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REVIEW

Development of stress tolerant transgenomic traits in sugar beet through biotechnological application

Sudeepta Pattanayak[1], Siddhartha Das[2]*, Sumit Kumar[3]

[1] Division of Plant Pathology, ICAR – Indian Agricultural Research Institute, Pusa Campus, New Delhi, India
[2] Department of Plant Pathology, Centurion University of Technology and Management, Parlakhemundi, India
[3] Department of Biotechnology, University Institute of Engineering and Technology, Kurukshetra University, Thanesar, India

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*Corresponding address:
siddhartha.das10@gmail.com

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Abstract

Sugar beet (*Beta vulgaris* L.) has emerged as an alternative to sugarcane. It is mainly utilized for sugar extraction and has significant industrial value with great nutritional impact. Different kinds of biotic and abiotic stresses are considered to be major barriers for sugar beet cultivation. As per the current scenario, every year sugar beet production suffers huge yield losses due to various stresses. The conventional breeding technique is a time-consuming lengthy procedure which can be replaced by a genetic transformation technique to bring new transgenic traits within a short period of time. Sugar beet has proven to be excellent sample material for in vitro culture of haploid plants, protoplast culture, somaclonal variation, and single cell culture, among others. *Agrobacterium* mediated and PEG-mediated transformations are the most effective genomic transformations in the case of sugar beet. Development of new traits in terms of fungus/virus, pest/nematode tolerance, herbicide and salt tolerance are the most frequently expected traits in the current scenario of sugar beet production. Potential transgenic plants are viable alternatives to traditional expression systems for end product (protein) development with more accuracy. So, transgenic production through genome editing/base editing is presently considered to be one of the best tools for sugar beet tolerant traits development. Food safety and environmental impacts are two major concerns of genetic transformation in sugar beet and need to be appropriately screened for public health acceptability.

Keywords: genetic transformation, stress tolerance, sugar beet, transgenic

Introduction

The cultivation of sugar beet began 200 years ago and it is considered to be a newer crop than other cultivated crops (Gurel *et al.* 2008). Sugar beet (*Beta vulgaris* L.) is popularly known for its industrial value for producing sugar or sucrose on an economical scale. According to the latest updates, sugar beet is responsible for one fourth of the world's sugar (Gurel *et al.* 2008; Zhang *et al.* 2016). Also, the processed wastes of beet crops are popular as major biofuel substitutes for production of bioethanol, fossil fuel, biofertilizer and food additives (Zhang *et al.* 2008; Ghaffari *et al.* 2021). The sucrose content of wild beet is reported to be 4 to 6% while it is 12% in sugar beet (Draycott 2006). The sucrose content of sugar beet has increased up to 20% in recent commercial cultivars via a series of breeding methods.

Several biotic and abiotic stresses such as weeds, diseases, pests, salinity and herbicides negatively affect the quality as well as quantity of beet production (Ma *et al.* 2020; Yolcu *et al.* 2021; Das and Pattanayak 2022). A combined approach of conventional and advanced biotechnological methods dramatically changed sugar beet cultivation by identifying novel traits related to various stresses. Conventional breeding methods are

more time consuming and primarily involve selection of uniform morphological traits of self-pollinated lines, qualitative seeds and economically useful properties (Lv *et al.* 2019). This remarkable achievement can be seen with double haploid production via ovule culture. However, its major drawback is high genotypic variation and non-reproducible regeneration protocols resulting in lower regeneration frequency (Gurel *et al.* 2008).

The broad field of biotechnology uses cellular and biomolecular processes to create technologies that enhance human health and quality of life (Flavell 2004). Biotechnology includes molecular and cellular biology and recombinant DNA techniques to identify and incorporate novel traits to produce better qualitative traits. Since advanced biotechnological approaches are less time consuming and involve novel traits, they are better than conventional approaches. Furthermore, these methods are more promising as they conserve and maintain more than one trait in a stabilized way. Advanced biotechnology involves several genetic transformation methods such as an *Agrobacterium* vector based on or directed by, e.g., somatic hybridization, electroporation, particle bombardment, sonication or polyethylene glycol (PEG) mediated techniques. In all the above-mentioned techniques, the most preferred materials are taken from intact leaves, shoots or cotyledon cells. Following these novel, promising transformation strategies, transgenic sugar beet hybrids have already been developed which are resistant or tolerant to herbicides, e.g., glyphosate, diseases like rhizomania root rot, nematodes, fungi, such as, *Cercosproa*, insects, salt stress, etc. This review will mainly focus on advanced biotechnological methods used to develop hybrids resistant to several kinds of stress, genetic transformation strategies, landmark achievements and some of the drawbacks encountered.

Novel genetic transformation strategies

Agrobacterium mediated transformation

Micro-organisms included under *Agrobacterium* spp. were considered to only be plant pathogens until the transfer of DNA to plant cells was discovered (Gelvin 2003). The unique ability to integrate a small portion of transfer DNA or T-DNA of *A. tumefaciens* and *A. rhizogenes*has shifted the traditional breeding approach to modern plant transformation strategies. Initially, this *Agrobacterium* mediated transformation approach was started in dicotyledonous plants and then it was also applied to a variety of other economically important plant species (Gelvin 2003). This novel transformation method is a highly complex method including genetic determinants of plant as well as bacteria cells. This method is more efficient and less expensive than other approaches. In this method, the opine and phytohormone biosynthesis genes are removed from the Ti and Ri (tumor inducing and root inducing) plasmids which will not disturb normal morphological plant development.

