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REVIEW ARTICLE

Applications and challenges of harnessing genome editing in oilseed crops

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Abstract

The ever-increasing demand for vegetable oil requirement has necessitated increasing the production of oilseeds crops. Productivity is compromised in these crops due to biotic and abiotic stresses. Albeit the substantial progress made in this direction through conventional breeding approaches, breeding for certain traits like stress tolerance is limited by the non-availability of genetic variability for these traits in primary germplasm, the time required for selection and the realization of a suitable genetic assemblage from the segregating populations, etc. This situation has necessitated adopting alternate approaches to achieve the objectives. Genome editing technology offers a solution to modify the genome precisely with least genetic perturbation in the least possible time frame and it has been adopted in several crops including oilseeds. Genome editing technology depends on the genetic transformation step for introducing the machinery required for altering the genome. However, the recalcitrance for in vitro manipulations observed in oilseed crops such as castor, sesame, jatropha, etc. has set a limit for exploiting this powerful technology in oilseed crops. In this review, we have summarized the genome editing work carried out in oilseed crops and also discuss the possibility of employing such technologies along with the promising gene targets that could be manipulated to generate required variants in oilseed crops.

Keywords Abiotic stress · Biotic stress · CRISPR/Cas9 genome editing · Oilseed crops · Target traits

Abbreviations

ZFNs	Zinc finger nucleases
TALENs	Transcription activator like effector nucleases
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
Cas	CRISPR associated system
PAM	Protospacer adjacent motif
NHEJ	Non-homologous end-joining
HDR	Homology-directed repair
SDN	Site directed nuclease
PTC	Plant tissue culture
RNPs	Ribonucleoproteins
MIR	miRNA genes

Introduction

More than twenty oilseed crops are cultivated across the world for human consumption as well as industrial uses. The major oleiferous crops include soybean [*Glycine max* (L.) Merr.], rapeseed and mustard (*Brassica spp.*), peanut (*Arachis hypogaea L.*), palm oil (*Elaeis guineensis* Jack.), sunflower (*Helianthus annuus* L.), coconut (*Cocos nucifera* L.), cottonseed (*Gossypium spp.*), niger [*Guizotia abyssinica* (L. f.) Cass.], sesame (*Sesamum indicum* L.), safflower (*Carthamus tinctorius* L.), camelina (*Camelina sativa* L.), castor bean (*Ricinus communis* L.), physic nut (*Jatropha curcas* L.), and linseed (*Linum usitatissimum* L.). In addition to the conventional oils, rice bran oil and corn kernel oil are important non-conventional sources of edible oils. Because of their nutritive values, maize kernal oil and rice bran oil are growing in popularity. Edible-oils and oilseed meals are rich in essential nutrients besides contributing up to about 40% of the calories in human diet. Growing population, changing dietary patterns, and improved living standards are increasing the demand for vegetable oil production. On the other hand, global warming, finite agricultural lands, abiotic and biotic stress factors are posing a

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threat for keeping pace with this demand. Hence, improving the quantity and quality of oilseeds is one of the key objectives for modern-day researchers to meet the global demand. Elite oilseed crop varieties are being created by either traditional, or mutational breeding methods, or through genomics assisted breeding approaches. However, these methods are tedious, time consuming and often coupled with undesirable trait combinations. Also, the conventional breeding approaches are limited by the available genetic variability for the traits of interest. Hence, there is a constant search for simple and precise methods that could alter the genome in a directed way with least or no perturbation to the rest of the genome. Site directed nucleases such as meganucleases, ZFNs (zinc finger nucleases) and TALENs (transcription activator like effector nucleases) have been used for targeted editing of the genomic content. However, the complexity of functionality as well as the fastidious requirements of these SDNs limited their widespread deployment in crops to create new directed genome variability. In the light of this, discovery of **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats (CRISPR)/**C**RISPR **a**ssociated **s**ystem (CRISPR/Cas) a microbial adaptive immune system, has offered a new tool for trait modifications (Jiang et al. 2013). The CRISPR/Cas system has many advantages over other SDN mediated modifications due to its diversity, robustness, and flexibility (Pickar-Oliver and Gersbach 2019; Walton et al. 2020). The ease with which this system could be adopted for precise genome editing across a wide variety of organisms including plants has opened up new vistas in the world of modern science (Zhang et al. 2020b). CRISPR/Cas is an RNA-guided endonuclease that specifically targets and cleaves the DNA at specific sites recognized through the protospacer adjacent motif (PAM) sequence near the edited site. Among several *Cas* genes reported (like *Cas9, Cas12, Cas13*, etc.), *Cas9* obtained from *Streptococcus pyogenes* (*SpCas*9) has been codon optimized for various crops and engineered with various PAM specificities and used in most of the studies. In this review, we discuss the applicability of the CRISPR/SpCas9 system in oilseed crops.

The double stranded breaks in DNA at the target site created by SDNs including Cas9 protein are repaired by two mechanisms, the error-prone, non-homologous end-joining (NHEJ) and homology-directed repair (HDR). The NHEJ leads to random nucleotide base insertions or deletions (InDels) at the cleavage site which in turn leads to the formation of mutated and more often non-functional or impaired protein. Based on the intended mode of editing, the genome editing has been grouped mainly into three groups: site directed nuclease 1 (SDN1), SDN2 and SDN3. SDN1 involves creation of InDels at the targeted sites via the NHEJ route, while SDN2 also creates specific changes at the target sites via the HDR mechanism. On the other hand, SDN3 introduces specific genes at the targeted site using HDR mechanism (Ahmad et al. 2021a). Among the three types, SDN1 has been exploited the most in plants owing to the increased frequency of NHEJ mediated repair compared to the HDR mediated one (Molla et al. 2021). Even though, developing edited lines in crops involves a genetic transformation step to introduce the editing machinery (CRISPR/Cas9) into the plant, as the site of action of this machinery is different compared to the site of insertion, in the subsequent generations the transgene free but genome edited lines could be realized though Mendelian segregation. Thus, the resultant edited lines will be without the introduced genes (transgenes), owing to which many countries including India, have categorized SDN1 and SDN2 derived genotypes as non-transgenic and thus exempted from going through cumbersome regulatory procedures before their release for commercial cultivation.

CRISPR/Cas9 has revolutionized the pace of plant biology research (Manghwar et al. 2019) and made precise plant genome editing a reality (Zhu et al. 2020). Thus, it has become an attractive and competitive field within a very short time and has been used to manipulate agronomic traits in many crops. Many versions of CRISPR/Cas9 with subtle but effective modifications have opened up novel ways of genome manipulations using base editors and prime editors (Molla et al. 2021; Das et al. 2022) and the field is evolving continuously bringing many more crops and traits under its fold of benefits. However, there are specific requirements to adopt genome editing approach in crops (Son and Park 2022) and they mainly include, (1) genome sequence information (2) functionally characterized target gene(s) (3) DNA transfer method (either biological such as Agrobacterium, or physical such as biolistic, electroporation, or chemical such as PEG, nanoparticles etc.) (4) suitable expression systems (either generic, modular or specific) and (5) a suitable regeneration system, preferably a genotype independent one to obtain edited plants from the transformed cells. Strategies are being developed to address these requirements or challenges (Son and Park 2022). Readers are referred to comprehensive and informative reviews on historical perspective of CRISPR/Cas system (Lander 2016), mechanism and modified versions of CRISPR/Cas (Das et al. 2022), applications in field crops (Zhu et al. 2020), horticultural crops (Kaur et al. 2021) and in basic research (Ledford 2021). Here our aim is to summarize the recent developments in the application of CRISPR/Cas9 in oilseed crops besides providing a perspective on future applications.

