Plant Archives Vol. 26, No. 1, 2026 pp. 1221-1232 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.no.1.166

FRONTIERS IN POTATO IMPROVEMENT: A REVIEW

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(Date of Receiving-23-01-2026; Date of Revision-14-03-2026; Date of Acceptance-31-03-2026)

ABSTRACT

Potato is a globally important staple crop, but its production is increasingly limited by biotic stresses such as late blight, bacterial and viral diseases, and abiotic stresses including drought, heat, and salinity. Although conventional breeding has improved yield and adaptability, progress is constrained by the crop's genetic complexity, tetraploid nature, and long breeding cycles. Biotechnological approaches, including tissue culture, marker-assisted selection, genetic engineering, and genome editing (e.g., CRISPR/Cas9), offer effective solutions for crop improvement. This review highlights key advances in potato biotechnology, such as meristem culture for disease-free planting material, micropropagation, molecular markers, genomics-assisted breeding, RNA interference, and genetic transformation, with emphasis on enhancing disease resistance, nutritional quality, and overall productivity.

Keywords: *Solanum tuberosum* L. Aeroponics · CRISPR/Cas9 · Genetic engineering. Tissue culture

Introduction

Potato (*Solanum tuberosum* L.) is an important non cereal food crop and plays a great role in the world's gross domestic products since it is one of the four main food crops (rice, wheat, and maize) grown worldwide and is considered one of the major food sources for humankind (Morais *et al*., 2018). It possesses one of the greatest genetic resources for the development of new varieties (Jacobs *et al*., 2011). It is estimated that between 690 and 783 million people in the world faced hunger in 2022 (FAO, 2023). To protect low-income nations from the hazards presented by growing global food costs, potato is a highly advised food security crop (Devaux *et al*., 2014). Most cultivated potatoes are propagated vegetatively, making them highly susceptible to numerous diseases (Tessema and Tesfaye, 2023). The high seed requirement (2.5–3.0 t/ha) accounts for 40–50% of total production costs, while the traditional method has a low multiplication rate (1:6) and is both costly and time-consuming (Awati *et al*., 2019). In India, viral diseases, particularly potato leaf roll virus, pose a major constraint to potato production (Mukherjee *et al*., 2003). Increased insect vector activity and virus incidence can lead to yield losses of 50% or more (Harahagazwe *et al*., 2018), and without proper seed maintenance, viral infection may reach 100% within three to four cropping seasons (Biniam and Tadesse, 2008). In order to produce plants free from diseases, biotechnological methods such as meristem culture has been successfully used in potato cultivation (Al-Taleb *et al*., 2011). In many nations, the production of seed potatoes now includes the quick multiplication of these disease-free clones employing micro-propagation in conjunction with traditional multiplication techniques (Donnelly *et al*., 2003). Techniques like Polymerase Chain Reaction (PCR) and Enzyme-Linked Immunosorbent Assay (ELISA) are also employed to test for viruses, ensuring that only pathogen-free plants are used for further propagation (Singh *et al*., 2024).

Merits of Contemporary Potato Improvement Approaches

The integration of biotechnological virus-elimination strategies, such as thermotherapy combined with

chemotherapy and meristem culture, enables the production of virus-free potato planting material, with eradication rates reaching up to 100% in some cases, thereby improving seed quality, crop health, and yield stability (Bettoni *et al.*, 2022). Biotechnological introgression of resistance genes from wild species like *Solanum chacoense* and *Solanum microdontum* has provided cultivated potatoes with durable resistance to devastating diseases such as potato virus Y (PVY) and late blight (*Phytophthora infestans*), improving crop stability and yield security (Machida-Hirano, 2015). Somatic hybridization through protoplast fusion enables the transfer of strong disease-resistance traits from wild relatives such as *Solanum palustre* (formerly *S. brevidens*) and *Solanum bulbocastanum* into cultivated potato, conferring resistance to major threats including potato leaf roll virus, potato virus Y (PVY), and early and late blight, thereby strengthening crop protection and yield stability (Bradshaw *et al*., 2006; Brown *et al*., 2006; Thieme *et al.*, 2010). Biotechnological somatic cell selection allows the efficient recovery of elite potato clones with durable resistance to common scab by selecting for Thaxtomin tolerance in cell culture, with resistance stably expressed in field trials, enhancing crop reliability, productivity, and resilience to soil-borne diseases (Wilson *et al*., 2009, 2010; Tegg *et al*., 2013).The adoption of transgenic potatoes such as New Leaf potato has led to major reductions in pesticide use and aerial spraying, lowering production costs, minimizing environmental impacts, and supporting more sustainable potato farming, including for growers in developing regions (Kaniewski and Thomas, 2004; Brookes and Barfoot, 2005; Curtis *et al*., 2004).

