Studying the Collection Accessions of Vegetable Crops to Expand the Range of Their Usage as Rootstocks

G S Martirosyan, I V Vardanian, L M Tadevosyan, A E Avagy and Z E Harutyunyan

Scientific Center of Vegetable and Industrial Crops of the Ministry of Economy of the Republic of Armenia, D.Ladoyan str., 38, vil. Darakert, Ararat marz, Armenia

E-mail: gayanemartirosyan@yahoo.com

Abstract. Local breeding tomato varieties released for cultivation in Armenia in the protected ground conditions have a relatively weak resistance towards disease and stress conditions, despite good taste and quality fruits indicators. This factor, among others, is the reason for the unprofitability of growing tomatoes in greenhouses, which in turn leads to an even greater intensification of production in terms of – pesticides use. The vegetative grafting of vegetable plants can be the problem solution, which helps to increase the disease resistance, yield and improve the fruits quality indicators. The effectiveness of grafted plants depends on the genetic rootstock potential. Collection accessions of tomato and eggplant lines have been evaluated for resistance to fusarium wilt using real-time PCR diagnostics. A preliminary phytosanitary assessment of collection accessions has made it possible to recommend the most disease-resistant samples for using as a rootstock for vegetative grafting. The grafted plants assessment according to a complex of agronomic characters has shown the advantage of using eggplant rootstocks comparing with tomato rootstocks.

1. Introduction

Tomato is one of the main vegetable crops in Armenia, which is cultivated both in open and protected ground. Today, about 4% of the total area under tomato is accounted for greenhouse facilities. The subsidy policies for constructing the greenhouse facilities pursued in the Republic contributes to expanding the area under tomatoes cultivated under cover, which, in turn, highlights the problem of introducing varieties which are resistant to exogenous and endogenous factors. In this regard, the need to develop and implement innovative cultivation technologies to increase crop yields and obtain high-quality and environmentally safe products is today one of the important directions in the field of vegetable growing. One of the modern innovative technologies in vegetable growing is vegetative grafting of vegetable plants, which helps to increase yields [1, 2], improve the quality fruits indicators [3], disease resistance [4] and resistance to unfavorable environmental factors [5]. Many authors’ studies indicate that the effectiveness of grafted tomato plants also largely depends on the chosen rootstock culture [3, 5, 6].

An important factor is using disease-resistant rootstocks in the grafting practice [1, 7]. The most common and harmful disease of a greenhouse tomato is fusarium wilt, which causes early plant death and a decrease in yield. The causative agents of the disease (Fusarium oxysporum f. sp. lycopersici and other species of p. Fusarium) penetrate from the soil into the vascular system of plants. High temperatures (27–28 °C), dry air and acidic soil (pH 5–5.6) contribute to the development of disease.
The pathogen enters the xylem waterworks through the roots, causing tissue necrosis. Gradually, the fungus mycelium clogs the vessels, disrupting the flow of aqueous solutions. As a result, the plant wilts [8, 9, 10].

In this regard, using the grafting on disease-resistant rootstocks is one of the highly effective methods for limiting the pesticides use [7].

Currently, the method of grafting a number of pumpkin and nightshade plants is widely used in Europe and Asia [11, 12, 13], but in Armenia this innovative method has not found wide application yet due to the poorly studied rootstock genetic material.

To study and disseminate the method of vegetative grafting in vegetable growing, the seed collection of the Scientific Center of Vegetable and Industrial Crops of the Ministry of Economy of Armenia, within the framework of regional cooperation, have been replenished with samples obtained from the gene bank of the World Vegetable Center, an international non-profit research institute.

The purpose of this work was to study and select promising rootstocks to increase the yield, quality and resistance to fusarium wilt of tomato fruits cultivated under cover.

2. Material and methodology
Collection accessions provided by the gene bank of the World Vegetable Center have been selected as the object of study: 5 eggplant accessions – VI045276, VI046101, VI046103, VI046104, VI034845 and 5 tomato ones – LO3708, LO5983, LO6176, LO6193, LO6194. As a standard, 2 options have been chosen: a greenhouse indeterminate hybrid of Armenian breeding – Lusarpi F1 (Standard 1) and a line in which the same Lusarpi F1 (Standard 2) has been used as a scion and rootstock.

