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Molecular Characterization of *Solanum tuberosum* L. ADG2 Fragment Associated with *Ryadg*

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Potato virus Y NTN (PVY^{NTN}) causes loss of potato yield and quality in Egypt and worldwide. This study aims to compare nucleotide sequence of ADG2 gene fragment in a wild potato line resistant to PVY^{NTN}-carrying *Ry$_{adg}$*, *Solanum tuberosum* L. subsp. *andigena* clone 17609 (R+adg), and line susceptible to PVY^{NTN} non-carrying *Ry$_{adg}$* (S-adg). We amplified ADG2 fragments from six potato lines with different genetic backgrounds by PCR and results showed one amplicon of 355 bp in all the examined potato lines. Two fragments from each resistant line (R+adg), and susceptible line (S-adg) were sequenced after purification using the ABI PRISM sequencer model 310. BLAST analysis of *Ry$_{adg}$* from the resistance line and the susceptible line matched 98.٤١ and 96.02% with the ADG2 gene in *S. tuberosum* recorded in GenBank, respectively. The sequence comparison of the ADG2 fragments revealed ten and three differences at nucleotide and amino acid sequence levels respectively, between the resistance line having *Ry$_{adg}$* and those lacking this gene. In addition, the 3D models of ADG2 proteins of R+adg and S-adg lines were evaluated by Ramachandran plot, which demonstrated that the homology modelling process was trusted. Therefore, the comparison of nucleotide sequences between the resistant line and the susceptible ones will lead to the identification of molecular markers, which will be used in potato breeding programs as marker-assisted selection to discriminate potato lines carrying *Ry$_{adg}$* from those lacking this gene.

Keywords: *ADG2* gene, alignment, Potato, Sequence, tertiary structure (3D), Ramachandran plot.

Introduction

Potato (*Solanum tuberosum* L.) is considered the third most important food crop in the world (Abo-Akel *et al*., 2024; El-Damarawy *et al*., 2025). Potato viruses are the greatest threat to potato production globally. One of the most serious viral diseases affecting cultivated potatoes is *Potato virus Y* (PVY) (Brunt *et al*., 1996; van der Sman *et al*., 2025). PVY spreads through the use of contaminated tuber seeds, mechanical contact, and aphid insect vector, and can result in potato yield losses up to 80% (Hooker, 1981). In domesticated and wild potato species, there are two main types of monogenically inherited resistance to PVY, hypersensitive resistance (HR) and extreme resistance (ER) (Ross, 1986). ER controlled by the *Ry* loci is effective against all PVY strains, while HR controlled by the *Ny* loci is often specific to PVY strain (Jones, 1990). In other instances, HR is not effective in preventing the spread of PVY in the plant host (Vidal *et al*., 2002). On the contrary, ER genes are broad-spectrum and refer to durable resistance, identified by the absence of visible symptoms after infection (Flis *et al*., 2005). *Ry$_{sto}$* in *S. stoloniferum* and *Ry$_{adg}$* in *S. tuberosum* subsp. *andigena* were discovered in both domesticated and wild potato species and are applied in potato breeding programs (Muñoz *et al*., 1975; Ross, 1986; Hämäläinen *et al*., 1998). Three molecular markers known to be associated with the resistance loci in *S. tuberosum* ssp. *andigena* are RYSC3, RYSC4, and ADG2. The RYSC markers are sequence-characterized amplified regions (SCAR) (Kasai *et al*., 2000) and the ADG2 marker is a cleaved-amplified polymorphic sequence (CAPS) (Sorri *et al*., 1994). Many studies assumed that managing main diseases by using of resistance loci could increase yields by more than 30%, and decreasing the use of pesticides (Gebhardt and Valkonen, 2001). This could be accomplished by applying novel breeding technologies to screen resistant lines that can then be used in novel crop genotypes (Armstrong *et al*., 2019).

This study aims to compare the nucleotide and amino acid sequences of ADG2 fragments in wild potato line resistant to PVY^{NTN} (carrying *Ry$_{adg}$*,) and

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line susceptible to PVY^{NTN} (non-carrying Ry_{adg}). Besides, the secondary (2D), tertiary (3D) structures, and Ramachandran plots of the ADG2 protein in the resistant and susceptible lines were determined.

Materials and Methods

Plant materials

Five potato varieties, Selena **(**Semi-early to medium-maturing**),** Spunta (**a** mid-early maturing and susceptibility to PVY^{NTN}), Cara (maturing later and susceptibility to PVY^{NTN}), Diamond (medium-maturity), and Nicola (medium-late and moderately susceptible to PVY) were obtained from the Brown Rot Project, Dokki, Giza, Egypt, as well as one wild genotype, *S. tuberosum* subsp. *andigena* 17609, supported by the Centre for Genetic Resources, Netherland (http://www.wur.nl). In our previous study, we assessed six potato lines for resistance against *Potato virus Y* tuber necrosis strain (PVY^{NTN}; accession no. EF 502038) by mechanical inoculation. It was observed that one out of six lines was highly resistant (HR), e.g., *S. tuberosum* subsp. *andigena* 17609, two were moderately resistant (MR), such as Cara and Diamond, one was resistant (R) to PVY like Nicola, and two were susceptible (S), i.e., Selena and Spunta (Mahfouze *et al*., 2009).

