OPEN ACCESS Freely available online

Journal of
Plant Pathology & Microbiology

Review Article

Marker-based Methods for Locating and Transferring of Viral Resistance Genes in Potato: A Review

Molla G. Taye[1]*, András P. Takács[2]

[1]*Department of Biotechnology, Tigray Biotechnology Center, Mekelle, Ethiopia*
[2]*The Hungarian University of Agriculture and Life Sciences, Institute for Plant Protection, Keszthely, Hungary, 8360*

ABSTRACT

Potato is a diverse and well-adapted food, feed, and industrially important non-cereal crop in the world. There are more than 40 viral diseases that have been recorded and have an inherent capacity of infecting potato universal. The yield reduction due to potato viruses can reach up to 80% with the possibility of total crop failure. Alleviating the potato virus diseases induced challenges involved various viral management options. However, breeding for viral-resistant potato varieties via efficient and accurate breeding techniques like marker-assisted selection (MAS) has made significant improvements in sustainable potato virus disease management. Therefore, this review gives some insight into what is known about the latest DNA-based mapping techniques and introgression approaches used in potato virus resistance breeding schemes in general. Eventually, this review briefly discusses and summarizes the properties of an ideal genetic marker, types of genetic markers, genetic linkage mapping, genome association mapping, and QTL detection, RNA-seq, whole-genome re-sequencing, pyramiding, marker-assisted selection, and CRISPR/Cas9 genome editing technology.

Keywords: Potato breeding; MAS; GWAS; RNA-seq; CRISPR/Cas9

INTRODUCTION

Potato is the third most principal non-cereal food crop in the globe, next to rice and wheat regarding human consumption. Potato's yearly production was a bit higher than 376 million tonnes from over 17 million hectares of cultivated land [1]. The potato tubers are a rich source of carbohydrates, vitamin C, B, potassium, and dietary fiber [2,3]. Presently, the potato is designated as a food and nutrition security crop, especially for the developing world. Regarding its genetic diversity, the potato has owned one of the richest gene pools of any other cultivated plants, as of 2021 there are more than 4,500 varieties of native potatoes and 100–180 known wild species [4], similarly previous study by Spooner DM and Hijmans RJ [5] has grouped about 200 tuber-bearing species in the section Petato of the genus Solanum. Besides, about 98,000 accessions were conserved *ex-situ* [6]. Within these, about 22,597 potato accessions are recorded in GENESYS. Despite its importance, the potato is affected by numerous biotic agents because both ware and seed producers have been facing varying magnitude of yield deterioration both in quantity and quality. Among the stringent biotic factors of potato production, viral impact negatively affected the overall profitability of the crop after late blight. To this end, more than 40 viral diseases have been recorded with an inherent capacity of infecting potatoes globally [7], only a few of them are inducing meaningful yield losses worldwide. The most damaging major potato viruses are PVY, PLRV, PVX, PVA, PVM, and PVS, of which PVY is the furthermost economically important and severe virus of all [8].

The crop yield losses due to PVY can reach up to 80% [9]. Similarly, Visser JC, et al. [10] have reported that a yield loss of 50%-100% due to severe PVY infection in different potato varieties. Likewise, potato virus X (PVX), which belongs to the genus potexvirus, causes yield losses of approximately 10%-20% [11]. However, the prevalence and economic importance of potato viruses differ concerning geographical locations, varieties in use, plant developmental stages, virus strain, environmental conditions, and management.

LITERARTURE REVIEW

As to alleviate the potato virus disease induced challenges various viral management options have been in place. In this regard, the use of clean planting materials, agrochemicals to control the potential vectors, applying phytosanitary measures, use of resistant potato varieties generated via different breeding schemes were some of the most frequently employed ones. However, breeding

Correspondence to: Molla G. Taye, Department of Biotechnology, Tigray Biotechnology Center, Mekelle, Ethiopia, Tel: +251960132552; E-mail: mogereme1982@gmail.com

Received: September 23, 2021; **Accepted:** December 23, 2021; **Published:** December 30, 2021

Citation: Taye MG, Takacs AP (2021) Marker-based Methods for Locating and Transferring of Viral Resistance Genes in Potato: A Review. J Plant Pathol Microbiol 12:588.

for viral-resistant potato varieties using efficient and accurate breeding techniques like marker-assisted selection (MAS) has made significant improvements in sustainable potato virus disease management. DNA marker technology can offer a great promise for potato virus-resistant breeding. Owing to linkage maps, allelic variation in the genes underlying virus resistance can be detected by DNA markers. Once a gene (s) is identified, they can be transferred from the donor to the recipient plants by the horizontal gene transfer approach, which is one the most dominant principal mechanism of gene transfer [12].

In this respect, plenty of major Ry and Ny genes has been identified in various potato cultivars in the form of extreme resistance (immunity) and hypersensitive response (HR) respectively. These major resistance genes are mainly sourced from S. tuberosum subsp. Andigena, *S. stoloniferum, S. acaule, S. megistacrolobum, S. tuberosum, S. housgasii* [13]. Following the identification of resistance genes from wild and cultivated potatoes, incorporation of R genes (major genes) for extreme resistance example to PVY into susceptible but adapted and high yielded potato varieties was possible even by conventional breeding [14].In the process, numerous genetic markers closely linked to various potato virus resistance genes have been localized to the different chromosomal locations and users, and some of them are Ryadg [15]; Rysto [16]; Rychc [17]; Rx1 [18] and Rx2 [19]. It is believed that marker-based methods for locating and transferring viral resistance genes in potato not only increase efficiency and precision but also fastens the breeding time. Therefore, this work gives some insight into what is known about the latest DNA-based mapping techniques and introgression approaches used in the potato virus resistance breeding scheme.

