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Advancements and prospects of CRISPR/Cas9 technologies for abiotic and biotic stresses in sugar beet

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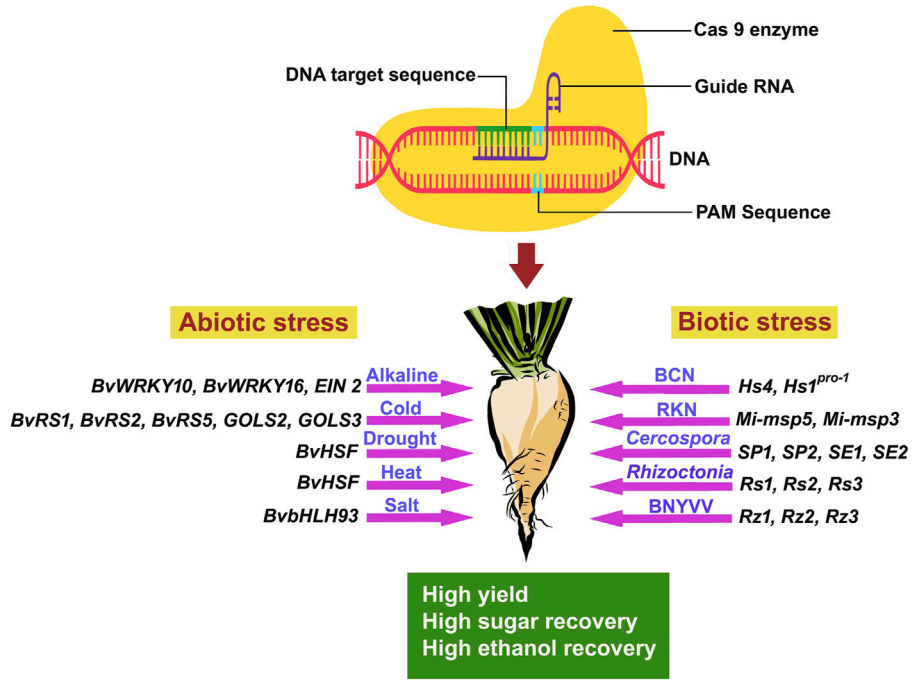
Sugar beet is a crop with high sucrose content, known for sugar production and recently being considered as an emerging raw material for bioethanol production. This crop is also utilized as cattle feed, mainly when animal green fodder is scarce. Bioethanol and hydrogen gas production from this crop is an essential source of clean energy. Environmental stresses (abiotic/biotic) severely affect the productivity of this crop. Over the past few decades, the molecular mechanisms of biotic and abiotic stress responses in sugar beet have been investigated using next-generation sequencing, gene editing/silencing, and over-expression approaches. This information can be efficiently utilized through CRISPR/Cas 9 technology to mitigate the effects of abiotic and biotic stresses in sugar beet cultivation. This review highlights the potential use of CRISPR/Cas 9 technology for abiotic and biotic stress management in sugar beet. Beet genes known to be involved in response to alkaline, cold, and heavy metal stresses can be precisely modified via CRISPR/Cas 9 technology for enhancing sugar beet's resilience to abiotic stresses with minimal off-target effects. Similarly, CRISPR/Cas 9 technology can help generate insect-resistant sugar beet varieties by targeting susceptibility-related genes, whereas incorporating *Cry1Ab* and *Cry1C* genes may provide defense against lepidopteron insects. Overall, CRISPR/Cas 9 technology may help enhance sugar beet's adaptability to challenging environments, ensuring sustainable, high-yield production.

KEYWORDS

abiotic, biotic, CRISPR/Cas, genes, genome editing, tolerance

1 Introduction

Sugar beet (*Beta vulgaris* L.) is cultivated in temperate regions of the world, however, its cultivation has spread to the tropical and subtropical zones of India (Mall et al., 2022a; Misra et al., 2022a). Sugar beet is well known for its sugar production in many countries of the world covering a significant area under cultivation (Table 1) and contributing to around 30% of the world's sugar requirement (Zicari et al., 2019). The root of sugar beet is an important source of natural sucrose as a sweetening agent (Mall et al., 2022b) and has diverse industrial applications (Misra et al., 2018). Sugar beet production faces major threats from biotic and abiotic stresses (Mulet, 2022). For instance, common viral (beet necrotic yellow vein virus, BNYVV (Ramchandran et al., 2021), bacterial (like *Pseudomonas aptata*), and fungal



GRAPHICAL ABSTRACT

diseases (like *Cercospora* (Misra et al., 2022c); *Alternaria* (Misra et al., 2020a), as well as nematodes (*Meloidogyne incognita*), and insects (like *Aphis fabae* (Mulet, 2022), *Spodoptera litura* (Santeshwari et al., 2020; Baitha et al., 2022)) hampers the production and productivity of the crop. Salinity, drought, heat (high temperatures), and cold are some of the abiotic stresses that severely impact sugar beet production all over the world (Misra et al., 2020b) (Table 2). Besides, other abiotic stresses like ozone build-up, flooding, nutritional deficiency, and heavy metal poisoning of the soil can also be challenging (Shabbir et al., 2022). In order for sugar beet plants to produce their optimum amount of sugar, enough moisture availability on a daily basis is necessary so as to allow for efficient transpiration and photosynthesis processes (Ober and Rajabi, 2010; Barratt et al., 2023). However, such ideal circumstances under natural environmental conditions have not been observed frequently owing to scanty rainfall or limited irrigating options. Despite the concerted efforts in developing tolerant varieties of sugar beet through conventional breeding and genome editing for improving the sugar and ethanol yield (Pattanayak et al., 2023), the scope still exists in the field of development of sugar beet varieties tolerance/resistance to abiotic and/or biotic stresses.

Molecular biology has witnessed a massive transformation due to the emergence and development of the CRISPR/Cas system as a biotechnological tool. The CRISPR/Cas 9 system is reportedly an efficient technology (Ahmad et al., 2021). The microbial adaptive immune system, CRISPR may target any genomic region by using a synthetic short guide RNA (sgRNA) (Jinek et al., 2012). Its strength comes in its capacity to effectively and precisely cause double-strand breaks in DNA at any location in the genome. CRISPR mediated genome editing can be used to change practically any sequence to expose its role in the genome (Asmamaw and Zawdie, 2021). On the basis of the genes encoding the effector modules CRISPR–Cas systems can be classified where different cas proteins have unique characteristics and functional roles (Chaudhuri et al., 2022) (Table 3). CRISPR/Cas 9 mediated genome editing requires a protospacer adjacent motif (PAM) for Cas nuclease for initiation of the cutting process. The PAM is located 3-4 nucleotides downstream from the specific site where cleavage needs to be done (Gleditzsch et al., 2019). The advances in genome editing techniques, particularly CRISPR/Cas 9 system, will benefit the cultivation of sugar beet by developing varieties resistant to abiotic and biotic stress conditions. This review discusses the current understanding of the mechanism of CRISPR/Cas 9 technology and its application in the improvement of sugar beet cultivars against abiotic/biotic stresses.

2 Site-directed nucleases (SDNs) and comparison of zinc finger nucleases, transcription activator like effector nucleases with CRISPR/Cas 9 technology

Targeted genome engineering has been emerged as an alternative to traditional plant breeding approaches, aiming to achieve a variety of crop improvement goals and sustainable food production (Misra et al., 2018). Genetic engineering (GE) techniques have been deployed to enhance the quality attributes of sugar beet (e.g., shelf life) (Monteiro et al., 2018) and improve its tolerance to biotic and abiotic stresses (Wan et al., 2021). In this context, the application of site-directed nucleases (SDNs) has evolved as a suitable GE technique for introducing desirable characteristics into plants (Aglawe et al., 2018). Zinc finger nucleases (ZFNs), transcription activator like

TABLE 1 Sugar beet area, yield, and production in major sugar beet producing countries of the world.

Country	Area (ha)	Yield (100 gm/ha)	Production (t)	References
China	229,300	342,386	7,850,900	FAO (2022)
France	491,880	855,116	34,365,390	
Germany	390,700	817,645	31,945,400	
Iran	91,803	560,650	5,146,924.8	
Italy	27,910	541,279	1,510,710	
Poland	250,570	609,564	15,273,850	
Russia	993,830	414,575	412,016,686	
Turkiye	288,940	631,620	18,250,000	
Ukraine	226,600	478,989	10,853,880	
United States	448,230	743,813	33,339,950	

TABLE 2 Impact of abiotic/biotic stresses on sugar beet yield.

Stresses		Reduction in sugar beet root yield (%)	References
Abiotic	Salt stress	49.3	Anagholi et al. (2018)
		10–50	Bybordi (2010)
	Cold	77 (in dry matter reduction)	Jalilian et al. (2017)
	Drought	27 (50% less irrigation); 21 (50% less irrigation)	Ghaffari et al. (2022)
		5 (Northern Europe)	Pidgeon et al. (2001)
		30 (Southern Russia)	
Disease resistance			
Biotic	Beet cyst nematode (*Heterodera schachtii* Schmidt)	25 to 50	Agrios (2005)
		21 (Italy)	Greco et al. (1993)
		60	Grujicic (1958); Cooke (1987)
		70	Pylypenko and Kalatur (2015)
	Cercospora leaf spot	30	Tan et al. (2023)
		40	Smith and Ruppel (1973); Esh and Taghian (2022)
		20–25 (India)	
	Beet curly top virus (BCTV)	30	AgResearch Magazine (2016)
	Beet necrotic yellow vein virus (BNYVV)	90	Johansson (1985); Casarini (1999)
Insect Pests			
	Armyworm (*Spodoptera* spp.)	>25 (Foliage damage)	DiFonzo et al. (2006)

(TAL) effector nucleases (TALENs), and Clustered regularly interspaced short palindromic repeats and CRISPR-associated (CRISPR/Cas) technology are instances of SDN methods. SDNs enable precise changes at predetermined places in a genome, avoiding any unintended random mutagenesis. They offer unparalleled control over targeted genome alterations, proving to be more cost-effective and efficient than conventional plant breeding and genetic engineering methods. SDNs have the potential to aid in crop improvement and enhance food security in many sugar beet producing countries (Table 4). SDNs are categorised into SDN1, SDN2, and SDN3 based on the outcomes of genomic alterations and double strand break repair (Ghouri et al., 2023). SDN1 does not require template and causes gene disruptions *via* InDels (small insertions or deletions of bases). SDN2 uses a homologous template to repair or modify the gene at one or more locations. SDN3 requires the use of a whole gene as a template and results in gene substitution or foreign DNA insertion (Chen and Gao, 2020).

TABLE 3 Different types or classes of Cas proteins, emphasizing their unique characteristics and functional roles.

