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EVALUATION OF SOME VARIETIES OF ALFALFA, *MEDICAGO SATIVA* (L.), FOR THEIR RESISTANCE TO THE ALFALFA STEM NEMATODE, *DITYLENCHUS DIPSACI* (KÜHN 1857) FILIPJEV 1936, UNDER LABORATORY CONDITIONS⁽¹⁾(²)

by

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The lucerne stem nematode, *Ditylenchus dipsaci* (Kühn), is a serious threat for cultivation of alfalfa in Iran (Abivardi and Sharafeh, 1973). According to observations in various other countries crop rotation, and other cultural practices or application of nematicides often give insufficient control or are too costly (Gil, 1961; Sherwood *et al.*, 1957). Therefore, the use of existing resistant varieties, and the development of new ones seem to offer the most promising possibilities of control of this nematode (Grundbacher and Stanford, 1962 a; Hunt *et al.*, 1972). There already is a considerable number of such varieties, but different factors have limited their value in certain areas. Susceptibility to bacterial wilt (Christie, 1959; Hanna and Hawn, 1965), insufficient adaptation to new environment (Griffin, 1968; Brown and Goodey, 1956), effect of temperature on resistance (Griffin, 1968; Grundbacher and Stanford, 1962 b), presence of the resistant-breaking populations (Allison, 1956; Smith, 1958), and susceptibility to other nematodes (Reynolds, 1955), have encouraged

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extensive and continuous efforts to combine other characters necessary for agricultural use with resistance to local stem nematode population (Burkart, 1934 and 1937; Gil, 1961; Godeck and Favret, 1965; Hawn, 1966; Lundin, 1967; Sherwood *et al.*, 1967; Smith, 1955).

This study, conducted in laboratory condition, was designed to determine: (i) rates of infection and growth of the seedlings after infestation with stem nematodes from a field of alfalfa in Khafr County; and (ii) fate of the nematodes after penetration in forty two varieties of alfalfa collected in Iran and other countries.

Materials and methods

Parts of the alfalfa plants severely infested with *Ditylenchus dipsaci* were collected in an infested field at Khafr County (Abivardi and Sharafeh, 1973), taken to the laboratory and processed by a combination of blender and Cobb sieving technique (Goodey, 1963). The nematodes were recovered from 100, 200, and 325 mesh sieves and then the suspension was passed through paper tissues. The tissues containing the nematodes were folded carefully and put in Petri dishes containing 20ml tap water (Fig. 1), and incubated at $25 \pm 1^\circ\text{C}$. The tissues were removed after 24 hours, and the nematode suspension was collected from the Petri dishes. Nematode suspension of 1-ml aliquots were counted and amplified to obtain an estimate of nematodes in the whole suspension. Then, the suspension was diluted to about 100 nematodes per ml which was used for inoculation.

Thirty alfalfa seeds of one of the varieties to be investigated were sown in a plastic pot of about 100 ml filled with heat sterilized clay-loam soil. The seeds were covered with a 4-mm layer of the same soil, and watered carefully with 30 ml of the diluted suspension. To ensure a humidity of about 75% (around the seedlings), the pots were kept in a plastic chamber at $20 \pm 2^\circ\text{C}$, and watered regularly with tap water. They were illuminated by four fluorescent lamps.

The experiment was conducted with a completely randomized design with five replicates (five pots per variety with 30 seeds per pot), and the results were analyzed by Duncan's multiple range test (Duncan, 1951).

The proportion of galled (swollen) seedlings was determined two weeks after sowing. Most of the seeds had germinated then. Numbers of nematodes that had entered the plants and their stage of development were determined one month after sowing. To do this, the largest gall of each replicate was collected with the surrounding