Properties of ideal genetic markers

A genetic marker to be supreme should meet most of the following parameters:

1. **Polymorphic:** variability is the backbone of breeding so that starting breeding materials should be genetically variable, then able to show polymorphism;
2. **Multi-allelic**: the frequency and range of polymorphism can be increased if plants owned two or more variant alleles;
3. **Codominant**: neither one of the alleles is recessive and the phenotypes of both alleles can be expressed in both parents at heterozygous and homozygote states;
4. **Non-epistatic**: since the appearance of a marker of an individual genotype can be recognized outwardly, irrespective of the area of the chose marker in the genome of an individual (codominance and a non-epistatic indication of a character can be defined as the shortfall of intra-and interlocus interaction, respectively);
5. **Neutral**: the substitution of alleles at the marker locus has no phenotypic or particular impact (polymorphism at the molecular level of DNA isoften impartial);
6. **Insensitive to environmental influences**: this ought to be shown in the relationship of the phenotype and the indication of a marker or marker characteristics, irrespective of the ecological openness.

Types of genetic markers

Markers are tightly associated with the gene of interest and they act as emblems or flags [20]. Genetic markers are usually classified into three core classes namely morphological, molecular, and biochemical markers. Typical classical markers are morphological, cytological, and biochemical, whereas some of the molecular markers are Restriction Fragment Length Polymorphism (RLFP), Amplified Fragment Length Polymorphism (AFLP), simple sequence repeats (SSRs), Single Nucleotide Polymorphism (SNPs), and Diversity Array Technology (DArT) markers [21].

Morphological markers : Morphological markers are commonly observable signs of phenotypically varying parameters of given crop plants. These can be a leaf and flower shape, color and size; seed size, color and shape; root system, and other physically distinguishable characteristics. Morphological markers are the most freely available and easiest to use markers. Alternatively, their detection requires neither a piece of special equipment nor reagents (Table 1). Their principal demerits are connected with their limited availability, constantly under the influence of the changing environmental conditions, rely on the developmental stage of the plant or organ or tissue specificity in which they originate [22]. Morphological markers have made an indispensable contribution towards genetics and plant breeding developments. Despite the aforementioned limitations, morphological markers remain genuine and worthwhile scientific tools in practical plant breeding [23]. There be occasions where morphological, biochemical, and molecular markers are used simultaneously to increase the efficiency of the breeding program or in some cases because both biochemical and molecular markers shared some common features (Table 1).

Biochemical markers : Isozymes are multi-molecular forms of enzymes coded by various genes, which have the same functions are termed as biochemical markers [24]. Isozymes are allelic dissimilarities of enzymes and consequently, gene and genotypic frequencies can be established with biochemical markers. Effective detection of genetic diversity, population structure, and gene flow, and population subdivision has been operated via biochemical markers [25]. These markers are co-dominant, easy to use, and cost-effective. However, they are less in number, they detect less polymorphism, and they are affected by various extraction methodologies, plant tissues, and different plant growth stages [26]. Adapted [27]

DNA molecular markers : DNA molecular markers are demarcated as a piece of DNA enlightening mutations that can be used to identify polymorphism between diverse genotypes or alleles of a gene for a specific sequence of DNA in a population. Such fragments are connected with a certain position within the genome and may be discovered utilizing certain molecular tools. DNA marker is a small region of DNA sequence displaying polymorphism (base deletion, insertion, and substitution) between different individuals. According to Southern EM [28], there are two basic methods to detect polymorphism namely a nuclear acid hybridization technique and Southern blotting. Some years later the PCR (polymerase chain reaction technique has been introduced by Mullis KB [29]. By using these advancements, the disparities in DNA samples or polymorphism for a specific region of DNA sequence can be recognized based on product features, such as band size and mobility. DNA markers/molecular markers have been playing a foremost role in molecular plant breeding. The comparison of the most widely used DNA markers is presented in Table 2.

Numerous markers linked to ER against many potato virus diseases have been reported. Some examples of DNA markers are the YES3-3A, YES3-3B and GP122 for Rysto [30-32] and the RYSC3 for

Table1: Genetic markers, their characteristics, and features.

Features	Markers		
	Morphological	Biochemical	Molecular
Detection	Visual	Dying in a gel via various dyes and substrates	Dying in a gel ethidium bromide, fluorescent, and radioactive tags
Level of detection	Phenotype	Proteins and metabolites	Nucleic acid (DNA, RNA)
Mode of inheritance	The dominant, recessive	Dominant, co-dominant	Dominant, co-dominant
Possibility of automation	No	No	Yes
Occurrence in genome	Low	Low	High
Specialized equipment required	Now	Yes	Yes
Cost	Low	Average	High

Table 2: Comparison of most widely used DNA marker systems in plants; adapted from and others.

