Evaluation of Maize Inbred Lines for *Iranian maize mosaic virus* (IMMV) Resistance

A. Estakhr¹, ², B. Heidari¹*, A. Dadkhodaie¹ and K. Izadpanah³

¹Department of Crop Production and Plant Breeding, College of Agriculture, 7144165186, Shiraz University, Shiraz, Iran.
²Research Center for Agriculture and Natural Resources of Fars Province, Iran.
³Plant Virology Research Center, 7144165186, Shiraz University, Iran.

**Authors’ contributions**

This work was carried out in collaboration between all authors. Author AE anchored the field study, gathered the initial data and performed preliminary data analysis. Authors BH and AD designed the study and wrote the proposal. Author KI edited the initial draft of manuscript and involved in technical supports. All authors read and approved the final manuscript.

**Article Information**

DOI: 10.9734/ARRB/2015/20058

Editor(s): (1) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.

Reviewers: (1) Inge Gazendam, ARC-Vegetable and Ornamental Plant Institute, South Africa. (2) Anonymous, Kansas State University, USA.

Complete Peer review History: [http://sciencedomain.org/review-history/10702](http://sciencedomain.org/review-history/10702)

**ABSTRACT**

In present study, the putative resistance capacity of thirty five maize inbred lines against *Iranian maize mosaic virus* (IMMV) was studied. Reaction to IMMV was analyzed under natural field infection and controlled conditions in a greenhouse in 2010 and 2011. In the greenhouse experiments, the maize plants were inoculated at two-leaf stage using the planthopper *Laodelphax striatellus*. In the field trials, an early sowing cultivation was used to facilitate a higher infection rate. The responses of inbreds to IMMV were assessed by symptom development and ELISA. The rate of natural infection of IMMV in susceptible control (SC704) was about 20%. MO17 showed about 40% infection in the first year. Both field and greenhouse results confirmed that MO17 was more susceptible to IMMV than SC704 and more reliable to be used as susceptible control in future studies of IMMV. Sowing one row of SC704 as vector spreader between every 5 rows of inbred lines caused sufficient vector propagation and virus transmission. Results of both field and greenhouse experiments showed little and no IMMV infection on K1263/1 and K3547/5.

*Corresponding author: E-mail: bheidari@shirazu.ac.ir;*
respectively. Hence, they were considered as IMMV-resistant. These lines with CIMMYT origin can also be used for production of resistant hybrids. Results showed that resistance to IMMV was not associated with maize maturity because resistance and susceptibility were found in both early and late matured inbred lines. Disease incidence and ELISA values were strongly correlated. Reduced plant height, ear weight and ear diameter and length and delayed silking were observed in plants infected with IMMV.

Keywords: IMMV disease; Incidence; Laodelphax striatellus; ELISA; K1263/1 line.

ABBREVIATIONS

IMMV-Iranian maize mosaic virus, MMV- Maize mosaic virus; ELISA- Enzyme-linked immunosorbent assay; OD-Optical density.

1. INTRODUCTION

Maize (Zea mays L.) is a natural host for over 30 viruses [1]. However, a limited number of viral agents from families potyviridae, rhabdoviridae and reoviridae seriously affect the maize growth globally [1-6].

Rhabdoviruses are single stranded negative sense (non-coding) RNA viruses with a monopartite genome and five major structural proteins. Plant rhabdoviruses are transmitted by cicadellid leafhoppers, delphacid planthoppers and aphids in a persistent propagative manner [7-9]. The rhabdovirus maize mosaic virus (MMV) causes an important disease of maize in the tropical and subtropical regions in Africa, South America, Hawaii and Australia [2,5,10,11]. MMV is transmitted by the planthopper Peregrinus maidis [8].

IMMV is one of the most widespread causal agents threatening maize cultivation in temperate regions of Iran (28° to 31° N) [17]. IMMV is mainly transmitted by the planthopper Laodelphax striatellus under Iran conditions [12]. Climatic changes and favorable temperatures for the planthopper vector exacerbate the problem. A delayed sowing till mid June and use of chemicals, however, are known to reduce vector transmission [13]. Yet, these methods are costly and have low efficiency. Use of varietal resistance is a cost-effective, environmental-friendly and convenient strategy to control plant viral diseases [14].

IMMV was first reported in 1979 from Fars province of Iran [15]. Despite some similarities to MMV, it appeared distinct in morphology, serology and biological characteristics [12,15-18]. IMMV has become epidemic since 2003 in temperate regions of Fars, Iran. This was mainly related to the wide distribution of the vector [12]. Maize fields are usually infested by viruliferous L. striatellus populations when seeds are sown early in May [13,19,20]. Conversely, delayed cultivation limits the propagation and the growth of the vector and inhibits viral transmission. Although this strategy is useful, the best method to control viral diseases is breeding for varietal resistant.

Natural and/or artificial inoculations have been used for maize resistance evaluation against various viruses, such as sugarcane mosaic virus and maize dwarf mosaic virus [21], maize stripe virus and maize mosaic virus [22,23], maize streak virus [24], maize rayado fino virus [25], maize rough dwarf virus [13,26,27] and other viruses [28]. But this work is the first report of maize resistance to IMMV under field and greenhouse conditions.

