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Intrinsic Onion Resistance against Bacterial Bulb Rots by Chemical Inducers

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Bacterial bulb rots of onion are among the most serious storage diseases that lead to great loss in the yield. In this study, sodium salicylate, ascorbic acid and thiamine hydrochloride were tested as resistance inducers on onion plants grown under *in vivo* condition to evaluate their potentials in controlling the bacterial rot diseases. The tested inducers were found to be effective in decreasing the bacterial rot incidence in stored onion bulbs. The treatments significantly increased the total phenols and significantly declined lipid peroxidation and the electrolyte leakage in onion bulbs indicating their potentials to induce the natural resistance. Moreover, the inducer-treated plants showed an increase in photosynthetic pigments content and foliar growth compared to the untreated control, justifying the role of chemical inducers in improving plant growth. It could be concluded that, the application of some chemicals to induce the intrinsic resistance in plants is a safe and effective tool in controlling plant diseases that can replace and minimize the hazardous effects of chemical pesticides.

Keywords: Ascorbic acid, onion bacterial rot, resistance, salicylic acid and thiamine hydrochloride.

Bacterial rot diseases of onion are among the most serious bulb storage diseases which may be recognized into at least three different syndromes, *i.e.* soft rot, caused by *Erwinia carotovora* subsp. *carotovora* or *Erwinia chrysanthemi*, slippery skin, caused by *Burkholderia gladioli* subsp. *allicola* and sour skin, caused by *Burkholderia cepacia* (McNab, 2004). Onion bacterial rots were tentatively determined to cause yield losses ranging from 5 to 50 % (Schwartz and Gent, 2004), thus the need for effective control is of paramount importance.

The induction of the natural defence mechanisms of plants against pathogens by external factors of various types (inducers) is a recent and safe approach in the field of disease control, the matter which called induced resistance that manifested upon subsequent inoculation, and depending on the mode of its expression, induced resistance can be local or systemic (Edreva, 2004).

Certain chemicals such as salicylic acid, 2,6-dichloroisonicotinic acid, potassium salts, amino butyric acid and Bion were reported to induce systemic acquired resistance in plants (Oostendorp *et al*., 2001). The use of chemical inducers of resistance has advantages over the exciting as a new perspective to supplement the classical chemical means of disease control by providing both effective and ecologically-friendly plant protection (Edreva, 2004), the exogenous application of these chemicals can induce the accumulation of pathogenesis-related proteins and lead to reduced incidence of several diseases in many crops (Gozzo, 2003).

The present work aimed to study the effect of some chemical inducers; as salicylic acid sodium salt (sodium salicylate), ascorbic acid and thiamine hydrochloride on controlling onion bacterial rots and to study some related physiological expressions in response to treatment of onion plants.

Materials and Methods

Pathogenic bacterial strains:

Two strains of the onion bacterial rot pathogens, namely *Erwinia carotovora* subsp. *carotovora* (Ecc) and *Burkholderia cepacia* (Bc) were isolated and identified in a previous study by Mansour *et al*. (2011).

Tested inducers:

Salicylic acid in its sodium salt (SS) and ascorbic acid (AA) were obtained from El-Nasr Pharmaceutical Chemicals Company (Egypt) and thiamine hydrochloride (TH) produced by MP Biomedicals, LLC, Germany, were used in this study.

In vitro antibacterial activity of inducers:

The antibacterial activity of the three chemical inducers against Ecc and Bc was tested using the plate-count technique. Concentrations of SS tested were 1, 3, 5, 10, 20, 30 mM, while those for AA and TH were 1, 5, 10, 20 mM.

In vivo experiments:

Pot experiment:

Onion transplants of Giza red cultivar were divided into four groups and their root systems were soaked separately overnight in the different treatments; SS at 3 mM, AA at 5 mM, TH at 1 mM (the candidate concentration of each material was selected according to previous preliminary experiments) and water as control, then air dried. Plastic pots of 17 cm diameter were filled with clayey: sandy soil (2:1), then planted with the treated. Onion transplants, two transplants per pot. Each group (treatment) had 18 pots and was subdivided into three subgroups, each had six pots. Two months later, all the treatments were treated for a second time with the same material by spraying on the onion plants. All the suitable cultural practices were carried out through the growing period. One month before the harvest, onion groups were inoculated with liquid bacterial cultures of rot pathogens (100 ml of the bacterial culture, (10^6 cfu/ml) were poured in the soil of each pot), each in subgroup as follow: 1) inoculated with Ecc, 2) inoculated with Bc and 3) treated with water as control. Onion was harvested after four months from planting (when 50% of the onion necks fallen down). Then onion was leaved in the open air for two weeks before being stored.

