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Full Length Research Paper

Effect of onion yellow dwarf virus (OYDV) on yield components of fall garlic (*Allium sativum* L.) in Serbia

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Production of garlic in Serbia is heavily affected by garlic diseases caused by viruses, among which economically most important is Onion Yellow Dwarf Virus (OYDV). An influence of infection of garlic with OYDV on yield components was evaluated. Using DAS ELISA test, OYDV was identified in 30.5% samples. Viral infection significantly reduced parameters of yield quantity and quality. Infected plants decreased height, mass and height of individual bulbs clove mass, as well as, content and yield of dry matter. Reduction of average bulb mass was 21.5% whereas, dry matter yield was 24.0% lower than in healthy plants. Pathogen did not influence number of leaves, number of outer membranous leaves and number of cloves. A positive correlation between visually rated disease intensity and results of DAS ELISA test was detected.

Key words: Onion yellow dwarf virus (OYDV), garlic, yield components.

INTRODUCTION

Garlic (*Allium sativum* L.) is one of the most significant vegetable crops from genus *Allium* and bulb plant of major importance which is used as a seasoning and medicinal plant (Rivlin, 2001; Singh and Singh, 2008). According to production areas, it is the second most distributed species from genus *Allium* after onion, with a yearly production of around 22 million tons (FAOSTAT, 2009). Due to exclusive vegetative reproduction, garlic was considerably affected by viral infections (Dovas et al., 2001, Gvozdanović-Varga et al., 2009a). More than eight viruses were detected in garlic, including Potyviruses, Carlaviruses and Allexiviruses, which are often found in viral complex that causes diseases known as garlic mosaic (Van Dijk et al., 1991; Van Dijk, 1993; Dovas and Volvas, 2003). Among them, the economically most significant ones are Potyviruses: Onion yellow dwarf virus (OYDV), Leek yellow stripe virus (LYSV) and Shallot yellow stripe virus (SYSV). Several estimates have proposed that garlic mosaic can generate up to 88% of losses in bulb weight (Lot et al., 1998). OYDV is distributed worldwide regularly caused heavy losses (Dovas et al., 2001; Mahmoud et al., 2008; Shahraeen et al., 2008). Previous investigations proved that OYDV is dominant on garlic in Serbia, whereas, LYSV occurs less frequently (Bagi et al., 2010). There are no data on the significance of Reovirus and Allexivirus in Serbia.

The aim of this research was to visually estimate virtually the virus infection intensity, to detect virus concentration using DAS ELISA test and to evaluate the relevant yield components. It will be discussed how viral infection affect and correlate with yield parameters.

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MATERIALS AND METHODS

Tested genotype of garlic

For this research, a clone of fall garlic K3/13, created at the Institute for Field and Vegetable Crops in Novi Sad (IFVCNS) Serbia was used. This clone was produced in breeding for high content of dry matter and since it represents progeny of a single plant, it has an homogenous genetic structure. The main characteristics of this clone are compact and large bulbs with uniformly large cloves and high amount of dry matter, from 36 to 38%, and average yield from 10 to 12 t/ha (Gvozdanović-Varga et al., 2009b).

Collection of samples

Samples of garlic for serological analysis were collected in May, 2011 from the experimental field of IFVCNS, Serbia, 220 days after planting, when garlic reached growth stage BBCH41.401 (Feller et al., 1995). In Serbian, agro climatic conditions, symptoms of viral diseases on garlic are the most distinctive during this stage of development. Upper leaf from randomly selected 82 plants was collected regardless of the disease and symptom intensity.

Visual evaluation of disease intensity

Visual evaluation of expressed disease symptoms was performed simultaneously with leaf sampling. The scale from 0 to 3 was used, where: 0 – no symptoms, 1 – mild mosaic, 2 - distinct mosaic, 3 – mosaic and leaf deformations (Bagi et al., 2010). Based on symptom ratings, average disease intensity was calculated as well as, disease index using McKinney's formula (McKinney, 1923).

