Investigation note

Vegetative characteristics of garlic plants with 'waxing' symptoms in Zacatecas

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Abstract

The state of Zacatecas is the main producer of garlic in Mexico. Recently, it was reported the presence of *Candidatus* Phytoplasma trifolii in this region affecting garlic plants that showed symptoms such as dwarfism, bright leaves and little or no differentiation of teeth, which are known as 'waxed' garlic. Considering that there is no information about the impact of this symptomatology on garlic plants, the purpose of this work was to determine its effect on vegetative characteristics of plants of four garlic genotypes (Tigre, Platero, Enc-4 and CH-6). 'Waxing' in garlic plants was found to be associated with consistent losses in plant height and leaf length and, to a lesser extent, in the weight of leaf and pseudostem. The rest of the variables showed erratic behavior between genotypes and sampling dates.

Keywords: *Candidatus* Phytoplasma trifolii, garlic genotypes, impact, symptoms.

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The state of Zacatecas is the main producer of garlic (*Allium sativum* L.) in Mexico, about 3 400 ha are sown with this bulb in the state, which represent 47% of the area and 60% of the national production (SIACON, 2018). Onion yellow dwarf virus (OYDV), Leek yellow stripe virus (LYSV), Shallot latent virus (SLV), Garlic common latent virus (GarCLV) and Tobacco etch virus (TEV) have been identified in plants with symptoms such as diffuse mosaic and deformations of the central vein (Velásquez-Valle *et al*., 2010a).

Since 2010, the presence of a group of symptoms known as 'waxing' (dwarfism, bright leaves leaning towards the ground and bulbs with little or no differentiation of teeth) has been reported in garlic plants, their incidence in commercial plots varied between 1 and 5%. Elisa results showed the presence of the viruses mentioned above, even in apparently healthy plants, so it was not possible to conclude about the association between these viruses and the observed symptomatology (Velásquez-Valle *et al*., 2010b). Recently, it was reported the presence of *Candidatus* Phytoplasma trifolii (16SrVI) (CPt) associated with garlic plants collected in this same region (Fresnillo, Zac.), which showed the symptomatology previously described (Reveles-Torres *et al*., 2018).

Infection of garlic plants by phytoplasmas has been mentioned in Argentina and the United States of America (Galdeano *et al*., 2009; Mollov *et al*., 2014), although the symptomatology exhibited does not completely agree with that observed in Zacatecas. Currently, there is no estimate of the impact of this symptomatology on the development of garlic plants, so the objective of this work was to determine the effect of the symptomatology known as 'waxing' on vegetative characteristics of plants of garlic genotypes.

The work was carried out on a plot located in the Zacatecas Experimental Field of the National Institute of Forestry, Agricultural and Livestock Research (INIFAP), in Calera de V. R., Zacatecas, Mexico. The plot was established on November 22, 2018 and received the management recommended by the Zacatecas Experimental Field (Reveles-Hernández *et al*., 2009), from this plot, 10 plants with symptoms of 'waxing' (S) and 10 apparently healthy plants (AS) were extracted from each of the four garlic genotypes (Tigre, Ch-6, Platero and Enc-4) sown in beds with four rows of plants, sampling was carried out on two different and representative dates of the development of the plants, March 14 and April 24, 2019 (112 and 153 days after sowing, respectively, which correspond approximately to the stages of the beginning of bulb formation and bulb filling approximately).

The following data were taken from each plant: height (cm), neck diameter (mm), weight (g) and equatorial diameter of the bulb (mm), number of leaves, as well as their length, width (cm) and weight (g) and pseudostem weight (g). A paired t-test (α: 0.05) was used to compare the information obtained with both types of plants (AS and S).

Tigre

On both sampling dates, the plant height, leaf length, leaf weight and pseudostem weight of AS was higher than that of S. In the variables of bulb diameter, bulb weight and number of leaves, no difference was found between the two types of plants on the two sampling dates. In the neck diameter variable, the S were higher than the AS on both sampling dates. Leaf width was favorable to AS on the first sampling date, but no significant difference was observed between the two types of plants in the second sampling (Tables 1 and Table 2).

Table 1. Comparison of mean values of characteristics of plants of four apparently healthy garlic genotypes (AS) and with symptoms associated with 'waxing' (S) in Zacatecas, Mexico. First sampling.

