VIRUSES OF SOME GARLIC ECOTYPES IN CROATIA

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Abstract
Recent, there is increasing demand for autochthonous ecotypes of garlic (*Allium sativum* L.) in Croatia. Many local ecotypes of garlic are developed due to specific environmental conditions and producer's selection. However, the average yield of garlic is not in the European average range, since the classical vegetative propagation from cloves usually results with virus infections that cause significant yield reduction. Viruses are considered significant garlic's pathogens. The research was set up to determine infection rate in plant material of different garlic ecotypes grown from cloves in different regions. Plants were collected from commercial fields in Zadar County (coastal part of Croatia) and Vukovar-Srijem County (eastern Croatia) and were tested by enzyme-linked immunosorbent assay (ELISA) on the presence of three viruses: *Onion yellow dwarf virus* (OYDV), *Leek yellow stripe virus* (LYSV) and *Garlic common latent virus* (GCLV). As a potential source of antigen leaf tissue was used and tests were conducted according to manufacturer's instructions (Bioreba AG, Switzerland). In plants from Zadar County dominant was OYDV, followed by GCLV and LYSV. All tested plants originating from Vukovar-Srijem County were infected with three tested viruses. Out of 316 analyzed plants only 4 (1.3%) showed to be free from viruses included in survey. Plants infected with OYDV showed symptoms of leaf yellowing and reduced growth, while those infected with LYSV expressed yellow stripes. Symptoms were most evident at the beginning of vegetation. Due to high infection rate, deteriorated sanitary status, and increased interest in use and production of local garlic ecotypes, work on sanitary selection will be important part of revitalization program.

Keywords: *Allium sativum* L., ELISA, *Onion yellow dwarf virus*, *Leek yellow stripe virus*, *Garlic common latent virus*.

Introduction

Garlic (*Allium sativum* L.) is one of the most important and widely widespread culinary plants used as a medicinal herb and spice (Abou El-Magd et al., 2013). According to FAO (2015) in period from 2000 to 2013 garlic is among top five food ingredients with annual production incensement of 7.7%, indicating its worldwide importance. In the Republic of Croatia production of garlic is insufficient for domestic consumption with no data about export, while most of the imported garlic comes from China (Dumičić et al., 2015). According to Toth et al. (2015) the scarcity of domestic production is a consequence of two major problems: poor quality of planting material and inadequate storage conditions. Garlic is produced in all agriculture regions, but mostly on small areas for local markets and personal consumption (Dumičić et al., 2015). By introducing virus-free planting material production could increase and represent a significant source of income (Radat, 2014). The average globally yield of garlic is about 10 t/ha (Lešić et al., 2016). Twice higher yields from world average have Egypt and USA, while in Croatia the average yield is 8.58 t/ha (Matotan, 2004). In Croatia most garlic cultivars are obtained by clonal selection of local ecotypes (Lešić et al., 2016). Transferring the ecotype of desirable properties in the new production area adaptive capacity of plant play significant role (Dumičić et al., 2015). The average bulb mass of domestic ecotypes is usually between 30 and 40 g, while the French non-virulent cultivars have an average mass of bulbs between 70 and 100 g (Lešić

et al. 2016). Viral diseases usually have influence on growth and yield of different crops (Hull, 2009). However, there is a little data about their impact on different crops in Croatia (Juretić, 2002). Species from the genus *Allium* are economically important cultures in the Mediterranean basin, and viruses are among the most important pathogens affecting their yield (Loebenstein and Lecoq, 2012). Since viral infections are usually systemic, they are problem in vegetative propagated crops. Vegetative propagation ensures the transmission of viruses to progeny and, when used on large scale without appropriate sanitary control, can lead to massive dissemination of viruses. Such material can serve as primary source of virus and led to secondary contaminations which, especially in case of viruses transmitted by insects, can lead to disease epidemic with significant consequences on crop growth and yield (Astier et al. 2006). Garlic can be infected with viruses from genera *Potyvirus*, *Carlavirus* and *Allexvirus*. These viruses are transmitted by vegetative propagation and different vectors (Chodorska et al. 2014). Some viruses, like *Onion yellow dwarf virus* (OYDV) and *Leek yellow stripe virus* (LYSV), cause mosaic symptoms and reduction of yield over 25% (Messianen et al. 1981; Vunsh et al. 1991). Other viruses are usually latent with no significant impact on yield (Van Dijk et al., 1991). However, simultaneous infection by *Carlavirus* or *Alexivirus* together with *Potyviruses* may have synergistic effect. It is estimated that viruses can reduce garlic yield during consecutive cultivation of infected plants up to 50% (Conci et al. 2003; Lot et al. 1998). In addition, using virus-free planting material resulted in 32-216% higher total bulb's mass in different garlic cultivars (Conci, 1997; Conci et al. 2003; Melo Filho et al. 2006; Walkey and Antill, 1989). The aim of this study was to determine occurrence of OYDV, LYSV, and *Garlic common latent virus* (GCLV) as the most common viruses of *Allium* species in Mediterranean region. Only OYDV has been previously reported in Croatia, but just on onion (Štefanac, 1977).