Research has already shown the *in vitro* susceptibility of sugar beet plants to *A. tumefaciens* (Zakharchenko *et al.* 2000) and *A. rhizogenes* (Moazami-Goodarzi *et al.* 2020). However, this can be avoided by preculturing the explants before inoculation or by extending the duration of co-culture. Novel hybrid varieties with increased yield can be developed by engineering the tolerance or resistance genes of beet related to a wide range of biotic stresses (Moazami-Goodarzi *et al.* 2020). Production of stress tolerant sugar beet varieties through modern breeding approaches can be considered to be a sustainable and cost-effective method for pest and disease management (Nyaboga *et al.* 2015). Using the bacterium *A. tumefaciens* with a binary vector containing beta glucuronidase or chloramphenicol acetyl transferase (cat) and the kanamycin resistance gene (*nptII*), the first effective regeneration of transgenic sugar beet plants was achieved (Gelvin 2003).

Similarly, the development of transgenic sugar beet lines also has been successful in conferring resistance to lepidopteran insects if the lines expressed *cry1Ab* and *cry1C* from *Bacillus thuringiensis* and *A. tumefaciens,* respectively (Kimoto and Shimamoto 2002; Sedighi *et al.* 2011).

Various beet explants such as leaves, callus or shoot bases can be useful. Cotyledon explants were reported to be more successful in sugar beet transformation (Zhang *et al.* 2008). Higher frequencies of transformation were observed by using some other explants such as a 6.2% transformation rate by leaf lamina explants obtained from shoots (Norouzi *et al.* 2005) and 30.6% from the tip of the bud of an immature flower having several shoot clumps (Yang *et al.* 2004).

Hs1pro-1, a novel gene conferring nematode resistance, was introduced through co-inoculation of hypocotyl explants and leaf petiole explants with *A. rhizogenes* 15834 and *A. tumefaciens* LBA4404 containing binary vector pAM 194 with GUS which resulted in increased root hairs. The increased root hair formation was assumed to be due to the action of the *tzs* (trans-zeatin secretion) gene which resulted in repeated cell multiplication by synthesized cytokinin secreted by the *Agrobacterium*. *Agrobacterium* mediated transformation is one of the fast-track methods to check several parameters in roots, for example, host and cyst nematode interaction in root cells, viral pathogenesis, insect interaction, the study of bacterial gene behavior, transgene expression, etc. (Cai *et al.* 2003; Menzel *et al.* 2003; Dimmer *et al.* 2004; Smigocki *et al.* 2007).

Particle bombardment

An alternate technique, particle bombardment-mediated transformation, is effective and suitable for a wide variety of plant species. It enables simultaneous multiple gene transfers without biological restrictions or host limits, allowing DNA transport into intact plant cells. Additionally, particle bombardment is used to transfer DNA directly into various tissues and, in temporary gene expression studies to examine plant gene expression. To deliver substances into cells and tissues, particle bombardment uses high-velocity microprojectiles. Foreign DNA is precipitated with calcium chloride and spermidine onto the surface of micron-sized tungsten or gold particles for genetic transformation (Smigocki *et al*. 2003; Ivic-Haymes and Smigocki 2005). The DNA separates from the particles once it has entered the cells. Transient expression and possible stable integration of the transgene into host chromosomes are probable outcomes if the foreign DNA enters the nucleus (Smigocki *et al*. 2003; Ivic-Haymes and Smigocki 2005). To produce genetically modified plantlets, a regeneration system must be used. This method uses some terminal DNA bases and a gene's coding region between the promoter and terminator as foreign DNA (Gurel *et al.* 2008).

Particle bombardment has already been successful in the stable transformation of a wide range of plant species. Earlier studies of genetic transformation of sugar beet mainly focused on optimizing several parameters by using transient expression (Onde *et al.* 2000). Testing of particle size and disk pressure through GUS expression in the first two cell layers of the apical dome was obtained by bombarding the apical meristematic region. This resulted in low expression of GUS in cells multiplying in the meristematic region (Gurel *et al.* 2008). The feasibility of callus tissue bombardment by using different sample plate distances and pressure was also checked but it showed less response than *in vitro* leaf tissue germinated seedlings (Onde *et al.* 2000). Bombardment regeneration of transgenic sugar beet plants was achieved for the first time applying embryogenic callus from the hypocotyl regions of the REL-1 regenerative line. This resulted in transformed sugar beet plants with enhanced resistance genes (cytokinin biosynthesis genes or pathogen defense related genes) to pathogens or insects. In another approach, this particle bombardment of the leaf disc, earlier cultured in the dark for 7 weeks showed a transformation efficiency of 0.9–3.7% (Smigocki *et al*. 2003; Ivic-Haymes and Smigocki 2005). Though many studies have been carried out by this transformation approach, it still has not gained the popularity of *Agrobacterium* mediated transformation.

