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Breeding strategies of virus resistance in solanaceous crops

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Abstract
Vegetables are regarded as protective foods and play a vital role in human nutrition. The vegetable sector has shown significant progress in production and productivity, emerging as a promising avenue to diversify agriculture, create employment opportunities, and bolster farmer's incomes. India holds the second position globally in vegetable production, trailing only behind China. Major vegetables cultivated in India include potato, tomato, chilly, and pepper, all belonging to the Solanaceous family. However, these crops are susceptible to various viral diseases due to their sensitive nature. Employing resistance breeding is crucial to mitigate losses. The backcross method stands out as the most effective conventional breeding approach for transferring resistance genes. Screening, such as the sap inoculation method used in tobacco for detecting Tomato spotted wilt virus by Mandal *et al*. (2008), is a pivotal technique. Hanson *et al*. (2016) conducted experiments on molecular marker-assisted selection and gene pyramiding for multiple disease resistance in tomatoes. Rai *et al*. (2014) employed molecular marker-assisted selection in interspecific crosses of Capsicum to confirm the presence of viruses. Biotechnological methods offer expedited solutions for developing disease-resistant varieties of Solanaceous crops, circumventing the lengthy duration required by conventional breeding methods. These non-conventional approaches facilitate the direct transfer or manipulation of specific genes enhancing efficiency. Gene pyramiding, coupled with marker technology enables the integration of desired traits into breeding programs mitigating the issue of linkage drag. Biotechnological interventions including gene cloning and transgene techniques enable the engineering of individual genes into commercial cultivars further enhancing disease resistance.

Keywords: Breeding strategies, virus resistance, solanaceous crops

Introduction
Presently, close to one billion individuals face malnutrition, with nearly twice that number lacking access to adequate nutrients and vitamins required for daily nutritional requirements. Losses incurred from plant diseases and pests occurring during both pre and postharvest stages exacerbate these deficiencies, particularly in developing nations. Production levels are dwindling due to various biotic and abiotic stressors. Biotic factors contribute to approximately 40% of these losses, with 15% attributed to insects, 10% to weeds, and 15% to other diseases and pathogens (Oerke and Dehne, 2004) [28]. Biotic stressors encompass fungi, bacteria, viruses, weed plants, parasites, nematodes, and insects. Viruses can infect plants, leading to symptoms like necrosis, chlorosis, leaf deformities, and stunted growth. The inherent characteristics of plant viruses often impede their control using conventional physical and chemical methods. Although the initial investment and time required to develop resistant varieties are substantial, resistance breeding proves to be an economically viable, environmentally friendly, and long-lasting approach for managing plant viruses. Moreover, vectors of plant viruses, such as insects, fungi, or nematodes, pose challenges for chemical control methods.

Prior to delving into breeding for virus resistance, it's essential to understand some fundamental concepts about viruses. Beijerinck (1898) [2] introduced the Latin term "VIRUS," meaning poison. His research involved examining filtered plant juices, which he observed caused healthy plants to fall ill. Subsequently, Wendell Stanley (1935) crystallized sap obtained from diseased tobacco plants, leading to the discovery that viruses consist of nucleic acid and protein (capsid). This complete viral entity is referred to as a virion.

Plant viruses can possess either DNA or RNA genomes, with the majority being RNA-based (Martelli, 1992) [25].

Viruses rank as the second most significant plant pathogens after fungi (Vidaver and Lambrecht, 2017) [37]. According to "Diseases: An Emerging Threat to Human, Animal, and Plant Health: Workshop Summary" (2011), 53% of plant viruses are transmitted by insects such as leafhoppers, aphids, and whiteflies, while the remainder spread through other methods like mechanical means (23%), nematodes (11%), or arachnids (23%). Plant viruses can interact with their insect hosts in various ways, including both non-persistent and circulative transmission. In non-persistent transmission, the virus is acquired by the vector after brief feeding sessions and is rapidly transmitted during short inoculation periods. The vector remains capable of transmitting the virus for only a short duration unless it feeds again on an infected plant. Conversely, in persistent transmission, the virus does not immediately infect healthy plants after the vector feeds on a diseased plant. Instead, the virus circulates within the insect's body before it can infect healthy plants, a process taking approximately 12-24 hours. Subsequently, the insect can transmit the virus to healthy plants for the remainder of its lifespan.

