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Effects of different levels of saline water on infection of tomato by *Botrytis cinerea*, the causal agent of gray mold

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ABSTRACT

A greenhouse experiment was conducted to investigate the effect of different levels of NaCl salt on tomato *upon B. cinerea infection* the causal agent of gray mold disease. The disease assessment was recorded after inoculation by using the scale based on percentage leaf area affected, and the growth of the plants was recorded for each treatment. Three weeks after inoculation by conidial suspension, the estimated disease severity on plants of tomato was 35.18% compared to the control. The highest incidence disease increase of gray mold (39.21%) was obtained with using 300 mM of NaCl after inoculation with *B. cinerea* compared with the other concentrations and as well as distilled water. Under severe salt stress (150 and 300mM) increased susceptibility of gray mold disease severity were observed in plants inoculated with *B. cinerea*, while under mild salt stress (50mM of NaCl) this effect was reversed. The treatment of plant by *B.cinerea* has reduced the growth of the aerial part of tomato plants (39.06%) after three weeks inoculation compared to the control. Three levels of NaCl (50, 100 and 150mM) increased respectively the plant height from 12.73 to 29.84%, 0.28 to 27.16% for the fresh eight and 5.75 to 33.35% for dry weight compared to the plants inoculated and irrigated by distilled water. NaCl addition at 300mM on plants inoculated with *B. cinerea* decreased the height, fresh weight and dry weight at 0.99, 4.45 and 11.01% respectively.

Key words: *Botrytis cinerea*, Gray mold, Incidence disease, *Lycopersicon esculentum*, NaCl.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is the most widely grown crop in the world. It is the second most important food crop in the developing world after potato, with an annual production of 164 million tonnes from 4.73 million hectares of area under cultivation in 2013 (FAOSTAT 2015). In Algeria, the tomato provides a key component in the so-called Algerian diet.

Several organisms such as fungi, bacteria and insect can cause diseases in tomato plant, resulting in significant yield losses (Mala *et al.*, 2016).

The tomato pathogen *Botrytis cinerea* causes gray mold, is ubiquitous fungi, widely distributed in soil, and other organic substrates, being pathogenic for a variety of plant species. Currently, *Botrytis cinerea* is one of the most severe diseases of tomatoes in Mediterranean basin (Elad *et al.*, 2004). In greenhouse, *B. cinerea* is capable of causing disease on virtually all parts of the tomato plant during any stage of plant growth (Carisse *et al.*, 2014).

Many studies concluded that exposure of plants to salinity stress can significantly increase the susceptibility of tomato to root knot nematode (Endongali and Ferris, 1982; Maggenti and Hardan, 1973), and to increase the susceptibility of citrus and chrysanthemum to Phytophthora root rots (Blaker and MacDonald, 1986; MacDonald, 1984; Swiecki and MacDonald, 1988). Soliman and Kostandi (1998) showed that maize had varying susceptibility to smut disease caused by *Ustilago maydis* under different concentrations of NaCl. The low levels of salinity (25-50mM) could increase the severity of *phytophthora* root rot of tomato with high Na:Ca ratios (10:1) (Bouchibi et *al.*, 1990); *Phytophthora* **(**Roubtsova and Bostock 2009; Sanogo, 2004), *F. oxysporum f. sp. vasinfectum* (Turco et *al.*, 2002), *F. oxysporum* f. sp. *radicis- lycopersici* (Triky-Dotan et *al.*, 2005), *Verticillium dahliae* and *Alternaria solani* (Regragui 2003., Nachmias *et al.*, 1993).

MATERIALS AND METHODS

Isolation of the pathogen: *B. cinerea* isolate was obtained from decayed tomato, collected from greenhouse in the Mostaganem, North-west, Algeria. Healthy and weakly infected leaf fragments were washed with tap water and cut in the middle. The leaf fragments were sterilized with sodium hypochlorite (2% available chlorine) for 5 min, rinsed three

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times in sterile distilled water, and dried filter paper. The leaf fragments showing lesions with a gray mold were placed on Potato Dextrose Agar (PDA).*B. cinerea* was incubated at 25°C and stored at 4°C. In order to harvest the conidia, the fungal culture plates were flooded with autoclaved, distilled water and gently rubbed with a Drigalski spatula under aseptic conditions. The liquid fraction was filtered. The concentration of conidia suspension was adjusted at 10^7 conidia /ml.

Cultivation of plants and inoculation: The experiment was conducted in a greenhouse at the Guelma University Station. Tomato cv. 532 variety was selected with good adaptation to all climatic areas in East Algeria, based on their economic importance to the state with important traits to consider include: potentially different disease resistance (The diseases we selected are *Fusarium oxusporum* (FO), *Alternaria solani* and *Phytophtora infestants*), yield potential and market opportunities.