Polyethylene glycol-mediated transformation

To obtain high transformation frequencies, protoplast populations optimized particularly for a single totipotent cell type produced from stomatal guard cells could be subjected to a polyethylene glycol (PEG)-mediated DNA transformation approach. PEG mediated transformation entails combining freshly extracted protoplasts with DNA and immediately adding PEG that has been dissolved in a solution containing divalent cations (mainly Ca ions). Protoplasts are washed and then placed in Petri plates for culture and growth after this mixture has been incubated for 30 minutes (Gurel *et al.* 2008). Due to considerably greater survival rates following treatment, PEG-mediated transformation is typically favored over electroporation for stable transformation of monocot protoplasts (Ivic--Haymes and Smigocki 2005). PEG causes the plasma membrane to become unstable, which in turn causes ionic macromolecules like DNA to precipitate across the cells and promote endocytosis. Of the total treated protoplasts 0.1 to 0.4% are generally transformed by PEG-mediated DNA absorption (Ivic-Haymes and Smigocki 2005). The ability of changed protoplasts to regenerate in a variety of environments is necessary for the production of transgenic plants (antibiotic resistant gene) (Gurel *et al.* 2008). The pat gene's ability to confer bialaphos resistance led to the development of a very effective selection mechanism. The uidA (GUS) reporter gene on pPGS and the distinctive phenotype of guard cell protoplasts were used in the polyethylene glycol (PEG)-mediated DNA import procedure (Hall *et al*. 1996). The greatest effect was shown to be an increase in the frequency of both transient expression and stable transformation at varied concentrations of PEG (13.3, 17.5, 20.0%) (Hall *et al*. 1996). Polyethylene glycol mediated genetic transformation in sugar beet was followed for crop improvement by developing resistance and tolerance against different biotic and abiotic stresses. This approach became popular after the totipotent nature of protoplast and stomatal guard cells came to light. Before conducting the transformation study, several parameters are taken into consideration such as the concentration of PEG and DNA, types of carrier DNA, incubation times, etc. It was observed that only the concentration of PEG can alter the stable integration and transient GUS expression (Gurel *et al.* 2008). This approach has recorded high frequencies of single copy insert, i.e., 23–36%, whereas multicopy insertion leads to co-suppression. Through this technique the derived transgenic plants have a slight soma clonal variation with some instances of tetraploidy. With this technique, BNYVV (beet necrotic yellow vein virus) resistant plants were developed by introducing a coat protein gene

(Lathouwers *et al.* 2005). The guard cell protoplast method was assumed to be genotype independent, able to develop transgenic plants in a short period (2 months), but the regeneration efficiency was reported to be low. Thus, an increase in regeneration efficiency can improve the application of this technique as somatic embryogenesis or, direct shoot organogenesis can reduce the soma clonal variation and culture time.

Selection strategies

Trans genomic sugar beet plants can also be achieved through the choice of selectable markers. Selecting suitable genes such as those with antibiotic resistance and herbicide tolerance in sugar beet has wider applicability with a trans genomic approach (Madsen and Sandoe 2001). Herbicide tolerance genes have the advantage of being transferred from pollen to wild relatives. Furthermore, they can strongly resist herbicide tolerant weeds (Bennett *et al.* 2004). Similarly, antibiotic resistant genes are nontoxic and capable of deactivating antibiotics, but they also have negative effects by being transferred to harmful bacteria (Meier and Wackernagel 2003). Some other genes can also be taken into consideration but they must have positive and desirable effects such as sucrose-phosphate synthase, phosphomannose isomerase (PMI), some nondestructive markers like yellow fluorescence proteins (YFP) or green fluorescence proteins (GFP) (Zhang *et al.* 2008).

Phosphomannose isomerase (PMI) selection was reported to be the first successful transformation achievement in sugar beet. This selection strategy has more positive effects than other markers as it avoids the accumulation of potentially harmful agents and their derivatives by converting mannose-6-phosphate to metabolizable fructose-6-phosphate (Privalle 2002). Higher transformation frequencies were reported if the promoters resulting in intermediate PMI expression level were used. The marker-free transgenic technique can also be used to create transgenic sugar beet plants. A pBSB, a selection marker free vector, and a maize SPS (sucrose-phosphate synthase) gene driven by rice Cab promoter through particle bombardment were required for this technique (Zhang *et al.* 2016). In several plant species, dual markers with pmi and gfp (one for selection and the other for visual observation) have recently been employed for transformation. Similarly, by combining the usage of a binary vector with a marker gene and a reporter gene driven by a promoter, a modified *Agrobacterium*-mediated transformation strategy can enhance the transformation frequency of sorghum embryos (Hiei *et al.* 2006).

Alternatives

Electroporation is listed as one of the top-rated alternative strategies for transformation of sugar beet. It was carried out for the first time by using an electrical pulse generating system to ease the protoplast uptake from the plasmid of a suspension cell. Protoplasts taken from mesophyll were reported to be more sensitive to electroporation damage. Protoplast fusion via chemical or electrical means is one of the best alternatives for developing somatic hybrids followed by transfer of cytoplasmic and nucleo genetic materials from one source to another source. Although regeneration of sugar beet plants cannot be achieved, production of somatic hybrid microcalli is possible in sugar beet (Gurel *et al.* 2008). Additionally, isolation of protoplast and fusion of mesophyll derived protoplast was achieved, but resulted in unsuccessful regeneration (Gurel *et al.* 2008). It was difficult to get regenerated plants through any of the alternative transformation strategies such as somatic hybridization or electroporation, but improved protocols via electroporation can surely increase the successful production of transgenic beet plants (Skaracis and McGrath 2005).

