Journal of Stress Physiology & Biochemistry, Vol. 10 No. 1 2014, pp. 230-243 ISSN 1997-0838

ORIGINAL ARTICLE

Faba Bean Can Adapt to Chocolate Spot Disease by Pretreatment with Shikimic and Salicylic Acids through Osmotic Adjustment, Solutes Allocation and Leaf Turgidity

Heshmat S. Aldesuquy*, Zakaria A Baka, Mohmad A. Abass,

Nahla T. Alazab

Botany Department, Faculty of Science, Mansoura University, Egypt, P.O. Box 35516

Fax No: +2- 050-2246254; Mobile No: +201006573700, +2- 050 -2223786

*E-Mail: heshmat-aldesuquy@hotmail.com, Aboneel@yahoo.com

Received October 27, 2013

This study investigated the effect of shikimic and salicylic acids at the concentrations of 0.4 and 0.7 mM, respectively, or their combination as phenolic compounds and Ridomil MZ at the concentration of 250 g/100 L as a fungicide on osmotic pressure (OP), solutes allocation, organic acids, inorganic ions and relative water content were quantified in *Vicia faba* leaves infected by *Botrytis fabae*. Pathogen induced noticeable decrease in osmotic pressure, total soluble sugar (TSS) and inorganic osmolytes (i.e. Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^-) while caused obvious increase in proline, total soluble nitrogen (TSN) and organic acids (i.e. Keto and citric acids) in water extract of the leaf of faba bean plants. Furthermore, pathogen caused marked decrease in relative water content (RWC) of infected leaves and as a consequence the saturation water deficit (SWD) was increased. Exogenous application of shikimic acid, salicylic acid or their combination could counteract the adverse effects of *B. fabae* on osmotic adjustment by inducing additional increase in proline, total soluble sugars, total soluble nitrogen and organic acids which in turn increase the osmotic pressure as well as relative water content in leaves of infected plants. Recovery of osmotic adjustment as well as leaf turgidity of infected host by using these chemical inducers may encourage the using of them as protective control means. The results of the present study showed also that the application of chemical inducers such as shikimic and salicylic acids or their interaction increased the resistance of *Vicia faba* against the chocolate spot disease.

Key words: Botrytis fabae, Salicylic acid, Shikimic acid, Osmotic adjustment, Leaf turgidity

ORIGINAL ARTICLE

Faba Bean Can Adapt to Chocolate Spot Disease by Pretreatment with Shikimic and Salicylic Acids through Osmotic Adjustment, Solutes Allocation and Leaf Turgidity

Heshmat S. Aldesuquy*, Zakaria A Baka, Mohmad A. Abass, Nahla T. Alazab

Botany Department, Faculty of Science, Mansoura University, Egypt, P.O. Box 35516

Fax No: +2- 050-2246254; Mobile No: +201006573700, +2- 050 -2223786

*E-Mail: **heshmat-aldesuquy@hotmail.com**, **Aboneel@yahoo.com**

Received October 27, 2013

This study investigated the effect of shikimic and salicylic acids at the concentrations of 0.4 and 0.7 mM, respectively, or their combination as phenolic compounds and Ridomil MZ at the concentration of 250 g/100 L as a fungicide on osmotic pressure (OP), solutes allocation, organic acids, inorganic ions and relative water content were quantified in *Vicia faba* leaves infected by *Botrytis fabae*. Pathogen induced noticeable decrease in osmotic pressure, total soluble sugar (TSS) and inorganic osmolytes (i.e. Na^{+}, K^{+}, Ca^{2+}, Mg^{2+} and Cl^{-}) while caused obvious increase in proline, total soluble nitrogen (TSN) and organic acids (i.e. Keto and citric acids) in water extract of the leaf of faba bean plants. Furthermore, pathogen caused marked decrease in relative water content (RWC) of infected leaves and as a consequence the saturation water deficit (SWD) was increased. Exogenous application of shikimic acid, salicylic acid or their combination could counteract the adverse effects of *B. fabae* on osmotic adjustment by inducing additional increase in proline, total soluble sugars, total soluble nitrogen and organic acids which in turn increase the osmotic pressure as well as relative water content in leaves of infected plants. Recovery of osmotic adjustment as well as leaf turgidity of infected host by using these chemical inducers may encourage the using of them as protective control means. The results of the present study showed also that the application of chemical inducers such as shikimic and salicylic acids or their interaction increased the resistance of *Vicia faba* against the chocolate spot disease.

Key words: Botrytis fabae, Salicylic acid, Shikimic acid, Osmotic adjustment, Leaf turgidity

Abbreviations: Relative water content= RWC, Salicylic acid= SA, Saturation water deficit= SWD, Shikimic acid = SH

Faba bean (*Vicia faba* L.) is one of the oldest and the seventh most important seed legumes in the world. In the Middle East it is a major source of protein in the human diet, whereas in Europe and other developing countries it is used primarily as animal feed. Faba bean is an attractive crop for farmers because of its potential to produce high seed yields in dry environments, for rotation benefits derived from breaking disease cycles of cereals, and for adding significant amounts of fixed nitrogen (N) to soils of declining fertility (Yakop, 2009). This crop is attacked by a number of plant pathogens and parasites. The most important fungal disease is chocolate spot caused by *Botrytis fabae.* Chocolate spot is the most destructive disease and causes considerable losses in the yield of faba bean in the northern region of the Nile Delta of Egypt, where low temperature and high relative humidity favoring its spread are prevailing. This disease is a limiting factor for faba bean production in north and middle Egypt (Attia *et al*., 2007).