Protein	Class	Type	Sub type	Process	Functions
Cas 1	1, 2	I, II, III, IV, V, VI	A, B, C, D, E, F, U	Spacer acquisition	DNAse, bind RNA; Cleavage at the specific site; Allow insertion of new spacers into CRISPR arrays
Cas 2	1, 2	I, II, III, IV, V, VI			Specific to U-rich regions; Homologous to mRNA interferase
Cas 3 (Signature)	1	I		Target interference	DNA helicase endonuclease; Cutting the target DNA at specific sites due to endonuclease domain
Cas 4	1, 2	I, II		Spacer acquisition and regulation	RecB-like nuclease homologous to RecB; Exonuclease activity and binds with RecBCD
Cas 5	1	I		crRNA expression and target binding	RAMP protein, crRNA biogenesis; (small subunit protein) Catalysation of crRNA processing; binds to a large subunit of Cas 8 in type I and Cas 10 in type III
Cas 6	1	I, III			RAMP protein, crRNA biogenesis; (nuclease activity) Repeat specific RNase involves in crRNA processing
Cas 7	1	I			RAMP protein, crRNA biogenesis; Co-transcriptional RNA cleavage during interference
Cas 8	1	I			Large protein with McrA/HNH-nuclease domain; Homologue of Cas10 protein; large subunit binding occurs with cas5 subunit
Cas 9 (Signature)	2	II	A, B, C	crRNA Target interference	Large multidomain protein with McrA-HNH nuclease domain; (Type II signature protein) crRNA dependent nuclease; Mediates RNA-guided DNA cleavage; widely used as DNA nucleases for inducing site-specific DNA breaks
Cas 10 (Signature)	1	III	A, B, C, D	crRNA expression and interference	HD nuclease domain, palm domain, Zn ribbon; Genetic manipulation
Cas 12a	2	V	A	Spacer acquisition	Cleaves the both complementary strands of the targeted DNA segment using a single RuvC nuclease domain
Cas 12b	2	V	B	Target interference	Dual-RNA-guided DNA nuclease and requires tracrRNA for further processing; higher efficiency for gene activation; wider target site for gene suppression; prefers T-rich protospacer adjacent motifs (PAMs)
Cas 12c	2	V	C	crRNA expression	Target binding rather than target degradation
Cas 12d (Signature)	2	V	D	Target interference	Catalyzes DNA cleavage with short complementary untranslated RNA
Cas 12e (Signature)	2	V	E	Target interference	Target DNA unwinding
Cas 13	2	VI	A, B, C, D	crRNA expression	Interference activity; Degradation of mRNAs; impart tolerance against plant viruses

The CRISPR/Cas system is a convenient replacement for ZFNs and TALENs in generating targeted genomic alterations (Ahmad et al., 2021). Both ZFNs and TALENs are utilized to mutate genomes at specific loci (Boti et al., 2023). However, these systems require two distinct DNA binding proteins flanking the region of interest, each having a C-terminal *Fok*I nuclease module. Custom proteins are necessary for targeting DNA sequences. The process of designing and constructing custom proteins in both these technologies (Zinc finger motifs in ZFNs, while DNA binding domains obtained from TALE in TALENs, are required) is laborious and time-consuming. In CRISPR-mediated genome editing, PAM is located 3-4 nucleotides downstream from the specific site where cleavage will occur (Gleditzsch et al., 2019). Therefore, accessibility to the protospacer adjacent motif (PAM) site, as the main determining factor for altering any functioning sequence, is easier (Akram et al., 2023). CRISPR technology depends on RNA-guided sequences, which can be designed effortlessly (Bajpai et al., 2023).

The target specificity in ZFNs and TALENs is more challenging as zinc fingers or TALEs identify short DNA sequences and require the combination of multiple modules to target the desired region, which may result in off-targets (Jyoti et al., 2023). The probability of off-targets is very low in CRISPR because it utilizes guide RNA to target the desired site, which can be easily programmed. Furthermore, the application of custom protein engineering in ZFNs and TALENs causes lesser adaptation and more laborious efforts (Kalaitzandonakes et al., 2022) while altering in sgRNA is easier for targeting the desired gene in CRISPR technology. The use of sgRNA in CRISPR technology is also beneficial in the delivery stage, making it a simple process compared to ZFNs and TALENs (Pankaj and Kumar, 2023). Due to the complexity involved in ZFNs and TALENs, including design and construction, target specificity, flexibility, and delivery), these methods are not preferred compared to CRISPR (Bhatia et al., 2023).

TABLE 4 Legislation of different sugar beet producing countries for genome editing crops.

Countries	Legislation	SDN 1	SDN 2	SDN 3	Year approved	Crops approved	References
United States	Classified SDN-1,2 genome-edited crops are equivalent to traditional breeds	Deregulated		Case by case	2018	Corn	Schmidt et al. (2020); Herrera et al. (2017); Chaturvedi (2004); Lombardo and Grando. (2020)
						Tomato	
					2017	Soyabean	
					2016	Mushroom	
					2017	Flax	
China	The Ministry of Agriculture announced preliminary recommendations for evaluating the safety of genome-edited plants that do not include exogenous DNA.	Under development			Not Applicable	No crops approved	Mallapaty (2022); Haque et al. (2018); Chaturvedi (2004); Wang et al. (2015a)
India	According to the memorandum, working with genome-edited plants must be done with extreme caution until exogenous inserted DNA is no longer present. The guidelines apply to SDN-1 and SDN-2 genome-edited plants	Under development			Not Applicable	No approved crops	Buchholzer and Frommer (2023); Lombardo and Grando. (2020); Chaturvedi (2004)
Russia	According to Resolution of 22 April 2019 no. 479, provision of funding for genome editing and defined transgene-free modified crops as equivalent to those produced through conventional breeding	New polices are expected			Not Applicable	No approved crops	Dobrovidova (2019)
UK	Initially, genome-edited crops will be free from GMO field trial rules. Field testing defines "qualified higher plants" as genome-edited plants that might have been developed using standard breeding procedures or may have occurred naturally. It is predicted that genome-edited plants will then be able to successfully enter field trials and acquire commercial approval without the need for case-by-case review	Case by case			Not Applicable	No approved crops	Stokstad (2021)
Brazil	Products obtained through site-directed random mutation involving the joining of non-homologous ends (SDN1 mutation) or site-directed homologous repair involving one or few nucleotides (SDN2 mutation) meet the criteria established in Normative Resolution No. 16 to be designated as non-GMO on a case-by-case basis. according to the resolution's provisions, site-directed transgene insertions (SDN3 mutation) are classified GM. If the product is labelled as GMO, the developer must meet all biosafety regulations and will be approved only after the CTNBio risk assessment. If the product is labelled non-GMO, it can be registered using the same methods as conventional items	Deregulated		Deregulated (If not transgenic)	NA	No approved crops	Schiemann et al. (2020); Chaturvedi (2004); Lombardo and Grando. (2020)

3 Application of CRISPR/Cas 9 technology for abiotic stress resistance in sugar beet

Sugar beet (*Beta vulgaris* L.) production is drastically affected by biotic and abiotic factors, which reduce the rate of photosynthesis, expansion of the canopy, development of the root system, and consequently, the accumulation of sucrose content in the plant (Misra et al., 2022a). Abiotic stresses, including temperature fluctuations, water scarcity, salinity, metal toxicity, and UV radiation, are harmful to the sugar beet crop, and severely affect its yield across the world (Yu et al., 2020). These stressors greatly restrict the distribution of sugar beet crops, affect their developmental processes, and decrease sugar beet productivity (Ober and Rajabi, 2010). Improved understanding of multiple molecular mechanisms, like pathway signalling, activation of transcription factors, transcript modification (post-transcriptional modification), translation of processed transcript, and protein

TABLE 5 Identified genes in other crops for providing abiotic stress tolerance through CRISPR/Cas technology.

Abiotic stress	Genes targeted	Crops	References
Alkaline stress	*OsPPa6*	*Oryza sativa*	Wang et al. (2019)
Cold stress	*OsPIN5b, GS3, OsMYB30, OsAnn5, OsAnn3, OsPRP1*	*Oryza sativa*	Zeng et al. (2020), Shen et al. (2017),
			Nawaz et al. (2019)
	AtWRKY34	*Arabidopsis thaliana*	Zou et al. (2010)
	SlCBF1	*Solanum lycopersicum*	Li et al. (2018)
	VvWRKY24	*Vitis vinifera*	Wang et al. (2014)
	BcWRKY46	*Brassica campestris*	Wang et al. (2011)
Heat stress	*OsPDS, OsHSA1, OsNAC006, OsNA C006, OsPyl14/6*	*Oryza sativa*	Nandy et al. (2019), Qiu et al. (2018), Wang et al. (2020), Miao et al. (2018)
	AtWRKY25/26, AtWRKY33, AtWRKY39	*Arabidopsis thaliana*	Li et al. (2011), Jiang et al. (2008), Park et al. (2005)
Drought stress	*AtOST2, AtAREB1, AtAVP1, AtmiR169a*	*Arabidopsis thaliana*	Osakabe et al. (2016), Roca Paixão et al. (2019), Park et al. (2017), Zhao et al. (2016)
	OsERA1, OsSAPK2, OsSRL1, OsSRL2, OsDST, OsNAC14, OsPUB67	*Oryza sativa*	Ogata et al. (2020), Lou et al. (2017), Liebe et al. (2020), Santosh Kumar et al. (2020)
	BnaA6.RGA	*Brassica napus*	Wu et al. (2020)
	BdWRKY36	*Brachypodium distachyon*	Sun et al. (2014)
	BcWRKY46, BnaA6.RGA	*Brassica campestris*	Wang et al. (2011), Wu et al. (2020)
	GmMYB118	*Soyabean*	Du et al. (2018)
	GmMYB118	*Chickpea*	Badhan et al. (2021)
Salt stress	*AtWRKY, AtWRKY4, AtACQOS*	*Arabidopsis thaliana*	Li et al. (2021a), Kim et al. (2021)
	OsDST, OsSPL10, OsRAV2, OsBBS1, OsNAC45, OsAGO2, OsVDE, OsRR22, OsSAPK2, OsPQT3, OsPIL14, OsBGE3	*Oryza sativa*	Santosh Kumar et al. (2020), Lan et al. (2019), Duan et al. (2016), Zeng et al. (2018), Zhang et al. (2020b), Yin et al. (2020), Wang et al. (2003), Zhang et al. (2019), Mo et al. (2020), Alfatih et al. (2020), Yin et al. (2017)
	VpWRKY1, VpWRKY2, VpWRKY3	*Vitis pseudoreticulata*	Li et al. (2010), Zhu et al. (2012)
Herbicide stress	*OsTB1, OsALS, OsACC*	*Oryza sativa*	Butt et al. (2018), Zhang et al. (2020b), Lyu et al. (2022)
Metal stress	*Atoxp1*	*Arabidopsis thaliana*	Baeg et al. (2021)
	OsARM1, OsNramp5, OsLCT1, OsHAK1, OsPRX2	*Oryza sativa*	Wang et al. (2017b), Tang et al. (2017), Lu et al. (2017), Nieves-Cordones et al. (2017), Mao et al. (2019)

modifications after the translation process, underlying stress responses of sugar beet crops at multiple levels, would be helpful in increasing the sugar beet production and sustainability through the application of CRISPR/Cas 9 (Yu et al., 2020; Misra et al., 2022a).