Field experiment:

This experiment was carried out in Dakahlyia Governorate, Egypt in a field naturally infested with the bacterial rot pathogens to investigate the role of SS, AA and TH in controlling onion bacterial rot diseases. The field was divided equally into

16 plots, each plot of 32.5 m^2 (6.5 m x 5 m). Red onion transplants (cv. Giza) were divided into four groups and their root systems were soaked overnight in the different treatments; SS at 3 mM, AA at 5 mM, TH at 1 mM (the candidate concentration of each material was selected according to previous preliminary experiments) and water as control. Onion transplants of different treatments were distributed randomly in the field plots, to make complete randomized design, four replicates (four plots) were used for each treatment. Two months after planting, all the treatments were sprayed for a second time with the corresponding treatment. All cultural practices were carried out as usual. Onion was harvested after four months from planting (when 50 % of the onion necks fell down). Then onion was left in open air for two weeks before being stored.

Preharvest estimations:

Three months after planting the photosynthetic pigments were estimated for both pot and field grown onion in the 3rd upper leaf according to Lichtenthaler and Wellburn (1985). Furthermore, some foliar growth parameters; number and length of leaves were determined in both pots and field grown onion.

Postharvest estimations:

In the beginning of storage period of the field grown onion, some parameters were estimated as disease resistance indices, these parameters included: total phenols content; was determined using Folin-Ciocalteau reagent according to the method of Singleton and Rossi (1965), anthocyanin content; was assessed following the method of Mancinelli *et al*. (1975), total soluble solids; were determined using hand refractometer as described by Andersen (2000), lipid peroxidation; was determined by the method of Shao *et al*. (2005) and electrolyte leakage; was estimated according to Goncalves *et al*. (2007) using an electrical conductivity meter. Also the disease incidence (bacterial rot incidence) was estimated for both pots and field grown onion after 3, 6, 8 and 10 months from the beginning of storage. After each time the diseased bulbs were discarded.

Statistical analysis:

Data were statistically analysed with CoStat software (CoStat, 2005). Data were first subjected to analysis of variance (ANOVA), then the multiple comparisons among means were made using Duncan's new multiple range test at $P \leq 0.05$.

Results

In vitro antibacterial activity of the tested chemical inducers against Erwinia carotovora subsp. carotovora and Burkholderia cepacia:

Sodium salicylate (SS), ascorbic acid (AA) and thiamin hydrochloride (TH), exhibited variable activities against Ecc and Bc. Sodium salicylate and ascorbic acid completely inhibited the growth of Bc at 20 and 10mM, respectively, while they didn't affect the growth of Ecc at any tested concentrations. TH didn't exhibit any activity against Ecc nor Bc *in vitro*.

In vivo experiments:
Pot experiment:
Preharvest estimations:

Table 1 shows that the tested chemical inducers increased the photosynthetic pigments content in onion leaves compared to the untreated control, TH exhibited the best effect in this regard hence it caused significant increase in chl A, chl B and carotene contents. It is observed that AA application significantly reduced chl B and carotene. It was also noticed that growth parameters, i.e. leaves number per plant and leaves length, responded positively and significantly to the application of the chemical inducers as compared with the control plants. Maximum leaves number per plant (9) was observed in the treatment of SS and AA and maximum leaves length (50 cm) was observed in SS treatment.

Table 1. Photosynthetic pigments concentration in the 3rd upper onion leaves and plant growth as affected by application of the chemical inducers under artificial inoculation condition in the pot experiment

Inducer* treatment	Photosynthetic pigment			Foliar growth parameter	
	Chl. A (µg/ gm)	Chl. B (µg/ gm)	Carotene (µg/ gm)	No. of leaves	Length of leaves (cm)
SS	719.3 ab	426.3 b	509.1 b	9 a	50.0 a
AA	672.3 ab	230.6 d	390.4 d	9 a	47.5 b
TH	766.7 a	626.9 a	612.4 a	8 a	43.5 c
Control	654.7 b	355.5 c	460.8 c	7 b	34.5 d

* SS= sodium salicylate, AA= ascorbic acid, TH= thiamin hydrochloride, Chl. A= chlorophyll A and Chl. B= chlorophyll B.

Notes: Values within a column followed by the same letters are not significantly different according to CoStat software at $p < 0.05$. Values are means of six replicates.