Identification and development of novel transgenic traits

Incorporation of transgenic traits related to herbicide tolerance

Herbicide tolerance is the most important trait in the genomic improvement of many crops such as wheat, rice, maize, and sugar beet (James 2013). Sugar beet producers documented weed control of around 55–59% in Minnesota and eastern North Dakota (USA) in 2020 (Thomas *et al.* 2020). Transgenic crops make up the majority of genetically modified plants cultivated with herbicide-tolerant species worldwide. Many countries produce GM crops such as South Africa, Sudan, and India (Mathur *et al.* 2017). In some research a variety of biotechnological approaches have been used to develop sugar beet plants with improved or novel features (Zhuzhzhalova *et al.* 2020). Herbicide tolerance (HT) crops were created by utilizing genes from microbes or higher plants which confer tolerance by combining the active site of a protein with a transformed cell that has been exposed to a herbicide and by using an enzyme that contains the herbicide's active components (Gurel *et al.* 2008).

Glyphosate is a frequently utilized herbicide in commercial agricultural crop production for herbicide-resistant, conventional and traditional crops. Herbicide-resistant sugar beet types express the

glyphosate-insensitive CP4 microbial gene for *Agrobacterium* sp. gene for a modified enzyme, CP4--EPSPS 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which was first released in 2007–2008 (Barker and Dayan 2019). Some glyphosate is converted into the major metabolite aminomethylphosphonic acid (AMPA) in soybean (*Glycine max*) and canola (*Brassica napus*) plants, while metabolic breakdown in various other plants is often slow or nonexistent (Correa *et al*. 016).

Glufosinate (ammonium salt of L-phosphinothricin, PPT) and bialaphos (PPT plus two alanines; L-phosphinothricinylL-alanyl-L-alanine) are highly toxic to plant cells; they act as competitive inhibitors of glutamine synthetase, which is critical for converting glutamic acid and ammonia into glutamine. Inhibition causes a hazardous build-up of ammonia, which causes cell death.

Two encoded enzymes, bar and pat, were identified from changed *Streptomyces* sp., in transgenic HT crops driven by two promoters, cauliflower mosaic virus (CaMV) and 35S, and these two TR1' and TR2', were also employed to obtain a glufosinate-tolerant sugar beet (*Beta vulgaris*) crop. A marker free approach was reported in transgenic sugar beet in 1997, at the root level. During the transformation of sugar beet into crystalline sugar, the destiny of glyphosate was tracked. The pressed pulp is separated from the diffusion juice by homogenizing the roots and filtering the homogenate.

A carbonation stage is used to eliminate proteins and generate thin juice, which is then concentrated into an evaporated thick liquid. The viscous fluid is subsequently crystallized, and the sugar is separated from the leftover molasses. The diffusion juice, which is the first product of processing, contained the most glyphosate. Finally, glyphosate is an excellent technique for controlling weeds in sugar beet production. Because of its capacity to translocate and exude from the roots, beets have relatively low amounts of herbicide by the time they are harvested. Furthermore, converting the juice into crystalline sugar removes all traces of the herbicide from the finished product (Barker and Dayan 2019).

Incorporation of transgenic traits related to viral resistance

The beet necrotic yellow vein virus (BNYVV) belongs to the *Benyvirus* genus, which is part of the Benyviridae family (Gilmer *et al*. 2017). Western yellows virus (BWYV) and beet necrotic yellow vein virus (BNYVV) are major viral diseases of sugar beet, transmitted by *Polymyxa betae*. Beet curly top virus (BCTV) is another important plant virus which infects sugar beet plants. Cross-kingdom RNAi, mediated by sncRNAs produced from pathogens, is critical for suppressing the expression of crucial host defense-related genes, particularly during plant-virus interaction. On the other hand, in addition to targeting important genes linked to disease, host plants also create sncRNAs (such as miRNAs) that can regulate the expression of genes relevant to host metabolism or defence. A thorough investigation found that transgenic plants expressing viral pathogen-derived genes frequently exhibit tolerance to the virus and its associated strains. These findings gave rise to the theory that the viral life cycle might be disrupted by the ectopic expression of genes encoding wild-type or mutant viral proteins. Recent research has shown that RNAi, which is crucial for plant antiviral defense, mediates this immunity. BNYVV is a pivotal agent of rhizomania disease and widely distributed in BNYVV which is accumulated in the root tissue of sugar beet. BNYVV by nature is an obligate parasite on roots, where it produces zoospores and long-living resting spores (Tamada and Kondo 2013). *Rz1* is the most important gene in sugar beet to control beet necrotic yellow vein virus (Liebe *et al*. 2020). *Rhizomania*-resistant sugar beet and its closely related wild Beta species – *B. maritima*, were created by harnessing disease-resistance genes with conventional breeding (Panella and Lewellen 2007).

Various coat proteins are used in sugar beet. *Cp21* is a gene silencing coat protein (Safar *et al.* 2020). Various techniques, such as expression of single-chain antibody fragments, partial replicase proteomics, and synthesis of antisense RNAs, have been tested, but not in sugar beets (Lathouwers *et al*. 2005).