Abiotic stress tolerance is a complex trait that is mediated by multiple genes. The components of metabolic, regulatory, and signalling networks in plants interact and crosstalk extensively under abiotic stress conditions (Garg et al., 2014; Mickelbart et al., 2015). Several abiotic stress resistance-conferring genes have been identified in plants (Table 5) and introgressed into related crops through the application of biotechnological tools (Razzaq et al., 2021).

Gene activation or repression occurs by targeting transcriptional activator or repressor complexes to particular sites within the gene promoter region with catalytically inactivated Cas endonuclease. For this purpose, CRISPR-based approaches like CRISPRa and CRISPRi could be beneficial in characterizing genes for abiotic stress tolerance (Osakabe et al., 2016). CRISPR activation, abbreviated as CRISPRa, is a CRISPR variation in which a catalytically dead (d) Cas 9 is coupled with a transcriptional effector to control target gene expression. When the guide RNA and the effector arm reach the genomic location, the dCas9 is unable to produce a cut, and the effector instead triggers downstream gene expression. CRISPRa technique is used to increase gene expression by targeting the promotor region upstream of the transcription start site (TSS), whereas the CRISPR interference (CRISPRi) approach suppresses gene expression by targeting the promotor region downstream of the TSS. The application of CRISPRa/i requires precise identification of

the TSS location (Davis et al., 2018; Thomas et al., 2019). CRISPRi is a technology that uses dCas9's programmable binding capacity to inhibit gene expression by preventing or interfering with RNA polymerase binding, transcription factor binding, and transcriptional elongation. sgRNA specific to a gene sequence's upstream regulatory region (e.g., promoter) or transcription initiation site could direct dCas9 to bind and inhibit transcription initiation or elongation, effectively silencing gene expression. To inhibit target gene expression, dCas9 is coupled with the transcription repression domain of the Kruppel associated box (KRAB). dCas9 alone or in combination with KRAB is an effective tool for knocking off one or more genes (Piatek et al., 2015). CRISPRi and CRISPRa regulate gene expression by increasing or suppressing RNA polymerase, respectively. Thus, CRISPRi and CRISPRa are promising approaches for exploring and regulating stress-regulatory genes, as well as developing abiotic stress tolerant varieties. CRISPRi/a has been utilized successfully in plants to modify expression by a factor of 1000 (La Russa and Qi, 2015). CRISPRi and CRISPRa technologies can also be used in sugar beet under abiotic stress conditions. Although there is a lack of information available on this aspect, there are prospects to be worked on. The application of these technologies can aid in regulating gene expression in response to abiotic stress conditions (McCarty et al., 2020). These could be correlated with gene expression in sugar beet under drought, salinity, or temperature stress to provide tolerance. Altering these gene expressions could produce resilient sugar beet varieties with respect to abiotic stress conditions. Inhibiting or activating the targeted stress-responsive genes in sugar beet under abiotic stress conditions will help in understanding the regulatory mechanism. This, in turn, will assist in developing sugar beet varieties tolerant to adverse environmental conditions.

Furthermore, Cas 12a could also be explored for targeting the specific genes contributing to abiotic stress pathways, like drought, salinity, temperature, etc. By altering the genes related to specific abiotic stress conditions in sugar beet, the plant's potential to adapt and survive under adverse conditions could be increased. For instance, Chen X. et al. (2018) reported the application of Cas12a in targeting drought-responsive genes (positive regulation of gene) in *Arabidopsis*. Altering these targeted drought-responsive genes through Cas 12a improved the plant's drought tolerance potential. Such plants exhibited better water retention capacity, reduction in wilting, and higher survivability under drought conditions.

The development and evolution of sugar beet varieties tolerant to abiotic stress induced by climate change and global warming are imperative (Yolcu et al., 2021). Therefore, CRISPR/Cas 9 technology is necessary to create highly resistant sugar beet varieties against biotic and abiotic stresses. The CRISPR/Cas 9 technology involves specific steps for developing novel sugar beet varieties for different abiotic stress tolerances (Figure 1).

3.1 Alkaline stress tolerance

Aside from ionic toxicity and osmotic stress, high pH in the alkaline soils disrupts cell pH stability, destroys cell membrane integrity, and reduces root vitality and photosynthetic activity (Fang et al., 2021). Under the condition of high saline stress, beet varieties that are cultivated along with wild types (naturally occurring/non-domesticated beet varieties) exhibited high antioxidant enzyme activities (Wang et al., 2017; Li B. et al., 2021). Wu et al. (2019) reported that with increasing sodium bicarbonate concentrations, Na^+ concentrations were enhanced significantly in shoots and roots of sugar beet plants under alkaline conditions while a steady level of potassium ion concentrations was observed. Maintenance of K^+ and Na^+ homeostasis could be a key strategy for sugar beets adjusting to alkaline stress. Several genes (like WRKY, NAC, MYB, etc.) have been reported to be involved in conferring abiotic stress tolerance in plants (Chinnusamy et al., 2006; Hennig, 2012; Khadiza et al., 2017).

Wu et al. (2019) reported 58 *WRKY* genes, and among them, 9 genes were found to be responsible for alkaline stress responses (~15 mM–100 mM $NaCHO_3$) in both shoot and root parts. It evidently proved the increased expression of the *BvWRKY10* gene (in the terminal and lateral shoots) and *BvWRKY16* gene (in roots) under alkaline stress. WRKY proteins have been known to be associated with the response of plants to biotic and abiotic stress conditions (Jiang et al., 2017). The quantitative alterations and tissue-specific expressions of different *BvWRKY* genes have clearly shown its implications for alkaline stress tolerance in sugar beet. Research to alter the expression of *BvWRKY* genes using CRISPR/Cas 9 technology is required for the development of stress-resistant varieties. The *BvWRKY* family genes are specific and play crucial roles in wider aspects of sugar beet development processes like germination, root development, photosynthesis, etc., including response to alkaline stress conditions (Wu et al., 2019). Therefore, these genes can be engineered through CRISPR/Cas 9 technology to develop alkaline stress-resistant varieties in sugar beet (Table 6). Furthermore, long noncoding RNAs (lncRNAs) in response to alkaline stress had also been identified and characterized in sugar beet leaves. Besides, the interactions of candidate genes and miRNAs with the lncRNAs under stress conditions have also been reported in sugar beet (Zou et al., 2020). The use of CRISPR/Cas 9 technology on these identified/characterized lncRNAs will be helpful in evaluating their function in sugar beet and its expression can be modified to provide alkali stress tolerance in sugar beet. Additionally, genes belonging to the bHLH (basic helix–loop–helix) family in sugar beet involved in salt stress tolerance have also been well known. Wang Y. et al. (2021) reported the *BvbHLH93* gene as a salt-responsive gene that has been shown to confer tolerance under salt stress conditions in sugar beet. By increasing antioxidant activity and decreasing ROS generation, the *BvbHLH93* gene modulates salt stress tolerance in sugar beets. Furthermore, the *BvbHLH93* gene's ability to reduce *RbohD* and *RbohF* gene expression through modulating polyamine metabolism warrants additional investigation in sugar beet. CRISPR/Cas 9 technology will help in understanding the role and expression of these genes in salt stress conditions. This will aid in knowing the regulation of this gene in conferring tolerance to sugar beet for salt stress conditions.

3.2 Cold stress tolerance

Chilling temperature affects sugar beet cultivation, production, and economic yield (Moliterni et al., 2015; Bhattarcharya, 2022).

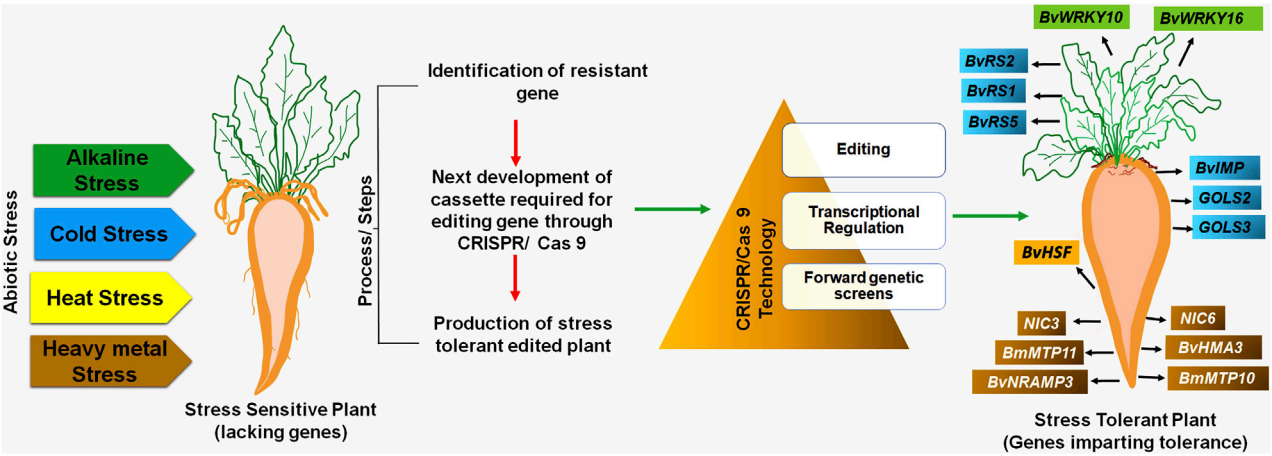


FIGURE 1
CRISPR/Cas 9 mechanism for abiotic stress tolerance in sugar beet.

TABLE 6 Transcription factor that can be engineered through CRISPR/Cas 9 in sugar beet to provide resistance to different abiotic stress conditions.

Abiotic stresses	Prospective transcription factor that can be targeted for CRISPR/ Cas 9	Gene function	Gene expression	Editing mechanism	References
Alkaline	*BvWRKY10*	Provides resistance to alkaline and saline stress	Upregulation	Prime and base editing	Wu et al. (2019)
	BvWRKY16				Li et al. (2020a)
	EIN 2	• Ethylene insensitive protein	Induce/Increase	Prime and base editing	Lei et al. (2020)
		• Regulates ethylene response			Zhang et al. (2008)
		• Provides tolerance even against green peach aphid			Zhou et al. (2020)
Salt	*BvbHLH93*	• Provides resistance to salt stress	High expression	Prime and base editing	Wang et al. (2021a); Wang et al. (2021b)
		• Modulates salt stress tolerance			
		• Reduces *RbohD* and *RbohF* gene expression			
Chilling/Cold stress	*BvRS1*	• Stress genes responsible for raffinose synthase	Induce	Prime and base editing	Kito et al. (2018)
		• Provide resistance			
		• Encodes protein (comprises 783 amino acids)			
	GOLS2	• Genes responsible for galactinol synthase	Upregulation	Prime and base editing	Keller et al. (2021)
		• Provide resistance			
	GOLS3	• Stimulated by cold stress	Upregulation	Prime and base editing	
		• Provide resistance			
Heat	*BvHSF*	• Genes responsible for higher expression of heat shock proteins during elevated temperature	Upregulation	Prime and base editing	Ismail et al. (2020)
		• Provide resistance			

