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Optimal inoculum levels for the resistance screening of sugar beet to root- knot nematode under greenhouse condition

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ABSTRACT

Sugar beet is a host plant for different root-knot nematode species. In this study, the effect of six inoculum levels including 250 + 250, 500, 500 + 250, 750, 500 + 500, and 1000 second instar larvae of *Meloidogyne javanica* was evaluated on susceptible cultivar Jolgeh in 450 cm^3 soil. Results showed significant effect of all inoculum levels on sugar beet infection and no significant effect was observed for the number of nodes caused by inoculum. Thus, 500 second instar larvae were considered as the lowest inoculum level for sugar beet infection in the greenhouse. In the second experiment, inoculum treatments were applied on Jolgeh and Pauletta (resistant to beet cyst nematode) cultivars and also sevenhalf-sib families derived from a population resistant to root-knot nematode (SB33). The number of nodes formed on the root was used as a resistance criterion. Based on the results, Jolgeh with a high average formed nodes (>100) was considered as asusceptible cultivarand half-sib families with the minimum number of formed nodes (<1) were classified as resistant plants. The commercial cultivar Paulettashowed high susceptibility to root -knot nematode.

Keywords: inoculation, resistance, root -knot nematode, sugar beet

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is an important industrial crop and is the second source of sugar production after sugar cane which provides 27% of the world's sugar consumption annually. Several root-knot nematode (*Meloidogyne* spp.) species, recognized as important sugar beet economic parasites, were identified in many parts of the world, especially in tropical and semi-tropical regions (Whitehead 1969; Janati et al. 1982; Arnold 1984; Ibrahim 2004). Economically, important sugar beet root knot species are *Meloidogyne arenaria* Chitwood, *M. incognita* Chitwood, *M. hapla* Chitwood, *M. javanica* Chitwood, *M. fallax* Chitwood and *M. chitwoodi* (Whitney and Duffus 1986; Franklin 1979).

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Root-knot nematode symptoms include node formation on both lateral and main root (Whitney and Duffus 1986) which dramatically influences the yield and quality ofsugar beet. Owing to the wide range of host plantsfor root-knot nematode, toxicity of nematicidesfor humans and environment and developmentof nematode resistance to nematicides, its control has become a crucial problem in sugar beet fields (Weiland and Yu 2003). Therefore, development of the resistant host is the important solution to overcome disease problems (Panella and Lewellen 2007). The initial inoculum level of the pathogen influences therate and amount of infectionin host plant. Determining the optimal level of inoculation improves the efficiency and accurate evaluation of the host resistance to this pathogen (Hashmi et al. 1994).

Little information is available on *Beta* species resistance to root -knot nematodes. Most studies are focused on sugar beet and root-knot nematode interaction with regard to cultivars performance and their susceptibility (Ismail et al. 1996; Maarg et al. 1998; Pathak and Keshari 2000; Korayem et al. 2012). To evaluate sugar beet genotypes resistance, different treatments including 1200 second instar larvae in polyethylene box with 110 cm^3 volume (Weiland and Yu 2003; Yu 2003), 1000 second instar larvae in 110 cm^3 pot (Yu 1995), and 500 second instar larvae in 170 cm^3 pot (Di Vito 1983) were used and 28, 40, 50, and 60 days after inoculation, root -knot numbers were counted. In Iran, different species including *M. incognita* (Akhiyani et al. 1993; Omidvar 1968), *M. javanica* (Akhiyani et al. 1993; Mehdikhani Moghadam et al. 1996; Niknam and Kheiri 1996; Karegar 2006; Ommati and Giti 2010), *M. hapla* (Ommati and Giti, 2010; Karegar 2006) and *M. arenaria* (Omidvar, 1968) were detected and isolated from sugar beet fields in Isfahan, Moqan plain, Mashad, and Hamedan; however, no study has been conducted to evaluate sugar beet cultivars susceptibility to the pathogen so far. Prerequisite of this study is the improvement of greenhouse evaluation methods. Therefore, the aim of this study was to evaluate the optimum level of root -knot nematode inoculation for determination of sugar beet genotypes resistance or susceptibility under greenhouse condition.

MATERIALS AND METHODS

Preparation of the nematode inoculum

Sugar beet roots infected to root -knot nematode were collected from a field in Joghtay city in Khorasan Razavi province, Iran. Purification was performed by inoculation of sole egg sac on Rotgerz tomato cultivar and reproduction on the susceptible cultivar Jolgeh. Formed egg bulks were hatched in a dark environment with 25-27^oC and second instar larvae were treated with 1% sulfate Stereptomycine for one hour and prepared for inoculation in a concentration of 500 larvae in one ml distilled water.

