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# Effect of the application of jasmonic acid and benzoic acid on grafted watermelons yield under greenhouse conditions in the southeast of Spain for mitigation of stress

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**Abstract**

One of the main problems of watermelon crops in Southeast Spain is the thermal difference because of climatic conditions that appear during the first stages of the crop. The objective of this work was to evaluate the effect of inducing the systemic acquired resistance (SAR) and the induced systemic resistance (ISR) through the application of jasmonic acid (JA) and benzoic acid (BA), respectively, to counter the abiotic stress. We assessed two treatments of JA and BA, $T_1$ (500 mg·kg$^{-1}$ + 500 mg·kg$^{-1}$) and $T_2$ (2000 mg·kg$^{-1}$ + 2000 mg·kg$^{-1}$), as well as a control test using an experimental design of randomized blocks with four replications. The results obtained for kg·m$^{-2}$, fruits/m², kg/plant and fruits/plant did not show statistically significant differences. However, we obtained statistically significant differences in the average fruit weight compared with the control test in the two experiments carried out in 2009 and 2010. The results showed that there was no metabolic cost in the plants when applying the assessed treatments of JA and BA.

***Key words:*** *Citrullus lanatus* T., abiotic stress, systemic acquired resistance, induced systemic resistance.

## Introduction

Natural protection of plants against pathogens and masticator insects is based partially in a series of barriers that are present in the plant before the real attack. The combined effect of all these barriers is known as constitutive resistance. Furthermore, plants can activate the mechanisms when they come into contact with an invader, which is called induced or acquired resistance [1]. It is important to determine if metabolic and physiological costs associated with induced resistance have impacts on crop yield, quality, or both [2].

The term *elicitor* has been used to refer to compounds that induce phytoalexin synthesis in plants [3]. Phytoalexin secretion is a plant defence mechanism because phytoalexins are toxic for a broad spectrum of fungi and pathogen bacteria. The use of elicitors in horticultural crops in greenhouses aims to provide better growth and development conditions for the plants, which face possible stresses, biotic as well as abiotic, although most of the yield losses are caused by these latter ones.

After infection, damage or stress, biochemical changes occur in the plants, and some of these changes have been related to phytoalexin expression [4]. Such phytoalexins are found in undetectable concentrations but are synthesized quickly after infection or stress initiaton [5].

Jasmonic acid (JA) and its derivatives, the jasmonates, increase their presence in plants after an injury, when an infection is caused by pathogens, or under stress conditions [6]. Jasmonic acid and jasmonates increase phytoalexin synthesis and favour cellular integration; therefore, they are included within the compounds called elicitors.

The main physiological processes in the development of plants where jasmonic acid participates are: root growth, tuberization, pollen development, tendril curling, fruit ripening and senescence [6-8].

There are reports about the use of jasmonic acid and jasmonates in different plant species, such as in *Nigella sativa* L. where it caused an increase in the accumulation of kalopanaxsaponin I., which is used as an anti-diabetic [4], and in *Taxus baccata* L., where it caused an increase in the accumulation of taxane, a substance used in chemotherapy [9]. In some tomato varieties jasmonates influence the infection by fungi in the arbuscular mycorrhiza area, because they reduce the fungi metabolism and, as a consequence, the colonization [10]. In sugar cane, the use of methyl jasmonate induced resistance to *Meloidogyne incognita* [11]. In tomato, the use of jasmonic acid increased resistance to *Oidium neolycopersici* [12]. In other experiments in artichoke (*Cynara scolymus*) seedlings, significant differences were found with the use of methyl jasmonate at low concentrations, because it caused increases in seedling height, fresh weight, length and root system growth. However, at higher concentrations, it caused a significant decrease of the mentioned parameters [13].

The effect of jasmonates in pear fruits in postharvest was that it limits the growth of *Penicillum expansum,* which causes blue mold [14]. In peach fruits, it reduced damage against stress caused by cold temperature in the refrigeration chambers [15]. There is very little information about the use of jasmonates in cucurbit plantations, thus it is necessary to carry out research about the effect of jasmonic acid on food and ornamental crops because of the economic importance of these crops. Benzoic acid is a biosynthetic precursor of salicylic acid [16] and has been tested in different crops. Fruits with a lower content of soluble solids were obtained in tomato (*Lycopesicum esculentum* M.). In potato (*Solanum tuberosum* L.), an increase in the number of tubers per plant was found. In soya, there was an increase in the growth of foliage and roots [17].

