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# Evaluating the Reaction of Semi-oriental Tobacco Cultivars to Potato Virus Y

**Musa Javi[1], Saeed Nasrollahnejad[2*], Abdolghaum Ebrahim[1]**

[1]*Department of Plant Pathology, Islamic Azad University, Gorgan Branch, Gorgan, Iran*

[2*]*Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran*

[*]***Corresponding Author**: Saeed Nasrollahnejad, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran, Tel: +989111164556; E-mail: S.nasrollanejad70@yahoo.com*

## ABSTRACT

*Because of the impact on the quality and quantity of the crop, tobacco viral diseases are very important. Potato virus Y (PVY) is one of the most important tobacco viruses and has a worldwide distribution. Studying the reaction of tobacco cultivars to PVY and identifying the possible sources of resistance is important and aimed at achieving resistant cultivars. Therefore the reaction of 31 tobacco cultivars was assessed in a completely randomized design with 10 replicates under greenhouse conditions. Seeds were planted in pots containing sterile soil and seedlings were transferred to individual pots. Seedlings were mechanically inoculated at four-leaf stage with a PVY isolate. The development of symptoms was recorded for one month by a grade from 0 (no symptoms) to 11 (for necrotic stem). The means were compared using Duncan's test. The cultivars with means above or below the total mean were selected as susceptible or tolerant cultivars, respectively. The final test on 7 selected tolerant cultivars were performed in the same way. Several samples were tested by ELISA to confirm the presence of infection. The results showed that in the preliminary experiment with comparison of the severity of symptoms and the concentration of virus, the cultivars Tr. 27, Tr.1, H.Tr.1, Samsoun katezini, Tr.23 and Tyk Kula were identified as tolerant cultivars.*

**Keywords:** Tobacco, PVY, Cultivars reaction, DAS-ELISA.

## INTRODUCTION

*Nicotiana tabacum* is a one-year-old herb of Solanaceae. The height of the plant is 1.5 m without inflorescence and more than 2 m with inflorescence in the conditions of the farm [1]. The history of tobacco cultivation is 3,000 years. The semi-eastern type of tobacco is commonly derived by two types of western and eastern, which range from the size of leaves, perfume, nicotine flavor and other intermediate chemical properties between western and eastern tobacco. Therefore, it is named Semi-oriental Tobacco that is commonly used as a filler or filler in cigars [2]. Performing a survey on the Semi eastern type of cultivars tobacco has done with the aim of increasing the yield strength, the balance of chemical compounds, the production of quality industrial goods, the resistance to most tobacco diseases in the bedding of seeds and farms. Tobacco is cultivated at least in 97 countries for commercial purposes. Although tobacco originates from subtropical regions, most types of it are currently produced in temperate regions. The total tobacco production in 1988 was about 6.5 mt, of which about 4.3 mh had been cultivated and about one-third of the production was from China [3]. The area under cultivation of tobacco was about 7,000 h in Iran for 2008 about 80 percent of which was in the provinces of Mazandaran, Golestan and the rest of its in provinces of Guilan, West Azarbaijan, and Kurdestan. Tobacco production is common in the provinces of Bushehr, Isfahan, Razavi Khorasan, Markazi and Fars [4]. The average yield of Semi-oriental Tobacco varieties is about 1125 kg per hectare [1].

Potato virus Y (PVY)belongs to the genus Potyvirus and Potyviridae family. This family is one of the largest groups of plant viruses [5]. The infection with the PVY reduces the quality and quantity of the tobacco product [6,7]. Every year in Iran, PVY cause 10 to 100 percent reduction in tobacco production [8]. Symptoms of PVY depends on the