DNA extraction and PCR amplification

Total genomic DNA was isolated from leaves of six potato lines using the DNeasy plant mini-prep kit (Qiagen, CA). The ADG2 fragment was amplified using gene specific primers as shown in Table (1) (Hämäläinen *et al*., 1998). PCR was performed in a thermal cycler (Biometra, biomedizinische Analytik GmbH) according to Watanabe *et al*., (2003). All the PCR-amplified fragments were electrophoresed on 1% agarose gel electrophoresis in 1X TBE buffer. The DNA fragments were stained with RedSafe Nucleic Acid Staining Solution (1/20,000) (iNtRON Biotechnology, Inc. Kr).

Purification, sequencing and analysis of the ADG2 fragment

The amplified DNA products were purified from genotype *S. tuberosum* sub sp. *andigena* 17609 (HR) which carry the dominant allele Ry_{adg} (R+adg), and the susceptible line Selan, which do not carry Ry_{adg} (S-adg), with the QIA quick PCR Purification Kit (Qiagen GmbH, Germany), according to the manufacturer's instructions. The two amplicons were sequenced on a Capillary Electrophoresis Sequencing (CES) automation system (ABI 3730xl System DNA Sequencer, Macrogen, South Korea).

Alignment of ADG2 fragment

The nucleotide and amino acid sequences of ADG2 fragments in the resistant line (R+adg) and the susceptible line (S-adg) were submitted to NCBI GenBank with accession numbers OR162002 and OR162003 respectively, and were aligned with corresponding sequences from the database using BLAST from the website http://www.ncbi.nlm.nih.gov/blast, to identify the sequence similarities, and conserved sites between two genes. Multiple alignments of nucleotide sequence and protein sequence were carried out by the CLC Main Workbench 8.0 program, Denmark.

Physio chemical analysis of ADG2 fragment

The CLC Main Workbench 8.0 software was used to determine a computational analysis of the physical and chemical characteristics such as molecular weight (MW), theoretical isoelectric point (PI, amino acid composition, and aliphatic index of ADG2 fragments in the lines (R+adg) and (S-adg).

Prediction of P-Loop and CC-NBS-LRR region

The Prabi server (https://npsa-prabi.ibcp.fr) was used to predict the coiled coil (CC) area of all ADG2 fragments in the susceptible line S-adg and the resistant line R+adg (Geourjon and Deleage, 1995). InterPro, an online website for functional analysis of proteins and identification of significant domains and sites, was used to predict the Nucleotide Binding Site (NBS), Leucine Rich Repeats (LRR), and P-loop of ADG2 proteins (Blum *et al*., 2021).

Table 1. Primer sequences used in this study.

Gene name	Forward primer	Reverse primer	Amplicon size (bp)
ADG2 fragment	ATACACTCATCTAAATTTGATGG	ACTTAACTGCTACTAGTTCAAG	355 bp

2D and 3D structures of ADG2 fragment

The SOPMA method (https://npsa-prabi.ibcp.fr) was used to estimate the secondary (2D) structure of the ADG2 fragments in the susceptible cultivar (S-adg) and the resistant line (R+adg) based on their primary amino acid sequence (Geourjon and Deleage, 1995). The Swiss-Model (https://swissmodel.expasy.org) servers were used

to identify the tertiary structure (3D) (Schwede *et al.,* 2003).

Ramachandran plot

A Ramachandran diagram or [ϕ (Phi; C-N-CA-C), ψ (Psi; N-CA-C-N)] plot, is a method to show energetically allowed regions for backbone dihedral angles ψ against ϕ of amino acid residues in proteins' structure in both R+adg and susceptible S-adg (Ramachandran *et al*., 1963). This plot was applied for theoretical estimation of various compositions of the ψ and ϕ angles that are potential for an amino-acid residue in a protein and structure validation by Swiss-Model software.

Results

Detection of the ADG2 fragment in potato lines

Six potato lines having different genetic backgrounds were utilized for the amplification of the ADG2 fragment by PCR using gene specific primers. All potato lines resistant or susceptible to PVY^{NTN} scored one amplicon with a molecular size of 355 bp (Fig. 1).