The seeds were sown in alveolar trays filled with peat on a one seed per mini-mound at a depth of 1 cm. After emergence, the transplantation was made in plastic pots of 16 cm diameter and 30 cm in height, the seedlings were transplanted on a one seedling per pot and filed in greenhouses with a 14-h light (24°C) and 10-h dark period (20°C) at 85% relative humidity. Every three days, the plants were irrigated regularly with Hoagland nutrient solution to 30% of the substrate holding capacity. The study was established with 20 plants per salt treatments (0, 50, 100, 150 and 300mM). After 60 days, the plants were exposed for one week to irrigation with different water salinity levels. The control plants were irrigated with distilled water. Then, tomato transplants were inoculated by applying 25 ml at 10^7 conidia /ml suspension of *B. cinerea* .For spray treatments, the spore suspension was applied to the entire plant, using an aerosol spray bottle, until plants were wetted. Non inoculated control plants received 25 ml of sterile water.

Disease assessment: Disease severity was recorded three week after inoculation by using the scale based on percentage leaf area affected as described by Cohen *et al*. (1991) in a sample of 30 seedlings per replicate. Disease severity scale from 0 to 4 was used, whereas, 0 = No leaf lesions, 1 = 1-25%, 2 = 26-50%, 3 = 51-75% and 4 = 76-100% infected area of tomato leaf. The coefficient of infection was calculated by multiplying of disease severity (DS) and constant values of infection for host response ratings of resistant (R), moderately resistant (MR), moderately resistant-moderately susceptible (M), moderately susceptible (MS) and susceptible (S), following Pathan and Park (2006). The length of the aerial part of the plants and the fresh weight and dry weight of plants were recorded for each treatment.

Statistical analysis: The results were expressed in the form of the mean ± SD. The analysis of variance was carried out with respect to each parameter measured [incidence (I), severity (S), coefficient of infection (Ci), height (cm), fresh weight, dry weight (g) and water content (%)]. Mean discrimination was performed applying the t test; statistically significant differences were determined at the probability level $p<0.05$.

RESULTS AND DISCUSSION

Results in Table 1 indicate that the isolate tested of *Botrytis cinerea* was able to infect tomato plants causing typical gray mold symptoms of disease at the temperature of 24°C and relative humidity of 85% at three weeks after inoculation (Fig 1). Significant difference was noted from pathogen-inoculated plants ($p<0.01$). In absence of salt stress (0mM), gray mold incidence was 35.18%, and severity was 4.21% (Table 1). However, the leaf that were inoculated and tested in lower levels of NaCl (50mM), decay incidence decreased from 35.18% to 16.66%, respectively. On the contrary, infection incidence and severity in leaf are higher at salt stress between 150 and 300mM than at other level ($P < 0.01$). Disease severity was increased by 4.87 and 5.02 at 150 and 300mM respectively, compared to plants inoculated with the pathogen and irrigated by sterile distilled water. With the same concentrations of salt (150 and 300mM), it is evidenced that the highest mean incidence was 54.16% to 57.88%.

The mean coefficient of infection in absence of salt stress was 8.42% and the highest mean coefficient of infection was 15.06% in 300 mM and lowest mean coefficient of infection was 1.49% in 50 mM. Also the inoculation of plants in the presence of NaCl has stimulated over time the incidence disease, severity and coefficient of infection at high levels of NaCl compared to plants inoculated with the pathogen and irrigated by sterile distilled water (35.18%).

Of the five groups of plants tested (0, 50, 100, 150 and 300mM), only two were confirmed to be resistant R" to combined effect of *B.cinerea* and salt stress (50 and 100mM), two groups (150, 300mM) were moderate susceptible "M" and once had moderate resistance "MR" in the plants inoculated with the pathogen and irrigated by sterile distilled water.

The plants were grown in the greenhouse and evaluations fresh weight, dry weight (g) and water content (%) were evaluated 30 days after inoculation.

The inoculation of tomato plants with *B. cinerea* reduced the height of plants (27.27), the mean inhibition in height of the plant was 39.06% compared with the control (44.75cm) Table 2.

It was observed from this study that the hybrid tomato cv. "532" is susceptible to response of low levels of salt stress after inoculation by increasing of morphological characteristics, but it still remains low compared to the control.