Determination of optimum nematode inoculation level for the evaluation of sugar beet resistance/susceptibility

Two months after seed sowing, seedlings were inoculated with purified second instar larvae of root -knot nematode. At the time of inoculation, three holes were made inside the soil and the transfer of water suspension plus nematode was conducted using syringe. After inoculation, holes were filled with soil. Water suspension was used as control. In this study, two greenhouse experiments were conducted. In first experiment, six second instar larvae levels including 250 + 250 (1), 500 (2), 500 + 250 (3), 750 (4), 500 + 500 (5), and 1000 (6) second instar larvae of *Meloidogynejavanica*were evaluated on Jolgeh at 450 cm^3 soilfor two times. In treatments 1, 3, and 5, second inoculation was applied one week after the first inoculation. In the second experiment and in order to evaluate the proper treatment of the first experiment, seedlings of seven half-sib families derived from a multigerm pollinator population (SB33) carrying root -knot nematode resistance gene together with a susceptible (Jolgeh) and resistant (Pauletta) controls were inoculated with 500 second instar larvae. Inoculated seedlings in both experiments were placed in greenhouse at 22±3 ^oC for 70 days. At the end of the experiments, pots were submerged in water, seedlings were pulled from the pots and rinsed. Root -knots were counted using stereomicroscope. Seedlings with less than 10 knots on root were considered as resistant and roots with 10 or more than 10 knots were considered as susceptible (Taylor and Sasser 1978).

Data analysis

Experiments were conducted based on completely randomized design. After root-knot counting, resistant and susceptible plants were identified (Taylor and Sasser 1978). Data were analyzed using one-way ANOVA and Duncan's multiple range test in the first experiment and non-parametric test of Kruskal-Wallis with Mann-Whitney and Binomial methods for mean comparison in the second experiment. SPSS software was used for data analysis.

RESULTS

Nematode species

After purification and reproduction of single egg mass, species identification wasperformed based on the characteristics of the mature larvae, second instar larvae, and male nematode using available sources (Jepsen 1987). Purified isolate belonged to *Meloidogyne javanica* species.

Optimum inoculation level for sugar beet infection to root-knot nematode in the greenhouse

Evaluation of the susceptible cultivar roots illustrated clear knot and egg sacs in all treatments (Figure 1). Number of formed nodes ranged from

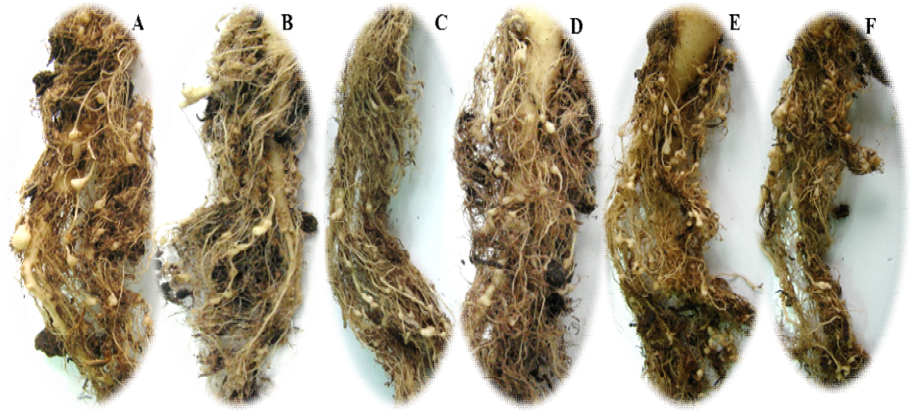

Figure 1. Nodes formed on Jolgeh root, (A) 70 days after inoculation with 250+250, (B) 500, (C) 500+250, (D) 750, (E) 500+500, and (F) 1000 second instar larva of *Meloidogynejavanica*.

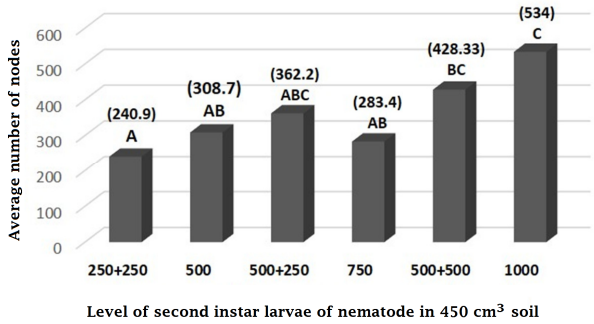

Figure 2. Effect of different *Meloidogyne javanica* inoculation levels on node formation in Jolgeh cultivar. Means with same letter are not significantly different.