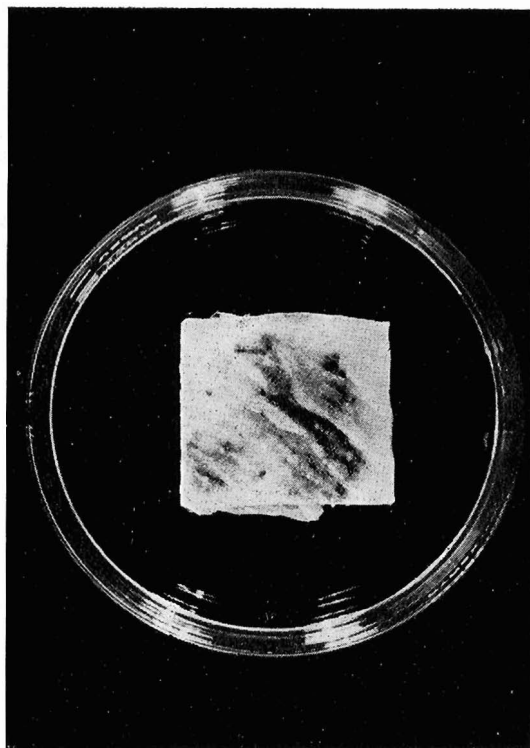


Fig. 1 - An extraction set (paper tissues and a Petri dish with 20 ml tap water) used for recovery of the alfalfa stem nematode after sieving the macerated plant tissues.

foliar parts (5 mm from each side of the gall), and was put in a few drops of water and squashed with another slide. The slides were examined under a compound microscope with a magnification of 25 (x) and 100 (x). Data on the fresh weights of the foliage were also recorded.

Results and discussion

Table I shows % plants galled, rate of reproduction of the lucerne stem nematode, and weight of foliage (g/pot) for each variety. According to % swollen seedlings, the varieties can be divided in three groups: (i) no swollen seedlings (Piaskowa of Poland), (ii) few swollen seedlings (Arnim's Altdeutsche 194 of Germany), and (iii)

Table I - Comparative resistance and tolerance of some varieties of alfalfa to the alfalfa stem nematode; determined by % swollen seedlings, nematode reproduction, and the fresh weight of the foliage (two weeks, one month, and one month after the treatment, respectively).

Variety (1)	Country of origin (2)	% plants galled (3)	Reproduction (4)	Weight of foliage (g pot)	Comments (5)
Piaskowa	Poland	—0— A	—	1.92	
Arnim's Altdeutsche 194	Germany	11.4 AB	—	2.73**	
Mohajeran-e-Hamadan	Teheran*	34.2 BC	—	1.54	
Moapa (A-27638)	USA	44.2 CD	+	2.50**	
Polycross-1	Badjgah (Shiraz)*	47.2 CD	+++	1.17	
Altfranken	Germany	47.2 CD	++	2.79**	
Culver (A-27638)	USA	51.0 CD	—	1.03	III & IV
Caliverde (A-27819)	USA	53.7 CD	?	1.22	I
Hamadani*	Hamadan*	54.6 CD	?	0.41	I
Kurmark-Ostsaat	Germany	55.4 CD	+++	3.19**	
Hamadani*	Teheran*	55.4 CD	?	0.23	I
Kashani	Kashan*	56.0 CD	?	1.00	I
Sechin-e-Hamadan	Teheran*	56.0 CD	—	1.39	
Palestra-Flocina	Greece	56.4 CD	++	1.55	
Vernal (A-17819)	USA	58.0 CD	++	1.69	
Lahontan (A-27819)	USA	58.0 CD	—	1.11	II
Arnim's Altdeutsche 1962	Germany	58.0 CD	+++	0.84	III
Jiroft	Jiroft*	61.0 CD	+++	1.28	III
Melissopetia Lamia	Greece	61.4 CD	—	1.67	III
Ghazvini	Teheran*	62.2 CD	+++	0.96	III
Bami	Shiraz*	64.8 CD	+++	2.04	
Polycross-2	Badjgah (Shiraz)*	65.0 CD	+++	0.84	III
Tuna	Sweden	66.2 CD	+++	0.90	II
Zia (A-27820)	USA	66.3 CD	++	1.47	
Lamia	Greece	67.0 CD	—	0.62	III & IV
Haftchin-e-Yazd	Yazd*	67.6 CD	+++	0.89	III
Yazdi	Teheran*	67.8 CD	+++	1.29	III

Table I continued.