Feature and description	RFLP	RAPD	AFLP	SSR	SNP
Genomic abundance	High	High	High	Moderate to high	Very high
Genomic coverage	Low copy-coding region	Whole-genome	Whole-genome	Whole-genome	Whole-genome
Expression/inheritance	Co-dominant	Dominant	The dominant/codominant	Co-dominant	Co-dominant
Number of loci	Small (<1,000)	Small (<1,000)	Moderate (1,000s)	High (1,000s – 10,000s)	Very high (>100,000)
Level of polymorphism	Moderate	High	High	High	High
Type of polymorphism	Single base changes, indels	Single base changes, indels	Single base changes, indels	Changes in length of repeats	Single base changes, indels
Types of probes/primers	Low copy DNA or cDNA clones	10 bp random nucleotides	Specific sequence	Specific sequence	Allele-specific PCR primers
Cloning and sequencing	Yes	No	No	Yes	Yes
PCR-based	Usually no	Yes	Yes	Yes	Yes
Radioactive detection	Usually yes	No	Yes or no	Usually no	No
Reproducibility/reliability	High	Low	High	High	High
Effective multiplex ratio	Low	Moderate	High	High	Moderate to high
Marker index	Low	Moderate	Moderate to high	High	Moderate
Genotyping throughput	Low	Low	High	High	High
Amount of DNA required	Large (5 – 50 µg)	Small (0.01–0.1 µg)	Moderate (0.5–1.0 µg)	Small (0.05 – 0.12 µg)	Small (≥ 0.05µg)
Quality of DNA required	High	Moderate	High	Moderate to high	High
Technically demanding	Moderate	Low	Moderate	Low	High
Time demanding	High	Low	Moderate	Low	Low
Ease of use	Not easy	Easy	Moderate	Easy	Easy
Ease of automation	Low	Moderate	Moderate to high	High	High
Development/start-up cost	Moderate to high	Low	Moderate	Moderate to high	High
Cost per analysis	High	Low	Moderate	Low	Low
Number of polymorphic loci per analysis	1.0 – 3.0	1.5 – 5.0	20 – 100	1.0 – 3.0	1.0
Primary application	Genetics	Diversity	Diversity and genetics	All purposes	All purposes

Ryadg [33]. In this regard, the use of genetic markers for selecting cultivars with desirable traits has proven to be time and cost-efficient in plant breeding [34]. Nevertheless, only markers that are closely associated with the desired gene have the potential for increasing selection efficiency [35,36].

Relative benefits of molecular markers

In conventional potato breeding, the development of new varieties takes 10–12 years. This classical breeding scheme starts with a huge breeding population following à series of phenotypic recurrent selections over numerous generations [37]. Contrarily, MAS offers prominent merits over classical breeding methods in such a way that it saves time by dropping the number of breeding steps, the selection at an early development stage, reduced number of lines to be tested, and individual plant selection based on their gene or marker [38]. However, there is still a controversy whether MAS offers a similar efficiency on polygenic, low heritable, and with possible large genotype and environment, interactions compared to monogenic and simple traits. As witnessed by Bouches A, et al [39]. MAS is most successful with relatively simple traits and those inherited in à Mendelian fashion.

Approaches for localizing potato virus resistance markers/genes

Genetic linkage mapping : The idea of the construction of a genetic linkage map was proposed in 1980, by Botstein D, et al [40] in humans based on restriction fragment polymorphisms (RFLPs). Then, they could successfully construct not alone the linkage map of humans but also other wide variety of organisms, these maps were found beneficial for countless genetic studies. However, most of the published RFLP maps were low and moderate density, which was considered one of the inherent limitations that could have been incredulous by the development of high-density maps. To this end, a high-density molecular linkage map consisted of more than 1000 markers with an average spacing between markers of approximately 1.2 cm (ca. 900 kb), has been constructed for the tomato and potato genomes via restriction fragment length polymorphism markers [41]. Likewise, amplified fragment length polymorphism (AFLP) markers have for potato been also developed and are used.This genetic map of potatoes integrating molecular markers with morphological and isozyme markers was constructed using a backcross population of 67 diploid potato plants [42]. Simple sequence repeat (SSR) markers have been developed and are used extensively in potato research and mapping approaches [43,44]. Sequencing of the potato genome enabled the discovery of many single-nucleotide polymorphisms (SNPs) within the potato genome [45].

As a result, many host resistance genes have been reported from wild and cultivated potato species.Examples of such host resistances are Rysto from *Solanum stoloniferum* that located at chromosome 12 [46,47] Ry_{adg} from *S. tuberosum* Group *andigena*, chromosome 11 [48] and Ry_{chc} from *S. chacoense*, chromosome 9 [49].Lately, the Rysto gene was isolated from the dihaploid clone Alicja and located to cypher a nucleotide-binding leucine–rich repeat (NB-LRR) protein with an N-terminal TIR domain, a structure common to several diverse plant infective agent resistance proteins[50].Expression of this protein in transgenic *S. tuberosum* cultivars (cv Maris Piper and Russet Burbank) rendered these plants immune to PVY infection, therefore demonstrating the quality of research to detect and map PVY resistance genes from dissimilar sources.

Genome association mapping and QTL detection : Genome-wide association study (GWAS) is an approach involving scanning of markers across DNA and genomes to identify genotype-phenotype associations and genetic variations associated with a particular disease [51]. Initially, bi-parental crosses were utilized to map quantitative trait loci (QTL) in plants, however, these materials were limited in allelic diversity and in having restricted genomic resolution [52]. Some challenges connected with the old-style of gene mapping have been resolved by the genome-wide association strategy (GWAS) are:

(i) Providing higher resolution, often to the gene level, and

(ii) Using samples from formerly well-studied populations in which frequently happening genetic variations can be linked with phenotypic variations. Besides all these advantages, the identification of QTLs/genes, and the interaction among them can be detected through GWAS.

GWAS has become progressively significant in crop genetic studies, and it is well-matched for applications such as QTL analysis and genotyping [53]. Recently, numerous new technologies such as GWAS, RNA-seq, and WGRS have been exploited for assessing potatoes under virus infection conditions. Because of the breeding process, resistances recognized as quantitative genetic traits and simple genetic traits in the viruses are reassigned to potatoes [54]. In potatoes, breeders have used numerous molecular markers, namely, SSR, SCAR, CAPS, and AFLP, to detect molecular markers connected with virus resistance genes. Based on the results found in this study, the SCAR marker RYSC3 and the CAPS marker GP122564 were linked with the PVY resistance gene Ryadg and Rysto, respectively. The locus for Rysto to PVY was mapped on chromosome XII and was co-segregating with an SSR marker in potato.