Because of hazards of pesticides in general, and fungicides in specific, on public health and environmental balance, a relatively recent direction of pest control management was introduced. The so called "induced resistance" is a promising modern approach with a broad spectrum in plant disease control. It could be induced in plants by applying chemical elicitors (Reglinski *et al*., 2001). Chemical elicitors (inducers) have been used to predispose the defense mechanisms in plants against diseases.

Induced resistance may affect other growth parameters; chlorophyll content, plant growth, accumulation of antifungal compounds and increasing activity of oxidative enzymes (Malolepsza and Urbanek, 2002; Fariduddin *et al*., 2003). Plants have developed various combating mechanisms to survive with the deleterious effects of stress. Among these, osmotic adjustment (OA) is one of the strategies that have been a potential defense toward salt stress (Aldesuquy *et al*., 2012). According to Blum *et al.* (1996), OA is usually defined as a decrease in cell sap osmotic potential resulting from a net increase in intracellular solutes rather than from a loss of cell water. The former may operate through the concentration accretion of inorganic and/or organic solutes (Ben Khaled *et al*., 2003). As a consequence, the cell's osmotic potential is diminished which in turn attracts water into the cell by tending to maintain turgor pressure (Pérez-Pérez *et al*., 2007). Furthermore, Munns (2005), compatible solutes like sugars, amino acids, organic acids and inorganic ions can contribute to this process as well as glycerol (Martnez et al., 2005) and fatty acids.

So far, limited studies on the effect of phenolic compounds on osmotic adjustment, solutes allocation and relative water content in infected leaves of host as a quick response to pathogen have been reported. The present investigation aimed to evaluate shikimic and salicylic acids as chemical elicitors compared with Ridomil MZ as a fungicide on osmotic adjustment and solutes allocation in response to infection of *Vicia faba* by *Botrytis fabae*.

MATERIALS AND METHODS

Planting and growth conditions. Seeds of faba bean (*Vicia fabae* L.) susceptible [G_{429}] were surface sterilized in 0.01% mercuric chloride for 3 min., subsequently rinsed with sterilized water several times. Sterilized seeds were divided into 4 sets. Seeds of the 1st , 2nd,3rd and 4th set were soaked in distilled water, 0.4 mM shikimic acid (SH), 0.7 mM salicylic acid (SA) and shikimic acid + salicylic acid (SH+SA), respectively, for 12 hrs. On 14 November 2008, seeds of each set were planted in plastic pots (5 seeds per pot) filled with 12-13kg mixed soil (clay and sand 2:1, *v/v*). All plants were watered regularly to near field capacity with tap water. Plants were maintained under natural conditions (day temperature 22^{0}C, night temperature 18^{0}C and 16 hrs photo period).

Forty five days later from planting, faba bean plants were inoculated with a spore suspension (40 x 10^4 spores ml^{-1}) of *B. fabae*. Untreated plants (control) were sprayed with sterilized water at the same time. Chemical fungicide Ridomil MZ at the rate of (250g 100 L^{-1}) was applied as spray treatments four times at 15-day intervals.

The experimental design can be summarized as follows:

Uninoculated treatment (control) (Cont)

B. fabae treatment (pathogen) (P)

Shikimic acid treatment (SH)

Shikimic acid + *B. fabae* treatment (SH+P)

Salicylic acid treatment (SA)

Salicylic acid + *B. fabae* treatment (SA+P)

Salicylic acid +Shikimic acid treatment (SA+SH)

Salicylic acid +Shikimic acid+ *B. fabae* treatment (SA+SH+P)

Fungicide (Ridomil MZ) (F)

10-Fungicide +B. fabae treatment (F+ P)

Samples from the 3rd compound leaf were harvested after 10 weeks from planting for measurements of osmotic pressure and other osmolytes.

The osmotic pressure of flag leaf sap was measured by the cryoscopic method (Walter, 1949).

Total soluble sugars was extracted and determined by anthrone method of Riazi *et al*. (1985).

The total soluble nitrogen was determined by the conventional semi micro-modification of Kjeldahl method (Pine, 1955).

The method adopted for estimation of proline was essentially that described by Snell and Snell (1954).

Keto-acids were determined according to the method adopted by Friedman and Haugen (1943). Furthermore, the method adopted for estimation of citric acid was essentially that described by Snell and Snell (1949).

The extracts of the experimental plants were analyzed for the cations: Na^+, K^+, Ca^{+2} and Mg^{+2} measured by flam emission spectrophotometery according to the method described by Chapman and Pratt (1978) and the anions Cl^- chlorides were determined by the $AgNO_3$ titration method as described by Hansen and Munns (1988).

Relative water content (RWC %) and saturation water deficit (SWD %) were determined according to the method adopted by Weatherly (1950).

Statistical analysis. A test for significant differences between means at $P \leq 0.05$ was performed using least significant difference (LSD) test (Snedecor and Cochran, 1976) using SPSS program.