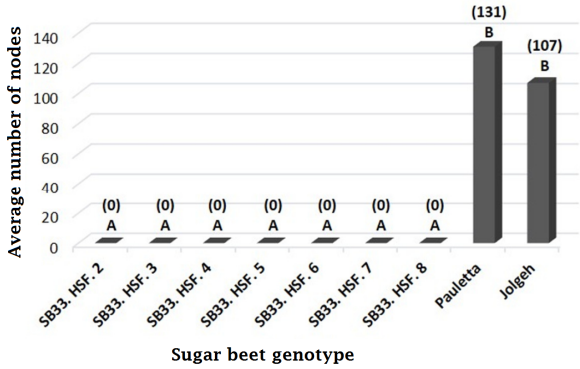

Figure 3. Mean comparison of node number formed on roots of different sugar beet genotypes inoculated with 500 second instar larva of *Meloidogyne javanica*in 450 cm^3 soil. Means with same letter are not significantly different.

65 (treatment 1) to 788 (treatment 6). Treatment 1 showed significant (P≤0.01) difference with treatments 5 and 6. The mean difference of node number between the lowest and highest treatments was significant (P≤0.01). Also, a significant (P≤0.01) difference was observed in the node number of treatments 1-4 and treatment 6. Results showed no difference in inoculation times (Figure 2). Therefore, the lowest inoculation level forsugar beet infection in greenhouse is 500 second instar larvae in one-time inoculation.

Evaluation of the optimal level of inoculum for sugar beet genotype selection

Pauletta and Jolgeh cultivars with node numbers of 131 and 107, respectively, were placed in susceptible group. All half-sib families derived from the SB33 population showed no node formation and were classified as resistant genotypes (Taylor and Sasser 1978, Figure 3).

DISCUSSION

Egg number or the second instar larva which is used for resistance evaluation against root-knot nematode in the greenhouse varies according to pot size, host plant susceptibility, and environmental condition. Therefore, it is essential to perform preliminary tests to determine the optimum inoculation level (Hussey and Janssen 2002). All isolates were not able to impose a pathogenic response. For example, the inoculum concentration for egg hatching percentage is 20-25%. The older the egg, the proportion of the eggs having embryos and their hatching will be higher (Ehwaeti et al. 1998). One to two larvae per cm^3 of soil is a good starting point. Using larvae as an infestation factor gives an accurate estimation of the time and infestation severity which is preferred in simple studies of the resistance. However, larvae are more susceptible to manipulation than the eggs. There is a linear relationship between resistance index evaluation and the amount of inoculum. Application of a low amount of inoculum in a susceptible plant treatment demands more time for the evaluation. Otherwise, allergic reaction may be wrongly attributed to resistance. Therefore, using the determined amount of specified pathogen is an important factor in the evaluation of ge-

netic material during screening programs for nematode resistance.

