Biotechnological Approaches in Sugar Beet Development

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Abstract

Sugar beet provides looming potential for sugar production globally supplementing sugarcane in the current scenario. The crop with the efficacy for bioethanol production from its pulp and molasses, minimal water requirement for its growth and shorter life cycle as compared to sugarcane is gaining importance. Its performance is influenced by various environmental and agronomic factors that ultimately decide the sugar yield. Genetic erosion of sugar beet is evident from the vast and prolonged use of varieties derived from similar parents. This hinders the selection process and renders it non-rewarding. The genetic diversity of the crop can be increased by the introgression of new alleles from its wild ancestors and wild relatives. Biotechnological tools like transgenics can help transfer the foreign gene even between two non-cross incompatible species. Effective genetic and genomic tools to screen and identify molecular tags conferring for important traits will help in the development of useful breeding material of sugar beet. Efforts to develop tolerance to biotic and non-biotic stress especially drought and cold is palpable. Genome sequencing through NGS and SMRT approaches helps in annotation of individual genes and deciphering phylogenic relationships among individuals. Incorporation of genetic transformation and *in vitro* technologies have been pertinent in producing salt-tolerant, herbicide-tolerant, disease-resistant, and pest-resistant cultivars.

Keywords

Genetic manipulations · OMICS · Regenerants · Sugar beet · Transgenics · Transcriptomics

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Abbreviations

AFLP	Amplified fragment length polymorphism
BNYVV	Beet necrotic yellow vein virus
CLS	Cercospora leaf spot
GOX	Glyphosate oxidoreductase
GSH	Glutathione
LD	Linkage disequilibrium
NGS	Next-generation sequencing
PIC	Polymorphism information content
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
SBP	Sugar beet pulp
SSR	Microsatellite
StGCS-GS	*Streptococcus thermophilus* γ-glutamyl cysteine synthetase-glutathione synthetase

5.1 Introduction

Sugar beet (*Beta vulgaris* L.) is a crop of global importance that stands second in prominence after sugarcane (Brar et al. 2015) and contributes 20% to the world sugar production (FAO 2009). Cultivated beets belonging to family Chenopodiaceae is thought to have originated from its wild progenitor "sea beet" scientifically called *B. vulgaris subsp. maritima* (Biancardi et al. 2012). Formally, sugar beet was likely domesticated as a pot herb and consumed for its leaves as the first harvest from its wild progenitor, sea beet [*B. vulgaris* L. subsp. *maritima* (L.) *Arcang*] for food (Biancardi et al. 2012; Ford-Lloyd et al. 1975; Lange et al. 1999). Later, the roots were used both as medicinal herbs and vegetables (Biancardi et al. 2012; Goldman and Navazio 2008). Root type sugar beet and its enlarged root was earlier documented in the Near East (Turkey, Iran, and Iraq) and eventually spread to the west (Europe) (Zossimovich 1940). Sugar beet is becoming an essential biofuel alternative to fossil fuel energy (Zhang et al. 2008). Sugar is widely used as livestock feed supplement that is largely produced by the sugar industry along with sizeable amounts of molasses and sugar beet annually as by-products (Olmos and Hansen Zúñiga 2012; Kracher et al. 2014). Sugar beet pulp (SBP) and molasses hold great potential for the production of energy-efficient bioethanol due to its high content of readily fermentable sugars (Rodriguez et al. 2010; Maung and Gustafson 2011). The crop further provides useful feedstock for alcohol, yeast, and pharmaceutical companies. Sugar beet is considered to be originated from indigenous Mediterranean *B. maritima*, a relatively young crop possessing a narrow genetic base (van Geyt et al. 1990) and has undergone significant genetic improvements since its cultivation about 200 years ago (Draycott 2006). Wild beets have 4–6% of sucrose content

whereas fodder beets have 12% sucrose content from which sugar beet was selected. The presently developed and cultivated cultivars have a much higher sugar content of 20% attributed to further improvements in the crop through conventional breeding. In India, it offers good potential to bridge the gap between projected and actual sugar production because of the high sugar content and production of useful by-products (Pathak et al. 2014). On a more recent development, advanced biotechnological methods alongside classical breeding approaches have been used to develop herbicide-tolerant, disease and pest-resistant cultivars. Sugar beet contest with sugarcane in sugar production at the global market. To compete with sugarcane and meet the high sugar demands of the global consumers, effective novel breeding technology and biotechnological interventions apart from the redundant breeding strategy are a necessity. Sugar beet diversity needs to be broadened by integrating wild alleles for useful traits from the wild species through skillful biotechnological methods as there exist crossability barriers between the cultivated and wild sugar beet species for effective selection and high-throughput molecular work establishment (Frese et al. 2001).

