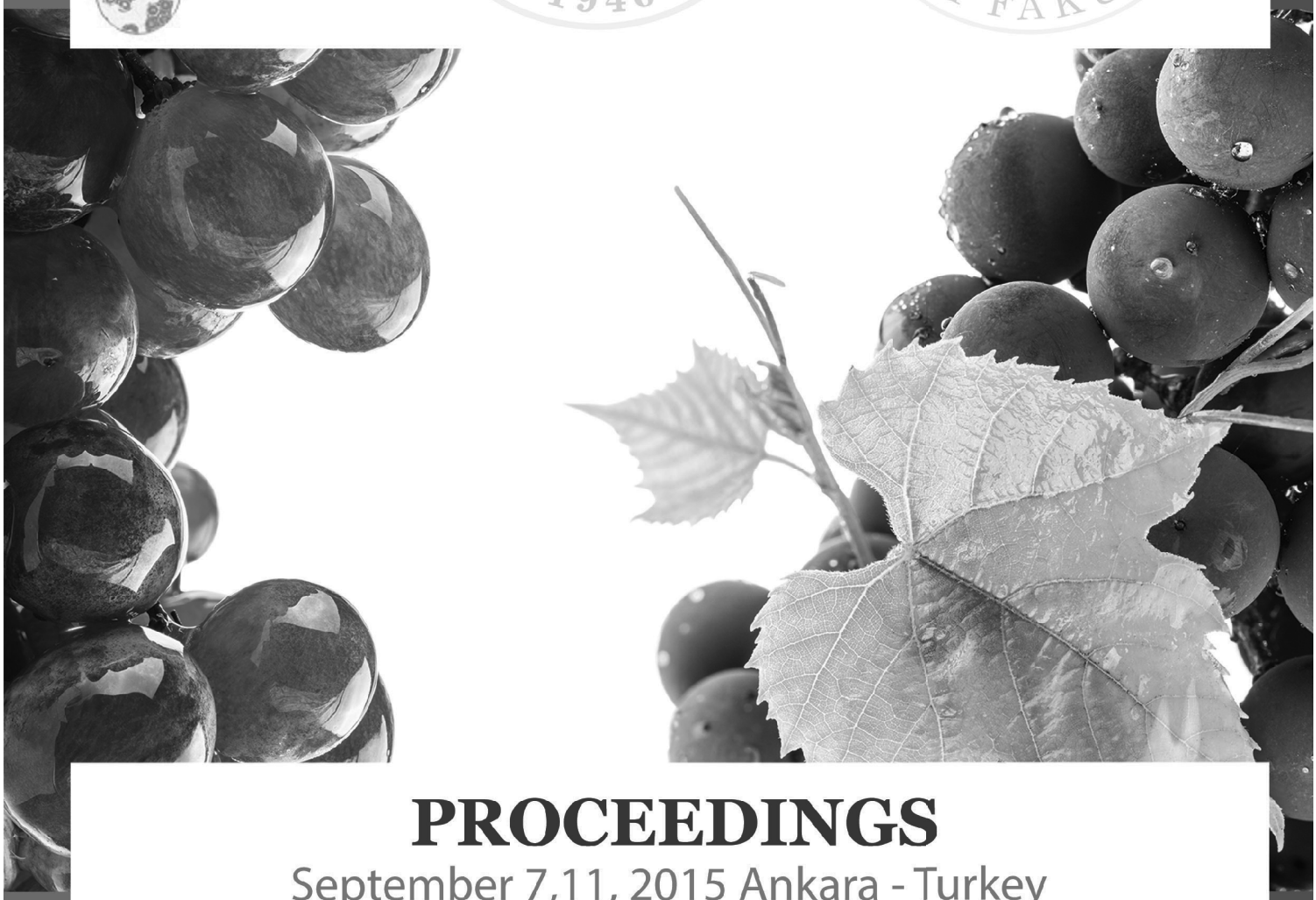
18th Congress of the International Council for the Study of **Virus and Virus-Like Diseases of the Grapevine (ICVG)**



PROCEEDINGS

September 7,11, 2015 Ankara - Turkey

PP 01 - Molecular characterization of Grapevine fan leaf virus from non Vitis hosts

M. Zakiaghl[1], K. Izadpanah[2], Z. Gholampour[1], M. Kargar[1], M. Mehrvar[1]

[1] *Department of Plant Pathology, College of Agriculture,Ferdowsi University of Mashhad, Iran*

Email: zakiaghl@ferdowsi.um.ac.ir

[2] *Plant Virology Research Center, College of Agriculture, Shiraz University, Shiraz, Iran*

** Corresponding author: izadpana@shirazu.ac.ir*

INTRODUCTION

Grapevine fanleaf virus (GFLV) (a member of the genus *Nepovirus*, family *Secoviridae*) is responsible for the for an economic disease of grapevines throughout the world (Andret-Link *et al.,*2004). It is naturally transmitted by the soil nematode *Xiphinema index*, with the coat protein determining transmission specificity (Schellenberger *et al.,*2011). Symptoms of the disease include fanleaf, mosaic, shortened internodes and in chromogenic strains severe leaf chlorosis. GFLV naturally infects grapevine (Andret-Link *et al.,*2004) as well as Bermuda grass and knotweed in Iran (Izadpanah *et al.,* 2003a, 2003b).

Despite ample information on molecular variability of GFLV isolates from grapevine, little is known about molecular aspects of this virus from other hosts. In this paper, we report new weed hosts of GFLV and . molecular variability of GFLV isolates from non-vitis hosts.