Different methods can be used to correctly diagnose plant diseases via symptoms. But precise diagnosis is very crucial when high numbers of genotypes are screened for resistance. ELISA is one of the most specific and easiest methods that provides rapid and precise virus detections [29-31]. ELISA is used to test a large number of genotypes in a relatively short period of time. Since its introduction by Clark and Adams [32], ELISA has accelerated the detection of viruses in plant materials, insect vectors, seeds, and vegetative propagules [32-35]. Therefore, accessing genetic diversity and using precise viral diagnosis methods are important for detection of varietal resistance.

The aim of this work was to investigate the responses of 35 maize inbred lines of Iranian maize breeding programs to IMMV using symptomatology and ELISA in greenhouse and field trials.
2. MATERIALS AND METHODS

2.1 Field Experiments with Natural Infection

Thirty five maize inbred lines were subjected to viral infections (Table 1). These lines were supplied from Seed and Plant Improvement Institute, Karaj, Iran. The SC704 cultivar was used as a susceptible local check for IMMV [13]. Field experiments were carried out at Fars Research Center for Agriculture and Natural Resources, Zarghan station (29° 47' N, 52° 43' E), southern Iran, over the years 2010 and 2011.

Inbred lines were arranged in a randomized complete block design with three replications. Genotypes were sown in 5 m single rows 0.75 m apart. Two seeds per hill were sown and seedlings were thinned to one at the two-leaf stage. Final density was 20 plants per row spaced 25 cm apart. A single row of SC704 was sown between every five rows of inbred lines as viral spreader. The best sowing time for maize grain production is 1-10 June in temperate regions of Fars [13]. However, to provide appropriate conditions for vector (L. striatellus) propagation and to enhance the rate of natural infection, seeds were sown on May 10th.

Table 1. Name of maize genotypes screened for IMMV resistance in 2010 and 2011

| Number | Genotype      | Source/Origin                                      |
|--------|---------------|----------------------------------------------------|
| 1      | K3547/3       | Srinagar8848                                       |
| 2      | K3547/5       | Srinagar8848                                       |
| 3      | K18           | Derived from MO17 modifications in Iran             |
| 4      | K3651/1       | SYN-Late                                           |
| 5      | K3545/6       | Tlaltizapan 8946                                   |
| 6      | KLM77002/10-1-1-1-2 | SYN-Late                         |
| 7      | K3653/2       | SYN-Late                                           |
| 8      | A679          | A B73 back-cross derived line [(A662 × B73)(3)]    |
| 9      | K166A         | Extracted from CIMMYT originated materials in Iran  |
| 10     | K74/1         | Extracted from unknown materials in Iran           |
| 11     | K19           | Derived from MO17 modifications                     |
| 12     | MO17          | CL.187-2× C103(CCB-C103)                           |
| 13     | K19/1         | Derived from K19 modifications                      |
| 14     | K1264/1       | Unknown CIMMYT source germplasm                     |
| 15     | K3640/3       | SYN-Late                                           |
| 16     | K166B         | Lines extracted from CIMMYT originated materials in Iran |
| 17     | M82           | Unknown                                            |
| 18     | K1263/1       | Unknown CIMMYT source germplasm                     |
| 19     | A188          | [(4-29*64)-29(4)]-NCB                              |
| 20     | TVA926        | Extracted from Yugoslavian source material          |
| 21     | L105          | Lancaster surecrop                                 |
| 22     | K615/1        | Unknown Iranian local source                        |
| 23     | K1264/5-1     | Unknown CIMMYT source germplasm                     |
| 24     | K3640/5       | SYN-Late                                           |
| 25     | K722          | Unknown of early maturity germplasm                 |
| 26     | Oh43          | Oh40B xW8(CCB-Oh43)                                |
| 27     | K1728/8       | Extracted from Yugoslavian source material          |
| 28     | B84           | Iowa Stiff Stalk, Synthetic Cycle 7 (BSSS germplasm) |
| 29     | B73           | Iowa Stiff Stalk, Synthetic Cycle 5 (BSSS germplasm) |
| 30     | K2816         | Unknown                                            |
| 31     | KE72012/12    | TL88B-6233H Pool93                                 |
| 32     | K1263/2-1     | Unknown CIMMYT source germplasm                     |
| 33     | S61           | O.P.Wigor(Poland)                                  |
| 34     | K3615/2       | SYN-Late                                           |
| 35     | K3493/1       | Unknown from CIMMYT material(EVT 16A)               |
| 36     | SC704         | MO17xB73                                           |
To supply the virus and its vector at the two-leaf stage, eight rows of SC704 were sown surrounding experimental plots on April 25th. Weeds in the border and spreader rows were not controlled and no chemical was used. Results of the first year showed that MO17 is a more susceptible line to IMMV than SC704. Thus, both MO17 and SC704 were used as spreader in the field experiment in the second year to enhance the natural infection.

Symptoms of IMMV visually and clearly were detected 40-50 days after sowing, prior to the silking. In that time, all plants with IMMV symptoms were counted in each replication. The incidence of disease was estimated as the percentage of plants with symptoms. Traits such as plant height, leaf number, ear weight, ear diameter, ear length, cob percent (cob weight×100/ear weight) and day to silking were also recorded.