5.2 Molecular Studies and Advances in Sugar Beet

5.2.1 Genetic Diversity in Sugar Beet

Exhaustive selection over time and widespread adoption of a genetically uniform crop varieties resulted in genetic stagnation and loss of genetic diversity in cultivated crops that hamper further crop improvement programs. The wild ancestors and wild relatives carry important traits including pest and disease resistance, drought tolerance, cold tolerance, salt tolerance, and nutraceutical properties that are essentially needed by the crops for its survival and good performance (Zhang et al. 2016). It is therefore imperative to replenish the lost alleles from the breeding pools through introgression of useful genes from its wild species counterpart (Ordon et al. 2005). Understanding the genetic diversity of a crop helps in framing appropriate selection strategy and breeding schemes for the overall refinement of the crop. Total genetic diversity of sugar beet along with other *Beta* species including other cultivated beet crops and its wild relatives is fairly high (Fievet et al. 2007). The genetic diversity of sugar beet is established hitherto through morphological traits, isozymes, and molecular marker study. Study of the sugar beet diversity with DNA marker systems such as RFLP (Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), and AFLP (Amplified Fragment Length Polymorphism) have been done in the early and mid-1990s (Jung et al. 1993; Barzen et al. 1995; Schondelmaier et al. 1996). Earlier attempts were made to understand the genetic relationship in *Beta Vulgaris* including table beet, sugar beet, and Swiss chard crop types using RAPD markers that revealed that table beet inbred lines clustered in an intermediate position between standard table beet germplasm and breeding lines of sugar beet, probably due to their origin from an introgression program designed to incorporate sugar beet genes (Wang and Goldman 1999). Linkage drag from introgressed genes

from sugar beet to table beet during the 1950s and 1960s might have caused a larger genetic distance between inbred lines derived from sugar beet and standard table beet (Goldman 1996).Genetic diversity study of 14 individual sugar beet plants within each parent analyzed using 18 microsatellites (SSR) markers revealed 75.5% of total phenotypic variation explained by the first two principal components (43 and 32.6% PV) for agro-morphological traits that could distinguish salinity-tolerant and drought-tolerant parents. Molecular analysis through SSR revealed 104 total alleles with 5.7 average number of alleles per primer pair and an average polymorphism information content (PIC) of 0.64 with the highest PIC belonging to ESTSSR *FDSB502* (Abbasi et al. 2014). A total of 243 amplicons were obtained which were further grouped into 88 alleles with an average of 17.36 amplicons/primer with distinct molecular weight ranging from 124 to 1222 bp and 4–10 alleles/SSR locus with moderate to high PIC ranging from 0.625 to 0.851 (Srivastava et al. 2017). Efforts were made to understand the genetic diversity of sugar beet pollinators. The total of alleles obtained were 129 alleles with an average of 3.2 alleles per SSR marker. The observed heterozygosity ranged from 0.00 to 0.87 (mean = 0.30). Expected heterozygosity and Shannon's information index and expected heterozygosity were highest for markers SB15s and FDSB502s and lowest for marker BQ590934.The same markers with PIC values of 0.70 and 0.69, respectively, were found most informative and were able to distinguish between genotypes. Maximum private alleles were identified in pollinator EL0204; two private alleles in C51pollinator; and one allele in NS1pollinators, C93035, and FC221. Intrapopulation variability (variation within the population) govern 77.34% of the total genetic variation resulting from molecular variance analysis (Taški-Ajduković et al. 2017). Extensively shared, non-unique genetic variation among different species of beets was attributed to the distribution of genetic variation in sugar beet. The phenomenon of apomorphy deciphered shared lineages within each species while differentiation within strong crop types was supported by principal components analysis. Sharing common ancestor and gene flow among the crop types through time indicated sharing of genome variation likely for important phenotypic characters that concealed a good demarcation of different species of beets. Table beet revealed greater genetic differentiation within the crop types. Table beet groups were well differentiated in comparison to the sugar beet species (Galewski and McGrath 2020).