Nucleotide sequencing of ADG2 fragment

The partial nucleotide sequences of the ADG2 fragment in the potato lines resistant to PVY^{NTN} (R+adg) and susceptible ones (S-adg) was aligned, and compared with the nucleotide sequences of the ADG2 fragments in the GenBank, using the BLAST search. BLAST sequence analysis found that the ADG2 fragment in potato line resistant (303 bp) had an identity ranging from 98.41 to ٨٥.٨٩% with *S. tuberosum* accessions recorded in GenBank (Fig. 2 and Table 2). However, the partial nucleotide sequence of the susceptible line Selena (303 bp) had a similarity ranging from 96.34 to ٨٢.٨٢% identity with R-genes in the GenBank (Fig. 3 and Table 2).

Genetic variations and base-pair substitutions of ADG2 fragment

The nucleotide sequence comparison of the ADG2 fragment in lines resistant (R+adg) and susceptible (S-adg) revealed two kinds of nucleotide substitutions that could be distinguished between two lines, as shown in Table (3). The first kind (I): Four nucleotides were substituted from a pyrimidine to another pyrimidine base. For example, adenine (A) and thimine (T) bases in line resistant (R+adg) were substituted for thimine (T) and adenine (A) bases in line susceptible (S-adg). The second kind (II): Four nucleotide bases were changed from pyrimidine bases to purine bases or vice versa. For instance, three thimine (T) bases in the line R+adg were replaced by guanine (G) or cytosine (C) base in the line S-adg. Also, adenine (A) base in PVY^{NTN}-resistant potato line was substituted with guanine (G) base in susceptible line. Finally, two purine bases were replaced to pyrimidine bases, e.g., two guanine (G) bases in the resistance line R+adg were changed to adenine (A) bases in the susceptible line S-adg (Fig. 4 and Table 3).

Table 2. Sequence alignments of ADG2 gene of *S. tuberosum* subsp. *andigena* and Selena using BLAST analysis.

Accession number	Gene name	% identity	
		S. tuberosum sub sp. *andigena* (resistant)	Selena cultivar (susceptible)
Y17789.1	Partial nucleotide sequence of ADG2 in *Solanum* sp. hybrid 84.194.30.	98.41	96.02
MH990904.1	Partial nucleotide sequence of RYSC3 gene in potato cultivar Sante.	97.48	96.22
XM_049523138.1	*S. stenotomum* TMV resistance protein N-like, mRNA.	97.07	96.34
AJ300266.1	*S. tuberosum* subsp. *andigena ry*-1 gene for resistance gene-like.	94.87	94.87
Y15297.1	partial nucleotide sequence of *S. tuberosum* subsp. *andigena* ADG2 gene.	95.22	95.22
AJ716161.1	*S. caripense* TIR-NBS-LRR, SC TNBS1-5.	85.89	82.82

Table 3. The nucleotide sequence substitutions between potato lines resistant and susceptible to PVY^{NTN}.

kinds of nucleotide substitutions	Resistant line (Ry_{adg})	Susceptible line (ry_{adg})
A pyrimidine to another pyrimidine base	A	T
	A	T
	T	A
	T	A
A pyrimidine to purine base	T	G
	T	C
	A	G
	T	C
A purine to pyrimidine base	G	A
	G	A

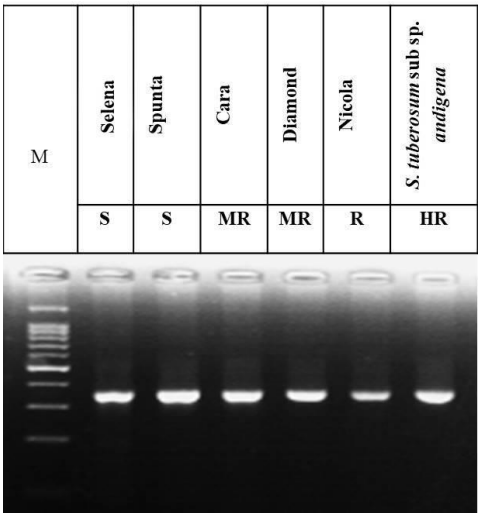

Fig. 1. DNA of six potato lines which amplify 355 bp in all tested potato lines (indicated by arrow). Lane M= 100 bp DNA ladder; S = susceptible; MR = moderately resistant; R = resistant; HR = highly resistant. TGGCAGC = Kinase 3a motif of P- loop = *R* gene.

TCCTTCTCTCTAAACTGGTAGGGGAAAAAGAAAATTGTGTGCATGATAAGGAGGACGGTAGGCACCTGATGGCTCGTAGACT
TCGTTTGAAGAAGGTTTTAGTTGTGCTTGATAACATAGATCATGAAGACCAATTGGATTACCTAGCAGGGGATCTTGGTTGGT
TTGGCAATGGCAGCAGAATTATTGCAACAACTAGAGACAAGCATTTCATACGGGAAAATGATGCTGTATATCCCGTGACTAC
TCTACTTGAACATGATGCAGTTAAGT

Fig. 2. Partial nucleotide sequencing of ADG2 gene fragment in *S. tuberosum* subsp. *andigena* resistant to PVY^{NTN} (*Ry$_{adg}$*-carrying). TGGCAGC = Kinase 3a motif of P- loop = *R* gene.