2.2 ELISA and Greenhouse Experiment

Greenhouse trial was performed at Plant Virology Research Center facilities, Shiraz University, Shiraz, Iran in 2011. Non-viruliferous vector planthopper (L. striatellus) was reared on barley. The planthoppers were placed on an infected maize plant for a week of acquisition feeding and transferred to susceptible barley plants for development of viruliferous colonies. Planthoppers from these colonies were used to inoculate maize seedling at two-leaf stage. Two seeds of each line were sown in a pot which were later thinned to one seedling per pot. The pots were individually kept in insect proof cages covered by nylon-mesh. Fifteen replications (pots) of each line were exposed to 14/10-h light/dark photoperiod. Inoculation of plants was carried out using 4-5 nymph viruliferous planthoppers at the two-leaf stage. Four weeks after artificial inoculation, samples of the newly emerged leaves were subjected to ELISA [35,36]. At this time the susceptible local check showed typical symptoms of IMMV. Leaf samples from healthy SC704 plants and their infected counterparts were used as negative and positive control, respectively. The alkaline phosphatase reactions were measured by determining absorbance at 405 nm with an MR 700 ELISA plate reader (Dynatech Laboratories, Chantilly, VA). Plants were considered ELISA-positive for the virus presence if the mean A405 absorbance was higher than $\overline{X} + 3SD$ in negative control where, $\overline{X}$ and SD denotes mean absorbance and standard deviation of the mean, respectively.

2.3 Statistical Analyses

The percentage of infected plants with IMMV in each plot of the field trials was considered as diseases incidence. Data were subjected to square root transformation, simple analysis of variance for each year and combined analysis of variance of two years using SAS software [37]. Biplot curves were drawn based on principal component analysis (PCA) to show the relationships between different traits and genotypes under natural infections and ELISA values using Genstat 12th edition software. ELISA data were analyzed based on randomized completely unbalanced design procedure. Mean comparisons were carried out using Duncan multiple range test. ELISA negative genotypes that had significant difference with susceptible check were considered IMMV-resistant.

3. RESULTS AND DISCUSSION

3.1 Appearance of Symptoms

Symptoms of IMMV began to appear on susceptible genotypes about 20 days after sowing and continued to develop until silking. IMMV early symptoms were chlorotic spots at the leaf base which developed as continuous/discontinuous chlorotic stripes on leaves and sheaths, accompanied by stunting of the plants and abortion of ears (Fig. 1). The symptoms were similar to those reported earlier [15,38].

3.2 Field Experiments

Simple analysis of variance showed that genotypes had significant variations for the incidence of IMMV in each year (Table 2). Incidence mean in the first year trial (9%) was higher than in the second year (6%). However, combined analysis showed that incidence rates in the two years were not significantly different (Table 3). IMMV incidence in some genotypes was higher than the local check, which means an extra source of IMMV inoculums was available for vector transmission.
Fig. 1. The symptoms of *Iranian maize mosaic virus* on a susceptible plant prior to tasseling stage. a: Chlorotic spots on leaf  b: Chlorotic streaks on leaf  c: Chlorotic streaks on whole plant

Table 2. Analysis of variance of IMMV incidence in maize genotypes in 2010 and 2011

| Source       | df | 2010 IMMV incidence | 2010 IMMV incidence transformed | 2011 IMMV incidence | 2011 IMMV incidence transformed |
|--------------|----|---------------------|---------------------------------|---------------------|---------------------------------|
| Replication  | 2  | 565.180             | 13.536                          | 79.897**            | 2.606**                         |
| Genotype     | 35 | 247.011*            | 4.948*                          | 66.420ns            | 2.475                           |
| Error        | 70 | 112.842             | 2.457                           | 44.397              | 1.393                           |
| Mean         |    | 9.09                | 2.58                            | 6.01                | 2.06                            |

* and **: Significant at 5% and 1% probability levels, respectively, ns: non-significant, IMMV: *Iranian maize mosaic virus*

Table 3. Combined analysis of variances of two-year data for IMMV incidence in maize genotypes

| Source        | IMMV Incidence | IMMV incidence transformed |
|---------------|----------------|----------------------------|
| Year          | 819.665ns      | 13.503**                   |
| Rep(Year)     | 319.254        | 7.893                      |
| Genotype      | 216.731*       | 5.270*                     |
| Genotype*year | 97.186ns       | 2.127ns                    |
| Error         | 79.477         | 1.942                      |
| Mean          | 7.09%          | 2.32%                      |

* and **: Significant at the 5% and 1% levels of probability, respectively, ns: non-significant, IMMV: *Iranian maize mosaic virus*

The mean of IMMV incidence in the two trials was 7.1%. MO17 (40.1%) and SC704 (16.1%) showed the highest IMMV incidence rates in 2010 and 2011, respectively. MO17 (26.7%) and SC704 (19.7%) had the highest IMMV incidences over two years mean (Table 4). This shows that these genotypes were susceptible to IMMV infection. Some lines had significant differences with susceptible genotypes. IMMV incidence in 7 lines was between 0 and 1.6% and significantly different from susceptible genotypes (MO17 and SC704) in 2010.

Natural incidence in the field trial in 2011 was less than that in 2010. Twelve lines with 0-1.9% of incidences had significant differences from both susceptible genotypes in 2011. Some inbred lines such as K74/1, K19, A679, K2816, K615/1 and TVA926 showed symptoms in one year and none in the other year. Three lines
(K3547/5, K1263/1 and S61) with no IMMV symptoms in both years and K19/1 with less than 2% incidence (0.9%) can be considered as IMMV-resistant. Resistance may be associated with decreased incidence of infection, decreased symptom severity or both [39]. Chen et al. [40] used disease incidence to show the response of maize inbred lines to maize rough dwarf virus (MRDV) under field conditions [40]. In their study, genotypes with 0 to 2% incidences were considered as highly resistant, while incidences of 10.1% to 20.0% and 20.1% to 100% were categorized as susceptible and highly susceptible, respectively.