RESULTS

Changes in osmotic pressure. Fungicide with pathogen as well as pathogen alone induced a noticeable decrease ($P \leq 0.05$) in osmotic pressure as compared to control. Treatment with salicylic acid, shikimic acid or their combination caused an obvious increase ($P \leq 0.05$) in osmotic pressure of infected plants or healthy plants. Application of salicylic acid had the most noticeable effect in increasing osmotic pressure than shikimic acid or shikimic + salicylic as shown in table 1.

Changes in organic osmolytes

Changes in proline. As compared to the control values, fungicide caused insignificant increase in proline content of faba bean leaf extract whereas, its application on infected plants caused a significant increase as well as pathogen alone. Application of salicylic acid or shikimic acid induced a marked increase in proline content either in infected or controlled plants while, their combination led to significant and insignificant increase in infected and controlled plants, respectively. Salicylic acid represents the most effective treatment in enhancing proline accumulation in faba bean leaf extract (Table 1).

Changes in total soluble sugars. The pattern of results in table 1 cleared that fungicide + pathogen as well as pathogen alone induced marked decrease in total soluble sugar. Moreover, application of shikimic acid, salicylic acid or their combination showed an additional increase ($P \leq 0.05$) in the total soluble sugars (TSS) of healthy faba bean leaf extract and insignificant decrease in TSS of infected plants as compared to control values. The effect of salicylic acid appeared to be the most effective treatment on production of total soluble sugars in faba bean leaf extract.

Changes in total soluble nitrogen. In relation to control values, *B. fabae* induced a significant increase ($P \leq 0.05$) in total soluble nitrogen in faba bean leaf extract (Table 1). Treatment with shikimic acid caused insignificant increase in total soluble nitrogen, while plants treated with salicylic acid or shikimic+salicylic showed a massive increase in the total soluble nitrogen. The infected plants treated with shikimic acid or salicylic acid and their combination showed additional increase in total soluble nitrogen. In general, the highest values were recorded with salicylic acid treatment.

Changes in organic acids

Changes in keto-acids. As compared with control values, data presented in table 1 showed that keto-acids were accumulated in response to infection. Shikimic acid, salicylic acid or their combination induced an obvious increase ($P \leq 0.05$) in keto-acids level in controlled and infected plants. The effect was more pronounced with salicylic acid treatment in comparing with all treatments.

Changes in citric acid. In relation to control values, fungicide shikimic acid alone as well as shikimic acid + salicylic acid showed insignificant increase in citric acid content of healthy faba bean plants (Table 1). On the other hand, pathogen led to significant increase in citric acid content and this effect was progressively increased in infected plants treated with shikimic acid, salicylic acid alone or their combination .Salicylic acid caused the higher production in citric acid content of faba bean plants as compared to other treatments.

Changes in ions content. As compared to control values, data obtained in table 2 showed that, the total content of all measured minerals were significantly decreased by the pathogen alone or in the presence of fungicide. Moreover, plants treated with shikimic acid, salicylic acid or their combination showed non-significant increase ($P \leq 0.05$) in Na^+ and Cl^- and obvious increase in K^+, Ca^+ and Mg^{++} content. Seed presoaking in shikimic acid, salicylic acid and shikimic acid + salicylic acid caused marked increases ($P \leq 0.05$) in K^+, Ca^+ and Mg^{++} content and insignificant increase in Na^+ and Cl^-. In the majority of cases, treatment with phenolic acids led to partial alleviation of this adverse effect in mineral content of infected plants, as compared with untreated infected plants. Furthermore, the highest values were recorded in the following order, salicylic acid > shikimic acid > shikimic acid + salicylic acid.

As shown in table 3 osmotic pressure appeared to be positively correlated with the TSS (r=0.81), ions (r =0.77-0.86) and has a weak correlation with (proline, TSN, keto-acid and citric acid).

Changes in leaf turgidity

Changes in relative water content (RWC %). The results in table 4 clearly showed that the pathogen as well as fungicide + pathogen induced significant decrease (P≤0.05) in RWC (%) of faba bean leaves as compared to control value. while, the reduction wasn't significant in case of fungicide alone. On the other hand, application of shikimic acid and salicylic acid caused significant increase (P≤0.05) in RWC of controlled plants and their combination had no significant effect. Comparing with control diseased plants, seed priming in shikimic acid, salicylic acid or their combination led to an obvious increase in RWC. The magnitude of increase was more pronounced with salicylic acid treatment than shikimic acid treatment or their combination in both controlled and infected plants.

Changes in saturation water deficit (SWD %). It is clear from results in table 4 that there is a massive increase (P≤0.05) in SWD in leaves of infected faba bean plants than those infected and sprayed with fungicide. On the other hand, SWD was significantly decreased in faba bean plants treated with shickimic acid and salicylic acid as compared to control plants. Furthermore, the application of salicylic acid and shikimic acid or their combination caused a marked reduction in SWD of infected plants as compared to untreated infected ones.

Table 1 : Effect of shikimic acid, salicylic acid and their combination on osmotic pressure (atm) and some osmolytes (proline, total soluble sugars, total soluble nitrogen and organic acids) (mg g^{-1}d wt) in leaf extract of faba bean plants infected with chocolate spot disease.

