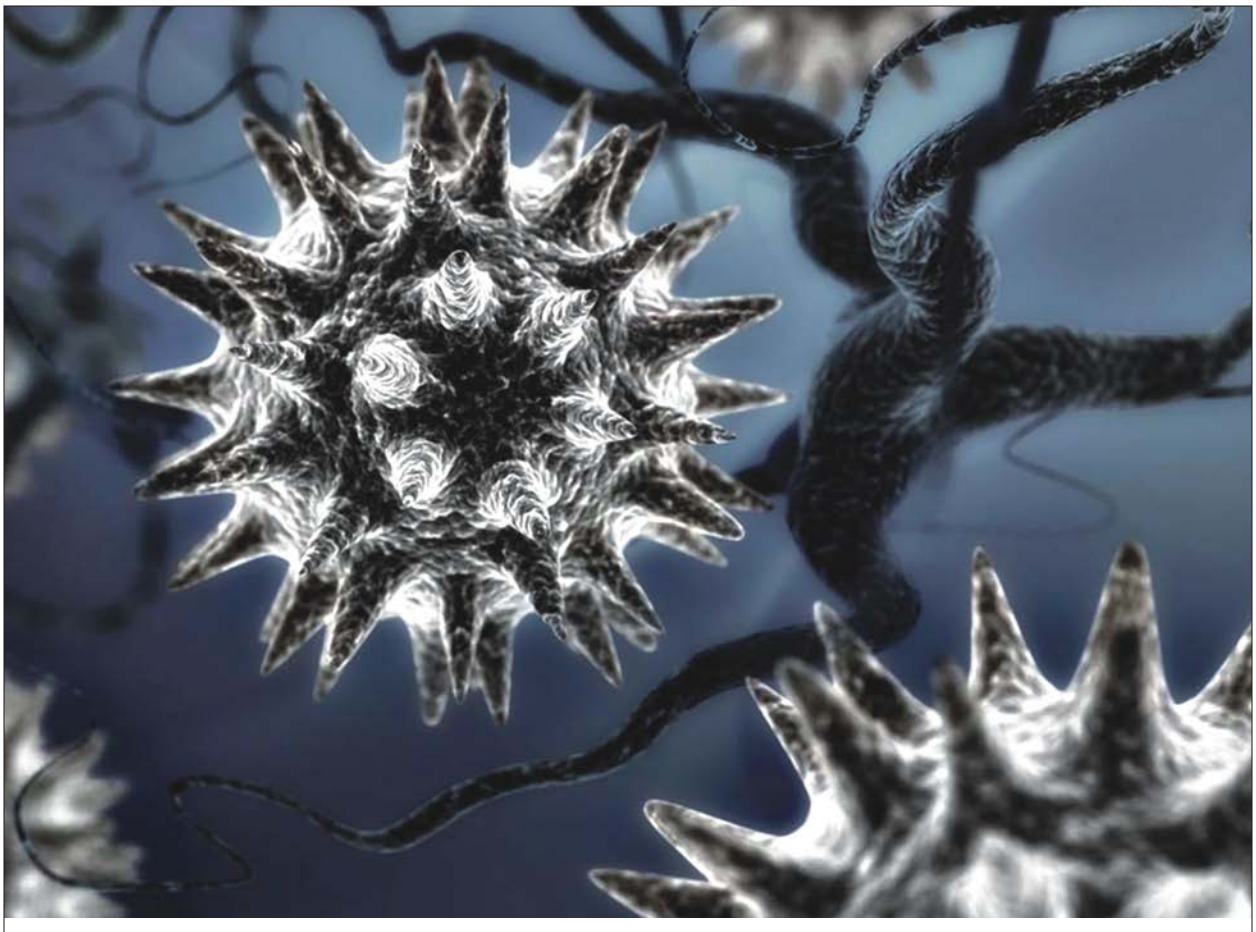

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Genetic Comparison Between Coat Protein Gene of *Alfalfa mosaic virus* Isolate Infecting Potato Crop in Upper Egypt and Worldwide Isolates

Osama A. Abdalla, Safynaz A. Mohamed, Amal I. Eraky and F.G. Fahmay
Department of Plant Pathology, Faculty of Agriculture, Assiut University, Assiut, Egypt