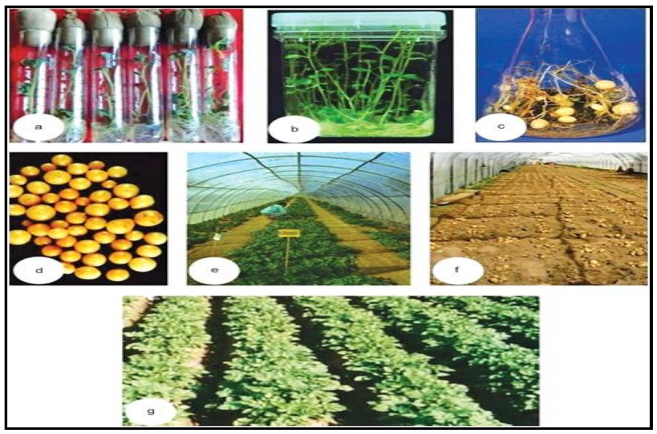

Fig. 1: Potential use of tissue culture in seed production of potato. (a) Micro-propagation of virus free plantlets (b) Micro-propagated plantlets further multiplied (c) Microtubers development in lab conditions (d) Microtubers harvested (e) Biotic stress free potato crop in net house (f) Harvesting of minitubers (g) Crop in open field conditions from minitubers. (Naik and Buckseth, 2018; Singh *et al*., 2019).

Tissue Culture in Potato

Tissue culture is an effective method for rapid production of high-quality, disease-free plantlets with genetic and physiological uniformity as shown in Fig. 1 (Naik *et al*., 2000). It involves sterilization of explants and their multiplication on nutrient media under aseptic conditions, followed by transplantation after sufficient growth (Lommen, 2024). In potatoes, various explants such as shoot tips, nodal stems, leaves, and embryos have been used since early reports of successful culture (Srivastava *et al*., 2012; Zhang *et al*., 2017; Santilago *et al*., 2012).

Micro-tuber production

Microtubers are genetically identical, high-quality, and pathogen-free, *In vitro* generated lab grown potato tubers (small seed potatoes) which are 0.5-1.5 cm in diameter that don't need to be acclimated like field-grown seed tubers do (Srivastava *et al*., 2012). Potato plants generated from micro-tubers are healthy and strong, and they may be utilised to produce original seed. Micro-tubers are also easier to handle and ship, which helps in their commercialization (Imani *et al*., 2010).

***In vitro* micro-tuber inducing agents**

(a) Lighting: Micro-tuberization efficiency increased when micro-propagated source plants grown under long days (16/8 h d/n) as compared to short days (8/16 h d/n) (Seabrook *et al*., 1993).

(b) Temperature: Micro-propagated plantlets develop more quickly at temperature between 20 and 25°C. The ideal temperature range for micro-tuber induction is typically between 15 and 18°C (Akita and Takayama, 1994).

(c) Medium Components: As the concentration of sucrose grew so did the percentage of micro-tuber-producing plants that were able to survive. At 10% sucrose, the average tuber diameter per plant and the best survival rate were discovered (Saha *et al*., 2013). An increase in sugar concentration to a certain extent can increase the production of micro-tubercules but large concentration (100 g) had detrimental effects on the plantlets' germination rate (Ali *et al*., 2018).

(d) Natural products and growth regulators: *In vitro*-tuberization can be marginally enhanced by kinetin-induced media. A combination of 2.5 mg/l kinetin, 90 g/l sucrose and 20 mg/l coumarin exhibited the most favourable features for *In vitro* micro-tuberization (Mohamed and Girgis, 2023).