In pepper, genome-wide association studies (GWAS) identified seven SNPs significantly connected with the virus population size at inoculation and systemic level on chromosomes 4, 6, 9, and 12. Two SNPs on chromosome 4 linked with both PVY population sizes map closely to the major resistance gene pvr2 encrypting the eukaryotic initiation factor 4E [55].The utilization of GWAS has delivered the identification of the SNPs linked to PVYO and PVY NTN. These results disclosed that NY (o,n) sto gene confers resistance to two viruses of potato [56]. Additionally, the Ry-fsto gene, another resistance gene resulting from S. stoloniferum, conferred broad-spectrum resistance to PVY [56]. Despite the advantages, there are also shortcomings for using GWAS such as:

1) Only restricted to traits with well-known biochemical and molecular data [57].

2) Likely to miss detection of essential yet unidentified genes involved in the desirable [58],

3) Inappropriate to minor-effect genes controlling complex traits [59].

Whole-genome re-sequencing: Whole-genome re-sequencing (WGRS) is a principal means for examining complete genomes with full well-known genome sequencing or all-inclusive genome sequencing to determine the whole DNA sequence of an organism's genome simultaneously [60]. The WGRS technique comprises the subsequent steps: many potato viruses tolerant genotypes are selected and planted; genomic DNA extraction is done from biweekly seedlings using the CTAB method; genomic DNA is cut into 500-bp fragments using sonication; DNA fragments are used to construct the DNA library; DNA library is sequenced using Illumina platform, and readings are found followed by re-sequencing. Re-sequencing includes the whole sequencing of the potato plant genome and its comparison with the reference genome sequence. Readings for each genotype are filtered and mapped, and the genes and virus-tolerant SNPs are recognized in potatoes. GWRS tactic involves sequencing all of an organism's chromosomal DNA to classify inherited disorders, depicting the mutation, recombination, and gene conversion in the genome [61]. WGRS can modernize QTL mapping, genome diversity, identification of selection signatures, and detection of candidate genes, and SNPs without a time-consuming and high cost [62].

RNA-seq: A transcriptome is the set of all RNA transcripts, comprising coding and non-coding, in an individual or a population of cells; it can also sometimes be used to refer to all RNAs or just mRNA. Transcriptome analysis is a potent approach to discern and deduce the gene content of a plant or an organism where the genome sequence is not available. Transcriptome can be studied by DNA microarray, a hybridization-based technique, and RNA-seq, a sequence-based approach [63].To this end, RNA-seq can investigate genes associated and expressed in varied mechanisms and tissue/cells of a plant [64]. The steps of RNA-seq comprise Phase 1 inclosing the mRNA is isolated from the susceptible and resistant

potatoes. The fragments are sequenced using one of the suitable sequencing platforms. These sequences can then be affiliated to a reference genome to reconstruct which genome areas were being transcribed. Though there is a reference genome is aligning, the de novo assembly can be used to reconstruct. Eventually, the sequences can be utilized to annotate were novel genes/transcripts.

Marker-assisted Selection

For effective MAS three important and interrelated considerations have been recognized. These consist of the traits of interest to be selected for genetic screening, the characteristics of molecular markers to be used and the marker technology to be mobilized. To this end, the trait of interest to be chosen should be unambiguous and has a discrete phenotype, like a trait conferred by a major gene. Furthermore, the efficiency of MAS can be enhanced by merging the screening for many traits employing plenty of markers immediately. In this regard, the DNA marker to be employed in MAS should be tightly linked to the trait of interest. This does not only provide a high level of confidence for selecting a few false negatives and positives among the chosen plants but also secures a high degree of accuracy. As reviewed by Bodo R and Trognitz Friederike C [65] markers work in various genetic backgrounds and can be applied with little technical effort. In reality, MAS will be economical when applied through comparatively safe, robust, and cheap technology.

Besides, MAS is one of the indirect selection methods, and its applicability relies on the association between genetic and phenotypic variations at a given locus as measured by specific markers; the trait marker correlation is highly advantageous. Noticeably, the phenotype of interest needs to be indisputably linked with the genomic region and thus determination of which is the prior center of QTL mapping systems [66]. Eventually, a molecular marker to be ideal should forecasts the phenotype of interest in a breeding population regardless of their genetic backgrounds. Practically, the smaller the physical distance between the traits of interest and a predictor marker locus, the larger the linkage disequilibrium is between marker and trait alleles, which rises the diagnostic value of the marker [67].

Recombinant DNA technology

Recombinant DNA was first accomplished in 1973 by Herbert Boyer, of the University of California at San Francisco, and StanleyCohen at Stanford University, who used E. coli restriction enzymes to insert foreign DNA into plasmids (www.genomenewsnetwork.org). Recombinant DNA is a form of synthetic DNA that is engineered via the combination or insertion of one or more DNA strands, thus merging DNA sequences that would not normally occur together. Likewise, recombinant DNA technology is an assembly of molecular genetic techniques that permit isolation, cloning, and expression of a gene from one organism in the same or, another organism [68].Under certain circumstances, a recombinant DNA molecule can enter a cell and reproduce there, either on its own or after it has been unified into a chromosome. These DNA molecules can be formed by laboratory methods of genetic recombination to get together genetic material from several sources, creating sequences that would not otherwise be found in the genome [69].

Recombinant DNA (rDNA) technology is a promising technology that has transformed various features of agriculture. So far, this technology has undeniably been recognized to progress the growth, development, yield, and plant-environmental interactions [70]. To this end, the potato is a clonally propagated crop via tissue culture techniques, making it amenable to gene integration by employing gene transformation technology [71].Almost all potato cultivars are capable of transforming using Agrobacterium tumefaciens and tissue regeneration. Agrobacterium is the most common method for stable transformation in a potato, although other methods such as particle bombardment, protoplast transformation, and microinjection are also successful. To this end, Monsanto had developed for the first time a potato transgenic variety called Russet Burbank, which is resistant to potato leafroll virus (PLRV) [72]. Recently, transgenic potato lines could overexpress the pepper resistance allele pvr12 and become resistant to the three (PVY^{O}, $PVY^{N:O}$, and PVY^{NTN}) predominant potato infecting strains of PVY [73].As reviewed by Brown KJ [74] only 6-12 months are needed to introduce a specific gene into potato using Agrobacterium, followed by regeneration of the whole plant.