5.2.2 OMICS Approaches in Sugar Beet

OMICS techniques encompass genomics, transcriptomics, proteomics, and metabolomics that functions to realize the molecular and biochemical structure and pathways of a plant genotype and effectively improve the crop for its overall usability (Fig. 5.1). In recent times, genomics evidence based on Next-Generation Sequencing (NGS), gene silencing, gene-editing systems, and over-expression methods have given a huge repository of genetic output to aid in deciphering both biotic and abiotic tolerance mechanisms in plants (Saad et al. 2013; Shan et al. 2013; Yin et al. 2014). An OMICS-driven unearthing of novel genes, proteins, and

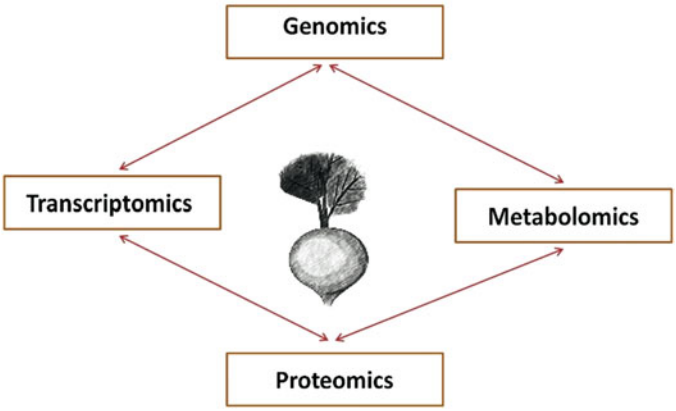

Fig. 5.1 Outline of OMICS studies in sugar beet

metabolites in sugar beet has aid in understanding the complex mechanisms underlying phenomena such as apomixis and tolerance to biotic and abiotic stresses. The knowledge harnessed is valuable for improving the tolerance of *B. vulgaris* to biotic and abiotic stresses and yield improvement of sugar beet for energy and food production (Zhang et al. 2016).

5.2.2.1 Genome Mapping for Useful Traits in *B. vulgaris*

Beta vulgaris is a diploid plant of $2n = 18$ chromosome number with an estimated genome size of 714–758 megabases. Efforts to genome map the chromosomes of sugar beet have been carried out (Laurent et al. 2007). The first reported linkage map in *B. vulgaris* was on the inheritance of the morphological markers for hypocotyl color (genes R and Y) and bolting behavior (B, annual vs. biennial), widely known as R–Y–B linkage association (Keller 1936; Owen and Ryser 1942), which is now mapped on Chromosome 2 of the Butterfass chromosome series. The crop shares an ancient genome triplication with other eudicot plants. The phylogenetic study revealed losses of gene family according to their lineages and further expansions and differentiation of Caryophyllales prior to the split of asterids and rosids (Dohm et al. 2014). The first linkage map with wide crosses in *B. vulgaris* between sugar beet and table beet mapped 23 new SSR makers (McGrath et al. 2007).

Leaf spot is known as one of the most widespread and devastating foliar diseases of sugar beet. It destroys the plant foliar structure and function and causes necrotic lesions (Holtschulte 2000). Further sugar recovery and yield of the sugar beet are greatly decreased by the disease. Four QTLs *viz*., qcr1, qcr4 qcr2, and qcr3 on chromosomes 3, 9, 4, and 6 underlying resistance to *Cercospora leaf spot* (CLS) was revealed through Composite Interval Mapping of RILs developed from a cross between a resistant line ("NK-310 mm-O") and a susceptible line ("NK-184 mm-O") (Taguchi et al. 2011). Another serious disease in sugar beet is Rhizomania, caused by Beet necrotic yellow vein virus (BNYVV) that lessens the sugar content and yield of beet. *Rz4,* a major QTL conferring resistance to BNYVV that explained

78% of the observed phenotypic variation was deciphered. RAPD marker Rz1 was mapped close to Rz4 in chromosome 3 which is also the previously identified mapped location for BNYVV resistance genes *Rz1*, *Rz2*, and *Rz3* (Lewellen et al. 1987; Paul et al. 1993; Scholten et al. 1996; Grimmer et al. 2007).

