single-chain variable fragments (scFv) directed against P21, the virus's main coat protein. They found that when transgenic sugar beet plants were infected with soil contaminated with rhizomania caused by BNYVV, their resistance levels correlated with their expressed levels of scFv antibodies. Pavli et al. (2010) tested three intron-hairpin constructs for their ability to provide resistance against rhizomania disease using a unique method involving sugar beet hairy roots transformed by *Agrobacterium rhizogenes* and expressing dsRNA from the BNYVV replicase gene. The nontransformed aerial parts of the same plants were found to be infected, whereas the genetically modified root systems of nearly all seedlings showed no or very low viral titers. This finding suggested that the hairy roots under investigation were successfully shielded from the virus (Pavli et al., 2010).

Numerous fungal diseases have a significant impact on sugar beet production. Two genetically modified diploid sugar beet varieties expressing the gene coding for polygalacturonase-inhibiting protein 2 of *Phaseolus vulgaris* (PvPGIP2)—which inhibits fungal polygalacturonase and prevents fungi from colonizing plant tissues—were created by *Agrobacterium tumefaciens*-mediated transformation in the study conducted by Mohammadzadeh et al. (2012) to generate plants resistant to fungi. Several transgenic plants exhibited varying degrees of inhibitory efficacy against *Fusarium phyllophilum* polygalacturonase (FpPG). In a further investigation conducted by the same group, *Phaseolus vulgaris* was used to isolate the gene encoding *PGIP1*, which was fused downstream of the leader sequence for extracellular production. This gene was then cloned into the expression vector pBI121 to facilitate sugar beet transformation by *Agrobacterium* and tested for activity against the phytopathogenic fungus *Colletotrichum*

lindemuthianum. They concluded that the *PGIP1* gene is a potent polygalacturonase inhibitor that will help increase the fungal resistance of sugar beets (Mohammadzadeh et al., 2015). Another study used a transgenic overexpression technique to demonstrate that the polygalacturonase-inhibiting protein (PGIP) encoded by the *PGIP*2 gene is active against the polygalacturonases of *Rhizoctonia solani*, the fungus that causes root and stem rot in sugar beets. This was carried out following the isolation and cloning of the *PGIP2* gene from beans. The antifungal properties of the *PGIP2* gene were validated by the findings (Kalantari et al., 2016). Sugar beet farms around the world suffer crop and economic losses due to leaf spot disease, which is caused by the fungus *Cercospora*. To enhance *Cercospora*-induced leaf spot resistance in sugar beet, the *cfp* gene—which codes a protein that exports phytotoxic cercosporins from *Cercospora*—was conjugally transmitted to the plant using *Agrobacterium tumefaciens* (Kuykendall et al., 2003).

Crop yields of sugar beet are severely constrained by insect pests. In a previous study, two sugar beet inbred lines were used to evaluate the optimization of the transformation process and the incorporation of four agronomically significant genes related to insect pests and glyphosate resistance (*cryIA105*, *cryIIIAa*, *CP4-epsps*, and *box* genes). The findings showed that the altered transformation strategy may effectively insert novel gene cassettes into sugar beet lines (Moazami-Goodarzi et al., 2020). In a prior investigation, sugar beet lines (using the MM1/2 line) were transformed with *Bacillus thuringiensis cry2A* and *cry1C* genes for plant resistance. They produced transgenic sugar beet lines that expressed the *cry2A* and *cry1C* genes, potentially providing resistance to *Lepidoptera* and *Diptera* insect pests (Lytvyn et al., 2014). A previous investigation described the transformation and expression of a *Nicotiana alata NaPI* gene, which codes for a serine proteinase inhibitor (PI) and has been demonstrated to successfully reduce the population of many insect pests in the hairy roots of sugar beets. According to their results, the altered sugar beet hairy roots expressed NaPI proteins (Smigocki et al., 2009). Jafari et al. (2009) used *Agrobacterium tumefaciens* expressing pBI35Scry, which carries a synthetic *cry1Ab* gene encoding a truncated endotoxin, to transform two diploid sugar beet genotypes of agronomical significance. As a result, transgenic sugar beet plants showed increased resistance to one of the most significant pests in sugar beet areas, *Spodoptera littoralis*, in bioassays conducted in vitro. *Agrobacterium rhizogenes*-mediated transformation was used to insert the sporamin gene *SpTI-1*, which encodes the sweet potato tuberous storage protein, a Kunitz-type trypsin inhibitor, into sugar beet in order to test the protein's potential as an antifeedant against the beet cyst nematode (*Heterodera schachtii* Schm.). The findings indicated that trypsin inhibitor activity plays a crucial role in preventing cyst nematode proliferation and development in sugar beet hairy roots that express the sporamin gene (Cai et al., 2003).

2.3. Omics technologies

2.3.1. Genomics

In 2014, Dohm et al. reported the complete genome sequence of cultivated beet using the cultivar KWS2320, assembled at the chromosome level for the first time (Dohm et al., 2014). They reported that the genome was composed of 567 Mb and concluded, based on mRNA evidence, that it contained 27,421 protein-coding genes (Dohm et al., 2014). Around 10 years later, the contiguous genome was published using the EL10 cultivar of sugar beet (McGrath et al., 2023). They reported that the assembled genome size was about 568 Mb, although the estimated genome size differed among individuals based on flow cytometry, ranging from 633 to 875 Mb. Moreover, they annotated 24,255 protein-coding genes based on RNA-seq data using the MAKER annotation pipeline (McGrath et al., 2023). The genomes of *Beta patula* and *Beta maritima*—wild beet species—were also sequenced in 2019 (Rodríguez del Río et al., 2019). It was estimated that the *B. patula* genome is 653 Mb and the *B. maritima* genome is 567 Mb, containing 25,127 and 27,662 genes, respectively (Rodríguez del Río et al., 2019).

In addition to nuclear genomes, the mitochondrial and chloroplast genomes of sugar beet were also reported. The complete chloroplast genome consisted of 149,637 bp (Li et al., 2014). Another study provided a similar estimate for the genome size, reporting it as 149,722 bp (Stadermann et al., 2015). Moreover, Li et al., (2014) reported that it contains 113 genes (79 protein-coding, 30 tRNA, and four rRNA), while Stadermann et al. (2015) found 114 genes (79 protein-coding, 28 tRNA, and seven rRNA). The mitochondrial genome, on the other hand, was reported to be 368,799 bp in length and to contain 59 genes (29 protein-coding, 25 tRNA, and five rRNA) (Kubo et al., 2000).

2.3.2. Transcriptomics

The response mechanism of plants to changing conditions starts with a switch in gene expression after the perception of new circumstances. These new conditions may arise due to abiotic factors, such as water scarcity and temperature changes, or biotic factors, such as diseases and pests. Researchers have been able to comprehensively study how gene activity changes under different conditions by analyzing all RNA molecules in a sample using next-generation sequencing technologies. Although little is known about how sugar beet reacts to biotic stresses, some studies have reported changes in transcriptome profiles caused by these stress factors.

In a very recent preprint study aimed at revealing the genes involved in the resistance mechanism against Aphanomyces root rot disease, which dramatically reduces

sugar yield, four sugar beet lines—two partially resilient (G06 and G12) and two sensitive (G01 and G17)—were inoculated with three different isolates of *Aphanomyces cochlioides*, the pathogen causing the disease (Rossi et al., 2024). They detected a total of 4262 DEGs—1987 (1149 upregulated and 838 downregulated) in G01; 2013 (1011 upregulated and 1002 downregulated) in G17; 547 (293 upregulated and 254 downregulated) in G06; and 2292 (999 upregulated and 1293 downregulated) in G12—according to RNA-seq data (Rossi et al., 2024). In addition, transcription factor (TF)-encoding DEGs were identified. Of the 181 TFs with altered expression, the susceptible genotypes G01 and G17 showed changes in 90 and 74 TFs, respectively, whereas the partially resistant lines G06 and G12 exhibited changes in 19 and 90 TFs (Rossi et al., 2024).

In an RNA-seq study investigating transcriptome changes in rhizomania-resistant transgenic (S3) and wild-type (WT) sugar beet varieties following beet necrotic yellow vein virus (BNYVV) infection, it was reported that less than 1% of genes were differentially expressed (Yousefiara et al., 2022). By comparing cDNA libraries of S3 and WT, researchers found that 343 out of 37,447 genes were differentially expressed, with 310 upregulated and 33 downregulated (Yousefiara et al., 2022). After GO and KEGG analysis, they concluded that these differentially expressed genes (DEGs) played roles in secondary metabolism, amino acid, lipid, and carbohydrate metabolism, as well as cellular processes and hormone signaling pathways (Yousefiara et al., 2022).

In another study, transcriptome profile changes caused by two different viruses, beet necrotic yellow vein virus (BNYVV) and beet soil-borne mosaic virus (BSBMV), in sugar beet were revealed (Gil et al., 2020). Based on mRNA-seq data, they discovered that 1076 DEGs were shared in plants infected with both viruses, while 1365 and 277 DEGs were specifically expressed in BNYVV- and BSBMV-infected plants, respectively (Gil et al., 2020). They also performed GO and KEGG analyses and reported that DEGs from BNYVV and BSBMV infections were significantly involved in 20 and 12 GO terms, and in 12 and three KEGG pathways, respectively (Gil et al., 2020). Moreover, differentially expressed transcription factors (TFs) were identified, belonging to 34 TF families, with more TFs stimulated by BNYVV than by BSBMV (Gil et al., 2020).