Horticultural crops in greenhouse represent a high percentage of the economic income that these crops produce worldwide. Watermelon is one of the most important crops in crop rotation in the province of Almería, Spain. The area used for this crop has been kept practically constant in the last decade. This trend has also been reflected in Spain, which is among the world's top 10 watermelon producers [18]; the province of Almería provides 60% of the national yield, which is estimated at 721 800 t.

During the crop cycle, watermelons in greenhouse are not exempted from facing different stresses, such as thermal differences or hydric deficit, which reduce their growth potential. With the purpose of mitigating these stresses, different elicitors have been used to encourage the plants to produce a stress-defence stimulus, but there is no published data regarding the case of combining the application of JA and BA in grafted watermelon crops. Therefore, this research was carried out during the 2008-2009 and 2009-2010 seasons and mainly aimed to document the applications of JA and BA, because they induce SAR and ISR, respectively, and to evaluate the effect on yield of grafted watermelon crops under greenhouse condition soil covered with sand in Southeast Spain.

## Materials and Methods

The experiment was conducted in the experimental plot UAL-ANECOOP, located in Retamar, in the municipality of Almería, in the place called "Los Goterones" (36.5177°N, 2.1708°W) at an altitude of 88 m above sea level (a.s.l) during the agricultural spring season in 2009 and 2010. The climate is Mediterranean arid, annual rainfall is very scarce (less than 300 mm/year); this is the most arid area of the Iberian Peninsula. Annual average temperature fluctuates between 15°C and 21°C, winters are mild, and the minimum average temperature is approximately 6°C in winter. In January and February, significant thermal differences are registered [19].

The experiment was conducted in the experimental station's U-3 unit, which faces east-west and has a greenhouse with a 1695 m² cultivable area. The greenhouse is a metallic structure with a symmetric bowed roof. This greenhouse has wind-speed sensors as well as temperature and rain sensors in order to automatically open and close the zenithal windows. It has two accesses, one to the east and the other to the west, connected by the central corridor with double doors to avoid the entry of pests, which can be transmitted by disease vectors. The covering material was made of 200 µm polyethylene. Grafted watermelon was transplanted on 6 February 2009 and 20 January 2010. Calcium carbonate (20 $g{\cdot}L^{-1}$) was applied to the greenhouse whole cover [20] on April 8$^{th}$ in 2009 and April 8$^{th}$ in 2010. Soil was covered with sand in both cycles as it is carried out traditionally in the area.

The plant material of *Citrullus lanatus* T. used was 'Reina de Corazones', cv. triploid which was grafted on RS-84 (interspecific hybrid of *C. maxima* x *C. moschata*) and the diploid cv. 'Dulce Maravilla' was used as a pollenizer. The plant density was 2500 plants/ha (0.25 plants/m²), mixing in the same line two plants of 'Reina de Corazones' and one plant of 'Dulce Maravilla'.

For insulating during the first three weeks of crop, 25 µm plastic tunnel was used with the purpose of improving the temperature conditions during the day and specially those during the night.

The irrigation lines were placed at a 90 cm distance. The self-compensating drippers were spaced every 50 cm, consuming 3.1 $L{\cdot}h^{-1}$, at a working pressure of 1.4 kPa. Seven irrigation lines were placed in each replication. To add the treatments, a fertilizer tank trademark Brot with 40-L capacity was used in each replication. The EC was fixed at 1.3 $dS{\cdot}m^{-1}$.