Virus determination using DAS ELISA test

In order to determine the concentration of OYDV and LYSV in garlic samples, DAS ELISA test was performed (Clark and Adams, 1977). This test was conducted according to protocol recommended by producer - Loewe Biochemica GmbH.

After homogenization in extraction buffer (Homex 6, Bioreba), each leaf sample sap was placed in a single microplate well in 3 replications. Positive and negative controls (obtained in DAS ELISA kit) were included in two and four replications, respectively. Since virus concentration was expressed by colour intensity in DAS ELISA test, light absorption in each microplate well was measured by spectrophotometer (Labsystem Multiscan Ex type 355) on wavelength of 405 nm. Measurement was done twice in 30 and 60 min after incubation. An infected sample considered with an average absorbance of 405 nm was twice higher than the average of the negative control, while all other samples were treated as healthy.

Yield components

The following yield parameters were determined in all tested plants: above-ground plant height (cm); HP, number of leaves-NL, bulb mass (g); BM, average clove mass per bulb (g); MC, average number of cloves per bulb; NC, number of outer membranous leaves (number of dry papery leaves to the first clover)-NOLB, bulb height (mm, from the top of the bulb to the stem)-HB, bulb width (mm, measured in the widest part of bulb)-WB, dry matter (%, measured by refractometer HR900, A. Krüss Optronic GmbH, Germany)-DM and dry matter yield by bulb (g, obtained by multiplication of bulb mass and % of dry matter)-YDM.

Determination of yield parameters was performed by methodology for recognition of onion and garlic cultivars (Official gazette of the Republic of Serbia, 2010). After harvest, prior to analysis, garlic samples were air-dried for 10 days at room temperature.

Statistical analysis

Results, separately for infected and healthy group of plants were presented as averages with standard deviation. In order to assess the influence of virus on tested traits, Spearman's coefficient of correlation was calculated (Spearman, 1904). Statistical analysis was done using software package Statistical ver.9.1 (StatSoft, Inc., Tulsa, Oklahoma, USA).

RESULTS

Average disease intensity for clone K3/13 (using a scale of 0 to 3) was 1.22, while McKinney's disease index was 40.6%. Out of 82 assessed plants, on 24 (29.3%) no symptoms were observed. A mild mosaic was observed on 33 plants (40.2%), distinct mosaic on 8 plants (9.8%), while leaf deformations in addition to mosaic were present on 17 plants (20.7%). DAS ELISA test showed that LYSV was not detected in sampled leaves, while OYDV was confirmed in 25 out of the 82 samples (30.5%). From 25 positive samples, eight samples were evaluated virtually to have distinct mosaic (rate 2) whereas, 17 plants had leaf deformations beside mosaic (rate 3).

Comparing results between healthy and infected group of plants, it was determined, that infection with OYDV causes significant yield reduction, both in quantity: bulb mass (21.5%), plant height (3.3%), bulb height (5.0%) and width (10.9%), clove mass (25.9%), and quality: dry matter (2.6%) and yield of dry matter (24.0%) (Table 1).

Based on the statistical analysis, absorption values that reflect virus concentration in plant tissue correlated positively with visual evaluation of disease intensity and negatively with plant height, bulb mass, height and width, clove mass, dry matter and yield of dry matter (Table 2). There was no correlation observed between the absorbance and number of leaves, number of outer membranous leaves and number of cloves within single bulb.

DISCUSSION

In Serbia, growers usually use part of their crop as seeds for the next year (Gvozdanović-Varga et al., 2009a). This practice enhanced the dissemination of viruses and other diseases across the country. Even plants from imported certified seed became infected after one or two years of reproduction due to the presence of virus infected plants in their close surroundings. The severity of OYDV of garlic and the great economic losses caused, necessitate the production of virus free seed (Kumar et al., 2010).

Previous research conducted during 2009 (Bagi et al., 2010), as well as, the current study confirmed that OYDV is the most significant garlic virus in Serbia which is

Table 1. Influence of OYDV infection on garlic yield components.