TCCTTCTCTCTAAACTGGTAGGGGAAAAAGAAAATTGTGTGCTTGATAAGGAGGACGGGAGGCACCTGATGGCTCGTAGACT
TCGTTTGAAGAAGGTTCTAGTTGTGCTTGATAACATAGATCATGAAGACCAATTGGATTACCTAGCAGGGGATCTTGGTTGGT
TTGACATAGGCAGCAGAATTATTGCAACAACTAGGGACAAGCATTTCATACGGAAAAATGATGCCGTATATCCCGTGACTAC
ACTACTTGAACATGATGCAGTTAAGT

Fig. 3. Partial nucleotide sequencing of ADG2 gene fragment in potato cultivar Selena susceptible to PVY^{NTN} (*Ry$_{adg}$*-non-carrying). TGGCAGC = Kinase 3a motif of P- loop = *R* gene.

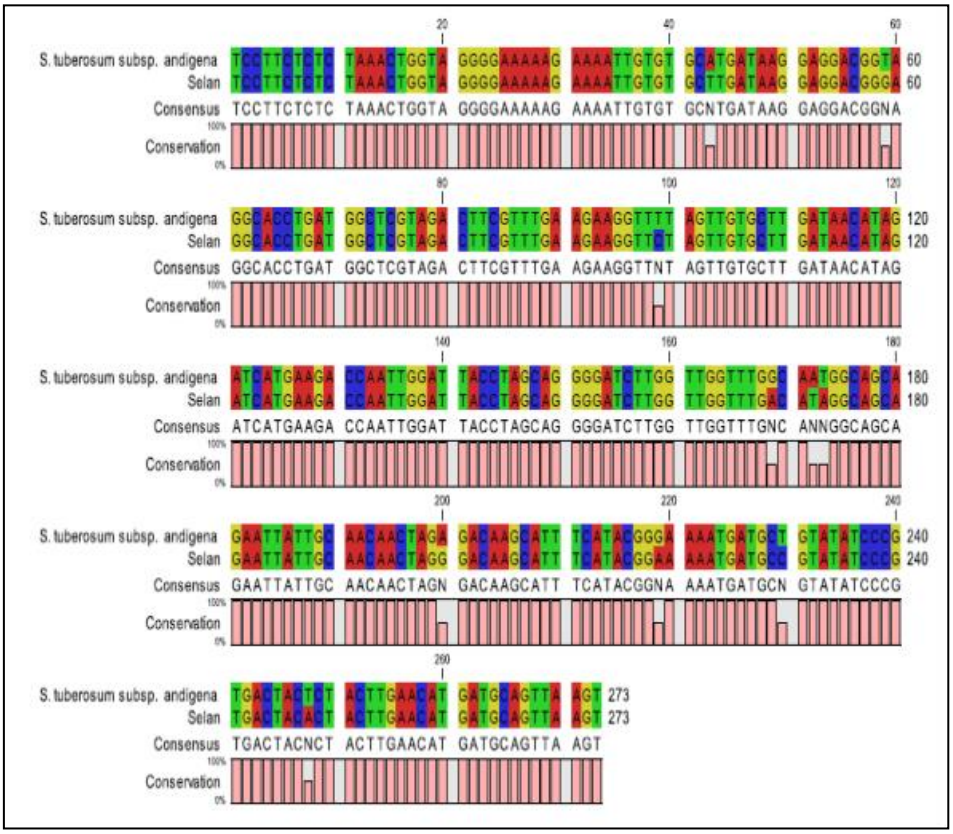

Fig. 4. Alignment of ADG2 fragment in resistant line *S. tuberosum* subsp. *andigena* and susceptible cultivar Selena.

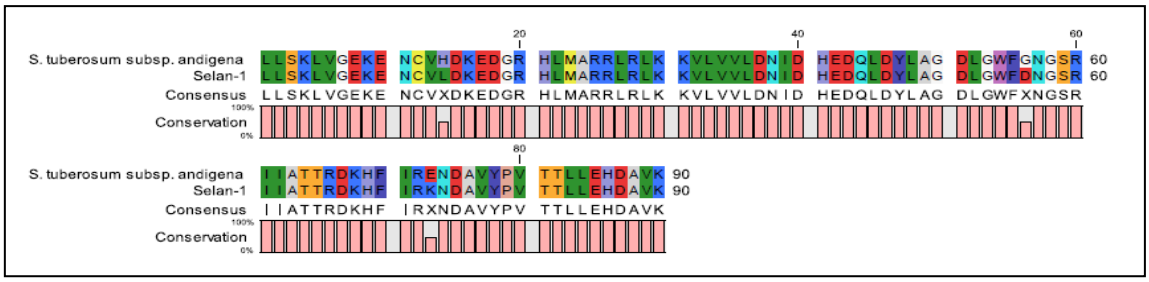

Fig. 5. Alignment of amino acid sequence (90 nucleotides) of ADG2 gene fragment in resistant line *S. tuberosum* subsp. *andigena* and susceptible cultivar Selena (translated, frameshift +1).