Postharvest estimations:

Table 2 shows that application of the chemical inducers reduced the disease incidence (DI) caused by Ecc and Bc three months after storage of pots grown onions compared to uninfected control plants. Ascorbic acid exhibited the best results in this regard followed by TH and SS hence the mean DI (DI mean of Ecc and Bc- infected onion) was recorded as 9.5, 14.5 and 22.7 % respectively. Furthermore, the disease incidence was zero when recorded after six, eight and ten months from storage.

Table 2. Bacterial rot disease incidence after three months from storage of pots grown onion bulbs as affected by the application of chemical inducers

Inducer treatment	DI of onion infected with Ecc (%)	DI of onion infected with Bc (%)	Mean DI (%)
SS	18.18	27.27	22.73
AA	9.09	10	9.55
TH	9.09	20	14.55
Control	20	33.33	26.67

SS= sodium salicylate, AA= ascorbic acid, TH= thiamin hydrochloride and DI= disease incidence.

Field experiment:
Preharvest estimations:

Table (3) indicates that onion treatment with the chemical inducers resulted in significant increase in the photosynthetic pigments content compared to the untreated control plants. Furthermore, foliar growth parameters (No. and length of leaves) measurements recorded significant increase. Maximum leaves number per plant (11) was observed in the treatment of SS and TH and maximum leaves length (60 cm) was observed in SS treatment.

Table 3. Photosynthetic pigments content in the 3rd upper onion leaves and plant growth as affected by application of the chemical inducers in the field experiment

Inducer treatment	Photosynthetic pigment			Foliar growth parameter	
	Chl. A (μg/gm)	Chl. B (μg/gm)	Carotene (μg/gm)	No. of leaves	Length of leaves (cm)
SS	681.7 a	397.9 a	483.8 a	11 a	60 a
AA	687.8 a	369.4 a	469.8 a	10 a	56.3 a
TH	680.9 a	333.1 ab	441.1 ab	11 a	59 a
Control	547.4 b	279.5 b	397.8 b	10 a	48.7 b

* As described in footnote of Table (1).

Postharvest estimations:

Table (4) refers to the potentials of the tested chemicals to induce onion resistance against bacterial rot diseases by assessing some disease resistance indices in field- grown onion bulbs including; total phenols, anthocyanin pigment, total soluble solids (TSS %), lipid peroxidation and electrolyte leakage percentage (ELP). It is evident that all treatments caused significant increase in the total phenols content in onion bulbs as compared with the untreated control plants. All treatments showed the same effect on anthocyanin and it was slightly higher than that of the control. The TSS % increased by SS and AA treatments, while it was comparable to the control in case of TH treatment. All treatments significantly reduced the malondialdehye (MDA) content indicating significant decrease in lipid peroxidation. The ELP decreased by all treatments, the significant decrease was by SS and AA.

Table 4. Disease resistance indices in onion bulbs after harvest as affected by chemical inducers application in the field experiment

Inducer* treatment	Total phenols (mg/100 gm)	Anthocyanin (A/gm)	Total soluble solids (%)	Lipid peroxidation (MDA= μmol/gm)	Electrolyte Leakage (%)
SS	36.75 c	0.8 a	13.9 a	1.8 b	57 b
AA	39.0 b	0.8ab	12.8ab	2.2 b	56 b
TH	41.2 a	0.8ab	12.5 b	1.7 b	66 a
Control	31.4 d	0.8 b	12.5 b	3.8 a	69 a

* As described in footnote of Table (1).

Table (5) reveals that after three months storage of the field- grown onion the disease incidence (DI) of the bacterial rot was decreased by SS, AA and TH treatments, the decrease was significant in case of AA and TH treatments. Significant decrease in the DI was detected in onion bulbs by all treatments after six, eight and ten months from storage. In general; the decrease in DI values trend was more pronounced throughout storage with time.

Table 5. Bacterial rot disease incidence throughout storage of field- grown onion bulbs as affected by the application of chemical inducers

Inducer* treatment	DI after 3 months (%)	DI after 6 months (%)	DI after 8 months (%)	DI after 10 months (%)
SS	11.9ab	5.9 b	1.2 b	0.04 b
AA	9.2 bc	5.6 b	0.7 b	0.07 b
TH	5.7 c	6.0 b	0.7 b	0.11 b
Control	16.3 a	11.6 a	3.9 a	0.78 a

* As described in footnote of Table (1).