Resistance to MMV has been previously reported in other studies [4,10]. Morphological, serological and molecular studies have confirmed that IMMV is distinct from MMV and other rhabdoviruses infecting gramineous plants [12,17,18,41]. Therefore, MMV resistance does not guarantee resistance against IMMV.

In the previous studies under natural infection conditions, maize genotypes displayed variable resistance to some viruses such as MRDV [26,42-44]. Resistance to MRDV was detected under natural infection conditions in China [45-47].

Table 4. Means of IMMV incidence in maize genotypes in 2010 and 2011

| Genotype         | IMMV incidence (%) |
|------------------|--------------------|
|                  | 2010      | 2011      | Combined data |
| K3547/3          | 13.7 a-d  | 1.9 cd    | 7.8 c-f      |
| K3547/5          | 0.0 d     | 0.0 d     | 0.0 f        |
| K18              | 3.7 cd    | 8.5 a-d   | 6.1 c-f      |
| K3545/6          | 4.8 cd    | 0.0 d     | 2.4 d-f      |
| KLM77002/10-1-1-1-1-2-3 | 5.1 cd | 0.0 d     | 2.6 d-f      |
| K3653/2          | 3.9 b-d   | 4.9 a-d   | 4.4 c-f      |
| A679             | 7.4 b-d   | 0.0 d     | 3.7 c-f      |
| K166A            | 7.3 b-d   | 1.2 d     | 4.3 c-f      |
| K74/1            | 0.0 d     | 6.8 a-d   | 3.4 c-f      |
| K19              | 0.0 d     | 4.8 a-d   | 2.4 d-f      |
| MO17             | 40.1 a    | 15.2 ab   | 27.6 a       |
| K19/1            | 0.0 d     | 1.9 cd    | 0.9 ef       |
| K1264/1          | 3.3 cd    | 8.3 a-d   | 5.8 c-f      |
| K3640/3          | 11.9 a-d  | 6.7 a-d   | 8.8 b-f      |
| K166B            | 8.6 b-d   | 2.3 a-d   | 5.4 c-f      |
| M82              | 6.9 b-d   | 7.2 a-d   | 6.9 c-f      |
| K1263/1          | 0.0 d     | 0.0 d     | 0.0 f        |
| A188             | 8.7 b-d   | 2.9 a-d   | 6.4 c-f      |
| TVA926           | 5.1 cd    | 0.0 d     | 2.6 d-f      |
| L105             | 23.5 a-c  | 5.7 a-d   | 14.6 a-c     |
| K615/1           | 9.1 b-d   | 0.0 d     | 4.5 c-f      |
| K1264/5-1        | 2.8 cd    | 2.2 b-d   | 2.5 c-f      |
| K3640/5          | 7.1 b-d   | 3.3 a-d   | 5.2 c-f      |
| K722             | 3.3 cd    | 14.5 a-c  | 8.9 b-f      |
| Oh43             | 13.5 a-d  | 9.6 a-d   | 11.5 a-e     |
| K1728/8          | 29.3 ab   | 4.2 a-d   | 16.7 a-c     |
| B84              | 11.1 b-d  | -         | 11.1 b-f     |
| B73              | 9.9 b-d   | 5.9 a-d   | 7.9 b-f      |
| K2816            | 7.5 b-d   | 0.0 d     | 3.8 c-f      |
| KE72012/12       | 1.6 d     | 9.3 a-d   | 5.4 c-f      |
| K1263/2-1        | 9.1 b-d   | 3.7 a-d   | 6.4 c-f      |
| S61              | 0.0 d     | 0.0 d     | 0.0 f        |
| K3615/2          | 15.9 b-d  | 6.1 a-d   | 10.9 b-f     |
| K3493/1          | 23.9 a-d  | 12.5 a-d  | 18.2 a-d     |
| SC704            | 23.3 a-c  | 16.1 a    | 19.7 ab      |

Means with the same letters are not significantly different
Natural infection was also used for identification of resistant genotypes to MMV [10]. However, the present work is the first report of resistance against IMMV.

Identification of virus resistance generally involves inoculating a genetically diverse array of germplasm with virus and identifying plants that show either no or low infection. Most frequently, infection is scored by the percent of symptomatic plants. However, it is useful to ensure that the resistance is associated with reduced virus titer using serological or molecular techniques [5]. In this study, resistance and susceptibility of genotypes in the field were confirmed based on determination of virus titer in a greenhouse trial.

3.3 Greenhouse Experiments

Although some of inbred lines showed no IMMV symptoms under field conditions, their reaction to viral infection was tested by ELISA under artificial inoculation in a greenhouse trial. IMMV is not transmitted by mechanical inoculation [1]. Thus, an artificial inoculation of IMMV was performed by *L. striatellus* planthopper under greenhouse conditions.