The amino acid sequence comparison of PVY^{NTN}-resistant and susceptible potato lines

The sequence identity ranged between 100-63.33% for the *Ryadg*-carrying resistant line, and the susceptible line was in the range of 98.89-67.78%. The deduced amino acid sequence between R+adg and S-adg lines showed three amino acid residue differences. The resistant potato line R+adg has three amino acids, e.g., histidine (H), glycine (G), and glutamic acid (E), which were substituted with the amino acids leucine (L), asparatic acid (D), and lysine (K), respectively, in the line susceptible S-adg (Fig. 5). On the other hand, amino acids conformation in both the resistant and susceptible lines was analyzed by the CLC Main Workbench 8.0 program (Table 4). Leucine (L) was the most frequent amino acid found in the sequence in both the resistant line R+adg (13) and the susceptible line S-adg (14). The percentage of leucine residues was 14.4 and 15.6% in the resistant line R+adg and susceptible line S-adg, respectively. On the contrary, Cysteine (C), Methonine (M), Proline (P), Glutamine (Q), and Tryptophan (W) were present to have the least frequent and percentage of amino acids (1; 1.1%) in both lines (Table 4).

Physico-chemical parameters of proteins

Physico-chemical properties, e.g., molecular weight (MW), isoelectric point (pI), and aliphatic index, were carried out using the CLC Main Workbench 8.0 program. The predicted molecular weight of two lines, R+adg and S-adg, was 10.336 kDa and 10.369 kDa, respectively. However, pI was detected to be acidic at 6.45 and 6.73 in the resistant line and susceptible ones, respectively. In addition, the aliphatic index of the resistant line was 105, while that of the susceptible line was 109.333.

Prediction of CC-NBS-LRR region and P-Loop

The coiled coil region of ADG2 proteins in R+adg and S-adg was predicted to be present in (14-17, 37-40, 51-53, 56, 59, 65-69, and 74-75), and (15-19, 30, 37-41, 51-52, 56, 59, 65-68, 72-75, and 83-85) amino acid positions of the terminal structure of the proteins, respectively. To identify the NBS, LRR, and P-loop of the ADG2 proteins in both PVY^{NTN}-resistant and susceptible lines, the InterPro protein families and domains database was used. Protein of both R+adg and S-adg lines have single C-terminal LRR at 1-70 and 1-71 amino acid positions, respectively. However, P-Loop (containing nucleoside tripfosphate hyrolyses) occurs at 9-74 and 9-80 amino acid sites in R+adg and S-adg, respectively. Besides, R+adg and S-adg lines contain Kinase 3a motif of P- loop. On the other hand, the resistant and susceptible lines have shown conserved NBS sites towards their N-terminal in InterPro analysis.

Secondary structure (2D) of ADG2 protein

The prediction of secondary structure (2D) of ADG2 protein in the lines R+adg and S-adg by the SOPMA method showed that line R+adg was composed of 40 α-helix (44.44%), 19 β-sheets (21.11%), 8 Beta-turn (8.89%), and 23 coil-coiled (25.56%). Whenever the line S-adg has 34 α-helix (37.78%), 24 β-sheets (26.67%), 6 Beta-turn (6.67%), and 26 coil-coiled (28.89%) (Figs. 6 and 7 and Table 5). Both R+adg and S-adg proteins showed around 71.11% sequence similarity with the NBS-LRR resistance protein in Protein Data Bank (PDB).

The tertiary structure (3D) of ADG2 protein in PVY^{NTN}-resistant and susceptible potato lines

SWISS-Model server was applied to build the 3D structures of the ADG2 protein in two lines R+adg and S-adg. The templates of two lines showed similarity for two proteins, as illustrated in Fig. (8). Thus, the crystal structure of ADG2 protein in lines R+adg and S-adg had monomers, composed of α-helix, β-sheets, Beta turn, and coiled–coil with a compact structure. On the other hand, Ramachandran plot showed the distribution of phi (φ) and psi (ψ) angles in the ADG2 protein in both lines R+adg and S-adg. The Ramachandran plot is an important way in the analysis of 3D protein structures. It is the 2D plot of the phi (φ) and psi (ψ) torsion angles of the polypeptide chains. This plot showed the amino acid residues in allowed, favoured and disallowed regions for backbone dihedral angles against amino acid residues in protein structure in both R+adg and S-adg lines (Fig. 9).