Three treatments with four replications were evaluated in a total design of randomized blocks: $T_0$ indicates the treatment of the control test, which consisted of the application of an ideal solution as balanced fertilizer (Table 1), $T_1$ consisted of the application of the described fertilizer + 500 $mg{\cdot}kg^{-1}$ JA + 500 $mg{\cdot}kg^{-1}$ BA, and $T_2$ consisted of the application of the described fertilizer + 2000 $mg{\cdot}kg^{-1}$ JA + 2000 $mg{\cdot}kg^{-1}$ BA. These concentrations were supplied by the manufacturers of the products, in order not to induce senescence. To measure the parameters evaluated, we proceeded in the following manner: We counted the number of fruits of each elemental plot once harvested; two harvests were carried out in 2009 and four harvests in 2010. We chose at random 10% of the fruits to obtain their average weight and considering the number of fruits counted, we obtained the total yield per plot; the result was divided into the area of each plot, repeating this procedure in each of the harvests.

**Table 1.** Nutrient solution used in treatments $T_0$, $T_1$, and $T_2$ ($mmol\,L^{-1}$).

| $NO_3^-$ | $H_2PO_4^-$ | $SO_4^{-2}$ | $HCO_3^-$ | $NH_4^+$ | $K^+$ | $Ca^{+2}$ | $Mg^{+2}$ |
|---|---|---|---|---|---|---|---|
| 15.00 | 1.50 | 2.00 | 0.50 | 0.50 | 8.50 | 6.00 | 2.00 |

To weigh the fruits, we used a scale (Gram Precision S.L model PM III, Spain) with weighing capacities from 0 to 30 kg and precision of 0.1 kg. The number of fruits was counted and they were divided into the area of each plot, in each of the cuts.

To determine the fruit yield, kg/plant, we divided the yield of each cut into the number of plants of each plot. We determined the number of fruits/plant by dividing the fruits harvested in each cut into the number of plants of each plot. In each harvest, 10% of the yield of each experimental plot was sampled to obtain the average fruit weight and to evaluate the parameter.

The data analysis was carried out using the computer program Statgraphics® Plus 5.1, 2001, for Windows® (Statistical Graphics Corporation USA). Analysis of variance and multiple range test Fisher's LSD at $p \geq 0.05$ were carried out.

## Results

***Total yield:*** The total yield expressed in $kg{\cdot}m^{-2}$ did not show significant differences between treatments during the two years that the experiment was carried out. Significant differences were not found for the values obtained in the different harvest dates of each cycle. However, the yield obtained compared with the control test was 9.65% higher and 2.16% lower for $T_1$ and $T_2$, respectively, within the first year of the experiment; in the second year, yields increased by 28.05% and 28.31% in $T_1$ and $T_2$, respectively (Table 2).

**Table 2.** Total yield of cv. Reina de Corazones.

| Treatment | kg·m$^{-2}$ | | | | |
|---|---|---|---|---|---|
| 2008-2009 | 109 dat | 116 dat | | | Accumulated |
| $T_0$ | 9.77 a | 2.25 a | | | 12.02 a |
| $T_1$ | 11.37 a | 1.81 a | | | 13.18 a |
| $T_2$ | 9.62 a | 2.14 a | | | 11.76 a |
| *P* value | 0.68 | 0.88 | | | 0.77 |
| 2009-2010 | 104 dat | 111 dat | 125 dat | 152 dat | Accumulated |
| $T_0$ | 1.95 a | 2.67 a | 0.42 a | 2.66 a | 7.70 a |
| $T_1$ | 2.89 a | 3.60 a | 0.53 a | 2.84 a | 9.86 a |
| $T_2$ | 2.24 a | 4.01 a | 0.62 a | 3.01 a | 9.88 a |
| *P* value | 0.13 | 0.25 | 0.62 | 0.86 | 0.30 |

Different letters represent significant differences at $P$<0.05. + dat (days after transplant).

***Number of fruits (fruits/m²):*** Significant differences were not found between treatments in the number of fruits/m² obtained during the two cycles of the experiment. However, in the first year, the results for $T_1$ and $T_2$ were respectively 3.57% and 17.86% lower than that of the control test. In the second year, $T_1$ and $T_2$ results were respectively 16.20 % and 19.72% higher than that of the control test (Table 3).