Parameter	Healthy plants	Infected plants	Decrease (%)[3]
Aboveground plant height (cm)	75.7[1] (8.55)[2]	73.2[1] (8.14)[2]	3.3
Number of leaves	10.2 (1.32)	10.2 (0.99)	0
Bulb mass (g)	75.3 (16.42)	59.1 (21.65)	21.5
Bulb height (mm)	42.3 (4.15)	40.2 (4.45)	5.0
Bulb width (mm)	59.9 (5.47)	53.4 (8.01)	10.9
Number of outer leaves	4.8 (0.84)	5.0 (0.95)	0
Clove mass (g)	5.4 (1.37)	4.0 (1.59)	25.9
Number of cloves	12.3 (2.97)	13.4 (2.65)	0
Dry matter (%)	38.0 (2.32)	37.0 (2.02)	2.6
Dry matter yield per bulb (g)	28.7 (5.71)	21.8 (8.38)	24.0

[1]Average values; [2]Standard deviation; [3]%Decrease=100-(I*100/H), I-average value for healthy plants, H-average value for OYDV infected plants.

Table 2. Spearman's correlation test between values of absorbance at 405 nm, visual ratings on disease intensity and yield parameters of garlic.

Parameter[1]	AD	VS	HP	NL	BM	HB	WB	NOLB	MC	NC	DM	YDM
AD	1.00											
VS	0.68*	1.00										
HP	-0.37*	-0.15	1.00									
NL	-0.06	0.04	0.53*	1.00								
BM	-0.43*	-0.26*	0.65*	0.47*	1.00							
HB	-0.35*	-0.28*	0.53*	0.36*	0.80*	1.00						
WB	-0.41*	-0.27*	0.57*	0.43*	0.87*	0.58*	1.00					
NOLB	0.06	0.11	0.26*	0.31*	0.28*	0.04	0.31*	1.00				
MC	-0.34*	-0.23*	0.33*	0.21	0.75*	0.60*	0.70*	0.26*	1.00			
NC	0.06	0.12	0.39*	0.38*	0.21	0.17	0.12	0.10	-0.41*	1.00		
DM	-0.23*	-0.23*	0.15	0.11	0.05	0.20	0.08	0.21	0.04	0.13	1.00	
YDM	-0.46*	-0.30*	0.64*	0.45*	0.99*	0.81*	0.85*	0.28*	0.75*	0.20	0.16	1.00

AD- absorbance at 405 nm; VS-visual score; HP-aboveground plant height; NL-number of leaves; BM-bulb mass; HB- bulb height; WB-bulb width; NOLB- number of outer leaves of the bulb ; MC- clove mass; NC-number of cloves; DM-dry matter; YDM-dry matter yield; * - significantly different from zero at 5%. [1]Values based on assessments of all tested sample.

consistent with the findings reported from other countries. Shahraeen et al. (2008) reported that OYDV was most common of all investigated viruses in different Iranian provinces and its average incidence varied between 40.3 and 65.5%. In south Italy, high disease intensity of 98% infected garlic plants with OYDV was reported (Dovas and Vovlas, 2003). Lunello et al. (2007) estimated that a percent of the diseased plants with OYDV in Argentina was between 58 and 100%.