Cold stress conditions have a serious impact on the sugar beets at various developmental stages, including early germination, sugar metabolism, growth, and bolting in the roots (Hoffmann and Kluge-Severin, 2011). The seedling stage of sugar beet is particularly prone to low temperature stresses. Cold stress during this stage leads to severe degeneration and growth retardation in the root system, consequently decreasing its sugar content (Moliterni et al., 2015; Jalilian et al., 2017). Porcel et al. (2018) revealed that overexpression of *BvCOLD1* gene in sugar beet exhibits cold tolerance potential along with other abiotic stress tolerance and overcoming boron deficiency. Keller et al. (2021) found that improved freezing-tolerant sugar beet genotypes accumulated more raffinose in the pith portion, which is vulnerable tissue to freeze damage. This finding demonstrated that raffinose and its precursors protect sugar beets from freezing damage. Recognizing the importance of raffinose in providing cold tolerance to sugar beet plants, Kito et al. (2018) isolated and characterized two genes, *BvRS1* and *BvRS2,* from the sugar beet plant. These genes code for the expression of raffinose synthase, which is a crucial enzyme during raffinose biosynthesis. An increase in transcription levels of *BvRS1* and *BvRS2* genes was observed in response to chilling stress in both leaves and roots (Kito et al., 2018). Furthermore, the production and build-up of oligosaccharides belonging to the raffinose family are particularly important during cold hardiness. Galactinol synthase (GolS) is considered a key regulator of the synthesis of such oligosaccharides and their accumulation (Vinson et al., 2020). The expression levels of *GOLS2* and *GOLS3* genes, responsible for galactinol synthase, as well as *BvRS2* and *BvRS5,* were high during chilling temperatures (Keller et al., 2021). Interestingly, the product of the *BvRS5* gene product and raffinose content increased exceptionally during freezing temperature in the taproots of tolerant sugar beet varieties, GT2 and GT3. In comparison with other sugar beet germplasm, GT2 exhibited high expression of *GOLS* and *RS* genes and raffinose content in roots, indicating chilling resistance in GT2 (Keller et al., 2021; Yolcu et al., 2021). Membrane proteins also frequently recognize cold stress conditions in sugar beet. These proteins activate a Ca^{2+} signal in the cytosol. Ca^{2+}-binding proteins may act as a bridge between the Ca^{2+} signal and several downstream transcription factors (Iqbal et al., 2022). It was reported that *B. vulgaris Integral Membrane Protein* gene resembles *AtERDL6*, which was previously reported for cold tolerance (Qi et al., 1995). Freezing conditions may be responsible for the increased transcription rate of *BvIMP* gene and vacuolar carbohydrates trafficking in sugar beet leaves, crucial for chilling stress response and germination of seed (Yu et al., 2020; Reyer et al., 2021).

Utilizing CRISPR/Cas technology, scientists have successfully enhanced cold stress tolerance in plants by editing genes allied with cold stress and raffinose synthesis. In the case of *Vitis vinifera*, the *VaDof17d* gene plays a critical role in the cold-responsive pathway and the production of raffinose family oligosaccharides. This is evidenced by the enhanced expression of galactinol synthase (GolS) and raffinose synthase genes. Mutating the *Dof17d-ED* gene through CRISPR/Cas 9 technology resulted in reduced cold tolerance and reduced levels of raffinose family oligosaccharides during cold stress (Wang et al., 2021). Therefore, genes including *BvIMP, BvRS1*, and *BvRS2* (Table 6) can be engineered in sugar beet through CRISPR/Cas 9 technology to develop site-specific mutants. These mutants could be instrumental in enhancing cold tolerance in sugar beet crops by boosting their expression.

3.3 Heat stress tolerance

Increased temperatures and water scarcity tend to drastically affect the water content in plants where excess transpiration decreases the rate of water intake and causes permanent wilting in the plant (Kumar et al., 2004). Sugar beet is greatly affected by altered climatic conditions and disturbances in weather (Abou-Elwafa et al., 2020). High temperatures hamper major metabolic activities such as germination of seed, seed viability and its vigor, etc., leading to a threat to the survival of crop plants (Stevanato et al., 2019). Critical physiochemical processes, including photosynthesis and photosystem (PSII) activity also greatly affected due to blockage in the electron transport chain under high temperature stress (Murakami et al., 2000; Moore et al., 2021). The recent expansion of sugar beet cultivation in tropic and sub-tropical regions has drawn attention to farming it during the summer season (Abou-Elwafa et al., 2020).

Recognizing the importance of sugar beet cultivation across the world during the summer season, Ismail et al. (2020) explored the role of the transcription factor *BvHSF* gene, which showed higher expression levels under heat stress. The sugar beet crop showed enhanced expression levels in response to water scarcity, heat stress, and drought stress. Heat shock transcription factors (HSFs) are pivotal transcription factors in plants, critical for their response to various abiotic stresses such as heat, cold, salt, and drought (Fan et al., 2021). These HSF family members act by binding to the reverse repeat region of heat shock elements (HSEs), facilitating the transcription of heat shock proteins (HSPs) and assisting in the plant's stress adaptation mechanisms (Guo et al., 2016; Jacob et al., 2017). Wu et al. (2022) demonstrated that CRISPR/Cas 9 knockout mutants, specifically targeting single copy *MpHSF* genes (*Mphsfa1V* and *Mphsfb1* mutants), resulted in different indel editing sites, showcasing enhanced thermotolerance in plants. Therefore, *BvHSF* genes can be engineered in sugar beet through CRISPR/Cas 9 technology to enhance their expression in sugar beet crops for increased tolerance towards heat resistance by creating in-dels (Table 6).

3.4 Drought stress tolerance

Drought stress also negatively impacts sugar beet root growth and development during the early phases of growth. Furthermore, the introduction of drought stress later in the growing season reduces leaf area and the number of leaves, ultimately resulting in lowered photosynthetic efficiency (Abou-Elwafa et al., 2020).

Generally, an increase in the expression of multiple drought-responsive genes and transcription factors enhances the plant's ability to tolerate drought conditions (Fang and Xiong, 2015; Kumar et al., 2020; Santosh Kumar et al., 2020). Conversely, upregulation of drought-sensitive genes in plants heightens their vulnerability to drought stress due to imbalances in hormonal levels, reduced antioxidant activities, and heightened production of reactive oxygen species (ROS). CRISPR/Cas 9 based genome

editing provides a promising avenue for enhancing drought tolerance in plants. This technique involves targeting negative regulators or drought-sensitive genes, allowing scientists to modify specific genetic elements and create crops that are more resilient to water scarcity. By precisely altering these genes, drought-resistant varieties can potentially be developed, ensuring sustainable agriculture in the face of changing environmental conditions. *WRKY* transcription factors are pivotal regulators of plant growth, development, and responses to both biotic and abiotic stresses. Among these factors, *WRKY3* and *WRKY4* genes in plants play a significant role in orchestrating the defense mechanisms against drought stress (Li B. et al., 2021). For instance, genetic manipulation of the *OsWRKY5* transcription factor has revealed significant insights into drought tolerance in plants. *OsWRKY5*, a key regulator, was found to hinder the plant's ability to withstand drought. During the seedling and heading phases, *OsWRKY5* was primarily expressed in growing leaves, and its expression decreased under drought stress conditions. Researchers conducted experiments using genome-edited loss-of-function alleles, *oswrky5-2* and *oswrky5-3*, to enhance drought tolerance. These edited alleles resulted in increased drought resistance, as evidenced by improved plant growth even under water scarcity (Lim et al., 2022). Conversely, when *OsWRKY5* was overexpressed in the activation-tagged line *oswrky5-D*, plants exhibited greater susceptibility to drought stress. Overexpression of *OsWRKY5* led to heightened sensitivity to abscisic acid (ABA), a plant hormone involved in stress response, and encouraged ABA-dependent stomatal closure. By editing the *OsWRKY5* genome, researchers successfully enhanced the plant's ability to produce grains even under drought stress conditions. This breakthrough offers valuable insights into improving crop resilience against water shortage, a critical factor in agricultural sustainability. Another example is for obtaining drought tolerance through CRISPR technology is enhancing the expression of *AREB1*, a specific transcription factor. In contrast, plants with a knocked-out *AREB1* gene exhibit increased sensitivity to drought stress (Singh et al., 2016). Roca Paixão et al. (2019) demonstrated enhanced drought stress tolerance through the utilization of CRISPR/dCas9 fusion with a Histone Acetyl Transferase (*AtHAT*) gene in *Arabidopsis*. These genes and transcription factors could also be targeted in sugar beet crops for attaining drought stress tolerance.

3.5 Heavy metal stress tolerance

Exposure of plants to toxic heavy metals causes different metabolic and physiochemical changes that depend on the concentration of these metals in soil, plant species, varieties, and abiotic conditions (Jamla et al., 2021; Thakur et al., 2022). Toxic metals like Pb damage the vacuolar membrane of sugar beet roots (Trela et al., 2012; Beata et al., 2022). Pb is one of the most toxic metals for plant cells, and it has a negative effect on the growth of plants, photosynthesis, respiration, and electron transport chain (Sharma and Dubey, 2005). Cd stress in *B. vulgaris* caused retarded growth, chlorosis, and increased root/plant ratio along with a decline in the rate of respiration in root-tips and photosynthesis (Greger and ögren, 1991; Larbi et al., 2002; Liu et al., 2022). Haque et al. (2021) observed that higher levels of Cd in sugar beet plants cause growth retardation due to an insufficient amount of Fe, resulting in decreased photosynthetic activity, and oxidative stress occurring in cells. Cd-treated plants display sensitivity to oxidative stress, leading to an increase in levels of O_2^- and H_2O_2 in roots and shoots. Additionally, Haque et al. (2021) reported the antioxidant defense mechanism in sugar beet under a higher concentration of toxic metal and observed that Cd stress enhances the activity of catalase (CAT) enzyme in the shoots, whereas the activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione reductase (GR) do not increase either in roots or in shoots. Genes involved in heavy metal stress tolerance in sugar beets have been explored and proven to be important. There are two MTP genes, *BmMTP10* and *BmMTP11,* reported for metal-resistant proteins from wild species of sugar beet (*B. maritima*). The detoxification process of Ni was controlled by genes from wild sugar beet (*B. maritim*a) named as toxic nickel concentration (NIC), i.e., *NIC3*, *NIC6*, and *NIC8* (Bozdag et al., 2014; Yolcu et al., 2021). It is estimated that all these genes are required for protection against Ni toxicity. In a similar study, under Cd toxicity, sugar beet roots showed higher expression of putative *BvHMA3* and *BvNRAMP3* genes, suggesting that these genes are involved in the Cd uptake process (Haque et al., 2021).

Heavy metal-associated proteins (HMPs) and natural resistance-associated macrophage proteins (Nramp) are vital for heavy metal transport and detoxification within plant cells (Singh et al., 2016; Li W. et al., 2020). In rice, essential transporter genes such as *OsLCT1* and *OsNramp5* have been identified as key players in the absorption of Cd by the roots (Chang et al., 2020). Through CRISPR/Cas 9 enabled gene-expression manipulation, significant strides have been made in reducing the levels of Cd and Pb in rice grains. Specifically, the knockout of *OsNRAMP1* using CRISPR/Cas9 technology, as demonstrated in studies by Chu et al. (2022) and Wang F. Z. et al. (2017), has led to a substantial decrease in Cd and Pb content. Therefore, *BvHMA3* and *BvNRAMP3* genes can also be engineered in sugar beet through CRISPR/Cas 9 technology to develop heavy metal resistant varieties in sugar beet by creating site-specific mutagenesis.