Discussion

Potato virus Y tuber necrosis strain (PVY^{NTN}) cause losses in potato yield up to 80% (Hooker 1981). Introgression resistance loci from PVY^{NTN}-resistant potato lines into domesticated potatoes will help in the management of the virus (Gebhardt and Valkonen 2001). Two kinds of monogenically inherited resistance loci were discovered in wild species and domesticated potatoes, e.g., *Ny* gene responsible for hypersensitivity reaction (HR) and the *Ry* gene referring to extreme resistance (ER) all PVY strains (Valkonen 1994). There are two types of *Ry* genes, the former is called Ry_{sto} from *S. stoloniferum*, and the second is known as *Ryadg* from *S. tuberosum* subsp. *andigenia* (Kasai *et al*., 2000). Some resistance gene-like fragments (RGL) were identified by PCR. In our previous study, six potato lines were evaluated for resistance against PVY^{NTN} by mechanical inoculation. It was observed that one out of six lines was highly resistant (HR), e.g., *S. tuberosum* subsp. *andigena* 17609; two were moderately resistant (MR), such as, Cara and Diamond, one was resistant (R), e.g., Nicola, and two were susceptible (S), i.e., Selena and Spunta (Mahfouze *et al*., 2009).

Table 4. Comparison between amino acid composition between the resistant and susceptible lines.

	Resistant line (Ry_{adg})		Susceptible line (ry_{adg})	
Amino acid	No. of amino acids	% of amino acids	No. of amino acids	% of amino acids
Alanine (A)	5	5.6	5	5.6
Cysteine (C)	1	1.1	1	1.1
Aspartic Acid (D)	10	11.1	11	12.2
Glutamic Acid (E)	6	6.7	5	5.6
Phenylalanine (F)	2	2.2	2	2.2
Glycine (G)	6	6.7	5	5.6
Histidine (H)	5	5.6	4	4.4
Isoleucine (I)	4	4.4	4	4.4
Lysine (K)	7	7.8	8	8.9
Leucine (L)	13	14.4	14	15.6
Methionine (M)	1	1.1	1	1.1
Asparagine (N)	4	4.4	4	4.4
Proline (P)	1	1.1	1	1.1
Glutamine (Q)	1	1.1	1	1.1
Arginine (R)	7	7.8	7	7.8
Serine (S)	2	2.2	2	2.2
Threonine (T)	4	4.4	4	4.4
Valine (V)	8	8.9	8	8.9
Tryptophan (W)	1	1.1	1	1.1
Tyrosine (Y)	2	2.2	2	2.2

Table 5. The secondary structure (2D) of ADG2 fragment in PVY^{NTN}-resistant and susceptible potato lines.

Protein structure (2D)	Resistant (Ry_{adg})	Susceptible (ry_{adg})
Alpha helix (Hh)	40 (44. 44%)	34 (37.78%)
Extended strand (Ee)	19 (21.11%)	24 (26.67%)
Beta turn (Tt)	8 (8.89)	6 (6.67%)
Coiled-coil (Cc)	23 (25.56%)	26 (28.89%)

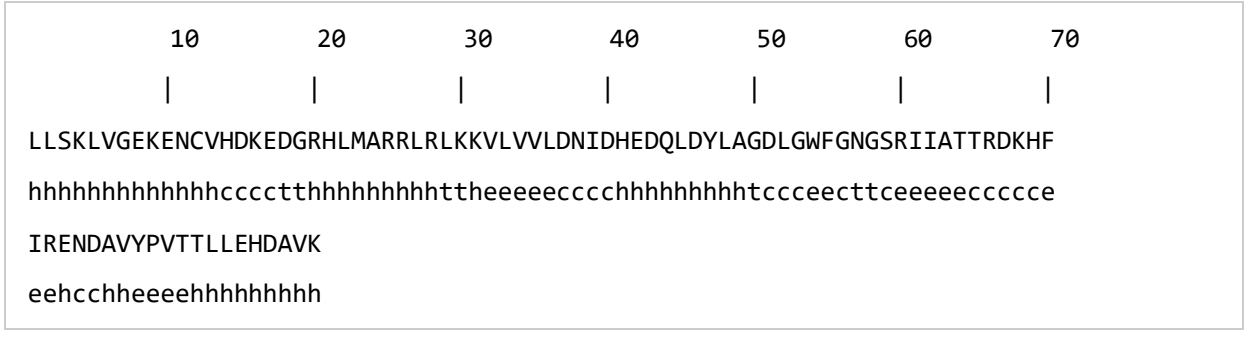

Fig. 6. The secondary structure (2D) of ADG2 fragment in *S. tuberosum* subsp. *andigena* resistant to PVY^{NTN} (Ry_{adg}-carrying).