Parameters / Treatments	Osmotic pressure (atm)	Proline (mg g^{-1} dwt)	TSS (mg g^{-1} dwt)	TSN (mg g^{-1} dwt)	Organic acid (mg g^{-1} dwt)	
					Keto-acid	Citric acid
Cont	1.44	0.311	138.24	7.58	0.533	7.79
P	1.33	0.475	80.62	8.48	0.639	9.01
F	1.42	0.323	133.33	7.66	0.541	7.85
F+P	1.36	0.488	92.24	8.66	0.643	9.42
SH	1.66	0.358	144.05	7.71	0.601	8.14
SH+P	1.52	0.537	139.66	8.91	0.678	10.05
SA	1.67	0.449	152.45	8.26	0.611	8.61
SA+P	1.61	0.576	142.11	9.53	0.712	11.54
SH+SA	1.54	0.333	145.34	7.95	0.578	7.99
SH+SA+P	1.47	0.521	139.92	8.78	0.650	9.86
LSD at P≤0.05	0.03	0.027	5.42	0.28	0.014	0.38

Table 2 : Effect of shikimic acid, salicylic acid and their combination on ionic content (mmole g^{-1} d wt) in leaf extract of faba bean plants infected with chocolate spot disease.

Parameters / Treatments	Ionic content (mmole g^{-1} d wt)				
	Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-
Cont	5.04	15.62	7.20	8.43	0.215
P	3.11	10.93	5.71	5.47	0.145
F	4.70	14.89	7.15	8.04	0.204
F+P	3.89	11.81	6.04	5.95	0.161
SH	5.08	16.91	7.94	9 .23	0.216
SH+P	4.62	13.87	6.96	8.44	0.193
SA	5.17	18.05	8.17	9.50	0.219
SA+P	4.89	14.66	7.16	9.01	0.210
SH+SA	5.10	15.83	7.80	8.96	0.217
SH+SA+P	4.06	13.41	6.55	8.72	0.184
LSD at P≤0.05	0.20	0.35	0.26	0.23	0.005

Table 3 : Coorelation coefficient (r) between osmotic pressure, ions and some osmolytes in leaf extract of faba bean plants infected with chocolate spot disease.

	Osmotic pressure
Variables	r
Osmotic pressure	1.00
Proline	0.03
TSN	0.04
TSS	0.81
Keto-acids	0.15
Citric acid	0.10
Na^+	0.77
K^+	0.83
Ca^{++}	0.86
Mg^{++}	0.86
Cl^-	0.78

Table 4 : Effect of shikimic acid, salicylic acid and their combination on relative water content (RWC %) and saturation water deficit (SWD %) of leaves of faba bean infected with chocolate spot disease.

Parameters / Treatments	**RWC (%)**	**SWD (%)**
Cont	95.11	4.89
P	75.73	24.27
F	93.94	6.06
F+P	83.72	16.28
SH	97.15	2.85
SH+P	93.31	6.68
SA	98.14	1.85
SA+P	95.09	4.91
SH+SA	96.52	3.47
SH+SA+P	94.78	5.22
LSD at P≤0.05	1.62	1.61

DISCUSSION

In the present study, pathogen induced a noticeable decrease in osmotic pressure, total soluble sugar (TSS) and inorganic osmolytes (i.e. Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^-) while induced obvious increase in proline, total soluble nitrogen (TSN) and organic acids (i.e. Keto and citric acids) in water extract of the 3rd compound leaf of faba bean plant. In this connection, Floerl *et al*. (2010) observed that infection of *Arabidopsis thaliana* with *Verticillium longisporum* resulted in a slight reduction in the osmotic pressure of infected leaves. On contrary to these results, Farrell *et al*. (1969) noticed that infection of potato by the late blight pathogen *Phytophthora infestans* induced the accumulation of soluble sugars contributing to an increase of the osmotic potential. In addition**,** infection by pathogenic fungi may lead to substantial changes in carbohydrates content of infected plants which may reflect an alteration in the different metabolic processes that are favourable or unfavourable for fungal development (Aldesuquy and Baka, 1992).

Results revealed that, *B. fabae* infection reduced the total soluble sugars content of faba bean plants as compared to control plants. These results are in a good agreement with the findings of El-Fallal and Migahed (2003). This reduction could be attributed to the diminished chlorophyll content (Baka *et al*., 2012) due to the infection which consequently led to the reduction of photosynthetic rate (Aly *et al*.,

2003) and/or the activities of carboxylase or dehydrogenase enzymes of CO_2 fixation. Another interpretation was coming from the fact that the diminished sugar content could also be derived for the higher supply of the fungus or due to the increase in respiration of infected plant (Aldesuquy and Baka, 1992). These results are in alliance with those of Baka and Aldesuquy (1992) who reported that the noticed decrease in glucose, sucrose and TSS in infected plants may be attributed to demands of host tissues for energy and for biosynthetic intermediates for growth and sporulation of the pathogen. The enhancement in chlorophyll formation will result in increasing the efficacy of photosynthetic apparatus with a better potential for resistance as well as decreasing photophosphorylation rate, which occurred after infection (Amaresh and Bhatt, 1998).