Incorporation of transgenic traits related to fungal resistance

Cercospora beticola leaf spot (CBLS) is one of the major fungal pathogens in sugar beet that has caused significant reduction in yield and quality. In recent years, due to the excessive amount of fungicide application, pathogens have become resistant to a specific chemical. Previously *Cercospora* was controlled by light fungicides but after some time it became evident that *C. beticola* leaf spot (CBLS) was resistant to a few chemicals. Therefore various IDM strategies have been applied to control the disease (Vogel *et al*. 2018). *Cercospora beticola* leaf spot affected 70–90% of the German and African sugar beet area in past years. Other infections such as syn. *E. polygoni* (powdery mildew), *Ramularia beticola, Erysiphe betae, Uromyces betae, Rhizoctonia solani* (*Rhizoctonia* foliar blight, and root and crown rot), and *R. solani* (*Rhizoctonia* foliar blight, and root and crown rot) were found on less than 15–20% of the sugar beet plants, thus having less economic significance (Brendler *et al*. 2008; Vasel *et al*. 2013). For breeding purposes, the key factor for fungal resistance is CBLS

in *B. vulgaris* (Gummert *et al.* 2015). The antifungal protein, like an encoding gene, plays an important role in *Rh. solani* defense responses in *B. vulgaris* (Holmquist *et al.* 2021). Because of the diverse soil-borne pathogen (*Pythium* and *R. solani*) mediated diseases, *Penicillium pinophilum's* ability in sugar beet has decreased (Kazerooni *et al.* 2019). *Rhisoctonia. solani* persists in soil as sclerotia or melanized mycelia, which are the predominant infection sources in the field at the time of seed germination (Boland *et al.* 2004).

Recently, carbohydrate active enzymes (CAZymes) in *B. vulgaris* were analyzed through omics database (dbCAN). Carbohydrate active enzyme is a carbohydrate related protein. Antifungal proteins (cysteine enriched proteins) from infested *B. vulgaris* leaves had defensive effects against fungicide resistance, an important source of fungal resistance in sugar beet. Research is underway to develop genetically modified *C. beticola* leaf spot (CBLS) sugar beet by inserting the *Cercospora* export gene, cfp (Kuykendall and Upchurch 2004). The pumpkin gene (chitinase gene) has been introduced to *Beta* germplasm, and signs of disease have been observed in genetically modified sugar beet plants. Some crucial genes like *SDR, GST1, GST2, AtMPK4, VSP1,* etc., are responsible for disease resistance of sugar beet and are shown in Table 1 and Figure 1.

Table 1. Novel genes involved in various biotic and abiotic stress responses in sugar beet (Coulbridge *et al.* 2007; Zhang *et al.* 2008; Lv *et al.* 2019; Yolcu *et al.* 2021)

Biotic stress			Abiotic stress
pathogen resistance	insect resistance	nematode resistance	salt and drought resistance
SDR	CEV1	$Hs1^{pro1}$	Prx
GST1	EIN2	$Hs2^{pro7}$	POX
GST2	$Hs1^{pro1}$	$Hs1^{web7}$	AOX
Rx1	BvSTI	Hs1-1	APX
Mi-1	JAR1	Mi	SnRK2
AtMPK4	COI1	Gpa2	SOS1
VSP1	HSP90		CMO
PAD4	SDR		Prx
EDS1			BvCK2
			BvM14-SAMDC
			KAT1
			PIPs

Incorporation of transgenic traits related to nematode resistance

The beet cyst nematode *Heterodera schachtii Schm* (BCN.) is an important pest influencing the yield of sugar beet (*Beta vulgaris* L.). Standard sugar beet traits are susceptible to parasites and support BCN reproduction. Therefore, the conventional breeding strategy of BCN resistant sugar beet (*B. vulgaris*) varieties is an alternative approach. Different monogenic nematode resistance has been introduced into sugar beet varieties. Nematode resistant BCN is a cytogenetic mutant that has been selected from the offspring (Reuther *et al.* 2017). Sugar beet has partial resistance to SBCN, while the procumbentes section of Beta, which includes *B. procumbens, B. patellaris,* and *B. webbiana*, has perfect resistance. These species, however, are not part of the sugar beet's basic gene pool, and gene transfer into

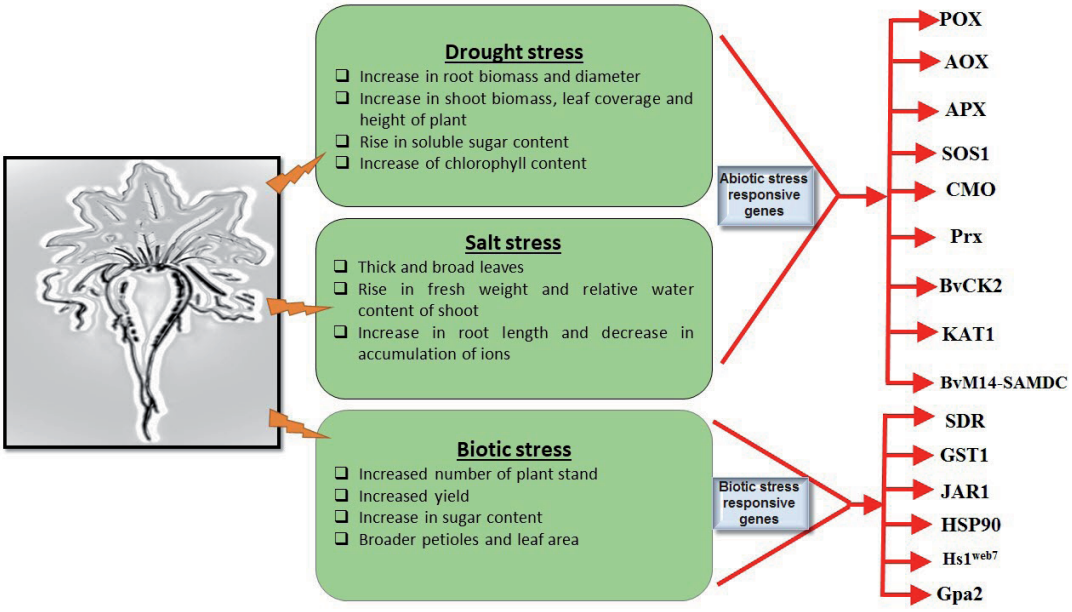

Fig. 1. Schematic representation of sugarbeet stress responsiveness and associated genes