Pyramiding

Plants' diseases can impose a reasonable crop yield reduction. On one hand, the durability of disease resistance is vital to achieving both universal food security, and agricultural sustainability [75]. Pyramiding (major) R-genes can be a way out to advance on both the level of resistance and on durability [76].Similarly, a wider range of resistance can be realized. Pyramiding is the build-up of (R)-genes into a particular genotype or cultivar and can be done using major R-genes, defeated R-genes, different alleles of one gene, or the same alleles. Diverse methods have been projected for sufficiently deploying major R genes and resistance QTL, to cumulate the durability of crop resistance to pathogens. The sustainable management of existing genetic resistance factors includes:

(i) The use of multi-line varieties or varietal mixtures harboring dissimilar R genes or QTL [77],

(ii) The rotation in space or time of various R genes [78], and

(iii) The combination (i.e., pyramiding) of R genes or QTL in the same genotype [79].

Breeding schemes for pyramiding major R genes have been comprehensively trialed [80] and resulted in the generation of resistant varieties that are broadly cultivated [81]. Approaches encompassing combinations of major genes, each conferring resistance to several unambiguous isolates, were recommended to increase resistance durability [82]. A study on potato plants from a hybrid population was attained by crossing the Mexican species Solanum neoantipoviczii sample, combining high resistance to late blight (gene R2-like) and Potato virus Y (PVY) (gene Ry_{sto}), with the variety Aurora selection. Aurora selection holds the recognized markers of genes responsible for resistance to nematode (Globodera rostochiensis) of Ro1 pathotype (gene H1) and late blight (gene R1). Genotyping data of this crossing the combination demonstrate the availability of the pathogen resistance gene pyramiding when crossing components complementing each other according to the detected markers [83]. In pepper, the major *pvr2*[3] resistance allele to *Potato virus* Y (PVY) was hurriedly stunned under controlled experimental conditions [84]. The combination of pvr23 with three incomplete resistance QTL considerably increased resistance durability under the same conditions.

CRISPR/Cas9 genome editing technology

Crop plant genome editing is a promptly developing technology that allows targeted changes to be introduced into a plant genome in a highly specific and precise manner. The method, for the most part, does not include transgenic changes and is significantly superior to chemical mutagenesis. To this end, accurate gene targeting and mutation including gene insertions/deletion, gene replacements, and single base pair conversions became possible with the help of the lately developed CRISPR/Cas 9 (Clustered regularly interspaced short palindromic repeats-associated protein 9) genome editing technology [85]. CRISPR/Cas9 was primarily discovered as an adaptive immune defense system in bacterial cells as a mechanism to defend against foreign DNA [86].When used for genome editing, the CRISPR/Cas9 mechanism essentially comprises two parts: a guide RNA (gRNA) and the Cas9 endonuclease. A gRNA is 20 nucleotides (nt) long and is an extremely gene-specific sequence [87]. Each gRNA is complementary and binds to an exact target DNA sequence that ends with a short DNA sequence, known as the proto spacer adjacent motif (PAM), which is often "NGG." The PAM region is indispensable for Cas9 binding and is found 3 bp downstream of the cleavage site of the Cas9 endonuclease [88]. Adjacent to the 3′ end of the 20 nt gRNA is an ~80 nt long gRNA scaffold sequence that is essential for Cas9 binding [89].

The precise cut (double-stranded break, DSB) is predominantly repaired by non-homologous end joining (NHEJ), which is commonly error-prone and results in insertion or deletion (indel) mutations at the cut site [90]. Such indel mutations lead to frame-shift mutations, affecting protein translation and thus disturbing a gene's function. Plant scientists have begun exploiting CRISPR/Cas9 gene-editing technology in both model and crop plants to deploy genetic pathways, improve various agronomic traits and produce pathogen-resistant crops.

CONCLUSION

Potato yields have been reduced by a reasonable amount in terms of quantity and quality due to a variety of potato virus infections. Many significant Ry and Ny genes have been identified in diverse potato cultivars in the form of immunity and hypersensitivity response, respectively, as a long-term solution. The majority of these significant resistance genes come from wild potato cultivars, such as *S. tuberosum* subsp. *Andigena*, *S. stoloniferum*, *S. acaule*, *S. megistacrolobum*, *S. tuberosum*, *S. housgasii*. Thanks to the use of closely related genetic markers and advances in molecular biology, it has become obvious to insert the extremely resistant R gene (s) of potato virus into high-yielding susceptible but adaptable potato cultivars.

Therefore, it is highly recommended for future work to find out new potato viral resistance genes with broad-spectrum and durable nature through a systematic combination of resistance breeding schemes like marker-assisted selection, CRISPER/Cas9, RNA sequencing, and genome-wide association study. In developing potato virus-resistant varieties genetic markers have to be wisely exploited in combination with the classical breeding schemes for identifying, localizing, introgressing, and validating the functionality of the gene (s) of interest. Overall, it is unadventurous to summarize that the use of viral resistant potato varieties is one of the best potato viral diseases while securing the farm productivity, economical feasibility, user-friendly and largely environmentally safe and sustainable.

AUTHOR CONTRIBUTION

The authors have acknowledged liability regarding the whole substance of this submitted review paper and endorsed submission.

RESEARCH FUNDING

The work was sponsored by the EFOP -3.6.3-VEKOP-16-2017-00008 project, which is co-financed by the European Union and the European Social Fund.