Corresponding Author: Osama A. Abdalla, Department of Plant Pathology, Faculty of Agriculture, Assiut University, Assiut, Egypt

ABSTRACT

Alfalfa mosaic virus is one of the most important viruses infecting potato worldwide. Genetic comparison between Coat Protein (CP) gene of AMV isolate infecting potato in Upper Egypt (AMV-Assiut) and worldwide isolates was carried out in this study. The AMV-Assiut isolate shared similarity in CP gene ranged from 90-95 and 95-97% in nucleotide and amino acid sequences of CP gene, respectively. The AMV-Assiut shared the highest similarity with Egyptian AMV isolates (Wady Elnatron) and AMV isolate from Croatia in case of nucleotide and amino acid, respectively. Phylogenetic analysis showed that AMV isolates tend to cluster into two main groups, with additional clustering of AMV isolates in each group into two subgroups, supporting the hypothesis of existence two main strains of AMV. No clear geographical or host origin structure was found among AMV isolates.

Key words: *Alfalfa mosaic virus*, coat protein gene, nucleotide sequence, amino acid sequence

INTRODUCTION

Potatoes are considered as one of the most important vegetable crops in Egypt (El-Helaly *et al*., 2012). It is being infected by several viruses causing great losses in potato production (Wangai and Lelgut, 2001). More than 25 viruses are reported to infect potato worldwide (Beemstar and Rozendaal, 1972). Among these viruses *Alfalfa mosaic virus* (AMV) is considered as one of the most important viruses infecting potato worldwide (Jaspars and Bos, 1980). It causes diseases in many economically important crops families including Solanaceae and Fabaceae (Hiruki and Miczynski, 1987).

Alfalfa mosaic virus (AMV) is the type member of genus *Alfamovirus* in Bromoviridae family of plat viruses (Parrella *et al*., 2000). The AMV particles are composed of icosahedral capsids measuring 30-57 nm in length and 18 nm in diameter (Thole *et al*., 1998). The genome of AMV consists of three single strand RNA molecules of plus sense polarity, conventionally numbered RNA 1-3 of decreasing size (Xu and Nie, 2006), encapsidated into B, M and T components, respectively (Jaspars, 1985). It is transmitted by aphids in non-persistent way (Hull, 1969). Sixteen species of aphids including *Myzus persicae* can transmit AMV (McDonald and Suzuki, 1983; Moreira *et al*., 2010). The AMV can also be transmitted by potato pollen and true seed (Crill *et al*., 1971; Zitikaite and Samuitiene, 2008).

Alfalfa mosaic virus (AMV) has a wide host range (Parrella *et al*., 2000; Al-Saleh and Amer, 2013). It can naturally infect many herbaceous and some woody plants and infect more than

430 plant species including several vegetable and woody crops in over 51 dicotyledonous families (Parrella *et al*., 2011). Potato is one of the most common hosts infected with AMV, which can cause various symptoms including mosaic, mottling and malformations, (Mughal *et al*., 2003; Bailiss and Ollenu, 1986). The yield of potatoes infected with AMV showing calico mosaic symptoms is reduced by about 20% (Miczynski and Hiruki, 1987).

In Egypt, AMV is considered as one of the most frequent viruses infecting potato in different locations (Gamal El-Din *et al*., 1994; El-Helaly *et al*., 2012).

This study was designed to genetically compare between AMV isolate infecting potato crop in Assiut governorate and other Egyptian and worldwide AMV isolates, estimate the genetic variability among AMV-Assiut isolate and worldwide isolates and also determine the genetic relationship among AMV-Assiut isolate and other Egyptian isolates as well as AMV worldwide isolates. These information will help to increase our understanding about AMV movement from country to country and mutation rate in virus genome and should put in consideration in any attempt to design along management strategy of this viral pathogen in Egypt.

MATERIALS AND METHODS

Source of viruses: Potato plant growing in experimental farm, Faculty of Agriculture, Assiut University showing typical AMV like symptoms including mosaic, mottling, malformation of leave and stunting were serologically tested and reacted positively with specific antibodies against AMV. The RNA extracted from these plants according to Ali *et al*. (2012) was tested in reverse transcriptase poly chain reaction using specific primer to amplify coat protein gene of AMV as described by Abdalla and Ali (2012a).