Present scenario of CRISPR/Cas applied to oilseed crops

CRISPR/Cas9 has been effectively employed in oilseed rape, soybean, camelina, peanut, cottonseed, and flax (Subedi et al. 2020b; He et al. 2021) to realize promising lines. A

summary of the genome editing work reported in oilseeds has been provided in Table 1. A perusal of the information in Table 1 indicates that most of the target genes chosen in these studies had been functionally validated through different approaches including gene silencing studies, either in the same crop or in heterologous or model crops, map-based cloning, transcriptome analysis, pathway analysis, etc. So far, only SDN1 type of genome editing has been adopted for trait manipulation. In oilseed crops, *CaMV 35S* has been used in most of the studies to drive the expression of cas9 while the sgRNA has been driven by promoters of small nuclear RNA (snRNA) genes such as *U3/U6* that are typically transcribed by class III RNA polymerases. In general, in other systems also, it has been opined those promoters with strong and constitutive expression patterns are employed to achieve balanced and high-level expression of Cas and gRNA. However, it has been demonstrated that crop-specific ubiquitin promoter (Feng et al. 2018) and meiotic cell-specific promoter *YAO* (Wolter et al. 2018) result in higher gene edits compared to *CaMV 35S*. In oilseeds also, some tissue specific promoters like *YAO* (Wang et al. 2022c), *EC1.2* (Lee et al. 2021), *AtEF1α* (Lyzenga et al. 2019) and *pM4* (Zhang et al. 2022b) have been used for driving the expression of cas9. In almost all the cases, Agrobacterium mediated transformation has been used to introduce the GE machinery and usually single to multiple sgRNA cassettes have been introduced. Homozygosity for the edited sites have been reported either in T_0 and T_1 generation indicating that biallelic edits have been achieved in T_0 generation. In a few cases, transgene free edited lines have been reported whereas in most of the cases the edited lines are reported to still carry the transgenes. Genome-editing efficiency ranging from 0.1 to 100%, depending on crops and their genotypes, has been reported. In *Brassica* species, the efficiency is reported to be 10–100% while it varied from 47.6 to 100% in cotton and 37–88% in soybean (Table 1).

In soybean, the main traits targeted have been biotic stress tolerance (insect, cyst, virus), agronomic (flowering time, increased yield, male sterility) and qualitative (enhanced fragrance). In rapeseed, genome editing efforts have targeted quality (glucosinolates, altered starch structure), and agronomic traits (self-incompatibility, seed number in siliques, plant architecture and male sterility). In Camelina, a model oilseed plant belonging to *Brassicaceae* family, and a plant for biodiesel production, GE for quality traits (reduced PUFA, altered fatty acid composition) has been reported. Reducing the allergen and increasing the nodule number are the traits targeted through genome editing in peanut. In cotton, altering the plant architecture has been achieved through genome editing. Many of the studies have reported testing of the feasibility of genome editing in respective crops. Allotetraploid oilseed rape and soybean have been successfully edited for increasing oil quality/quantity and biotic/ abiotic stress tolerance (Du et al. 2016; Xu et al. 2019; Huang et al. 2020).

Based on the literature available across all the crops and considering the specific research objectives of oilseed crops (as enumerated in Table 2), possible target traits and the genes that could be edited in oilseed crops are illustrated in Fig. 1 and they are discussed briefly here.

Increasing the seed oil content and altering the oil quality

As oilseeds are predominantly cultivated for seed oil purpose, the main breeding objective of these crops is increasing the oil production per unit area. This can mainly be achieved by increasing the seed yield as well as the oil content. However, the synthesis and accumulation of oils is controlled by complex gene networks and the exact interplay among these networks is still unclear and needs to be further understood. Nevertheless, there is significant information regarding the genetic control of seed oil formation and the genes involved in this process (Kumar et al. 2020; Yang et al. 2022), and these genes are being functionally validated in the model plant Arabidopsis as well as other oilseed crops (Subedi et al. 2020b). Oils are stored basically as triacylglycerol (TAG) and key genes involved in oil accumulation (such as the enzymatic Kennedy pathway genes, Fatty Acid Synthases, oleosins etc., and the transcription factors like *WRKY, LEC 1, LEC 2, FUS 3, GL 2* etc.) have been identified and functionally validated using different approaches including developing transgenic lines that overexpress or silence these genes. Cotton seed oil content was enhanced by 7.3% and 16.7% when genes such as *PEPC1* and *PEPEC2A,* known to play negative role in lipid biosynthesis, were silenced (Xu et al. 2016b; Zhao et al. 2018). These genes could be targets of genome editing when oil content is to be enhanced. There are reports of InDels in TAG biosynthesis genes that have led to increased oil content, e.g., one amino acid change in *DGAT-1* in maize led to increased oil content (Zheng et al. 2008), and therefore such genes could be targeted for editing. Interestingly, there are many transcription factors such as *MYB* genes (*MYB78, MYB89, MYB118, MYB123*), *WRKY6*, *AP 2, TT 8*, etc. which act as negative regulators of oil accumulation (Kumar et al. 2020) and these genes are the possible targets for genome editing to manipulate the seed oil content trait (Zafar et al. 2019).

Oil quality, which is primarily decided by the fatty acid profile and the antioxidants present in the oil are also the targets of specific breeding programmes (Subedi et al. 2020a). There are reports of developing such lines in oilseed crops through different approaches including genome editing. Metabolic engineering of genes from other plant sources have also been used to alter the oil content and fatty acid profile in oilseed crops such as brassica, safflower, cotton, peanut

Table 1 List of some important recently targeted traits/genes in various oilseed crops through CRISPR/Cas9*

Crop	Target gene	The role of the gene	How was the gene selected	Trait and phenotype achieved	Transformation approach and type of sgRNA	Promoter Expressing		Off target effects	Homozygosis reached in (generation)	Number of edited lines of Percentage of success	Whether transgene clean edited plants, if so how achieved	References (for the gene source and the report)
						Cas9	gRNA					
Glycine max L	UDP-glycosyl transferase	Flavonoid biosynthesis	Insect-resistance QTLs	Insect (*Helicoverpa armigera* and *Spodoptera litura*) resistance	AM & single gRNA	*GmUbi3*	*GmU6*	ND	T1	5 edited lines obtained	TC & by segregation	Zhu et al. (2006) and Zhang et al. (2022a)
	Betaine aldehyde dehydrogenase 2	Aroma synthesis	Heterologous system followed by gene silencing studies	Enhanced fragrance in seeds for value addition	AM & single gRNA	*35S*	*GmU6*	NM	T1 and T2	30 edited lines obtained	NM	Arikit et al. (2011) and Qian et al. (2022)
	Night light-inducible and clock-regulated 2 (*LNK 2*) & four homologs	Flowering-time control	Heterologous system and genome wide transcriptome analysis	Advanced flowering time and pod production time under long-day (LD) conditions	AM & Multiplexing	*35S*	*U3b, U3d, U6-1*	NM	T1	6.5%	TC & by segregation	Rugnone et al. (2013) and Li et al. (2021b)
	Aborted microspores 1 (*AMS 1*)	Tapetal and microspore development	Heterologous system and T-DNA mutation	Male sterility (Sporophytic)	AM & single gRNA	*35S*	*ZmU3*	NM	T1	25%	T	Sorensen et al. (2003) and Chen et al. (2021a)
	Time of Flowering 16 (*Tof16*)	Flowering time and yield control	Heterologous system and positional cloning of QTL	Improved grain yield under short-day (SD) conditions	AM	NM	NM	NM	NM	NM	NM	Dong et al. (2021)

Table 1 (continued)