CONFLICT Of INTEREST STATEMENT

The authors pronounce no irreconcilable circumstance in regards to this article.

REFERENCES

1. FAOSTAT. https://www.statista.com/statistics/192966/us-potato-production-since-2000/. 2019, Retrieved 4 5, 2021.
2. McGill CR, Kurilich Anne C, Davignon J. The role of potatoes and potato components in cardiometabolic health: A review. Ann Med. 2013;45(7):467-473.
3. Camire ME, Kubow S, Donnelly DJ. Potatoes and human health. Crit Rev Food Sci Nutr. 2009; 49(10):832-840.
4. CIP (International Potato Center). Retrieved 4 5, 2021.
5. Spooner DM, Hijmans RJ. Geographic distribution of wild potato species. Am J Bot. 2001;88(11):2101-2112.
6. FAO (Food and Agriculture Organization of the United Nations). The second report on the state of the world's plant genetic resources for food and agriculture.2010.
7. Valkonen J. Potato viruses: Economical losses and biotechnological potential. Potato biology and biotechnology: Advances and perspectives. 2007;619-641.
8. Zhang W, Bai Y, Gao Y, Sheng Y, Fan G. A survey on occurrence frequencies of potato viruses in major-producing provinces in China. Heilogjiang Agri Sci. 2010;4:71-73.
9. Ahmadvand R, Takacs A, Wolf I, Polgar Z, Taller J. Potato viruses and resistance genes in potato. Acta Agronomica Hungarica. 2012;60:283-298.
10. Visser JC, Bellstedt DU, Pirie MD. The recent recombinant evolution of a major crop pathogen, potato virus Y. PLoS One. 2012;7:e50631.
11. Palukaitis P. Resistance to viruses of potato and their vectors. Plant Pathol J. 2012;28:248-258.
12. Jin G, Nakhleh L, Snir S, Tuller T. Inferring phylogenetic networks by the maximum parsimony criterion: A case study. Mol Biol Evol. 2007;24(1):324-337.
13. Barker H. Extreme resistance to potato virus Y in clones of Solanum tuberosum that are also resistant to potato viruses Y and A: Evidence for a locus conferring broad-spectrum resistance. Theor Appl Genetic. 1997; 95:1258-1262.
14. Simko I, Shelley J, Stephenson S, Spooner D. Genetics of resistance to pests and disease. In potato biology and biotechnology. Elsivier. 2007;117-155.
15. Hamalainen J, Watanable K, Valkonen J, Arihara A, Plaisted R. Mapping and marker-assisted selection for a gene for extreme resistance to potato virus Y. Theor Appl Genet. 1997;94:192-197.
16. Cernak I, Decsi K, Nagy S, Wolf I, Polgar Z. Development of a locus-specific marker and localization of the Ry_{sto} gene based on linkage to a catalase gene on chromosome XII in the tertraploid potato genome. Breeding Sci. 2008;58:309-314.

17. Sato M, Nishikawa K, Kormura K. Potato virus Y resistance gene Rychc mapped to the distal end of potato chromosome 9. Euphytica. 2006;149:367-372.

18. Gebhardt C, Bellin D, Henselewski H, Lehmann W, Schwarzfischer J. Marker-assisted combination of major genes for pathogen resistance in potato. Theor appl genet. 2006;112(8):1458-1464.

19. Ritter E, Debener T, Salamini F, Gebhardt C, Barone A. RFLP Mapping on potato chromosome of two controlling extreme resistance to potato virus X (PVYX). Mol Gen Genet. 1991;227(1):81-85.

20. Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica. 2005;142:169-196.

21. Jiang GL. Molecular markers and marker-assisted breeding in plants. Plant Breeding from Laboratories to Fields. 2013.

22. Winter P, Kahl G. Molecular marker technologies for plant improvement. World J Microbiol. Biotechnol. 1995;11(4):438-448.

23. Eagles H, Bariana H, Ogbonnaya F, Rebetzke G, Hollamby G. Implementation of markers in Australian wheat breeding. Aust J Agric Res. 2001;52:1349-1356.

24. Bayley D. Isozymic variations and plant breeder's rights. In: Tanksley SD, OrtonTJ, editors. Isozymes in plant genetics and breeding. Amsterdam: Elsevier. 1983.

25. Mateu-Andres I, De Paco L. Allozymic differentiation of the *Antirrhinum majus* and *A. siculum* species groups. Ann Bot. 2005;3:465-473.

26. Mondini L, Noorani A, Pagnotta M. Assessing plant genetic diversity by molecular tools. Diversity. 2009;1(1):19-35.

27. Chesnokov YV, Kosolapov VM, Savchenko IV. Morphological genetic markers in plants. Russ J Genet. 2020;56:1406-1415.

28. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol. 1975;98(3):503-517.

29. Mullis KB. The unusual origin of the polymerase chain reaction. Sci Am. 1990;262(4):56-65.

30. Flis B, Hennig J, Strzelczyk-Zyta D, Gebhardat Ch, Marczewski W. The Ry-fsto gene from *solanum stoloniferum* for extreme resistance to potato virus Y maps to potato chromosome XII and is diagnosed by PCR marker GP122718in PVY resistant potato cultivars. Mol Breeding. 2005;15:95-101.

31. Song Y, Schwarzfischer A. Development of STS markers for selection of extreme resistance (Ry_{sto}) to PVY and maternal pedigree analysis of extremely resistant cultivars. Am J Po Res. 2008;85:159-170.

32. Valkonen JPT, Wiegmann K, Hamalainen JH, Marczewski W, Watanable KN. Evidence for utility of the same PCR-based markers for selection of extreme resistance to potato virus Y controlled by Ry_{sto} of *Solanum stoloniferum* derived from different sources. Ann Appl Biol.2008;152:121-130.

