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Influence of the application of jasmonic acid and benzoic acid on watermelon fruit quality

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Abstract

In watermelon crops in Southeastern Spain, important thermal differences appear during the first stages of plant development that can affect them. This work shows the effect of applying jasmonic acid and benzoic acid (JA+BA), inductors of systemic acquired resistance (SAR) and induced systemic resistance (ISR), respectively, on fruit quality parameters from a crop in a greenhouse in Southeastern Spain, where crops face a remarkable abiotic stress. We assessed two treatments of JA+BA, T1 (500+500 ppm), T2 (2000+2000 ppm) and a control test using an experimental design of randomized blocks with four replications. The results obtained for the parameters assessed (ºBrix, flesh firmness, rind thickness, polar and equatorial diameter) did not show statistically significant differences. The results showed that there was no metabolic cost in the plants when applying the assessed treatments of JA+BA.

Key words: *Citrullus lanatus*, *Cucurbita maxima* x *Cucurbita moschata*, abiotic stress, induced resistance, acquired resistance.

Introduction

Plants have a series of barriers that protect them against pathogens and chewing insects. The combined effect of all these barriers is known as constitutive resistance. Furthermore, plants activate defense mechanisms when they come into contact with invaders, which is called induced or acquired resistance [1]; it would be interesting to know whether the metabolic and physiological costs associated with induced resistance have an impact on crop quality [2].

Induction to phytoalexin synthesis in plants can be carried out through compounds called elicitors [3]. The use of elicitors in plants aims to improve growth and development conditions for the plants to face possible stresses, biotic as well as abiotic. After one infection, damage, or stress, biochemical changes occur in the plants associated with defense mechanisms, producing substances called phytoalexins [4]. Such phytoalexins are found in undetectable concentrations if there is not a stress situation, but in a stress situation, they are synthesized quickly, and they are toxic for a broad spectrum of pathogens [5].

When an injury or infection is caused by pathogens or under stress conditions in plants, there is also an increase in jasmonic acid and jasmonates, increasing the phytoalexin synthesis; therefore, they are included among the compounds called elicitors. Jasmonic acid participates in physiological processes such as root growth, tuberization, pollen development, tendril curling, fruit ripening and senescence [6-8]. In tomatoes, it has been observed that jasmonates influenced infection by fungi in the roots when arbuscular mycorrhiza are about to spread because they reduce the fungi metabolism and, as a consequence, colonization by mycorrhiza [9]; it also increases resistance to *Oidium neolycopersici* [10]. In sugar cane crops, the use of jasmonates induced resistance to *Meloidogyne incognita* [11]. In artichoke (*Cynara scolymus*) seedlings, the use of methyl jasmonate at low concentrations caused greater growth in seedling height, fresh weight and length of root system; however, when the methyl jasmonate concentration increased, the opposite effect was produced in all the parameters mentioned by Martín-Closas [12].

In harvested products, studies have been conducted regarding the effect of jasmonic acid on pears, and it was observed that it limits the growth of *Penicillum expansum* [13]. In peaches, it reduced stress-induced damage due to cold in refrigeration chambers [14]. Currently, there is very little information about the use of jasmonic acid and jasmonates in food and ornamental crops, although these crops have great economic importance [15].

Benzoic acid is a biosynthetic precursor of salicylic acid [16] and has been tested in various crops, such as tomato (*Lycopersicum esculentum* M); bigger fruits with a lower content of soluble solids were obtained in tomato crops [17]. In soya crops, an increase in the growth of foliage and roots has been reported [18].

Many vegetable crops are grown under protective systems such as greenhouses, mesh, etc. The profitability of these production systems is higher than that of non-protected systems. Eight crops are being used in the high-yield horticultural system in Almería, and the cultivated area used for watermelons has been kept practically constant over the last 10 years. Spain is among the world's top 10 watermelon-producing countries [19], and the province of Almería accounts for 60% of the national yield, which is estimated to be 721,800 t.

The crop cycle carried out in the Spanish southeast for this cucurbit causes it to face thermal and/or hydric stresses, which reduce its productive potential. In an attempt to minimize this loss, various elicitors have been used to stimulate plants and overcome stress to maintain the quality of harvests. In our case, we have applied a combination of jasmonic and benzoic acid, and we are not aware of the existence of data published regarding its effect on watermelon fruit quality.

