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Research Paper

The role of some elicitors on the management of Roumy Ahmar grapevines downy mildew disease and it's related to inducing growth and yield characters

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ABSTRACT

Many elicitors (silicon, chitosan, bion or fungicide) were investigated on grapevines grown under natural infestation to evaluate their capacity on inducing defense from downy mildew disease and enhancing growth, yield, berries quality as well as some physiological and anatomical characteristics of Roumy Ahmar grapevines.

Foliar application of any elicitors used, in special, silicon was significantly increased yield per vine, cluster weight, 100 berry weight, soluble solids%, total anthocyanin and total phenols while reducing the total acidity% in berries as well as increasing leaf area and potassium percentage in leaves and antioxidant enzyme activities comparing with control. Chitosan application was significantly superior than using other elicitors in increasing shoot length, nitrogen and phosphorous percentage, photosynthetic pigment, ascorbic acid, proline and phenolic compounds in the leaves, and decreased hydrogen peroxide and lipid peroxidation leading to improved membrane permeability. All tested elicitors, in special, Si reduced downy mildew disease severity as compared with untreated control vines. Also, the chloroplast exhibited different degrees of malformation and lysis. The mitochondria were highly vacuolated and the nucleus was misshapen in infested vines.

It could be suggested that spraying Roumy Ahmar grapevine with 1000 mg/l silicon four times controlled downy mildew disease in grapevine as well as improving its growth, yield and quality.

1. Introduction

In Egypt, grape (*Vitis vinifera*.) is considered the second major fruit crop after citrus, occupied about 152,983 feddans "4200 m^2" and produced about 1.389 million tons (FAO, 2013). Roumy Ahmar grape is one of the most popular seeded cultivar grown in Egypt, marketable period exceeds from September to November.

Grapevine is attacked by many diseases, in special, downy mildew. Downy mildew caused by *Plasmopara viticola* L (Berk. & M.A. Curtis) (Beri & Detoni). is a very destructive viticulture disease, that are responsible for growth reduction, delayed fruit ripening, young berries falls off the cluster easily and finally decreased the yield and quality (Wong et al., 2001). In response to pathogen infection, plants have evolved a multilayered immune system to defend themselves against pathogens, including the generation of free radical oxygen (FRO). FRO likewise has coordinate antimicrobial activity, inducing lignin formation and phytoalexin production (Baker and Orlandi, 1995). Then again, FRO over-produced may also deleteriously affect the host cells themselves, by interacte with a lot of molecules, causing chlorophyll degradation, lipid and protein oxidation, and nucleic acid damage leading to cell death (Mittler et al., 2004). Due to their highly reactive nature, FRO are kept as low as possible in plant cells by an efficient FRO-scavenging system, including enzymatic antioxidant and low molecular antioxidant solutes (Baker and Orlandi, 1995; Mittler et al., 2004; Farouk et al., 2012). Traditionally, synthetic fungicides, the mainstay of fungal pathogen management for decades, are coming under increasing scrutiny as concerns about their impact on non-target organism mounts, especially in highly populated areas as well as harmful to human health. However, in spite of the great advantages they have brought to grapevine, the excessive use fungicides have taken its toll environmentally and on human health, including the development of resistant strains, high cost, environmental pollution, breaking up the ecological balance of the soil (Bautista-Baños et al., 2006). Thusly, there is a worldwide trend to investigate new alternative that control plant disease in ecological farming, giving priority to strategies that reduce disease incidence and avoid negativity and side effects on

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Table 1
Mechanical and chemical analysis of the experimental soil during the two growing seasons.

Properties	Sand%	Silt%	Clay%	Texture	O.M.%	$CaCO_3$%	pH (1:25)	EC “dSm^{-1}” (1:5 soil water extract)	Available ions ppm		
									N	P	K
1st season	26.43	24.19	49.38	Clay	2	1.94	8	0.66	40	13	274
2nd season	26.44	24.16	49.40	Clay	2.10	1.95	7.95	0.71	42	14	285

human health (Li et al., 2013).

Induced resistance seems to be a promising and environmentally friendly option for crop protection against a broad range of pathogens (Vallad and Goodman, 2004). Using plant defense elicitors in grapevine protection deserves specific consideration due to their low environmental impact and of their ability to improve some quality attributes of plant foodstuffs. Several abiotic and biotic agents (elicitors) can promote plant growth and/or induced plant resistance to pathogens as a good and safe means of disease control (Farouk et al., 2012; Prakongkha et al., 2013; Tohamey and El-Sharkawy, 2014). In recent years, responses mediated by chitosan, bion, and silicon proposed that these substances have valued biochemical roles and great potential as elicitor and mediators of resistance signal transductions.