4 Application of CRISPR/Cas 9 technology for improving biotic stress resistance

4.1 Pathogen resistance mechanism

The CRISPR/Cas 9 adaptive immune system for viral, bacterial, fungal resistance operates in three steps (Figure 2), regardless of the various shapes: 1) adaptation, 2) expression and maturity, and 3) interference. Protospacers, unique short DNA snippets from the invasive pathogen, are recognized by the Cas protein and inserted into CRISPR repeats as new spacers during the adaptation process. The host can then establish immunological memory and be equipped to recognize the same invasive infections in the future concerning this new spacer, which also serves as a genetic record (Paul et al., 2021).

For some CRISPR/Cas 9 systems to acquire the protospacer, the target DNA must have a short PAM (of 3-5 nucleotides) (Bolotin et al., 2005; Deveau et al., 2008; Shah et al., 2013). The CRISPR array

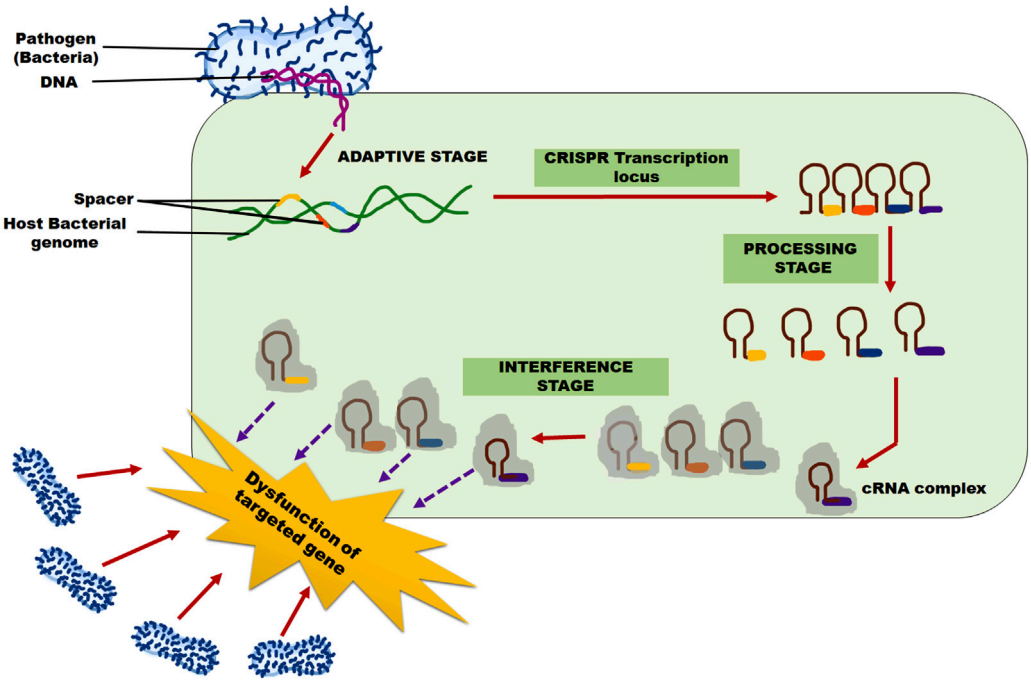


FIGURE 2
CRISPR/Cas 9 system mechanism for pathogen (Bacterial/Fungal/Viral) resistance in the plants. Adaptive, processing, and interference stages are three steps involved in this system.

is translated into a precursor-CRISPR RNA (pre-RNA), which is then processed to produce short mature CRISPR RNA (crRNA) through endo-nucleolytic cleavage and contains the sequences of the invading pathogen that have been learned (Carte et al., 2008; Haurwitz et al., 2010). At its 5′ end, each crRNA has a single spacer (a brief RNA segment that complements the DNA sequence of the foreign genetic material), and at its 3' end, it has a CRISPR repeat sequence.

An active Cas-crRNA effector complex is formed when the mature crRNA combines with one or more Cas effector proteins. This complex searches for and attacks the cell's foreign nucleic acids during the interference phase. Using the specific PAM sequence either upstream or downstream of the protospacer, and relying on Watson-Crick base pairing, the crRNA component of the complex serves as a guide to identify the target DNA (Semenova et al., 2011; Jiang et al., 2013; Westra et al., 2013; Fineran et al., 2014; Zetsche et al., 2015). Once the target DNA is successfully recognized, the Cas nuclease cleaves and digests it. Different classes of the CRISPR immune system, based on various effector Cas proteins and PAM recognition sequences, have emerged in recent years.

Invading foreign DNA fragments of virus particles are recognized and eliminated by the CRISPR/Cas 9 system, allowing it to identify and remove DNA or RNA sequences that facilitate continued invasion (Barakate and Stephens, 2016). CRISPR/Cas 9 technology modifies the plant's inherent defense mechanism by detecting and removing harmful genes hidden within plant viruses. It can also be utilized to develop agricultural cultivars that are more resistant to specific plant viruses. This approach has fundamentally transformed virus resistance research because of its ability to use sequence-specific nucleases (Hsu et al., 2014).

4.2 CRISPR tools for disease diagnosis in sugar beet

The efficient implementation of control measures or management strategies heavily relies on the correct and timely identification of diseases and causative organisms. Consequently, disease diagnosis is critical and serves as the starting point for disease management. In this regard, Cas proteins play a significant role in managing diseases at the initial stages in plants. Cas proteins are attractive candidates for repurposing nucleic acid detection due to their programmability and extreme selectivity in binding and cleaving nucleic acids. Advances in understanding various Cas proteins have paved the way for the development of ultrasensitive, mobile, and cost-effective nucleic acid-based point-of-care (POC) testing equipment. Cas 9 proteins have been utilized to create robust and reliable nucleic acid detection technologies, but the recent discovery of Cas 13a and Cas 12a, with guaranteed cleavage activity, has revolutionized the field of nucleic acid detection (Gootenberg et al., 2017; Chen J. S. et al., 2018; Gootenberg et al., 2018; Azhar et al., 2021; Jiao et al., 2021).

A new molecular diagnostic approach known as DETECTR (DNA Endonuclease Targeted CRISPR Trans Reporter) technology (Chen J. S. et al., 2018), based on the CRISPR-Cas 12a system had been utilized for detecting BNYVV in sugar beet roots (Ramachandran et al., 2021). In this diagnostic method, Cas 12a cleaves any surrounding single-stranded DNA without regard for its target. This trait is known as collateral activity, and it has been exploited to develop DETECTR. In this approach, a complementary guide RNA first directs Cas12a to a target dsDNA (Figure 3). When Cas 12a binds to the correct target, it cleaves ssDNA reporter

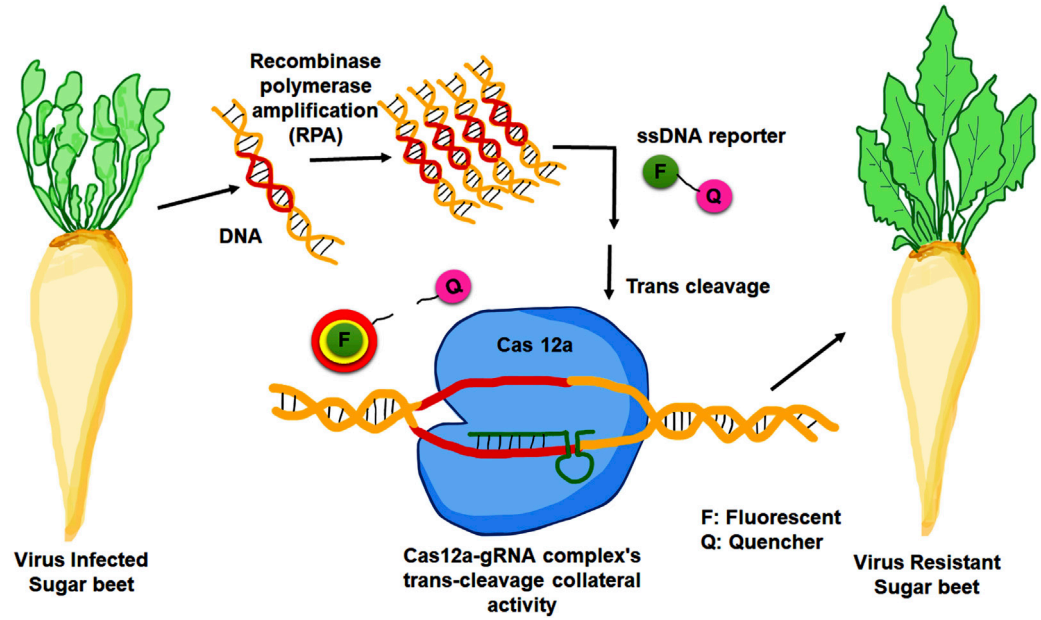


FIGURE 3
Development of viral resistance in sugar beet through DETECTR technology. The Cas12a-gRNA complex identifies target DNA that was amplified by recombinase polymerase amplification (RPA). When the target is recognised, it fragments the surrounding FQ-labeled ssDNA reporters, allowing the fluorescence to be recovered.

molecules coupled with a quencher and a fluorophore. A fluorescence quencher (FQ)-labeled reporter was employed to monitor the trans-cleavage activity induced by the Cas12a-gRNA complex binding to the guide-complementary target DNA. In the presence of target DNA, the Cas 12a-gRNA complex's trans-cleavage activity is triggered, leading to the cleavage of surrounding FQ-labeled ssDNA reporters (Chen J. S. et al., 2018). The fluorescent signal generated by the separation of the quencher and the fluorophore detects the indiscriminate cleavage (Kocak and Gersbach, 2018). DETECTR exhibits enhanced sensitivity when combined with RPA preamplification. This diagnostic technique requires the amplification of viral fragments from template DNA under isothermal conditions. The one-step reverse transcriptase recombinase polymerase amplification (RPA) method was employed in sugar beet. The precise sensitivity of this method is its standout feature, as it can detect a single molecule of viral particle within a microliter of the sample (Kocak and Gersbach, 2018).

Another common CRISPR-based technology applied in sugar beet for viral resistance is nucleic acid sequence-based amplification CRISPR cleavage (NASBA). This technology helped the plants achieve total viral resistance. This technique involves targeting viral DNA using a guide RNA (gRNA) and cutting the viral DNA with the Cas 9 enzyme. The trans-activating crRNA (tracrRNA) base pairs with the repeat sequence in the crRNA to form a unique dual RNA hybrid structure guide that directs Cas9 to cleave the target DNA. A chimeric sgRNA combines crRNA and tracrRNA into a single RNA transcript. The two nuclease domains (RuvC and HNH) present in Cas 9 cut the target and non-target DNA strands, respectively. A short trinucleotide PAM is also required for the initial target sequence identification; without it, the target sequence cannot be recognized. Successful identification results in a double-strand upstream of the 3′-NGG PAM (Li J. et al., 2022). This method is based on Cas 9 selective cleavage of target DNA and the toehold switch principle. This approach can distinguish between genotypes as it can identify a single base difference based on the presence or absence of a PAM sequence. This method utilizes sequence-based amplification, PAM-dependent target detection, Cas 9 cleavage, and a toehold sensor (Pardee et al., 2016).