Crop	Target gene	The role of the gene	How was the gene selected	Trait and phenotype achieved	Transformation approach and type of sgRNA	Promoter Expressing		Off target effects	Homozygosis reached in (generation)	Number of edited lines of Percentage of success	Whether transgene clean edited plants, if so how achieved	References (for the gene source and the report)
						Cas9	gRNA					
	GmJAG1	Zinc finger protein	Map-based cloning of QTL	Increased yield (9% than wild type)	Transformation approach NM & 2 gRNAs	NM	NM	GmJAG2 also edited	T1	NM	TC & by segregation	Fang et al. (2013) and Cai et al. (2021)
	γ-SNAP	Vesicle trafficking	Fine mapping of QTL	Soybean cyst nematode (SCN) resistance	AM & 2 gRNAs	*35S*	*MtU6*	NM	NM	NM	NM	Kim et al. (2011) and Butler et al. (2021)
	GmF3H1, GmF3H2 and *GmFNSII-1*	Isoflavonoid metabolic pathway enzymes	Isoflavonoid metabolic pathway	Soybean mosaic virus (SMV) resistance	AM & Multiplexing	*GmUbi3*	*GmU6, GmU3*	NM	T2	6.25% to 12.50%	TC & by segregation	Zhang et al. (2020c)
	Pod dehiscence 1	Dirigent (DIR) family protein	QTL mapping	Pod shattering tolerance	AM & Multiplexing	*pM4*	*GmU6*	NM	T1	NM	TC & by segregation	Zhang et al. (2022b)
Brassica napus L	*Bna*A06.*GTR2*	Glucosinolate transporters	Heterologous system and genome wide transcriptome analysis	Low seed glucosinolate	AM & Multiplexing	*2X35S*	*U6-29p, U6-26p*	ND	T0	Three edited lines obtained	T	He et al. (2022)
	*Bn*S6-*SMI2*	Gene silencing	Classical genetics	Self-incompatible phenotype	Transformation approach NM & 3 gRNAs	NM	NM	NM	T1	Ten edited lines obtained	TC & by segregation	Yasuda et al. (2016) and Dou et al. (2021)
	BnaMS5	Uncharacterized protein	Spontaneous mutant	Male-sterility (Sporophytic)	AM & 2 gRNAs	*2X35S*	*AtU6-26p*	NM	T0	Eight edited lines obtained	T	Xin et al. (2020)

Table 1 (continued)

Crop	Target gene	The role of the gene	How was the gene selected	Trait and phenotype achieved	Transformation approach and type of sgRNA	Promoter Expressing		Off target effects	Homozygosis reached in (generation)	Number of edited lines of Percentage of success	Whether transgene clean edited plants, if so how achieved	References (for the gene source and the report)
						Cas9	gRNA					
	BnaEOD3	Member of cytochrome P450/ CYP78A6	Heterologous system & Silencing studies	Increased number of seeds of silique (13.9% than wild type)	AM & Multiplexing	*2X35S*	*AtU3d, AtU3b, AtU6-1, and AtU6-29*	ND	T0	49.1%	T	Qi et al. (2017) and Khan et al. (2020)
	BnaA03.BP	knotted1-like homeobox gene	Heterologous system & transposon tagging	Semi-dwarf and compact architecture (Height reduced by 15.8%–16.9%)	AM & 2 gRNAs	NM	NM	NM	T0	79%	T	Lincoln et al. (1994) and Fan et al. (2021)
	BnaSBE	Starch branching enzymes	Pathway analysis	Altered starch structure (Starch branching frequency, higher starch-bound phosphate content, and altered pattern of amylopectin chain length distribution)	AM & Multiplexing	Embryo sac expressed *YAO*	*AtU3d, AtU3b, AtU6-1*, and *AtU6-29*	ND	T0	80.5%	T	Wang et al. (2022c)
Camelina sativa L	*Fatty acid desaturase 2*	Synthesizes linoleic acid from oleic acid	Heterologous system and T-DNA mutation	Enhanced monounsaturated fatty acids by 60%	AM & single gRNA	Egg-specific *EC1.2* and *35S*	*AtU6*	ND	T1	0.4 to 84.1%	T	Okuley et al. (1994) and Lee et al. (2021)

Table 1 (continued)

Crop	Target gene	The role of the gene	How was the gene selected	Trait and phenotype achieved	Transformation approach and type of sgRNA	Promoter Expressing		Off target effects	Homozygosis reached in (generation)	Number of edited lines of Percentage of success	Whether transgene clean edited plants, if so how achieved	References (for the gene source and the report)
						Cas9	gRNA					
	Cruciferin C	Seed storage protein	Heterologous system and proteomic, transcriptomic analysis	Altered fatty acid composition (Increased relative abundance of saturated fatty acids)	AM & two gRNA	*AtEF1α*	*AtU6-26p*	NM	T2	18%	T	Higashi et al. (2006) and Lyzenga et al. (2019)
Arachis hypogaea	*Ara h 2*	Allergen gene	Proteomics followed by gene silencing studies	Proof of concept	AM & Multiplexing	*35S*	*CmYLCV*	NM	NM	0.13% to 0.8%	T	Dodo et al. (2008) and Biswas et al. (2022)
	AhFatB	Acyl-Acyl Carrier Protein Thioesterase	Heterologous system and T-DNA mutation	Low palmitic and high oleic acid (oleic acid increased from 46.82% to 62.74% and linoleic acid decreased from 36.46% to 22.60%)	AM & Multiplexing	*CBh*	*AtU6*	NM	T3	1.63% to 2.86%	T	Bonaventure et al. (2003) and Tang et al. (2022)
	AhNFR5	Nod Factor Receptor (NFR) genes	Heterologous system & map-based cloning	Nodule formation (Proof of concept)	AM & Multiplexing	*2X35S*	*MtU6*	NM	NM	NM	T	Madsen et al. (2003) and Shu et al. (2020)
	AhFAD2	Fatty acid desaturases	Spontaneous mutant	Proof of concept	AM & two gRNA	*2X35S*	*MtU6*	NM	NM	21%	NM	Yuan et al. (2019)

Table 1 (continued)

Crop	Target gene	The role of the gene	How was the gene selected	Trait and phenotype achieved	Transformation approach and type of sgRNA	Promoter Expressing		Off target effects	Homozygosis reached in (generation)	Number of edited lines of Percentage of success	Whether transgene clean edited plants, if so how achieved	References (for the gene source and the report)
						Cas9	gRNA					
Gossypium hirsutum L	*GhPEBP*	Phosphatidyl ethanolamine-binding protein	RNAi silencing studies	Plant Architecture (Compact cotton plant architecture)	AM & Base-editing	*OsUb*	*GhU6.7*	ND	T0 and T1	64%	TC & by segregation	McGarry et al. (2016) and Wang et al. (2022b)
	MIR482	A negative post-transcriptional regulator of NLRs	Heterologous system & Bioinformatics	Resistance against *Verticillium dahlia*	AM & Multiplexing	*35S*	*GhU6.9*	ND	T0 and T1	0–100%	T	Li et al. (2012) and Zhu et al. (2022)
	DsRed2 and *GhCLA1*	Phenotype (red color embryo & albino) genes	Heterologous system and T-DNA mutation	Proof of concept	AM & Multiplexing	*35S*	*GhU6.9*	ND	T0 and T1	66.7–100%	T	Mandel et al. (1996) and Wang et al. (2018)
	Rec, Rep	NA	NA	To develop base lines of recombinase mediated gene stacking	AM & single gRNA	*35S*	*AtU6-26S*	NM	NM	NM	T	Aslam et al. (2022)
	GhCLA1 and *GhVP*	Cloroplastos alterados 1 and vacuolar H + -pyrophosphatase genes	NA	Proof of concept	AM & two gRNA	*2X35S*	*AtU6-26S*	ND	NM	47.6–81.8%	T	Chen et al. (2017)
	Gh14-3-3d	Regulatory proteins	Proteomics & RNAi studies	Fungal resistance against *Verticillium dahliae*	AM & single gRNA	*35S*	*AtU3*	ND	T1	More than 150 lines	TC & by segregation	Gao et al. (2013 and Zhang et al. (2018)

Table 1 (continued)

