ESTIMATION OF APICAL NECROSIS IN JUNGLANS REGIA L. WALNUTS HARVESTED IN MOLDOVA

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Abstract: Blight caused by *Xanthomonas pv. Juglandis (Xaj)* is a bacterial disease of Persian Juglans regia L. walnut. This article refers to an international bibliographic study on microbial risk caused by this bacterium. Recent studies indicate that the bacterium *Xantbomonas arboricola pv, juglandis* is the most frequently microorganism associated with apical necrosis and can produce initial infections in young nuts. This bacterium was isolated on fruit orchards from Austria, Bulgaria, France, Germany, Greece, Portugal, and Romania. Also, in this work there are characterized and other microorganisms such as *Alternaria* spp. and *Fusarium* spp. isolated from „apical necrosis". Apical necrosis was estimated in walnuts Juglans regia L. harvested in Moldova.

Key words: apical necrosis, walnuts, Juglans regia L., *Xantbomonas arboricola,* Brown Apical Necrosis (BAN)

INTRODUCTION

Xanthomonas arboricola pv. Juglandis is the causal agent of walnut blight, one of the most important and widespread diseases of Persian (English) walnut (Juglans regia L), causing severe damage to leaves, twigs and nuts [7]. The apical necrosis was first observed in the late 90s following an intense early fruit drop and was found to affect the main walnut production in Spain [1, 2], France [4, 5], Italy [3], and Turkey [9]. Apical necrosis infections are more frequent and severe in the early fruit developmental stages. Initial infections occur after fruit set and in environmental conditions are favorable, infection spreads externally and internally trough the walnut fruit tissues [9].

Environmental conditions favorable to apical necrosis are not yet clearly determined. However, local wet and warm conditions during the walnut fruit initial growing period enhance the disease incidence and severity. Preventive sprays with copper derive applied according to the schedule proposed for walnut blight control [8] contributes to reduce the incidence of apical necrosis. Removal of mummified nuts from the trees and dropped fruits from the orchard ground reduces infestation *Xanthomonas arboricola pv. Juglandis,* prevent further yield losses. Appropriate soil characteristics and walnut fertilization help to reduce the damage [6].

DISCUSSIONS AND APPROACHES

Global walnut (Juglans regia L.) production increases from year to year. Bacterial diseases are threatening these nut crops all over the World. *Xhanthomonas arboricola pv. juglandis (Xaj*) is the agent of walnut bacteriosis, and has been associated also to brown apical necrosis. Several walnut genotypes have shown a range of tolerance to this disease in diverse countries. Deep bark canker (*Brenneria rubifaciens*) and Shallow bark canker (*Brenneria nigrifluens*) are the other bacterial diseases affecting walnut [5].

The main bacterial diseases that threaten both crops and possibilities to control them by using genetic resources are discussed in several studies [1-9].

Xanthomonas arboricola pv. Juglandis (Xaj) is the causal agent of walnut blight, the most important bacterial disease of Juglans regia and other Juglans species, which affects a high percentage of flowers and fruits, but does not kill bearing trees [1, 5, 7]. This bacterium has been also isolated from tissues affected by bacterial apical necrosis [3]. Blight has been recorded from Europe (Austria, Bulgaria, Denmark, France, Germany, Greece, Italy, Moldova, Netherlands, Poland, Portugal, Romania, Russia, southern Russia, Slovenia, Spain, Switzerland, UK, Ukraine and former Yugoslavia) Asia, [Azerbaijan, China, (Hubei, Henan, Jiangsu, Shaanxi, Shandong), Georgia, India, (Himachal Pradesh, Uttar Pradesh) etc [5].

To investigate the genomic variability of *X. Aboricola pv.juglandis*, 66 isolates obtained from different countries (England, France, Italy, The Netherlands, Romania, Spain, USA, and New Zealand) were analyzed using the Amplified Fragment Length Polymorphism (AFLP) technique. AFLP analysis proved to be reproductive, reliable, and sufficiently sensitive to reveal the individual genomic variability within *X. Arboricola pv juglans* isolates used in this study. Result indicates that geographic location could be partly responsible for the genomic heterogeneity [7].

Being polyphenols involved in tolerance to bacterial diseases, it has been shown that the gene jrPPO1 is the sole polyphenol oxidase PPO gene in walnut able to encode a *jrPPO* enzyme that is expressed in the leaves, hulls and flowers of walnut trees. It can also happen that some modifications in the anatomical traits of the epidermis of walnut progenies.

Figures 1-2 are apical necrosis of walnut fruit Juglans regia L, Figure 3 - colonies of *Xanthomonas arboricola pv. juglandis* isolated from apical necrosis lesions in walnut fruits, growing on modified Tween Medium [9]

Fig. 1. External symptoms of apical necrosis in young fruits collected from walnut tree (A). Internal symptoms of apical necrosis in fruits collected from walnut tree (B) or dropped to the soil (C) [9].