The optimum amount of inoculum and its relationship with measurable symptoms should be evaluated at the beginning of the evaluation programs. Different studies showed that among five inoculum levels of 20, 100, 200, 1000 and 2000 larvae and eggs of the root-knot nematode, tomato resistance can be evaluated in the greenhouse at 32.5 °C with 200 eggs and larvae (Araujo et al. 1982). In addition, the optimum level of root-knot nematode inoculation for evaluation of tomato and peach resistance in tissue culture medium was recognized as 75 and 200 second instar larvae, respectively (Hashemi et al. 1994). To study sugar beet genotypes resistance, 1200 second instar larvae in polyethylene pot with 110 cm^3 volume (Weiland and Yu 2003; Yu 2003), and 110 cm^3 pots (Yu 1995) and 500 second instar larvae in 170 cm^3 pots (Di Vito 1983) were used at 28, 40, 50 and 60 days after inoculation on root knot. In another study, 500 second instar larvae of *M. hapla* were used in pots with 350 cm^3 volume for resistance evaluation. Their results showed that among the 500, 750, and 1250 treatments, 500 larvae inoculation for two times made more knots than one-time inoculation. Whilst at the higher inoculation levels, formed knots of the one-time inoculation were higher than two-times inoculation; the reason was lower pathogenic effect of *M. halpa* compared to the other species (Yu et al. 1999). Therefore, at low levels of inoculation, primary inoculation predisposes plants to secondary invasion by the parasite. Unlike viral diseases, plants are not immune against nematodes, so the initial treatment with parasite will reinfest the plant (Jatala and Jensen 1976). In general, the results of this study showed that two-times inoculation compared with one-time inoculation had no effect on *Meloidogyne javanica* knot formation (Figure 3) and 500 second instar larvae is the proper inoculation level for the evaluation of sugar beet response to root-knot nematode. However, other researchers used a greater number of larvae of the same species for the screening of the resistance (Di Vito 1983; Yu 1995, 2003; Weiland and Yu 2003). The SB33 population carry the resistance gene to root-knot nematode (Yu and Lewellen 2004). Since the gene is dominant, all the half-sib families produced from this population have the resistance gene. The evaluation of the sugar beet resistance against root-knot nematode is done based on the knots formed on the root. Plants with 10 or less root knot numbers are considered as resistant and plants with more than 10 knots are considered as susceptible. Nematode reproduction has a direct relationship with this root knot number and it is a criterion for the evaluation of sugar beet resistance to root-knot nematode (Taylor and Sasser 1978; Yu 1995; Yu et al. 1999; Weiland and Yu 2003; Gohar and Maareg 2009). Results of these experiments showed that greenhouse evaluation can be used for the differentiation of resistant and susceptible genotypes. For all susceptible plants, the number of knots formed on the root was more than 10 but their number depends on the plant growth condition. The growth and reproduction of *Meloidogyne* species demand healthy plants. In plants with better growth and more roots, the number of knots are higher than the same genotype with weak growth and less root number. Therefore, the number of knots formed on Jolgeh cultivar in the two experiments, with similar inoculation level of 500 second instar larvae, was different. Nevertheless, in both experiments this cultivar was classified in a susceptible group. The screening method for the identification of resistant lines to root-knot nematode should be able to differentiate genotypes. In spite of this, greenhouse evaluation for the identification of produced lines resistant to root-knot nematode requires the preparation of purified culture for the inoculum of susceptible plants. The amount of nematode inoculation level is of the extensive evaluation limit under greenhouse condition. For this reason, the lowest level of the inoculum capable of screening resistant genotypes is of more attention. In this study, the lowest level of root-knot nematode inoculation for resistance screening was 500 second instar larvae in 450 cm^3 soil. However, other researchers suggested more second instar larvae for the evaluation of sugar beet resistant genotypes.

REFERENCES

Akhiyani A, Damadzadeh M, Ahmadi AR. Identification of plant parasitic nematodes in sugar beet fields in Esfahan. Proceedings of the 11th Iranian Plant Protection Congress, Guilan University, Rasht Iran. 1993. P. 123.

Araujo MT, Dickson DW, Augustine JJ, Bassett MJ. Optimum initial inoculum levels for evaluation of resistance in tomato to *Meloidogyne* spp. at two different soil temperature. Journal of Nematology. 1982; 14(4): 536-540.

Arnold ES. Nematode parasites of sugar beet. In: W.R. Nickle, eds. Plant and Insect Nematodes. New York, Marcel Decker Inc. 1984; pp. 507-569.

Di Vito M. Reaction of *Beta* spp. to root knot nematodes. Journal of Nematology. 1983; 15(1): 144-145.

Ehwaeti ME, Phillips MS, Trudgill DL. The viability of *Meloidogyne incognita* eggs released from egg masses of differ-

ent ages using different concentrations of sodium hypochlorite. Nematologica. 1998; 44: 207-217.

Franklin MT. Economic importance of *Meloidogyne* in temperature climates. In: F. Lamberti and C.E. Taylor, eds. Root-knot Nematodes (*Meloidogyne* species) Systematic, Biology and Control. London and New York: Academic Press. 1979. pp. 331-339.

GoharIMA, Maareg MF. Effect of inoculum level, type, plant age and assessment date on evaluating sugar beet resistance methods for root- knot nematode, *Meloidogyne incognita*. Journal of Agricultural Science. 2009; 34(5): 5401-5419.

Hashmi G, Huettel RN, Hammerschlac FA, KrusbergLR. Optimal levels of *Meloidogyne incognita* inoculum for infection of tomato and peach in vitro. Journal of Nematology. 1994; 26(4): 531-534.

Hussey RS. Janssen GJW. Root-knot nematodes: *Meloidogyne* species. In: J.L. Starr, R. Cook and J. Bridge, eds. Plant resistance to parasitic nematodes. CABI publishing, Wallingford, UK. 2002; pp. 43-70.