Crop	Target gene	The role of the gene	How was the gene selected	Trait and phenotype achieved	Transformation approach and type of sgRNA	Promoter Expressing		Off target effects	Homozygosis reached in (generation)	Number of edited lines of Percentage of success	Whether transgene clean edited plants, if so how achieved	References (for the gene source and the report)
						Cas9	gRNA					
Jatropha curcas	*Phytoene desaturase*	Photobleached phenotype	Heterologous & Gene silencing studies	Proof of concept	AM & two gRNA	*35S*	*AtU6*	NM	NM	30%	T	Cunningham and Gantt (1998) and Arockiasamy et al. (2021)
	JcCYP735A	Cytokinin metabolic gene	Pathway analysis	Altering cytokinin levels	AM & single gRNA	*35S*	*AtU3d*	NM	T1	NM	T	Cai et al. (2018)
Linum usitatissimum	*EPSPS*	Biosynthesis of aromatic amino acids	Pathway analysis	Herbicide Tolerance	AM & single gRNA	*MAS*	*AtU6*	ND	T1	2.4% to 3.7%	T	Sauer et al. (2016)

*All resulted genetic modifications belong to SDN-1, *AM* Agrobacterium mediated, *35S* CaMV 35S, *NM* Not mentioned, *ND* Not detected, *TC* Transgene-clean, *T* Transgenic

Table 2 List of genome sequenced oilseed crops, tissue culture methods and their important traits to be targeted by CRISPR/Cas

Sl No	Crop	NCBI ID	Traits that could be addressed immediately	Present status of transformation protocols
1	Soybean (*Glycine max*)	NCBI: txid90675	Beany flavour elimination, Kunitz trypsin inhibitor and lipoxygenase null lines, high oleic content, virus resistance, nematode resistance, herbicide tolerance, moisture stress (both less and high) tolerance, precocious germination, herbicide tolerance, increasing oil content	Genetic transformation methods are established (Xu et al. 2022)
2	Rapeseed (*Brassica napus*)	NCBI:txid3708	Glucosinolates, pod shattering, aphid resistance, orobanche resistance, architecture, seed and oil yield, resource and nutrient use efficiency, drought tolerance, herbicide tolerance	Genetic transformation methods are established (Cao Chu et al. 2020)
3	Peanut (*Arachis hypogaea*)	NCBI:txid3818	PSND and PBND viral resistance, stem rot resistance, tikka disease resistance, photosynthesis efficiency, water use efficiency, partitioning of the photosynthates, herbicide tolerance, biofortification, drought tolerance	Genetic transformation methods are established (Biswas et al. 2022)
4	Palm oil (*Elaeis guineensis*)	NCBI:txid51953	Oil yield, alteration in fatty acid composition, low temperature stress, tree architecture, basal stem rot resistance	Still problems in tissue culture method and selection of transgenic plants (Yarra et al. 2019)
5	Sunflower (*Helianthus annuus*)	NCBI:txid4232	sunflower necrosis disease, fungal disease resistance, self-incompatibility, drought tolerance, oil quality and quantity improvement, herbicide tolerance	Genotype dependent, Low levels of transformation and regeneration (Darqui et al. 2021)
6	Coconut (*Cocos nucifera*)	NCBI:txid13894	Yellowing disease resistance, cadang-cadang disease, alteration in fatty acid composition	Low levels of transformation and regeneration (Nguyen et al. 2015)
7	Cottonseed (*Gossypium hirsutum*)	NCBI:txid3635	Increasing seed oil content, gossypol removal, plant architecture	Genetic transformation methods established (Kalbande and Patil 2016; Qandeel-e-arsh et al. 2021)
8	Linseed (*Linum usitatissimum*)	NCBI:txid4006	Flax quality improvement, herbicide resistance, oil quality (low alpha-linolenic acid) & quantity improvement, herbicide tolerance, reducing anti-nutritional factors	Low levels of transformation (Beyaz et al. 2016)
9	Sesame (*Sesamum indicum*)	NCBI:txid4182	Shattering resistance, phyllody resistance, uniform maturity of capsules, water logging resistance, herbicide tolerance	Low efficiency of plant regeneration and genetic transformation (Teklu et al. 2022)
10	Camelina (*Camelina sativa*)	NCBI:txid90675	Seed yield, biofortification, herbicide tolerance, alteration in fatty acid composition	Low efficiency of plant regeneration and genetic transformation (Sitther et al. 2018)
11	Castor bean (*Ricinus communis*)	NCBI:txid3988	Plant architecture, determinate pattern, synchronous maturity of primary and secondary spikes, ricin and RCA bio-detoxification, non-shattering, herbicide tolerance	Recalcistrant to genetic transformation (Xiao et al. 2022)
12	Safflower (*Carthamus tinctorius*)	NCBI:txid4222	Oil quality and quantity improvement, plant architecture (increased basal branches) a greater number of capitula, more seeds per capitula, drought tolerance, seed size and number, herbicide tolerance	Genotype independent regeneration methods need to be developed (Nitnaware et al. 2021)
13	*Jatropha curcas*	NCBI:txid180498	Oil yield, non-shattering, architecture, bio-diesel compliant oil	Recalcistrant to genetic transformation (Al-Khayri et al. 2022)
14	*Niger* (*Guizotia abyssinica* Cass.)	Genome sequence is not available	Self incomtability, increasing the harvest index, plant architecture, shattering resistance	Recalcistrant to genetic transformation as well as regeneration. Only two reports available claiming genetic transformation to date (Murthy et al. 2003; Dangat and Patil 2011)