In addition to transcriptome studies on rhizomania, there are studies examining the molecular response of sugar beet to the beet cyst nematode (BCN) at the transcriptome level. Ghaemi et al. (2020) compared mRNA-seq data obtained from BCN-sensitive (7112*SB36) and resistant (Nemakill) beet cultivars at two time points (4 and 10 days after inoculation [dai]) under both diseased and control conditions. They reported that the sensitive variety activated most of its defense-related genes at the early stage (4 dai) but deactivated them later (10 dai). In contrast, the resistant variety continuously induced them at both the early and late stages. Moreover, several genes potentially contributing to Nemakill's resistance were identified, including those associated with the phenylpropanoid pathway and encoding proteins such as cysteine-rich transmembrane module (CYSTM) domain-containing proteins, F-box proteins, chitinase, galactono-1,4-lactone dehydrogenase, and CASP-like proteins (Ghaemi et al., 2020).

Rhizoctonia, (meaning "root killer") is another disease that causes yield losses in sugar beet. A transcriptomic comparison of different sugar beet cultivars (two partially resistant and two sensitive) revealed that three genes containing nucleotide-binding sites and leucine-rich repeat (NBS-LRR) domains were induced just two days after infection (Holmquist et al., 2021). Several other potential defense genes were also identified, including those encoding major latex protein-like (MLP; *BvMLP1–3*) proteins (Holmquist et al., 2021). Furthermore, they cloned those genes into *Arabidopsis thaliana* to demonstrate their function in the disease response. Notably, overexpression of *MLP1* and *MLP3* has been shown to slow disease development in transgenic *Arabidopsis* compared to *Atmlp1/Atmlp3* double mutants (Holmquist et al., 2021).

In addition to mRNA-seq studies, the roles of microRNAs (miRNAs) were examined in sugar beet under infection using NGS. Using two beet curly top virus (BCTV)-resistant lines (KDH13 and KDH4–9) and one BCTV-sensitive line (KDH19–17), it was shown that differentially expressed miRNAs altered the expression of their target genes, including carboxylesterase, leucine-rich repeat (LRR) receptor-like proteins, serine/threonine protein phosphatases, and pyruvate dehydrogenase (Majumdar et al., 2022). Moreover, the expression levels of these genes were higher in resistant lines than in the sensitive line. Interestingly, the virus expressed some miRNAs that targeted sugar beet transcripts, such as those encoding LRR, 7-deoxyloganetic acid glucosyltransferase, and transmembrane emp24 domain-containing proteins, and affected their expression (Majumdar et al., 2022). In contrast, sugar beet varieties were found to express miRNAs targeting capsid- and replication-related viral genes, with different expression levels between resistant and susceptible varieties (Majumdar et al., 2022).

2.3.3. Proteomics

In addition to genome and transcriptome studies, proteome research offers significant advantages in elucidating plant responses to biotic stress. By providing information about expressed proteins at a given time, proteomics enables the direct identification of molecular players involved in defense mechanisms. This approach complements transcriptomic studies by revealing posttranscriptional modifications and protein abundance,

which often correlate more closely with phenotypic outcomes. Moreover, proteomics facilitates the discovery of novel proteins and protein interactions, expanding our understanding of complex defense networks. Ultimately, this knowledge can be applied to develop resilient crop varieties through targeted breeding or genetic engineering strategies. Proteomic studies on sugar beet biotic stress responses are very limited. Here are some examples:

In a study, to assess the impact of genetic modification and RNA silencing on sugar beet root protein composition, researchers employed two-dimensional gel electrophoresis to compare protein profiles between two transgenic (6018-T3:S6–44 [S6] and 219-T3:S3–13.2 [S3]) rhizomania-resistant cultivars and wild-type (9597) cultivar (Hejri et al., 2021). The results revealed substantial differences in protein abundance, with 25 and 24 proteins exhibiting altered levels in the two transgenic lines, respectively. A more detailed analysis indicated that 15 protein spots experienced significant upregulation or downregulation (Hejri et al., 2021). Additionally, ten spots were consistently affected in both transgenic lines, while seven displayed variable changes. Subsequent mass spectrometry analysis identified these differentially abundant proteins, many of which were implicated in metabolic pathways and stress responses (Hejri et al., 2021).

To investigate how BNYVV evades plant defenses, researchers performed a comparative analysis of protein expression between an Rz1 resistance-breaking strain and traditional A-type strain (Webb et al., 2015). Using quantitative proteomic analysis, the researchers reported significant changes in protein profiles depending on the presence or absence of resistance genes and viral resistance-breaking variants (Webb et al., 2015).

A comprehensive analysis of protein alterations in sugar beet roots infected with beet necrotic yellow vein virus (BNYVV) was conducted using multidimensional liquid chromatography (Larson et al., 2008). Among the thousands of protein peaks detected in root extracts, a substantial proportion (7.4% and 11% for resistant and susceptible cultivars, respectively) displayed changes in response to the virus. Through tandem MALDI-TOF-MS analysis, 65 proteins were discovered, and their differential expression was confirmed using RT-PCR for selected candidates. The proteomic data revealed potential roles for systemic resistance mechanisms in the Rz1-mediated resistance response and implicated phytohormones in the development of disease symptoms (Larson et al., 2008).

2.3.4. Metabolomics

Plant genotype and phenotype are linked through the analytical technique known as metabolomics, which enables the qualitative and quantitative characterization of primary and secondary metabolites in a living system (Webb et al., 2020). Future developments in plant breeding, biotechnology, and crop protection will depend heavily on understanding how plants respond to biotic stressors and how their metabolism can be improved. The development of disease-resistant crop cultivars may benefit from a deeper understanding of the metabolites generated by plants in response to infection. Although the number of metabolomics studies conducted on sugar beet genotypes under biotic stress is limited, the following examples are available:

A group of sugar beet varieties with variable levels of resistance to Cercospora leaf spot (CLS), caused by the fungal pathogen *Cercospora beticola*, were found to have differentially accumulated metabolites in a previous study using ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS). The findings showed that 15 metabolites could more accurately distinguish between sensitive and resistant varieties (Heidari et al., 2019). Another study described the metabolic characteristics of field-grown sugar beet leaves sampled at four growth stages—seedling, early growth, root enlargement, and disease development—using nuclear magnetic resonance (NMR), for a subset of genotypes with varying degrees of resistance to Cercospora leaf spot disease. The results showed that choline concentrations declined, while sugar and glycine betaine levels increased at later stages. Furthermore, before inoculation experiments, susceptible and resistant genotypes could be distinguished using γ-aminobutyric acid (GABA) and Gln (glutamine) (Sekiyama et al., 2017). In another study, host genotypes with differing levels of disease susceptibility were used to define metabolite response patterns to fungal infection and to develop a noninvasive, presymptomatic technique for pathogen detection. Metabolite analysis showed that the host had altered levels of L-DOPA, 12-hydroxyjasmonic acid, 12-O-β-D-glucoside, pantothenic acid, and 5-O-feruloylquinic acid as part of a strong defense response. It appears that the resistant genotype functions as a continuously protected system due to the formation of glucosylvitexin (Arens et al., 2016).

Targeted phytohormone measurement in conjunction with nontargeted metabolome monitoring was used to investigate the metabolic and signaling networks linked to BNYVV infections that result in rhizomania disease in sugar beet. Regardless of plant genotype, distinct variations in metabolites were found after pathogen infection, especially those connected to the lipid content of the host or other membrane-associated processes. The differential production of metabolites suggested that both primary and secondary metabolites play a role in the interactions between BNYVV infection and sugar beet roots. They also probably play a role in the occurrence of rhizomania symptoms and the initiation of the sugar beet defense system (Webb et al., 2020).

One significant sugar beet disease is Rhizoctonia crown and root rot (RCRR), which is caused by *Rhizoctonia solani* Kühn AG 2–2 IIIB. Nontargeted metabolomic characterization by reversed-phase UHPLC-MS and GC-MS was used to compare and describe the metabolic alterations that occurred during interactions between resistant and susceptible sugar beet cultivars with *R. solani*. The metabolome showed distinct differences based on tissue type, genotype, and response to *R. solani*. A total of 143 compounds were annotated, and numerous metabolites linked to plant resistance against fungi were detected in both germplasm samples (Webb et al., 2016).

Apart from these, several studies have revealed the comparative metabolome profiles of sugar beet genotypes under abiotic stress conditions such as salt, drought, temperature, and light. In addition, metabolomic alterations in postharvest sugar beet roots (Fugate et al., 2024) and metabolic concepts underlying sugar beet storability (Gippert et al., 2022) were analyzed in recent studies.

3. Breeding techniques for development of biotic stress-resistant sugar beet

3.1. Classical breeding

Cultivated forms of *Beta vulgaris* were obtained from wild forms in the Vulgares section, namely *Beta maritima*. It was first reported in 1608 that the juice of red beet resembled sugar syrup. Later, when the same characteristic was observed in fodder beet, this juice was used to feed bees. White Silesian beets were obtained by selecting types suitable for sugar factories from very heterogeneous populations (sugar content 5%–7%) (Gurel et al., 2008; McGrath and Panella, 2018). Vilmorin started selection studies and applied the progeny test method in beet breeding in 1837. After World War II, polyploid varieties were bred, and tetraploid (4x = 36) sugar beet varieties were obtained (Schwanitz, 1938). These varieties were widely used in the 1950s and 1960s and were preferred for their superior agronomic properties, easy fragmentation of seed balls, suitability for obtaining technical monogerm varieties (75%–90% single plant), and facilitation of mechanization (Gayon and Zallen, 1998).