Characteristic	Tigre		Ch-6		Platero		Enc-4	
	AS[1]	S[2]	AS	S	AS	S	AS	S
Height (cm)	33.3	13.9 *	32.5	17.1 *	32.3	17.7 *	33.6	17.1 *
Neck[3] (mm)	1.2	1.3 *	1.22	1.6 *	1.3	1.7 ns	1.4	1.8 *
Bulb[4] (mm)	2.2	2.1 ns	2.1	2.8 *	2.3	2.9 *	2.2	2.7 *
Bulb weight (g)	11	8.9 ns	10.2	13.7 *	12.5	15.8 ns	11.5	14 ns
No. of leaves	5.7	4.9 ns	5.8	5 *	5.9	5.7 ns	6.2	5.8 ns
Leaf length (cm)	33.7	18.8 *	28.8	17.1 *	31.6	20 *	31.5	19.1 *
Leaf width (cm)	2	1.4 *	2.1	1.7 *	2.1	1.9 ns	2.1	1.9 *
Leaf weight (g)	16.4	4.6 *	16.8	6.3 *	20	10.5 *	21.4	10 *
Pseudostem (g)	8.9	3.1 *	8.7	4.6 *	9.2	6.5 ns	10.9	7.2 *

[1]= apparently healthy; [2]= symtomatic to 'waxed'; [3, 4]= diameter; ns= non-significant difference; *= significant difference.

Table 2. Comparison of mean values of characteristics of plants of four apparently healthy garlic genotypes (AS) and with symptoms associated with 'waxing' (S) in Zacatecas, Mexico. Second sampling.

Characteristic	Tigre		Ch-6		Platero		Enc-4	
	AS[1]	S[2]	AS	S	AS	S	AS	S
Height (cm)	64.7	25.2 *	70	30.8 *	63.9	26.5 *	59.7	28.1 *
Neck[3] (mm)	1.4	1.8 *	1.7	2 ns	1.5	1.9 ns	1.3	1.6 ns
Bulb[4] (mm)	2.9	3.2 ns	3.6	3.7 ns	3.4	3.6 ns	3.5	3.4 ns
Bulb weight (g)	25.3	26.4 ns	39.3	35.8 ns	35.6	34.5 ns	33.6	33.7 ns
No. of leaves	6.8	6.4 ns	7.3	5.9 *	7	7.2 ns	7.6	7 ns
Leaf length (cm)	41.7	24 *	46	26.6 *	39.6	26.8 *	36.9	25.6 *
Leaf width (cm)	2.1	1.9 ns	2.5	2.2 ns	2.2	2.1 ns	2	1.8 ns
Leaf weight (g)	30.6	18.5 *	44.1	20.6 *	32.4	23.5 ns	29	22 ns
Pseudostem (g)	26.2	15.4 *	37.8	19.8 *	29	16.4 *	23.8	15.4 *

[1]= apparently healthy; [2]= symtomatic to 'waxed'; [3, 4]= diameter; ns= non-significant difference; *= significant difference.

Ch-6

The mean values of the variables plant height, number of leaves, leaf length, leaf weight and pseudostem weight in the AS were higher than those of S on both sampling dates. The variables neck diameter, bulb diameter, bulb weight and leaf width were greater in S during the first sampling but showed no significant difference in the second sampling (Tables 1 and 2).

Platero

Only the variables plant height and leaf length were higher in AS on both sampling dates. No differences were detected in the values of neck diameter, bulb weight, number of leaves and leaf width of diseased and apparently healthy plants on the two sampling dates. The bulb diameter was higher in S on the first sampling date, but there was no difference between the two types of plants on the second sampling date. Leaf weight in the first sampling was favorable to AS, while in the second sampling no difference was detected, an inverse situation occurred with the pseudostem weight, which during the first sampling did not manifest differences between both types of plants but was superior in AS during the second sampling (Tables 1 and 2).

Enc-4

The variables plant height, leaf length and pseudostem weight in AS were greater than those of S on both sampling dates. The values of bulb weight and number of leaves were similar between both types of plants and dates of sampling. The variables leaf width and leaf weight were higher in AS during the first sampling but showed no differences in the second sampling. The values of neck diameter and bulb diameter were higher in S during the first sampling but showed no difference in the second sampling (Tables 1 and 2).

Phytoplasmas are microorganisms whose habitat is restricted to the phloem tissues of their hosts due to their high content of fructose and glucose, which causes a physiological imbalance that leads to the induction of a wide range of symptoms that point towards an alteration in the content of the phloem and loss of hormonal balance (Arismendi *et al*., 2010). Some of the symptoms observed in garlic plants in Zacatecas such as dwarfism, short leaves and thickening of the pseudostem and bulb could be a direct result of CPt infection.