**Table 3.** Number of fruits/m² of cv Reina de Corazones.

| Treatment | Fruits/m² | | | | |
|---|---|---|---|---|---|
| 2008-2009 | 109 dat | 116 dat | | | Accumulated |
| $T_0$ | 1.46 a | 0.50 a | | | 1.96 a |
| $T_1$ | 1.48 a | 0.41 a | | | 1.89 a |
| $T_2$ | 1.19 a | 0.42a | | | 1.61 a |
| *P* value | 0.37 | 0.83 | | | 0.54 |
| 2009-2010 | 104 dat | 111 dat | 125 dat | 152 dat | Accumulated |
| $T_0$ | 0.23 a | 0.42 a | 0.06 a | 0.71 a | 1.42 a |
| $T_1$ | 0.34 a | 0.52 a | 0.09 a | 0.70 a | 1.65 a |
| $T_2$ | 0.29 a | 0.57 a | 0.07 a | 0.77 a | 1.70 a |
| *P* value | 0.26 | 0.34 | 0.33 | 0.17 | 0.28 |

Different letters represent significant differences at $P$<0.05.+ dat (days after transplant).

***Fruit yield per plant (kg/plant):***The yield (kg/plant) results show that there were no significant differences at all; however, when accumulating the yield in the first cycle of the crop (2009), $T_1$ was 9.62% higher and $T_2$ 2.65% lower than the control test. In the second cycle of the crop (2010), results for T1 and $T_2$ were 29.27% and 26.96% higher, respectively, compared with the control test (Table 4).

**Table 4.** Yield per plant (kg/plant) of cv. Reina de Corazones.

| Treatment | kg/plant | | | | |
|---|---|---|---|---|---|
| 2008-2009 | 109 dat | 116 dat | | | Accumulated |
| $T_0$ | 29.31 a | 6.76 a | | | 36.07 a |
| $T_1$ | 34.11 a | 5.43 a | | | 39.54 a |
| $T_2$ | 28.85 | 6.41 a | | | 35.26 a |
| *P* value | 0.41 | 0.96 | | | 0.45 |
| 2009-2010 | 104 dat | 111 dat | 125 dat | 152 dat | Accumulated |
| $T_0$ | 5.90 a | 8.09 a | 1.29 a | 8.08 a | 23.37 a |
| $T_1$ | 8.78 a | 10.92 a | 1.90 a | 8.60 a | 30.21 a |
| $T_2$ | 6.78 a | 12.15 a | 1.60 a | 9.12 a | 29.67 a |
| *P* value | 0.13 | 0.25 | 0.61 | 0.89 | 0.18 |

Different letters represent significant differences at $P$<0.05. + dat (days after transplant).

***Number fruits per plant (fruits/plant):*** The results found do not show statistically significant differences between treatments within the two years of the experiment; however, in the first cycle (2009), the values for $T_1$ and $T_2$ were 3.39% and 17.80% lower, respectively, compared with those of the control test. In the second year of the experiment, the values obtained in $T_1$ and $T_2$ were 13.93% and 18.26% higher, respectively, compared with those of the control test (Table 5).

**Table 5.** Number fruits per plant (fruits/plant) of cv Reina de Corazones.

| Treatment | Fruits/plant | | | | |
|---|---|---|---|---|---|
| 2008-2009 | 109 dat | 116 dat | | | Accumulated |
| $T_0$ | 4.40 a | 1.50 a | | | 5.90 a |
| $T_1$ | 4.47 a | 1.23 a | | | 5.70 a |
| $T_2$ | 3.59 a | 1.26 a | | | 4.85 a |
| *P* value | 0.58 | 0.85 | | | 0.53 |
| 2009-2010 | 104 dat | 111 dat | 125 dat | 152 dat | Accumulated |
| $T_0$ | 0.72 a | 1.28 a | 0.20 a | 2.16 a | 4.38 a |
| $T_1$ | 1.03 a | 1.55 a | 0.27 a | 2.13 a | 4.99 a |
| $T_2$ | 0.87 a | 1.73 a | 0.23 a | 2.34 a | 5.18 a |
| *P* value | 0.19 | 0.43 | 0.76 | 0.91 | 0.51 |

Letters represent significant differences at $P$<0.05. + dat (days after transplant).