The seeds were sown in cells in the first half of January. Vegetative grafting of seedling was carried out in the phase of two true leaves, when the stem diameter was 1.6–2.0 mm. To ensure a high accretion percentage, the grafted plants seedlings were placed in a special chamber, where +26–28 °C temperature and relative air humidity of more than 85–90% were maintained, and shading was provided. On days 4–5, the process of gradual adapting the grafted plants for the greenhouse began. Planting the grafted tomato plants into the greenhouse was carried out in the second half of March in the phase of 9 true leaves (at the age of 55 days). The experiments were carried out in 4-time repetition, with the randomization method, according to the 90+60x50cm scheme, 2.5 plants per 1 m². The plant care was carried out according to the methodology generally accepted for tomatoes [14]. The greenhouse was equipped with a cooling system, which made it possible to stably provide the daytime temperature of 25–28 °C and the nighttime temperature of 16–18 °C, the relative humidity of 70–75%, depending on the phase of plant development. Soil acidity – pH 6.0–6.5, the EC of irrigation solution – 2.5. The plants were provided with all the necessary nutrients in the proper amount (based on the data of soil analysis and the plant development phase).

Phenological observations, description of morphological features and yield elements registration were carried out throughout all vegetation. The dry substances content was determined by a refractometer, sugars – according to Bertrand, the vitamin C content was determined according to Murry [15], nitrate nitrogen was determined using an ion-selective electrode according to the Vdovina-Medvedeva method.

Experimental research, data collection and analysis were carried out according to the field-plot technique (with the basics of research results statistical processing) [16] and according to the Methodology of experimentation in vegetable growing and melon growing [17].

In the course of the work, a phytosanitary assessment of the rootstock plants resistance according to a percentage-point scale [18] and the Fusarium oxysporum pathogen identification by PCR [19] were carried out. The studies were carried out in an artificial infection background. To obtain an inoculum, the sporulating colonies in pure culture were grown on a potato-glucose medium. The experiments were carried out with 3-time repetition.

Diagnostics and identification of the Fusarium oxysporum pathogen was carried out by real-time PCR. Infected plant stems and leaves were used as samples. Positive and negative controls were used for each series of samples. PCR assays were performed in the final volume of 20 mcI of reaction mixture
according to the manufacturer’s instructions. The fluorescence level was determined by the FAM channel. DNA amplification was carried out in the following mode: 1) 5 min at 94 °C (1 cycle); 2) 30 sec – 94 °C, 10 sec – 72 °C (40 cycles), 3) 10 sec – 62 °C (1 cycle). Using the software of the LightCycler 96 amplifier (Roche, Germany), the values of the reaction threshold cycle Ct (according to the threshold value Ct=35), the standard RT-PCR curve, the mean inclination value, the amplification efficiency, and the mean value of the correlation coefficient were determined.

3. Results and discussion

It should be noted that we have previously studied tomato and eggplant rootstocks in terms of biomorphological, phenological characteristics and productivity. Studies of eggplant rootstocks (VI045276, VI046101, VI046103, VI046104, VI034845) showed that the period from mass sprouts to 50% of flowering was 84–93 days. The period up to 50% of fruiting ranged from 108–114 days. Yield indicators, depending on the line of the rootstock, varied from 7kg/plant up to 12kg/plant, with the average fruit weight of 155.0–290.0 g. Among the studied eggplant lines obtained from AVRDC, two samples VI046104 and VI045276 were distinguished in terms of yield.

Biomorphological features of tomato lines (LO3708, LO5983, LO6176, LO6193, LO6194) and their efficiency as rootstocks for the Big Beef variety were studied in detail [20].

Studies to assess the phytosanitary state of tomato and eggplant rootstocks to fusarium wilt were carried out in experimental greenhouses of the Scientific Center and the Laboratory of Phytopathology, Biotechnology and Plant Physiology. The experiments showed that under the artificial infection conditions in plants of eggplant VI046101, VI046103, VI034845 and tomato LO3708, LO5983, LO6194 lines, a lag in growth was already observed in the early development period, in comparison with VI046104, VI045276 and LO6176, LO6193 lines, which indicated the onset of the course of the disease. The first symptoms of the disease appeared in the form of the lower leaves yellowing, which spread to the upper leaves. Affected leaves gradually withered and died, wilt progressed up the stem. When the stem was cross-sectioned, the vessels were browned.