The vast majority of sugar beet varieties cultivated worldwide are hybrids, and these are developed in two stages. In breeding programs, hybrid production involves fertilizing an inbred male-sterile line (CMS) as the main parent with an unrelated inbred O-type. Seeds are harvested from the CMS, and the resulting F1CMS plants are crossed with inbred multigerm lines. The variety is obtained by crossing the diploid (2n) monogerm F1CMS main parent with a diploid (2n) or tetraploid (4n) male pollinator (Figure 1). Due to its disadvantages, the use of triploids (3n) has been rapidly abandoned, and they are being replaced by new diploid varieties with high yield, quality, and disease resistance (Bosemark, 2006; McGrath and Panella, 2018).

Sugar beet is a cross-pollinated plant; therefore, it produces either no seeds or very few seeds under good isolation conditions. However, self-incompatible plants may produce a limited number of seeds after selfing. This pseudocompatibility, or pseudoself-fertility, results from the breakdown of self-incompatibility in different genotypes and is strongly influenced by environmental conditions, particularly temperature. The presence of a dominant self-fertile (*SF*) gene enables self-fertilization. In cross-pollinated sugar beet, the flowers are very small, pollen can travel long distances, and pollination occurs mainly by wind (Biancardi et al., 2010).

With the continuous use of commercial beet varieties and breeding lines in breeding programs, the gene pool of sugar beet has gradually narrowed (Bosemark, 2006). Therefore, research on the identification, evaluation, and utilization of beet species that form the genetic basis of breeding programs has gained importance. Primitive forms and wild relatives of sugar beet have significant potential for breeding studies due to the genetic variation they contain (Monteiro et al., 2013). Especially wild beet species have been considered by breeders for certain morphological traits, resistance to stress factors, diseases, and pests, as well as genetic characteristics.

Commercial hybrid varieties are heterozygous for disease resistance, and their resistance is lower than that of the source population. In recent years, marker-assisted breeding has become increasingly important to support breeders in developing commercial sugar beet hybrids.

3.1.1. *Beta* genus and subclassification

The genus *Beta* belongs to the family Amaranthaceae (formerly Chenopodiaceae), and its species are widespread in the Mediterranean region, Europe, and parts of Asia. *Beta vulgaris* is one of the most important agricultural species and includes subspecies such as sugar beet, leaf beet, fodder beet, and garden beet.

The section *Beta* generally has a diploid chromosome number, and the basic chromosome number is x = 9. Beets belonging to the section *Beta* can easily cross-fertilize, and abundant seeds are obtained in their hybrids.

3.1.2. Flower structure

The flower structure and reproductive biology of sugar beet are of great importance in breeding studies. While the self-incompatibility mechanism ensures the preservation of genetic diversity, pollination and fertilization contribute to the development of new varieties. In breeding programs, consideration of factors affecting the flowering process plays a critical role in the development of high-yielding and disease-resistant lines.

Sugar beet is a cross-pollinated plant, and pollination is generally by wind, but insect pollination can also occur.

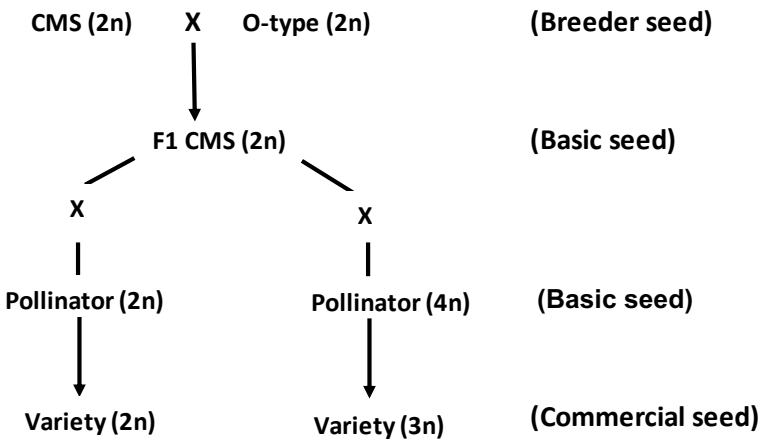


Figure 1. Variety development in sugar beet.

Pollen is carried to the stigma surface under the influence of environmental factors, enabling fertilization. Sugar beet has a self-incompatibility mechanism, which promotes cross-pollination. Sugar beet flowers are usually small, greenish, and have five petals. The flowers are in tight clusters (inflorescences), and each flower contains five stamens and one pistil (Milford, 2007). Sepals protect the flower from external influences and support its development during the bud stage. In sugar beet, sepals merge with the petals during development and become apparent when the flower opens (McGrath et al., 2011). In sugar beet, petals are small and greenish, and may serve to attract insects. However, since the main pollination occurs by wind, this structure is usually not prominent. Pollen is carried by wind and insects, thereby enabling fertilization (Bosemark, 2006).

3.1.3. Incompatibility system

Sugar beet is a cross-pollinated plant. The male organs mature before the female organs. Pollen matures (protandry) and disperses 24–48 h before the female organ reaches fertilization maturity. The self-incompatibility system in sugar beet is a mechanism developed to maintain genetic diversity and increase fertilization success. This system promotes cross-pollination by enabling the plant to recognize and reject its own pollen. Self-incompatibility in sugar beet is based on the gametophytic self-incompatibility mechanism. In this system, the genetic components in the pistil (stigma) are compared with those in the pollen, and if they share the same genotype, pollen germination is prevented (McGrath et al., 2011). In the self-incompatibility system, receptor proteins in the stigma interact with certain proteins on the pollen surface and prevent germination. This process is controlled by specific genes (e.g. *S-locus* genes). In sugar beet, *S-locus*–encoded proteins recognize and selectively reject pollen on the stigma surface (Jung et al., 2018). There are two types of incompatibility in sugar beet: (i) complete self-incompatibility, which ensures fertilization with foreign pollen, and (ii) partial self-incompatibility, which allows self-fertilization under certain conditions. Some environmental factors, such as high temperature and low humidity, can reduce the effectiveness of the self-incompatibility system and enable self-fertilization (Biancardi et al., 2010).

The self-incompatibility system in sugar beet is an important factor that enhances genetic diversity in breeding programs. Breeders can create heterozygous lines and develop high-yielding hybrid varieties using this system. The self-incompatibility mechanism is used to develop hybrid varieties by crossing genetically distinct parents. Self-incompatibility also helps prevent narrow gene pools and supports sustainable agriculture in planting areas (Bosemark, 2006). In recent years, advances in molecular biology and genetic engineering have improved understanding of the self-incompatibility mechanism in sugar beet. Gene editing techniques (e.g., CRISPR-Cas9) can be used to manipulate the self-incompatibility system and accelerate breeding processes.

3.1.4. Monogermity

The naturally occurring multigerm seed structure in sugar beet results in the presence of more than one embryo in a seed cluster (Figure 2). However, monogerm sugar beet varieties have been developed to facilitate mechanical planting and optimize agricultural production. In 1948, Russian geneticist V.F. Savitsky identified five monogerm plants in the seed fields of the Hybrid-18 variety during his studies in Michigan, USA. The seeds propagated from these were designated as SLC101 and SLC107 and were used to introduce the monogerm seed trait in subsequent studies. Monogermity is controlled by a single recessive gene (m) in sugar beet. While the genotype is MM or Mm in polygerm plants, the mm genotype is observed



Figure 2. Multigerm and monogerm flowers, and germinating seeds in the sugar beet plant.

in monogerm plants (Owen, 1945). Monogerm varieties developed from SLC101 and SLC107 are widely used worldwide, but multigerm varieties are still cultivated in countries where field output and labor costs are low (e.g., North Africa and China) (Biancardi et al., 2010). This gene ensures the development of only a single embryo in the flower cluster, leading to the formation of monogerm seeds (Figure 2). When multigerm and monogerm plants are crossed, all F_1 offspring are multigerm. If F_1 offspring are selfed, multigerm and monogerm plants segragate at a ratio of 3:1 (multigerm:monogerm). In sugar beet, the monogerm trait is determined by a pair of alleles designated as "Mm", and monogerm occurs under homozygous recessive (mm) conditions. Heterozygous plants (Mm) are multigerm, but they produce fewer fruits per ball than homozygous multigerm plants (Biancardi et al., 2010). The monogerm trait provides a major advantage in sugar beet breeding. The need for thinning during seed planting is eliminated, agricultural labor costs are reduced, and precision in field planting is increased (Richardson, 2011). However, it is also known that monogerm varieties have lower competitive ability and may be more susceptible to diseases compared to polygerm varieties (McGrath et al., 2011). For this reason, breeders focus on developing hybrid varieties that combine the monogerm trait with disease resistance and high yield. Multigerm plants are used as pollinator parents in sugar beet. Multigerm parents are open-pollinated and are mostly selected by mass selection for the disease resistance trait. It is easy to transfer dominant or codominant resistance alleles to these parents used as pollinators. For this reason, commercial hybrid varieties are heterozygous for the disease resistance trait, and their resistance is lower compared to the source population. However, it is quite difficult to incorporate new traits into the monogerm seed parent.

Monogerm seeds are easy to plant and are well-suited for mechanization during spring planting and maintenance operations in sugar beet cultivation. Hand thinning after emergence is important to prevent competition between plants and to ensure uniform plant distribution, providing equal space for each plant (80,000–100,000 plants/ha). Today, genetic mapping and marker-assisted selection (MAS) techniques are used to identify the genomic regions carrying the monogerm gene, enabling more effective breeding studies. Gene-editing technologies such as CRISPR-Cas9 aim to modify this trait with greater precision.