Discussion

In the present study three chemical inducers were employed to induce onion resistance against postharvest bacterial rot diseases through preharvest application. The inducers used were sodium salicylate (SS) as salt of salicylic acid, ascorbic acid (AA) and thiamine hydrochloride (TH). Beside the basic role of the chemical inducers in stimulating the natural diseases resistance of plant, they improved the plant growth which can be indicated by an increase in the photosynthetic pigments content and increasing the foliar growth parameters; as shown by an increase in the number and length of onion leaves. These results are in agreement with those of Abd El-Hai *et al*. (2009) who found that salicylic acid at 10 mM significantly increased the photosynthetic pigments content in sunflower and had a role in enhancing its morphological characters, as plant height and No. of leaves per plant. Also Alhakimi and Alghalibi (2007) estimated the enhancement of the photosynthetic pigments content in broad bean under application of salicylic acid and thiamin (vitamin B1). The increment in the photosynthetic pigments content upon the application of the inducers may be due to stabilizing and protecting the photosynthetic pigments and the photosynthetic apparatus from being oxidized (Hamada and Hashem, 2003). Ascorbic acid (vitamin C) was believed to play a vital role in improving plant growth; De Cabo *et al*. (1996) gave an explanation that ascorbic acid is involved in the control of plant cell growth and development, for instance, it regulates the biosynthesis of hydroxyproline-rich proteins required for the progression of G1 and G2 phases of the cell cycle in several plant roots. In this regard, Shahda (2000) found that AA and SA when used as soil drench at 20 mM exhibited growth promoting effect on tomato; they increased shoot and root length and dry weight.

In the present study SS and AA were found to completely inhibit the growth of *Burkholderia cepacia* (Bc) at 20 and 10 mM, respectively, the matter which confirm their antimicrobial activity and clarify one mode of action in controlling plant diseases by direct inhibitory effect on the growth of phytopathogenic microorganisms, these results confirmed by Matthew and Alexander (1999). In the same concern El-Mougy (2002) stated that salicylic acid (SA) and acetylsalicylic acid decreased significantly the mycelium linear growth of some plant pathogenic fungi, their growth was completely inhibited at 20 mM of SA and ASA, also SA and ASA showed significantly inhibitive effect against some plant pathogenic bacteria; *Bacillus polymyxa*, *Erwinia carotovor a*s subsp. *carotovora* and *Pseudomonas solanacearum* which failed to grow when SA and ASA were added to the growth liquid medium at concentration of 30 mM. Also, Shahda (2000) found that SA and AA significantly reduce the linear growth of *Fusarium oxysporum*, *F. solani* and *Rhizoctonia solani* and the spore germination of *Fusarium* spp. at 20 mM.

The current study demonstrated that sodium salicylate (SS) at 3 mM reduced the onion bacterial rot incidence throughout storage of pots and field-grown onion bulbs. This is in harmony with Al-Mughrabi (2008) who established the potential of the exogenous application of salicylic acid and its derivatives in controlling plant diseases through induction of plant's own defence against pathogens. SA is critical for the survival of plants under abiotic and biotic stress by acting as one of the main signalling compounds required for the activation of defence reactions. SA is a part of complex signalling cascade, which begins by the recognition of pathogen or other external inducers and leads to the activation of ultimate defence genes, *e.g.* those encoding pathogenesis- related (PR) proteins (Hukkanen, 2008). SA is accumulated at high concentrations in the immediate vicinity of incompatible infection sites and is considered a key endogenous regulator of defence responses, being involved both in localized defences and systemically acquired resistance (Chen *et al*., 1995). Our results showed that treatment of onion with SS significantly increased the total phenol contents in onion bulbs, also significantly reduced extend of lipid peroxidation and electrolyte leakage (Table 4) confirming its resistance inducing activity. According to Matern and Kneusal (1988), the first step in the defence mechanism in plants involves a rapid accumulation of phenols at the infection site, which restricts or slows the growth of the pathogens. This effect might be due to the impact of these substances on the enzymatic activity and translocation of the metabolites. Phenolics are well-known as antifungal, antibacterial and antiviral compounds occur naturally in plants (Sivaprakasan and Vidhyasekaran, 1993). Our results regarding the potentiality of SS in reducing disease incidence is in concord with Zhang *et al*. (2007) who established the ability of SS in reducing fusarium head blight of wheat in the greenhouse, and Abo-Elyousr *et al*. (2009) who used SS at 5 mM as seed dresser to control cotton root rot disease. Hajhamed *et al*. (2007) used SA for suppression of the bacterial soft rot disease of potato caused by *Erwinia carotovora* subsp. *carotovora*, the disease severity was completely reduced when SA was applied at 0.9 mM, before or at the same time or after inoculation with the pathogen. Yao and Tian (2005) found that the efficacy of inducing resistance in sweet cherry fruit pre-harvest-treated with SA to *Monilinia fructicola* was better than that of fruit with post-harvest treatments, as the pre-harvest treatment induced β-1,3-glucanase, phenylalanine ammonia-lyase (PAL) and peroxidase (POD) activities during the early storage time.