Association mapping is budding as a novel molecular tool in plant genomics (Myles et al. 2009) and is currently used in the molecular analysis of populations from applied breeding programs (Reif et al. 2010; Würschum et al. 2011). The technique helps in identifying major and minor QTLs that confers the traits of interest. It will be pivotal to acknowledge the existence of inherent population structure in the plant populations that may pose a potential problem while running the analysis. Presence of any non-functional correlations between the population structure and the trait will be projected as QTL (Zhao et al. 2007). Association mapping is based on the concept of linkage disequilibrium (LD), a non-random association of alleles of different loci between the QTL, and examined molecular markers associated with the trait. Linkage disequilibrium is an accurate indicator of the population genetic forces that structure a genome. Association mapping for traits is anticipated to have higher mapping resolution in contrast to classical linkage mapping as it excavates all the historical recombination events in the mapping population. The strength and extent of LD is dependent on the structure of the population, therefore, is population-specific and influenced by many genetic factors (Flint-Garcia et al. 2003). Moreover, the LD strength is highly variable across the genome. The extent of association between the QTL and marker determines the power and precision in detecting QTL conferring for the trait. The association is measured by r^2 value which establishes the marker and QTL correlation. Lower r^2 values will only allow the discernment of QTL with large effects (major QTLs) whereas high r^2 values are requisite to detect medium and small size QTL. LD is expected to be higher in the plant breeding population in contrast to the natural populations on account of the shorter history of the germplasms and selection of favorable genotypes over time. Trait associated markers with explained genotypic variance and QTL in *B. vulgaris* for important characters viz., nitrogen content, sodium content, potassium content, the proportion of impurities, sugar content, white sugar content, beet yield, root yield, sugar yield, and white sugar yield were studied (Weber et al. 1999; Schneider et al. 2002; Reif et al. 2010; Stich et al. 2008a, b, Würschum et al. 2011).

5.2.2.2 Next-Generation Sequencing and Other Sequencing Applications in Sugar Beet

The NGS technology has provided a platform for locating molecular tags of trait phenotype accurately. It has effectively aided forward genetics in the discerning causative variation of a phenotype easy and precise. NGS technologies have made molecular study easier offering high-throughput sequencing data as compared to Sanger sequencing with a 99% read accuracy. NGS also reduces the cost incurred in sequencing in comparison to sangers making the genomic study more affordable. The whole-genome sequencing of sugar beet was completed and reported by Dohm et al. (2014). Based on transcription data and sequence homology annotation of the

genome, a total of 27,421 protein-coding genes were envisaged (Dohm et al. 2014). Reports on the complete sequence of mitochondrial genome ((Kubo et al. 2000) and chloroplast genome (Li et al. 2014; Stadermann et al. 2015) of sugar beet (*Beta vulgaris* L.) are available. The genome size of Mt is about 368,799 bp encompassing 29 proteins, 25 Trna, and 5 Rrna, also found in *Arabidopsis thaliana*. A novel $tRNA^{cys}$gene (trnc2-GCA) was deciphered that actually transcribes into mature Trna unlike the native $tRNA^{cys}$ gene (trnc1-GCA) that functions as a pseudogene (Kubo et al. 2000). SMRT sequencing of the sugar beet chloroplast genome revealed 79 genes encoding for an mRNA (i.e., proteins), 7 encode rRNA, and 28 encoding tRNAs in a total of 114 individual genes. Nine genes were located within the inverted repeat (IR) regions that conferred 5 mRNAs, 3 tRNAs, and 1 rRNA (Stadermann et al. 2015).

5.2.2.3 Transcriptomics and Proteomics Study in Sugar Beet

Transcriptomics and proteomics study revealed differentially expressed proteins involved in several processes and various biological pathways (Li et al. 2009; Zhu et al. 2009). A study on salt stress through proteomics revealed the involvement of cystatin (Wang et al. 2012), glyoxalase I (Wu et al. 2013), CCoAOMT, and thioredoxin peroxidase (Zhang et al. 2016) in salt resistance mechanism of M14, a high salt tolerance monosomic addition line of sugar beet. Proteins regulating drought stress through oxidative stress, signal transduction, and redox regulation were identified (Hajheidari et al. 2005). Genetic and non-genetic SSR has been deciphered in sugar beet through transcriptomics that has a good amount of polymorphism and demarcates clearly between genotypes. Forty of such primer-pairs were revealed with high polymorphic distinguished diversity present among eight diverse *B. vulgaris* genotypes. The transcriptomic data and identified SSR markers will make useful public domain genomic resources for understanding functional elements of the genome of sugar beet. It will further facilitate RNA-sequencing-based expression research, enable the discovery of novel genes, and propel selective breeding and genetic research in sugar beet (Fugate et al. 2014).