3.1.5. Ploidy

All members of the Beta section are diploid, with 2n = 2x = 18 chromosomes. The wild form *Beta macrocarpa*, a subspecies of *Beta maritima*, contains both diploid and tetraploid populations (2n = 4x = 36). Artificially induced tetraploids using colchicine were employed in sugar beet breeding in Europe in the early 1940s. Varieties termed polyploid or anisoploid, consisting of mixtures of tetraploid (2n = 4x = 36), triploid (2n = 3x = 27), and diploid (2n = 2x = 18) plants, were developed (Figure 3). In the 1960s, these varieties were replaced by triploid hybrid cultivars.

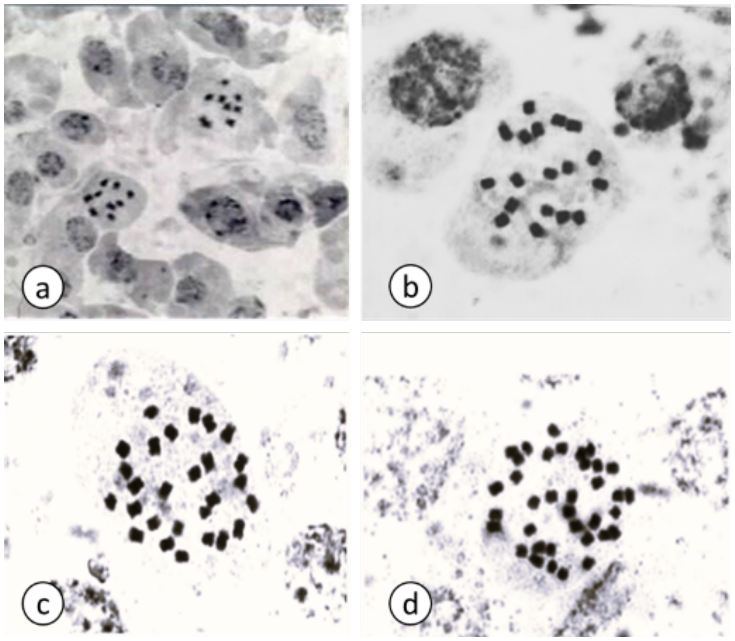


Figure 3. Chromosome numbers in sugar beet cells: (a) haploid (x = 9), (b) diploid (2x = 18), (c) triploid (3x = 27), and (d) tetraploid (4x = 36).

The genetic structure of sugar beet determines many key traits, including plant growth rate, disease resistance, and sugar content. Diploid plants are most commonly used to obtain commercial sugar beet cultivars resistant or tolerant to pests and diseases. In addition, diploid plants provide genetic stability (Jung et al., 2018). Triploid plants have advantages in terms of root yield and sugar content and are especially preferred in commercial cultivars.

Aneuploid individuals occur among tetraploid (2n = 4x = 36) and triploid (2n = 3x = 27) sugar beet plants. Since aneuploid plants have 4x = 32–39 or 3x = 25–29 chromosomes, their root yield decreases and their overall development is weak. Anisoploid varieties contain mixtures of diploid (2x), triploid (3x), and tetraploid (4x) chromosome sets. Colchicine, discovered in 1937, was used to double the chromosome number of plants, leading to the production of tetraploid plants.

Plants with 2n = 4x = 36 chromosomes exhibit larger cells, broader leaves, more rounded roots, and a longer vegetation period. They are more resistant to stress conditions. However, their generative reproduction capacity is low, and difficulties may arise in seed production. These plants are mostly used as male parents in crosses with diploids to increase genetic diversity (Shepherd, 2003).

Regulation of ploidy levels is employed in the development of male-sterile lines and in crossbreeding programs. Modern biotechnological tools have made ploidy manipulation in sugar beet more precise, such as:

i) Tetraploid plants can be obtained by using colchicine to block cell division (Skaracis and Biancardi, 2000).

ii) Lines with different ploidy levels can be generated through tissue culture techniques (Stevanato et al., 2015).

iii) Genetic profiles of individuals with different ploidy levels can be analyzed using DNA markers (Jung et al., 2018).

3.1.6. Hypocotyl and root color

Hypocotyl color in sugar beet is a genetically determined trait with important applications in breeding programs. Its simple inheritance and easy identification make it a valuable marker for selection, hybridization tracking, and genetic studies. In cultivated sugar beet, as well as in some fodder beets and many types of chard, the roots of *Beta* beets are typically white, red, or yellow in fodder beets, and red in table beets. This coloration is caused by betacyanin and betaxanthin pigments dissolved in the cell sap. Although the roots of all sugar beet plants are white, hypocotyl coloration in seedlings can be pronounced (Ries et al., 2016). In sugar beet, hypocotyl color is an important phenotypic marker in breeding programs. The hypocotyl, the stem region between the cotyledons and the root, exhibits color variations—primarily red and green—controlled by genetic factors. The inheritance of hypocotyl color in sugar beet is mainly controlled by a single gene locus, the R locus. The dominant allele at this locus produces red hypocotyls, while the recessive allele results in green hypocotyls. This monogenic inheritance pattern enables simple phenotypic selection in breeding

programs. Research has shown that pooling genetically diverse breeding lines with contrasting phenotypes enables high-resolution mapping of the causal gene in crop species, as demonstrated by studies on the R locus in *Beta vulgaris*. Using hypocotyl color as a marker provides several advantages in sugar beet breeding. Hypocotyl color increases selection efficiency in breeding programs. The clear visual distinction between red and green hypocotyls allows rapid identification of desired genotypes during selection. Hypocotyl color helps monitor the inheritance of specific traits in hybridization experiments, ensuring accurate transfer of desired characteristics. The R locus associated with hypocotyl color serves as a reference point in genetic mapping studies, aiding in the identification of linked traits and the construction of high-density genetic maps (Ries et al., 2016; McGrath and Panella, 2018).

3.1.7. Male sterility

3.1.7.1. Cytoplasmic male sterility

The discovery of cytoplasmic male sterility (CMS) and the maintenance of corresponding genotypes are crucial steps for the success of hybrid breeding programs in sugar beet. F.V. Owen first identified CMS plants in sugar beet in an old variety called US1. Owen reported that male sterility resulted from the interaction of two recessive nuclear genes (x and y) with sterile cytoplasm. He defined the maintainer plants as having the xxzz genotype with normal cytoplasm. Therefore, these plants were termed Owen type (O-type) plants (Owen 1945). Because the production of hybrid sugar beet varieties was initially dependent on a single CMS source, this system was referred to as Owen CMS. To date, additional CMS sources have been identified in wild beet populations, expanding the genetic base of the cytoplasm. Three CMS sources in beet have been reported, each associated with mitochondrial genes. As in other plants, the CMS trait in sugar beet results from interactions between nuclear genes and the mitochondrial genome (Matsuhira et al., 2012).

To obtain completely sterile offspring, CMS plants must be pollinated with maintainer plants that carry the appropriate nuclear genes for male sterility while possessing a normal, unaltered mitochondrial genome. Plants with the appropriate maintainer genotype are rare in natural populations and can be identified through test-crossing with CMS plants (Biancardi et al., 2010).

Sterile cytoplasm (S) arises when two pairs of alleles are homozygous recessive, and male-sterile plants are expressed as (S)xxzz, as described by Biancardi et al. (2010). Accordingly, CMS plants are represented by the (S)xxzz genotype. Other allele combinations produce fertile or semifertile offspring. Normal cytoplasm (N) consistently results in fertile offspring. For the propagation of CMS lines, maintainer plants with N cytoplasm should be used, carrying homozygous recessive x and z genes. Such maintainer types are known as O-type plants. In open-pollinated populations, the proportion of O-type plants is typically less than 0.5%. Initially, both CMS and monogerm inbred lines are weak, but their characteristics can be improved through crossbreeding and seed production (Biancardi et al., 2010).

3.1.7.2. Mendelian male sterility

Genetic male sterility (NMS) in sugar beet was first described by Owen (1945), who reported that sterility was controlled by a single recessive nuclear gene (a1). Because it does not allow populations with 100% male-sterile plants, the use of this system in commercial hybrid production is limited, and it is applied only in certain specialized breeding programs.

3.1.8. Biennial growth

Although all cultivated beets are biennial, they require short- or long-term exposure to low temperatures to transition fully from the vegetative to the generative stage. Most wild Mediterranean Beta species (*Beta maritima*, *Beta macrocarpa*, *Beta patula*) are annual, although biennial types also exist. Flowering is controlled by the bolting gene (B) (Milford et al., 2010). Plants carrying this gene produce seeds under long-day conditions at specific temperatures. Heterozygous plants develop more slowly and carry modified alleles derived from the biennial parent. Annual plants are important for accelerating backcrossing programs, conducting genetic research, and testing sterility in breeding programs. The bolting rate in cultivated sugar beet should not exceed 0.1% (Sadeghian and Johansson, 1992).

Hybridization between annual and biennial beets revealed that annual traits are dominant and monogenic. The B gene is known as the bolting gene. Depending on whether the B gene is dominant or recessive, plants are annual or biennial: BB (annual), Bb (annual), and bb (biennial). Because the B gene reduces yield and quality, it is desirable for cultivated beets to carry the recessive allele (Kuroda, 2024). Mass selection is highly effective against early bolting. First, seeds are sown in spring; then seeds are produced from beets resistant to bolting, selected from breeding lines and populations (Milford et al., 2010).

Sugar beet has a 2-year growth cycle and is a biennial plant. Depending on latitude, annual weather patterns, and sugar factory demand, it is typically planted in spring and harvested in autumn in temperate climates. During its 1st year, extending from spring to October, the plant develops vegetative structures such as leaves and roots. If cultivated as a winter crop, sugar beet remains in the field during the colder months.