sugar beet necessitates the translocation of a chromosomal fragment containing the resistance gene (Panella and Lewellen 2007). Interspecific crosses with *B. procumbens* were used to introduce a resistance gene (*Hs1pro1*). However, the instability of the wild beet chromosome fragment, undesired-unpaired linked sequences encoding leaf- and root bulgeness and wart formation, and the multi-top phenotype considerably reduces production even in the absence of severe nematode infestations which passively limits the applicability of this alien source (Panella and Lewellen 2007). Recent studies were carried out on the stem nematode pest *Ditylenchus dipsaci*, found in European sugar beet production. In India, Germany and France, stem nematode pest harm and also *D. dipsaci* damage in topographical areas of sugar beet were found (Storelli *et al.* 2021b). Under in vivo condition aforesaid method has been used on stem nematode and to investigate *D. dipsaci* interaction with sugar beet (Storelli *et al.* 2021a) Testing sporamin (sweet potato derived storage protein), as an antifeedant for SBCNs was used as an alternate way of nematode-resistance development. Some nematode resistant genes like $Hs1^{pro1}$, $Hs2^{pro7}$, $Hs1^{web7}$, *Gpa2* are represented in Table 1 and Fig. 1. In the case of genetically modified tobacco and cauliflower, sporamin which is a Kunitz-type trypsin inhibitor, alters the pattern of insect resistance. At the time of sporamin gene (*SpTI-1*) introduction and insertion in *B. vulgaris* by *A. rhizogenes*, several secondary root like structures expressing sporamin were found and assayed for nematode resistance; seven to eight clones expressly suppressed the development of female nematodes under such conditions. Inhibition in the roots was related to trypsin inhibitor activity but not to sporamin levels, indicating that sporamin might be used as an anti-feedant to suppress SBCNs (Cai *et al.* 2003).

Incorporation of transgenic traits related to insect resistance

The sugar beet root maggot (SBRM), *Tetanops myopaeformis* (Röder) is an important insect pest of sugar beet. In July of 2017, the US Environmental Protection Agency approved the registration of Movento HL insecticide for use in sugar beet. The addition of this product is encouraging from an insect resistance management perspective because spirotetramat, the active ingredient in Movento, belongs to the lipid biosynthesis inhibitors (LBIs), and has a completely different insecticide mode of action than the ACHE inhibitors (Mark and Jacob *et al.* 2021). *Cry1C* and *Cry2A* genes express in insect pests in sugar beet. These two genes are identified through reverse transcription PCR analysis (Litvin *et al.* 2014). *Nicotiana plumbaginifolia* leaf extract was transferred with the ipt gene in sugar beet (Smigocki *et al.* 2003). The cytokinin insect resistance gene may be able to control the sugar beet root maggot. In addition, a gene for a serine (trypsin) protease inhibitor (BvSTI) was extracted from a sugar beet line that was somewhat resistant to sugar beet root maggot, and two promoters, CaMV and 35S, were combined to introduce the gene into hairy root cultures.

A two- to threefold increase in the concentration of trypsin inhibitor activity was transformed in root culture of sugar beet. BvSTI may be involved in resistance. Biotic stress responsive novel genes are shown in Figure 1 and Table 1. Research on the impact of trypsin inhibitor activity on insect mortality and growth rates is also underway (Smigocki *et al.* 2007).

Incorporation of transgenic traits related to bolting resistance

Water, thermal treatment (i.e., vernalization), and a nutritional breeding development program in sugar beet commences with bolting in the world-wide changing situation after winter cold (stem elongation) (Hoffmann and Kenter 2018; Abou-Elwafa *et al.* 2020). Bolting during first-year growth generates significant output reductions (0.5% sugar yield for 1% field area of bolted beets) due to decreased spring temperatures (Skaracis and McGrath 2005). The genetic foundation for sugar beet bolting control and how to improve it have been extensively researched (Dally *et al.* 2014). Bolting gene-B, which increases the beginning of bolting during long days without preceding vernalization, was shown to be responsible for sugar beet's yearly pattern (Abou-Elwafa *et al.* 2020). Harvesting and sugar extraction operations may suffer from mild to severe for bolting. Early spring sowing is not possible due to the bolting problem, which would be beneficial because early establishment leads to larger sucrose yields. Flowering genes, notably those involved in gibberellic acid (GA) biosynthesis, regulate bolting resistance. Inhibition of GA production using genetic engineering appears to postpone bolting in sugar beets, according to research on *A. thaliana*. To date, no success has been reported in down regulating GA1 and GA4 by over-expressing pumpkin GA20-oxidase or utilizing antisense constructs for GA20-oxidase and GA3-β-hydroxylase.