Materials and Methods

This study was carried out during the 2009 and 2010 seasons in grafted watermelon plants in greenhouses on soil covered with sand and drip irrigation. The experiment was conducted in the UAL-ANECOOP experimental plot during the agricultural spring seasons of 2009 and 2010. It is located in Retamar, in the municipality of Almería, in a place called "Los Goterones," West longitude 2°17'08" and North latitude 36º51'77", at an altitude of 88 m above sea level (a.s.l.). 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. The annual average temperature fluctuates between 15ºC and 21ºC, with a minimum average temperature of approximately 6ºC during the months of January and February, indicating important thermal differences.

The experiment was carried out in unit U-3 of the aforementioned experimental station; the unit runs east-west and has a greenhouse with a cultivable area of 1695 m². The greenhouse is a metallic structure with a symmetrical, bowed roof. This greenhouse has wind speed, temperature and rain sensors, used to open and close the zenithal windows automatically. It has two access points connected by the central corridor and two sets of doors to prevent the entry of pests, which can even be disease vectors. The covering material is made of 800-gauge polyethylene. Grafted watermelon plants were transplanted on 6 February 2009 and 20 January 2010. Calcium carbonate was applied to the whole cover of the greenhouse [20] on 8 April 2009 and 2010. Soil was covered with sand in both cycles, as it is done traditionally in the area.

The plant material used was Reina de Corazones, cv. triploid, which was grafted onto RS-841 (interspecific hybrid of C. *maxima x C. moscata*), and the diploid cv. Dulce Maravilla was used as the pollenizer. The plant density used was 2500 plants/ha (0.25 plants/m²), mixing in the same line two Reina de Corazones plants and one Dulce Maravilla plant. A 100-gauge plastic tunnel was used for insulation during the first three weeks of cultivation for the purpose of improving the temperature conditions during the day and especially the night temperatures.

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

Three treatments with four replications for each one were evaluated in a total design of randomized blocks. T0 was the treatment for the control test, which consisted of the application of an ideal solution as a balanced fertilizer (Table 1). T1 consisted of the application of the described fertilizer + 500 ppm JA + 500 ppm BA, and T2 consisted of the application of the described fertilizer + 2000 ppm JA + 2000 ppm BA. These concentrations were supplied by the manufacturers of the products, in order not to induce senescence. The fruit were collected on two harvesting dates, 109 dat and 116 dat in 2009 and 104 dat and 111 dat in 2010.

The parameters were measured on three fruits chosen at random per treatment, replication, harvest and year. The parameters measured were the content of soluble solids, flesh firmness, flesh color, pH, rind thickness, and polar and equatorial diameters.

The content of soluble solids, expressed in °Brix, was measured in the fruit juice using a refractometer Atago® ATC-1E, 0-32% (ATAGO JAPAN CO. LTD.).

To measure firmness, we used a penetrometer Force Gauge PCE-FM200 (England), cutting first along the fruit equator and taking three measures of each fruit in different areas. We took as reference the average of the measures assessed.

The fruit juice pH was measured using a pH meter, Delta HD 8602, with a precision of $\pm$0.02 pH (Italy). Rind thickness was calculated as the average of three measurements taken in fruits opened previously using a digital caliper MITUTOYO Absolute Digimatic, model 500-172-20, with a precision of $\pm$0.01 mm (USA). To determine the flesh color, a conventional color scale table was used, with values ranging from 1 to 4, 1 corresponding to pale pink, 2 corresponding to pink, 3 corresponding to light red, and 4 corresponding to red. The measurements of the polar and equatorial diameters were taken with a conventional tape measure.

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

Results

Soluble solids °Brix: The total content of soluble solids was similar for the two years of the experiment (2009 and 2010); statistically significant differences were not found. T1 and T2 obtained in the first year of the experiment (2009) had lower values, 0.55% and 5.32%, respectively, than the control test. In the second cycle of the experiment (2010), lower values were obtained for T1 and T2 of 1.12% and 2.13%, respectively, compared to the control test (Table 2).