Plants exhibit various interactions with viruses, and the form of inheritance of resistance, whether major or minor gene, dominant or recessive, monogenic or polygenic, significantly influences breeding strategies for developing resistant crop cultivars. Resistance governed by monogenic or oligogenic factors is termed vertical resistance or race-specific, while resistance controlled by polygenic factors is termed horizontal resistance or race-nonspecific. Generally, horizontal resistance tends to be more durable compared to vertical resistance, which is relatively short-lived. Despite more than 200 R genes identified to act against viruses, only 22 have been cloned and characterized (de Ronde *et al*., 2014) [6].

Solanaceous crops are susceptible to numerous viral diseases, including Potato leaf roll virus (PLRV), Tobacco etch virus (TEV), Potato virus Y (PVY), Tomato ring spot virus, Tomato yellow leaf curl virus (TYLCV), Tomato spotted wilt virus, Tomato mosaic virus, Cucumber mosaic virus, Potato virus X (PVX), Alfalfa mosaic virus (AMY), Potato spindle tuber viroid (PSTV), Beet curly top virus (BCTV), Pepper mottle virus, Pepper mild mottle virus, Chilly leaf curl virus, Little leaf of Brinjal, Eggplant mottled dwarf virus (EMDV), and Eggplant mild leaf mottle virus (EMLMV).

In resistance breeding, a resistance source is always required. This can be a known variety, germplasm collection, related species, mutation, or unrelated organism that possesses resistance traits. A program involving backcrossing to the susceptible parent and selecting resistant phenotypes results in plants resembling the susceptible parent but possessing the desired resistance.

Despite the optimism surrounding conventional breeding methods for disease and insect pest resistance, advancements in new technologies like gene pyramiding, transgenic breeding, somaclonal variation, RNAi technology, and marker-assisted selection have emerged. Diagnosing viral diseases based on symptoms is more challenging compared to other pathogens (Lievens *et al*., 2005) [22]. At times, symptoms may not be visually evident because plant virus infections can occur asymptomatically (Bove *et al*., 1988) [5].

Screening method

Prior to initiating a breeding program aimed at developing disease-resistant crops, screening techniques are essential to determine the presence or absence of disease. These methods encompass various approaches such as field screening, vector-mediated screening, sap inoculation method, grafting method, and agro-inoculation-based screening. Currently, the agro-inoculation technique is increasingly favored as a screening method. It has been widely employed to introduce viruses into plants for several purposes, including validating both mono- and bipartite viruses as causative agents of disease, characterizing novel viruses and their genomes through mutagenesis, conducting recombinatorial analyses between related viruses, and facilitating transient RNA interference (RNAi) and virus-induced gene silencing (VIGS) studies.

Table 1: Sources of resistance for viral disease in Solanaceous crops

Crops	Disease	Source of resistance	Reference
Tomato	Tomato spotted wilt virus	*S. chilense* (LA 130, LA 2753, LA 1938)	Lima *et al*., (2003) [23]
		S. peruvianum (PI -126935, PI – 126944, LA 444/1 and LA 371)	Lima *et al*., (2003) [23]
		S. habrochaites (PI 134417, PI 127826)	Maluf *et al*., (2010) [24]
		S. peruvianum	Stevens *et al*., (1992) [35]
	Tomato leaf curl virus	*S. peruvianum* (L00735, L00671, L00887, L06138), *S. chilense* (TL 02226), *S. pimpinellifolium* (L03708, TL 02213)	Sain *et al*., (2016) [33]
	Tomato yellow leaf curl virus	*S. Pimpinellifolium* *S. chilense* *S. habrochaites*	Zamir *et al*., (1994) [40] Hanson *et al*., (2000) [11]
	Tomato mosaic virus	*S.hirsutum* *S. peruvianum* *S. peruvianum*	Pelham, (1966) [29]
Capsicum	Tomato spotted wilt virus	*C. chinense* (AC09-207), *C. baccatum* (PIM26-1)	Ngoc Huy *et al*., (2013) [27]; Soler *et al.* (2015) [34]
	TSWV and CaCV	*C. chinense*	Persley *et al*., (2006) [30]
Brinjal	Eggplant mosaic virus	*S.hisidum*	Rao, (1980) [32]
	Little leaf of brinjal	*S. integrifolium*	Khan *et al*., (1978) [19]
Potato	PVX PVY	*S. acaule, S. chacoensis, S. chacoensis,* *S. stoloniferum*	Hawkes,1990 [13]