It is evident from the present data that all the tested phenolic compounds surpassed the control of all tested metabolic aspects. This could be attributed to the effect of these compounds, which may increase TSS content via increasing chlorophyll content, and the IAA hormone by inhibiting the enzyme IAA oxidase. This leads to the inhibition of chlorophyll breakdown as IAA protects chlorophyll and thus increasing in chlorophyll content may increase the amount of CO_2 fixed during photosynthesis hence carbohydrates accumulation may also increase, as was reported by Abou-Grab *et al*. (1997). Furthermore, this increase may be attributed to the antagonistic effect of shikimic acid or salicylic acid and their combination on *B. fabae*. Chandra and Bhatt (1998) reported that SAR-protected plants when infected with a pathogen by exhibiting morphological and biochemical changes such as increasing glucose and fructose concentrations in systemic tissue.

Soluble sugars are involved in the responses to a number of stresses, and act as nutrient and metabolite signaling molecules that activate specific or hormonal- crosstalk transduction pathways, resulting in important modifications of gene expression (Couée *et al*., 2006). In the present study, the increase of soluble sugars in plants treated with phenolic compounds could be related to the high chlorophyll content (Baka *et al*., 2012) and consequently increased photosynthetic rate. These results revealed that accumulation of soluble sugars in infected faba bean plants especially when treated with phenolic compounds indicated the relationship between sugar regulation and activation of the systemic resistance.

Results of the present study showed that, proline content of faba bean plants was increased by *B. fabae* infection and application of the phenolic compounds (shikimic acid, salicylic acid or their combination) caused additional increase in proline content. These results are confirmed by Ali *et al*. (2007) who observed an increase in proline content in ginseng root treated with salicylic acid. Furthermore, similar results were obtained by Faheed *et al*. (2005) who reported that proline accumulation seems to be greater when stimulated by applying low concentration of salicylic acid on tomato plants. This conclusion is in agreement with other studies, which documented the importance of salicylic acid in pathogen-induced disease resistance and hypersensitive cell death (Fabro *et al*., 2004). Furthermore, According to Fabro *et al*. (2004); Souza *et al*. (2004)**,** proline accumulation in plant tissues may increase the plant tolerance to several stresses, such as biotic or abiotic stress. In addition, proline accumulation was faster and stronger when stimulated by adding low concentration of salicylic acid to plants.

The rapid breakdown of proline upon relief of stress may provide sufficient reducing agents that support mitochondrial oxidative phosphorylation and generation of ATP for recovery from stress and repairing of stress-induced damages (Hare *et al*., 1998).

Proline concentration in foliar tissues of pepper plants inoculated with *Verticillium dahliae* increased significantly between days 21 and 28 after inoculation, while they did not change in the leaves of control plants (Goicoechea *et al*., 2000) whereas, in cotton, higher proline levels were detected in leaves from inoculated plants with *Verticillium* than in those from controls (Tzeng *et al*.,1985). Accumulation of large amounts of osmolytes (proline) is an adaptive response in plants exposed to stressful environments (Jaleel *et al*., 2007). Ashry and Mohamed (2011) suggested that the amino acid proline may act as a potent scavenger may also function as a protein compatible hydrotrope and as a hydroxyl radical scavenger. In any case the higher proline accumulation in diseased tissue might be related to pathological disorder of ROS and this property of proline might prevent the induction of programmed cell death by ROS.

The present results indicate that, infection led to massive increase in total soluble nitrogen in leaves of faba bean plants. These results are in consistence with the finding of Reddy *et al*. (2005) who reported that the increase in soluble nitrogen may be ascribed to increased hydrolysis of proteins.

In the present study, application of phenolic compounds caused a significant increase in total soluble nitrogen which are consistent with those of Abd-Alla (1994) who concluded that phenolic compounds at low concentration (100 µM) can be used to improve soybean crop performance by stimulating nodulation, plant growth and ammonia-assimilation in addition to enhancing nitrate reductase and glutamate dehydrogenase activities.

The presented data showed that, the organic acid increased after infection. In this respect, organic acid is not only important osmotic regulator (Morgan, 1984) but also, is an important negative charge contributors, playing important roles in ionic balance and pH adjustment (Venekamp *et al*., 1989). The increase in citric acid content might be explained on the fact that the role of citric acid decarboxylation to increase CO_2 concentration in mesophyll during the day while night time citric acid accumulation increases the buffer capacity of the vacuole (Franco *et al*., 1992).

The variation in mineral ions (Na^+, K^+, Ca^{++}, Mg^{++} and Cl^-) concentrations in plant leaves under infection by *B. fabae* as well as treatment with shikimic acid or salicylic acid and their combination could be related to the influence of these treatments on the uptake and/or the metabolism of such minerals by the faba bean plants. The adverse effect of *B. fabae* on mineral accumulation in the plant might be due to the consumption of such minerals by the fungus to build its own metabolites as it was suggested by Mahmoud *et al*. (2004). Moreover, water stress developed as a result of plant infection apparently inhibits the mechanism of uptake of nutrients (Hsiao 1973).