Incorporation of transgenic traits related to drought tolerance

Drought is one of the most significant abiotic stress factors that affect plant development and productivity (Ferweez and Bashandy 2021). Morphological characterization and screening are not enough for drought tolerance. Sugar beet verieties are more susceptible to ecofriendly changes (Fufa *et al.* 2005). Consequently, in conventional breeding approaches, molecular markers have been used to identify the polymorphism of traits

which are not influenced by environmental effects. Simple Sequence Repeat (SSR), Inter Simple Sequence Repeat (ISSR), Random Amplified Polymorphic DNA (RAPD) and ISSR markers has been widely used for detecting multilocus regions of genomes by using the primer and microsatellite sequence. Additionally, these can identify the location of DNA (Tatikonda *et al.* 2009). Drought tolerance can cause several types of morphological conditions such as wilting of leaves, photosynthetic pigments, transpiration, root biomass, and leaf weight (Skorupa *et al.* 2019; Wisniewska *et al.* 2019). In recent studies, non-symbiotic hemoglobin (BvHb2) genes have been identified in sugar beet plants which are expressed on leaf tissues and showed osmatic stress tolerance in the case of *Arabidopsis* and tomato. Overexploitation of the *BvHB2* gene is found to be effective in drought tolerance (Gisbert *et al.* 2020). Some of the important drought stress tolerant genes like *BvM14-SAMDC*, *KAT1, BvCK2*, *Prx,* etc., are shown in Figure 1 and Table 1.

Also, heat shock factors (HSFs) are transcription factors related to various abiotic stress conditions (Guo *et al.* 2016). The expression of heat shock factor (BvHSF) gene of *B. vulgaris* (also involved in drought stress) was raised under PEG-induced water stress (Ismail *et al.* 2020). Increasing the cellular concentration of osmolytes and osmoprotectants has resulted in drought-tolerant transgenic plants in various species. The expression of a *Bacillus subtilis* gene, *SacB*, used to encode bacterial fructans, resulted in significant drought tolerance in sugar beet. Compared to non-transgenic beets, transgenic plants grew better under drought stress and had larger total dry weights (+25–35%).

Incorporation of transgenic traits related to salt tolerance

Beta vulgaris, which can tolerate a salinity level of up to ~45 to 120 mM, is well known for its high salt tolerance potential. Sugar beet has good tolerance to water scarcity in comparison to various other crop plants (Pinheiro *et al.* 2018). Various types of sugar beet genes are involved in salt tolerance, including the recently discovered sugar beet *bHLH93 (BvbHLH93)* gene, whose helix frame is involved in salt tolerance or response. Over expression of *bHLH93 (BvbHLH93)* gene in Arabidopsis Na+ concentration is lower, enzyme concentration is higher except for the Ca- ion concentration of two gene families, RbohD and RbohF (Wang *et al.* 2021). Another, SnRK2 homologs protein was obtained in beet plants, using an omics tool, at the transcriptomic level. BvSnRK2 protein was amplified under salinity conditions which shows a potential salt response. This (SnRK2s) protein kinase belongs to the Ser/Thr kinase family (Wu *et al.* 2021). The *BETA1* gene, which is a homolog of the *Arabidopsis SAH7* gene, was developed by screening a *B. oceanic* cDNA library. *BETA1* gene ancestral articulation was instigated by salt responses in leaves and foundations of a wild primitive beet variety. The capacity of this *BETA1* gene is not known, however, it may be involved in salt resistance in wild sugar beet (Uysal *et al.* 2017). The AtNHX1 gene, extracted from Arabidopsis and expressing a Na+/H+ antiport localized to the vacuolar membrane, was used to create transgenic sugar beet plants with dramatically increased salt tolerance (Yang *et al.* 2005). Salt responsive stress tolerant novel genes like *POX, AOX, APX, SOS1,* etc., are shown in Table 1 and Fig. 1.

Incorporation of transgenic traits through modification of carbon metabolism

A gene is said to be differentially expressed if there is a statistically significant difference or change in read counts or expression levels/indices between two experimental conditions. Using FRKM (Fragments Per Kilobase of exon model per Million mapped reads), the expression of each gene was determined. A variety of tools, including Cuffdiff2, were employed to find the genes that were expressed differently in the two samples. The p-value criterion was established using the false discovery rate (FDR). The range of differentially expressed genes (DEGs) is significant if the FDR value reaches 0.05 and $|\log2FC| \geq 1$ or FRKM ≥ 1. Recent studies have identified the DEGs, which are activated under salt stress conditions via amino acid biosynthesis and carbon metabolism.

Salt stress in sugar beet further activates sugar metabolism by regulating nitrogen and carbon metabolism. Compared to contrasting genotypes of sugar beet one is T710MU and the second is S710 under salt stress conditions (Geng *et al.* 2019). In the case of fructose, low molecular weight fructans were synthesized enzymatically from sugar beet or sugar cane sucrose using *Aspergillus niger* fructosyl transferase, but manufacturing costs were greater. It was seen that sugar beet was transformed with a l-sucrose: sucrose fructosyl transferase gene (1-sst) from *Cynara cardunculus* driven by CaMV and 35S promoter, which mediates the first steps of fructan synthesis, converting sucrose to low molecular weight fructans GF2, GF3, and GF4 using PEG-mediated transformation of stomatal guard cell protoplasts. Sucrose was converted to low molecular weight fructans in transgenic plants' tap root cells (>90%). Leaves had trace quantities of GF2, GF3, and GF4. Despite significant metabolic changes, transgenic plants grown in a greenhouse had no apparent abnormalities in their taproots. Sugar beet transformed with two fructosyl transferase genes from onion, 1-sst and 6g-fft (fructan:fructan 6G-fructosyl transferase)

singly or conjointly, successfully converted sucrose into fructans in taproot parenchyma cells without losing carbohydrate content (Weyens *et al*. 2004).