ELISA results showed that the absorption values of most inbred lines were not significantly different from that of infected SC704 as positive control. Two lines (K3640/5 and MO17) with the highest ELISA absorbance had significant differences with positive control (Table 5). Thus, these inbred lines were more susceptible to IMMV than SC704. The OD values of K1263/1 (0.00), K3547/3 (0.065), K3547/5 (0.036), K3545/6 (0.071), KLM77002/10-1-1-1-1-2-3 (0.069), TVA926 (0.116), K166B (0.157), S61 (0.162) and K19/1 (0.181) were less than 0.190, i.e. the mean value of negative control plus 3 standard deviations (Table 5). These lines had no significant differences with the healthy check SC704 as a negative control (OD=0.09). Except S61 and K19/1, other inbreds had significant difference with the infected SC704 as positive control (OD=0.289) (Table 5). This means that the viral titer in K1263/1, K3547/3, K3547/5, K3545/6, KLM77002/10-1-1-1-1-2-3, TVA926 and K166B was quite low and they could be considered resistant. Two or three folds of mean absorbance value of negative control [48,49] or negative control mean plus two to three folds of standard deviations [30,49,50] have been used as negative threshold. In the present study, mean ELISA value of negative controls plus 3 times of standard deviation (0.19) which is more reliable was used for data analysis and detection of resistance. A genotype with OD less than 0.19 was considered IMMV-resistant if it's OD was also significantly different from the OD of susceptible positive control.

The OD of K1263/1, K3547/3, K3547/5, K3545/6, KLM77002/10-1-1-1-1-2-3 were significantly lower than infected positive control (0.289) and non-significantly lower than that of healthy SC704, as a negative control (0.09) (Table 5). Their OD values also were lower than 0.19. IMMV incidences of these inbred lines in field trials were lower than the susceptible genotypes (SC704 and MO17) which show their resistance to IMMV. Greenhouse trial confirmed field results, because K1263/1 and K3547/5 with no IMMV symptoms in the field had also low ELISA values. These two lines could be used as IMMV resistant for further genetic studies in breeding programs. The genotypes K3640/5, MO17, Oh43, A679, B73, L105, K722, K1728/8, K3615/2 and K18 are widely used in Iran in maize breeding programs but they are highly susceptible to IMMV. MO17 was the most susceptible genotype to IMMV. MO17 and B73 are the parental lines of SC704 hybrid, and have been reported as susceptible lines to other viruses [22,23,51,52].

Combined analysis of variance showed that infected and non-infected plants were significantly different for plant height, ear weight, ear diameter, ear length, cob percent and day to silking. Except cob percent and day to silking, infected plants had lower values for interested agronomic traits compared to non-infected plants (Table 6). The significance of genotype effect for traits implies the existence of suitable genetic diversity among maize inbreds. Leaf number was not significantly different between infected and non-infected plants. Plant height reduction of infected plants is probably due to internodes reduction by IMMV. IMMV caused delaying in silking date. Plant height reduction and delaying in silking and kernel yield reduction from viral diseases has been reported earlier [12,13]. A study on MMV has indicated that maize plants can be dwarfed below 50 cm in height with no kernels produced under severe epiphytotics of the virus [53].
opposite direction with cob percent. This means was in the same direction with leaf number diameter (ED) a correlated. Ear weight (EW) strongly correlated OD and incidence show that they are strongly relationships of traits and genotypes (Fig. 2).

Principal component biplot showed the relationships of traits and genotypes (Fig. 2). Tight and acute angles between the vectors of OD and incidence show that they are strongly correlated. Ear weight (EW) strongly correlated with some ear characteristics such as ear diameter (ED) and ear length (EL) and its vector was in the same direction with leaf number vectors. The vectors of EL, ED and EW were in opposite direction with cob percent. This means that lower cob percent in ear causes higher kernel yield. Incidence and ELISA OD were related to reducing ear weight and other ear traits. Also longer day to silking seems related to higher incidence and higher OD. MO17 being in the vicinity of OD and incidence vectors shows its susceptibility to IMMV. In general, genotypes in right upper box in the vicinity of OD and incidence vectors are very susceptible to IMMV. K1263/1, S61 and TVA926 located in lower left box were far from OD and incidence vectors, which shows their resistance to IMMV (Fig. 2).