Yildirim et al. (2019) utilized CRISPR/Cas 9 technology to confer various resistances in sugar beet against two curly top viruses (Beet curly top viruses (BCTV) and beet curly top Iranian viruses (BCTIV)). These viruses belong to two separate genera within the Geminiviridae family, specifically curtovirus and be-curtovirus families, respectively. The gRNA/cas-9 endonuclease system was transiently overexpressed to check BCTV and BCTIV in sugar beet plants. Sugar beet plants overexpressing the gRNA/Cas 9 constructs exhibited decreased viral DNA accumulation, and this accumulation was also observed to be delayed compared to plants without the overexpression of gRNA/Cas 9 constructs. The CRISPR/Cas 9 system used in providing resistance to sugar beet plants against viral diseases, specifically targets the dsDNA of a geminivirus with gRNA. This approach helps restrict virus reproduction by disrupting critical replication genes (Khatodia et al., 2017). Ji et al. (2015) demonstrated the application of CRISPR technology against geminivirus in plants. Efficient antivirus sgRNAs can be identified to recognize specific sites in the viral genome. This approach has high potential for developing multiple resistances to all Geminiviruses. The viral resistance mechanism in sugar beet through the CRISPR/Cas 9 system is illustrated in the figure below (Figure 4).

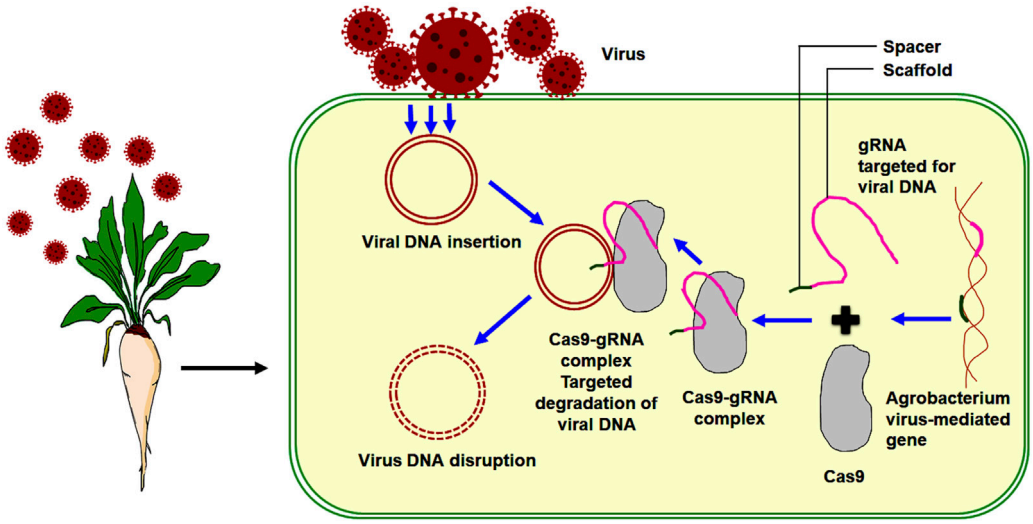


FIGURE 4
Viral resistance in sugar beet through CRISPR/Cas 9 system (Nucleic acid sequenced based amplification CRISPR cleavage).

The advent of CRISPR/Cas 9 has opened up numerous prospects for developing superior crop varieties through genome editing with great precision and accuracy. CRISPR techniques usher in a new era of breeding systems in which plant immunity is enhanced by disrupting the compatible connection between infections and hosts (Karmakar et al., 2022). CRISPR-based available tools like SHERLOCK, FLASH, and LEOPARD wherein Cas 12 and Cas 13 proteins have been utilized, will be helpful in the identification, diagnosis and management of diseases in sugar beet.

4.3 Beet cyst nematode (*Heterodera schachtii* schmidt) resistance

The host range of the beet cyst nematode (BCN) is wide and includes numerous species from abundant plant families, including Chenopodiaceae and Brassicaceae. In sugar beet cultivation (*Beta vulgaris* L.), *Heterodera schachtii* is a serious pest. It is known that cultivated *Beta* species lack the genes that provide resistance to nematodes. *Beta procumbens*, a wild species, and its allied species (*B. webbiana* and *B. patellaris*) are the sources of resistance genes (Cai et al., 1997). BCN is resistant to the $Hs1^{pro-1}$ locus. The native $Hs1^{pro-1}$ gene encodes a 282-amino acid protein with incomplete leucine-rich repeats and a potential membrane-spanning region. This protein is produced in sugar beet roots. The expression of the matching complementary DNA gave resistance to BCN infection in susceptible sugar beet (Cai et al., 1997). The $Hs1^{pro-1}$ (Table 7) promoter promotes nematode feeding site-specific GUS expression in both sugar beet and *Arabidopsis*, indicating a shared mechanism for regulating $Hs1^{pro-1}$ expression in these two species (Thurau et al., 2003).

Kumar et al. (2021) identified the resistance gene, *Hs4* (about 230 kb-sized area). CRISPR-Cas-mediated deletion (CRISPRi) and overexpression (CRISPRa) in susceptible sugar beet roots were used to characterize a candidate gene. The gene encodes a rhomboid-like protease predicted to be bound to the endoplasmic reticulum. CRISPR Cas mutagenesis in the resistant sugar beet cultivar, NEMATA, deleted the ORF1. The roots of the knockout clones were extremely susceptible as huge numbers of J4 females and cysts packed with eggs observed in these roots. The expression of ORF in sugar beet roots determined nematode resistance/susceptibility. Beet roots expressing ORF 1 were resistant to nematodes while low ORF expression led to moderate susceptibility. Roots that did not express were susceptible to the nematode. Thus, CRISPRa can be a potential tool for the overexpressing targeted genes, which can confer tolerance to abiotic and biotic stress conditions (Horlbeck et al., 2016; Rai et al., 2019).

4.4 Beet necrotic yellow vein virus resistance

The multipartite genome of Beet necrotic yellow vein virus (BNYVV) consistis of five positive-stranded RNAs, providing an enticing framework for the production of several foreign proteins (Jiang et al., 2019). Additionally, *NbPDS* guide RNAs were delivered through BNYVV-based vectors to transgenic plants expressing Cas9 for genome editing. This delivery resulted in a photobleached phenotype in systemically infected leaves. The BNYVV-based vectors will facilitate the expression and production of multiple proteins in sugar beet and related crop plants. gRNA can be delivered using BNYVV-based vectors for CRISPR/Cas 9 plant genome editing (Jiang et al., 2019).

In a breeding line developed by the Holly Sugar Company in the USA, partial resistance to BNYVV was found to be caused by a single dominant gene (*Rz1*) (Lewellen et al., 1987; Scholten et al., 1996). Accessions WB42 (*Rz2*) and WB41 (*Rz3*) of *Beta vulgaris* subsp. maritima from Denmark have also been identified as having BNYVV resistance (Lewellen et al., 1987; Gidner et al., 2005). Compared to the *Rz1* gene, the dominant *Rz2* gene seems to confer a higher level of resistance (Paul et al., 1993).

Detection of BNYVV is extremely sensitive and specific to the infected roots, as measured by the reporter signal. Ramachandran et al. (2021) established isothermal RT-RPA and CRISPR-based

TABLE 7 CRISPR/Cas 9 technology application in major sugar beet diseases for pathogen (bacterial/fungal/viral) resistance.

Diseases	Causal organism	Potential genes imparting tolerance/resistance	Gene function	Yield loss (%)	References
Nematode associated diseases					
Beet cyst nematode	*Heterodera schachtii*	*Hs1^{pro-1}*	Nematode resistance	Up to 60*	Cai et al. (1997)
		Hs4	Located on the wild beet translocation. Works together or independently of Hs1^{pro-1}		Kumar et al. (2021); *Ghaemi et al. (2020)
Root-knot nematode	*Meloidogyne incognita*	*R6m-1*	Nematode resistance	Up to 50%	Bakooie et al. (2015)
		Mi-sbp-1	Regulator of lipogenesis		Shivakumara et al. (2019)
		Mi-cpl-1	Interaction between plants and nematodes		Dutta et al. (2015)
		Mi-msp3	Nematode resistance		Joshi et al. (2020)
		Mi-msp5			
		Mi-msp18			
		Mi-msp24			
		Hs1^{pro-1}	General stress signalling genes and Nematode resistance		Abo-Ollo et al. (2018)
		HSPRO2			
		Mi-1.2	Nematode resistance		
		R6m-1			Bakooie et al. (2015)
Fungal diseases					
Cercospora leaf spot	*Cercospora beticola*	*SP1*	Acid chitinase activity	40*	Nielsen et al. (1994); Harvenson, (2013)
		SP2			Nielsen et al. (1994)
		SE1	Chitinase activity		Nielsen et al. (1993)
		SE2	Exochitinase activity		Nielsen et al. (1993)
		qcr1	(Qualitative Trait Loci) QTL disease resistance		Taguchi et al. (2011)
		qcr4			
Rhizoctonia root rot	*Rhizoctonia* sp.	*Rs1*	QTL disease resistance	50*	Lein et al. (2007); *Barry (2006)
		Rs2			
		Rs3			
Fusarium root rot	*F. oxysporum*	*BvSP2*	SNP markers	40–50	Yerzhebayeva. (2018)
		BvSE2	Chitinase activity		
Powdery mildew	*Erysiphe betae*	*Pm 1*	Partial resistance	Up to 35*	Francis and Luterbacher (2003); Lewellen and Schrandt (2001); Grimmer et al. (2007); *Neher and Gallian (2013); *Francis (2002)
		Pm 2			
		Pm 3	Complete resistance to disease		
		Pm 4	Partial resistance		
		Pm 5			
		Pm 6	Stronger resistance than others		
Aphanomyces seedling disease (Black rot or Black leg)	*Aphanomyces cochlioides*	*Acr 1*	Resistant gene	0–100	Taguchi et al. (2010); *Windels (2000)

(Continued on following page)

TABLE 7 (*Continued*) CRISPR/Cas 9 technology application in major sugar beet diseases for pathogen (bacterial/fungal/viral) resistance.