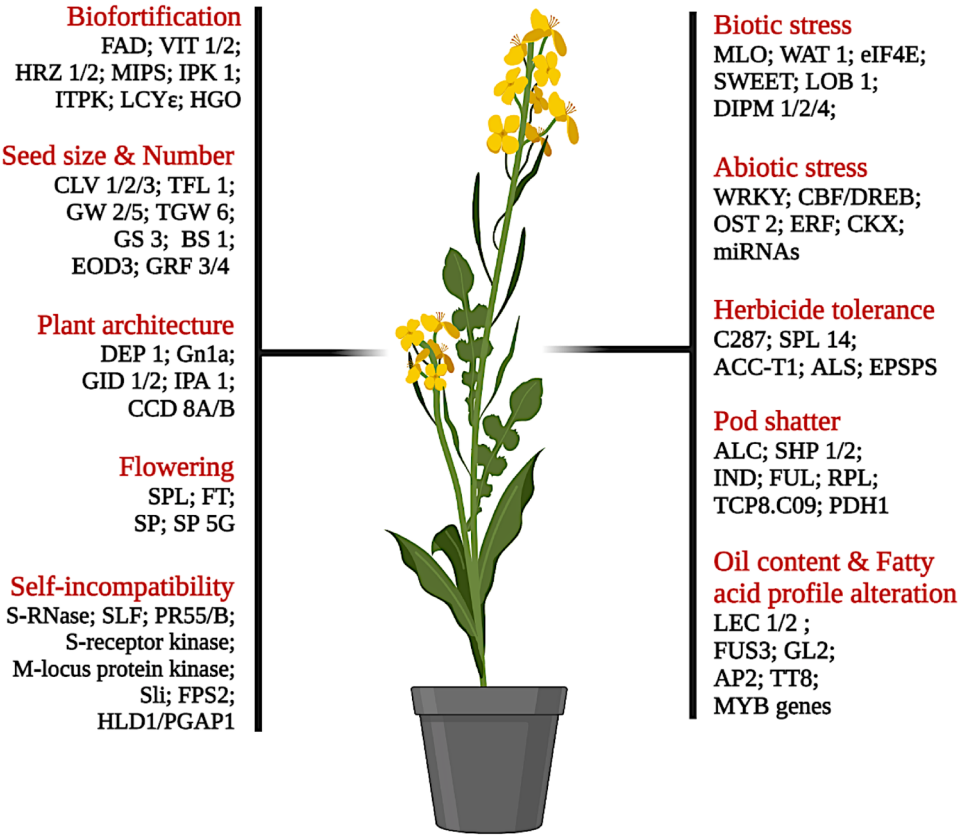

Fig. 1 Promising traits and probable gene targets for editing by CRISPR/Cas in oilseed crops. *MYB*, Transcription factors; *TT 8*, Transparent Testa 8; *AP 2*, Apetala 2; *GL 2*, Glabra; *FUS 3*, Fusca 3; *LEC 1/2*, Leafy Cotyledon 1/2; *PDH 1*, Pod Dehiscence 1; *RPL*, Replumless; *FUL*, Fruitfull; *IND*, basic helix-loop-helix gene Indehicent; *SHP 1/2*, MADS-box genes Shatterproof 1/2; *ALC*, Alcatraz; *EPSPS*, encodes 5'enolpyruvylshikimate 3-phosphate synthase; *ALS*, acetolactate synthase; *ACC-T1*, acetyl-coenzyme A carboxylase; *SPL 14*, Squamosa Promoter Binding-Like 14; *C287*, C287 mutant of acetolactate synthase; miRNAs, micro RNAs; *CKX*, cytokinin oxidase/dehydrogenase; *ERF*, ethylene responsive factor; *OST 2*, Open Stomata 2; *CBF/DREB*, C-repeat/DRE binding factor/ Dehydration Responsive Binding Element; *WRKY*, encodes transcription factors; *DIPM 1/2/3*, DspE-interacting proteins of Malus 1/2/4; *LOB 1*, Lateral Organ Boundaries 1; *SWEET*, sugar will eventually be exported transporter; *WAT 1*, Wall Are Thin 1; *eIF4E*, eukaryotic translation initiation factor 4E; *MLO*, Mildew Resistance Locus O; *HGO*, homogentisate dioxygenase; *LCYε*, lycopene epsilon-cyclase; *ITPK*, inositol triphosphate kinases; *IPK1*, inositol-1,3,4,5,6-pentakisphosphate 2-kinase 1; *MIPS*, myo-inositol-3-phosphate synthase; *HRZ 1/2*, hemerythrin motif-containing really interesting new gene (RING)- and zinc-finger protein 1/2; *VIT 1/2*, Vacuolar Iron Transporter 1/2; *FAD*, fatty acid desaturases; *CLV 1/2/3*, Clavata 1/2/3; *TFL 1*, Terminal Flower 1; *GW 2/3*, Grain Weight 2/5; *TGW 6*, Thousand-Grain Weight 6; *GS 3*, Grain Size 3; *BS 1*, Big Seed 1; *EOD3*, Enhancer 3 Of Da 1; *GRF 3/4*, Growth Regulating Factor 3/4; *DEP 1*, Dense And Erect Panicle 1; *Gn1a*, Grain Number 1a; *GID 1/2*, Gibberellin Insensitive Dwarf 1/2; *IPA 1*, Ideal Plant Architecture 1; *CCD 8A/B*, carotenoid cleavage dioxygenase 8A/8B; *SPL*, Squamosa Promoter Binding Protein-like; *FT*, Flowering Locus T; *SP*, Self-Pruning; *SP 5G*, Self-Pruning 5G; *SLF*, F-box protein; *PR55/B*, PP2A 55 kDa B regulatory subunit; *Sli*, S-locus inhibitor; *FPS2*, farnesyl pyrophosphate synthase; *PGAP1*, post-GPI attachment to proteins 1. Picture created by Biorender.com

and camelina through transgenic approach (Wu et al. 2022; Porokhovinova et al. 2022). Tinkering the pathway genes by SDN1 modifications and incorporating novel genes by SDN3 modifications can be done by genome editing in order to change the fatty acid composition in oils.

Enhancing biotic stress tolerance

Plants co-exist with a myriad of microbes and pests. Plant photosynthetic efficiency is drastically impeded by pathogens thus causing around 20–40% of yield losses globally. Plants do harbour a set of genes called susceptibility genes (*S*-genes) that predispose the plants for pathogen attack and they could be edited or modified to make plants resist the pathogens. It has been demonstrated that the *S*-gene products are essential for initial establishment, growth and proliferation of phytopathogens (van Schie and Takken 2014). Hence, disrupting these genes can break the host–pathogen compatibility and render resistance to plants (Garcia-Ruiz et al. 2021). This phenomenon has been demonstrated using gene silencing or knock-out studies, for e.g., gene knock-out of *Mildew Resistance Locus O (MLO)* has conferred resistance

to various powdery mildew causing fungus in crop species like wheat, grape and tomato (Zaidi et al. 2018). Mutation of *Walls Are Thin1 (WAT1)* enhances broad-range resistance to vascular pathogens such as *Ralstonia solanacearum* and *Verticillium dahliae* in Arabidopsis and cotton respectively (Denancé et al. 2013; Tang et al. 2019). Knocking-out of the eukaryotic translation initiation factor, *eIF4E* and *SWEET* genes conferred resistance to virus and bacterial pathogens respectively in several crops (Zaidi et al. 2018). Promoter disruption of *Lateral Organ Boundaries 1 (CsLOB 1)* gene decreased the citrus-canker disease severity by 83.2–98.3% in *Citrus sinensis* (Peng et al. 2017). Similarly, gene editing of *DIPM-1, DIPM-2*, and *DIPM-4* in Apple conferred resistance to fire blight disease caused by *Erwinia amylovora* (Malnoy et al. 2016). *S*-gene homologs have been reported in many crops including oilseeds. The *SWEET* gene and *WAT 1* orthologs have been identified and validated for their role as *S*-genes in both soybean and cotton respectively (Koseoglou et al. 2022). Apart from *S*-genes, there are many other genes that could be targeted for disease resistance (Schenke and Cai 2020). Still, the pleiotropic effects and to what extent these *S*-genes identified in model crops are functionally conserved in other oilseed crops are unanswered questions and only after these issues are addressed could they be used as targets for genome editing in oilseed crops. Therefore, it is likely that the CRISPR/Cas-mediated targeting of homologs of such *S*-genes may confer resistance to pathogens in oilseed crops (Ali et al. 2022).

Enhancing abiotic stress tolerance

Abiotic stress like drought, temperature and salinity causes major threat for oilseed production especially in India as more than 70% of oilseed cultivation in India is under rainfed condition and in marginal lands. As indicated by several basic studies earlier, there are negative regulators of abiotic stress tolerance in crops and if these genes are silenced or disrupted, it might lead to abiotic stress tolerance (Singh et al. 2019). Many attempts have been made to edit signalling cascade genes for abiotic stress responses in model crops (Kaur et al. 2022). Editing of gene encoding *Open Stomata 2 (OST 2),* a proton pump in Arabidopsis increased drought tolerance (Osakabe et al. 2016; Joshi et al. 2020). Manipulation of the cytokinin levels by silencing of *cytokinin oxidase/dehydrogenase (CKX)* gene in roots is shown to increase the drought tolerance in many crops (Zalabak et al. 2013). Several *cis*-regulatory sequences act as negative regulators of abiotic stress tolerance. Transcription factors (TFs) like *WRKY (GhWRKY17, GmWRKY13,* and *ZmWRKY17*), ethylene responsive factor (ERF) and CBF/DREB, bind to these sequences and negatively regulate abiotic stress tolerance (Zafar et al. 2020). Such genes have been reported in brassica, cotton, and peanut (Luo et al. 2021; Shazadee et al. 2022; Wang et al. 2022a). Several genes such as *GhNAC79, GhRaf19, GhWRKY6, GhABF2, GhRaf19, GhMKK3, GhWRKY27a, GhMAP3K65, G18431620 (GH 3.5)* and *AtHUB2* were successfully confirmed by virus-induced gene silencing (VIGS) in cotton for their role in heat, drought, salt and cold stress (Singh et al. 2019). VIGS mediated silencing of *AhABI4s* conferred the salt tolerance in peanut (Luo et al. 2021). All these genes are potential target genes for conferring abiotic stress tolerance. MicroRNAs are also known to mediate abiotic stress tolerance (Begum 2022). Usually, microRNAs silence the target genes by binding to their targets and marking them for cleavage. Such target genes could be the candidate genes for genome editing (Gao et al. 2022). Apart from this, the *MIR* genes (microRNA encoding genes) can be a potential target for genome editing if these microRNAs are negative regulators of stress tolerance (Basso et al. 2019). Some miRNAs such as miR414, ghr-miR399, ghr-156e, miR319, ghr-miR5272a, miR156a/d/e, miR167a, miR169, miR397a/b, miR399a, miR535a/b, miR827b and many more are playing a role in various abiotic stress tolerance in cotton, brassica, and soybean (Chaudhary et al. 2021; Tiwari and Rajam 2022; Begum 2022). Therefore, editing the main target genes of these miRNAs would confer tolerance in oilseed crops.