Reduction in K^+ and Ca^{2+} contents in infected faba bean tissues might be related to toxins produced by the fungi which affected K^+ uptake and stomata function leading to uncontrolled transpiration and excessive loss of water (Aducci *et al.* 1997). While phenolic compounds increased K^+ contents in infected plants could be via increasing the level of K^+ impede the flow of nutrients from host to pathogen and retard the spread of the pathogen and its toxins (Nafie 2003). Also, an adequate K^+ supply dose promote cell wall thickening that helps plant to resist disease (El-Khallal 2007a). However, low level of Ca^{2+} content in all infected plants as compared with non-infected plants (healthy control) might be responsible for decreasing the integrity of cell membranes and hence increase their permeability and induced leakage from tissues involved by *B. fabae*.

The present results revealed that the application of shikimic acid, salicylic acid or their combination could be effective in enhancing the uptake of some inorganic nutrients which play a role in decreasing the incidence of chocolate spot disease. Thus, improvement in plant nutrition can enhance plant development and also might increase the plant resistance against the effect of disease. Similar results were obtained by El-Khallal (2007) who found that salicylic acid improve the uptake of some inorganic element by tomato plants infected with *Fusarium oxysporum*. In the majority of cases, treatment with phenolic acids led to partial alleviation of this adverse effect in mineral content of infected plants, as compared with untreated infected plants. Furthermore, the highest values were recorded in the following order, salicylic acid > shikimic acid > shikimic acid + salicylic acid.

Salicylic acid is presumed to play a role in controlling ion uptake by roots and stomatal conductivity (Raskin 1992). Mori *et al*. (2001) reported that application of salicylic acid stimulates stomatal closure in many plants. In addition, results revealed that, osmotic pressure appeared to be positively correlated with TSS as well as inorganic ions.

The present investigation clearly showed that RWC was greatly reduced by infection with *B. fabae* and as a consequence the SWD was increased. Similar results were obtained by El-Hendawy (1999) who found that inoculation of cucumber seedlings with *Xanthomonas campestris* resulted in reduction of relative water content (RWC) of infected cotyledons. The deficiency of water in infected plants was directly proportional with disease severity. In relation to this, Aldesuquy and Baka (1992) concluded that, the imbalance in water relations resulted from the alterations in cell permeability which appeared as characteristic of diseased plants. Furthermore, Mayek-Perez *et al*. (2002) noticed slight reduction in relative water content in common bean (*Phaseolus vulgaris* L.) infected with *Macrophomina phaseolina* than controlled uninfected plants. Shabana *et al*. (1997) attributed the increase in SWD in infected water hyacinth plants than healthy ones to alteration caused by the pathogen in the nutrient, mineral, photosynthetic and hormonal relations of the plant.

Application of shikimic acid, salicylic acid or their combination increased the leaf turgidity of healthy and infected faba bean plants by increasing its RWC. These results are similar to those obtained by Aldesuquy *et al*. (1998) using wheat plants grown under salinity conditions and treated with antitranspirants (sodium salicylate or ABA).

As shown in table 3, osmotic pressure appeared to be positively correlated with the TSS (r=0.81), ions (r =0.77-0.86) and has a weak correlation with (proline, TSN, keto-acid and citric acid).

It can be concluded from this study that seed presoaking in phenolic compounds such as shikimic acid, salicylic acid or their combination displayed a positive role in increasing the resistance of infected faba bean plant towards *B. fabae* infection by alleviating the stress induced by the pathogen on plant growth vigor and internal ultrastructure of mesophyll cells of faba bean plants (Baka *et al*., 2012). Furthermore, the positive effects of the applied phenols on osmotic adjustment as well as leaf turgidity.

REFERENCES

Abd-Alla, M.H. (1994) Some phenolic compounds enhance nodulation and nitrogen fixation in a soybean / *Bradyrhizobium japonicum* system. *Phyton Ann Rei Bota*., **33:** 249-256.

Abou-Grab, O.S., Kady, F.A., El-Kafrawy, A.M. (1997) Effect of some aromatic compounds as antitranspiration on growth yield, chlorophyll content, water consumptive use and leaf blight disease for maize plant. *Zagazig J Agric Res*., **24:** 393-406.

Aducci, P., Ballio, A., Marra, M. (1997) Phytotoxins as molecular signals. In: Aducci, P. (Ed.): Signal Transduction in Plants. *Birkhauser Verlag, Basel* pp. 83-105.

Aldesuquy, H.S., Baka, Z.A.M. (1992) Physiological and biochemical changes in host leaf tissues associated with the growth of two biotrophic fungi growing in Egypt. *Phyton* **32:** 129-142.

Aldesuquy, H.S., Makarios, A.T., Awad, H.A. (1998) Effect of two antitranspirants on growth and productivity of salt treated wheat plants. *Egypt J Sci Physiol.,* **22:** 189-211.

Aldesuquy, H.S., Baka, Z.A.M., El-Shehaby, O.A., Ghanem, H.E. (2012) Efficacy of seawater salinity on osmotic adjustment and solutes allocation in wheat (*Triticum aestivum*) flag leaf during grain filling. *Inter J Plant Physiol and Biochem*., **4:** 33-45.

Ali, M.B., Hahn, E.J., Paek, K.Y. (2007) Methyl jasmonate and salicylic acid induced oxidative stress and accumulation of phenolics in *Panax ginseng* bioreactor root suspension cultures. *Molecules* **12:** 607-621.