Sequencing: Sequencing was carried out in both directions using Big-Dye terminator cycle sequencing according to Sanger *et al*. (1977) at the core facility of Molecular Biology Unit, Assiut University, Assiut, Egypt using a sequencing instrument DNA Sequencing Applied Biosystem.

Phylogenetic analysis: Neighbor joining trees were generated with bootstrap 1000 from both nucleotide and amino acid sequence of AMV-Assiut isolate and worldwide AMV isolates available in the GenBank database from other geographical locations (Table 1) using the MEGA 5.02 program (Tamura *et al*., 2011).

RESULTS

Comparison between nucleotide sequences of AMV isolates from Assiut and worldwide isolates: Neighbor-joining tree generated from 20 nt sequences of Assuit-AMV and worldwide AMV isolates (Fig. 1), showed that AMV isolates clustered into two main groups (group I and II) and Egyptian isolates fall in both of these groups. Assiut-AMV isolate fall in group II with another Egyptian isolate from Wady Elnatron (Accession number: HG315522), while another Egyptian isolate from Elmonyfeya governorate fall into group I. The majority of AMV isolates in group I originated from countries in North, South America, Europe and Australia including USA, France, Italy, Canada, Chile, Mexico and Brazil, beside AMV isolate from Elmonyfeya-Egypt. While the majority of isolates in group II originated from Asian countries including China, Korea and Japan, beside isolates form Egypt (Assiut and Wady Elnatron).

Table 1: *Alfalfa mosaic virus* (AMV) worldwide isolates in GeneBank data base (used to compare coat protein gene of AMV-Assuit isolate and worldwide isolates

Accessions	Countries	Hosts	References	Year
L00162	USA	*N. glutinosa*	Koper-Zwarthoff *et al*. (1977)	1977
AJ130709	France	*N. glutinosa*	Parrella *et al*. (2000)	1998
AJ130707	France	*N. glutinosa*	Parrella *et al*. (2000)	1998
AJ130703	Italy	*N. glutinosa*	Parrella *et al*. (2000)	1998
JN256026	USA	*Glycine max*	Khatabi *et al*. (2012)	2011
JN256025	USA	Soybean	Khatabi *et al*. (2012)	2011
JN209847	Australia	*T. repens*	Emmerling *et al*. (2004)	
KJ504107	Croatia	Lavandin	Stankovic *et al*. (2014)	2013
JQ691234	Spain	*S. oleraceus*	Bergua *et al*. (2014)	
JQ691229	Spain	*C. album*	Bergua *et al*. (2014)	2005
U12510	New Zealand	*M. sativa*	Unpublished	1994
AF294433	Korea	*S. tuberosum*	Unpublished	2000
LK937168	China	*Nicotiana*	Unpublished	2014
EF427449	Spain	*V. lucidum*	Cebrian *et al*. (2008)	
FJ858265	Brazil	*M. sativa*	Moreira *et al*. (2010)	2009
HQ185569	USA	*Glycine max*	Fajolu *et al.* (2010)	2006
JN040542	Chile	*V. tinus* L.	Pena *et al*. (2011)	2011
HQ288892	Egypt-1	*S. tuberosum*	EL-Helaly *et al*. (2012)	2010
JQ673587	Iran	*M. sativa*	Unpublished	2008
JX154092	USA	*Hydrangea*	Lockhart *et al*. (2013)	2012
HF570950	Italy	*A. sericifera*	Parrella *et al*. (2013)	2012
JX996119	Croatia	*Lavandula* x	Vrandecic *et al*. (2013)	2012
JQ691184	Spain	*M. sativa*	Bergua *et al*. (2014)	2005
FJ858264	Brazil	*Carica papaya*	Moreira *et al*. (2010)	2010
AF215664	New Zealan	*S. tuberosum*	Timmerman-Vaughan *et al*. (2001)	2000
DQ314756	Canada	*S. tuberosum*	Xu and Nie (2006)	2005
DQ314755	Canada	*S. tuberosum*	Xu and Nie (2006)	2006
DQ124429	USA	*P. paniculata*	Unpublished	2005
JQ691222	Spain	*B. officinalis*	Bergua *et al*. (2014)	2000
JQ691212	Spain	*S. lycopersicum*	Bergua *et al*. (2014)	1984
JQ691204	Spain	*Capsicum annuum*	Bergua *et al*. (2014)	1981
JQ691202	Spain	*Medicago sativa*	Bergua *et al*. (2014)	2007
KC569797	Saudi Arabia	*S. tuberosum*	Al-Saleh and Amer (2013)	2013
AY340071	USA	*Phaseolus vulgaris*	Shah *et al*. (2006)	2002
AY957607	Mexico	*L. nepetaefolia*	Unpublished	2005
KJ855273	Saudi Arabia	*S. oleraceus*	Al-Shahwan *et al*. (2003)	2013
KJ855272	Saudi Arabia	*S. tuberosum*	Al-Shahwan *et al*. (2003)	2013
AB451173	Japan	*S. tuberosum*	Maoka *et al*. (2010)	2010
FN667967	Italy: Liguria	*L. stoechas*	Parrella *et al*. (2010)	2010
HG315522	Egypt	*S. tuberosum*	Unpublished	2005
JX857635	China	*Medicago sativa*	Unpublished	2011
Y09110	Italy	*L. esculentum*	Unpublished	1996
KF959874	Mexico	*Vicia faba*	Unpublished	2010
JX112759	Australia	*Medicago sativa*	Jones and Pathipanawat (1989)	2001
JX112758	Australia	*Medicago sativa*	Jones and Pathipanawat (1989)	2001