Flesh firmness kg cm^{-2}: The results obtained for the fruits flesh firmness were similar in the two experiments, and statistically significant differences were not found. In the first experiment (2009), T1 and T2 had 2.03% and 10.81%, respectively, higher values compared to the control test. In the second experiment (2010), T1 had a 3.12% higher value than the control test, with T2 being 5.10% lower than the control test (Table 2).

pH: In the 2009 experiment, in the first harvest, T1 had the highest value with statistically significant differences with respect to the control test (T0). In the second harvest, significant differences were not found. In the 2010 experiment, in the first harvest,

Table 1. Nutrient solution used in treatments T0, T1, and T2 (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

Table 2. Fruit quality, cv. Reina de Corazones.

Treatment	Soluble solids (°Brix)			Firmness (kg cm^{-2})		
2009	109 dat	116 dat	Average	109 dat	116 dat	Average
T0	11.38 a	10.44 a	10.91 a	1.43 a	1.53 a	1.48 a
T1	11.35 a	10.34 a	10.85 a	1.36 a	1.67 a	1.51 a
T2	10.55 a	10.10 a	10.33 a	1.54 a	1.74 a	1.64 a
P value	0.42	0.31	0.35	0.48	0.32	0.29
2010	104 dat	111 dat	Average	104 dat	111 dat	Average
T0	9.72 a	9.97 a	9.85 a	1.45 a	1.76 a	1.60 a
T1	10.03 a	9.45 a	9.74 a	1.75 a	1.54 a	1.65 a
T2	9.27 a	10.01 a	9.64 a	1.54 a	1.50 a	1.52 a
P value	0.22	0.13	0.80	0.52	0.25	0.43

Values followed by the same letter in the columns are not significantly different at $P< 0.05$. + dat (days after transplant).

significant differences were not found; however, T1 and T2 were 0.96% and 1.35%, respectively, lower than the control test. In the second harvest and in the average values, the control test obtained higher values with significant differences (Table 3).

Table 3. The pH of fruit, cv. Reina de Corazones.

Treatment	pH		
2009	109 dat	116 dat	Average
T0	5.46 b	5.69 a	5.58 a
T1	5.73 a	5.60 a	5.67 a
T2	5.43 b	5.70 a	5.57 a
P value	0.02	0.45	0.51
2010	104 dat	111 dat	Average
T0	5.20 a	5.58 a	5.39 a
T1	5.15 a	5.15 b	5.25 b
T2	5.13 a	5.37 b	5.25 b
P value	0.22	0.00	0.04

Values followed by the same letter in the columns are not significantly different at $P< 0.05$. + dat (days after transplant).

Rind thickness: The values obtained in the two experiments (2009 and 2010) did not show statistically significant differences, however; in 2009, the average values obtained for T1 were 3.82% lower and 8.27% higher for T2 compared to the control test (T0). In the 2010 experiment, T1 and T2 had higher values than T0 at 7.06% and 7.73%, respectively (Table 4).

Flesh color: The results obtained in the first experiment (2009) did not show statistically significant differences. The average values obtained for T1 and T2 were 16.18% and 11.27% lower compared with the control test. In the 2010 experiment, the values of T1 and T2 were higher than that of the control test, showing statistically significant differences (Table 5).

Table 4. Rind thickness (mm), cv. Reina de Corazones.

Treatment	Rind Width (mm)		
2009	109 dat	116 dat	Average
T0	12.38 a	13.26 a	12.82 a
T1	13.60 a	11.06 a	12.33 a
T2	15.22 a	12.54 a	13.88 a
P value	0.27	0.45	0.61
2010	104 dat	111 dat	Average
T0	17.26 a	17.26 a	17.98 a
T1	19.47 a	17.03 a	19.25 a
T2	20.42 a	18.31 a	19.37 a
P value	0.26	0.33	0.13

Values followed by the same letter in the columns are not significantly different at $P< 0.05$. + dat (days after transplant).

Polar and equatorial perimeter: Measures of polar perimeter in 2009 were higher for T2 in the first and second harvest, showing an average value with a statistically significant difference compared to the control test (T0). In the 2010 experiment, the values were significantly higher than those corresponding of the control test (T0). The difference was higher in T1 during the first harvest and in the average values, and in the second harvest, the difference was higher in T2 (Table 6).

With respect to the values for equatorial perimeter, in the 2009 experiment, T0 was lower than T1 and T2 with statistically significant differences. T2 had the highest values. In the 2010 experiment, statistically significant differences were not obtained (Table 6).