33. Kasai K, Morikawa Y, Sorri V, Valkonen J, Gebhardt C, and Watanable K. Development of SCAR markers to the PVY resistance gene Ryadg based on a common feature of plant disease resistance genes. Genome. 2000;43(1):1-8.

34. XuY, Crouch JH. Marker-assisted selection in plant breeding: From publications to practice. Crop Sci. 2008;48:391-407.

35. Semagn K, Bjornstad A, Skinnes H, Maroy A, Tarkegne Y, William M. Distribution of DArT, AFLP, and SSR markers in a genetic linkage map of a doubled-haploid hexaploid wheat population. Genome. 2006;49(5):545-555.

36. Xu P, Wu X, Wang B, Liu Y, Qin D. Development and polymorphism of *Vigna unguiculata* ssp *unguiculata* microsatellite markers used for phylogenetic analysis in asparagus bean (*Vigna unguiculata* ssp. *sesquipedialis* (L) Verdc). Mol Breeding. 2010;25:675-684.

37. Gebhardt C. Bridging the gap between genome analysis and precision breeding in potato. Trends Genet. 2013;29(4):248-256.

38. Slater A, Cogan N, Forster J. Cost analysis of the application of marker-assisted selection in potato breeding. Mol Breed. 2013;32:299-310.

39. Bouches A, Hospital F, Causse M, Gallais A, Charcosset A. Marker-assisted introgression of favorable alleles at quantitative trait loci between maize elite lines. Genetics. 2002;162(4):1945-1959.

40. Botstein D, White M, Skolnick M, Davis R. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet. 1980;32(3):314-331.

41. Tanksley S, Ganal M, Prince J, De Vicente M, Bonierbale M. High-density molecular marker linkage maps of the tomato and potato genomes. Genetics. 1992;132(4):1141-1160.

42. Jacobs JME, Van Eck HJ, Arens P, Stiekema WJ, Jacobsen E, et al. A genetic map of potato (*Solanum tuberosum*) integrating molecular markers, including transposons, and classical markers. Theor Appl Genet. 1995;91:289-300.

43. Feingold S, Lioyd J, Norero N. Mapping and characterization of new EST-derived microsatellites for potato (*Solanum tuberosum* L). Theor Appl Genet. 2005;111(3):456-466.

44. Ghislain M, Nunez J, Herrera M. The single Andigenum origin of neo-tuberosum potato materials is not supported by microsatellite and plastid marker analyses. Theor Appl Genet. 2009;118(5):963-969.

45. Uitdewilligen J, Wolters A, D'hoop B, Borm T, Visser R. Correction: A next-generation sequencing method for genotyping-by-sequencing of highly heterozygous autotetraploid potato. PLoS One. 2015;10: e0141940.

46. Flis B, Hennig J, Strzelczyk-Zyta D. The Ry-fsto gene from *solanum stoloniferum* for extreme resistance to potato virus Y maps to potato chromosome XII and is diagnosed by PCR marker GP122718in PVY resistant potato cultivars. Mol Breeding. 2005;15:95-101.

47. Song Y, Hepting L, Schweizer G, Hartl L, Wenzel G. Mapping of extreme resistance to PVY (Ry_{sto}) on chromosome XII using anther-culture-derived primary dihaploid potato lines. Theor Appl Genet. 2005;111: 879-889.

48. Hamalainen J, Watanable K, Valkonen J, Arihara A, Plaisted R. Mapping and marker-assisted selection for a gene for extreme resistance to potato virus Y. Theor Appl Genet. 1997;94:192-197.

49. Sato M, Nishikawa K, Kormura K. Potato virus Y resistance gene Rychc mapped to the distal end of potato chromosome 9. Euphytica. 2006;149:367-372.

50. Grech-Baran M, Witek K, Szajko K, Witek A, Morgiewicz K. Extreme resistance to potato virus Y in potato carrying the Ry_{sto} gene is mediated by a TIR-NLR immune receptor. Plant Biotechnol J. 2020;18(3):655-667

51. Tam V, Patel N, Turcotte M, Bosse Y, Pare G. Benefits and limitations of genome-wide association studies. Nat Rev Genet. 2019;20(8):467-484.

52. Borevitz J, Nordborg M. The impact of genomics on the study of natural variation in Arabidopsis. Plant Physiol. 2003;132(2):718-725.

53. Miller M, Tang Y. Basic concepts of microarrays and potential applications in clinical microbiology. Clin Microbiol Rev. 2009;22(4):611-633.

54. Gallois J, Moury B, German-Retana S. Role of the genetic background in resistance to plant viruses. Int J Mol Sci. 2018;19(10):2856.

55. Tamisier L, Szadkowski M, Nemouchi G, Lefebvre V, Szadkowski E.

Genome-wide association mapping of QTLs implied to potato virus Y population sizes in pepper: Evidence for widespread resistance QTL pyramiding. Mol Plant Pathol. 2020;21(1):3-16.

56. Van Eck H, Vos P, Valkonen J, Uitdewilligen J, Lensing H. Graphical genotyping as a method to map Ny $(o,n)_{sto}$ and *Gpa5* using a reference panel of tetraploid potato cultivars. Theor Appl Genet. 2017;130:515-528.

57. Hall D, Tegstrom C, Ingvarsson P. Using association mapping to dissect the genetic basis of complex traits in plants. Brief Funct Genomics. 2010;9(2):157-165.

58. Stich B, Urbany C, Hoffmann P, Gebhardt C. Population structure and linkage diseqilibrium in diploid and tetraploid potato revealed by genome-wide high-density genotyping using SolCAP SNP array. Plant Breed. 2013;132:718-724.