The degree of variation in nucleotide sequences of CP genes of AMV isolates (Table 2) was up to 10%. Assiut-AMV isolate shared highest similarity in CP gene with isolated from Egypt (Wady Elnatron), followed by AMV isolates form China and Egyptian isolates form Elmonyfeya, as the degree of variation were only 4,8, 5.1 and 5.6%, respectively (Table 2). While, Assiut-AMV isolate shared the less degree of similarity in nucleotide sequences of CP gene with isolates from Brazil then isolates from Australia, as the degree of variation were 9.9 and 7.5%, respectively (Table 2).

Comparison between amino acid sequences of AMV isolates from Assiut and worldwide isolates: Neighbor-Joining (NJ) tree generated from 20 aa sequences of CP genes of Assuit-AMV isolate and worldwide isolates, showed that AMV isolates also formed two clusters,

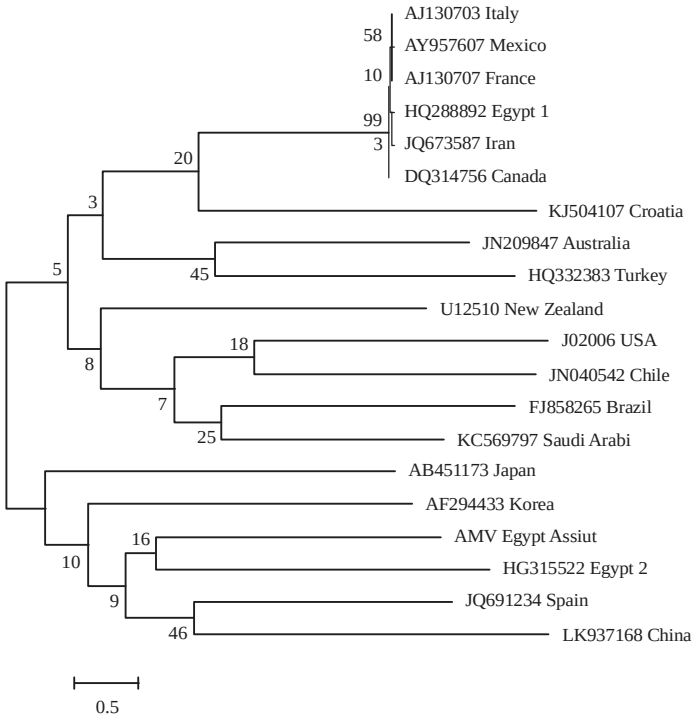

Fig. 1: Neighbor joining tree construct from amino acid sequences of AMV-CP gen of AMV isolate from Egypt and 19 worldwide AMV isolates

Table 2: Pairwise distance among nucleotide sequences in coat protein gene of *Alfalfa mosaic virus* isolates from Assiut (Egypt) and worldwide AMV isolates