Discussion

We do not know of published information regarding the effects of the exogenous application of JA+BA on watermelons. The lack of this metabolic cost is observed in the parameters where T1 and T2 were not significant compared with the control test, specifically the content of soluble solids, flesh firmness, and equatorial perimeter. In the case of color and pH, the data obtained were not consistent because, in one of the experiments, the values were higher than the control test, and in the other experiment, the opposite results were obtained. There were significant differences only in the parameter polar diameter.

In other experiments carried out post-harvest in other crops using jasmonates and methyl jasmonates, the results are consistent with those we have obtained. Yilmaz [21] experimented with two strawberry varieties receiving doses of 210, 105, 57.5, and 21 ppm JA, obtaining a decrease in the content of soluble solids (TSS); except for the lowest dose, all doses caused earlier fruits than the control, as well as a significant increase in fruit size within the first two harvest weeks. Zhang [13] reported that the total soluble solids in pear fruits were reduced by 7.22% at post-harvest, using a dose of 45 ppm of methyl jasmonates (MeJA). However, other authors, such as Wang and Sheng [22], after applying a dose of 22.4 and 2.24 ppm MeJA to pre-harvest raspberries, obtained an increase in the content of soluble solids (TSS). Fan [23] also showed that apples treated with 210 ppm JA after 2 weeks at 20°C

Table 5. Flesh color, cv. Reina de Corazones.

Treatment	†Flesh Color (1-4)		
2009	109 dat	116 dat	Average
T0	1.75 a	2.33 a	2.04 a
T1	1.17 a	2.25 a	1.71 a
T2	1.42 a	2.25 a	1.89 a
P value	0.32	0.44	0.39
2010	104 dat	111 dat	Average
T0	2.25 a	2.08 b	2.16 b
T1	2.75 a	2.33ab	2.54 a
T2	2.41 a	2.66 a	2.54 a
P value	0.13	0.04	0.03

Values followed by the same letter in the columns are not significantly different at $P< 0.05$. † A scale of 1 to 4 was used to assess the color of the watermelon. Flesh 1 is slightly pink, 2 is pink, 3 is light red, and 4 is red. + dat (days after transplant).

Table 6. Fruit quality, cv. Reina de Corazones.

Treatment	Polar Perimeter (cm)			Equatorial Perimeter (cm)		
2009	109 dat	116 dat	Average	109 dat	116 dat	Average
T0	75.83 b	61.61 b	71.09 b	71.75 b	60.85 b	68.12 b
T1	82.04 a	63.22 b	75.77 ab	75.60 a	61.30 b	70.86 b
T2	83.32 a	69.51 a	78.71 a	77.82 a	68.08 a	74.57 a
P value	0.02	0.04	0.02	0.03	0.02	0.03
2010	104 dat	111 dat	Average	104 dat	111 dat	Average
T0	81.07 a	75.56 a	78.31 b	76.87 a	71.92 a	75.35 a
T1	82.15 a	76.89 a	79.52 a	77.22 a	74.55 a	75.89 a
T2	77.22 b	77.15 a	77.19 b	75.88 a	74.43 a	75.15 a
P value	0.00	0.09	0.01	0.36	0.67	0.53

Values followed by the same letter in the columns are not significantly different at $P< 0.05$. $^+$ dat (days after transplant).

The flesh firmness obtained in our experiments was higher in the plants to which JA+BA was applied than in the control test, although significant differences were not found. Our results are consistent with those of Zhang [13], who reported an increase in pear firmness of 8.27% using a dose of 45 ppm of methyl jasmonates (MeJA) at post-harvest. Our results, without being statistically significant, show a trend toward greater firmness in the case of the fruits to which JA+BA was applied. This data can improve the handling of fruits post-harvest.

With respect to flesh color, our results are consistent with those obtained by De la Cruz [24], who applied jasmonates in *Ananas comosus* cv. MD-2 (pineapple) and obtained an increase in exterior and interior fruit color.

Conclusions

The effect assessed with respect to the application of jasmonic acid + benzoic acid (JA+BA) under the conditions of our experiment did not show statistically significant differences in the quality parameters (°Brix, flesh firmness, and rind thickness). The pH, flesh color, and equatorial perimeter parameters yielded contradictory results in statistical significance, possibly due to the interference of the application of JA+BA with the particular climatological variables (climatological differences over the year) in each experiment.

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