Currently, available data analyzes influence of garlic viruses on yield, primarily, the decrease of bulb mass in the infected plants. Carvalho et al. (1981) observed that during two consecutive years of research, the mean weight of bulbs of the California early cultivar decreased with about 12.9% in the first year and 13.1% in the second year. Conci et al. (2005) reported that viral infections might decrease the mass of garlic bulb up to 78%. In addition, Lot et al. (1998) determined that OYDV reduces bulb mass from 39 to 60% depending on the genotype. Lunello et al. (2007) registered significant losses in garlic yield due to viral infection. According to them, LYSV decreased bulb mass for 28% and bulb width for 9%, whereas, in the case of multiple virus infection, these parameters were 74 and 37%, respectively. Bulb mass reduction up to 68% was caused by garlic virus complex as reported by Walkey and Antill (1989). Furthermore, it is thought that OYDV exhibits more severe disease symptoms and yield reductions than LYSV (Takaichi et al., 2001). Yield losses from OYDV as high as 25 to 54% have been reported by Barg et al. (1997), Lot et al. (1998) and Dovas et al. (2001). Research conducted in Argentina indicated that the decrease of yield in infected plants varied between 66 and 216% in bulb weight and from 13 to 51% in bulb perimeter (Conci et al., 2003). According to Melo (2006), the use of healthy garlic seed can increase yield by more than 100% when compared to the standard garlic seed used by farmers. Our research also reported decrease in bulb mass in OYDV infected plants (21.5%), as well as, other yield components, such as; plant height, bulb height and width, clove mass and percent dry matter and yield per bulb. However, there were no differences in number of leaves, number of papery leaves and number of cloves between infected and healthy plants, and hence, these components were not suitable characteristics for the detection of viral infection, which correspond to results of Melo (2006).

Results from visual disease rating and DAS ELISA test coincided and we therefore, discussed results based on groups: i) symptomless and mild mosaic, which were considered as healthy; ii) distinctive mosaic and mosaic with deformations, which were OYDV infected. Regardless of this finding, we cannot distinguish healthy plants from infected ones with complete certainty based on disease symptoms only, since sometimes they may not be clearly observed (Alves et al., 2008). In order to obtain reliable results from DAS ELISA test, tips of the first expanded leaves from the top of the plant were sampled. Dovas et al. (2002) also reported that both tested viruses (OYDV and LYSV) were found in the highest concentrations in two latest fully developed leaves.

The occurrence of OYDV, a dominant viral pathogen of garlic, severely compromises production of this crop in Serbia. It occurs in high intensity and may significantly reduce the economic value of the yield since it significantly decreased relevant yield components. Further investigations and planned screening of genetic material used in garlic production in Serbia is needed since differences between garlic genotypes were detected in the intensity of response to the OYDV and LYSV infection (Lot et al., 1998, Bagi et al., 2010).

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REFERENCES

Alves MJ, Marraccini FM, Melo Filho PA, Dusi AN, Pio-Ribeiro G, Ribeiro BM (2008). Recombinant expression of Garlic virus C (GARV-C) capsid protein in insect cells and its potential for the production of specific antibodies. Microbiol. Res., 163(3): 354-361.

Bagi F, Gvozdanović Varga J, Budakov D, Stojšin V, Janićijević M, Šantić M, Jasnić S (2010). Infection level of garlic by *Onion yellow dwarf virus* and *Leek yellow stripe virus*. X Serbian Plant Protection Meeting, Zlatibor: pp. 57-58.

Barg E, Lesemann DE, Vetten HJ, Green SK (1997) Viruses of *Allium* and their distribution in different *Allium* crops and geographical regions. Acta Hortic. 433: 607–616.

Carvalho MG, Shepherd RR, Hall DH (1981). Virus from clones of garlic without symptoms and cleared of Garlic yellow stripe virus . Fitopatol. Bras., 6:236.

Clark MF, Adams AN (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol., 34: 475-83.

Conci VC, Canavelli A, Lunello P, Rienzo JDi, Nome SF, Zumelzu G, Italia R (2003). Yield losses associated with virus-infected garlic plants during five successive years. Plant Dis., 87: 1411-1415.

Conci VC, Perotto MC, Cafrune E, Lunello P (2005). Program for intensive production of virus-free garlic plants. Acta Hort., 688: 195-200.

Dovas CI, Hatziloukas E, Salomon R, Barg E, Shiboleth Y, Katis NI (2001). Incidence of viruses infecting *Alliums spp*. in Greece. Eur. J. Plant Pathol., 107: 677-684.

Dovas CI, Mamolos AP, Katis NI (2002): Fluctuations in concentration of two potyviruses in garlic during the growing period and sampling conditions for reliable detection by ELISA. Ann. Appl. Biol., 140: 21-28.

