

STUDIES ON TOMATO WILT CAUSED BY *FUSARIUM OXYSPORUM* F.SP. *LYCOPERSICI* IN KAZACHESTAN. IA: EFFECT OF EXOGENOUS APPLICATION OF SALICYLIC ACID AND RIBOFLAVIN AS RESISTANCE INDUCER TREATMENTS ON THE WILT DISEASE INCIDENCE AND SOME PLANT GROWTH PARAMETERS

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ABSTRACT

In this study, seedlings of tomato cultivar Carolina Gold were treated by salicylic acid (SA) and riboflavin (R) using different application methods and inoculated with *Fusarium oxysporum* f.sp. *lycopersici*. The obtained results revealed that the wilt disease severity (DS) was significantly affected by inducer treatments and application method/treatment interactions. Concerning interactions, the highest significant reduction in DS was recorded by IR/SA @ 0.1mM (94.1%) followed by IR/SA @ 10.0mM, SS/SA @ 10.0mM and IR+SS/R @ 10.0mM (88.2% reduction), IR/R @ 10.0mM, IR+SS/R @ 0.1mM and IR+SS/SA @ 10.0mM (82.4% reduction), IR/R @ 0.1mM and IR+SS/SA @ 0.1mM (70.6% reduction), respectively comparing with the control treatment. Using IR/SA @ 0.1mM caused the highest increase in plant height (53.4%) whereas some interactions such as , IR/R @ 0.1mM, SS/R @ 10.0mM and IR+SS/SA @ 0.1mM showed no significant effects on plant height when compared with the untreated control. The root length recorded the highest significant increase by using SS/SA @ 10.0mM (67.7%) and SS/R @ 0.1mM (65.6%). The lowest significant increase in RL was induced by IR+SS/R @ 0.1mM (21.5%) whereas, IR/R @ 0.1mM and SS/R @ 10.0mM only showed no significant effect on the RL comparing to the untreated control.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the world's most important crops due to the high value of its fruits both for fresh market consumption and in numerous types of processed products (Giovanni *et al.*, 2003). One of the main constraints to tomato cultivation is damage caused by pathogens, including viruses, bacteria, nematodes and fungi, which cause severe losses in production (Barone and Frusciante, 2007). *Fusarium oxysporum* f. sp. *Lycopersici* (FOL) has an extensive presence in all continents (Menzies *et al.*, 1990, Brayford, 1996 and Eraky *et al.*, 2007) and become one of a limiting factor in the production of tomato and accounts for yield losses annually (Abogharsa1, *et al.*, 2006). It has become one of the most prevalent and damaging diseases wherever tomatoes are grown intensively because the pathogen persists indefinitely in infested soils (Gordon and Martyn, 1987). The control of the pathogen spread mainly involves in three strategies: husbandry practices, application of agrochemicals and use of resistant varieties (Barone and Frusciante, 2007). The methods used to control vascular wilt are either not very efficient or are difficult to apply. The pathogen has increased in the infested soil and become resistant to chemical fungicides. For this reason, alternative methods with emphasis on biological control using the resistance inducers for controlling the disease have been studied by several researchers to reduce fungicide application and decrease cost of plant production. Recently, there have been many reports stated that some plant extracts and safe chemicals become a necessary to make a fungicide to control the soil borne diseases including tomato *Fusarium* wilt (Tzeng and DeVay, 1984; Dong and Beer, 2000; Aba AlKhail, 2005; Deepak, *et al.*, 2005; Abogharsa1, *et*

al., 2006; Taheri and Hofte, 2006; Mandal *et al.*, 2009). This work was conducted to evaluate effect of applying salicylic acid (SA) and riboflavin (R) @ 0.1 and 10.0mM concentrations on suppressing development of the tomato Fusarium wilt disease under glasshouse condition in Kazakhstan. Effects of tested treatments on % wilted plants, wilt disease severity, plant height and root length were investigated.