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        10        20        30        40        50        60        70
         |         |         |         |         |         |         |
LLSKLVGEKENCVLDKEDGRHLMARRLRLKKVLVVLDNIDHEDQLDYLAGDLGWFDNGSRIIATTRDKHF
hhhhhhhhhhheeeccccchhhhhhhhhhceeeeeeccccchhhhhhhhtcceeecttceeeeeccccee
IRKNDAVYPVTTLLEHDAVK
ecccchheeeehhcccthhhh
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Fig. 7. The secondary structure (2D) of ADG2 fragment in cultivar Selena susceptible to PVY^{NTN} (non-*Ry$_{adg}$*-carrying).

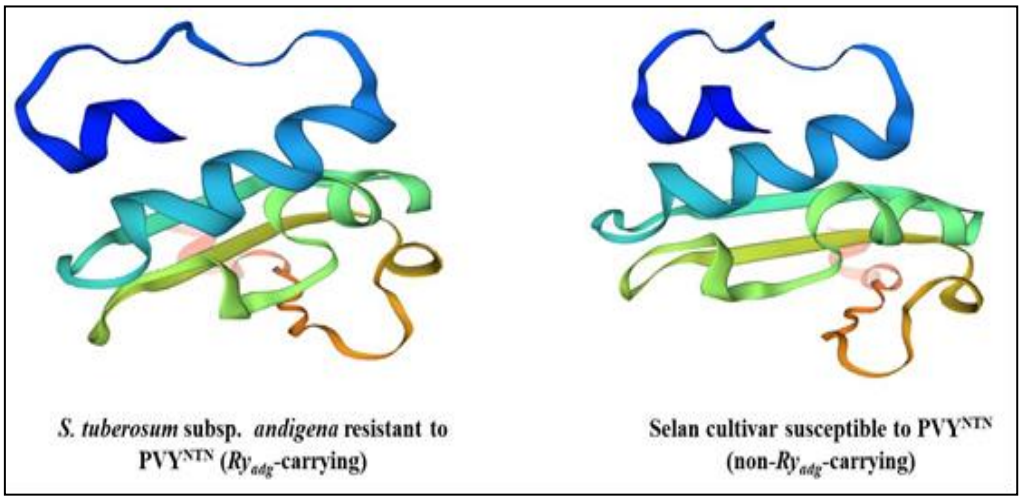

Fig. 8. Predicted 3D structure model of ADG2 protein *Ryadg*-carrying resistant line (R_{adg}) and *Ryadg*-lacking susceptible cultivar (S_{adg}).

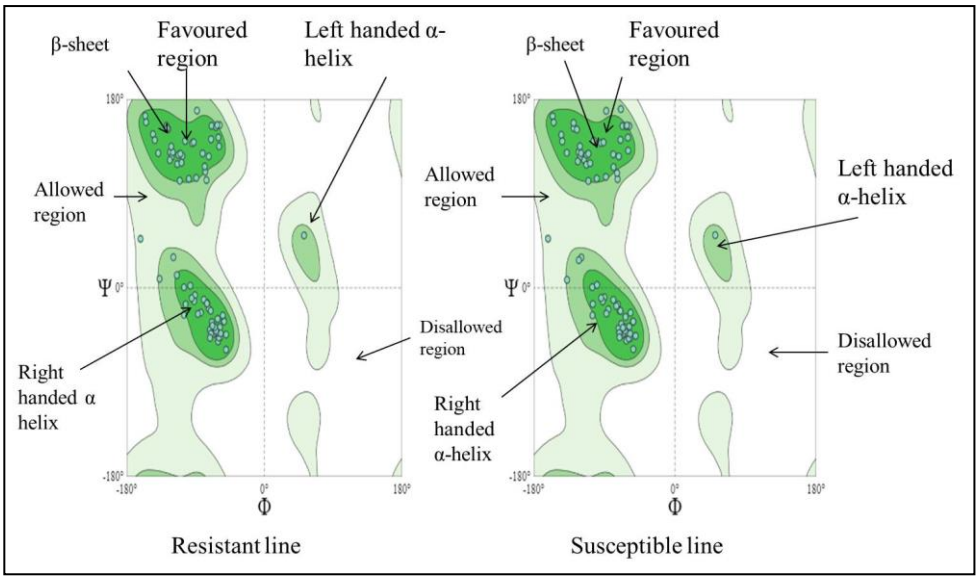

Fig. 9. Ramachandran plot of ADG2 protein in *Ryadg*-carrying resistant line (R+adg) and *Ryadg*-lacking susceptible line (S-adg). Most favoured regions in the Ramachandran plot are shown in dark green; allowed regions are shown in light green; and disallowed regions are shown in white color. Dots represent (φ, ψ) angles for each residue of the predicted structure.