Micro-tuberization in bioreactor Systems

Bioreactor systems enable the efficient, cost-effective production of high-quality plant propagules and secondary metabolites from cell and root cultures (Ziv, 2005). Several bioreactor types have been developed for plant micropropagation, including balloon-type bubble bioreactors, bubble column bioreactors, and automated temporary immersion systems (Shohaeland Paek, 2013), with proven potential for large-scale potato micro-tuber proliferation (Donnelly *et al*., 2003). These systems offer precise control of culture conditions, optimized nutrient and growth regulator supply, improved aeration, stage-specific medium changes, reduced contamination, and enhanced formation of buds or somatic embryos (Ziv, 2005). In Bangladesh, standardized protocols using national potato cultivars and optimized culture conditions have further improved micro-tuber production (Hossain, 2005; Hoque, 2010). Nutrient mist bioreactors are mainly applied to hairy root cultures but can also support other plant propagules (Weathers *et al*., 2008).

Mini Tuber

Mini-tubers produced from micro-tubers planted in high density on nursery beds (Naik, 2005). According to Struik (2007), mini-tubers are the tubers that are produced *In vitro* from virus-free potato plantlets. This contrasts with ordinary seed potatoes, which may carry pathogens that can lead to reduced crop yields and quality (Tolessa, 2021). Bigger mini-tubers of 5 to 25 mm size are becoming increasingly common in contemporary systems.

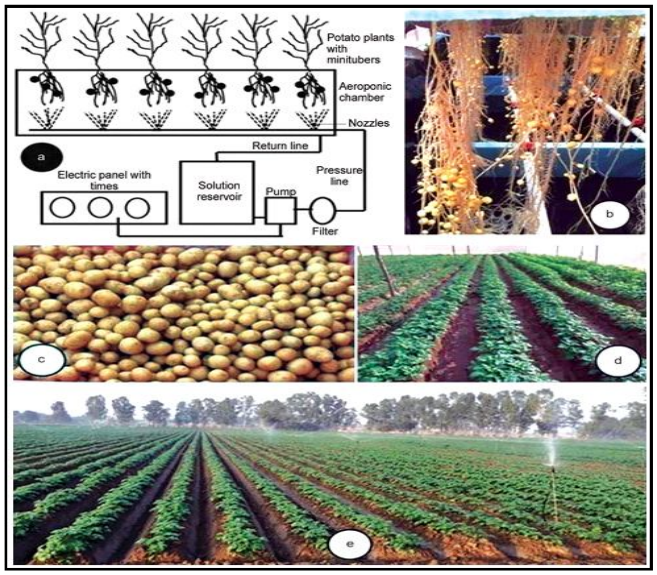

Fig. 2: Efficient use of aeroponics technique for virus free potato seed production. (a) Diagrammatically explained aeroponic system. (b) Minitubers developed (c) Harvested minitubers. (d) Minituber crop in net house, and (e) Minituber crop in the field (Naik and Buckseth, 2018; Singh *et al*., 2019).

According to (Struik, 2007), this size range equates to a weight range of 0.1-10 g or more. Techniques for producing soilless mini-tubers have been suggested to boost output volume. A range of soilless, hydroponic, aeroponic and bioreactor-type production techniques have been developed (Ritter *et al*., 2001; Kämäräinen-Karppinen *et al*., 2010). The hydroponic production method demonstrated to be effective in increasing the quantity of mini-tubers produced. The simplest method for producing mini-tubers from *In vitro* plants that are suited for the plains of North India disclosed by Naik (2005).

Aeroponics in potato

Aeroponics is a soilless cultivation method in which plant roots are suspended in air and supplied with nutrients via atomized sprays (Lakhiar *et al*., 2018). Globally, soilless systems have shown promising results for sustainable food production (Sardareand Admane, 2013; Gruda, 2019), offering environmentally safe rhizosphere control with reduced water use (Ritter *et al*., 2001; Farran and Mingo-Castel, 2006; Buckseth *et al*., 2016). Aeroponics is considered an optimal system for potato due to efficient nutrient delivery and rapid root development (Factor *et al*., 2007; Buckseth *et al*., 2016), supported by sterile, oxygen-rich growth chambers that accelerate plant growth (Calori *et al*., 2017). Successful production depends on precise control of nutrient concentration, pH, EC, humidity, spray intervals, oxygen, temperature, and light (Lakhiar *et al*., 2018), though cultivar-specific optimization is still required (Tierno *et al*., 2014; Buckseth *et al*., 2016). Aeroponics improves mini-tuber yield and quality, producing comparable phenolic, flavonoid, and antioxidant levels to soil-grown plants while achieving higher productivity (Ritter *et al*., 2001; Farran and Mingo-Castel, 2006). Plants may grow up to three times faster, enabling shorter harvest cycles (Kuncoro *et al*., 2021), while using far less water than field production (Otazu, 2010). Yield increases under optimized temperature regimes have been reported (Ritter *et al*., 2001), with reduced fertilizer runoff and improved nutrient efficiency (Nichols, 2006), and healthier, pest-free plants at higher densities (Waters *et al*., 2002).