Accessions	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
AMV_Egypt_Assiut																			
J02006_USA	6.9																		
AJ130707_France	5.7	6.5																	
AJ130703_Italy	5.1	6.5	0.0																
JN209847_Australia	7.5	7.1	5.3	5.3															
KJ504107_Croatia	6.8	5.9	4.0	4.0	6.3														
JQ691234_Spain	6.3	7.5	6.8	6.8	7.9	6.7													
U12510_NewZealand	6.4	4.2	5.1	5.1	4.7	8.2	6.3												
AF294433_Korea	5.5	4.8	6.7	6.7	7.6	4.5	6.4	5.8											
LK937168_China	5.1	5.3	8.0	8.0	7.2	7.1	4.7	10.0	5.3										
FJ858265_Brazil	9.9	4.7	5.7	5.7	5.3	7.6	7.3	7.4	10.0	10.0									
HQ332383_Turkey	7.6	7.4	5.2	5.2	4.3	6.5	7.2	7.2	5.7	6.3	6.8								
JN040542_Chile	7.3	4.4	6.0	6.0	7.0	5.6	6.8	7.2	7.6	6.3	4.6	7.8							
HQ288892_Egypt_1	5.6	6.7	0.4	0.0	5.3	4.2	6.8	5.0	6.6	7.9	5.4	5.1	6.0						
JQ673587_Iran	5.6	6.4	0.4	0.0	5.2	4.1	6.7	5.1	6.8	7.8	5.7	5.2	6.1	0.3					
DQ314756_Canda	5.5	6.5	0.3	0.0	5.1	4.1	6.7	5.1	6.8	7.8	5.6	5.2	6.1	0.3	0.0				
KC569797_SaudiArabi	6.8	6.6	5.5	5.5	4.2	8.0	4.7	3.9	6.9	7.9	4.0	7.4	5.0	5.4	5.4	5.3			
AY957607_Mexico	5.7	6.4	0.1	0.0	5.1	4.1	6.9	5.0	6.8	8.0	5.6	5.2	6.0	0.5	0.0	0.0	5.4		
AB451173_Japan	7.4	10.0	5.9	5.9	6.6	7.3	4.7	5.4	5.4	7.5	7.2	7.1	7.0	6.0	5.8	5.8	6.8	5.8	
HG315522_Egypt_2	4.8	5.9	7.1	7.1	10.0	4.9	5.3	6.6	4.8	5.3	5.3	7.4	8.3	7.4	7.3	7.3	10.0	7.3	5.3

with additional clustering of AMV isolates in each groups into two separate subgroups (Fig. 2). But the difference between NJ tree constructed from nucleotide sequences and that one generated from amino acid sequences was the affiliation of each isolates in each groups, as some isolates which fall in group I in case of nucleotide tree fall into group II in case of amino acid tree. Egyptian isolates also fall in both groups. However, the affiliation of the isolates in groups was different than

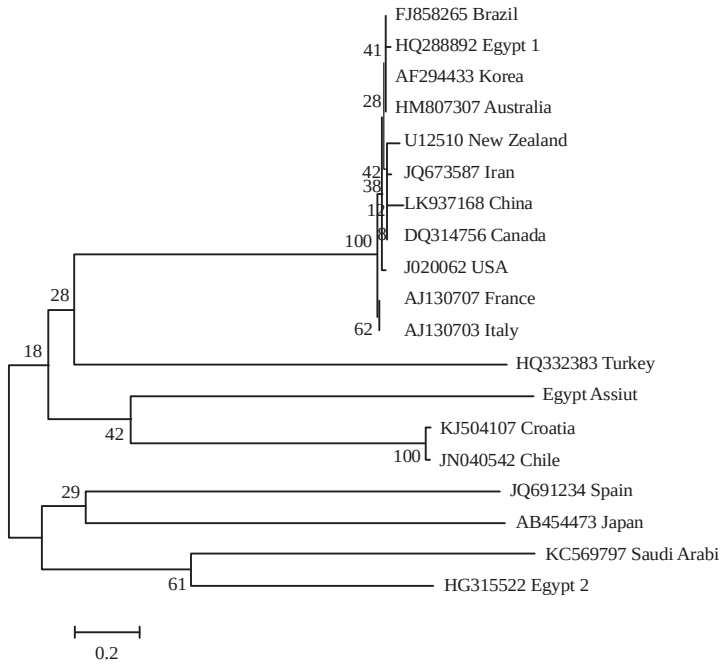

Fig. 2: Neighbor joining tree construct from amino acid sequences of AMV-CP gen of AMV isolate from Egypt and 19 worldwide AMV isolates