When sugar beet is grown for root production, flowering during the 1st year is considered undesirable. However, 1st-year flowering is deliberately induced during seed production. Ongoing research on flowering

regulatory mechanisms has led experts to reevaluate breeding methods in sugar beet. Artificial vernalization techniques have been developed for sugar beet breeding, enabling seed production within a year under controlled temperature conditions. Photothermal treatment is well known to induce bolting in immature sugar beet plants under nearly all conditions. Depending on genotype and breeding objectives, this procedure involves exposing plants to temperatures of 5–8 °C with 16 h of light for 8–14 weeks. This approach enables completion of a full seed-to-seed cycle within a single year, thereby greatly accelerating breeding progress (Bosemark, 2006).

Sugar beet bolting consists of two components. In field conditions, bolting can be detrimental due to prolonged daylight and low temperatures. Nevertheless, bolting can be mitigated through agronomic practices such as seed preparation, selection of suitable cultivars, and adherence to recommended planting dates. A thorough understanding of sugar beet flowering physiology is essential for breeders to develop bolting-resistant varieties. Developing bolting-resistant sugar beet cultivars and hybrids requires collaboration among molecular biologists, biotechnologists, physiologists, and breeders. Furthermore, a better understanding of the principles governing the transition from vegetative to generative phases of development will accelerate breeding and seed production processes (Kroupin et al., 2023). In several sugar beet genotypes, photothermal techniques are commonly used to induce bolting and flowering. This method involves prolonged vernalization in climate-controlled rooms or specialized greenhouses with extended daylight. With speed-breeding techniques, seeds can be produced in less than a year.

3.2. Molecular breeding

Sugar beet is one of the most important crops worldwide. Sugar beet is also crucial for its applications in industrial sectors beyond sugar production. These applications include the production of biofuels, biogas from molasses, syrups, pectin, cellulose, bioplastics, livestock feed, and pharmaceutical products. As in other crops, sugar beet production faces threats that can be classified as biotic and abiotic. Biotic stresses are major threats that negatively impact plant health and agricultural productivity. Although sugar beet is an important global source of sugar, its production is often reduced by various biotic stress factors.

Fungal, bacterial, and viral pathogens, as well as insect pests, can cause severe yield losses. Therefore, developing sugar beet varieties resistant to biotic stresses is crucial for both production efficiency and food security. One of the main approaches to developing resistant varieties against biotic factors is molecular breeding. Since the 1990s, sugar beet breeding has incorporated molecular biology, enhancing the efficiency and reliability of selection processes.

Molecular markers, representing polymorphisms and genomic variations, were used to construct genetic linkage maps, facilitating genotyping and marker-assisted selection (MAS) (Bradshaw, 2010). Genetic linkage maps are valuable tools in plant breeding, showing the positions and relative distances of polymorphic traits along chromosomes. These maps are based on the principle that genes and markers segregate during meiosis, with loci that are closer together more likely to be inherited together than those farther apart. One of the earliest linkage maps in sugar beet was constructed using restriction fragment length polymorphism (RFLP) markers. Random amplified polymorphic DNA (RAPD) markers were also employed to create a genetic map in sugar beet (De Lucchi et al., 2021).

To enhance molecular breeding, genomic research combined with recent advances in sequencing and genotyping technologies is essential. Single nucleotide polymorphisms (SNPs) are ideal molecular markers for these purposes. SNPs are abundant and evenly distributed across the genome, occurring in both coding and noncoding regions. Within coding regions, SNPs are classified as synonymous (silent) mutations, which do not alter the protein, or nonsynonymous mutations, which cause amino acid substitutions. SNPs are widely used to construct genetic linkage maps, with haplotype-based maps proving especially useful for marker-assisted selection, as they provide more reliable information than individual SNPs (Broccanello, 2016).

Higher SNP density simplifies breeding selection, lowers costs, and improves the identification of both desirable and undesirable traits. High-density haplotype analysis can help address segregation distortions, which remain difficult to predict (Würschum, 2012). Rapid SNP identification is commonly achieved through next-generation sequencing, enabling efficient and low-cost discovery of numerous variants. This approach also facilitates genotype comparisons within the same species to detect nucleotide variations in specific genomic regions. Once validated, SNPs can be applied in genotyping, phylogenetic analysis, marker-assisted selection, quantitative trait locus (QTL) mapping, genomic selection, bulk segregant analysis, and genome-wide association studies.

Advanced biotechnological techniques have been integrated with traditional breeding to develop cultivars tolerant to herbicides and resistant to diseases and pests. Various transformation strategies have been explored, including vector-based methods (*Agrobacterium tumefaciens*, *Agrobacterium rhizogenes*) and direct methods such as particle bombardment, polyethylene

glycol (PEG)-mediated transformation, electroporation, sonication, and somatic hybridization. Intact cells from leaf, cotyledon, and shoot-base tissues of in vitro-grown shoots are typically used for Agrobacterium-mediated and particle bombardment gene transfers. For PEG-mediated transformation, protoplasts from mesophyll or stomatal guard cells are often the most effective (Gurel et al., 2008). Molecular biology has been transformed by the development of the CRISPR/Cas method, particularly CRISPR/Cas9, which is highly effective. Advances in genome editing, especially CRISPR/Cas9, are expected to significantly improve sugar beet cultivation by generating varieties resistant to both abiotic and biotic stresses (Ahmad et al., 2021; Misra et al., 2023). The CRISPR/Cas9 adaptive immune system provides resistance against viruses, bacteria, and fungi through three main stages: i) adaptation, ii) expression and maturation, and iii) interference.

During the adaptation stage, Cas proteins recognize protospacers—short, unique DNA sequences from invading pathogens—and integrate them into CRISPR arrays as new spacers. This process enables the host to acquire immunological memory, allowing recognition and defense against the same pathogen in future encounters. The newly integrated spacer also serves as a genetic record (Paul et al., 2021). CRISPR/Cas9 technology holds great promise for addressing biotic stress in sugar beet, which is severely affected by pathogens and pests that reduce both quality and yield. Traditional breeding methods lack the precision and speed required for targeted genetic modifications, whereas CRISPR/Cas9 provides a groundbreaking tool to enhance sugar beet resistance to these stresses. Although CRISPR/Cas9 has been effective in controlling diseases in major crops, its potential for managing insect pests remains underexplored. A major challenge for developing insect resistance is the limited availability of suitable target genes. To address this limitation, researchers must explore resistance sources, evaluate germplasm, and apply multiomics approaches to identify stress-responsive genes. The successful application of CRISPR/Cas9 for pest resistance will depend on advances in targeted gene editing and on overcoming regulatory barriers (Misra et al., 2023).

In conclusion, sugar beet plays a crucial role not only in sugar production but also in diverse industrial applications, including biofuels, biogas from molasses, syrups, and bioplastics. However, its cultivation is threatened by both biotic and abiotic stressors, with biotic stresses—particularly those caused by pathogens and pests—posing major challenges to yield and production. To address these challenges, molecular breeding techniques have been employed, with CRISPR/Cas9 emerging as a particularly promising tool. This advanced genome-editing system offers a transformative approach to enhancing sugar beet resistance to biotic stresses and improving overall crop resilience. While traditional breeding methods are limited in precision and speed, CRISPR/Cas9 offers a more effective alternative, although its application in insect pest control remains underutilized. Continued research to identify resistance sources, evaluate germplasm, and apply multiomics techniques will be essential for developing pest-resistant varieties. The successful integration of CRISPR/Cas9, together with overcoming regulatory challenges, will be key to advancing sugar beet cultivation and ensuring its continued contribution to global food and industrial needs.

3.3. Speed breeding

The concept of speed breeding involves optimizing light intensity, light quality, crop-specific temperature, and daylength control. This approach enhances photosynthesis, promotes early flowering, accelerates seed maturity, and ultimately shortens generation time. Combined with single-seed descent, speed breeding is used to develop efficient breeding and inbred lines, enabling faster production of improved crop varieties and providing a cost-effective alternative to doubled haploid production. Additionally, speed breeding supports gene insertion (common haplotypes) of distinct phenotypes following marker-assisted selection (MAS) in hybrid lines (Sowndarya and Tyagi, 2021).

Sugar beet follows a biennial life cycle. In the 1st year, it develops substantial leaf and root biomass. After exposure to cold during winter, the plant typically enters its reproductive phase in the 2nd year. However, in the 1st year, plants may prematurely produce flowering shoots ("bolting") due to vernalization and long-day conditions. Such bolting reduces both root yield and sugar productivity. Under controlled climate conditions, speed breeding stimulates bolting and flowering in sugar beet, accelerating breeding and seed production by enabling faster "seed-to-seed" cycles (Kroupin et al., 2023).

A major cause of sugar beet bolting is early spring planting, particularly during prolonged cold spells with rapid temperature drops and insufficient rainfall. Under such conditions, seeds may remain in the soil for up to 40 days, undergoing vernalization. Vernalization (Latin *vernus*, "of the spring") is the process by which seeds acquire or enhance their ability to bolt and flower after prolonged exposure to low temperatures simulating winter conditions. Vernalization protocols—including prevernalization and postvernalization, acclimatization, and bolting phenotyping—have been examined in detail and are summarized in Kroupin et al. (2023).

Speed breeding faces several key challenges: (i) optimizing environmental factors such as temperature, light intensity, duration, and spectral composition across different phases (prevernalization, vernalization, acclimatization, and postvernalization) to accelerate

reproductive development; (ii) refining chemical treatments—including mineral nutrition, epimutagens, and hormones—to further enhance this transition; (iii) establishing optimal conditions to produce sufficient viable seeds; (iv) exploring in vitro vernalization of embryos through embryo rescue techniques; and (v) enabling positive selection for bolting in segregating populations under speed-breeding conditions (Kroupin et al., 2023).