As it can be seen from the data shown in Table 1, the damage degree to eggplant rootstocks VI046103, VI046101, VI034845 varied from 14.2±1.0% to 16.1±0.8%, while plants of VI045276 and VI046104 lines showed high disease resistance.

Along with visual observation of the disease development, PCR assay was carried out. Moreover, in cases of positive amplification, the efficiency of the latter was E=5.1%, the slope of straight line was (-3.513), R²=1. In the samples of VI045276 and VI046104 lines, the pathogen was not detected, while in VI046103, VI046101 and VI034845 lines the Ct value was 28.26±0.11, 25.48±0.50, and 24.22±0.45, respectively.

When evaluating tomato rootstocks, it is necessary to isolate rootstocks LO6176 and LO6193, in which there were no visible fusarium wilt symptoms, however, a weakly positive result was recorded during PCR assay: Ct –35.62±0.67 and 34.41±0.82, respectively, with the number of DNA pathogen copies 1.20·10² and 1.47·10². This result once again proves that using RT-PCR in the pathogen diagnosis allows us to accurately and identify the pathogen in the shortest time possible, determine its content in samples, thereby regularly monitoring the disease development [21].

Disease resistance was observed in tomato plants of LO3708, LO5983 and LO6194 lines, while the damage degree was low and amounted up to 18.7±1.1, 12.3±0.7 and 21.1±0.5%, respectively. When PCR assaying, the Ct values recorded in the samples were 22.6±1.12, 31.8±0.77 and 23.18±1.02, the number of DNA copies in the reaction mixture was 1.37·10⁶, 1.65·10⁷ and 1.58·10⁷, respectively.

Therefore, all the studied samples showed resistance to fusarium wilt, however, according to the expression of high disease resistance, the eggplant rootstocks VI045276, VI046104 and tomato LO6176, LO6193 should be distinguished.

Further studies were carried out with selected eggplant VI045276, VI046104 and tomato LO6176, LO6193 lines as rootstocks and Lusarpi F₁ as a scion.
Phenological observations have shown that in grafted plants the development phases are accelerated in comparison with the standards: flowering began 4–5 days earlier in plants grafted on tomato rootstocks (Lusarpi F1/LO6176, Lusarpi F1/LO6193) and 2–3 days earlier – on eggplant ones (Lusarpi F1/VI045276, Lusarpi F1/VI046104). Regardless of the culture of the rootstock used, the method provided the development processes acceleration. So, the first fruit harvest from tomato plants was on day 100–102, which is 8–14 days earlier than according to the standards 1 and 2, and when using eggplant rootstocks – 98–99 days (11–13 days earlier) (table 2).

As can be seen from table 2, the grafted plants exceeded both standards in yield and fruit quality. The grafted plants yield, regardless of the rootstock culture, was comparatively higher than that of ungrafted ones. The lines were distinguished by high fruits tradability (97.0–98.7%). The variants grafted on eggplant stood out with a particularly high tradable yield, the indicators of which were: Lusarpi/vI046104 – 21.2 kg/m² and Lusarpi F1/vI045276 – 19.6 kg/m², which exceeded the standard 2 by 5.0 kg/m² and 3.4 kg/m², respectively. Among the tomato rootstocks, the Lusarpi F1/LO6193 sample turned out to be high-yielding, with a tradable yield of 19.3 kg/m², which is 4.0–4.1 kg/m² more than standards 1 and 2.

The study results have shown that the grafted plants yield also depends on the average fruit mass. In all grafted tomato lines Lusarpi F1/LO6176 and Lusarpi F1/LO6193 this indicator was 30.5–54.5 g higher compared to standards 1 and 2. The eggplant rootstocks lines Lusarpi F1/vI045276 and Lusarpi F1/vI046104, in which the average fruit mass was 285.0 and 307.1 g, respectively (table 2).

Yield indicators of standard 2 (Lusarpi F1/Lusarpi F1) were slightly lower than those of ungrafted plants of standard 1 (Lusarpi F1). This is due to their later development and the degree of plant adaptation after grafting. Therefore, it is not recommended to use the same variety/hybrid as a scion and rootstock.