Traits conferred by genetic engineering in potato

Since potato is the world's fourth most important crop in production and demand, improving this crop is important for both developing and developed countries (Dolnièar, 2021). Traditional breeding is limited because potatoes are vegetatively propagated and usually tetraploid (Bradshaw, 2022). As a result, genetic modification has helped develop new potato varieties with better yield,

Table 1: Association of markers with viruses, late blight, and potato cyst nematode resistance genes in potato.

Sr. No.	Marker Type	Marker Name	Forward sequence	Reverse sequence	Gene	ER
1	SSR	STM 0003	GGAGAATCATAACAACCAG	AATTGTAACTCTGTGTGTGTG	Rysto	PVY
2	ESTS	Yes3-3A	TAACTCAAGCGGAATAACCC	AAATTCACCTGTTTACATGCT TCTTGTG	Rysto	PVY
3	ESTS	Yes3-3B	TAACTCAAGCGGAATAACCC	CATGAGATTGCCTTTGGTTA	Rysto	PVY
4	SCAR	RYSC3	ATACACTCATCTAAATTT GATGG	AGGATATACGGCATCATTTTT CCGA	Ryadg	PVY
5	AFLP	M6	ACATGATATAAGTTGATATGG AGAAT	GTGCTTTGTCTTTTCTGCATGTA	Ryadg	PVY
6	AFLP	M45	GACTGCGTACATGCAGCT	GATGAGTCCTGAGTAAGGA	Ryadg	PVY
7	STS	RY186	TGGTAGGGATATTTTCCTTAGA	GCAAATCCTAGGTTATCAACTCA	Rychc	PVY
8	SCAR	Ry364	CTATTATAAGTCTGGTACTAG GACG	GGCTATATGTTCAATGAATTC ATGCTAA	Rychc	PVY
9	PCR	5Rx1	TCAGGGCAAAACCCTAACAC	ATCGGCCTAGAGTGACATCG	Rx1	PVX
10	PCR	PVX	ATCTTGGTTTGAATACATGG	CACAATATTGGAAGGATTCA	Rx1	PVX
11	PCR	106Rx2	GGAGAAATCCTGCAATGTAAC	CTTGTCAAAGAAAGAAGG CCT	Rx2	PVX
12	RFLP	GP21	GGTTGGTGGCCTATTAGC CATGC	AGTGAGCCAGCATAGCATTACTTG	Rx2	PVX
13	CAPS	SC811	CGAACAAAATACGTAATGCAT TGAATAA	GACCTATATCAGTCCCTTCT AATCCACTAT	Ns	PVS
14	SCAR	SCG17-321	ACGACCGACACTCAAATTTGT ACAAGAAA	GATGCCCCGACAGAGGAAG	Ns	PVS
15	SCAR	N127	TAGAGAGCATTAAGAAGCTGC	TTTTGCCTACTCCCGGCATG	PLRV1	PLRV
16	SSR	R1	CACTCGTGACATATCCTCACTA	CAACCCTGGCATGCCACG	R1	LB
17	SSR	R2	ATGGCTGATGCCTTTCTATCA TTTGC	TCACAACATATAATTCCGCTTC	R2	LB
18	SSR	SHa	ATCGTTGTCATGCTATGAGAT TGTT	CTTCAAGGTAGTGGGCAGTAT GCTT	R3a	LB
19	SSR	R3b4	GTCGATGAATGCTATGTTTCT CGAGA	ACCAGTTTCTTGCAATTCCAG ATTG	R3b	LB
20	SCAR	CosA	CTCATTCAAAATCAGTTT TGATC	GAATGTTGAATCTTTTTGTGA AGG	R1	LB
21	SSR	45/ XI	AGAGAGGTTGTTTCCGAT AGACC	TCGTTGTAGTTGTCATTC CACAC	Rpi-Smira1	LB
22	SSR	184–81	CCACCGTATGCTCCGCCGTC	GTTCCACTTAGCCTTGTCTTG CTCA	Rpi-Smira2	LB
23	SCAR	N146	AAGCTCTTGCCTAGTGCTC	AGGCGGAACATGCCATG	H1	PCN
24	SCAR	N195	TGGAAATGGCACCCACTA	CATCATGGTTTCACTTGTCAC	H1	PCN
25	SCAR	57R	TGCCTGCCTCTCCGATTTCT	GGTTCAGCAAAAGCAAGGACGTG	H1	PCN
26	SCAR	Gro1-4	TCTTTGGAGATACTGATTCTCA	CGACCTAAAATGAAAAGC ATCT	Gro1-4	PCN
27	STS	Gpa2-2	GCACTTAGAGACTCATTCCA	ACAGATTGTTGGCAGCGAAA	Gpa2	PCN