strains of the virus and the tobacco genotype including clearing of veins and mosaic to severe leaf necrosis and necrotic spots on leaves and stems [3]. Based on localized or systemic symptoms on a number of tobacco and potato cultivars, PVY strains are classified into three main groups which are important respectively PVY-C, PVY-N and PVY-O [9]. The strains of PVY-C and isolates PVY-O and PVY-N were identified from the response samples PVY of Daland and Gorgan peppers respectively on tobacco cultivar (cv.) samsoun using monoclonal antibodies [10]. Symptoms of PVY were first observed in Iranian tobacco fields during a review in 1961-1962 [11]. The first report of the presence of a necrotic strain of PVY was on potatoes and tobacco fields in Mazandaran and Golestan provinces during the surveys conducted at Tirtash Research and Training Center [12]. Currently, PVY is one of the most harmful tobacco agents in the world. This is confirmed by CORESTA's organization on tobacco viruses. The damage of PVY has spread especially the necrotic strains in many countries [13]. PVY transmits in a non-persistent manner by several aphid species and also the mechanical transmission of PVY is easily through abrasives [3]. The reaction of 4 potato cultivars to PVY-O strain was studied.

Most control methods of PVY include 1- Avoiding contamination such as planting when there is no carrier or its population is low. 2. It is better to plant different varieties in fields. 3. The infection plants must be destroyed quickly before disease transmitted by manner. 4. Spraying mineral oils to reduce virus transmission [14]. In this regard, one of the most successful methods is the use from tolerant or resistant varieties to PVY [3]. Some PVY strains produced the severe symptom of disease as necrosis in tobacco genotypes that contain the root-knot resistance gene while these strains had mild symptom on tobacco genotypes without root-knot resistance gene [15]. The use of resistant varieties in the areas where the virus is present is considered to be the best way to control the virus. Resistant varieties are grown for several years [16]. In this survey, the response of different varieties of tobacco to the PVY were evaluated to determine potential genetic sources of resistance that is one of the best ways to reduce the damage to the disease.

## MATERIALS AND METHODS

The survey was done in July 2016 in greenhouse condition of Gorgan University of Agricultural Sciences and Natural Resources. PVY-O strain which is isolated from the tobacco cultivar (cv.) was previously inoculated mechanically in greenhouse conditions for testing of PVY. In this research, Semi-oriental Tobacco varieties were used from the seed bank of Tirtash Research and Education Center. These cultivars are given in Table 1. Tobacco seeds were planted in pots containing sterile soil under controlled conditions and away from contamination sources. They were seeded in pots containing sterilized, and perlite with a ratio of 1:1:3. After reaching the appropriate stage the greenhouse was sprayed every two weeks with primicarb and imidacloprid insecticides to fight aphids and flies (Figure 1).

**Figure 1:** Planting semi-oriental tobacco varieties in the greenhouse.

**Table 1**: The name Semi-oriental tobacco varieties were used from the seed bank of Tirtash Research and Education Center.

| Cultivar | Number | Cultivar | Number |
|---|---|---|---|
| Tr.1 | 17 | Ch.T.266-1 | 1 |

| | | | |
|---|---|---|---|
| Albors.22 | 18 | Ch.T.266-6 | 2 |
| Albors.23 | 19 | Ch.269-12 | 3 |
| F.K.40-1 | 20 | Ch.T.269-12.A | 4 |
| S.S.289-2 | 21 | Ch.T.269-12.C | 5 |
| Samsoun | 22 | Ch.T.269-12.D | 6 |
| Samsoun.H | 23 | Ch.T.269-12.E | 7 |
| Samsoun.1 | 24 | Ch.T.283-8 | 8 |
| Samsoun.959 | 25 | Ch.T.273-3.B | 9 |
| Samsoun.Dere | 26 | Ch.T.283-6 | 10 |
| Samsoun Katezini | 27 | H.Tr.1 | 11 |
| Samsoun S.1 | 28 | T.S.8 | 12 |
| T.K.23 | 29 | Tr.21 | 13 |
| Tyk Kula | 30 | Tr.23 | 14 |
| XanthiaN.C | 31 | Tr.27 | 15 |
| | | Tr.93 | 16 |

Typical symptoms of viral infection were selected. 0.15 mm phosphate buffer solution was prepared with pH=7.5 (first, 17.5 g D-potassium hydrogen orthophosphate ($K_2HPO_4$) was dissolved in 1 L of distilled water, then, 3.4 g potassium orthophosphate ($KHPO_4$) was dissolved in 250 ml of distilled water and two solutions were combined to obtain a pH of 7.5. This solution was kept at 4°C until transferred to the plant [17]. After making a wound on a leaf, the finger impregnated with the virus was inspected slowly on the surface of two leaves of each plant [18].