5.2.2.4 Genetic Manipulation Through Transgenics in *Beta vulgaris*

Non-crossability among different species has driven the wheel of transgenics where a foreign gene of interest is transported through a medium like bacterial pathogen *Agrobacterium tumefaciens* to the genome targeted for incorporation and expression of the trait in the host plant. Stable integration and safe transformation of the transferred DNA are essential in the plant nucleus for the successful expression of the trait. Alternatively, transient transformation may occur wherein the foreign DNA does not integrate but transiently remain in the nucleus and is transcribed to produce desirable gene products. *Agrobacterium tumefaciens* is an essential core tool of plant biotechnology and numerous interactions with plants studied and elucidated (Hwang et al. 2017). In sugar beet, transformation is achieved for some traits and illustrated by different studies for *A. tumefaciens* transformation (D'halluin et al. 1992; Elliott et al. 1996; Krens et al. 1996) and peg-mediated guard cell protoplast transformation (Hall et al. 1995). Progress through transformation techniques using *A. tumefaciens*-

mediated transformation has found success in sugar beet (Fry et al. 1991; Konwar 1994). Stable transformation is shown to be dependent on different factors including genotype (von Wordragen and Dons 1992) acetosyringone or phenolic compounds present in the plant tissue (Jacq et al. 1992). Expression of the introduced gene is determined by the transgene copy number that further enables their positive or negative association (Hobbs et al. 1993; Linn et al. 1990; Matzke and Matzke 1993).

Sugar beet is moderately salt tolerant. Lack of efficient gene transformation has limited the breeding of varieties in saline conditions for salt tolerance. Positive transformation of *GUS* gene in sugar beet is reported and has shown effective expression through *Agrobacterium*-mediated transformation (Lindsey and Gallois 1990; Krens et al. 1996; Hisano et al. 2004). Further, improved salt tolerance was observed in transgenic sugar beets expressing *AtNHX1* gene (Yang et al. 2005). The constitutive expression of *AtNHX3 gene in* sugar beet provided salt tolerance and improved sugar synthesis in transgenic plants.

Efforts have been put forth to develop glyphosate resistance sugar beet through genetic transformation. The chemical name of glyphosate is N-(phosphonomethyl)glycine, an active ingredient for the herbicide Roundup. Two transformants (HIAB1: 1 and HIAB2: 2) introduced with *CP4 EPSPS* gene showed high tolerance to Roundup that did not manifest any phytotoxic or morphological effects after treatment with the maximum dose of glyphosate (Mannerlöf et al. 1997). Reports on the transformation of *glyphosate oxidoreductase* (GOX) for tolerance to herbicide were also given (Steen and Pedersen 1993; Steen and Pedersen 1995a, b; Brants et al. 1995; Tenning et al. 1995; Mannerlöf et al. 1997).

Heavy metal pollution poses a serious environmental threat globally. The phytoremediation process is viewed as an ideal curbing mechanism to ameliorate heavy metal pollution given its high efficiency and absence of secondary environmental pollution. Phytoremediation should have higher proliferation rates in vivo, high biomass, and faster growth. Three transgenic sugar beet (*Beta vulgaris* L.) lines (s2, s4, and s5) introduced with novel *Streptococcus thermophilus* γ-glutamyl cysteine synthetase-glutathione synthetase (StGCS-GS) that synthesizes *glutathione* (GSH) gives enhanced tolerance to different concentrations of zinc, cadmium, and copper. These transformed lines have increased root length, biomass, and relative growth in comparison to wild-type plants (Liu et al. 2015).

5.2.3 Plant Tissue Culture Techniques in Sugar Beet

Plant tissue culture is an indispensable component of plant biotechnology. Tissue culture is becoming an alternative in vitro means to vegetative propagation of plants. As in vitro plants are propagated in sterile conditions, it is essentially free from bacterial and fungal diseases and can be reproduced at a faster rate in cultures. The individual plants produced through tissue culture are highly uniform within a clone population that allows commercial production of clonal cultivars (Krishna and Singh 2013). The presence of genetic variation however is seen in isolated protoplasts, undifferentiated cells, calli, tissues, and morphological characters of in vitro-raised

plants (Bairu et al. 2011; Currais et al. 2013). Apart from being a useful biotechnological tool, plant tissue culture approaches have gained industrial importance in recent years for plant propagation, plant improvement, production of secondary metabolites, and disease elimination (Hussain et al. 2012). Further, in vitro cultures can help understand the physiological mechanism of injury caused by environmental stress (Dix et al. 1983; Van Swaaij et al. 1986).