MATERIALS AND METHODS

In this study, salicylic acid (SA) and riboflavin (R) each @ 0.1 and 10.0 mM concentrations were used as chemical resistance inducer treatments for treating 4 weeks-old tomato (*Solanum lycopersicum*) seedlings (Carolina Gold cv.) immediately before transplanting into plastic pots (30cm. in diameter) each containing 11 Kg of natural soil mixture consisted of clay and sand at rate of 2:1 (by weight). Each inducer treatments was performed by immersing roots (IR) for 10 min., spraying shoots (SS) until dropping or combination between IR and SS application methods (IR+SS). The plain water was used instead of inducer treatments for treating tomato seedling in the control treatment. Spore suspension of an aggressive isolate of *Fusarium oxysporum* f.sp. *lycopersici*, which was isolated from wilted tomato plants grown under glasshouse conditions in Kazakhstan, was prepared and adjusted according to Beshir, 1991 and Amini, 2009 and immediately used for inoculating 4-weeks old tomato seedlings by pouring 20 ml of spore suspension (10^6 spores/ml) over stem base of each seedling one week after transplanting. All pots were irrigated and maintained at 25-30°C and 70% relative humidity under glasshouse conditions. After tow months from inoculation, the wilt disease severity (DS) for each treatment was determined using a visual scale of 0-4 as following: **0**= No wilting symptoms (healthy plant); **1**= Plant slightly wilted, vascular discoloration found in main root region; **2**= Plant moderately wilted, yellowing of old leaves, spreading vascular browning; **3**= Plant severely wilted, dying of all leaves except end leaves; and **4**= Dead plant, seedling entirely wilted (Vakalounakis and Fragkiadakis, 1999). The wilt disease severity was determined according to Song *et al.*, (2004) meanwhile; percentage of disease reduction (efficiency) was calculated according to Elhenawy, *et al.*, 2007. At the same time, plant height and root length were measured in all tested treatments.

All data were subjected to analysis of variance according to Snedecor and Cochran, 1982. The least significant difference at 0.05 was calculated.

RESULTS

Percentage of wilted plants and wilt disease severity:

Percentage of wilted tomato plants was significantly affected only by tested treatments but not by methods or method/treatment interactions. Using SA @ 10.0mM and R @ 10.0mM were the most effective, reduced % wilted plants by 73.3% followed by SA @ 0.1mM (60.0%) and R @ 0.1mM (53.3%) compared

Table (1): Effect of salicylic acid (SA) and riboflavin (R) at 0.1 and 10.0 mM using different application methods on % wilted tomato plants under stress of infection with *F. oxysporum lycopersici*

Treatments	* Application methods			Mean	Efficiency %			Mean
	IR	SS	IR+SS		IR	SS	IR+SS	
SA @ 0.1mM	16.7	50.0	33.3	33.3	80.0	40.0	60.0	60.0
SA @ 10.0mM	33.3	16.7	16.7	22.2	60.0	80.0	80.0	73.3
R @ 0.1mM	50.0	33.3	33.3	38.9	40.0	60.0	60.0	53.3
R @ 10.0mM	33.3	16.7	16.7	22.2	60.0	80.0	80.0	73.3
Control	83.3	83.3	83.3	83.3	0.0	0.0	0.0	0.0
Mean	43.3	40.0	36.7		48.0	52.0	56.0	

L.S.D. at 5% for:

Methods	NS
Treatments	14.93
Interaction	NS

* IR = immersing roots, SS = spraying shoots

with the control treatment (**Table, 1**). However, wilt disease severity (DS) was significantly affected by inducer treatments and application method/treatment interactions (**Table, 2**). Using SA @ 10.0mM caused the highest reduction in DS (86.3%) followed by R @ 10.0mM (74.5%), SA @ 0.1mM (72.5%) and R @ 0.1mM (70.6%), respectively comparing to the untreated control (0.0% reduction). Concerning interactions, the highest significant reduction in DS was recorded by IR/SA @ 0.1mM (94.1%) followed by IR/SA @ 10.0mM, SS/SA @ 10.0mM and IR+SS/R @ 10.0mM (88.2% reduction), IR/R @ 10.0mM, IR+SS/R @ 0.1mM and IR+SS/SA @ 10.0mM (82.4% reduction), IR/R @ 0.1mM and IR+SS/SA @ 0.1mM (70.6% reduction), respectively comparing with the control treatment.

Table (2): Effect of salicylic acid (SA) and riboflavin (R) at 0.1 and 10.0 mM using different application methods on wilt disease severity (%) under stress of infection with *F. oxysporum lycopersici*

Treatments	* Application methods			Mean	Efficiency %			Mean
	IR	SS	IR+SS		IR	SS	IR+SS	
SA @ 0.1mM	1.4	11.1	6.9	6.5	94.1	52.9	70.6	72.5
SA @ 10.0mM	2.8	2.8	4.2	3.2	88.2	88.2	82.4	86.3
R @ 0.1mM	6.9	9.7	4.2	6.9	70.7	58.8	82.4	70.6
R @ 10.0mM	4.2	11.1	2.8	6.0	82.4	52.9	88.2	74.5
Control	23.6	23.6	23.6	23.6	0.0	0.0	0.0	0.0
Mean	7.8	11.7	8.3		67.1	50.6	64.7	