The present investigation proved that AA at 5 mM decreased the onion bacterial rot incidence throughout storage compared to the control. It is evident that AA has been found to alter the redox potential and to improve plant tolerance under various types of stress and to induce plant resistance against pathogens attack (De Cabo *et al*., 1996 and Shahda, 2000). Treatment of onion with AA resulted in significant increase in the total phenols content and significant decrease in the extend of lipid peroxidation and the electrolyte leakage, the matter which proves the ability of AA to induce the natural resistance in plant and its antioxidant activity which can resist the oxidative stress in plant subjected to pathogen infection. These results are in harmony with Shahda (2000) who found that AA significantly reduced tomato damping- off when used as soil drench at 20mM and Saber *et al*. (2003) who demonstrated that AA decreased the disease incidence and severity of strawberry fruit rots.

Application of thiamine hydrochloride (TH) at 1mM in the current study on onion resulted in significant reduction in the bacterial rot diseases throughout storage, the matter which can be explained by the significant increase in the total phenols content and the decrease in the lipid peroxidation and electrolyte leakage in the TH-treated plants comparing with the untreated control plants. These results confirm the successful role of TH in inducing the natural resistance of plant against diseases and agreed with Hashem and Hamada (2002) who used TH for controlling root rot disease of wheat under field condition and found that thiamin significantly reduced the disease severity during seedling, flowering and ripening stage. Also Alhakimi and Alghalibi (2007) used thiamin for controlling broad bean rot disease caused by *Fusarium solani* and *Rhizoctonia solani*. Ahn *et al*. (2005) reported that thiamine induces systemic acquired resistance (SAR) in plants. Thiamine-treated rice, arabidopsis and vegetable crop plants showed resistance to fungal, bacterial, and viral infections. Thiamine treatment potentiates stronger and more rapid pathogenesis-related (PR) genes expression and the up-regulation of protein kinase C activity. The effects of thiamin on disease resistance and defense-related gene expression mobilize systemically throughout the plant.

In conclusion, the chemical inducers used in this study; sodium salicylate, ascorbic acid and thiamin hydrochloride played a successful role in inducing onion resistance against bacterial rot diseases, so they are recommended to be used as alternatives and safe chemicals for plant diseases control toward effective and ecologically-friendly plant protection and hence for sustainable agriculture.

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استحثاث مقاومة البصل ضد أمراض الأعفان البكتيرية للأبصال باستخدام المستحثات الكميائية

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تعد أمراض الأعفان البكتيرية من أخطر الأمراض التى تصيب محصول البصل أثناء التخزين حيث أنها تؤدى إلى فقد كبير فى الإنتاج. فى هذه الدراسة تم اختبار كلا من ساليسلات الصوديوم كأحد أملاح حمض السالسليك ، وحمض الأسكوربكوهيدوكلوريد الثيامين كمستحثات كميائية للمقاومة على نبات البصل تحت ظروف الحقل لتقييم قدرة هذه المواد على مقاومة الأعفان البكتيرية. وقد وجد بالفعل أن لها تأثير واضح فى إختزال نسبة حدوث أمراض الأعفان البكتيرية فى الأبصال المخزنة. كما برهنت النتائج على أن هذه المعاملات أدت إلى زيادة معنوية فى كمية الفينولات الكلية وإلى نقص معنوى فى أكسدة الدهون ونسبة تسرب الألكتروليتات فى الأبصال المعاملة الأمر الذى يدل على قدرتها على استحثاث المقاومة الطبيعية فى النبات. كذلك قد وجد أن النباتات المعاملة بهذه المستحثات أدت إلى زيادة فى كمية أصباغ البناء الضوئى والنمو الخضري بمقارناً بالنباتات الغيرمعاملة مما يؤكد دور هذه المستحثات الكميائيه فى تحسين نمو النبات. من هذه الدراسه يمكن إستنتاج أن تطبيق بعض المواد الكميائية لإستحثاث المقاومة فى النبات يعد وسيلة أمنة وفعالة فى مقاومة أمراض النبات مما يساعد فى الحد من التأثيرالضار للمبيدات الكميائية.