Amaresh, C., Bhatt, R.K. (1998) Biochemical and physiological response to salicylic acid in relation to the systemic acquired resistance. *Photosynth*., **35:** 255-258.

Attia, M.F., Abou-Zeid, N.M., Abada, K.A., Coleman, M.H., El-Badawy, N.F. (2007) Isolation of chitinase gene induced during infection of *Vicia faba* by *Botrytis fabae*. *Arab J Biotech*., **10:** 289-300.

Ashry, N.A., Mohamed, H.I. (2011) Impact of secondary metabolites and related enzymes in flax resistance and or susceptibility to powdery mildew. *World J. Agric. Sci*., **7:** 78-85.

Baka, Z.A., Aldesuquy, H.S. (1992) Studies on powdery mildew fungus *Spherotheca fuliginea*: Ultrastructure and host responses. *Acta Bot Hung*., **37:** 15-31.

Baka, Z.A., Aldesuquy, H.S. , Abass, M.A., Alazab, N.A. (2012) Effect of phenolic compounds and fungicides on the ultrastructure of chloroplasts of *Vicia faba* infected by *Botrytis fabae*. *J Environ Sci*., **41:**37-54.

Ben Khaled, L., Morte-Gomez, A., Honrubia, M., Oihabi, A. (2003) Effet Cu stress salin en milieu hydroponique sur le trèfle inoculé par le Rhizobium. *Agronomie* **23:** 553- 560.

Blum, A. (1996) Crop response to drought and the interpretation of adaptation. *Plant Growth Regul*., **20:** 135- 148.

Chandra, A., Bhatt, R.K. (1998) Biochemical and physiological response to salicylic acid in relation to the systemic acquired resistance. *Photosynth*., **35:** 255-258.

Chapman, H.D., Pratt, P.F. (1978) Methods of analysis for soils, plants and waters. *Univ. California, Div. Agric. Sci.*

Couée, I., Sulmon, C., Gouesbet, G., El-Amrani, A. (2006) Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. *J Exp Bot*., **57:** 449-459.

El-Fallal, A.A., Migahed, F.F. (2003) Metabolic changes in broad bean infected by *Botrytis fabae* in response to Mushroom spent straw. *Asian J Plant Sci*., **2:** 1059-1068.

El-Hendawy, H.H. (1999) Water stress in cucumber cotyledons infected with *Xanthomonas compestris* pv. *Cucurbitae*. *Folia Microbiol*., **44:** 530-534.

El-Khallal, S.M. (2007) Induction and modulation of resistance in tomato plants against *Fusarium* wilt disease by bioagent fungi (*Arbuscular Mycorrhiza*) and/or hormonal elicitors (jasmonic acid & salicylic acid): 1- changes in growth, some metabolic activities and endogenous hormones related to defence mechanism. *Aust J Basic Appl Sci*., **1:** 691-705.

Fabro, G., Kovacs, I., Pavet, V., Szabados, I., Alvarez, M.E. (2004) Proline accumulation and ATPCS2 gene activation are induced by plant pathogen incompatible interactions in *Arabidopsis*. *Amer Phytopath Soc*., **17:** 343-350.

Faheed, F.A., Abd-Elaah, G.A., Mazen, A. (2005) Alleviation of disease effect on tomato plants by heat shock and salicylic acid infected with *Alternaria solani*. *Int J Agric Biol*., **7:** 783-789.

Fariduddin, Q., Hayat, S., Ahmad, A. (2003) Salicylic acid influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity and seed yield in *Brassica juncea*. *Photosynth*., **41:** 281-284.

Farrell, W.M. (1969) Effects of infection by *Phytophthora infestans* (Mont.) de Bary on the stomata of potato leaves. *Ann Appl Biol*., **63:** 265-275.

Floerl, S., Druebert, C., Aroud, H.I., Karlovsky, P., Polle, A. (2010) Disease symptoms and mineral nutrition in *Arabidopsis thaliana* in response to *Verticillium longisporum* VL43 infection. *Plant Pathol J*., **92:** 693-700.

Franco, A.C., Ball, E., Lüttge, U. (1992) Differential effects of drought and light levels on accumulation of citric and malic acids during CAM in Clusia. *Plant Cell Environ*., **15:** 821-829.

Friedman, T.E., Haugen, G.E. (1943) Pyruvic acid. II. The determination of keto acids in blood and urine. *J Biol Chem*., **147:** 415- 442.

Goicoechea, N., Aguirreolea, J., Cenoz, S., Garcia-Mina, J.M. (2000) *Verticillium dahliae* modifies the concentrations of proline, soluble sugars, starch, soluble protein and abscisic acid in pepper plants. *Eur J Plant Pathol*., **106:** 19-25.

Hansen, E.M., Munns, D.N. (1988) Effect of $CaSO_4$ and NaCl on mineral content of *Leucaena leucocephala*. *Plant and Soil* **107:** 101- 105.

Hare, P.D., Cress, W.A., Staden, J.V. (1998) Dissecting the roles of osmolyte accumulation during stress. *Plant Cell Environ*., **21:** 535-553.

Hsiao, T.C. (1973) Plant responses to water stress. *Ann. Rev. Plant Physiol*., **24:** 515-570.