ER Extreme Resistance, PVY potato virus Y, PVX potato virus X, PVA potato virus A, PVS potato virus S, PLRV potato leafroll virus, LB late blight, PCN potato cyst nematode (Islam *et al*., 2024)

disease resistance, and nutritional quality, using tools such as CRISPR/Cas9, TALENs, transgenesis, cisgenesis, and RNA interference (Malzahn *et al*., 2017).

(a) Starch composition: Potato starch contains about 80% amylopectin and 20% amylose in tuber amyloplasts (Brummell *et al*., 2015). Extracting amylose needs chemical pre-treatment, which is costly and can harm the environment (Veillet *et al*., 2019). When the gene that makes amylose is knocked out, starch still looks normal but its chemical properties change (Brummell *et al*., 2015). Amylose-free potatoes were first made using RNA interference (RNAi) to silence the granule-bound starch synthase gene (Brummell *et al*., 2015). More recently, CRISPR/Cas9 was used to mutate all four alleles

of this gene in cv. Kuras without stable integration, mainly causing small deletions of 1–10 bp (Andersson *et al*., 2017).

(b) Acrylamide Content: Acrylamide forms when reducing sugars react with free asparagine in potato tubers, and it is carcinogenic in rodents (Chawla *et al*., 2012). Because of possible health risks, researchers are trying to reduce acrylamide in potatoes. The genes Asn1, Asn2, and Vlnv help control acrylamide buildup (Chawla *et al*., 2012). Changing the expression of asparagine synthetase genes on chromosomes 6 and 4 lowered asparagine levels (Clasen *et al*., 2016), but silencing Asn2 caused developmental problems (Chawla *et al*., 2012). Another method targets sugar-related genes; silencing Vlnv in cv. Katahdin reduced its expression by 97% (Bhaskar *et al*., 2010; Zhu *et al*., 2016).

(c) Resistance to Phytophthora infestans: Potato late blight is caused by the oomycete Phytophthora infestans, which infects plant tissue through zoospores (Adolf *et al*., 2020). Studies on wild potato relatives have found about 50 resistance (R) genes against late blight (Adolf *et al*., 2020). These R genes are often found in clusters and change frequently in the genome (Ghislain *et al*., 2019). One strong way to improve resistance is gene pyramiding, where several R genes are combined (Mundt, 2014). The DuRPh programme aims to move R genes from wild species into commercial potatoes using cis-genesis (Holme *et al*., 2013). Introducing three R genes from wild Solanum species gave full resistance in farmed potatoes (Ghislain *et al*., 2019), and new gene-editing tools can further improve R genes for better pathogen recognition (Nadakuduti *et al*., 2018).

(d) Resistance to Colorado potato beetle: The Colorado potato beetle is a major pest of potatoes, as both adults and larvae eat leaves and reduce yield (Kadoic *et al*., 2020). Insecticides can control it, but they also kill helpful insects, increase costs, and harm the environment (Kadoic *et al*., 2020). The Bt protein from the Bacillus thuringiensis CryIIIA gene kills the Colorado potato beetle and related insects. Monsanto™ developed the first Bt potatoes called NewLeaf™, and in 1998 released NewLeaf Plus™ from cv. Russet Burbank with added resistance to potato leafroll virus (PLRV). Bt sprays are less effective because they break down in sunlight and wash off easily, and insects can become resistant (Kadoic *et al*., 2020).

Table 2: Correlation of SSR markers with drought and cold tolerance in potato. (Islam *et al*., 2024).