One of the symptoms that characterize the disease is the thickening of the neck and bulb, both symptoms occurred in the four garlic genotypes studied. During the first sampling, the mean values of these variables were significantly higher in Tigre plants, Ch-6 and Enc-4 for neck diameter, and for Ch-6, Platero and Enc-4 for bulb diameter. During the second sampling, only the neck diameter in the S of the Tigre genotype exceeded those of AS. For the four genotypes and on both sampling dates, the mean values of plant height and leaf length of AS were consistently higher than those of S. Dwarfism in garlic plants infected by phytoplasmas has previously been mentioned by Mollov *et al*. (2014).

The loss in height of S was consistent in both samplings for the S in the four genotypes. On the first sampling date, the percentage of reduction in this variable ranged from 50.9 (Enc- 4, first sampling) to 61.1 (Tigre, second sampling). During the second sampling these percentages increased marginally for all genotypes, although Enc-4 and Platero were the ones that resulted with the lowest (2%) and highest (3.7%) increase, respectively (Table 3).

Table 3. Characteristics of plants of four garlic genotypes with symptoms of 'waxing' during 2018 in Zacatecas, Mexico.

Characteristic	Tigre		Ch - 6		Platero		Enc - 4	
	March 14	April 24	March 14	April 24	March 14	April 24	March 14	April 24
Height (cm)	-58.2[1]	-61.1	-52.6	-56	-54.8	-58.5	-50.9	-52.9
Neck[2] (mm)	5.7	32.3	35.2	0	0	0	28.6	0
Bulb[3] (mm)	0	0	33.3	0	26.1	0	22.7	0
Bulb weight (g)	0	0	34.3	0	0	0	0	0
No. of leaves	0	0	-13.8	-19.2	0	0	0	0
Leaf length (cm)	-44.2	-42.4	-40.6	-42.2	-36.7	-32.3	-39.4	-30.6
Leaf width (cm)	-30	0	-19	0	0.0	0	-9.5	0
Leaves (g)	-71.9	-39.5	-62.5	-53.3	-47.5	-27.5	-53.3	-24.1
Pseudostem (g)	-65.2	-41.2	-47.1	-47.6	0	-43.4	-33.9	-35.3

[1]= means with negative sign indicate the loss (%) in a given variable for plants with symptoms of 'waxing', means with positive numbers indicate the percentage of gain in a given variable for diseased plants and means at zero indicate non-significant difference between apparently healthy and diseased plants. [2, 3]= diameter.

The length of the leaves of AS was greater than that of S in all cases (genotypes or sampling date), the loss in leaf length, in terms of percentage, was from 30.6 (Enc-4, second sampling) to 44.2% (Tigre, first sampling). The variables leaf weight and pseudostem weight partially showed the same trend: in most cases the values of AS were higher than those of S. The losses in these variables were severe, especially for the Tigre genotype in the first sampling, where the S experienced a reduction of 71.9 and 65.2% in the weight of leaves and pseudostem, respectively (Table 3).

Only in three genotypes the leaf width values of AS were higher than those of S, leading to variable losses between 9.5 (Enc-4, first sampling) and 30% (Tigre, first sampling), in the rest of the genotypes no differences were found between AS and S or between sampling dates. Notably and in both samplings, the number of leaves in AS was higher than in S in the CH-6 genotype. The loss in number of leaves in S was 13.8% and 19.2% for the first and second sampling respectively (Table 3).

The variables of neck diameter, bulb diameter and bulb weight expressed differences in favor of S, especially in the first sampling. For the Tigre genotype, the values of the neck diameter variable favored S on both sampling dates, which meant that the diameter of its bulbs was 5.7% and 32.3% higher than that of AS, in the first and second samplings respectively (Table 3). This contrasts with what was mentioned by Mollov *et al*. (2014), who point out that garlic plants infected by phytoplasmas of the 16Srl group (Aster Yellows) had bulbs of smaller than normal size.

Conclusions

The symptomatology called 'waxing' in four genotypes of garlic was found to be associated with consistent losses in plant height and leaf length and to a lesser extent, in the weight of leaves and pseudostem. The rest of the variables evaluated showed erratic behavior between genotypes and sampling dates.

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