Table 3: Pairwise distance among amino acid sequences in coat protein gene of *Alfalfa mosaic virus* isolates from Assiut (Egypt) and worldwide AMV isolates

Accessions	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Egypt_Assiut																		
J020062_USA	2.5																	
AJ130707_France	2.4	0.0																
AJ130703_Italy	2.4	0.3	0.0															
HM807307_Australia	2.9	0.1	0.4	0.4														
KJ504107_Croatia	2.1	2.1	2.1	2.1	2.1													
JQ691234_Spain	2.7	2.7	2.7	2.7	2.7	2.5												
U12510_NewZealand	2.0	0.6	0.6	0.6	0.5	2.1	2.7											
AF294433_Korea	2.5	0.1	0.4	0.4	0.0	2.1	2.7	0.5										
LK937168_China	2.5	0.8	0.8	0.8	0.6	2.3	2.7	0.9	0.6									
FJ858265_Brazil	2.5	0.1	0.4	0.4	0.0	2.1	2.7	0.5	0.0	0.6								
HQ332383_Turkey	3.0	2.3	2.3	2.3	2.3	2.7	3.2	2.3	2.3	2.4	2.3							
JN040542_Chile	2.1	2.1	2.1	2.1	2.1	0.3	2.5	2.1	2.1	2.3	2.1	2.7						
HQ288892_Egypt_1	2.5	0.3	0.5	0.5	0.5	2.1	2.7	0.0	0.1	0.8	0.1	2.3	2.1					
JQ673587_Iran	2.5	0.4	0.4	0.4	0.3	2.3	2.7	0.5	0.3	0.6	0.3	2.3	2.3	0.0				
DQ314756_Canda	2.5	0.3	0.3	0.3	0.1	2.1	2.7	0.4	0.1	0.5	0.1	2.3	2.1	0.3	0.1			
KC569797_SaudiArabi	2.7	2.7	2.7	2.7	2.7	3.6	2.7	3.0	2.7	2.7	2.7	2.4	3.6	2.7	2.7	2.7		
AB451173_Japan	4.3	2.5	2.5	2.5	2.5	3.0	2.5	2.7	2.5	2.5	2.5	3.0	3.0	2.5	2.5	2.5	3.6	
HG315522_Egypt_2	2.4	2.5	2.5	2.5	2.5	2.1	2.5	2.5	2.5	2.5	2.5	3.0	2.1	2.7	2.5	2.5	1.8	2.1

nucleotide tree. Two Egyptian isolates (from Assiut and Elmonyfeya) fall in group I, but in different subgroups Ia and Ib, respectively (Fig. 2). While, Egyptian isolate (Wady Entron) fall into group II. The degree of variation in CP amino acid sequences of AMV isolates (Table 3) was less than degree of variation in nucleotide sequences and it was up to 4.3% (Table 3).

Assiut-AMV isolate shared highest similarity in aa sequences of CP gene with isolates from Croatia and Chile followed by AMV isolate form Egypt (Wady Elnatron), as the degree of variation