**Figure 2:** Mechanical inoculation of tobacco seedlings at the 4$^{th}$ stage with PVY.

The control treatment was only inoculated with potassium phosphate solution. This experiment was conducted in a completely randomized design with 31 treatments (cultivars) in 10 replicates. Indicator plants, the tobacco cultivar (cv.) samsun were kept in the greenhouse after inoculation (Figure 2). They were evaluated 4 weeks post inoculation and symptoms were recorded based on the following index. For the severity of the disease, zero degrees (no symptoms 2), 1 (mild mosaic), 3 (mosaic), 5 (intense mosaic), 7 (one to two leaves with necrotic arteries), 9 (three to five leaves with veins necrotic (VN)) and 11 (stem necrosis) were used [18]. To confirm the presence of PVY, samples from the tested tobacco were used in the DAS-ELISA. ELISA test results were recorded using the ELISA

Reader (Biotek, ELX 800). Plants with no symptoms were ELISA negative and plants with very severe symptom have high virus concentration the extract of healthy plant and the extract of the infected plant were used respectively as a negative control and a positive control. The samples with the mean of an optical absorption value three times above that of the healthy control were considered as positive.

This experiment was conducted in two preliminary stages using a completely randomized design to investigate the reaction of Semi-oriental Tobacco varieties to PVY and their strains. According to the degree of symptoms registered in the evaluations, Statistical analysis was performed. If there was a significant difference, the mean of the treatments was checked based on the ELISA test. The cultivars with the absence of infection were identified by ELISA as resistant cultivars, treatments with a mean significantly lower than the total mean were considered as tolerant cultivars and treatments with a mean significantly higher than the mean total were considered sensitive. Experiments of the varieties that were identified as resistant or tolerant were repeated. In the preliminary stage, 31 Semi-oriental tobacco varieties were evaluated. In the main stage, in order to ensure non-infection, cultivars with low mean disease severity were once again evaluated by resistance and tolerance method.

## RESULTS

The results revealed a significant difference between the means of different cultivars for PVY concentration (p0.01<) (Table 2).

The average severity of the symptoms varied from 1.2 to 5. Also, the results of this study showed that the lowest mean of symptoms were Tr.27 (1.2), Tr.93 (1.6), Tr.1 (1.6), H.Tr.1 (1.8), Katezini Samsoun (1.8), Tr.23 (2), Tyk Kula (2), Samsoun Dere (2.2), Xanthia NC (2.4) and the highest ones were Ch.T.266-1, Ch.T.269-12.A, Ch.269-12, Ch.T. 269-12.C, Ch.T.269-12.E, and Ch.T.269- 12.D were with a mean value of 5. Mean degree of symptoms were compared on the basis of Duncan's multivariate test, and the treatments that had different with a mean of the total (2.1047) were determined (Table 3).

Accordingly, in the preliminary experiment cultivars of Ch.T.269-12.C and Ch.T.269-12.D were detected sensitive and 9 cultivars Tr.27, Tr.93, Tr.1, H.Tr.1, Katezini Samsoun, Tr.23, Tyk Kula, Samsoun Dere, and Xanthia NC. Average of other cultivars did not have a significant difference with a mean of the total.

**Table 2**: The ELISA results for the tested cultivars (mean±SD);** significant at a probability level of one percent.

| Source | DF | MM | MS | F |
|---|---|---|---|---|
| Treatment | 30 | 31.6046 | 1.0534 | 16.99** |
| Error | 277 | 17.1798 | 0.062 | |
| Total | 307 | 48.7844 | | |
| Cv | 11.79 | | | |

**Table 3**: Comparison of the average degree of symptoms of 31 varieties of semi- oriental tobacco cultivars inoculated with PVY.