Material and methods

Survey was conducted on four garlic ecotypes (E1-E4) originating from Zadar County and one ecotype (E5) from Vukovar-Srijem County. Plants were divided in three categories: I) plants/ecotypes E1-E4 grown from cloves collected from commercial fields located in Zadar County; II) plants/ecotypes E1-E4 originating from Zadar County, but grown from cloves in Zagreb (experimental field of University of Zagreb Faculty of Agriculture); III) plants/ecotype E5 originating from Vukovar-Srijem County, but grown from cloves in Zagreb. Collecting of leaves, used as a potential source of antigen, was done in the stage 3-5 well developed leaves. Collected leaves, symptomatic or asymptomatic, were tested by enzyme-linked immunosorbent assay (ELISA) on the presence of three viruses: OYDV, LYSV and GCLV. ELISA was performed using commercial kits provided by Bioreba (Switzerland) according to manufacturer's instructions. Each sample was tested twice on each virus. Final results were measured on spectrophotometer EL800 (Biotek, USA) two hours after adding the substrate. Samples with spectrophotometric values at least two times greater than average value of negative controls were considered positive. After laboratory tests, more detailed analysis of the symptoms and their correlation with the results of laboratory tests was done.

Results and discussion

ELISA results showed high infection rates of all five garlic ecotypes, especially with OYDV. Garlic collected in Zadar County showed prevalence of OYDV (93%), while GCLV (9.3%) and especially LYSV (2.3%) had significantly lower occurrence. Only in 3 plants (out of 43 tested) from that region presence of viruses was not confirmed. Similar situation was confirmed in garlic originating from Zadar, but grown in Zagreb, with prevalence of OYDV (99.4%) and presence of GCLV (21.9%) and LYSV (5.8%) with only 1 plant (out of 155) free of tested viruses. The highest infection rate was confirmed in Zagreb on plants originating from Vukovar-Srijem County where all 118 plants included in survey showed mixed infection with three viruses. Significant difference in virus composition and infection rate was discovered in material originating from Zadar County, while planting material from Vukovar-Srijem County showed significantly higher rate of GCLV and LYSV when compared to

material from Zadar County. Detailed review of sanitary status determined in this survey is given in Table 1. Infected plants showed reduced growth that was most evident at the beginning of vegetation. Additionally, plants infected with OYDV expressed symptoms of leaf yellowing, while yellow stripes were present on plants infected with LYSV. Yellowing, reddening and necrosis of leaf tips were detected on plants simultaneously infected with three viruses (Figure 1.). According to literature three viruses determined in this survey have worldwide distribution and are commonly found in all garlic grown regions, especially without adequate sanitary selection (Loebenstein and Lecoq 2012). In Europe, viruses infecting garlic have been reported from France (Lot et al. 1998), Greece (Dovas et al. 2001), Czech Republic (Klukácková et al. 2004; Smékalová et al. 2010), Poland (Chodorska et al. 2014) and Spain (Lunello et al. 2005). The vast majority of information relates to viruses economically important for production region of mentioned countries. Klukáčková et al. (2007) showed that five tested garlic varieties in Czech Republic were infected in average 75.4% by OYDV, 31.2% by LYSV and 99.6% by GCLV. Total, 80.9% of examined garlic plants had visible symptoms of virus infection on leaves. Dovas et al. (2001) and Dovas and Vovlas (2003) confirmed presence of OYDV in almost all tested plants from Greece and Italy. Symptoms observed in this survey are in accordance with those described by other authors (Van Dijk 1993, 1994). Although negative impact of viral infections is well documented in other countries (Loebenstein and Lecoq 2012), effect on Croatian garlic ecotypes is unknown. According to obtained results sanitary selection will be necessary for revitalization of the production of Croatian autochthonous garlic ecotypes either by finding virus-free plants from production field or elimination of viruses by cryotherapy, thermotherapy and/or meristem-tip culture.

Table 1. Virus infection rates determined in different garlic ecotypes (E1-E5) from Croatia

Origin	Ecotype	Total number of analyzed samples	GCLV (%)	OYDV (%)	LYSV (%)	Virus free (VF) (%)
Zadar County	E1	2	2 (100)	2 (100)	1 (50)	0
	E2	14	1 (7.1)	12 (85.7)	0	2 (1.4)
	E3	9	0	9 (100)	0	0
	E4	18	1 (5.6)	17 (94.4)	0	1 (5.6)
	Total	43	4 (9.3)	40 (93)	1 (2.3)	3 (6.9)
Zagreb *	E1	20	8 (40)	20 (100)	5 (25)	0
	E2	31	4 (12.9)	31 (100)	0	0
	E3	15	5 (33.3)	15 (100)	0	0
	E4	89	17 (19.1)	88 (98.9)	4 (4.5)	1 (1.1)
	Total	155	34 (21.9)	154 (99.4)	9 (5.8)	1 (0.7)
Zagreb**	E5	118	118 (100)	118 (100)	118 (100)	0
TOTAL	5	316	156 (49.4)	312 (98.7)	128 (40.5)	4 (1.3)

Legend: GCLV - *Garlic common latent virus;* OYDV - *Onion yellow dwarf virus;* LYSV - *Leek yellow stripe virus;* Zagreb*- plants grown in Zagreb, but cloves originating from Zadar County; Zagreb** - plants grown in Zagreb, but cloves originating from Vukovar-Srijem County

Figure 1. Symptoms of yellowing, reddening and leaf tip necrosis on garlic infected with *Garlic common latent virus*, *Onion yellow dwarf virus* and *Leek yellow stripe virus*.

Conclusions

Survey gives additional knowledge about occurrence and distribution of three viruses of garlic indicating deteriorated sanitary status of Croatian garlic ecotypes originating from Zadar and Vukovar-Srijem County. In the future adequate clonal and sanitary selection should be undertaken in order to give producers choice of using virus-free planting material as a prerequisite for stable production. This could have positive impact on revitalization and enhancement of production based on Croatian autochthonous garlic ecotypes which represent original Croatian value and are important segment of Croatian heritage.

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