Table 2: Mapped TYLCV resistance loci identified from wild *Solanum* species

Gene	Genetic source		Chromosome	Reference
	Accession			
	Line	Species		
Ty-1	LA1969	*S. chilense*	6 (pericentromere region)	Zamir *et al*., 1994 [40]
Ty-2	B6013	*S. habrochaites*	11	Hanson *et al*., 2006 [10]
Ty-3	LA1932, LA2779	*S. chilense*	6 (long arm)	Ji *et al*., 2007 [15]
Ty-4	LA1932	*S. chilense*	3	Ji *et al*., 2009 [16]
Ty-5	TY17	*S. peruvianum*	2	Anbinder *et al*., 2009 [1]

Backcross

Backcross breeding is employed to introduce simply inherited traits from donor parents that may not be adapted into recipient lines. This method entails repeated cycles of crossing with the recipient line, also known as the recurrent parent, followed by selection of the specific trait being transferred (Kenaschuk, 1975) [18]. The success of the backcross method relies on the transferred trait maintaining its effectiveness over several generations, requiring a sufficient number of backcrosses to recover the desirable traits of the recurrent parent (Harlan *et al*., 1992) [12]. In 1922, Harlan and Pope first advocated for the use of backcrossing in developing crop plants, highlighting its longstanding use in animal breeding to confer fixed traits and underscoring its underappreciated value in agriculture (Harlan *et al*., 1992) [12]. Through one round of backcrossing, they demonstrated that a portion of the resulting progeny exhibited the phenotypic characteristics of one parent while possessing the desired genetics of the other. This provided breeders with an effective and cost-efficient means of introducing desired traits into the preferred genetic background (Fehr, 1991) [7].

Procedure for backcross method

The process begins with crossing the donor parent, which carries the gene of interest, with the recurrent parent, an elite line that stands to benefit from the addition of the gene of interest. The resulting offspring from this initial cross are then crossed back to the recurrent parent, hence the term "backcross." The progeny of this cross are carefully selected for the desired trait and subsequently crossed back again to the recurrent parent. This iterative process continues through multiple backcrosses until a line is developed that closely resembles the recurrent parent but possesses the desired gene from the donor parent. By the fourth backcross generation (BC4), the lines are typically over 96% identical to the recurrent parent. Marker-assisted backcrossing, also known as background selection, can often expedite this process by allowing for more efficient selection of individuals with the desired genetic background. Additionally, the backcrossing method is commonly employed for resistance gene pyramiding, where multiple resistance genes are incorporated into a single line.

Demerits

1. The new variety will typically not surpass the recurrent parent in traits other than those specifically transferred.
2. The process requires extensive crossing efforts, with 6-8 backcrosses often proving challenging and time-consuming.
3. Occasionally, undesirable genes may inadvertently accompany the desired trait during transfer.
4. Over the course of the backcrossing program, the recurrent parent may potentially be supplanted by other varieties that exhibit superior yield and other desirable traits.

Gene pyramiding

The concept of gene pyramiding was initially proposed by Watson and Singh in 1953. Gene pyramiding involves the process of combining multiple desirable genes or QTLs (quantitative trait loci) from different parents into a single genotype, aiming to enhance specific or multiple traits through conventional breeding methods.