When combined with complementary approaches, speed breeding shortens the breeding cycle and accelerates the development of new cultivars within a much shorter timeframe. Integrating speed breeding with other breeding technologies can result in rapid genetic gains. Speed breeding also provides an alternative strategy for improving challenging crops, including short-day species such as maize and biennial species such as sugar beet. The combination of speed breeding with genetic tools such as genome editing, marker-assisted backcrossing, and transgenic approaches—together with cost-effective genotyping and rapid phenotyping—facilitates the development of homozygous inbred lines. These inbred lines can then be crossed to enable rapid cycling, enhance genetic gains, and accelerate the development of improved crop varieties. Therefore, the integration of speed breeding with multiple complementary techniques can be applied to improve orphan crops, legumes, vegetables, leafy greens, and forage crops (Sowndarya and Tyagi, 2021).

3.4. Haploid induction breeding

With rising demand for agricultural products, there is an urgent need for technologies that accelerate the selection of cultivated crops. The primary goal of breeding sugar beet is to develop highly productive domestic hybrids through linear methods. The efficiency of hybrid development increases markedly when biotechnological methods are applied to generate new breeding material. The economic importance of *B. vulgaris* has stimulated strong interest in optimizing in vitro micropropagation, creating haploid forms, and deriving homozygous lines for breeding purposes.

A haploid plant, whether diploid- or polyploidy-derived, exhibits a gametophytic chromosome number, meaning it contains a single set of unpaired chromosomes (n). Haploid plants may occur naturally or be induced artificially through various methods. These methods include in vivo approaches such as parthenogenesis, and in vitro approaches such as androgenesis (via microspore and anther culture) and gynogenesis (via ovule and ovary culture) (Aflaki et al., 2017). Haploid production is a valuable research tool with applications in plant breeding, genetic manipulation, and genome or gene mapping. A key advantage is achieving complete homozygosity in a single generation, whereas traditional breeding requires over five generations with selection to reach acceptable levels (Gerald et al., 2013). This process may take even longer in biennial crops. Additionally, haploid plants enable the simultaneous expression of recessive alleles normally masked in heterozygous states, facilitating the identification, evaluation, and selection of desirable traits (Aflaki et al., 2017).

Haploid production in plants can be achieved through multiple methods, each providing specific advantages depending on the species and breeding objectives. One approach involves pollinating plants with pollen from the same species, using haploid inducers, where the resulting haploids are categorized as paternal or maternal depending on their genetic origin. Another method is interspecific hybridization, for example crossing wheat with corn, which has proven particularly effective in generating haploids in several *Triticum* species (Kolesnikova et al., 2021). Pollination with irradiated pollen is another technique, particularly applied in cucumber breeding, where it facilitates haploid production that requires subsequent chromosome doubling for use in breeding programs (Gurel et al., 2021). The CENH3-mediated haploid induction method has also been studied, but so far it has shown limited success in most crops, with maize being a notable exception (Watts et al, 2016). Haploids can also be produced through androgenesis or gynogenesis, in which isolated anthers, microspores, or ovules are cultured in an artificial nutrient medium (Kolesnikova et al., 2021).

Biotechnology has accelerated the development of new sugar beet hybrids, especially through the production of doubled haploids (DHs), which significantly reduces the time and resources needed to develop elite lines. The most effective method for producing haploids in *B. vulgaris* is induced gynogenesis, in which nonpollinated ovules are cultured in vitro, resulting in plants with a haploid chromosome set. To develop doubled haploid (DH) lines, haploid chromosomes are doubled using antimitotic agents, followed by ploidy control and the cultivation of microclones under greenhouse conditions, ultimately leading to the production of DH plant plugs (Gurel et al., 2000). However, tissue culture processes for sugar beet—particularly the induction of haploid (H) and DH forms—still require further investigation. Literature indicates that improving the efficiency and reproducibility of doubled haploid production in *B. vulgaris* requires further investigation and refinement using novel approaches. Biotechnology laboratories must be capable of producing haploids at scale, requiring improvements in explant culture methods, diploidization, rooting, and the acclimatization of regenerants from sterile in vitro conditions to soil. Optimizing each stage of this process is essential for improving both the quality and quantity of the resulting homozygous material (Kolesnikova et al., 2021).

4. Sugar beet diseases

4.1. Rhizomania (root beard) disease

Beet necrotic yellow vein virus (BNYVV) is the causal agent of rhizomania, one of the most devastating diseases affecting sugar beet production globally. First identified in Italy in the 1950s, rhizomania has since spread to nearly all major sugar beet-growing regions worldwide (Canova, 1959). The disease first appears as yellow patches in fields, spreading via infected soil carried by ploughs and farm machinery. Infected roots lose efficiency in water and nutrient uptake, producing pale yellow leaves with elongated petioles. Root symptoms vary from mild to severe, commonly including dense hairy secondary roots, darkened vascular tissue, reduced taproot size, and in some cases root rot. Rhizomania can reduce sugar content in beets from 17% to 10% and decrease root yield by as much as 90% (Asher, 1993).

Among viral diseases affecting sugar beet, BNYVV is the most destructive, surpassing beet western yellows virus (BWYV) and beet yellows virus (BYV). BNYVV is transmitted via soil by the widely distributed fungus *Polymyxa betae* Keskin, causing severe stunting and extensive lateral rootlet formation on the main root. In susceptible varieties, the disease can reduce root yield, sucrose content, and juice quality by up to 80% (Stevens et al., 2006). To counter this, rhizomania-resistant varieties have been developed through conventional breeding, using resistance genes from sugar beet and its wild relative *Beta maritima* (Gurel et al., 2008). In 1983, U.S. researchers identified the dominant resistance gene *Holly* (Lewellen et al., 1987). Resistance or susceptibility of genotypes was found to depend on the presence or absence of one or two of five genes, which show different expression levels in the heterozygous state. These genes—*Rz1*, *Rz2*, *Rz3*, *Rz4*, and *Rz5*—are located on chromosome III, with *Rz1*, *Rz4*, and *Rz5* representing alleles of the same gene. The resistance genes *Rz2* and *Rz3*, acting individually or in combination, can completely prevent BNYVV infection, and are thus considered distinct entities (Nalbandyan et al., 2019).

To combat rhizomania, one of the biotechnological approaches is marker-assisted selection (MAS), based on identified resistance genes. Breeders use this approach to select resistant varieties. Genetic engineering offers another option, enabling the transfer of resistance genes from wild relatives into cultivated species through transgenic techniques. In addition, RNA-silencing mechanisms against BNYVV in sugar beet have been reported (Lennefors et al., 2006). For example, dsRNA-mediated silencing targeting the BNYVV replicase gene was achieved via *Agrobacterium rhizogenes*-mediated transformation (Pavli et al., 2010). Beyond these techniques, new technologies offer promising opportunities for the future. One such example is CRISPR/Cas9 genome-editing technology. It is being applied in domestication, yield improvement, and the development of crops resistant to biotic and abiotic stresses.

4.2. Cercospora (leaf spot) disease

Cercospora leaf spot (CLS), caused by *Cercospora beticola* Sacc. (class: Ascomycota; order: Dothideales; family: Mycosphaerellaceae), is the most important foliar disease of sugar beet, leading to severe yield and quality losses. The pathogen was first described by Saccardo in (1876) (Khan et al., 2009). Leaves infected with *C. beticola* develop tan to gray circular lesions with centers surrounded by tan-brown to reddish-purple rings. As the disease progresses, lesions coalesce to form larger necrotic areas. Severe infection ultimately leads to leaf death. The lesions contain black spore-bearing structures in their centers. These structures are known as pseudostromata. Symptoms first appear on older leaves and then spread to younger ones (Rangel et al., 2020). The *C. beticola* reference genome has been reported to contain 12,281 genes, 12,495 transcripts, and 28,389 exons (De Jonge et al., 2018).

Polymerase chain reaction (PCR) is a specific and sensitive method for identifying potential risks of CLS. Primers CBACTIN915L (5' GTAAGTGCTGCCACAATCAGAC 3') and CBACTIN915R (5' TACCATGACGATGTTTCCGTAG 3') were designed for PCR detection (Lartey et al., 2003). Management can be achieved through traditional methods such as crop rotation. Fungicide application is another management strategy for CLS, but repeated use of the same fungicide can lead to the development of resistant pathogen strains. Available fungicidal chemicals include dithiocarbamates, nitriles, fentin derivatives, benzimidazoles, demethylation inhibitors (DMIs), amines, and quinone outside inhibitors, some of which are used for protective and/or curative purposes (Ioannidis et al., 2010). Beyond traditional and chemical methods, molecular techniques are valuable tools for selecting more resistant sugar beet cultivars. Studies have shown that randomly amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR), and sequence-related amplified polymorphism (SRAP) markers are highly polymorphic in sugar beet and can distinguish resistant cultivars from susceptible ones. In addition, biochemical, protein, and isozyme markers are useful for detecting resistant cultivars. Knowledge of molecular and biochemical techniques can facilitate the selection of resistant cultivars and reduce the time required compared to traditional breeding approaches.

Biotechnological approaches hold great potential for controlling Cercospora disease. However, their commercialization and widespread use face both technical and regulatory challenges. Advances in genetic and microbiome engineering may play a key role in overcoming these challenges. Furthermore, the environmental impacts

and public acceptance of these biotechnological methods should be prioritized in future research.