Transgenic sugar beets with increased sucrose synthesis by expressing sucrose-enhancing genes such as sucrose phosphate synthase, sucrose synthase, and sucrose transporters have long been research goals. A chimeric antisense sucrose non-fermenting (*SNF*1) gene has been found which encodes a protein kinase that regulates cellular glucose levels to hairy root cells and reduces the *SNF*1 homologue activity. A rice Cab promoter-linked maize sucrose-phosphate synthase (SPS) gene was also inserted, and it was hypothesized that increased levels of SPS, a crucial enzyme in sucrose biosynthesis, would improve sucrose synthesis. Despite increased leaf and taproot cytokinin levels, transgenic and non-transgenic plants had similar leaf sucrose levels. More crucially, transgenic taproot development and growth were greatly hampered, resulting in lower sucrose levels (Hashimoto and Shimamoto 2001). After root damage, carbon metabolisms in the TCA cycle were changed, showing systemic effects of injury on carbon metabolism. Wounding was connected to increases in glucose 6-phosphate, fructose 6-phosphate, glucose 1-phosphate, and ADP concentrations in the internal tissue of roots during the course of the experiment, with an increase in citrate concentration occurring only on the 3rd and 4th days following injury (Lafta and Fugate 2011).

Conclusions and future prospects

Several biotic and abiotic stresses of sugar beet are the key problems and primary concern of global sugar beet producers. The absence of successful management strategies against these stresses has increased the complications in sugar beet cultivation among producers as well as industries all over the world. Prolonged studies on stress biological constraints have developed novel techniques in beet production technology. Different breeding approaches have played a vital role in developing new resistant and tolerant varieties, but they have certain drawbacks such as being time consuming, labor intensive, requiring skilled laborers, etc. Thus, there is an immense need to develop new varieties within a short period of time which can target more than one stress simultaneously. The recent shifting of research from conventional breeding to a biotechnological approach or speed breeding has been one of the most successful achievements in sugar beet production. The main objective of this review was to focus on new biotechnological approaches to introduce novel genes to combat various stresses.

Novel biotechnological methods introduce and enrich useful traits which cannot be achieved through traditional breeding. Genetic engineering can be used for improving the multilayered defenses of plants against different microbial diseases. Transgenic expression of positive and negative strands of viral genomes, inverted repeats for the production of artificial micro RNAs were found to be the most effective through RNAi technology. CRISPR-Cas9 was found to be the best genetic engineering tool against DNA and RNA virus related host defence. Resistance (R) gene stacking can be used for achieving broad spectrum disease resistance. Multiple R genes can group together through molecular stacking or targeted gene insertion, which makes the subsequent genetic segregation process easier. Comparative genomic tools like R gene enrichment sequencing (RenSeq) facilitates high altered screening of germplasms or mutants for rapidly detecting candidate R genes by spotting core effectors. Genetic transformation can potentially act against several stresses through the enhancment of resistance to disease, drought, salt, and nematodes, as well as improvement of weed control related genes and by capturing nutrients and other biosynthetic compounds to increase the yield dramatically. Advanced biotechnological methods which can identify major genes related to various stresses can lead to a diverse, cost effective and sustainable beet cultivation technology. Transformation yields vary significantly between different biotechnological tools. For example *Agrobacterium* mediated transformation carries – 6.2% to 30.6%; particle bombardment carries – 0.9–3.7%, etc. From a practical point of view *Agrobacterium* mediated transformation (AMT), protoplast mediated transformation (PMT), and genetic bombardment (GB) showed high transformation efficiency and good yield. In addition to this, introducing these advanced technologies in beet breeding and seed industries can be helpful for improving the quality of seed material.

Restrictions and protocols should be followed strictly while maintaining the *in vitro* propagation and transformation techniques to avoid any further harmful impacts on humans and the environment. However, the cultivation of transgenic sugar beet has not been approved by any of the countries unlike the USA. Despite all the positive impacts and minimal risk, the transgenic beet has more potential negative effects which can result in consumer dissatisfaction and product rejection. Genetic engineering in sugar beet research enhances and improves the efficiency and movability towards stress and disease resistance like rhizomania and cyst nematodes. Furthermore, modified or altered biosynthetic pathways enriched the capacity of sugar beet roots to produce valuable compounds. Modern genetic engineering technologies, coupled with traditional breeding and future advances in identification

of genes and their functions or insertion or deletion of the gene of interest, could lead to major advances that will make sugar beet more productive, highly cost effective, more diverse and sustainable. As with all transgenic crops, concerns about their commercialization are many, including food safety, possible damage to the environment, and economic and consumer acceptance. Therefore food safety rules from advanced countries and their acceptance of genetically modified crops are of top priority in this field. More focus on lessening any negative impact and increasing the public awareness of positive effects can surely lead to more advanced and sustainable beet cultivation in the future.

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