Table 5. Means ELISA values of maize genotypes inoculated with IMMV

| Genotype  | OD value | Number of tested plants | Genotype  | OD value | Number of tested plants |
|-----------|----------|-------------------------|-----------|----------|-------------------------|
| K3547/3  | 0.065<sup>n</sup> | 15 | TVA926 | 0.116<sup>n</sup> | 9 |
| K3547/5  | 0.036<sup>n</sup> | 14 | L105 | 0.289<sup>c,h</sup> | 19 |
| K18      | 0.375<sup>d</sup> | 16 | K615/1 | 0.385<sup>b,d</sup> | 11 |
| K3651/1  | 0.369<sup>b</sup> | 13 | K1264/5-1 | 0.398<sup>b,d</sup> | 11 |
| K3545/6  | 0.071<sup>k,n</sup> | 15 | K3640/5 | 0.513<sup>a</sup> | 9 |
| KLM77002/10-1-1-1-1-2-3 | 0.069<sup>n</sup> | 15 | K722 | 0.310<sup>g</sup> | 11 |
| K3653/2  | 0.358<sup>d</sup> | 30 | Oh43 | 0.406<sup>a-c</sup> | 16 |
| A679     | 0.404<sup>c</sup> | 16 | K1728/8 | 0.307<sup>c,g</sup> | 8 |
| K166A    | 0.197<sup>f</sup> | 9 | B84 | - | 18 |
| K74/1    | 0.323<sup>g</sup> | 15 | B73 | 0.379<sup>b,d</sup> | 15 |
| K19      | 0.208<sup>f</sup> | 17 | K2816 | 0.271<sup>c,i</sup> | 12 |
| MO17     | 0.450<sup>b</sup> | 12 | KE72012/12 | 0.374<sup>b,d</sup> | 17 |
| K19/1    | 0.181<sup>f</sup> | 15 | K1263/2-1 | 0.384<sup>b,d</sup> | 16 |
| K1264/1  | 0.264<sup>e,i</sup> | 11 | S61 | 0.162<sup>i</sup> | 12 |
| K3640/3  | 0.288<sup>h</sup> | 10 | K3615/2 | 0.333<sup>b,e</sup> | 16 |
| K166B    | 0.157<sup>h,m</sup> | 10 | K3493/1 | 0.325<sup>b</sup> | 8 |
| M82      | 0.291<sup>c,h</sup> | 15 | SC704 (infected check) | 0.289<sup>c,h</sup> | 14 |
| K1263/1  | -0.006<sup>n</sup> | 17 | SC704 (healthy check) | 0.085<sup>n</sup> | 17 |
| A188     | 0.352<sup>d</sup> | 9 | |

Means with the same letter are not significantly different (p>0.05). ELISA: Enzyme-linked immunosorbent assay, IMMV: Iranian maize mosaic virus, OD: Optical density

Table 6. Combined analysis of variance for agronomic traits of maize genotypes infected with IMMV

|                      | PH     | LN     | EW     | ED     | EL     | CP     | Silking |
|----------------------|--------|--------|--------|--------|--------|--------|---------|
| Year                 | 104171.95** | 4.02<sup>ns</sup> | 169070.97** | 75.88** | 762.82** | 3793.38** | 105.71** |
| Rep (year)           | 498.91 | 0.95   | 2224.49 | 0.23   | 12.08   | 152.05  | 22.43   |
| Genotype             | 1788.86** | 15.17** | 7623.29** | 1.00** | 33.75** | 236.21** | 53.71** |
| Year*Genotype        | 563.66** | 2.25** | 2270.71** | 0.64** | 9.45**  | 109.94<sup>ns</sup> | 5.78<sup>ns</sup> |
| Error                | 126.78 | 0.94   | 566.45  | 0.24   | 5.70    | 73.60   | 5.67    |
| C.V.%                | 8.6    | 6.2    | 24.9    | 13.0   | 17.5    | 36.4    | 3.4     |

Mean squares

**ns**: non -significant, * and **: significant at 0.05 and 0.01,
PH: Plant height, LN: Leaf number, EW: Ear weight, ED Ear diameter, EL: Ear length, CP: Cob percent of ear,
Silking: Day to silking
Fig. 2. The principal component biplot for the association of agronomic traits and maize genotypes. PH: Plant height, ED: Ear diameter, EL: Ear length; EW: Ear weight, LN: Leaf number; CP: Cob percent, Silking: Silking date, Incidence: IMMV incidence, OD: Optical density in ELISA, numbers refer back to the name of genotypes in Table 1

4. CONCLUSION

The present study was conducted to screen 35 maize inbred lines for their reaction to IMMV. This work is the first combined field and greenhouse evaluation of maize genotypes.

The data obtained proved resistance of K1263/1 and K3547/5 and susceptibility of MO17, K3640/5, Oh43, K3651/1, B73, L105, K722, K1728/8, K3615/2 and K3493/1 to IMMV. The genotypes K1263/1 and K3547/5 are the first Iranian IMMV-resistant inbreds with CIMMYT origin that can be used in production of resistant hybrids. K1263/1, K3547/5 and MO17 are genetically homozygous, which is a prerequisite for production of segregating populations and suitable for genetic studies via QTL analysis. K1263/1 is the parental line of an early maturing hybrid, SC260, known as the commercial cultivar Fajr [19]. This hybrid can probably be used in delayed sowing patterns for vector avoidance in temperate regions.

Plant height, ear weight and ear diameter and length were reduced in plants infected with IMMV, but cob percent increased and silking delayed in IMMV-infected plants. As this experiment is the first of its kind in Iran, it lays the foundation for further studies of maize viral diseases programs. Detected resistant lines could be used further as a check control genotype to confirm resistance of a genotype and incorporation in hybrid producing programs as parental lines.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lapierre H, Signoret PA. Viruses and Virus Diseases of Poaceae (Gramineae). INRA Editions. Paris. 2004;857.
2. Gordon DT, Bradfute OE, Gingery RE, Knoke JK, Nault LR, Scott GE. Introduction: history, geographical distribution, pathogen characteristics and economic importance. In: Virus and Virus-like Disease of Maize in the United States. 247th ed. in: Gordon DT, Knoke JK, Scott GE, eds. Southern Cooperative Series Bulletin, Wooster, Ohio; 1981.
1. Gordon DT, Thottappilly G. Maize and sorghum. In: Loebenstein G, Thottappilly G, eds. Virus and virus-like diseases of major crops in developing countries. Kluwer Academic Publishers, The Netherlands; 2003.