4.3. Cyst nematode disease (*Heterodera schachtii Schmidt.*)

The sugar beet cyst nematode (BCN) was among the earliest plant-parasitic nematodes to be identified. It is a small plant-parasitic nematode that can drastically reduce crop yields. It can infect more than 200 plant species, including sugar beet, garden beet, table beet, and canola. BCN has been reported in more than 87 countries worldwide, affecting all major sugar beet-growing regions (Dewar and Cooke, 2006). *Heterodera schachtii* is responsible for about 90% of all nematode-related infestations in sugar beet worldwide. High soil temperature is critical for the growth of *H. schachtii* during the vegetation period. *H. schachtii* may gain an advantage due to global warming, which can result in decreased sugar beet yield (Sielemann et. al., 2023). Standard sugar beet cultivars traditionally used are sensitive and vulnerable to BCN (Daub, 2021). Efforts are underway to reduce these losses by developing resistant and tolerant varieties through breeding.

4.3.1. Description and life cycle

The botanist Herman Schacht first observed *H. schachtii* in Halle, Central Germany, in 1859. Later in 1871, Adolf Schmidt formally described the species and named it in honor of its discoverer (Daub, 2021).

The most distinctive stage of *H. schachtii* is the lemon-shaped cyst, formed from the cuticle of the deceased female, which can contain up to 600 eggs. After hatching, second-stage juveniles (J2) exit the cyst through the vulval or oral orifice, enter the soil, and penetrate the roots of their host plants. Cysts in the soil may hatch even in the absence of host plants; however, root exudates stimulate further hatching and attract larvae to the roots (Dewar and Cooke, 2006). After that, J2 enter root cells with the aid of enzymes such as cellulases and pectinases, secreted by gland cells that modify the cell wall, allowing them to migrate along positional gradients within the root. Juveniles establish a feeding site called syncytium, where they settle. Once a syncytium is properly established, each J2 feeds within it and becomes sedentary. Smaller syncytia are typically associated with males, which later emerge from the roots to fertilize females. After the female nematode dies, its body wall tans and hardens, forming a protective cyst around the eggs (Fosu-Nyarko et al., 2016). Egg development is strongly influenced by soil temperature, which must rise to around 30 °C for the life cycle to be completed (Dewar and Cooke, 2006). Even without host plants, 400–500 eggs enclosed in protective cysts can persist in the soil for several years, demonstrating the remarkable resilience of nematode eggs under adverse conditions (Gurel et al., 2008).

4.3.2. Damage and symptoms

Beet cyst nematodes (BCN) are sedentary endoparasites that feed within the roots of their host, where they induce highly specialized feeding structures in the vascular system, causing significant yield losses (Gurel et al., 2008). Nematodes invade plant roots and cause damage. Disrupted root growth and development lead to water and nutrient deficiencies in the plant. Consequently, susceptible cultivars exhibit wilting and stunted growth (Daub, 2021). In severe BCN infestations, older leaves turn yellow due to manganese deficiency. Damage typically peaks about 2 months after sowing. Symptoms vary depending on the growth stage and may include excessive lateral root proliferation, taproot stunting, abnormal leaf growth, and premature wilting of foliage (Dewar and Cooke, 2006).

Severe root infections can cause lateral root death, root deformation, and "bearding" of storage roots. These effects increase plant susceptibility to wilting and reduce growth and yield (Fosu-Nyarko et al., 2016).

4.3.3. Protection from cyst nematode

H. schachtii is classified as a major quarantine pest in many countries. It causes severe damage to sugar beet and poses a substantial threat to production.

One management strategy against cyst nematodes is the use of nematicides. These chemicals kill plant-parasitic nematodes and are fast and effective; however, repeated applications by trained personnel are required. Moreover, they are toxic and may enter the food chain (Tóthné Bogdányi et al., 2021). The EPA's restrictions on chemical nematicides are largely driven by environmental health concerns. Nonfumigant nematicides can be applied as preplanting treatments, but they do not provide the same level of protection as fumigants.

Induced resistance is an alternative to nematicides for protecting sugar beet against BCN. This method offers a safer solution compared to nematicides. Induced resistance plays a critical role in defense priming. Defense priming occurs in two steps. In the first step, elicitors or priming agents are applied to activate defense responses. In the second step, when a stress factor such as BCN attacks the plant, it triggers the defense system to respond more quickly and strongly. Plant defense priming is ecologically low-cost and triggers strong, broad-spectrum defense responses (Singh et. al., 2020). In studies conducted on *Arabidopsis*, salicylic acid was found to reduce BCN infections (Wubben et al., 2008), while jasmonic acid enhanced plant defenses and conferred resistance against BCN (Kammerhofer et al., 2015). Another study showed that jasmonate and ethylene applications enhanced the basal defense responses of sugar beet plants against BCN infection (Ghaemi et al. 2020).

An important protection strategy, particularly for breeders, is the introduction of resistance genes into

cultivars. Effective resistance genes against BCN are absent from the sugar beet genome. The *Procumbentes* section of *Beta*—including *B. procumbens*, *B. patellaris*, and *B. webbiana*—contains these genes (*Hs1*$^{pro-1}$). Researchers succeeded in introgressing this gene into sugar beet through interspecific crossing with *B. procumbens*; however, this introgression resulted in significant yield reductions (Gurel et al., 2008). The *Hs1*$^{pro-1}$ gene limits syncytium growth, thereby aggressively destroying invasive young nematodes. Kifle et al. (1999) introduced the *Hs1*$^{pro-1}$ gene into sugar beet via A. *rhizogenes*. The study reported highly stable transformation of the *Hs1*$^{pro-1}$ gene and resistance to *H. schachtii*. Unfortunately, these resistant types suffer from low yield and are therefore not widely cultivated.

Recent studies have identified additional resistance genes against BCN. The *HsBvm*$^{-1}$ gene was identified in *Beta vulgaris* ssp. *maritima*. It protects beet yield under BCN infection and mitigates the damage caused by the pathogen (Stevanato et al., 2015). Sielemann et al. (2023) identified *BvNLP7* genes via mapping-by-sequencing, which may influence tolerance to *H. schachtii*.

Farmers also employ early sowing, extend crop rotations to 3–5 years, and avoid host species in rotations, which allow for natural population decline and reduced damage from cyst nematodes. However, extended crop rotations are economically impractical.

4.4. Rhizoctonia root rot disease

Rhizoctonia crown and root rot, caused by *Rhizoctonia solani*, is a fungal species complex consisting of different anastomosis groups (AGs). It is significant in both agriculture and forestry. The optimum temperature range for infection is between 18 °C and 30 °C (Bolton et al., 2010). The pathogen causes a wide range of symptoms and diseases in major crops, including root, crown, and stem rot, damping-off, and wilting. The fungus can persist for long periods in soil or plant residues as mycelium or sclerotia, which makes disease management particularly challenging. Root rot symptoms may range from small brown to black lesions on the root surface to complete root decay. The disease often first appears in isolated patches within a field. However, when inoculum levels are high—often as a result of continuous cultivation of susceptible crops—the disease can devastate entire sugar beet fields under favorable conditions.

Chemical control (e.g., azoxystrobin) is used in some countries, but available options remain limited (Bolton et al., 2010; Rafiei et al., 2023). Therefore, alternative approaches such as crop rotation with nonhost plants, tillage practices, and the use of resistant cultivars are being implemented (Rafiei et al., 2023). Effective control of the disease primarily relies on cultivating resistant sugar beet varieties. Breeding programs for root rot-resistant cultivars began in 1957 at Fort Collins (Gaskill, 1968). These programs relied on mass selection and recurrent field selection under artificially induced disease conditions (Gaskill, 1968; Panella, 1998) . Since 1966, many resistant lines have been developed and released. Nearly all of these resistant breeding lines are believed to originate from the open-pollinated variety GWS 359–52R, developed by the Great Western Sugar Co. (Panella, 1998).

Sustainable breeding for *R. solani* resistance in sugar beet depends on the continued identification of resistance genes for incorporation into new cultivars. Improved disease management can be achieved by using tolerant or resistant cultivars selected with molecular markers such as single nucleotide polymorphisms (SNPs). One such marker, RsBv1 (Chromosome 6, position 9,000,093 bp, C/T), is associated with Rhizoctonia resistance and has been validated across three geographically diverse plant materials (Ravi et al., 2021). The development of resistance-linked markers can greatly support breeding programs for Rhizoctonia resistance. A major objective of breeding programs is to advance markers for marker-assisted selection and to study resistant cultivars. Therefore, it is essential to identify new markers, as in the cited study, and to integrate resistant variants into breeding programs.

4.5. Sugar beet root maggot disease

The sugar beet root maggot (SBRM), *Tetanops myopaeformis,* (von Röder), is a major pest of sugar beet in the United States and Canada, particularly in the Red River Valley of North Dakota and Minnesota (Theurer et al., 1982; Campbell et al., 2011). This species is distributed throughout North America. Its range includes Alberta and Manitoba in Canada, and U.S. states such as California, Colorado, Idaho, Minnesota, Nebraska, North Dakota, and Utah. Severe larval infestations damage plant roots and prevent adequate nutrient uptake. As a result, yield losses can range from 10% to 100% (Li and Smigocki, 2018).

4.5.1. Description and life cycle

Adult SBRM flies are about ¼ inch long. Their bodies are shiny black with a few hairs. They have two transparent wings. Female abdomens are pointed, whereas male abdomens are rounded. The eggs are white and slightly curved in shape. The larvae are white and legless. Pupae are brown to flesh-colored capsules (Hein, 1995). Pupae are smaller than fully grown larvae. Eggs, larvae, and pupae all occur in the soil (Bechinski et al., 1994).