Introducing herbicide tolerance

Herbicides are used to restrict weeds which affect productivity owing to competitiveness with crops. Establishment of oilseed crops is affected badly by the weeds especially during the initial phase of crop establishment and removal of them at that stage is crucial for crop growth. This is more pronounced in small seeded crops like sesame, niger, and mustard. Manual weed control is not only costly but also time consuming. Herbicide application is a viable alternative if there are herbicide tolerant genotypes in oilseeds. As most of the herbicides inhibit specific enzymes involved in amino acid metabolism pathways, if the target enzymes are modified in such a way that they are not acted upon by herbicides, then the plants become herbicide tolerant. Such mutant forms of target enzymes have been reported in different crops and they are being exploited by deploying them as transgenes conferring herbicide tolerance. CRISPR/Cas9 system has been successfully employed to introduce herbicide tolerance in model crops like rice, wheat, tomato, potato, brassica and watermelon by editing the key genes such as *5-enolpyruvylshikimate- 3-phosphate synthase (EPSPS*) and *acetolactate synthase (ALS)* gene (Hussain et al. 2018) in such a way that they are not affected by herbicides. Genome editing approach needs to be explored for other classes of herbicides including those inhibiting *protoporhyrinogen oxidase* and *4-hydroxyphenyl pyruvate dioxygenase* (Kaur et al. 2022). Herbicide tolerance has

been demonstrated in soybean against chlorsulfuron (Li et al. 2015) and in flax against glyphosate (Sauer et al. 2016; Hussain et al. 2021). Base-editing of genes like *C287, SPL 14* and *ACC-T1* has been successfully exploited to introduce herbicide resistance in rice (Mishra et al. 2020) and this could be adopted successfully in oilseed crops (Fig. 1).

Minimizing pod shattering damage

Pod dehiscence accounts for a major pre-harvesting as well as post harvesting yield loss in oilseed crops such as soybean, sesame, and oilseed rape. Minimizing the shattering loss is an important objective in breeding of oilseed crops and mutant studies have identified the underlying genes for shattering resistance. Biotechnological efforts to minimize the pod shattering is a pressing priority. The genetic network that expresses in silique dehiscence zone (DZ) is well documented in Arabidopsis (Ballester and Ferrándiz 2017; Ogutcen et al. 2018). Four TFs i.e., *Shatterproof 1 (SHP 1)* and *SHP 2* upregulate downstream TFs i.e., *Indehiscent (IND)* and *Alcatraz (ALC)* at DZ zone. Mutation in *shp1 shp2* and *ind* genes led to the production of fully indehiscent silique in Arabidopsis (Liljegren et al. 2000). Editing of *ALC* resulted in more shatter resistance in oilseed rape (Braatz et al. 2017). Additionally, two transcription factors, *Fruitfull (FUL)* and *Replumless (RPL)* that express in the valves and replum respectively, also regulate the expression of the DZ genes. However, there is a need for understanding or deciphering the genes involved in pod dehiscence so that it will provide a handle for manipulations. For example, in *Brassica napus*, an integrated approach led to identification of *BnTCP8.C09* as the gene responsible for pod shattering (Chu et al. 2021). Elimination of *Pod dehiscence 1 (PDH 1)* gene resulted in pod shattering tolerance in soybean (Zhang et al. 2022b). Overall, tweaking of the homologs of any of the genes reported to be associated with pod shattering in other crops, could minimize the pod shattering in oilseed crops (Fig. 1).

Tuning the genes related to seed size and number

Oilseeds are a storehouse of oils and manipulating the traits such as inflorescence branching, silique structure, size and number of grains produced by plant will have a huge impact on the quantity of seeds produced. Multilocular phenotype has been achieved by precise editing of homologues of development related genes *Clavata (CLV 1/2/3)* in *B. napus* (Yang et al. 2018). Similarly editing the gene *BnnEOD3* led to increased number of seeds in silique in rapeseed (Khan et al. 2020). The loss-of-function of Arabidopsis mobile regulator *Terminal Flower 1 (TFL 1)* produced large seeds compared to wild type (Zhang et al. 2020a). Knock-out of four rice genes i.e., *Grain Size 3 (GS 3)*, *Grain Weight 2/5 (GW 2/5),* and *Thousand-Grain Weight 6 (TGW 6),* that negatively regulate rice grain weight led to the improvement of the grain weight (Xu et al. 2016a). Deletion of *Big Seeds 1 (BS 1),* a negative regulator of organ size, significantly enhanced the grain size in both leguminous plants *Medicago* and soybean (Ge et al. 2016). Base-editing of *GRF 3/4* by adenine base editor (ABE) resulted in increasing grain size and yield in rice (Hao et al. 2019). Thus, there is enormous scope for increasing the sink capacity in oilseeds, especially in crops like sesame, mustard, niger and linseed if the homologs of the genes negatively controlling seed size are disrupted (Fig. 1).

Tweaking the genes related to plant architecture

The principle behind the success of green-revolution is plant architecture. The structure of crops affects many important agricultural traits, especially yield. Plant ideotype concept has been developed in many crops to suit different conditions of cultivation. The central theme in such manipulations is to alter the plant architecture by increasing or decreasing the number of branches, altered height, basal or top branching types, reduced duration, etc. to suit the resource availability during the length of growing period. In oilseed crops like castor, there is a requirement to develop plant architecture suitable for mechanical harvesting. In other crops, like sesame, no branching types with erect plant type to encourage high density planting is a requirement. There are genes reported in crops that are known to alter the plant architecture. Plant hormone gibberellic acid (GA) plays a vital role in growth and development. GA acts by degrading the DELLA protein which in turn is regulated by two proteins i.e., the gibberellin receptor GID 1 (*Gibberellin Insensitive Dwarf 1*) and the F-box protein GID 2 (*Gibberellin Insensitive Dwarf 2*). Loss-of-function of *GID 1* and *GID 2* displayed a greater number of branches and leaves in rice (Wu et al. 2020). In another report, editing of *carotenoid-cleavage dioxygenase 8A* and *8B* (*CCD 8A, CCD 8B*) also produced similar patterns in rice and *N. tabacum* (Gao et al. 2018; Liu et al. 2020). Similarly, deletion of *Grain Number 1a (Gn1a), Dense And Erect Panicle 1 (DEP 1),* and *Ideal Plant Architecture 1 (IPA 1)* lead to the improvement of grain number, panicle architecture, grain size, and architecture in rice (Li et al. 2016). In an interesting study in castor bean, candidate gene involved in dwarfism has been identified (Wang et al. 2021). Any of these target genes could be used for modifying the plant architecture through genome editing.