Ibrahim IKA. Nematode Parasites of Field and Horticulture Crops. (In Arabic). Dar El-Maaref, Alexandria, Egypt. 2004; 330 pp.

Ismail AE, Aboul-Eid HZ, Besheit SY. Effects of *Meloidogyne incognita* on growth response and technological characters of certain sugar beet varieties. Afro-Asian Journal of Nematology. 1996; 6(2): 195-202.

Janati A, Aouragh EH, Meskine M. The root- knot nematodes *Meloidogyne* spp. in Morocco. 3th Research and Planning Conference on Root Knot Nematodes *Meloidogyne* spp. Coimbra, Portugal; 1982. P. 85-93

Jatala P, Jensen HJ. Self-interaction of *Meloidogyne hapla* and *Heterodera schachtii* on *Beta vulgaris*. Journal of Nematology. 1976; 8(1): 43-48.

Jepsen SB. Identification of Root Knot Nematode (*Meloidogyne*) species, CABI international Wallingford, UK. 1987; 265 pp.

Karegar A. Identification of plant-parasitic nematodes associated with sugar beet and their distribution in Hamadan province, Iran. Iranian Journal of Plant Pathology. 2006; 42(1): 159-178. (in Persian).

Korayem AM, El-Bassiouny HMS, Abd El-Monem AA, Mohamed MMM. Physiological and biochemical changes in different sugar beet genotypes infected with root-knot nematode. Acta Physiologiae Plantarum. 2012; 34: 1847-1861.

Maareg MF, Hassanein MA, Allam AI, Oteifa BA. Susceptibility of twenty six sugar beet varieties to root knot nematodes *Meloidogyne* spp. in the newly reclaimed sandy soils of Al-Bostan region. Egyptian Journal of Agronematology. 1998; 2(1): 111-125.

Mehdikhani Moghadam E, Kheiri A, Okhovat M. Morphological and morphometrical study of three endoparasitic nematodes of sugar beet in Mashhad region. Iranian Journal of Plant Pathology. 1996; 32(1-2): 1-15. (in Persian).

Niknam G, Kheiri A. Identification of plant parasitic nematodes (Tylenchida) in Moghan fields. Journal of Agricultural Science. 1996; 7(1,2): 1-33.

Omidvar AM. Plant Parasitic Nematodes, Behavior, Biology, Systematics and their Control, Ministry of Agriculture, Tehran, Iran. 1968; 192 pp.

Ommati F, Giti M. Identification and spread of sugar beet parasitic nematodes of Semnan province. Proceedings of the 19th Iranian Plant Protection Congress, Iranian Research Institute of Plant Protection, Tehran, Iran. 2010. P. 5495.

Panella L, Lewellen RT. Broadening the genetic base of sugar beet: introgression from wild relatives. Euphytica. 2007; 154: 383-400.

Pathak KN, Keshari N. Effect of inoculum levels of *Meloidogyne incognita* (Kofoid and White, 1949) Chitwood, 1919, on seed germination, seedling emergence and plant growth of red beet (*Beta vulgaris* var. *crassa*). Pest Management in Horticultural Ecosystems. 2000; 6(2): 118-123.

Taylor AL, Sasser JN. Biology, Identification and Control of Root-Knot Nematodes (*Meloidogyne* Species). Raleigh: North Carolina State University Graphics. 1978. 111 pp.

Weiland JJ, Yu MH. A cleaved amplified polymorphic sequence (CAPS) marker associated with root-knot nematode resistance in sugar beet. Crop Science. 2003; 43: 1814-1818.

Whitehead AG. The distribution of root-knot nematode, *Meloidogyne* spp. in tropical Africa. Nematologica. 1969; 15: 315-333.

Whitney ED, Duffus E. Compendium of Beet Disease and Insects. APS Press, USA. 1986; 76 pp.

Yu MH, Heijbroek W, Pakish LM. The sea beet source of resistance to multiple species of root-knot nematode. Euphytica. 1999; 108: 151-155.

Yu MH, Lewellen RT. Registration of root-knot nematode resistant sugar beet germplasm M6-2. Crop Science. 2004; 44: 1502-1503.

Yu MH. Development of root-knot nematode resistant sugar beet. 1st Joint IIRB-ASSBT Congress, 26th Feb.-1st March. USA: San Antonio; 2003; pp. 763-65

Yu MH. Root-knot nematode development and root gall formation in sugar beet. Journal of Sugar Beet Research. 1995; 32(1): 47-58.