Advantages of gene pyramiding

1. Improving trait expression through the combination of two or more mutually supportive genes.
2. Addressing deficiencies by incorporating genes from alternative sources.
3. Enhancing longevity or sustainability.

Types of gene pyramiding

1. Conventional technique
2. Molecular technique

Marker-assisted selection (MAS) is a technique used to efficiently introduce desirable traits into new cultivars. Molecular markers, also known as DNA tags, are especially valuable for incorporating genes that are strongly influenced by environmental factors, as well as genes conferring resistance to diseases and pests. They are also instrumental in accumulating multiple resistance genes for specific diseases and pests within the same cultivar, a process known as gene pyramiding.

At AVRDC, a three-parent cross, [(CLN2777G × G2-6-20-15B) × LBR-11], was created in October 2008 and coded as CLN3241 by June 2012 using the pedigree selection method. CLN2777G carries homozygous resistance genes for Bwr-12 (bacterial wilt), Ty-2 (tomato yellow leaf curl disease), and Tm22 (TMV). G2-6-20-15B is homozygous for Ty-3 (tomato yellow leaf curl disease resistance), while LBR-11 carries homozygous resistance genes for Ph-2 and Ph-3, I2 (resistance to race 2 of the fusarium wilt pathogen), and Sm (resistance to the gray leaf spot pathogen). Through this breeding program, five F7:8 lines were developed, exhibiting high yield and resistance to multiple diseases. These lines hold promise for potential release as inbred line cultivars, hybrid parental lines, or as breeding stock (Hanson *et al*., 2016) [10].

Agrobacterium mediated gene transfer

Agrobacterium tumefaciens is a soil-dwelling phytopathogen that naturally invades plant wounds, causing crown gall disease by transferring DNA (T-DNA) from bacterial cells into host plant cells. This process has made A. tumefaciens the most widely used tool for plant

transformation, facilitating the production of genetically modified organisms through DNA technology.

The process relies on two genetic components located on the bacterial Ti-plasmid: the T-DNA, which contains 25-base pair imperfect repeats, and the virulence (vir) region, consisting of at least seven major loci that govern the infection process. Additionally, two plant hormones, auxin and cytokinin, not only contribute to tumor growth but also influence Agrobacterium physiology and gene expression (Hwang *et al*., 2010) [14]. The interaction between Agrobacterium and plants is a complex process involving several functional steps, extensively reviewed elsewhere (Bhattacharya *et al*., 2010) [3].

Initially, the plasmid is extracted from the bacterium, and the T-DNA is excised by restriction enzymes. Simultaneously, the foreign DNA is cut using the same enzyme and then inserted into the T-DNA of the plasmid, resulting in the formation of a recombinant Ti plasmid. This recombinant Ti plasmid is subsequently reintroduced into the bacterium. Consequently, the bacterium serves as a vehicle to transfer the T-DNA, carrying the foreign gene, into the chromosome of the plant cell. The plant cells are then cultured, and the plant is regenerated, containing the foreign gene. This resulting plant is commonly referred to as a genetically modified plant.

Gene silencing technology

Gene silencing technology disrupts the central dogma process by targeting mRNA strands and breaking them down, thereby preventing protein production. Small interfering RNAs (siRNAs) are short RNA molecules typically comprising 19 to 22 nucleotides. These siRNAs are produced through the cleavage of double-stranded RNA (dsRNA) templates by an RNAse III ribonuclease called DICER. Once generated, the siRNAs are incorporated into a complex known as the RNA-induced silencing complex (RISC) and unwound into single-stranded molecules. Subsequently, these single-stranded siRNAs guide the RISC complex to target mRNAs for degradation, initiating RNA interference. This process results in the specific breakdown of messenger RNAs prior to protein synthesis, leading to reduced expression of the targeted gene (Read, 2001) [36]. The extent of gene expression suppression depends on the level of siRNA expression and its inhibitory efficiency. Consequently, the expression of the target gene can be completely blocked or significantly suppressed. This technology enables researchers to investigate the function of genes, particularly those that would cause lethality upon complete knockout. Gene silencing holds considerable potential for elucidating the functions of identified genes (Lacomme *et al*., 2005) [20].