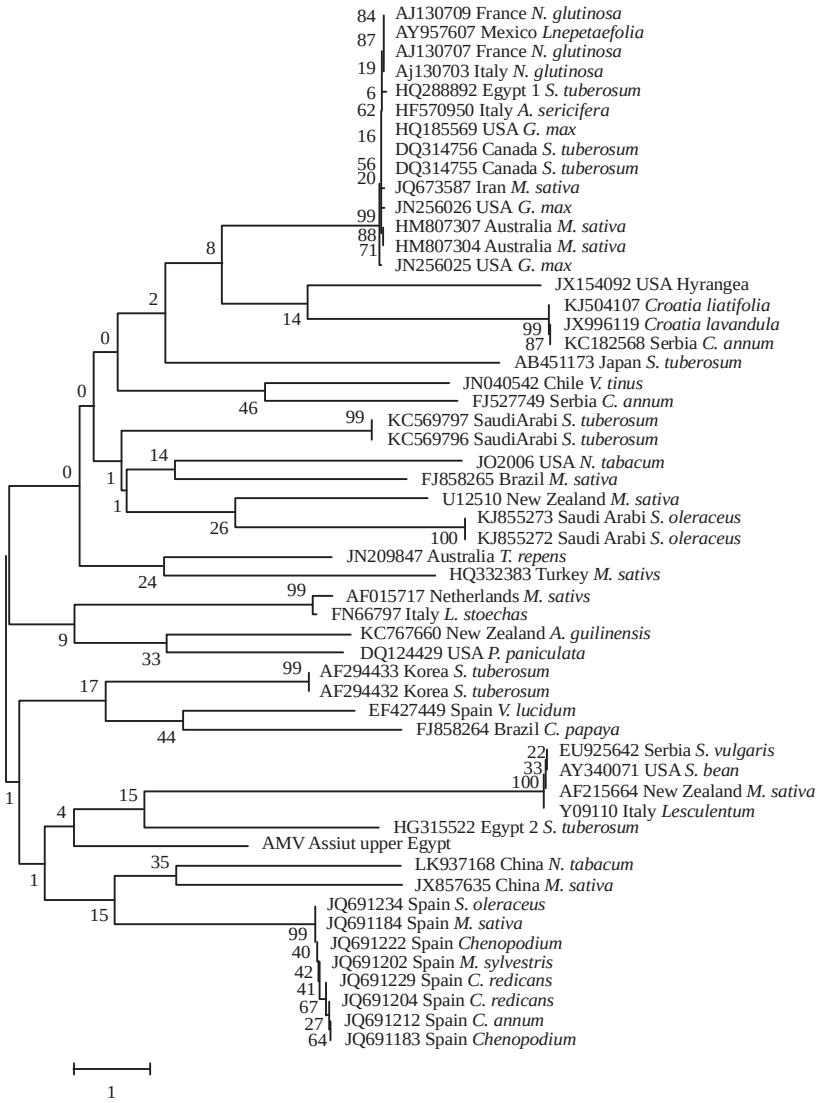

Fig. 3: Neighbor joining tree construct from nucleotide sequences of AMV-CP genes of AMV isolate from worldwide AMV isolates to study the effect of host factor on virus divergence

which were only 2.1, 2.1 and 2.4%, respectively (Table 3). While, Assiut-AMV isolate shared the less degree of similarity in aa sequences of CP gene with isolates from Japan followed by an isolate from Brazil, as the degree of variation were 4.3 and 3%, respectively (Table 3).

Effect of host and geographic origin on relationship among AMV isolates: Results revealed that AMV has a wide host range and to study whether, host and geographical origin of each isolate has any effect on variation, a neighbor joining tree was constructed from CP gene 50 AMV worldwide isolates.

The NJ tree (Fig. 3), showed that AMV clustered into two main groups, with additional clustering of AMV in each groups into subgroups (a and b). Phylogenetic analysis showed neither clear structure among AMV isolates according to their geographical origin nor to their host origin. However, each subgroup contained isolates belong to different and far geographic origin and

different host. The AMV-Assiut showed tendency to cluster according to geographic and host origin, as AMV-Assiut which was closely related to an isolate from Egypt (Wady Elnatron) and both isolates were originated from potato plants, supporting the close relation between these isolates, but the same subgroup also included isolates from different countries and different hosts, This fact contradict with the hypothesis of presence clear AMV structure according to geographic or host origin.

DISCUSSION

Alfalfa mosaic virus (AMV) is one of the most important viruses worldwide and has a very wide host range (Parrella *et al*., 2011; Jaspars and Bos, 1980). It causes severe disease in potatoes (Bailiss and Ollenu, 1986; Miczynski and Hiruki, 1987). In Egypt, AMV appeared on potato plants in several location causing severe lose (El-Helaly *et al*., 2012). Information about degree of variation in AMV and comparison among AMV strains from other countries will lead to better understanding of the similarities and differences among AMV isolates and will help to design a proper approach for detection and management of this virus (Xu and Nie, 2006).

An AMV isolate infecting potato crop in Assiut governorate (AMV-Assiut) was isolated and identified. Sequencing of CP gene of AMV-Assiut isolate showed that this isolate shared high degree of variability with other AMV isolates, this variability ranged from 4.8-10% in case of AMV isolate form Egypt (Wade Elnatron) and isolate from Brazil, respectively.