Increase the levels of water *salinity (50 to 150mM)* with inoculated of tomato plants enhanced the plant height, fresh

Fig 1: Effect of different levels (NaCl) on the disease severity on tomato, cv. 532 variety. (A) Control; (B) plant inoculated after 2 weeks. (C); plant inoculated and irrigated with saline water (50mM), (D); plant inoculated, irrigated with saline water (100mM), (E); plant inoculated, irrigated with saline water (150mM), (F); plant inoculated irrigated with saline water (300mM).

Table 1: Effect of different levels of NaCl on the: incidence (I), severity (S) and coefficient of infection (CI) of *Botrytis cinerea* on tomato. The results are expressed as the mean ± SD.

Treatments NaCI (mM)	Incidence (I)	Severity (S)	Coefficient of infection (Ci)	Observed epidemic group
0	35.18±4.24 [a]	4.21±0.51[a]	8.42±1.35[a]	MR
50	16.66±1.39[b]	1.49±0.12[b]	1.49±0.11[b]	R
100	31.01±2.12	2.79±0.19[b]	2.79±0.14[b]	R
150	54.16±1.39[a]	4.87±0.12	14.61±2.53[a]	M
300	57.88±2.14[a]	5.02±0.18[b]	15.06±3.81[a]	M

Table 2: Effect of different levels of NaCl and *Botrytis cinerea* on the height, fresh weight, dry weight and water content on tomato: The results are expressed as the mean ± SD.

Treatments NaCl (mM)	Height (cm)	Fresh weight (g)	Dry weight (g)	Water content (%)
Control	44.75±0.95	164.75±1.25[a]	24.2±0.62 [a]	85.38±0.48[a]
0	27.27±1.47[a]	88.75±1.20[a]	11.8±0.24 [b]	86.85±0.27[b]
50	31.25±4.99[b]	89±1.82[b]	12.52±2.09[b]	85.94±2.13
100	34.25±0.59	101.87±4.68[b]	16±0.96[b]	84.26±1.31
150	38.87±0.90[a]	121.85±3.22[b]	17.75±0.69 [b]	85.52±0.68
300	27±1.41[a]	84.8±3.22[b]	10.5±0.53 [b]	87.61±0.55[a]

weight and dry weight compared to plants inoculated with the pathogen and irrigated by sterile distilled water. There was a significant increase in plant height from 12.73 to 29.84%, 0.28 to 27.16% for the fresh weight and 5.75 to 33.35% for dry weight measured respectively by 50 to 150mM. When plants of tomato were inoculated with *B. cinerea* and i*rrigated* by *water* of higher *salinity* (300mM), the mean inhibition were 0.99, 4.45 and 11.01% on the height, fresh weight and dry weight respectively. Plants inoculated at 300mM increased the water content compared to the control.

Among the great number of abiotic stress affecting plants, salinity in soil or water, especially in arid and semi-arid regions, is the most severe and stronger one that limits crop productivity (Freitas *et al*., 2011). Furthermore, salinity negatively affects plant physiology as a consequence of the toxic effects of sodium and chloride, which induce nutrient imbalances in the plant and alter osmotic potentials hindering water uptake by the roots (Munns and Gilliham 2015). From literature, plants exposure to salt stress can causes a range of adverse effects in plants, it imposes osmotic stress by imbalances and ultimately interfering with various metabolic processes (Munns and Tester, 2008). Also, it leads to an increase of diseases caused by various species pathogen (Bouchibi *et al*., 1990).

The primary goal of this study was to determine the effects of *saline irrigation water* on incidence and severity of gray mold caused by *B. cinerea* on tomato plants.

Here, we report that NaCl application at 150 to 300mM can increase disease incidence and severity. These results were consistent with those of Ouazzani *et al* (2014), who found the stimulator effect of salinity was observed on the mycelia growth, conidia production and conidia germination of the tested stain of *V. dahliae* respectively in concentrations 170, 120 and 256mM. In the same way, salinity effects were reported for: *Phytophthora sojae Kaufm, P. capsici, Fusarium solani, Phytophthora citrophthora, Fusarium oxysporum* f. sp. *radicis-lycopersici* (Canaday *et al*., 2010; Sanogo, 2004; Firdous and Shahzad, 2001; Sulistyowati, 1993; Triky-Dotan *et al*., 2005) respectively. The same result was also showed by Elmer (2002), which found no reductions in the severity of *Fusarium wilt* caused by *F. oxysporumf.sp. cyclaminis* when NaCl were applied.