When analyzing the fruit quality of the Lusarpi F1/vI045276 and Lusarpi F1/vI046104 eggplant lines, the average variability values of dry matter and ascorbic acid were higher compared to the standards and amounted up to 7.0 and 7.3%, 22.0 and 21.5 mg%, respectively. A similar situation with tomato lines Lusarpi F1/LO6176 and Lusarpi F1/LO6193. (table 3).

Studies have shown that the total sugars content in fruits of both standard 1 (Lusarpi F1) and standard 2 (Lusarpi F1/ Lusarpi F1) was higher than that of all grafted combinations. The data obtained coincide with the data of Schwarz’s studies, in which grafting also led to a decrease in the sugar content and an increase in acidity [22].
Table 2. Economic assessment of grafted plants.

| Rootstocks               | Days from planting to | Tradable yield, kg/m² | Fruit tradability, % | Average fruit mass, g |
|--------------------------|-----------------------|------------------------|----------------------|-----------------------|
|                          | Flowering             | Fruitification         | 1st harvest          |                       |
|                          | beginning             |                        |                      |                       |
| Tomato rootstocks        |                       |                        |                      |                       |
| Lusarpi F₁ st.1          | 63±1.2                | 84±0.9                 | 110±0.7              | 16.3                  | 95.8                  | 250.0                |
| Lusarpi F₁/              | 64±1.1                | 86±0.8                 | 112±0.5              | 16.2                  | 94.4                  | 245.5                |
| Lusarpi F₁/ Lusarpi F₁, st.2 | 59±1.0                | 79±0.8                 | 102±0.9              | 17.9                  | 97.0                  | 280.5                |
| Lusarpi F₁/ LO6193       | 58±1.3                | 77±1.1                 | 100±0.8              | 19.3                  | 97.2                  | 300.5                |
| Eggplant rootstocks      |                       |                        |                      |                       |
| Lusarpi F₁/ VI045276     | 60±1.1                | 82±0.9                 | 98±1.1               | 19.6                  | 98.5                  | 285.0                |
| Lusarpi F₁/ VI046104     | 61±1.2                | 80±0.7                 | 99±0.9               | 21.2                  | 98.7                  | 307.1                |
| HCP₀.₀₅                  | 1.4                   | 1.2                    | 1.2                  | 1.4                   | 4.2                   |

In all the combinations studied, the nitrate nitrogen content in fruits was below the permissible amount limit and varied from 50 to 61 m/kg (table 3).

Table 3. Indicators of the fruit quality of tomato and eggplant grafted lines in a greenhouse in the winter-spring turnover.

| Rootstocks               | Dry matters, % | Total sugars, % | Ascorbic acid, mg/% | Acidity, % | Nitrate nitrogen, mg/kg |
|--------------------------|----------------|-----------------|---------------------|------------|-------------------------|
|                          |                |                 |                     |            |                         |
| Tomato rootstocks        |                |                 |                     |            |                         |
| Lusarpi F₁ st.1          | 5.29           | 4.40            | 17.05               | 0.49       | 50                      |
| Lusarpi F₁/              | 5.33           | 4.38            | 19.4                | 0.50       | 57                      |
| Lusarpi F₁, st.2         |                |                 |                     |            |                         |
| Lusarpi F₁/ LO6176       | 6.70           | 3.90            | 21.7                | 0.51       | 60                      |
| Lusarpi F₁/ LO6193       | 6.65           | 4.08            | 20.9                | 0.51       | 61                      |
| Eggplant rootstocks      |                |                 |                     |            |                         |
| Lusarpi F₁/ VI045276     | 7.0            | 4.10            | 22.0                | 0.48       | 58                      |
| Lusarpi F₁/ VI046104     | 7.3            | 4.25            | 21.5                | 0.50       | 55                      |
| HCP₀.₀₅                  | 0.15           | 0.2             | 1.1                 | 0.05       | 2.1                     |
| Vₜ, %                    | 6.2            | 7.0             | 10.4                | 5.5        | 11.5                    |

4. Conclusion

Therefore, among the collection tomato LO3708, LO5983, LO6176, LO6193, LO6194 and eggplant VI045276, VI046101, VI046103, VI046104, VI034845 lines; LO6176, LO6193 and VI045276, VI0454196 lines have been selected as promising rootstocks according to the economically valuable traits and resistance to fusarium disease. Phyto sanitary assessment of these lines has shown high disease resistance: real-time PCR diagnostics has shown the absence of the Fusarium oxysporum pathogen DNA in eggplant samples VI045276, VI046104 and in tomato samples LO6176, LO6193, in the absence of visible disease expression, the result has been weakly positive (Ct –35.62±0.67 and 34.41±0.82).