***Average fruit weight:*** In the 2009 experiment cycle, the highest value was obtained in the first harvest of treatment $T_2$, and difference was statistically significant compared with the control test. The yield of this harvest represented 82% of the total experiment harvest. In the second harvest, significant differences were not found, but $T_1$ and $T_2$ obtained higher values (by 3.24% and 13.66%, respectively) compared with the control test. The average value of the first experiment (2009) $T_2$ showed statistically significant differences compared with the control test.

In the 2010 experiment, significant differences were found in the second and third harvest of $T_1$ compared with the control test. Yield from these harvests represent the 45% of the whole harvest obtained. The average value $T_1$ showed statistically significant difference compared with the control test (Table 6).

**Table 6.** Average fruit weight (kg/fruit) of cv. Reina de Corazones.

| Treatment | Average fruit weight | | | | |
|---|---|---|---|---|---|
| 2008-2009 | 109 dat | 116 dat | | | Average |
| $T_0$ | 6.65 b | 4.32 a | | | 5.49 a |
| $T_1$ | 7.73 a | 4.46 a | | | 6.09 ab |
| $T_2$ | 8.03 a | 4.91 a | | | 6.47 b |
| *P* value | 0.01 | 0.57 | | | 0.05 |
| 2009-2010 | 104 dat | 111 dat | 125 dat | 152 dat | Average |
| $T_0$ | 8.10 a | 6.23 a | 6.23 a | 3.73 a | 6.07 a |
| $T_1$ | 8.50 b | 7.03 b | 7.01 b | 4.02 a | 6.64 b |
| $T_2$ | 7.77 a | 7.02 b | 7.02 b | 3.87 a | 6.42 ab |
| *P* value | 0.17 | 0.01 | 0.00 | 0.42 | 0.05 |

Different letters represent significant differences at $P$<0.05.+ dat (days after transplant).

## Discussion

The production results obtained in the present experiments showed that there was no metabolic cost in watermelon crop for any of the doses of JA+BA evaluated (500 mg·kg$^{-1}$ + 500 mg·kg$^{-1}$ and 2000 mg·kg$^{-1}$ + 2000 mg·kg$^{-1}$). The lack of this metabolic cost was shown for all the yield parameters evaluated (kg/m$^2$, fruits/m$^2$, kg/plant and fruits/plant). Furthermore, increases of fruit weight were obtained for both doses in the two experiments.

Redman *et al.* [21] have evaluated the effect of JA (210 mg·kg$^{-1}$ and 2100 mg·kg$^{-1}$) on tomato plants through foliar application twice weekly, concluding that a significant metabolic cost is produced for the plant, characterized by a yield reduction of 26.1% compared with the control for the highest experimental dose, a significant reduction by 35.7% of the number of fruits per plant and a significant increase by 14.9% of fruit weight.

Yilmaz *et al.* [22] treated two strawberry varieties using doses of 210.0, 105.0, 57.5 and 21.0 mg·kg$^{-1}$ JA; except for the lowest dose, the rest of the doses caused earlier fruits than the control, strawberry size was increased within the two first harvest weeks and the total yield per plant was also increased.

Gutiérrez *et al.* [17] reported an increase by 31% in the number of tubers of *Solanum tuberosum* (potato) using 12.2 mg·kg$^{-1}$ BA. Herrera-Medina *et al.* [10] reported a statistically non-significant increase in fruit weight (2.7%), number of fruits per plant (15.7%) and production per plant (18.8%) compared with the control test in tomato treated by 12.2 mg·kg$^{-1}$ BA.

## Conclusions

The evaluated effects of the application of JA+BA did not show statistically significant differences in any of the yield parameters evaluated (kg·m$^{-2}$, fruits/m$^2$, kg/plant, fruits/plant), which indicates that the watermelon crops did not show a metabolic cost to the application of the JA+BA treatments evaluated. However, higher fruit weights were obtained, which were statistically significant in both cycles.It makes no sense for producers of watermelon grafted to use JA + BA to increase crop yield parameters.

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