Dovas CI, Vovlas C (2003). Viruses infecting *Allium* spp. in southern Italy. J. Plant Pathol., pp. 85-135.

FAOSTAT (2009). Food and Agricultural Organisation. http://faostat.org.

Feller C, Bleiholder H, Buhr L, Hack H, Hess M, Klose R, Meier U, Stauss R, Van Den Boom T, Weber E (1995). Phenological Stages of vegetables: I. onion, root, tuber and leafy vegetables. Deut. Pflanzenschutzd., 47, 193-206.

Gvozdanović-Varga J, Vasić M, Takač A, Bugarski D, Jovićević D, Červenski J, Stojšin V (2009a). Garlic production from the aspect of planting material. A periodical of scientific research on field and vegetable crops 46 (1): 99-109.

Gvozdanović-Varga J, Vasić M, Červenski J, Takac A. (2009b). Evaluation of fall garlic ecotypes for yield and quality. Acta Hort., 830: 173-178.

Kumar S, Baranwal VK, Joshi S, Arya M, Majumder S (2010). Simultaneous Detection of Mixed Infection of Onion yellow dwarf virus and an Allexivirus in RT-PCR for Ensuring Virus Free Onion Bulbs. Indian J. Virol., 21: 64-68.

Lot H, Chovelon V, Souche S, Delecolle B (1998). Effects of Onion Yellow Dwarf and Leek Yellow Stripe Viruses on Symptomatology and Yield Loss of Three French Garlic Cultivars. Plant Dis., 82: 1381-1385.

Lunello P, Rienzo JD, Conzi VC (2007). Yield loss in garlic caused by Leek yellow stripe virus Argentinean isolate. Plant Dis., 91: 153-158.

Mahmoud SYM, Abo-El Maaty SA, El-Borollosy AM, Abdel-Ghaffar MH (2008). Identification of *Onion yellow dwarf potyvirus* as One of the Major Viruses Infecting Garlic in Egypt. Int. J. Virol., 4(1): 1-13.

McKinney HH (1923). Influence of soil temperature and moisture on infection of wheat seedling by *Helminthosporium sativum*. J. Agric. Res., 26(5): 195-217.

Melo Filho PA, Resende RO, Torres Cordeiro CM, Buso JA, Torres AC, Dusi AN (2006). Viral reinfection affecting bulb production in garlic after seven years of cultivation under field conditions. Euro. J. Plant Pathol., 116: 95–101.

Official gazette of the Republic of Serbia (2010). Law on the registration of agricultural plant varieties. p. 30.

Rivlin RS (2001). Historical perspective on the use of garlic. J. Nutr., 131: 951S–954S.

Shahraeen N, Lesemann DE, Ghotbi T (2008). Survey for viruses infecting onion, garlic and leek crops in Iran. Bulletin OEPP/EPPO Bulletin 38: 131-135.

Singh VK, Singh DK (2008). Pharmacological Effects of Garlic (*Allium sativum* L.). Annu. Rev. Biomed. Sci., 10: 6-26.

Spearman C (1904). The proof and measurement of association between two things. Amer. J. Psychol., 15: 72–101.

Takaichi M, Nagakubo T, Oeda K (2001). Mixed virus infections of garlic determined by a multivalent polyclonal antiserum and virus effects on disease symptoms. Plant Dis., 85(1): 71-75.

Van Dijk P (1993). Carlavirus isolates from cultivated *Allium* species represent three viruses. Neth. J. Plant Pathol., 99: 233-257.

Van Dijk P, Verbeek M., Bos L (1991). Mite-borne virus isolates from cultivated *Allium* species, and their classification into two new rymoviruses in the family *Potyviridae*. Neth. J. Plant Pathol., 97: 381-399.

Walkey DGA, Antill DN (1989). Agronomic evaluation of virus-free and virus-infected garlic (*Allium sativum* L.). J. Hort. Sci., 64: 53–60.