

Effect of hydrogen peroxide on broccoli seedlings (*Brassica oleracea* var. *italica*)

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Resumen

El estado de Guanajuato es el principal productor de brócoli (*Brassica oleracea* var. *italica*), este cultivo como un todo se produce bajo el sistema de plantación, uno de los problemas que enfrentan los productores es la pérdida en el trasplante debido a las plantas de estrés sufren al hacerlo. La aplicación del peróxido de hidrógeno (PH) aumenta la resistencia de las plantas al estrés biótico y abiótico, por lo que el objetivo de esta investigación es evaluar el efecto de las aplicaciones foliares de PH en plántulas de brócoli, ya que se plantó la variedad Avenger 8 días después de la siembra se realizó la aplicación de PH a dosis de 0,6, 1,4 y 1,8 mM con adhesivo al 1% (Bionex®) en un diseño completamente al azar con 10 repeticiones y testigo de que sólo se aplicó adhesivo, Unidad consistía en una planta, la aplicación se hizo cada 8 días durante 3 semanas. Las variables evaluadas fueron: longitud total de la plántula, longitud del tallo, longitud de la raíz, diámetro del tallo, peso fresco, peso seco y biomasa. Las aplicaciones de peróxido de hidrógeno a dosis de 1,8 mM cada ocho días de brócoli, aumentan la longitud del tallo y el peso fresco, mientras que la dosis de 1,4 mM aumenta la biomasa de las plántulas

Peróxido de hidrógeno, plántulas, altura de la planta, longitud de la raíz

Abstract

The state of Guanajuato is the leading producer of broccoli (*Brassica oleracea* var. *italica*), this crop as a whole is produced under the plantation system, one of the problems facing producers is the loss in transplantation due to stress plants suffer when you do it. The application of hydrogen peroxide (PH) increases the resistance of plants to biotic and abiotic stress, which is why the aim of this research is to evaluate the effect of foliar applications of PH in seedlings of broccoli, for it was planted the Avenger variety in trays, eight days after sowing the application of PH at doses of 0.6, 1.4 and 1.8 mM was performed with 1% adherent (Bionex®) in a completely randomized design with 10 repetitions and a witness to that I was only applied adhesive, the experimental unit consisted of a plant, the application was made every 8 days for 3 weeks. The variables evaluated were: total length of seedling, stem length, root length, stem diameter, fresh weight, dry weight and biomass. Applications of hydrogen peroxide at doses of 1.8 mM each eight days broccoli seedlings, increase stem length and fresh weight, whereas the dose of 1.4 mM increase the biomass of seedlings

Hydrogen peroxide, seedlings, plant height, root length

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Introduction

Mexico is among the world's leading producers and exporters of vegetables, ranking fourth in the world (Financiera Rural, 2008). On the other hand, Guanajuato is one of the main producers of broccoli with a contribution of 60.16% (SAGARPA, 2010).

Cruciferae like broccoli, multiply by sexual seed; for the establishment of open field crops, the preparation of seedlings is required. Production begins in the seedbed, which can be an area of land or containers (trays) properly adequate to deposit the seeds and provide the optimal conditions of light, temperature, fertility and humidity, to obtain the best emergency and development in initial states until the transplant to the field (Jaramillo 2006).

The use of defense mechanisms in plants, particularly the expression and regulation of broad-spectrum resistance associated with ISR (systemic resistance induced) and SAR (acquired systemic resistance) phenomena, may serve as a viable alternative for the design of management strategies Seedlings (Mejía, 2014). Hydrogen peroxide (H₂O₂) is considered a signal molecule and a regulator of the expression of some genes in cells. When it accumulates in plants, it activates transcription factors that regulate different physiological processes, inhibits plant growth and development, and stimulates defense mechanisms to biotic and abiotic stress (Yu et al., 2002, Pnueli et al. 2003; Hung et al., 2005).

Due to the characteristics of this compound (highly reactive, short half-life and potent reductive oxidation of cellular components) there are few works where its exogenous application in plants is experienced. Previous experiments on different cultures revealed that H₂O₂ sprays inhibited stem growth and increased starch and lignin content (Foyer et al., 1997; López-Delgado et al., 1998, 2005); in others the yield and quality of production increased (Romero-Romero and Lopez Delgado, 2009). In the present work, the effect of hydrogen peroxide (H₂O₂) on agronomic variables of broccoli plants.

Methodology

The present investigation was carried out in the greenhouses Agro Vida, Celaya; Gto; For the evaluation of hydrogen peroxide in broccoli seedlings the hybrid Avenger (Sakata®) was used, the seeding was performed mechanically in trays of 338 cavities of 7.5 mL of capacity of each well, the trays were previously disinfected with formaldehyde To 38%. As substrate was used professional Growing Mix (Sungro®) and perlite with a ratio of 2: 1 approximately, to cover the seed already emerged was used vermiculite (Sunshine®). The trays were stowed, sewed and sprinkled with Kaisen® (Metamidofos). Two days after the seimbra were uncovered and covered with vermiculite to pass to the greenhouse and placed in 32 trays per lane and the first irrigation was performed.