| Mean of symptoms* | Cultivar |
|---|---|
| 5:00 AM | Ch.T.269-12.C |
| 5:00 AM | Ch.T.269-12.D |
| 5:00 AM | Ch.T.269-12 |
| 5:00 AM | Ch.T.269-12.A |
| 5:00 AM | Ch.269-12.E |
| 5:00 AM | Ch.T.266-1 |
| 4.8 ab | Ch.T.266-6 |
| 4.8 ab | T.S.8 |
| 4.8 ab | Ch.T.273-3.B |
| 4.6 ab | Ch.T.283-8 |

| | |
|---|---|
| 4.4 ab | Samsoun S.1 |
| 4.4ab | Samsoun.H |
| 4.2ab | T.K.23 |
| 4.2ab | Samsoun.1 |
| 4.2ab | F.K.40-1 |
| 4.2ab | Albors.23 |
| 4 ab | Samsoun.959 |
| 4 ab | S.S.289-2 |
| 3.6 abc | Ch.T.283-6 |
| 3.5 bc | Samsoun |
| 3.4 bc | Tr.21 |
| 2.6cd | Albors.22 |
| 2.4 cd | Xanthia N.C |
| 2.2 ed | Samsoun.Dere |
| 2ed | Tr.23 |
| 2ed | Tyk Kula |
| 1.8 ed | H.Tr.1 |
| 1.8 ed | Samsoun Katezini |
| 1.6 ed | Tr.1 |
| 1.6 ed | Tr.93 |
| 1.2 e | Tr.27 |

The means with non-identical letters have a significant difference in the probability level of 1% in the Duncan test.

The reaction cultivars to inoculation with PVY revealed in Figure 3 a comparison between healthy, mosaic and intense mosaic. A cultivar of Tr.23, Tr.27, Tr.93, Tyk Kula, Samsoun Katezi, Xanthia N.C Tr.1, Samsoun revealed mild mosaic and Ch.T.269-12.C, Ch.T.269-12.D revealed intense mosaic (Figure 3).

The average severity of symptoms varied from 1.2 to 5. Ch.T.269-12, recognized with average 5 severity of disease as most sensitive. Considering that infection from 9 tolerant cultivars in the initial experiment was confirmed only in the Samsoun Katezini, Tr.1, Samsoun. Dere, H.Tr.1, Tr.23, Tr.27 and Tyk Kula cultivars were confirmed in the ELISA test, therefore, the original experiment was carried out in 7 cultivars.

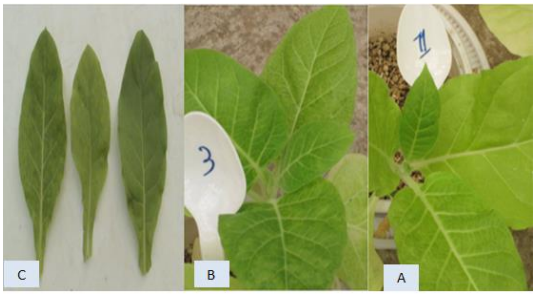


**Figure 3**: The Symptoms of PVY on different semi- oriental tobacco cultivars. A: Mild mosaic, B: Intense mosaic and C: Comparision between health and infection leaf that has shown mild and intense mosaic.

**Table 4**: The results of data analysis of variance in the evaluation of the reaction of semi- oriental tobacco cultivars relative to PVY in the original experiment;** Significant at a probability level of one percent.

| Source | DF | MM | MS | F |
|---|---|---|---|---|
| Treatment | 20 | 19.42092 | 1.61841 | 39/79** |
| Error | 117 | 4.75863 | 0.040672 | |
| Total | 129 | 24.17956 | | |
| cv | 13.12 | | | |

The results of variance analysis of the data regarding the degree of symptoms of H.tr.1, Katezini Samsoun, Tr.23, Tyk Kula, Tr.27, Tr.1 and Samsoun Dere cultivars in original experiment revealed significant differences between all cultivars with a mean total (5362/1) at 1% level (P<0.01) (Table 4). The results of the average comparison of cultivars using Duncan's test showed that all the cultivars except Samsoun Dere were in the same group. As a result of the seven tolerant varieties in the original experiment, there were no significant differences between the six cultivars, only Samsoun Dere had a significant difference with other, so it was sensitive rather than other 6 cultivars (Figure 4).