Diseases	Causal organism	Potential genes imparting tolerance/resistance	Gene function	Yield loss (%)	References
Viral diseases					
Viral Diseases	Virus Family	Vector	Potential Genes Imparting tolerance/ resistance	Yield loss (%)	References
Beet severe curly top virus (BSCTV)	Geminiviridae	Leafhopper	*Rep*	100*	*Agrios (2005); Strausbaugh et al. (2017)
			Ty		Gupta et al. (2021)
Beet necrotic yellow vein virus (BNYVV)	Benyvirus	*Polymyxa betae*	*Rz1 (the Holly gene)*	90*	Lewellen et al. (1987); Paul et al. (1993); Liebe et al. (2020)
			Rz2		Scholten et al. (1999); Wetzel et al. (2021)
			Rz3		Francis and Luterbacher (2003); *Pferdmenges (2007)

virus diagnostic techniques for detecting BNYVV in sugar beet roots with rhizomanial disease. Using this CRISPR-based technique, BNYVV in sugar beet roots baited for rhizomania can be identified, generating a readily identifiable fluorescence signal as compared to healthy reference root samples. The BNYVV RNA-1 sequence was chosen as the target since it is one of the least diverged components of BNYVV. The CRISPR-Cas 12a-based BNYVV detection approach has paved the way for a sensitive, focused, and high-throughput detection platform for the assessment of rhizomania (Ramachandran et al., 2021).

The creation and validation of such CRISPR-based BNYVV diagnostic approaches for sugar beet roots offer advantages in terms of sensitivity and resilience in isothermal circumstances. Hence, it would be a helpful tool for the companies involved in evaluating viruses to drive disease control strategies (Ramachandran et al., 2021). Furthermore, the technology established for virus identification in underground root tissue can be applied to create a CRISPR-based detection platform for viruses and soil-borne disease-causing pathogens in other crops as well.

4.5 Beet curly top virus resistance

Beet curly top virus is a single stranded DNA virus that belongs to the genus Curtovirus and has been known to cause outbreaks in sugar beet as a disease. This virus has a strong impact on sugar beet yield, reducing it to 30% and above. The beet leaf hopper (*Circulifer tenellus*) is the vector of this disease (Strausbaugh et al., 2012). Yildirim et al. (2019) utilized 20 gRNAs which targeted viral DNAs of this virus, and the plant was transformed with Cas 9 enzyme including vector (pKIR1.1) to provide antiviral resistance. Viral movement is inhibited by the gRNA/Cas 9 construct. Overexpressing the gRNA/cas-9 constructs in sugar beet plants resulted in a delayed and diminished accumulation of both viral DNA. In this manner, sugar beet was harnessed to build full viral resistance in the species.

Yildrim et al. (2022) used a genome-based characterization technology of beet curly top Iranian virus in sugar beets and isolated and identified the Turkish variants of this virus along with its pathogenicity. Ebrahimi et al. (2022) explained the presence and pathogenicity of beet curly top Iranian virus in sugar beet. This helps in the management of this viral disease. Furthermore (Yildrim et al., 2023), reported that first-time broad-spectrum resistance against *Becurtovirus* using CRISPR technology where four gRNAs, involved in beet curly top Iranian virus, were cloned into vector consisting of Cas 9 and later agroinoculated into the virus infected leaves of sugar beet. Briddon et al. (1989) demonstrated that the genes for the capsid protein (CP), *C4*, and *C2* are essential for viral pathogenesis and the emergence of disease symptoms in plants. The CP gene mutation caused the virus to lose its infectiousness and ability to propagate. Targeting these genes through the CRISPR/Cas 9 tool in sugar beet will help in the management of this viral disease.

The importance of miRNAs in sugar beet curly top virus resistance (BCTV) has been shown. Majumdar et al. (2022) determined that differentially expressed (DE) miRNAs are, in certain cases, only present in the R lines. Future functional evaluation of these potential miRNAs, either by overexpression in germplasm that is BCTV sensitive or by employing them as molecular markers to scan various sugar beet genomes, will aid in establishing BCTV resistance. Furthermore, Eini et al. (2022) demonstrated the development of very effective geminiviral replicons (GVR) from BCTV with a broad host range for recombinant gene expression in plants.

Prior to the advent of molecular markers, only a single disease resistance gene (C gene) for BCTV resistance had been mapped in sugar beet (Mutasa-Gottgens et al., 2000). Studies have successfully developed markers linked to resistance genes for BNYVV (Luterbacher et al., 1998; Luterbacher et al., 2000), BCN (Owen and Ryser, 1942; Scholten et al., 1997) powdery mildew (Uphoff and Wricke, 1992) and to quantitative trait loci against *Cercospora* leaf spot.

4.6 *Cercospora* leaf spot resistance

In response to infection by the leaf-spot fungus *Cercospora beticola*, two isoforms of acid chitinase (*SE1* and *SE2*) were found in the leaves of sugar beet. However, only one of the isoforms (*SE2*) had exochitinase

TABLE 8 Genes that can impart tolerance to sugar beet crop against insect pests (larval stage) *via* CRISPR/Cas 9 technology.

(a) Some genomes successfully edited in insects by the CRISPR/Cas tool kits						
Order	Insect name (common name)	Gene Imparting tolerance/ resistance	Gene function	Editing	Outcome of the editing	References
Lepidoptera	*Spodoptera litura* Fabricius (Armyworm)	*Slit PBP3*	Sex pheromone perception	Chimera mutation	Destroyed pest insect mating	Zhu et al. (2016)
		siSe2 (Serine protease 2)	Sperm movement and activity	Knockout	Induces male sterility	Bi et al. (2022)
		Abdominal-A (slabd-A)	• Embryonic development gene • Body segmentation	Knockout	Defected body segmentation and irregular pigmentation	Bi et al. (2016); Sun et al. (2017)
		SlitBLOS2	Acts as a marker gene	Knockout	Coloration of the integuments, a marker gene for functional studies and pest control strategies	Zhu et al. (2017)
	Spodoptera frugiperda J.E. Smith (Fall armyworm)	*BLOS2* (Biogenesis of lysosome-related organelles complex 1 subunit 2)		Knockout	Translucent mosaic integument	Zhu et al. (2020)
		TO (Tryptophan 2, 3-dioxygenase)			Olive eye color	
		E93	A key ecdysone-induced transcription factor that promotes adult development		Larval-pupal intermediate phenotypes	
		ABCB1	Susceptibility to chemical pesticides or Bt toxins	Knockout	Susceptibility to emamectinbenzoate, beta-cypermethrin and chlorantraniliprole	Li et al. (2022b)
	Spodoptera exigua (Beet armyworm)	*CYP9A186*	Restoration of Emamectin benzoate (EB) [(4″R)-4″-deoxy-4” -(methylamino) avermectin B1 benzoate] susceptibility	Knockout	Susceptibility to emamectin benzoate (EB)	Zuo et al. (2021)
		Ryanodine receptor	Regulates calcium release from intracellular stores and other cellular processes, *viz.*, muscle contraction, gene transcription, neurotransmitter release, hormone secretion and cell proliferation	Substitution	Controlled insect population and resistance to various insecticides	Zuo et al. (2017)
		P-glycoprotein gene	Unknown	Knockout	Susceptibility to abamectin and emamectin benzoate	Jin et al. (2020)
		Sea6 (*Spodoptera exigua* a-6-nicotinic acetylcholine receptor (nAchR))	Role in Spinosyns insecticide interaction	Knockout	Resistance to spinosyn insecticides	Zuo et al. (2018); Zuo et al. (2020)
	Spodoptera littoralis (Egyptian cotton leafworm)	Orco (Odorant receptor co-receptor)	Impairs feeding, mating and egg-laying behavior	Knockout	Reduced survival rate	Koutroumpa et al. (2016)
		SlitOrco	plant odor and sex pheromone olfactory detection	Knockout	Investigated the function of the *Orco* gene in the non-model insect *Spodoptera littoralis*	Cui et al. (2017); Koutroumpa et al. (2016)
	Agrotis ipsilon Rott. (Cutworm)	*Yellow-y*	Involved in body pigmentation and play a role in waterproofing	Knockout	Pigmentation plays a vital role in insect survival and reproduction	Chen et al. (2018a)
		AiTH (*Agrotis ipsilon* tyrosine hydroxylase)	Insect melanin and catecholamine biosynthesis pathway	Knockout	• Narrowing in the eggshell • Pigmentation of epidermis and newly hatched larval development	Yang et al. (2018)

(Continued on following page)

TABLE 8 (*Continued*) Genes that can impart tolerance to sugar beet crop against insect pests (larval stage) *via* CRISPR/Cas 9 technology.

(a) Some genomes successfully edited in insects by the CRISPR/Cas tool kits						
Order	Insect name (common name)	Gene Imparting tolerance/ resistance	Gene function	Editing	Outcome of the editing	References
		Aidsx	Embryonic development	Knockout/ Disruption	Sexually dimorphic development and behavior	Chen et al. (2019)
		AiMasc (*Masculinizer gene*)	Sex determination		Induced expression of male specific double sex isoform	Wang et al. (2019)
	Helicoverpa armigera Hübner (Gram pod borer)	*GmUGT* (QTL-M)	Alteration of flavonoid biosynthesis pathway	Insertion	Resistance to insect	Zhang et al. (2022)
		HaCad (*Helicoverpa armigera* cadherin)	As receptor for Bt Cry 1A Toxin	Knockout	Resistance to Bt toxin Cry1Ac	Wang et al. (2016)
		Cluster of nine P450 genes	Defense mechanism against natural/synthetic insect toxins	Knockout	Identification of the key players in the insecticide metabolism	Wang et al. (2018)
		CYP6AE		Knockout	Regulation of detoxification enzymes	Wang et al. (2018)
		OR16	Pheromone antagonist	Knockout	Destroyed pest insect mating	Chang et al. (2017)
		Tetraspanin	Cell migration, signal transduction, and intracellular trafficking	Knockout	Resistance to Bt toxin cry1Ac	Jin et al. (2018)
		HaABCA2	Involved in the resistance mechanism for Cry2Ab	Knockout	Resistance to cry2Aa and cry2Ab	Wang et al. (2017a)
		White	Differential distribution of eye pigments	Knockout	Patterns of pigmentation	Khan et al. (2017)
		Brown			No phenotypic effects on viability or pigmentation	
		Scarlet			Increase amount of pteridines or ommochromes	
		ok	Analogous to that of brown gene in *Drosophila*			
		NPC1b	Growth of *Helicoverpa armigera* larvae and dietary cholesterol uptake	Knockout	Upregulation in gene expression during early larval instars	Zheng et al. (2020)

(b) Some genomes successfully edited in plants by the CRISPR/Cas tool kits against insects							
Order	Insect Name (Common Name)	Gene Imparting tolerance/ resistance	Crop reported in	Gene Function	Editing	Outcome of the editing	References
Lepidoptera	*Spodoptera frugiperda* J.E. Smith (Fall armyworm)	*Cry1Fa*	Field crop	Practically resistance	Knockout	SeABCC2 has a major role and SeCad1 a minor role in mediating toxicity of Cry1Ac and Cry1Fa	Huanga et al. (2020); Flagel et al. (2018); Jin et al. (2020)
		SfABCC2		Resistance to *Cry1F* but no alteration in susceptibility to small molecule pesticides	Knockout	Cry1F due to mutations in the SfABCC2 gene do not affect susceptibility to the synthetic and semisynthetic small molecule pesticides	Abdelgaffar et al. (2020); Jin et al. (2021)

(Continued on following page)

TABLE 8 (*Continued*) Genes that can impart tolerance to sugar beet crop against insect pests (larval stage) *via* CRISPR/Cas 9 technology.