Silicon (Si) is the second most abundant constituents in the earth's crust after oxygen and involves 31% of its weight (Ma, 2005). It has been reported that Si increases plant growth, maintaining plant water balance and photosynthetic activities, yield and enhancing fruit quality as well as conferring plant resistance to pathosystems and unfavorable climatic conditions on several crops (Tohamey and El-Sharkawy, 2014; Hilal et al., 2016; Qin et al., 2016; Ratnayake et al., 2016). However, the mechanism by which Si provides protection against fungal plant pathogens is still indistinct. Some authors agree that Si acts as a physical barrier in cell walls, preventing the penetration of fungal hyphae into host tissues (Bowen et al., 1992), while others believe Si is related to specific plant defense reactions (Rodrigues et al., 2004). In this concern, silicon application was found to markedly control downy mildew in grapevine (Bowen et al., 1992) and bitter gourd (Ratnayake et al., 2016).

Chitosan (β-1,4-linked glucosamine) is a deacetylated derivative of chitin, has pulled in enormous consideration as potentially important biological resources due to its biological properties including biocompatibility, non toxicity and biodegradability (Xing et al., 2015). Recently, a few specialists detailed that chitosan enhanced plant growth and development (Farouk et al., 2012). For a few years, many endeavors have also been made to use chitosan against a broad spectrum of phytopathogens (Farouk et al., 2012; Saberi and Panah, 2015). The mechanism of inducing disease resistance by chitosan remains obscure. Two biological roles can be ascribed to this compound. First, at defined concentrations, it presents antifungal properties as appeared by its inhibitory action on the mycelia growth and spore germination of various pathogenic fungi (Xing et al., 2015). Secondly, it acts as a potent elicitor, therefore enhancing plant resistance against pathogens (Xing et al., 2015).

Bion (Acibenzolar-S-methyl; BTH) (1,2,3,-benzothiadiazole-7-thiocarboxylic acid S-methyl ester) is a promising defense system applied exogenously to activate resistance in many crop plants against plant pathogen as well as enhancing vegetative growth and yield (Harm et al., 2011; Sood et al., 2013). It is well reported that, BTH weekly application reduced the incidence of *P. viticola* in grapevine up to 88% (Dagostin et al., 2006).

The present study was planned to evaluate the efficacy of some elicitors (sodium metalsilicate, chitosan and bion as well as a bellis fungicide), on growth, yield, fruit quality and induced resistance for controlling downy mildew disease of Roumy Ahmar grapevines under natural infection in the field condition. Additionally, some physiological, biochemical constituents and anatomical changes were determined as a probable response to tested elicitors.

2. Materials and methods

Two field experiments were carried out under naturally infection during 2015 and 2016 successive seasons on thirteen years old Roumy Ahmar grapevine cultivar grown in a private vineyard at kafr El-shabrawy village, Aga Center, Dakahlia Governorate, Egypt, to assess the role of elicitors (sodium metasilicate, chitosan, bion and bellis fungicide) and/or water as control treatment on Roumy Ahmar grapevine growth, yield and its quality as well as some biochemical and anatomical attributes. The experimental soil is clay, its physiochemical characteristics of the soil samples (0–90 cm depth) were determined according to Page (1982). The obtained data are appeared in Table 1.

The chosen uniform vigor and healthy vines were trained according to spur pruned system using quadrilateral cardon trellis with supporting by three vertical wire systems. Pruning was carried out in the 1st week of February in the first and second seasons by leaving 5 spurs with three eyes on each,the total load was 60 buds. A completely randomized blocked design consists of 5 treatments, each treatment, including 3 replicates; each one has 3 vines spaced at 2 × 2 m. The vines were sprayed separately with an aqueous solution (2.5 l/vine) of 1000 mg/l sodium metasilicate (“SI” Sigma Che. Co., USA), 500 mg/l chitosan (“CHI” Sigma Che. Co., MO, USA), 150 mg/l bion (“BTH” Syngenta, Basel, Switzerland) or systemic fungicide bellis (Basf Co, the active ingredient pyraclostrobin (strobilurine) 128 g/kg and boscalid (carboxanilide) 252 g/kg), at the rate 0.4 g/l, beside water as control after added tween 20 as a wetting agent, four times at vegetative growth beginning, before bloom stage, at fruit set stage, and two weeks after fruit set stage.

2.1. Sampling date and data recorded

In both growing seasons, vegetative growth parameters at four weeks after fruit set stage, as well as yield and its quality (total soluble solid, acidity, anthocyanin and phenol in berries) at harvesting time were determined. In addition, disease assessment was determined in both growing seasons twice at the fruit set stage and four weeks after fruit set. Meanwhile, physiological characteristics were evaluated at 4 weeks after fruit set in both growing season. On the other hand, anatomical changes were studied in the second season only at 4 weeks after fruit set.

2.2. Vegetative growth parameters

Five shoots were tagged on each experimental grapevine from a non fruiting shoot and determined average shoot length (cm) as well as average leaf area (cm^2). Sixth and seventh leaves per shoot from the tip were picked and their area was measured with Montero et al. (2000) method.

2.3. Physiological characteristics

Ion percentage was determined in the ground dry leaves. Dry leaves (opposite to cluster) were wet digested with $HClO_3/H_2SO_4$ mixture (1:1 v:v) as described by Chapman and Pratt (1978). Total nitrogen was