Over 90% of plant viruses are RNA viruses, which replicate using a double-stranded RNA (dsRNA) intermediate. Consequently, RNA interference in plants has developed partly as a defense mechanism against viral infections and the proliferation of retrotransposons (Waterhouse *et al*., 2001; Voinnet, 2001) [39, 38]. Gene silencing techniques can be utilized to confer virus resistance in plants by inserting a modified DNA fragment containing a small portion of a virus into a plant's genome.

Confirmation of virus

At the conclusion of the breeding program, it is essential to conduct confirmation work to verify the presence or absence of the virus. The most reliable method for confirmation is marker analysis technique.

Marker Assisted Selection (MAS): Involves the indirect selection of desired plant traits based on the banding pattern of molecular (DNA) markers linked to those traits. MAS operates on the principle that the presence of a marker tightly linked to a gene of interest can infer the presence of that gene. Resistance genes against potato virus Y, root cyst nematode, Potato virus X, and potato wart are combined using CAPS, SCAR, and PCR markers (Gebhardt *et al*., 2006) [8]. Similarly, resistance to Tomato spotted wilt virus is attained through CAPS markers (Langella *et al*., 2004) [21], and monogenic recessive resistance to Pepper leaf curl virus in interspecific crosses of Capsicum is developed with the assistance of markers (Rai *et al*., 2014) [31].

MAS relies on PCR techniques for virus detection, wherein DNA specific to the organism is extracted and amplified, enabling the multiplication of DNA molecules exponentially. This amplification process makes DNA analysis much simpler by providing a larger amount of DNA for analysis. The technique typically involves three steps.

1. The DNA sample is initially denatured at 94°C, where it is heated to unravel and separate the double DNA helix.
2. At 54°C, annealing occurs, during which primase and polymerase enzymes, along with selected primers, identify DNA sequences and generate copies of them. Ionic bonds form and break between the single-stranded primer and the single-stranded template. The polymerase attaches itself to the double-stranded DNA (composed of template and primer) and initiates the copying process. Once a few bases are synthesized, the ionic bond between the template and the primer becomes strong and remains intact.

Extension takes place at 72°C, wherein the bases complementary to the template are coupled to the primer on the 3' side. The polymerase adds dNTPs (nucleotides) from the 5' to 3' direction, reading the template from the 3' to 5' direction, to ensure complementarity.

Table 3: Achievements in India

Crop	Disease	Variety/ hybrid	Institute
Tomato	Tomato leaf curl virus	a) Arka Rakshak (Tolcv+BW+EB) b) Hisar Anmol c) Arka Ananya	IIHR HAU IIHR
Chilly	Leaf curl virus	a) Pusa Sadabahar b) Jawahar218	IARI JNTU
	Tobacco mosaic virus	a) Punjab Lal b) Panjab Guchhedar	PAU
Potato	Potato virus X	Kufri Badshah (Kufri Jyoti X Kufri Alankar	CPRI

Conclusion

1. Unconventional methods represent highly effective techniques for enhancing crop traits.
2. Integrating gene pyramiding with marker technology enables the incorporation of multiple genes into existing plant breeding programs, facilitating the transfer and combination of genes.
3. Marker-assisted selection (MAS) prolongs the durability of resistant cultivars by combining multiple resistance genes through pyramiding and reintroducing the desired genotype into the recurrent parent via background selection.
4. Biotechnological interventions offer solutions to the problem of linkage drag. It is now feasible to clone individual genes and integrate them into the genome of commercial cultivars using transgene techniques.

Future thrust

1. Identifying and mapping new sources of disease resistance genes in germplasm or wild relatives of major or minor crops is essential for gene pyramiding.
2. Future resistance breeding initiatives should also seek to leverage horizontal resistance controlled by polygenes.
3. Identifying specific regions and seasons for efficient screening of large populations under natural epidemic conditions is crucial. For instance, screening for tomato leaf curl disease proves highly effective during the autumn season (August planting) in the North Indian plains due to the high prevalence of viruliferous whiteflies.

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