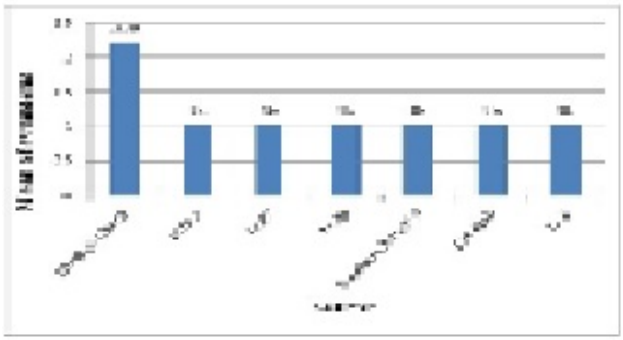

**Figure 4**: Results of comparison of mean symptoms of tolerant cultivars selected in the main experiment.

## DISCUSSION

This was the same with the results of Shukla regarding the mosaic symptoms of PVY-O strain in tobacco [19]. Also, the results of the present study were the same with the results Dorozewska and Fisher, which measured the resistance of Samson varieties to all three isolates of PVY with mechanical inoculation and reported the susceptibility of these varieties to all three isolates of the virus. Statistical analysis of the data from both the preliminary and original tests showed that there was a significant difference between the treatments at the 1% probability level. Due to the uniformity of the test conditions for all treatments, this difference can be related to genetic resistance. Zinatifakhrabady and Nasrollanejad Investigated in evaluating the resistance to PVY-O strain in four potato cultivars, Satina cultivar was the most susceptible to the index of symptoms relative to the total mean, but based on the results Indirect-ELISA, the highest concentration of the virus was observed in Bourne cultivar [20,21]. In evaluating 90 Eastern and semi-eastern tobacco varieties, the Erzeogonia and KB101 genotype had the highest and lowest concentration [22]. Evaluation results in term of the severity of the symptoms of PVY and the rate of absorption of the virus in a sensitive cultivar, the tolerant cultivars of semi-oriental and healthy plant revealed no correlation between the severity of the symptoms and the concentration of the virus in the plant. Also in this study, based on the Duncan test, the concentration of viral in the original experiment had a significant difference except for Samsoun. Dere and Tr.21 cultivars although the severity of the symptoms of these two varieties was significant. According to the Duncan test in the original experiment cultivars of Tr.27, Tr.1, H.Tr.1, Samsoun katezini, Tr.23, and Tyk Kula had a significant difference to mean of the total, so they can be categorized as a virus-tolerant cultivar, and it focuses on racial issues in areas where the infection is high. The result revealed that there was no complete resistance to PVY between cultivar. According to the results of the ELISA test in the original experiment, H.Tr.1 and Samsoun Katezini showed the least amount of infection to the PVY, these two cultivars, due to their good tolerance, can be considered in the breeding in high infection areas. These cultivars should also be tested on field conditions and be used in breeding programs if they have the appropriate crop characteristics. In identifying SSR markers related to PVY resistance in tobacco germplasm, three sites (PT30260, PT30061 and PT30046) were identified from the 6, 8a and 11 groups of the reference tobacco map [23]. Moradi using ELISA test showed the extremely limited spread of PVY in different potato cultivars except indigenous [24]. Pobeda 3, Zichna, Plovdiv 58 and Ourmia 205. Pobeda 3, Zichna,

Plovdiv 58 and Ourmia 205 had significant differences too. The results of Stoddard and Hunger showed that there was no correlation between the severity of the symptoms and the amount of the virus in the plant [25,26]. In addition, there can be no correlation between the increase in the virus in the plant and the exacerbation of the symptoms of the disease. The reaction of different varieties to PVY depends on the various compounds present in different varieties of tobacco, as well as viral strains. Based on the amount of protein and phenolic compounds in leaves of sensitive and resistance cultivars infected with PVY, Peroxidase and polyphenol oxidase activity of VAM cultivar was less than that of K326 cultivar at 1% probability levels after all time inoculation. Also, the absorption rate of K326 was significantly higher than the VAM cultivar, which showed a positive correlation between peroxidase activity and polyphenol oxidase activity (R2=0.546) [27]. The virus has a high genetic diversity and so far in many parts of the world, there are isolates of PVY that have been able to overcome the genetic resources of resistance. Therefore, the probability of occurrence of these isolates as well as the condition of the patient should be continuously evaluated.

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