2. Redinbaugh MG, Jones MW, Gingery RE. The genetics of virus resistance in maize (Zea mays L.). Maydica. 2004;49(3):183-190.

3. Redinbaugh MG, Pratt RC. Virus resistance. In Bennetzen JL, Hake SC (eds) Handbook of maize: Its biology. 251-268. New York, Springer; 2009.

4. Ali F, Yan JB. Disease resistance in maize and the role of molecular breeding in defending against global threat. Journal of Integrative Plant Biology. 2012;54:134-151.

5. Ammar ED, Nault LR. Virus transmission by leafhoppers, planthoppers and treehoppers (Auchenorrhyncha, Homoptera). Advances in Botanical Research. 2002;36:141-167.

6. Nault LR, Knoke JK. Maize vectors. In: Knoke JK, Gordon DT, Scott GE (eds) Virus and virus-like diseases of maize in the United States. South. coop. Ser. Bull., Wooster, Ohio; 1981.

7. Redinbaugh MG, Houghton W, Salomon R, Creamer R, Hogenhout SA, Gordon DT, Ackerman J, Meulia T, Seifers DL, Abt JJ, Styer WE, Anderson RJ. Maize fine streak virus, a new leafhopper-transmitted rhabdovirus. Phytopathology. 2002;92:1167-1174.

8. Ming R, Brewbaker JL, Pratt RC, Musket TA, McMullen MD. Molecular mapping of a major gene conferring resistance to Maize mosaic virus. Theoretical and Applied Genetic. 1997;95:271-275.

9. Thottappilly G, Bosqueperez NA, Rossel HW. Viruses and virus diseases of maize in tropical Africa. Plant Pathology. 1993;42:494-509.

10. Izadpanah K, Ahmadi AA, Parvin S, Jafari SA. Transmission, particle size and additional hosts of the rhabdovirus causing maize mosaic in Shiraz, Iran. Journal of Phytopathology. 1983;107:283-288.

11. Estakhr A, Choukan R. Effect of planting date on grain yield and its components and reaction to important maize viruses in Fars Province in some exotic and Iranian maize hybrids. Seed and Plant Production Journal. 2011;27(3):313-333.

12. Kang BC, Yeam I, Jahn MM. Genetics of plant virus resistance. Annual Review of Phytopathology. 2005;43:581-621.

13. Izadpanah K, Parvin S. Occurrence of maize mosaic virus in corn fields around Shiraz. Iran. J. Plant Path. 1979;15:78-82. (In Persian).

14. Ammar ED, Gomez-Luengo RG, Gordon DT, Hogenhout SA. Characterization of Iranian maize mosaic virus and comparison with Hawaiian and other isolates of maize mosaic virus (Rhabdoviridae). Journal of Phytopathology. 2005;153:129-136.

15. Izadpanah K. Purification and serology of the Iranian maize mosaic rhabdovirus. Journal of Phytopathology. 1989;126:43-50.

16. Milne RG, Masenga V, Conti M. Serological relationships between the nucleocapsids of some planthopper-borne rhabdoviruses of cereals. Intervirology. 1986;25:83-87.

17. Estakhr A, Dehghanpour Z. Determination of the suitable planting date for new early maturity maize hybrids in second cropping in temperate regions in Fars Province. Seed and Plant Production Journal. 2010;26(2):169-191.

18. Estakhr A, Heidari B, Ahmadi Z. Evaluation of kernel yield and agronomic traits of European maize hybrids in the temperate region of Iran. Archives of Agronomy and Soil Science. 2015;61(4):475-491.

19. Kuntze L, Fuchs E, Grünzig M, Schulz B, Klein D, Melchinger AE. Resistance of early-maturing European maize germplasm to sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV). Plant Breeding. 1997;116:499-501.

20. Dintinger J, Boissot N, Chiroleu F, Hamon P, Reynaud B. Evaluation of maize inbreds for maize stripe virus and maize mosaic virus resistance: Disease progress in relation to time and the cumulative number of planthoppers. Phytopathology. 2005;95:600-607.

21. Dintinger J, Verger D, Caiveau S, Risterucci AM, Gilles J, Chiroleu F, Hamon P. Genetic mapping of maize stripe disease resistance from the Mascarene source. Theoretical and Applied Genetics. 2005;111(2):347-359.

22. Vivek BS, Odongo O, Njuguna J, Imanywoga J, Bigirwa G, Pixley K. Diallel analysis of grain yield and resistance to
seven diseases of 12 African maize (Zea mays L.) inbred lines. Euphytica. 2010; 172(3):329-340.

25. Zambrano JL, Francis DM, Redinbaugh MG. Identification of resistance to Maize rayado fino virus in maize inbred lines. Plant Disease. 2013;97:1418-1423.

26. Chen YK. Germplasm evaluation and quantitative trait loci identification of resistance to maize rough dwarf virus in maize. Dissertation, Xinjiang Agricultural University; 2006.

27. Wang F, Qin G, Sui Z, Wang Z, Wang Z, Yu J, Zhang J. Improved method for assaying maize plant resistance to maize rough dwarf disease by artificial inoculation and real-time RT-PCR. European Journal of Plant Pathology. 2006;116:289–300.

28. Zambrano JL, Jones MW, Francis DM, Tomas A, Redinbaugh MG. Quantitative trait loci for resistance to maize rayado fino virus. Molecular Breeding. 2014;34(3):989-996.