Variety (1)	Country of origin (2)	% plants galled (3)	Reproduction (4)	Weight of foliage (g/pot)	Comments (5)
Afghani	Abadan*	68.0 CD	?	0.66	I
Williamsburg (A-27638)	USA	68.2 CD	?	0.90	I
Borazjani	Borazjan*	68.6 CD	?	0.85	I
Atlantic (A-27819)	USA	68.8 CD	++	1.68	
Baghdadi	Abadan*	69.2 CD	+++	0.66	III
Hamadani*	Shiraz*	70.2 CD	?	0.67	I
Grimm	USA	71.2 CD	—	0.91	III
Momtaz-e-Tehrani	Teheran*	72.6 CD	+++	0.83	III
Shirazi	Shiraz*	73.0 CD	+++	1.12	III
Esfahani	Isfahan*	74.0 CD	+++	1.54	III
St. Louise	USA	78.3 DE	+++	0.78	III
Bassreie	Abadan*	79.0 DE	?	0.46	I
Hairy Peruvian (A-27328)	USA	79.0 DE	?	0.87	I
Hassanabad-e-Esfahan	Isfahan*	79.2 DE	++	1.40	III
Klezczevska	Poland	80.0 DE	+++	0.14	III
Tehrani	Teheran*	80.0 DE	++	1.21	III
Arabi	Abadan*	82.0 DE	?	—0—	V

- 1 - Number in parentheses have been designated by the USDA for the seeds mailed to us through their department. (*) Same variety from different localities.
- 2 - (*) Cities located in Iran.
- 3 - Means of five replicates. Means not followed by the same letter or determined by (**) are different at the 1% level.
- 4 - (—), no; (+), few; (++), 5-7; (+++), 10-20 or more gravid females and eggs per gall; and (?) means: no gall was present, at the end of the experiment, for microscopical observations.
- 5 - (I), poor stand; all galled seedlings died during the experiment; (II), poor germination; (III), many infested plants died during the experiment; (IV), few dead larvae surrounded by browning tissues; and (V), all seedlings died during the experiment.

many swollen seedlings (all other varieties). However, microscopical examinations of the galls revealed that in spite of many swollen seedlings in Mohajeran-e- Hamadan from Iran, Culver, Lahontan, and Grimm from USA; *Melissoptia Lamia*, and *Lamia* from Greece, no female had been produced one month after sowing. In another variety (Sechin-e- Hamadan of Iran) only few males and non-gravid females were observed in some galls. Therefore, these varieties may

be called resistant to the population used in this study. Considering the records on death of many infested seedlings of these varieties (except Lahontan), and the presence of few larvae surrounded by browning cells similar to those reported by Barker and Sasser (1959) in Culver and Lamia, the resistance is believed to be mainly attributed to hypersensitivity of the tissues to the invasion. As the reactions of the mentioned varieties were extensive, the cultivars are not probably suitable for direct application in the infested fields. However, they might be used as sources for the breeding programs.

The practical value of Piaskowa is questionable because it is unproductive; while Arnim's Altdeutsche 194 may be considered a promising candidate variety for screening programs.

Moapa from USA, Altfranken and Kurmark-Otsaat from Germany tolerated the nematode attack in the course of our study, resulting in high yields (in spite of having many swollen seedlings and active nematode reproduction).

In our investigation Lahontan gave more swollen seedlings than in those of Bingefors (1961) and Smith (1958); while Vernal, Atlantic, and Grimm resulted in lower number of galls when compared to those reported by these investigators. This may be due to the differences in the nematode populations used in these studies. Similar differences were found when resistant varieties from Argentina were tested with the population in California (Grundbacher and Stanford, 1962 a). According to Grundbacher and Stanford (1962 b), Lahontan gave more swollen seedlings at 15.5°C and 21°C than at 11°C; and that after 25 days at 21°C, the infested plants were practically free of nematodes although they showed distorted leaves and petioles 12 days after inoculation. This report is in accordance with our findings in regard to more swollen seedlings of this variety under our conditions ($20 \pm 2^\circ\text{C}$), and the presence of few larvae at the end of the study (after one month).