In terms of phenological development phases, yield indicators and fruit quality, the lines Lusarpi F₁/LO6176, Lusarpi F₁/LO6193 and Lusarpi F₁/VI045276, Lusarpi F₁/VI046104, grafted on these rootstocks, have surpassed the standards. The lines have been distinguished by high fruit tradability.
(97.0-98.7%). The variants grafted on eggplant have been distinguished by particularly high tradable yield, the indicators of which were: Lusarpi/VI046104 – 21.2 kg/m² and Lusarpi F₁/VI045276 – 19.6 kg/m², which exceeded standards 1 (Lusarpi F₁) and 2 (Lusarpi F₁/Lusarpi F₁) by 3.3–5.0 kg/m². Among the tomato rootstocks, the Lusarpi F₁/LO6193 sample has turned out to be high-yielding, with a tradable yield of 20.3 kg/m², which is 4.0–4.1 kg/m² higher than the standard.

References

[1] Marsic N K and Osvald J 2004 Acta agriculturae slovenica 83 (2) 243–49
[2] Turhan A, Ozmen N, Serbeci M S and Seniz V 2011 Hort. Sci. Prague 38 (4) 142–49
[3] Mavlyanova R F, Lyan E E, Karimov B A and Dubinin B V 2020 IOP Conf. Series: Earth and Environmental Science 613 012077
[4] Ahmed M A and Mahmoud 2014 Journal of Horticultural Science & Ornamental Plants 6(3) 109–15
[5] Bahadur A, Rai N, Kumar R, Tiwari S K, A K Singh, Rai A K, Singh U, Patel P K, Tiwari V, Rai A B, Singh M and Singh B 2015 Vegetable Science 42 (2) 82–7
[6] Karimov B A, Liang E E, Mavlyanova R F and Aramov M H Potatoes and vegetables 11 17–9
[7] Bolandnazar S, Moghbeli E M, Panahandeh J and Arzanlou M 2014 Acta Horticulturae 1041 (1041) 127–32
[8] Singha I M, Kakoty Ye, Unni B and Kalita M C 2011 World Journal of Microbiology and Biotechnology 27 (11) 2583–89
[9] Ignjatov M, Milošević D, Nikolic Z and Gvozdanović-Varga J 2012 Pestic. Phytomed (Belgrade) 27 (1) 25–31
[10] Abdesselem Si M, Hamini-kadar N, Mebrouk K and Jamal E H 2016 African Journal of Microbiology Research 10 (30) 1156–63
[11] Kumar P, Rouphael Y, Cardarelli M and Colla G 2017 Front. Plant Sci. 8 1130
[12] López-Marín J et al. 2017 Sci. Hortic. 214 9–17
[13] Musa I et al. 2020 Plants (Basel) 9 (11) 1583
[14] FAO 2018 Guidelines for organizing tomato production in heated greenhouses in the Republic of Armenia (Moscow) 32 p
[15] Ermakov A I 1972 Methods of plants biochemical research (Kolos: Leningrad) 350 p
[16] Dospekhov B A 1985 Field experiment technique (Agropromizdat: Moscow) 351 p
[17] Belik V F 1992 Experimental methodology in vegetable growing and melon growing (Agropromizdat: Moscow) 319 p
[18] Khokhryakov M K 1979 Guidelines for the experimental study of phytopathogenic fungi (VIZR: Leningrad) 78 p
[19] 2018 Guidelines for the diagnosis of phytopathogens by polymerase chain reaction with fluorescence detection of results using diagnostic kits manufactured by OOO (Agrodiagnostics: Moscow) 29 p
[20] Martirosyan G, Kirakosyan G and Hakobyan A 2017 Bulletin of National Agrarian University of Armenia 1 8–112
[21] Lievens B, Brouwer M, Vanachter Alfons C R C and Bruno P A 2006 Plant Science 171 (1) 155–65
[22] Schwarz D, Öztekin G, Tüzel Y, Brückner B and Krumbein A 2013 J. Scientia Horticulturae 149 70–79