(b) Some genomes successfully edited in plants by the CRISPR/Cas tool kits against insects							
Order	Insect Name (Common Name)	Gene Imparting tolerance/ resistance	Crop reported in	Gene Function	Editing	Outcome of the editing	References
		Cry1Ac	Field crop	(SfCad) cadherin protein	Knockout mutant strain	Targets different exons of the *SfCad* gene	Zhang et al. (2020a)
		Cry1Ab		Toxin protein			Zhang et al. (2020a); Kimoto and Shimamoto (2001); Hernández-Rodríguez et al. (2013); Jin et al. (2020)
		Cry1C		(SfCad) cadherin protein			
		ABC transporters		As receptor for Bt Cry1Fa and Cry1Ab toxins	Knock out	• Toxicity of two *bacillus* thuringiensis cry1 toxins to the pest	Jin et al. (2020)
						Resistance to both cry1fa and cry1ab toxins	
	Spodoptera littoralis (Egyptian cotton leafworm)	*Cry1Ab*	Sugar beet	Toxin protein	Insertion	• Strong anti-feedant effect on insect	Sedighi et al. (2011)
						• Increase in the developmental time and mortality	
	Helicoverpa armigera Hübner (Gram pod borer)	*Cry1Ac*	Field crop	Cadherin as a receptor, Toxin protein	Insertion	Saturable, high-affinity binding to insect	Stewart et al. (2001); Wang et al. (2005); Wang et al. (2016); Chen et al. (2018b); Karim et al. (2000)
		Cry2Ab			Knockout	High levels of resistance	Chen et al. (2018b); Wang et al. (2017a)
		GmUGT (QTL-M)	Soyabean	Alteration of flavonoid biosynthesis pathway	Insertion	Overexpression of GmUGT produced sensitive soybean varieties against *H. armigera*	Zhang et al. (2022)
		Cry2Aa	Pigeon pea	Mediate toxicity	Knockout	High levels of resistance	Wang et al. (2017a); Singh et al. (2018)
Diptera	All species	*Cry2*	Multiple crops	Toxin protein	• Potential gene that can be targeted for CRISPR/ Cas 9		Yuan et al. (2007)
					• Transcription repression causes lack in ability to repress Clock: cycle mediated transcription		
	Tetanops myopaeformis (Sugar beet root maggot)	Delta-endotoxin genes	Sugar beet	Target an insect's digestive system	Insertion	Resistance	Wilhite et al. (2000); Smigocki et al. (2003)
Coleopteran	All species	*Cry3*	Multiple crops	Toxin protein	• Potential gene that can be targeted for insecticidal *Bt* toxins using CRISPR/Cas 9		Orduz et al. (1996); Pauchet et al. (2016)

activity and could successfully hydrolyze chito-oligosaccharides (Nielsen et al., 1993). In resistant vs. susceptible cultivars, the sugar beet *SE2* gene (*B. vulgaris SE2*) is expressed at a substantially higher level following *Cercospora* infection (Nielsen et al., 1993). The leaves of sugar beet infected by *C. beticola* also contained two isoforms of another acid chitinase (*SP1* and *SP2*), which are similar to *SE1* and *SE2*. Infected sugar beet plant leaves had very high levels of *BvSP2* (*B. vulgaris* SP2) gene expression, however, *BvSP2* protein accumulation was only found in the vicinity of the infection sites (Nielsen et al., 1994).

Sugar beet leaves responded vigorously when exposed to a cell-wall protein solution made from the non-pathogenic oomycete *Pythium oligandrum* isolate. This response involved a substantial increase in

BvSE2 gene expression, which peaked at 4 h following the vaccination (Takenaka and Tamagake, 2009). However, since oomycetes lack chitin, *BvSE2* may be produced as part of a coordinated response to other proteins involved in the disease (Collinge et al., 1993).

4.7 Insect-pest resistance in sugar beet

Generally, resistance in insect-pest populations occur due to mutations in genes, which facilitates the encoding for receptor molecules and disrupt the interaction between the insect and the toxin. In rice, CRISPR/Cas9-dependent knockout of *CYP71A1* mutant gene enables it encode a functional tryptamine 5-hydroxylase (Lu et al., 2018). Tryptamine 5-hydroxylase is responsible for transforming tryptamine to serotonin and increases plant resistance against plant hoppers. A similar approach could be applied to the sugar beet plants to develop resistance against leaf chewing insects like *H. armigera* and *S. litura*. CRISPR/Cas 9-mediated mutagenesis of *GmUGT* led to the development of transgenic plant (Zhang et al., 2022). The *Arabidopsis ugt72b1* mutant exhibited aggravated cell wall lignification and an increase in flavonoid content (Lin et al., 2016). Both cell wall lignification and flavonoids contribute to resistance against leaf-chewing insects. Cell wall lignification serves as the first physical barrier against leaf-chewing insects (War et al., 2012). Hence, the enhanced resistance against leaf-chewing insects due to GmUGT mutations could be attributed to aggravated cell wall lignification and altered flavonoids (Zhang et al., 2022). Sugar beet plants transformed with the *cry1Ab* gene exhibited powerful defense against lepidopteron insects (Jafari et al., 2009). Sedighi et al. (2011) also reported a similar success story against an Egyptian leafworm (*Spodoptera littoralis*) infestation. Sugar beet plants have been transformed with *Cry1Ab* and *Cry1C* which exhibit resistance against the cabbage armyworm (*Spodoptera frugiperda*) (Kimoto and Shimamoto, 2001; Kimoto and Shimamoto, 2002) and on lepidopteron with *Cry1C* and *Cry2A* as well (Lytvyn et al., 2014). Regev et al. (1996) described that the *Cry1C* protein controls larvae of *Spodoptera* spp.

One of the most efficient methods for pest control is utilizing the toxic qualities of Cry protein's present in the Gram-positive pathogenic bacterium *Bacillus thuringiensis*. Different families of *Cry* proteins (or *Bt* proteins) exhibit highly selective toxicity against members of specific insect orders (Lytvyn et al., 2014). The order Lepidoptera exhibits a toxic response against proteins encoded by *Cry1* and *Cry9* genes (Table 8). Diptera and Lepidoptera show toxicity against proteins encoded by *Cry2* genes. Coleopteran insects exhibit a toxic response against proteins encoded by *Cry3* genes. Diptera also show a toxic response against the protein encoded by the *Cry2* gene (Yuan et al., 2007).

5 Conclusion and future prospects

Global laboratories are increasingly turning to CRISPR/Cas 9 editing as their instrument of choice for determining how genes work and how they might be used in other contexts. This technology is being utilized in various crop development efforts to reduce biotic and abiotic stressors. The great precision, efficacy, efficiency, cost-effectiveness, and time efficiency of editing procedures have led to their development as useful tools. The advancement in molecular tools like CRISPR/Cas 9 has opened up new approaches for genome editing in sugar beet. This technology can be used for the generation of resistant/tolerant sugar beet breeding lines/germplasm to withstand abiotic/biotic stress. In sugar beet, specific genes can be silenced or knocked out to change their functionality. The plant may benefit and adapt to the abiotic stress environment. Tolerance to such circumstances may be linked to adjustments in their physiological and biochemical mechanisms. The subsequent breeding cycles produces sugar beet cultivars that are more resilient to such challenges due to the adoption of carefully chosen tolerant breeding lines. Under drought stress conditions, this improvement becomes apparent in the plant, highlighting the plant's enhanced water usage efficiency. This can also be tested for sugar beet. Furthermore, investigations into the use of CRISPR/Cas 9 to create novel quantitative features/traits with gain-of-function mutations through replacements in sugar beet could be seen. CRISPR/Cas 9 technology promises to make a significant contribution to understanding the gene regulatory networks underlying abiotic stress response/adaptation and crop improvement initiatives to create stress-tolerant plants.

Additionally, CRISPR/Cas 9 technology applications hold great potential for addressing the challenges faced in sugar beet crops during biotic stress. Biotic stresses, resulting from pathogen and pest occurrences, significantly affect sugar beet production and yield. Traditional breeding methods have certain limitations in achieving rapid and precise genetic modification. CRISPR/Cas 9 technology has emerged as a promising solution with revolutionary approach to improve sugar beet tolerance to biotic stresses. Despite success of CRISPR/Cas 9 technology in controlling biotic stress, particularly diseases in economically significant crops, its use in insect management has not been fully utilized. Modest success has been achieved despite the intellectual exercise in creating techniques for insect pest resistance in both insects and plants. In contrast to other stresses, the main drawback has been the scarcity of target genes. Therefore, it is crucial for scientists to focus on finding sources of resistance that might serve as a foundation for insect control. To achieve this, it is necessary to evaluate the available germplasm, including wild relatives of certain crops, for pest response and to identify stress-responsive genes using multi-omics techniques. Targeted mutations turning susceptible plants into those that can control their respective pests are not far off in space or time, with such studies already in vogue. These factors, along with regulatory restrictions on gene-edited crops, may help the technique succeed in advancing not only science but also societal acceptance.

Author contributions

VM: writing, drafting, origin of the concept and compilation; AM: editing and writing, proof reading; HP: writing, compilation, concept; SS: writing and review of literature; AS: compilation of information for table. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Glossary

aaNAT	Arylalkylamine-N-acetyltransferase
Ag	Silver
APX	Ascorbate Peroxidase
As	Arsenic
BBMV	Brush-border membrane vesicles
BCTV	Beet Curly Top Virus Resistance
BNYVV	Beet Necrotic Yellow Vein Virus
CAT	Catalase
Cd	Cadmium
Co	Cobalt
CP	Capsid Protein
Cr	Chromium
CRISPR/Cas	Clustered Regularly Interspaced Short Palindromic Repeats
CRISPRa	CRISPR Activation
CRISPRi	CRISPR Interference
crRNA	CRISPR RNA
CRY	Crystalline Protein
DE	Differentially Expressed
DETECTR	DNA Endonuclease Targeted CRISPR Trans Reporter
dsRNA	Double-Stranded RNA
Fe	Iron
FQ	Fluorescence Quencher
GR	Glutathione Reductase
GVR	Geminiviral Replicons
HD-RNAi	Host-Delivered RNA Interference
HIGS	Host-Induced Gene Silencing
HSFs	Heat Shock Transcription Factors
HSPs	Heat Shock Proteins
HSEs	Heat Shock Elements
ICPs	Insecticidal Crystal Proteins
KRAB	Kruppel Associated Box
lncRNAs	Long noncoding RNAs
NADA	*N*-acetyl dopamine
Ni	Nickel
NIC	Toxic Nickel Concentration
PAM	Protospacer Adjacent Motif
Pb	Lead
PI	Protease Inhibitor
PM	Peritrophic Membrane
POC	Point-of-Care
Pt	Platinum
RKN	Root-Knot Nematode
ROS	Reactive oxygen species
RPA	Recombinase Polymerase Amplification
SBRM	Sugar beet root maggot
sgRNA	Short Guide RNA
SOD	Superoxide Dismutase
TALENs	Transcription Activator Like Effector Nucleases
TSS	Transcription Start Site
VIP	Vegetal Insecticidal Protein
ZFNs	Zinc-Finger Nucleases
Zn	Zinc