Almost all swollen seedlings of Caliverde, Hamadani, Kashani, Afghani, Williamsburg, Borazjani, Bassreie, Hairy Peruvian, and Arabi became necrotic and died during the investigation.

Further studies are required to evaluate the practical value in Iranian Agriculture of the varieties found resistant in these investigation.

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S U M M A R Y

Fortytwo varieties of alfalfa from Iran and other countries were screened for their resistance to the alfalfa stem nematode, *Ditylenchus dipsaci* (Kühn 1857) Filipjev 1936. Piaskowa of Poland produced no, Arnim's Altdeutsche 194 of Germany few and the remaining varieties many swollen seedlings. However, the galls of Mohajeran-e-Hamadan from Iran; Culver, Lahontan, and Grimm from USA; Melissopetia Lamia and Lamia from Greece did not contain female nematodes. In another variety (Sechin-e-Hamadani from Iran) only a few males and non-gravid females were observed in some galls. Moapa from USA, Altfranken and Kurmark-Otsaat from Germany tolerated the nematode attack; while almost all swollen seedlings of Caliverde, Hamadani, Kashani, Afghani, Williamsburg, Borazjani, Bassreie, Hairy Peruvian, and Arabi became necrotic and died during the investigation.

R I A S S U N T O

Valutazione, in condizioni di laboratorio, della resistenza di alcune varietà di Erba medica verso il nematode degli steli, Ditylenchus dipsaci (Kühn 1857) Filipjev 1936.

Sono state saggiate quarantadue varietà di Erba medica provenienti da diversi paesi per la resistenza al nematode degli steli *Ditylenchus dipsaci* (Kühn) Filipjev. La varietà polacca Piaskowa e la varietà tedesca Arnim's Altdeutsche 194 hanno prodotto piantine rispettivamente senza o con leggeri ingrossamenti, mentre molto distorte ed ingrossate apparivano le piantine delle altre varietà. Tuttavia, le galle delle varietà Mohajeran-e-Hamadan (Iran), Culver, Lahontan e Grimm (U.S.A.) e Melissopetia Lamia e Lamia (Grecia) non contenevano femmine del nematode. Nella varietà Sechin-e-Hamadani (Iran) sono stati osservati solo maschi e femmine non gravide. « Moapa » (U.S.A.), « Altfranken » e « Kurmak-Otsaat » (Germania) hanno tollerato l'attacco del parassita, mentre la quasi totalità delle piantine ingrossate di « Caliverde », « Hamadani », « Afghani », « Williamsburg », « Borazjani », « Bassreie », « Hairy Peruvian » ed « Arabi » sono morte nel corso della prova.

R E S U M É

Evaluation de la résistance de quelques variétés de luzerne envers le nématode des tiges, Ditylenchus dipsaci (Kühn 1857) Filipjev 1936, en conditions contrôlées.

La résistance au nématode des tiges, *Ditylenchus dipsaci* (Kühn) Filipjev, de quarante-deux variétés de luzerne provenant de divers pays, a été éprouvée. La variété Piaskowa (Pologne) a donné des plantules sans symptômes. La variété de l'Allemagne, Arnim's Altdeutsche 194 n'a montré que des légères galles et les plantules des autres variétés étaient très déformées et renflées. Dans les galles des variétés Mohajeran-e-Hamadan (Iran), Culver, Lahontan et Grimm (U.S.A.),

Melissopetia Lamia et Lamia (Grèce) on n'a pas trouvé des femelles du nématode. La variété Sechin-e-Hamadan (Iran) présentait seulement des mâles et des femelles non gravides. « Moapa » (U.S.A.), « Altfranken » et « Kurmak-Ostsaat » (Allemagne) ont toléré l'attaque du parasite. La presque totalité des plantules renflées de « Caliverde », « Hamadani », « Afghani », « Williamsburg », « Borazjani », « Bassreie », « Hairy Peruvian » et « Arabi » sont mortes au cours de l'épreuve.

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