29. Astier S, Albouy J, Maury Y, Robaglia C, Lecoq H. Principles of plant virology: Genome, pathogenicity, virus ecology. Science Publishers, ISBN: 1578083168, New Hampshire, USA; 2007.

30. Naidu RA, Hughes JDA. Methods for the detection of plant virus diseases. In: Hughes JDA, Odu BO, eds. Plant virology in sub-Saharan Africa. 233–260. Proceedings of a Conference Organized by IITA, International Institute of Tropical Agriculture, ISBN 9781312149, Nigeria; 2001.

31. Purcifull DE, Hiebert E, Petersen M, Webb S. Virus detection – Serology. In: Maloy OC, Murray TD (eds) Encyclopedia of Plant Pathology (2)1100–1109. John Wiley & Sons Inc. ISBN: 0-471-29817-4; 2001.

32. Clark MF, Adams AN. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. Journal of General Virology. 1977;34:475–483.

33. Koenig R, Paul HI. Variants of ELISA in plant virus diagnosis. Journal of Virological Methods. 1982;5:113-125.

34. Bashir M, Hampton RO. Detection and identification of seedborn viruses from cowpea (Vigna unguiculata (L.) walp) germplasm. Plant Pathology. 1996;45: 54-58.

35. Clark MF, Bar-Joseph M. Enzyme immunosorbent assays in plant virology. In: Methods in virology, edited by Maramorosch K, Koprowski H. Academic Press, New York, USA; 1984.

36. Converse RH, Martin RR. ELISA methods for plant viruses. In: Hampton RO, Ball EM, DeBoer SH, eds. Serological methods for detection and identification of viral and bacterial plant pathogens, a laboratory manual. APS Press; 1990.

37. SAS Institute. SAS 9.1.3. SAS. Institute Inc. Cary, NC; 2002-2005.

38. Izadpanah K, Masumi M, Kamran R. Alternative hosts of the Iranian maize mosaic rhadovirus Iran. Journal of Plant Pathology. 1993;29:89-90.

39. Pratt RC, Anderson RJ, Louie R, Mcmullen MD Knoke JK. Maize responses to a severe isolate of maize chlorotic dwarf virus. Crop Science. 1994;34:635–641.

40. Chen YK, Li XH, Xiao MJ, Li MS, Yuan SX, Wang XD, Zhang SH. Genetic variation in sixty-four maize inbred lines in relation to maize rough dwarf virus. Acta Agronomica Sinica. 2006;32(12):1848–1854.

41. Massah A, Izadpanah K, Afsharifar AR, Winter S. Analysis of nucleotide sequence of Iranian maize mosaic virus confirms its identity as a distinct nucleorhabdovirus. Archives of Virology. 2008;153:1041-1047.

42. Bar-tsurs A, Saadi H, Antignus Y. Resistance of corn genotypes to maize rough dwarf virus [in Israel]. Maydica. 1998;33:189–200.

43. Guo QT, Li Z, Dong Z. The observation and analysis of varietal resistance of maize rough dwarf virus disease. Plant Protection. 1995;1:21–23.

44. Lu YG, Deng F, Miao H, Tian L, Di D, Zhang ZF. Identification of the disease resistance of maize cultivars to maize rough dwarf disease and the relationships between disease indices and yield loss. Plant Protection Beijing. 2007;33(6):90.

45. Liu ZZ, Chi SM. Resistance of corn genotypes to maize rough dwarf virus. Journal of Maize Science. 1996;4:68–70.

46. Lu YG, Di D, Miao H, Tian L. Identification and analysis on resistance of introduced foreign and domestic maize inbreds to MRDV. Journal of Agricultural University of Hebei. 2001;5:22–25.

47. Shi LY, Hao ZF, Weng JF, Xie CX, Liu CL, Zhang DG, Li MS, Bai L, Zhang SH. Identification of a major quantitative trait locus for resistance to maize rough dwarf virus in a Chinese maize inbred line X178
using a linkage map based on 514 gene-derived single nucleotide polymorphisms. Molecular Breeding. 2012;30(2):615-625.

48. Ilbagi H, Rabenstein F, Habekuss A, Ordon F, Çıtır A. Incidence of virus diseases in maize fields in the Trakya region of Turkey. Phytoprotection. 2006; 87:115-122.

49. Sutula CL, Gillett JM, Morrissey SM, Ramsdell DC. Interpreting ELISA data and establishment the positive negative threshold. Plant Disease. 1986;70: 722-726.

50. Sharma K, Misra RS. Molecular approaches towards analyzing the viruses infecting maize (Zea mays L.). Journal of General and Molecular Virology. 2011; 3(1):1-17.

51. Liu XH, Tan ZB, Rong TZ. Molecular mapping of a major QTL conferring resistance to SCMV based on immortal RIL population in maize. Euphytica. 2009; 167(2):229-235.

52. Permet A, Hoisington D, Franco J, Isnard M, Jewell D, Jiang C, De León DG. Genetic mapping of maize streak virus resistance from the Mascarene source. I. Resistance in line D211 and stability against different virus clones. Theoretical and Applied Genetics. 1999;99(3-4): 524-539.

53. Brewbaker JL. Disease of maize in the wet lowland tropics and the collapse of the classic Maya civilization. Economic Botany. 1979;33:101-118.