MATERIALS AND METHODS

Samples of grapevine and herbaceous plants were randomly collected from vineyards of Iran during growing season of 2012-2014.Total RNA was extracted from the samples using CTAB-PVPP method (*Gibbs* and *Mackenzie 1997*). cDNA was synthesized using reverse transcriptase (*RevertAid*™) and oligo-dT primer. PCR reactions were performed in a final volume of 25µl with *Taq* DNA polymerase (Amplicon Red PCR master mix, Denmark), using a primer pair designed in this work (5'GGATTAGCTGGTAGAGGAG3'/5'CACAAACAACACACTGTCGCC3'), based on sequence of the Iranian isolates of GFLV, targeting the capsid protein (CP) gene. Amplicons were ligated into InsT/A Clone PCR Product Cloning Kit (Fermentas,Thermo Scientific, Inc.) and transformed to *E. coli* XLBlue competent cells. Recombinant plasmids were purified from bacterial cells using Prime Prep Plasmid DNA Isolation Kit (Genetbio-Korea). Recombinant clones were sequenced in both directions. The sequences were aligned using Muscle and phylogenetic trees were constructed using maximum likelihood algorithm performed with MEGA version 5.1.

RESULTS

A fragment of 1515bp was amplified from the herbaceous and grapevine samples by RT-PCR. Bermuda grass, Knotweed, Johnson grass, Raspberry, *Melilotus* sp., *Plantago lanceolata* were found to be naturally infected with GFLV .

Pairwise alignment of the sequences revealed 79-99% identity of herbaceous isolates with grapevine isolates of GFLV at nucleotide and amino acid levels . *Rubus* isolates of the virus were the most divergent.

In the maximum likelihood tree the Iranian isolates of GFLV formed a distinct cluster. They consisted of two sister clades of North East and North West isolates reflecting their geographical separation.

Herbaceous isolates from Bermuda grass, knotweed, Johnson grass, *Melilotus* and *Plantago* and *Rubus* plants from North West and *Rubus* isolates of Sothern Iran were closely related to grapevine isolates from North West of Iran. Surprisingly, Bermuda grass isolates of Sothern Iran showed similar properties to grapevine isolates of other countries (Fig 1).

DISCUSSION

Bermuda grass, knotweed, and raspberry were reported previously as non-Vitis hosts of GFLV in Iran (Izadpanah *et al.,* 2003a, 2003b), Here we report Johnson grass, *Melilotus* sp. and *Plantago lanceolata* as new hosts of GFLV in this country.

Previous analyses based on the MP and CP genes of GFLV have demonstrated that Iran GFLV isolates have distinct

phylogenetic position (Sokhandan-Bashir *et al.,* 2007, 2009). Weed isolates of GFLV show similar molecular properties to grapevine isolates. Also, geographical isolation has significant effect on their phylogenetic relationships. The level of genomic variation suggests that GFLV genomes may consist of a genetically diverse collection of variants, in the manner of a quasispecies (Roossinck 1997). Surprisingly we found two distinct populations of GFLV among Iranian isolates. A divergent isolate showed different evolutionary pathway and formed a separate clade in the phylogentic tree, whereas the others had similar properties to GFLV-F13. This gives further support to the previous hypothesis that GFLV has originated in this region from where it has spread to other parts of the world (Vuittenz1970).

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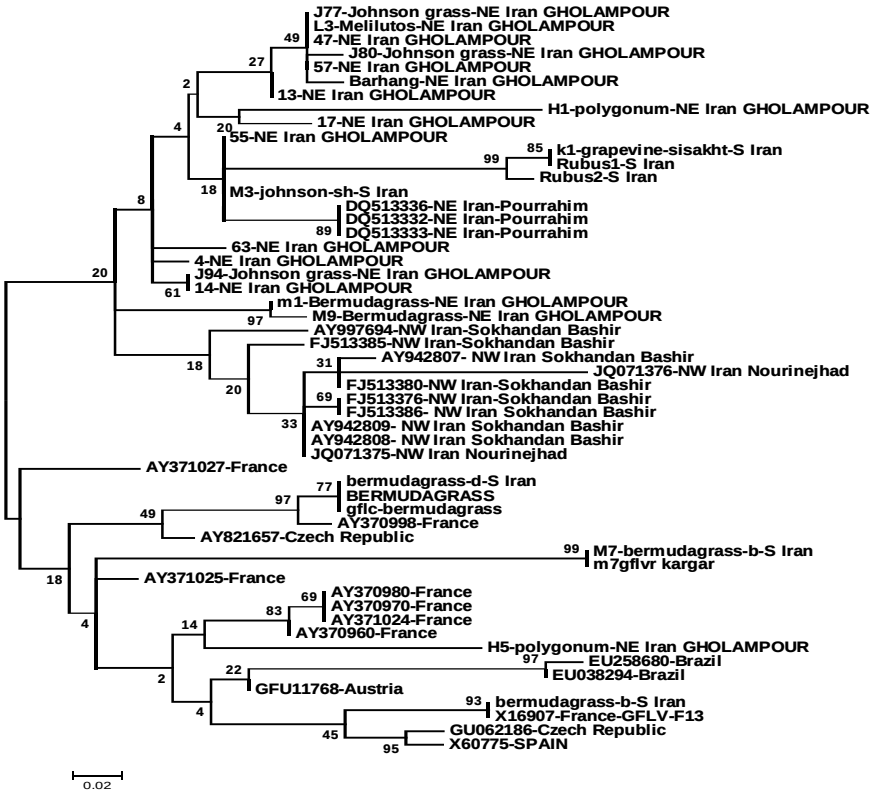

Figure 1: Maximum likelihood tree represent phylogenetic relation of weeds isolates of GFLV with other isolates of GFLV