In the current study, we detected the ADG2 gene fragment in six tested potato lines using gene specific primers. All the studied potato lines exhibited one amplified fragment of 355 bp. Two fragments of ADG2 from potato line resistant to PVY^{NTN} *S. tuberosum* subsp. *andigenia* (R+adg), and

susceptible line Selena(S-adg) were sequenced, and % similarity of the nucleotide sequences of the two fragments ranged between (98.41-85.89%) and (96.02-82.82%) respectively, compared with reference sequences in the GenBank. The sequence alignment revealed many base-pair substitutions between the R+adg line and susceptible line S-adg. It has been found that there is little polymorphism at nucleotide (ten nucleotides) and amino acid (three residues) levels between the PVY^{NTN}-resistant (R+adg) and susceptible line (S-adg). Sorri *et al.,* (1999) amplified ADG2 fragment of *Ryadg*-carrying resistant and *Ryadg*-lacking susceptible potato lines. They differed in 12 nucleotides and four amino acid residues. Mahfouze *et al.,* (2022) indicated the presence of differences in the nucleotide sequences of the *R3a* and *R8* loci in *S. tuberosum* lines resistant and susceptible to late blight, which led to the alterations in amino acid sequence. Besides, the greatest number of substitutions was scored in recessive allele *r8* of cultivars 'Bellini' and 'Cara' (16 substitutes). However, the least number was found in dominant allele *R3a* of cultivar 'Cara' (4 substitutes).

To identify the NBS, LRR, and P-loop of the ADG2 proteins in both R+adg and S-adg lines, the InterPro protein families and domains database was used. Motif analysis showed variability in positions CC, NBS, LRR, and P-loop (containing nucleoside tripfosphate hyrolyses) between two lines. In Ry_{adg}, CC domain is responsible for cell death autonomously. However, a nucleotide binding pocket is formed by the NBS region, which functions as a molecular switch to control signal transmission through conformational changes (Wang et al., 2021). A second conformational shift in the NBS-CC domain and additional activation of the signalling cascade are triggered by the recognition of the pathogen by the LRR region, which results in a conformational change that is transduced through the LRR to NBS II and allows the exchange of ADP for ATP (DeYoung and Innes 2006). In this work, sequence analysis has shown that they contain LRR, which is characteristic of plant proteins involved in protein-protein interactions.

In the present investigation, Prabi and Swiss-Model softwares were used to predicate the secondary (2D) and tertiary structures (3D) of the ADG2 protein, respectively (Geourjon and Deleage 1995; Schwede *et al.,* 2003). The results showed a little variation in the number α-helix, β-sheets, Beta-turn, and coil-coiled. Thus, the R+adg line consisted of α-helix (40; 44.44%), β-sheets (19; 21.11%), Beta-turn (8; 8.89%), and coil-coiled (23; 25.56%). Whenever the S-adg line has α-helix (34; 37.78%), β-sheets (24; 26.67%), Beta-turn (6; 6.67%), and coil-coiled (26; 28.89%). β-sheets is part of the LRR domain, which shares in the immune specificity (DeYoung and Innes 2006). The 3D model of the ADG2 fragment in both the resistant and susceptible lines is a monomer composed of an alpha helix and several beta pleated sheets with a compact conformation. Although the two are similar in crystal structure, the functions are different. The 3D models of ADG2 proteins of R+adg and S-adg lines were evaluated by the Ramachandran plot. These models have favoured, and allowed, and disallowed amino acids. The Ramachandran plot information is crucial and demonstrated that the homology modelling process was trusted. This ensuring of the structure can contribute in comprehension the strategy of protein folding. Therefore, these models are confident and can be used for further analysis. This is the first report describing the ADG2 protein isolated from R+adg and S-adg potato lines. In this research, we supplied data on the 3D model of the ADG2 protein linked to the R resistance proteins. The characterization of the crystallography of ADG2 proteins will aid in understanding the protein-protein reactions between the R protein of the potato and the virus protein (Chattopadhyaya and Pal 2008). Data on the tertiary structure (3D) of proteins will aid in the comprehension of biological processes in plant host cells. Proteins carry out their biological functions by folding into compact, three-dimensional shapes determined by their amino acid sequences (Anfinsen 1973; Mahfouze *et al.,* 2017).

Conclusions

The sequence comparison of the ADG2 fragments between the resistance line ($Ry+_{adg}$) and the susceptible line (S-adg) showed they varied in ten nucleotides, and three amino acid residues. Therefore, the comparison of nucleotide sequence between two lines will lead to the identification of DNA-based markers, which will be applied in potato breeding programs as marker-assisted selection to discriminate potato lines bearing Ry_{adg} from those lacking this locus. Besides, the characterization of the crystallography of ADG2 proteins will aid in understanding the protein-protein reactions between the R protein of the potato and the virus proteins.

Conflict of Interest

The authors do not have any conflicts of interest to disclose.

Data Availability

The data sets generated and/or analysed during the current study are available in the NCBI repository (https://www.ncbi.nlm.nih.gov) with accession numbers OR162002, OR162003, WLQ22389, and WLQ22390.

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