Jaleel, C.A., Gopi, R., Sankar, B., Manivannan, P., Kishorekumar, A., Sridharan, R., Panneerselvam, R. (2007) Alterations in germination, seedling vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seedlings under salt stress. *South Afris J Bot*., **73:** 190-195.

Mahmoud, Y.A.G., Ebrahim, M.K.H., Aly, M.M. (2004) Influence of some plant extracts and microbiaogents on some physiological traits of faba bean infected with *Botrytis fabae*. *Turk J Bot*., **28:** 519-528.

Malolepsza, U., Urbanek, H. (2002) O-Hydroxyethylorutin-mediated enhancement of tomato resistance to *Botrytis cinerea* depends on a burst of reactive oxygen species. *J Phytopathol*., **150:** 616-624.

Martínez, J.P., Kinet, J.M. and Bajji, M., Lutts, S. (2005) NaCl alleviates polyethylene glycol induced water stress in the halophyte species (*Atriplex halimus* L.). *J Exp Bot*., **56:** 2421- 2431.

Mayek-Pérez, N., García-Espinosa, R., López-Castañeda, C., Acosta-Gallegos, J.A., Simpson. J. (2002) Water relations, histopathology and growth of common bean (*Phaseolus vulgaris* L.) during pathogenesis of *Macrophomina phaseolina* under drought stress. *Physiol. Mol. Plant Pathol*., **60:** 185-195.

Morgan, J. (1984) Osmoregulation and water stress in higher plants. *Ann Rev Plant Physiol*., **35:** 299-319.

Mori, I.C., Pinontoan, R., Kawano, T., Muto, S. (2001) Involvement of superoxide generation in salicylic acid-induced stomatal closure in *Vicia faba*. *Plant Cell Physiol*., **42:** 1383-1388.

Munns, R. (2005) Genes and salt tolerance: Bringing them together. *New Phytolog*., **167:** 645- 663.

Nafie, E.M. (2003) The possible induction of resistance in *Lupinus termis* L. against *Fusarium oxysporum* by *Streptomyces chibaensis* and its mode of action: 1. Changes in certain morphological criteria and biochemical composition related to induced resistance. *Int J Agric Biol*., **4:** 463-472.

Pérez-Pérez, J.G., Syvertsen, J.P., Botía, P., García-Sánchez ,F. (2007) Leaf water relations and net gas exchange responses of salinized Carrizo citrange seedlings during drought stress and recovery. *Annals Bot*., **100:** 335-345.

Pine, N.W. (1955) Proteins. In: Modem methods of plant analysis. (Peack, K. and Tracey, M. V., eds) IV, 23, *Springer Verlage, Berlin*.

Raskin, I. (1992) Role of salicylic acid in plants. *Annu. Rev. Plant Mol. Biol*., **43:** 439- 463.

Reddy, M.N., Sridevi, N.V., Devi, M.C. (2005) Changes in the nitrogen fractions and amino acid metabolism of turmeric (*Curcuma longa* L.) roots infected with *Fusarium solani*. *Plant Pathol Bull*., **14:** 221-226.

Reglinski, T., Whitaker, G., Cooney, J.M., Taylor , J.T., Pooles, P.R., Roberts, P.B., Kim, K.K . (2001) Systemic acquired resistance to *Sclerotinia sclerotiorum* in kiwi fruit vines. *Physiol. Mol. Plant Pathol*., **58:** 111-118.

Riazi, A., Matsuda, K., Arslan, A. (1985) Water stress induced changes in concentrations of proline and other solutes in growing regions of young barely leaves. *J Exp Bot*., **36:** 1716- 1725.

Shabana, Y.M., Baka, Z.A., Abdel-Fattah, G.M. (1997) *Alternaria eichhorniae*, a biological control agent for waterhyacinth: mycoherbicidal formulation and physiological and ultrastructural host responses. *Eur J Plant Pathol*., **103:** 99-111.

Snedecor, G.W., Cochran,W.G. (1976) Statistical Methods. 6th Ed. *Oxoford IBH Publishing Co. New Delhi*.

Snell, F.D., Snell, C.T. (1949) Colorimetric methods of analysis. Volume II. D. *Van Nostrand Co. Inc., New Yourk.*

Snell, F.D., Snell, C.T. (1954) Colorimetric methods of analysis. Volume IV. D. *Van Nostrand Co. Inc., New Yourk.*

Souza, G.M., Cardoso, V.J.M., Goncalves, A.N. (2004) Proline content and protein patterns in *Eucalyptus grandis* shoots submitted to high and low temperature shocks. *Braz Arch Biol Technol.* **47:** 355-362.

Venekamp, J.H., Lampe, J.E., Koot JT (1989) Organic acids as sources for drought induced proline synthesis in field bean plants *Vicia faba* L. *J Plant Physiol*., **133:** 654-659.

Walter, H. (1949) Gundlagen der flanzen verlientung. *Eintubring, in di pflanzengeographie- fur studierends der hocholen, standorstlehre: Stuttgart Ulmer.*

Weatherly, P.E. (1950) Studies on the water relations of the cotton plants. I. The field measurement of water deficits in leaves. *New Phytolog*., **49:** 81- 97.

Yakop, U.M. (2009) Variation in the resistance of faba bean (*Vicia faba* L.) to ascochyta blight. *Crop Agro*., **2:** 145-151.