Sr. No.	Marker type	Marker name	Gene name*	Tolerance
1	SSR	HRO_ACS3_1	ACS3	Drought
2	SSR	HRO_ALDH_H	ALDH	Drought
3	SSR	HRO_ETRTF_5a_D	ETRTF3	Drought
4	SSR	HRO_PARGH_1A_B	PARG	Drought
5	SSR	HRO_PP2C_1_B	PP2C	Drought
6	SSR	S215	-	Cold
7	SSR	S165	-	Cold
8	SSR	STHSF004_F_R	StHsf004	Cold
9	SSR	STHSF007_F_R	StHsf007	Cold
10	SSR	STHSF009_F_R	StHsf009	Cold
11	SSR	STHSF014_F_R	StHsf014	Cold
12	SSR	STHSF018_F_R	StHsf018	Cold
13	SSR	STHSF019_F_R	StHsf019	Cold
14	SSR	STHSF022_F_R	StHsf022	Cold

Marker-Assisted Selection (MAS) in potato breeding

Molecular markers allow breeders to pinpoint specific genes associated with desirable traits, improving the precision of selection processes. This is particularly beneficial in crops where traditional breeding methods may be less effective due to complex traits influenced by multiple genes (Woodward *et al*., 2022). The idea of molecular marker-assisted selection (MAS) developed because of extensive research into the potential of selecting genotype rather than phenotype following the discovery of several molecular markers for plant genome analysis. MAS involves the use of molecular markers—such as DNA, RNA, or biochemical markers—that are associated with traits of interest (e.g., disease resistance, abiotic stress tolerance). By identifying these markers, breeders can select individuals that carry favourable alleles linked to beneficial traits, thereby improving the likelihood of developing superior genotypes(Unêda-Trevisoli *et al*., 2017). There have been reports of using molecular markers in potato breeding for viruses, fungal diseases, nematodes and different abiotic stresses as shown in Table 2 and 3.

CRISPR/Cas9

The CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats) system consists of an endonuclease (Cas9) and a guide RNA (gRNA). According to Hille *et al*., (2018), gRNA binds the target gene and directs Cas9 to cut it. Cas9 contains two domains: HNH, which cleaves the complementary DNA

Table 3: Applications of CRISPR/Cas systems for both basic research andagronomic/agroindustrial traits improvement in potato. (Nahirñak *et al*., 2022).

DNA repair pathway	Delivery approach	Target gene	Objective
NHEJ	Agrobacterium tumefaciens	Phytoene desaturase (PDS) and StAA2 gene (encoding an Aux/IAA protein)	Proof of concept
	Agrobacterium tumefacienswith either a conventional T-DNA or a modified geminivirus T-DNA	Acetolactate synthase (ALS)	Proof of concept
	Agrobacterium tumefaciens	Transcription factor gene MYB44	Functional genomics
	Protoplast transfection with DNA vector	Granule-bound starch synthase (GBSS)	Modification of starch composition. High amylopectin.
	Agrobacterium tumefaciens	S-locus RNase (S-RNase)	Elimination of reproductive SI
	Agrobacterium tumefaciens	S-locus RNase (S-RNase)	Elimination of reproductive SI
	Agrobacterium rhizogenes	Steroid 16α-hydroxylase (St16DOX)	Nutritional quality.
	Protoplast transfection with RNP	Granule-bound starch synthase (GBSS)	High amylopectin.
	Agrobacterium tumefaciens	Granule-bound starch synthase (GBSS)	Optimization of Cas9 expression
	Agrobacterium tumefaciens	S-genes (StMLO1, StHDS, StTTM2, StDND1, StCHL1, StDMR6-1 and StDMR6-2)	Biotic stress tolerance.
	Protoplast transfection with RNP	Starch branching enzymes (SBE1 and SBE2)	High amylose and longer amylopectin chains.
NHEJ and base	Protoplast transfection with	Granule-bound starch synthase	Modification of starch composition.
Base editing	DNA vector and Agrobacterium tumefaciens	(GBSS)	High amylopectin.
	Agrobacteriumtumefaciens-mediated transient expression	Acetolactate synthase (ALS)	Herbicide resistance
NHEJ and base editing	Agrobacterium tumefaciens	Granule-bound starch synthase (GBSS) and Downy Mildew Resistant 6 (StDMR6-1)	Proof of concept

strand, and RuvC, which cleaves the non-complementary strand, together creating double-strand breaks (Jinek *et al*., 2014).