5.2.3.1 Sugar Beet Micropropagation

Micropropagation can be obtained within a short period of time in a confined space (Krishna et al. 2008). In sugar beet, limited in vitro culture techniques are available despite the importance of the crop which is unfortunate. Shoot cultures maintained in vitro (Hussey and Hepher 1978), but regenerated from callus (Saunders and Daub 1984; Tetu et al. 1987; Freytag et al. 1988; Ritchie et al. 1989) tends to be inconsistent, occurring at low frequency and strongly cultivar dependent that limits its usability either for in vitro selection or clonal propagation. Success however has been reported in some cultivars where it was possible to obtain regenerated lines from hormone-treated autonomous cell cultures (Van Geyt and Jacobs 1985). Most of the undifferentiated culture regeneration is seen from adventitious shoot initiation and seldom from somatic embryos (Freytag et al. 1988). Protoplast culture and plant regeneration have also been seen rarely as the process is highly genotype dependent. The first successful culture has been reported in diploid beet (Krens et al. 1990). Direct organogenesis has been reported as the most effective way to produce true-type regenerants in sugar beet (Bekheet et al. 2007). Micropropagation of sugar beet has been carried successfully with a good percentage of regenerants (Mikami et al. 1985; Goska and Szota 1992; Sullivan et al. 1993; Grieve et al. 1997; Bekheet et al. 2007; Morsi et al. 2019).

5.2.3.2 Somaclonal Variation in Sugar Beet

Somaclonal variation, a term coined by Larkin and Scowkraft in 1981, denotes plant variants derived from any form of cell or tissue culture. Genetic variability is obtained quicker through tissue culture without any sophisticated technology. An added advantage is that the screening for desirable traits can be obtained in lesser time and space. Somaclones have ample applications in genetic improvements and recovery of novel variants with enhanced characteristics. Suitable in vitro selection might further aid the recovery of novel variants (Jain 2001; Lestari 2006). Somaclonal variants in sugar beet are most commonly seen through indirect regeneration from callus derived from petiole, leaf lamina, or hypocotyl explants (Saunders and Doley 1986; Brears et al. 1989; Jacq et al. 1992). There are reports also on protoplasts regeneration (Steen et al. 1986; Lenzner et al. 1995; Jazdzewska et al. 2000) and direct regenerants from explants (Harms et al. 1983; Dikalova et al. 1993; Zhong et al. 1993). Somaclonal variation in sugar beet for root rot resistance *F. oxysporum var. orthoceras* was reported with a frequency of shoot depending on the genotype of 1.0–12.5% and multiple shoot formations on the explants (Urazaliev et al. 2013). Rearrangements of mitochondrial DNA induced by cell suspension, culture, and regeneration were also reported.

5.3 Future Prospects

The OMICS information can further be applied to improve sugar beet stress tolerance and enhance yield and energy output (bioethanol) with an accumulation of useful metabolites, for example, betalains and glycine betaines.

5.4 Conclusion

Biotechnological intervention and the genomic study provide in-depth information on the whole genome of sugar beet and the structure and functions of genes underlying useful agronomical traits. OMICS study helps understand the molecular workings and biosynthetic pathways involved in response to tolerance to biotic and abiotic stress in sugar beet. Genomic information helps facilitate and engineer important metabolites. Apomixis and stress tolerance mechanism has been studied to great extend in unique sugar beet germplasm M14 through proteomics and transcriptomics to identify the genes and proteins underlying this traits. Transformation study has been successful in constitutive expression of *AtNHX3* gene for salt tolerance, CP4 EPSPS gene for tolerance to Roundup, and novel StGCS-GS that synthesizes GSH for phytoremediation. However, poor transformation success, expression of the gene due to low regeneration, genotype dependency, and practical applications of in vitro culture technologies in sugar beet being still at nascent stage limits sugar beet research and improvement. This stipulates ample scope for the application of sugar beet, a high economic value crop in food, bioenergy, and pharmaceutical industries through progressive genetic study and effective biotechnological protocol.

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