Fig. 2 External and internal extent of necrosis is not always correlated [9]

Fig.3. Colonies of *Xanthomonas arboricola pv. juglandis* isolated from apical necrosis lesions in walnut fruits, growing on modified Tween Medium [9]

Table 1 summarizes the information from different countries on tolerance/resistance/ low susceptibility to *Xaj* in J. regia. *Polyphenols and Xaj*. It is known that polyphenol oxidase (PPO) catalyzes the oxidation of polyphenols to quinones, which are very reactive against disease agents [5].

Table 1. Juglans regia accessions resistant/tolerant to *Xaj*. found in native walnut population.

Country	Locality	Selections or cultivars	Resistance/ tolerance	References
China	Taigu, Shanxi	Jinboxiang 3	*Xaj* resistance in the open	Tian et al., 2008
	Kunming, Yunnan	'Yunxin Gaoyuan', out of J. sigillata x J.regia 'Yulin A7', cross made in 1977	Resistant to *Xaj* in the open	Xi et al., 2007
	Kunming, Yunnan	'Yunxin 90303' derived from J. sigillata cv. 'Santai' x J. regia cv. 'Xinzao No. 13'	Diseases resistant	Zhao et al., 2007
	Tai'an, Shandong	'Luguo 4', open pollinated native genotype	*Xaj* resistant in the open	Zhang et al., 2009
	Ghengxian county, Gansu	'Longnan 15'	*Xaj* resistant in the open	Guo et al., 2009
Greece		'Amigo', 'Ashley', 'Serr', 'Eliana', 'Grand Jean', 'EK-1', 'EH-28', 'EH-29'	The lower *Xaj* susceptible in the trial conditions	Tsiantos et al., 2007
Italy	Pignataro Maggiore, area of Caserta	Ex situ collection	All *Xaj* susceptible	Piccirillo, 2003 Piccirillo and Petriccione, 2006
Romania	Oltenia	550 genotypes out of native population	Several genotypes are *Xaj* tolerant	Botu et al., 2001
	Valcea	Valcor, Valmit and Valrex, out of native population	Texting in orchard for *Xaj* and other traits	Botu et al., 2007
	Valcea	'Portval' (syn. 'VL 26 B'), seedling rootstock	Good resistance to diseases, *Xaj* not specified	Achim et al., 2007
Spain	Galicia	>550 natural genotype tolerant in the open were evaluated for *Xaj*, susceptibility	'MBLu-20', 'MBLu-21' and 'MBC-45', did show low *Xaj* susceptibility in lab.	Aleta et al., 2001
	Cantabria	Native seedlings aged over 15-years-old	28 were selected as *Xaj* free symptoms. In	Arrieta and Diaz, 2007; Lopez et

			lab all were susceptible	al., 2007
	Murcia	Ex situ collection	Segregation to *Xaj* tolerance were found in an ex situ collection	Frutos et al., 2008, 2009, 2010; Ruiz et al., 2010
Slovenia		24 genotypes preselected	Most promising (*Xaj* resistant) are late leafing genotypes 'Z-62', 'Krnc' and 'Z-60'	Solar and Stampar, 2006
Turkey		19 genotypes	All genotypes *Xaj* resistant	Akca and Ozongun, 2004
		Sen 2, C440, 77 H-1, Kaplan 86, Tokat 1, Kaman 1 and Bilecik	All selections *Xaj* low susceptible	Özaktan et al., 2007
Yugoslavia		Nbs. 10/88, 40/92, 30/93, 28/94, 9/96, selected from native population	Highly resistant to diseases	Mitrovic, 2003
		Nbs. 10/88, 40/92, 30/93, 28/94, 9/96	All resistant to Xaj.	Mitrovic et al., 2007

Microorganisms associated to *Brown Apical necrosis* BAN of walnut:

- *Xanthomonas arboricola pv. juglandis*, isolated from all walnut fruit affected tissues in more than 36% fruits.
- *Fusarium spp*.(*F. moniliforme, F. solani*, etc.) isolated from exocarp and seed in 4% fruits (depending on fruit tissue)
- *Alternaria spp*. only isolated from exocarp at the rate of 1% (more frequent in exocarp and mesocarp).
- *Xaj* was isolated from external and internal symptoms of walnut fruits in sampling period and produced typical Ban symptoms on detached walnuts.
- Some *Fusarium spp*. Isolates were pathogenic on detached nuts.

CONCLUSION

This paper includes a bibliographic study of accidents apical necrosis of walnut fruit Juglans regia L from different regions. The incidence of walnut blight can vary markedly between cultivars, locations and years with the development of damaging epidemics when weather conditions are favorable. The most important bacterial diseases in walnut seem to be due to *Xaj*, have a worldwide distribution.

In Moldova we also observed incidence of apical necrosis walnuts. *Xanthomonas arboricola pv. Juglandis* is the causal agent of walnut blight, one of the most important and widespread diseases of Persian (English) walnut (Juglans regia L), causing severe damage to leaves, twigs and nuts. It is necessary to study this effect in order to minimize losses crop and reduce quality walnuts and nut products.

In this context this paper is a first step in this direction.

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