Fully grown larvae overwinter 12–14 inches deep in the soil of the previous year's fields. As temperatures rise, larvae move toward the soil surface and pupate. Between the middle of May and the beginning of June, adult flies emerge and reach peak abundance. Unseasonably cold weather reduces fly emergence, whereas warm conditions significantly increase their numbers. Because the flies are weak fliers, they disperse only to nearby fields. Adults

begin laying eggs at 3–10 days of age. Females lay eggs in clusters of 20–40 on the soil surface near sugar beet plants. Each female lays an average of 100–140 eggs. Moist soil is essential for egg survival. The maggots feed on sugar beet roots until late July or early August, progressing through three larval instars. Once fully developed, they enter diapause and overwinter until the following spring (Bechinski et al., 1994; Hein, 1995).

4.5.2. Damage

SBRM causes damage only during its larval stage. Larvae feed by scraping the root surface of sugar beet with their rasping mouth hooks. Thus, SBRM reduces both root yield and sucrose content in sugar beet (Bechinski et al., 1994). Root damage negatively affects plant nutrition. Heavy infestations can cause severe wilting or even death of sugar beet plants. As the plant's defense weakens, it may become more vulnerable to other pathogens (Hein, 1995; Campbell et al., 2008). The extent of damage depends on plant size and the degree of larval infestation. Small seedlings are the most susceptible because their roots can be cut more easily.[1]

4.5.3. Protection from sugar beet root maggot

Crop rotation alone is insufficient because flies can move among neighboring fields. Early planting of sugar beet provides an advantage against root maggots. This is because sugar beet has passed its delicate growth stage by the time the maggots begin feeding. These two approaches are known as cultural methods and can reduce damage in sugar beet; however, they may not be sufficient under severe infestations.

Another control method is the use of chemical insecticides to reduce larval populations in the field. Chlorpyrifos and terbufos have been used for over 35 years to reduce SBRM larval feeding. These chemicals act against SBRM by inhibiting acetylcholinesterase (Campbell et al., 2008). Chemical insecticides may have adverse effects on the environment and, at times, on the sugar beet plant itself. SBRM may develop resistance to chemical insecticides in the future, which could negatively impact sugar beet production.

Resistance genes against root maggots in sugar beet have been studied since the 1980s (Theurer et al., 1982). To date, only a few resistant germplants appear to be publicly available, such as F1015 (PI 605413), F1016 (PI 608437), and F1024 (PI 658654) (Campbell et al., 2000; Campbell et al., 2011). These resistant sugar beet lines can reduce damage by approximately 40% (Li and Smigocki, 2018).

5. Future prospects

5.1. Regulatory framework

CRISPR-Cas technology enables precise genetic modifications in both model and crop plants to address challenges such as disease resistance, heat tolerance, and improved nutritional profiles. The technique offers heritable and predictable modifications, unlike traditional genetic engineering methods, which has led to a debate on whether CRISPR-edited crops should be classified as genetically modified (GM) (Prado et al., 2024). Regulatory bodies such as the FDA, EFSA, and WHO conduct extensive safety assessments of GM crops before approving them for commercial use, deeming them safe for human consumption. Countries such as India and China have exempted CRISPR-edited crops from biosafety regulations, demonstrating a growing acceptance and adoption of this technology for crop improvement (Das et al., 2023), while others have deregulated CRISPR-edited crops such as SDN1 and SDN2 due to the absence of foreign DNA. The EU considers all gene-edited crops GM. Changing regulations underscore the need for a universal and transparent framework to regulate gene-edited crops and ensure consumer acceptance and environmental safety. Countries are rethinking regulatory frameworks for genetically modified organisms and relaxing regulatory barriers for edited plants, reflecting the need to adapt regulations to accommodate advances in gene editing technologies such as CRISPR (Das et al., 2023).

5.2. Future of biotechnology and breeding technologies in sugar beet

This review outlines the advancements in breeding and biotechnology that have been achieved in sugar beet cultivation to date. Sugar beet, an alternative to sugarcane, is mostly used for sugar extraction and possesses high industrial value (sugar, ethanol, and fodder industries) as well as substantial nutritional importance. Abiotic and biotic stress factors are considered the primary obstacles to sugar beet cultivation. Sugar beet breeding has advanced rapidly, resulting in the development of stress-resistant plants through progress in traditional breeding, biotechnology, transgenic methods, and genome editing technologies. Although traditional breeding alone is time-consuming, combining classical breeding with genetic transformation and genome editing technologies has enabled the development of new traits in sugar beet in a short time. Sugar beet and its wild relatives' genomes have provided researchers with valuable sources of information for gene transfer and genome editing in sugar beet as well as other plants. The discovery of genes that regulate sugar beet traits such as size, disease resistance, and drought/salt tolerance has made it feasible to create new sugar beet varieties that are healthier for the environment and for humans, thanks to these cutting-edge technologies.

A range of biotic and abiotic stressors, including pests, diseases, weeds, salinity, and herbicides, negatively affect

[1] Hein GL (1995). Sugarbeet Root Maggot Management. Historical Materials from University of Nebraska-Lincoln Extension. Paper 1074. [online] Website http://digitalcommons.unl.edu/extensionhist/1074 [accessed 16 February 2025].

both the yield and quality of beet production (Yolcu et al., 2021). Traditional methods are the oldest and most labor-intensive approaches in sugar beet breeding. Nonetheless, they are still used today, mainly focusing on selecting self-pollinated lines with stable morphological traits, high-quality seeds, and economically valuable attributes (Lv et al., 2019). Molecular marker systems, genetic transformation, and genome editing techniques are examples of advanced biotechnological tools that require less time compared to classical methods. These approaches are more promising as they allow the stable preservation and enhancement of multiple traits (Pattanayak et al., 2023). Genome-wide association studies (GWAS) enable the detection of genome-wide polymorphisms to uncover genetic variation by comparing genotypes and phenotypes across diverse populations. A recent comprehensive study resequenced 306 important cultivars and breeding lines originating from China, the USA, and Europe. After applying a minor allele frequency (MAF) threshold of >0.05, more than 8.3 million SNPs were retained for analysis. The GWAS was carried out for 26 agronomic traits using the Genomic Association and Prediction Integrated Tool (GAPIT) based on a mixed linear model (MLM), producing Manhattan and QQ plots to visualize the results. From this analysis, nearly 4,000 genes showed significant associations with the examined traits, with their chromosomal distributions systematically mapped. Although thousands of loci were detected, subsequent functional validation highlighted six key candidate genes with strong relevance to major agronomic traits (Wang et al., 2023). These findings illustrate that while GWAS provides a broad set of associations, only a subset can be confirmed as causative, and modern biotechnological tools such as CRISPR and genetic transformation can be employed to further explore and utilize these genes in breeding programs. In sugar beet, Agrobacterium- and PEG-mediated transformations are the most effective methods of genomic modification (Pattanayak et al., 2023). In current sugar beet production, the most desirable traits are resistance to fungi, viruses, pests, and nematodes, as well as herbicide and salt tolerance. Transgenic sugar beet plants offer a more precise way of developing end products (e.g., proteins) compared to conventional methods. Gene editing technologies can help farmers produce more nutritious crops and address the food demands of an increasingly populated world. Beyond agriculture, CRISPR technology may also be applied more broadly to elucidate genetic structures and functions in plants, including sugar beet (Baloglu et al., 2022).

Future sugar beet breeding programs could utilize genetic transformation and/or CRISPR technologies to improve several key agronomically important traits, such as resistance to biotic and abiotic stresses, enhanced nutritional quality, and increased sugar content (Misra et al., 2023). This review highlights recent studies demonstrating the widespread application of next-generation biotechnologies, such as omics, GWAS, genetic transformation, and CRISPR, to confer desirable traits on sugar beet. However, further advancements are needed to improve the efficiency of regeneration and transformation systems, establish reliable screening and selection protocols, and develop multifunctional vector systems.

A few potential solutions to these issues in the near future are summed up as follows:

Sugar beet still has limited regenerative ability; however, the development of a faster, one-step (monophasic) process for simultaneous shoot and root production should take precedence (Subrahmanyeswari and Gantait, 2022). Further research is also needed on the use of synthetic seed technologies and somatic embryogenesis for sugar beet regeneration.

New techniques, such as delivering transformation vectors into germline cells, identifying sugar beet lines with high regeneration capacity, and optimizing protoplast-mediated transformation, could be developed to improve transformation efficiency.

CRISPR has made it possible to modify the sugar beet genome in novel ways. This technique can be applied to the development of sugar beet germplasm and breeding lines tolerant to abiotic and biotic stresses. Specific genes, such as $Hs1^{pro-1}$ for nematode resistance and *Rz1*, *Rz2*, and *Rz3* for beet necrotic yellow vein virus (BNYVV) resistance, can be altered by silencing or targeted mutageesis.

Extended field testing should be conducted to evaluate the performance of genome-edited plants before commercialization, even though CRISPR eliminates the possibility of foreign DNA being present in the final crop (Baloglu et al., 2022).

GWAS could be used to identify and characterize functional genes at the molecular level that influence valuable agronomic traits in sugar beet.

Several crucial genes associated with pathogen resistance (*SDR, GST1, GST2, Rx1, Mi-1, AtMPK4, VSP1, PAD4, EDS1*), insect resistance (*CEV1, EIN2, BvSTI, JAR1, COI1, HSP90, SDR*), and nematode resistance (*Hs1 pro−1, Hs2 pro−7, Hs1 web−7, Hs1-1, Mi, Gpa2*) (Lv et al., 2019; Yolcu et al., 2021) should be further examined using different omics technologies to clarify their functional roles in biotic stress mechanisms in sugar beet.

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