59. Wood A, Esko T, Yang J, Vedantam S, Pers T. Defining the role of common variation in the genomic and biological architecture of adult human height. Nat Genet. 2014;46(11):1173-1186.

60. Tadmouri G, Nair P, Obeid T, Al Ali M, Al Khaja N. Consanguinity and reproductive health among Arabs. Reprod Health. 2009;6:17.

61. Mooney S. Progress towards the integration of pharmacogenomics in practice. Hum Genet. 2015;134(5):456-465.

62. Deschamps S, La Rota M, Ratashak J, Biddle P, Thureen D. Rapid genome-wide single nucleotide polymorphism dicovery in soybean and rice via deep resequencing of reduced representation libraries with the Illumina genome analyzer. Plant Genome. 2010;3(1):53-68.

63. Zhong W, Mark G, Michael S. RNA-seq: A revolutionary tool for transcriptomics. Nat Rev Genet. 2009;10(1):57-63.

64. Garg R, Jain M. RNA-seq for transcriptome analysis in non-model plants. Methods Mol Biol. 2013;1069:43-58.

65. Bodo R, Trognitz Friederike C. Increasing the efficiency of potato breeding through marker-assisted selection-general thoughts. Molecular markers for late blight resistance-when applied for breeders. Plant Breeding and Seed Science. 2004;50:96-105.

66. Miles C, Wayne M. Quantitative trait locus (QTL) analysis. Nat Educ. 2008;1:208.

67. Firidin S. Recombinant DNA technology. Yunus Res Bull. 2010;10:16-19.

68. Guleria P, Kumar V. Impact of recombinant DNA technology and nanotechnology on agriculture. In: Lichtfouse E. (eds) sustainable agriculture reviews 32. Sustainable agriculture reviews.Springer. 2018.

69. Wan B. Transgenic pyramiding for crop improvement. In: Al-Khayri JM, Jain SM, Johnson DV (eds) advances in plant breeding strategies: Breeding, biotechnology, and molecular tools. 2015:369-396.

70. Chakravarty B, Wang-Pruski G, Flinn B, Gustafson V, Regan S. Genetic transformation in potato: Approaches and strategies. Am J Potato Res. 2007;84:301-311.

71. Kaniewski W, Thomas P. The potato story. AgBioforum. 2004;7:41-46.

72. Cavatorta J, Perez K, Gray S, Van Eck J, Yeam I. Engineering virus resistance using a modified potato gene. Plant Biotechnol J. 2011;9(9):1014-1021.

73. Halterman D, Chakowski A, Vercho J. Potato, viruses, and seed certification in the USA to provide healthy propagated tubers. Pest Tech. 2012;1:1-14.

74. Brown KJ. Durable resistance of crops to disease: A Darwinian perspective. Annu rev phytopathol. 2015;53:513-539.

75. Nelson R. Stabilizing racial populations of plant pathogens by use of resistance genes. J Environ Qual. 1972;1:220-227.

76. Sapoukhina N, Paillard S, Dedryver F, Vallavieille-Pope C De. Quantitative plant resistance in cultivar mixtures: Wheat yellow rust as a modeling case study. New phytol. 2013;200(3):888-897.

77. Papaix J, Goyeau H, Du Cheyron P, Monod H, Lannou C. Influence of cultivated landscape composition on variety resistance: An assessment based on wheat leaf rust epidemics. New phytol. 2011;191:1095-1107.

78. Mundt CC. A key to sustainable management of pathogens and pests. Infect Genet Evol. 2014;27:446-455.

79. Ordon F, Kuhne T. Response to viral pathogens: In: Kumlehn J., Stein N. Berlin, Heidelberg: Springer. 2014;69.

80. Ellis JG, Lagudah ES, Spielmeyer W, Dodds PN. The past, present, and future of breeding rust resistant wheat. Front Plant Sci. 2014;5:641.

81. Feechan A, Kocsis M, Riaz S, Zhang W, Gadoury M D, et al. Strategies for *RUN1* development using *RUN2* and *REN2* to manage powdery mildew informed by studies of race specificity. Phytopathol. 2015;105.

82. Zoteyeva H, Klimenko N, Khyutti A. Pyramiding of pathogen resistance genes via a crossing of Mexican potato species *Solanum neoantipoviczii* with a selection from 'Aurora' variety. Plant Prot News. 2019;4:16-22.

83. Quenouille J, Paulhiac E, Moury B. Quantitative trait loci from the host genetic background modulate the durability of a resistance gene. Heredity. 2014;112(6):579-587.

84. Dong O, Yu S, Jain R, Zhang N, Doung P. Marker-free carotenoid-enriched rice generated through targeted gene insertion using CRISPR-Cas9. Nat Commun. 2020;11:1178.

85. Jinek M, Chylinski K, Hauer M, Dounda J, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Sci. 2012;337(6096):816-821.

86. Gao Y, Zhao Y. Self-processing of ribozyme-flanked RNAs into RNAs *in vitro* and *in vivo* for CRISPR-mediated genome editing. J Integr Plant Biol. 2014;56(4):343-349.

87. Ran F, Hsu P, Wright J, Agarwala V, Scott D, et al. Genome engineering using the CRISPR-Cas9 system. Nat Protoc. 2013;8:2281-2308.

88. Jiang F, Doudna J. CRISPR-Cas9 structures and mechanisms. Annu Rev Biophys. 2017;46:505-529.

89. Zhang Y, Showalter A. CRISPR/Cas9 genome editing technology: a valuable tool for understanding plant cell wall biosynthesis and function. Front Plant Sci. 2020;11:589517.

90. Wang C, Liu Q, Shen Y, Hua Y, Wang J, et al. Clonal seeds from hybrid rice by simultaneous genome engineering of meiosis and fertilization genes. Nat Biotechnol. 2019;37:283-286.