This degree of variation was similar to the degree of variability reported before by Al-Saleh and Amer (2013), who mentioned that AMV isolates from Saudi-Arabia shared similarity ranged from 90.3-99.3% with AMV worldwide isolates and Xu and Nie (2006), who revealed that the level of variation in CP among AMV isolates was up to 7% in amino acid sequence and up to 10% in nucleotide sequences. Moreover these data was in agreement with Parrella *et al*. (2010), who showed that variation in CP genes of AMV worldwide isolates was up to 7% at the nucleotide level. While Parrella *et al*. (2011) mentioned that the percentage of diversity in CP gene of AMV isolates ranged between 4.0-6.1 and 4.3-5.5% in nucleotide and amino acid sequences, respectively.

This high degree of variation in AMV isolates was reported before as AMV is one of the most biologically variable plant viruses with numerous natural variants having different pathogenicity (Crill *et al*., 1970; Massumi and Pour, 2010; Hajimorad and Francki, 1988).

This level of variation in AMV isolates was similar to degree of variation reported in other RNA viruses like *Papaya ring spot virus*, which was up to 12% at nt sequence (Abdalla and Ali, 2012b), but is still lower than nt variation reported in other RNA like *Yam mosaic virus* (YMV) (Bousalem *et al*., 2000) and *Rice yellow mottle virus* (RYMV) (Pinel *et al*., 2000), which showed nt variation in CP up to 28 and 22.4%, respectively.

The higher variation is character of all RMV viruses which are characterized by rapid mutation and evolution rate (Domingo and Holland, 1997). High variation in CP gen may suggest that coat protein may not the major gene responsible for symptoms se induced by AMV (Van der Vossen *et al*., 1996).

The degree of variation in amino acid sequences among AMV isolates was less than degree of variation in nucleotide sequences, as AMV-Assiut isolate shared a degree of variability ranged from 2.1-4.7% in case of AMV isolates from Croatia and Japan, respectively. This could be explained as some changes in nucleotide did not affect amino acid sequences (Wang *et al*., 1994). This agrees with other studies that reported this natural pressure is keeping virus primary structure as conserved as possible (Hema and Prasad, 2004).

The result of this study is in partial agreement with data reported before about AMV in Egypt by El-Helaly *et al*. (2012), who revealed that Egyptian isolate from Elmonyfeya governorate was closely related to other worldwide AMV isolates and showed similarity with other worldwide isolates which ranged from 97-98%. This difference in data may be due to that the current study used bigger number of isolates than number of isolates which were used in the aforementioned analysis, in addition that may be AMV-Assiut isolate is more distinctive than AMV isolate from Elmonyfeya, However more sequences of AMV isolates from different regions in Egypt is still required to present clear idea about variation among AMV isolates in Egypt.

Phylogenetic analysis, showed that AMV worldwide isolates tend to cluster in two main groups, in case of neighbor joining trees generated from either nucleotide or amino acid sequences (with additional formation of two subgroups in each main group), this tendency may indicate to presence of two distinctive strains of AMV two pathway of evolution of AMV isolates. Similar results were mention before as by Parrella *et al*. (2000), who reported that the topology of the trees obtained with two methods was essentially showing that AMV isolates clustering in two monophyletic groups close clustering of Italian strains in subgroup I and French strains in subgroup. This data in partial agreement with finding published by Kraal (1975), who mentioned that AMV could by divided into three different groups on the basic of amino acid sequences and Xu and Nie (2006), who divided AMV isolates into four different groups.

Study the effect of geographic or host origin on AMV variation and relationship did not show clear relation between either geographic origin or host origin and clustering (relation) among AMV isolates. The AMV isolates from different geographic and host origin tended to cluster in the same subgroup. These data contradict with the hypothesis suggested before about the effect of geographic distinctiveness of evolutionary dynamics of these AMV strains (Parrella *et al*., 2000).

The current study confirmed the high degree of variation in CP gene among MV isolates. This degree of variation should put in consideration in any attempt to control AMV, especially in long term management strategy. The current result revealed that AMV isolates could be divided into two main groups and AMV isolates from Egypt fall into both groups, indicating the possible existence of two strains of AMV isolates in Egypt. Additional molecular and serological studies are required to present more information about AMV isolates infecting potato in Egypt.

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