This study demonstrated that low salinity (50 to 100mM) associated with *B.cinerea* decreased incidence disease. Furthermore, disease severity of infection in plants subjected to decrease compared to the plants inoculated and irrigated by distilled water. These results are in accordance with those of Kumar et *al*, (2010); with increase in salinity stress the severity of early leaf spot (*Cercospora arachidicola*), late leaf spot (*Phaeoisariopsis personata*) and rust (*Puccinia arachidis*) decreased. The same author showed that higher salinity affected the crop growth and there was no corresponding increase in pod yield. The author concluded that although groundnut is a sodium sensitive crop, it can be grown profitably up to a threshold salinity stress of 2.0 to 2.5 dS m-1 at this salinity the severity of foliar diseases were less and the pod yield was maximum. According to Jeffrey *et al*., (1985), the concentration of 10.25mM eliminates the production zoospores of *Lagenidium giganteum* able to germinate. In fungi, a low osmotic potential decreases spore germination and the growth of hyphae and changes the morphology (Juniper and Abbott, 2006) and gene expression (Liang *et al*., 2007). Sodium decreased the severity of cotton root rot (*Phymatotrichum omnivorum* Duggar) (Lyda and Kissel, 1974), yellow rust of greenhouse-grown wheat (*Puccinia striiformis* Westend) (Russell, 1978), and powdery mildew of wheat (*Erysiphe graminis* DC.) (Leusch and Buchenauer, 1989).

The reduction in disease severity in lower levels may be due to the metabolic changes in the host under salt stress causes stimulation the activity of enzymes involved in disease development, of which cellulase and polygalacturonase are favored by alkaline reactions.

Application of NaCl at concentrations on 50 at 150mM with inoculated by *B.cinerea* enhanced the plant height, fresh weight and dry weight compared to the plants inoculated and irrigated by distilled water, which may be increase tolerance or plant response to salt. Other results that support what has been shown here, are those by Amira and Abdul Qados (2011) with his study on *Vicia faba*; Hamada (1995) on maize *Zea mays* L; Memon *et al*. (2010) in their study on *Brassica campestris* L; and ûnally by Misra *et al*. (1997) with their study on rice seedlings *Oryza sativa* L. vr. Damodar, where they showed that the use of low levels of salt stress led to increases in plants lengths. The increase in fresh weight and dry weight may be due to the ability of the plant to increase the size of its sap vacuoles, which allows for the collection of a lot of water and saltions (Munns, 2002). Also, increased in fresh weight and dry weight that corresponded with the increased in chemical content. This reading was registered as well, including the study done by Chao *et al*. (1999). They noticed an increase of protein content of the tomato plant *Lycopersicon esculentum* (L.) in response to salt stress. Some plants can develop a lot of mechanisms to support low salinity, as example the development of internal water deficit, which can be important for some crops such as coffee and mango in order to trigger phenological events such as flower bud release, height, fresh weight and dry weight. Greenhouse experiments by Elmer (1997) conducted on two sugar beet cultivars showed that application of NaCl (10 g/l) increased plant dry weight by 12% in rhizoctonia-infested soils and by 40% in non-infested soils as compared with plants that did not receive NaCl treatment. The agronomical parameters used for salt

tolerance are yield, survival, plant height, leaf area, leaf injury (Parvaiz and Satyawati 2008), however, salt tolerance in plants may be explained by functional and structural adaptations, such as growth regulations, osmotic adjustment, regulation of stomatal conductance, changes in cell wall elasticity, mineral nutritional and hormone balance, all of which may help alleviate the harmful effects of stress (Suárez, 2011).

On the contrary, application of high salinity (300mM) with inoculated by *B.cinerea* has been shown to decrease the agronomical parameters (plant height, fresh weight and dry weight). Many studies have shown that salinity affects physiological processes of plants, causing a nutrient imbalance, altering the levels of growth regulators, inhibiting photosynthesis and protein synthesis, all of which lead to reduced plant growth (Hashem *et al.* , 2015; Hamed *et al.*, 2014; Zaid *et al.*, 2014). An increase in the fresh and dry weights of the shoot system are affected, negatively by changes in salinity concentration, type of plant species or type of salt (Rui *et al*., 2009; Taffouo *et al*., 2010; Memon *et al*., 2010). Thus, salinity inhibition of plant growth was the result of osmotic and ionic effects and the different plant species have developed different mechanisms to cope with these effects (Munns and Tester, 2008).

CONCLUSION

All though the mechanisms by which stress increases disease severity are not yet known, recent work with salinity stress has provided some information. This study is relevant to management of *B. cinerea* under saline conditions in that it provides an understanding of the direct and indirect effects of salinity on gray mold, and their interaction. In light of the results from this study, the salinity in water may predispose susceptible tomato plants to infection by *B. cinerea*. However, further work is necessary with the selected salts to evaluate their efficacy against several strains of *B. cinerea* in several tomato cultivars in order to establish whether NaCl could eventually be increased gray mold of tomato.

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