Tinkering the flowering

Duration taken for flowering as well as determinate v/s indeterminate flowering habits, both are very crucial in crop

plants to enable them to fit into different cropping seasons and systems. Since oilseeds are grown predominantly as rainfed crops in India, these traits are very crucial in breeding programmes. Also, in some crops like sesame, breeding for thermo and photo-insensitive genotypes is important to allow the cultivation of the crop in different seasons as well as regions. Therefore, depending on the case, genome editing of appropriate gene(s) would help in accomplishing these objectives. With respect to inducing early flowering, editing of two genes *Self-Pruning (SP)* and *Self-Pruning 5G (SP 5G)*, that work as floral repressors, led to early flowering genotypes in tomato (Soyk et al. 2017). Interestingly, in soybean double mutants of *Gm*FT2a/*Gm*FT5a, the homologs of *Flowering Locus T (FT)* and transcription factor *Squamosa Promoter Binding Protein-like (SPL),* displayed more seed number (~250%) compared to wild type under short day condition. But the results were opposite when the same was adopted in *B. napus* and *B. juncea.* So, careful empirical assessment of the target genes would be crucial in translating the work from one system to the other (Subedi et al. 2020b).

Biofortification

Nutritionally enhanced food crops with increased bioavailability of the essential nutrients, both microminerals (e.g., Minerals such as iron, zinc, copper, etc.) and macronutrients (such as amino acids and macrominerals like Ca, K, S, etc.) are expected to address the malnutrition of human population in a very effective way. Bio-fortification of commercial crops for these nutrients is gaining importance and they are being addressed through breeding, biotechnology and agronomy practices. Transgenics have been used for biofortification of crops with respect to vitamins, minerals, essential fatty acids and amino acids, antioxidants, and starch (Garg et al. 2018). Gene(s) involved in increasing either the quantity of the nutrients or their bioavailability have been the targets for manipulation. The gene FAD encodes *fatty acid desaturases* that convert high value oleic-acid to low value product linoleic acid. Researchers have commercially exploited this gene in camelina, peanut, soybean, cotton, rice and brassica for creating high oleic acid crops (Abe et al. 2018; Chen et al. 2021b; Siddique 2022). The existence of the fad2 gene in the maize genome suggests a potential future possibility to produce maize kernel oil rich in oleic acid (Mikkilineni and Rocheford 2003). Editing of phytic acid metabolism genes such as *myo-inositol-3-phosphate synthase (MIPS), inositol-1,3,4,5,6-pentakisphosphate 2-kinase (IPK)* and *inositol triphosphate kinases (ITPK)* augmented micronutrients such as zinc, calcium, phosphate and magnesium in rapeseed and soybean (Tian et al. 2022; Jianing et al. 2022; Siddique 2022). In soybean, researchers successfully enhanced the Vitamin-E content by mutating *homogentisate dioxygenase (HGO)* gene (Stacey et al. 2016). In cotton, RNA interference (RNAi) mediated attenuation of the expression of genes encoding stearoyl-ACP desaturase 1 (SAD1), and ketoacyl-acyl carrier protein synthase (KASII) led to a considerable change in the fatty acid composition of seed oil (Wu et al. 2022). Similarly, down-regulation of cadinene synthase led to gossypol-free (gossypol is an anti-nutritional factor that naturally present in cotton plant) cottonseed (Sunilkumar et al. 2006). Apart from oilseed crops, functional disruption of *Vacuolar Iron Transporter 1 and 2 (VIT 1/2)* displayed increased Fe/Zn accumulation in rice seeds (Zhang et al. 2012). *Hemerythrin motif-containing Really Interesting New Gene (RING)- and Zinc-finger protein 1 (OsHRZ 1)* and *OsHRZ 2* edited rice plants accumulated iron in their shoots and grains (Kobayashi et al. 2013). With the accumulating information on genes that could be manipulated for biofortification in different crops, there is a large scope for adopting genome editing for improving this trait in oilseed crops.

Self-incompatibility

Pollination and fertilization lead to seed production, which is the economic part in oilseeds. Self-incompatibility (SI) hampers the creation of inbred lines and breaking of SI is a breeding objective in oilseed crops such as niger, and brassica. Self-compatibility (SC) has been successfully achieved by editing some of the crucial genes like, *S-RNase, F-box protein (SLF), PR55/B, S-receptor kinase* and *M-locus protein kinase* in potato, cabbage and oilseed rape (Ahmad et al. 2021b; Shin et al. 2022; Kardile et al. 2022) as well as through introgression of *S-locus inhibitor (Sli)* in potato (Kardile et al. 2022). Knocking out of *PGAP 1*, post-GPI attachment to proteins 1 genes disrupted SI in Arabidopsis without developmental defects (Lin et al. 2022). In contrast, self-incompatibility was restored in citrus by tweaking the *FPS2* gene to trigger parthenocarpy (Qin et al. 2018). Present gene-editing technologies have widened the possibilities to overcome the SI/SC in oilseed crops to create novel breeds.

Domestication of wild oilseed crops

Crop wild relatives (CWRs) form a treasure trove of many important agronomic traits but as such these CWRs are not suitable for intensive cultivation due to the presence of a few undesirable characteristics including wild and weedy nature, lower harvest index, etc. To make the CWRs suitable for extensive cultivation, scientists have started modifying the domestication traits in them using genome-editing tools that results in the development of new variants or genotypes that have the required agronomic traits compared to CWRs. Presently, de novo domestication of wild crops has been shown as an innovative crop breeding strategy to address future

food challenge (Kumar et al. 2022). Wild tomato (*Solanum pimpinellifolium*), groundcherry (*Physalis pruinosa*), and, most recently, allotetraploid rice (*Oryza alta*) have all been successfully bred for a variety of agronomic traits (Lemmon et al. 2018; Zsogon et al. 2018; Yu et al. 2021). The domestication of the day neutral wild tomato is closely examined by editing of SELF-PRUNING 5G (SP5G) gene that led to the creation of compact growth habit and rapid flowering variety (Soyk et al. 2017). De novo domestication may be enabled by mutations in the SP5G gene in day-neutral wild relatives of oilseed crops like groundnut, sunflower, and safflower. To create neo-domesticated cultivars, it may be possible to target genes that are related to survival, spread, and fitness related traits like pod shattering, plant architecture, grain size and number, flowering, and photoperiodism. Among all oilseed crops, high-quality whole-genome assemblies of CWRs of soybean (*Glycine soja*), peanut (*Arachis duranensis* and *Arachis ipaensis*), and pangenome assemblies are available for Brassica sp., sunflower, and cotton (Bohra et al. 2022). It might not be a simple path for all oilseed crops to achieve de novo domestication, because, an annotated reference genome of CWRs, knowledge of domestication-related genes, are still unavailable for many wild relatives of oilseed crops sesame, linseed, niger, and safflower, just to cite as examples.

Caveats and challenges for applying CRISPR/Cas9 mediated GE in oilseed crops

All the genome editing approaches depend on the transformation procedure for delivering the editing machinery into plant cells and are thus dependent on plant tissue culture (PTC) and transformation procedures. Therefore, the biggest bottleneck in genome-editing of oilseed crops is efficient delivery of gene editing reagents into the plant regenerative cells. PTC is a lengthy, costly, labour-intensive and is possible in limited number of plant species (Anjanappa and Gruissem 2021). Even though, there are reports of genetic transformation and regeneration in each of the oleiferous crops, repeatable and genotype independent protocols for routine applications of in vitro manipulations as adoptable by different labs are still missing in many of these oilseed crops (eg. sunflower, Darqui et al. 2021; cotton, Kalbande and Patil 2016; coconut, Nguyen et al. 2015; linseed, Beyaz et al. 2016; camelina, Sitther et al. 2018; sesame, Teklu et al. 2022; castorbean, Xiao et al. 2022; safflower, Nitnaware et al. 2021; jatropha, Al-Khayri et al. 2022) (Table 2). Therefore, developing repeatable and efficient transformation protocols should be the primary line of activities in successfully adopting GE technology in these crops. The bottlenecks of regeneration and transformation are specific to crops and delving into all these aspects is beyond the scope of this review article and therefore, not elaborated.