The rapid adoption of the CRISPR/Cas system in plant science has enabled targeted gene modifications in crops such as Arabidopsis, wheat, and rice (Nekrasov *et al*., 2013; Shan *et al*., 2013; Matres *et al*., 2021). According to Sevestre *et al*. (2020), potatoes are a major food crop and a key source of raw materials for the food processing sector, making them an important target for genome editing. Accordingly, potatoes have become a significant model and application system for CRISPR/Cas-based genetic engineering, with extensive use of this technology reported in Table 4 (Wang *et al*., 2015).

Potato has major social and economic importance, but self-incompatibility limits breeding. S-RNase controls this trait, and CRISPR-Cas9–mediated removal of S-RNase generated self-compatible diploid potatoes (Ye *et al*., 2018). Targeted S-RNase mutagenesis also produced stable self-compatible lines (Taylor, 2018; Enciso-Rodriguez *et al*., 2019). In addition, haplotype-resolved genomes of tetraploid and heterozygous diploid potatoes now provide a foundation for genome-editing-based breeding (Zhou *et al*., 2020; Sun *et al*., 2022). CRISPR/Cas gene editing accelerates potato breeding for resistance to biotic and abiotic stresses. Disruption of StPHO1 in the CRISPR-edited tMYB44 mutant impaired phosphate transport in potato (Zhou *et al*., 2017). In addition, CRISPR/Cas9 knock outs the S genes (StDND1, StCHL1, and StDMR6-1) leading to increased resistance to late blight in potato (Kieu *et al*., 2021).

CRISPR-based systems have enabled durable disease resistance in potato, including persistent viral resistance using CRISPR/Cas13a (Zhan *et al*., 2019). Potatoes face multiple biotic stresses throughout growth, such as aphids and major diseases (Savary *et al*., 2012), causing 30–60% yield losses (Brown, 2011). Transgenic potato lines expressing Cas13a/sgRNA reduced PVY accumulation and disease symptoms (Zhan *et al*., 2019). In addition, CRISPR-Cas9–mediated disruption of StPHO1 in the tMYB44 mutant impaired phosphate transport in potato (Zhou *et al*., 2017). GBSS has been widely targeted to reduce amylase content in potato (Zong *et al*., 2018; Johansen *et al*., 2019; Tuncel *et al*., 2019; Wang *et al*., 2019; Zhao *et al*., 2021; Toinga-Villafuerte *et al*., 2022), making it a promising genome-editing target for quality improvement. CRISPR/Cas9 deletion of St16DOX eliminated SGA accumulation in potato hairy roots (Nakayasu *et al*., 2018). Yield improvement has been achieved by introducing AtPAP2 (Zhang *et al*., 2014), inserting an Agrobacterium auxin biosynthetic gene (Kolachevskaya *et al*., 2015), downregulating stsu4 (Chincinska *et al*., 2008), and mutating StIT1 using CRISPR/Cas9, which increased stolon branching and tuber yield.

CRISPR/Cas offers advantages in ease of use, adaptability, efficiency, and cost over TALENs and ZFNs (Cho *et al*., 2013; Wang *et al*., 2013; Castro *et al*., 2021; Urnov *et al*., 2010). Its application in crop improvement is widely reviewed (Chen *et al*., 2019; Massel *et al*., 2021), and modified Cas9 proteins have enabled precise base and prime editing (Molla *et al*., 2021). CRISPR/Cas9 has been successfully applied in crops such as Oryza sativa, Nicotiana tabacum, Sorghum bicolor, and Arabidopsis thaliana (Jiang *et al*., 2013; Hussain *et al*., 2018; Chen *et al*., 2019).

Conclusion

Biotechnological tools have significantly advanced potato improvement, enabling higher yield, better nutrition, and enhanced stress tolerance through approaches like tissue culture, genetic engineering, marker-assisted breeding, and CRISPR/Cas9. These technologies address key challenges such as diseases and environmental stresses, supporting the development of resilient varieties. Expanding their use, especially in developing countries, is essential for sustainable agriculture and global food security.

Author contribution: Arusha Sharma, Davinder Singh, Surender Kumar Dhankhar: literature search and writing; Vikash Kumar: drafting and revising the manuscript; MK Rana, Yogita Chaudhary: design and editing; Shrawarna Sarma: critical discussions and revisions of the manuscript.

Conflict of interest: There is no conflict of interest between authors.

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