L.S.D. at 5% for:

Methods	NS
Treatments	4.12
Interaction	12.36

* IR = immersing roots, SS = spraying shoots

Plant height and root length:

The plant height was significantly affected by application methods, inducer treatments as well as by method/treatment interactions (**Table, 3**). The IR method recorded the highest increase in plant height (150.2 cm) followed by SS method (141.6 cm) and IR+SS method (136.2 cm), respectively. As for treatments, using SA @ 0.1mM recorded the highest % increase in plant height (30.6%) followed by R @ 10.0mM (20.0%) and SA @ 10.0mM (19.69%) without significant difference between the latter two treatments whereas, the lowest % significant increase (4.1%) was recorded by using R @ 0.1mM comparing to the untreated control. Among tested interactions, IR/SA @ 0.1mM was best in this respect (53.4%) whereas some interactions such as , IR/R @ 0.1mM, SS/R @ 10.0mM and IR+SS/SA @ 0.1mM showed no significant effects on plant height when compared with the untreated control. Also, the root length (cm)/plant (RL) was significantly affected by tested application methods, inducer treatments as well as by the interaction in between (**Table, 4**). The SS method recorded the highest significant increase in the RL (21.3 cm) followed by IR and IR+SS methods (19.5 cm). All tested inducer treatments induced significant increases in the RL. The highest % significant increase was produced by SA @ 10.0mM

Table (3): Effect of salicylic acid (SA) and riboflavin (R) at 0.1 and 10.0 mM using different application methods on on plant height (cm) under stress of infection with *F. oxysporum lycopersici*

Treatments	* Application methods			Mean	Efficiency %			Mean
	IR	SS	IR+SS		IR	SS	IR+SS	
SA @ 0.1mM	190.5	171.5	124.7	162.2	53.4	38.1	0.4	30.6
SA @ 10.0mM	148.2	153.5	144.2	148.6	19.3	23.6	16.1	19.7
R @ 0.1mM	127.8	133.5	126.5	129.3	3.0	7.5	1.9	4.1
R @ 10.0mM	160.3	125.2	161.7	149.1	29.1	0.8	30.2	20.0
Control	124.2	124.2	124.2	124.2	0.0	0.0	0.0	0.0
Mean	150.2	141.6	136.2		21.0	14.0	9.7	

L.S.D. at 5% for:

Methods	0.74
Treatments	1.23
Interaction	3.69

* IR = immersing roots, SS = spraying shoots

(53.0%) followed by SA @ 0.1mM (36.2%), R @ 10.0mM (30.5%) and R @ 0.1mM (28.8%), respectively without significant difference between the latter two treatments comparing to the untreated control. Except IR/R @ 0.1mM and SS/R @ 10.0mM which showed no significant effect on the RL, all remained method/treatment interactions significantly increased RL. In this respect, the highest increase was produced by SS/SA @ 10.0mM (67.7%) and SS/R @ 0.1mM (65.6%) whereas; the lowest significant increase was induced by IR+SS/R @ 0.1mM (21.5%) comparing to the untreated control.

Table (4): Effect of salicylic acid (SA) and riboflavin (R) at 0.1 and 10.0 mM using different application methods on root length (cm) under stress of infection with *F. oxysporum lycopersici*

Treatments	* Application methods			Mean	Efficiency %			Mean
	IR	SS	IR+SS		IR	SS	IR+SS	
SA @ 0.1mM	23.0	22.3	18.0	21.1	48.4	44.1	16.1	36.2
SA @ 10.0mM	21.7	26.0	23.5	23.7	39.8	67.7	51.6	53.0
R @ 0.1mM	15.9	25.7	18.8	20.1	2.4	65.6	21.5	29.8
R @ 10.0mM	21.7	17.2	21.8	20.2	39.8	10.8	40.9	30.5
Control	15.5	15.5	15.5	15.5	0.0	0.0	0.0	0.0
Mean	19.5	21.3	19.5		26.1	37.6	26.0	

L.S.D. at 5% for:

Methods 0.41

Treatments 0.68

Interaction 2.03

* IR = immersing roots, SS = spraying shoots

DISCUSSION

Induction of plant defense against pathogen attack is regulated by a complex network of different signals. Elicited by a local infection, plants respond with a salicylic-dependent signaling cascade that leads to the systemic expression of a broad spectrum and long-lasting disease resistance that is efficient against fungi, bacteria and viruses. Changes in cell wall composition, de novo production of pathogenesis-related-proteins and synthesis of phytoalexins are associated with resistance, although further defensive compounds are likely to exist but remain to be identified (**Heil and Bostock, 2002**). Concerning method/treatment interactions, the present results indicated that using IR/SA @ 0.1mM recorded the highest significant reduction in the wilt disease severity (DS) (94.1%) followed by IR/SA @ 10.0mM, SS/SA @ 10.0mM and IR+SS/R @ 10.0mM (88.2%), IR/R @ 10.0mM, IR+SS/R @ 0.1mM and IR+SS/SA @ 10.0mM (82.4%), IR/R @ 0.1mM and IR+SS/SA @ 0.1mM (70.6%), respectively comparing with the control treatment. These findings were in agreement with **El- Khallal, 2007** who reported that, spraying tomato plants infected by *Fusarium oxysporum* 3 times with jasmonic acid (JA) and SA significantly reduced % of disease incidence. **Mandal, et al., (2009)** mentioned that, SA-treated tomato plants challenged with *Fusarium oxysporum* f.sp. *lycopersici* (Fol) exhibited significant reduction in the vascular browning and leaf yellowing wilting. Significant increase in basal level of SA in control plants indicated that tomato root system might have the capacity to assimilate and distribute SA throughout the plant. The results indicated that the induced resistance observed in tomato against Fol might be a case of SA-dependent systemic acquired resistance. SA is associated with systemic acquired resistance (SAR) and priming for defense potentiation in plants (**Delaney et al., 1994; Ryals et al., 1996**). Application of exogenous SA was correlated not only with the induction of SAR and pathogenesis-related (PR) protein expression (**Friedrich et al., 1996; Dempsey et al., 1999; Métraux, 2001**), but also with plant tissues potentiated to respond rapidly and effectively with a variety of defense mechanisms after pathogen challenge or elicitation (**Kauss et al., 1993; Mur et al., 1996; Katz et al., 1998**). Also, the role of riboflavin as an elicitor of systemic resistance and an activator of a novel signaling process in plants was demonstrated. **Saikia, et al., 2006** stated that, riboflavin caused

induction of systemic resistance in chickpea against *Fusarium* wilt and charcoal rot diseases. The dose effect of 0.01 to 20 mM riboflavin showed that 1.0 mM concentration was sufficient for maximum induction of resistance; higher concentration did not increase the effect. Riboflavin induced plants found accumulation of phenols and a greater activities of phenylalanine ammonia lyase (PAL) and pathogenesis related (PR) protein, peroxidase was observed in induced plant than the control. Riboflavin is an antioxidant (**Packer, et al., 1996** and **Upreti, et al., 1991**), and other antioxidants induce disease resistance in plants (**Norris, 1991**). These features are consistent with the concept that riboflavin might play a role in plant disease resistance. We ruled out the possibility that riboflavin might directly inhibit pathogens. Both molecular and phenotypic data showed that riboflavin substantially induced systemic disease resistance and did so by activating distinct signal transduction pathway. In addition, no direct effect of the vitamin was detected on the growth of the culturable pathogens, suggesting that riboflavin is not likely to directly inhibit pathogens. Riboflavin-induced resistance was effective against *A. alternata*, which has not yet been considered as a target of induced resistance (**Hammerschmidt and Kud, 1995**; **Ryals, et al., 1996**).

Recent studies of riboflavin (**Dong and Beer, 2000**) indicate the function of the compound in mediating resistance signal transduction. Riboflavin is a cofactor of enzyme flavoproteins, some of which catalyze lipid peroxidation, a main process in producing reactive oxygen intermediates (ROIs) that serve as a signaling network in plant immune responses. The role of riboflavin in peroxidation is antagonistic to its role in antioxidation. Balance between both reactions should be a part of the signaling mediation and may affect whether programmed cell death occurs. Exploration of the existence of similar receptors and their roles as signal transducers in plants responding to riboflavin and other elicitors of resistance would be very worthwhile. The universal existence of flavin kinases, required to activate flavoproteins, may be linked with protein kinase cascades, which are a typical mode of signal transduction. Therefore, there is a reasonable basis for riboflavin to mediate a distinct signal transduction pathway. We hope that this report will stimulate additional studies of the novel roles of riboflavin and mechanisms of resistance signal transduction in plants.

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