Among the different genome editing tools reported, CRISPR/Cas9 system is the most followed method due to its versatility and options. However, it has some limitations as well. Large size of Cas nucleases is not much suitable for Agrobacterium mediated transformations thus discovering or engineering smaller Cas variants is inevitable. Limiting PAM specificity, random off-target mutations, selection of sgRNA, balanced in-vivo expression of sgRNA and Cas cassettes in host are some of the concerns/limitations and they need to be addressed for realizing high editing efficiency. Identification and optimization of promoters suitable for crop is needed for effective expression of Cas and sgRNA. Genome sequenced oilseed crops and their immediate targetable traits have been listed in Table 2. Selecting the specific functionally characterized target genes for manipulating the trait is crucial. In oilseeds, availability of such characterized genes has been a limitation and therefore, either this has to be empirically determined or they need to be taken from the heterologous systems as indicated in Table 1.

Construct design is a vital step in genome-editing (Hassan et al. 2021). Three components i.e., (1) selection of Cas nucleases, (2) design of gRNA, and (3) promoters that are used to express Cas protein and gRNAs need to be selected carefully. Despite the wide-ranging use of SpCas9, it does come with certain limitations such as off-targets, limited 5′-NGG-3′ PAM sequence, and bigger in size (Nadakuduti et al. 2018). To address this, several natural and engineered variants of Cas9 have been developed (Cebrian-Serrano and Davies 2017). Codon optimized Cas genes for each host species work better than wild type as demonstrated in soybean (Michno et al. 2015). Design of effective gRNA is another crucial step in genome-editing. There are many web tools available for gRNA design among which CRISPOR (Concordet and Haeussler 2018), CRISPR-P (Liu et al. 2017), RGEN Cas designer (Park et al. 2015), and CHOPCHOP (Labun et al. 2019) are widely used and suitable for oilseed crops. Availability of whole genome sequence of the crop is necessary for designing the effective sgRNA using online web tools. As the editing efficiency is mainly dependent on sgRNA selection and promoters used for expressing Cas-nuclease and sgRNA, these need to be empirically determined in crops.

Many of the cultivated oilseed crops are polyploids (soybean, rapeseed, peanut, cotton and camelina). In comparison to diploid crops, the editing effectiveness has varied greatly, in particular, in polyploid crops owing to the genome complexity. The challenge of simultaneous elimination of all copies of genes including the homoeologs with the same function is necessary especially when paralogs and orthologs have redundant functions (Zaman et al. 2019). Alebit this perceived difficulty, thanks to the cutting-edge technologies

(Li et al. 2021a), genome editing has been successfully carried out in rapeseed, soybean, peanut, cotton, and camelina (Table 1). The editing effectiveness may be directly influenced by the careful design of gRNA, choice of promoter, optimized/newly discovered Cas nucleases, and multiplexing (Zaman et al. 2019).

Future prospects of genome editing in oilseed crops

As outlined in this review, genome editing has been demonstrated in a few oilseed crops for a few agronomic traits. But, considering the success seen in other crops, genome editing could become a main tool in the armoury of genetic engineering options for oilseed crops as well. Genome sequence of many oilseed crops have been deciphered (Table 2) and thus offers a platform to select the target gene sequences. There are crop specific traits (as listed in Table 2) that could be improved through genome editing in oilseed crops. Establishing repeatable transformation protocols, dissecting the plant/cellular processes involved in trait manipulation, understanding the functionality of the implicated genes thoroughly to obtain expected phenotype, overcoming the limitations of off-target effects would all lead to harnessing the benefits of GE in oilseed crops. Among these limitations, the biggest bottleneck in genome-editing of oilseed crops is the efficient delivery of gene editing reagents into the plant regenerative cells. To circumvent this limitation, PTC-free genome editing has been achieved through many methods such as polyethylene glycol (PEG) mediated delivery of pre-assembled Cas/gRNA, ribonucleoproteins (RNPs) to protoplasts (Banakar et al. 2020), particle bombardment delivery of CRISPR/Cas reagents (Demirer et al. 2020; Banakar et al. 2020), de-novo meristematic microinjection of developmental regulators with gene-editing reagents (Maher et al. 2020), use of viral vectors (Ellison et al. 2020) and CRISPR-combo system (Pan et al. 2022). Furthermore, as our understanding of regulatory genes' functions in plant genetic transformation and regeneration expands, we may someday be able to harness these genes to develop a single universal genotype-independent plant tissue culture approach for all oilseed crops (Maren et al. 2022). As has been demonstrated in recent studies, nanovectors/nanocarriers promise to offer a solution to the issue of delivering the elements of CRISPR/Cas mediated genome editing directly to the target tissue(s) in plants. Nanomaterials like mesoporous silica particles (MSNs), gold nanoparticles (AuNPs), carbon nanotubes (CNTs), and layer double hydroxides (LDHs) have been used as promising cargo carriers and deliver the editing reagents in a highly effective and species-independent manner and bring in the editing of the target genes (Vats et al. 2022; Zhi et al. 2022; Savage 2022). These methods are technically challenging, less effective, and have not yet been adopted for oilseed crops on a large scale. Once these techniques are well established, many of the legislative and public concerns raised due to transgene integration (Teferra 2021) will also be allayed. This is only the beginning of PTC free GE and we can expect to see a rapid advance in this field in future and this will help oilseed researchers to a great extent.

Functionality of genes and their role in trait development are being established through many approaches such as allele mining, phenotypic analysis of chemical induced, T-DNA, and transposon mutants, map-based cloning of QTLs, candidate gene-based phenotyping, transgenics–both silencing and overexpressing types, transcriptome studies, pathway analysis, and others in both model and related plant species. This information could be used for selecting the target genes for manipulation through gene editing.

There is an urgent need to develop climate smart high-yielding oilseed crop varieties owing to many snags challenged by dynamic climate. There is immense scope to apply state-of-the-art technologies like CRISPR/Cas to improve their productivity. Editing of many genes that act as negative regulators for growth and development offer a great potential. Knowledge accrued from related model crops like Arabidopsis, tobacco, tomato and rice might help in developing suitable strategies to realize high yielding varieties in other oilseed crops as well. There is substantial literature available on genes and non-coding RNAs which play pivotal role as negative regulators of growth, yield and stress responses in model crops (Ojolo et al. 2018; Begum 2022) and this information shall be effectively used in genome editing to manipulate the traits.

Another important area of future development would be to exploit editing to modify MIR genes. It is well known that miRNAs that are up-regulated under stress (biotic/abiotic) conditions are expected to downregulate the target genes which in turn are negative regulators of the stress tolerance whereas the miRNAs that are down-regulated under stress conditions are expected to up-regulate the target genes which are positive regulators of the stress tolerance (Zhang 2015). Hence, if miRNAs are up-regulated in the given stress, the target gene of that cognate miRNA could be edited and if miRNAs are down-regulated during a particular stress, then MIR genes encoding them could be edited to enhance the stress tolerance. Therefore, literature on the role of miRNAs in stress responses offer a great potential for creation of climate-smart crops by genome-editing (Begum 2022).

The continuous methodological improvements of CRISPR/Cas toolbox particularly, discovery of tiny Cas variants (Savage 2019), reducing off-targets (Movahedi et al. 2022), epigenetic modifications (Gardiner et al. 2022), base-editing, prime-editing (Molla et al. 2021), CRISPR-combo system (Pan et al. 2022) and near-PAMless (Walton et al. 2020) specificity should expand the scope of genome

editing. Taken together, it clearly hints that genome-editing approaches have opened up exciting possibilities for improving oilseed crops and in future we are expected to reap rich dividends from this research.

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Declarations

Conflict of interest The authors declare no competing interests with respect to the content of this article.

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