At 8 days after germination the treatments were applied, using a completely randomized design with 10 replicates and each experimental unit consisted of one plant. A 1.0 L manual sprayer (Trupper®) was used for the application of the treatments, applications were made once a week for three weeks. Each dose was applied 1 mL of adherent (Bionex®) per liter of water, the control was applied only water and adherent.

No. Trat	Treatment	Concentration
1	Hydrogen peroxide	0.6 mM
2	Hydrogen peroxide	1.4 mM
3	Hydrogen peroxide	1.8 mM
4	Witness	--

Table 1 Concentration of hydrogen peroxide applied to broccoli seedlings.

The irrigations were performed daily and every third day were fertilized with the formula 20-20-20

The variables evaluated were performed 2 times per week and 10 plants per experimental and labeled unit were chosen.

Fresh Weight (g). The plants were first removed from the trays and washed well from the root, weighed and then placed in a sanitary napkin with their respective number.

Measurement of root and stem. Once the plant was already washed and weighed, the length of the root (cm) was measured

with a rule of 30 cm (Baco®), it was stretched in its entirety and for measuring the length of the stem (cm) was considered from the base of the stem to the first branch

Diameter of stem (mm). A vernier (Truper®) was used for this, the measurement was performed in the middle part of the stem.

Dry weight (g). After all previous measurements were taken, each seedling was placed in toilet towels and placed in an oven at 72 ° C for 3 days, after which it was weighed on an analytical balance (Adam®). Biomass (g). It was determined by difference between fresh weight and dry weight.

With the results of plant height, stem length, root length, stem diameter, fresh weight,

dry weight and biomass, a variance (ANOVA) analysis was performed with 10 treatments and a control to indicate differences between treatments With PH applications. When ANOVA indicated significant differences between treatments, the Tukey's test ($p \leq 0.05$) was applied for the separation of means. For the statistical analysis the SAS system program for Windows ver. 9.0 (2002).

Results and discussion

The effect of foliar applications of hydrogen peroxide (PH) on the variables of seedling height, stem length, root length and stem diameter of broccoli seedlings are shown in the middle square in Table 2, where The effect of the treatments was significant for the stem length variable, for the variables of plant height, root length and stem diameter no effects were observed, these values coincide with those reported by Ramírez et al. (2006) did not observe effects of treatments with salicylic acid, abscisic acid and chitosan in broccoli plants in these same variables. Sampling significantly affected all evaluated variables, whereas; The interaction of treatment by sampling affected highly to stem length and significantly to plant height and root length.

FV	G L	CM ¹			
		Height Plant	Height Stem	Root Length ²	Stem Diameter ³
Trat ⁴ (T)	3	3.16	0.18*	1.41	0.02
Muest ⁵ (M)	5	189.13*	2.72*	35.38*	0.86**
M x T	15	3.48*	0.24*	3.51*	0.01
Repetición	9	1.56	0.02	0.96	0.005
Error	20 7	2.00	0.03	1.80	0.008
Total	23 9				
R ²		0.71	0.70	0.79	0.71
CV		10.81	10.65	20.17	7.45

¹Current Medium, ²Length, ³Diameter, ⁴Treatment, ⁵Sampling

Table 2 Average squares for plant height, stem height, root length and stem diameter of broccoli seedlings as a result of the exogenous application of hydrogen peroxide.

The values of the coefficient of determination ranged from 0.70 to 0.79, while the coefficient of variation was less than 21% for root length and 7.45% for stem diameter.

Table 3 shows that treatments with PH had a highly significant effect on strawberry weight and biomass of broccoli seedlings, not observing effect on dry weight, this value is in agreement with Casierra-Posada et al. (2010) Who did not observe effect on the dry weight of broccoli seedlings with zinc applications in the soil.

While for the variables of fresh weight, dry weight and biomass the effect was highly significant for sampling, the interaction treatment by sampling was highly significant for all variables under study.

The coefficient of determination was greater than 0.89 for the variables under study and the coefficient of variation observed was 16.14, 15.9 and 16.41 for the variables of fresh weight, dry weight and biomass, respectively.

FV	GL	CV		
		Peso Fresco	Peso Seco	Biomasa
Trat (T)	3	0.16**	0.0005	0.15**
Muest (M)	5	7.27**	0.074**	5.90**
M x T	15	0.07**	0.0005**	0.06**
Repetición	9	0.02	0.00007	0.02
Error	207	0.02	0.0001	0.01
Total	239			
R ²		0.89	0.90	0.89
CV		16.14	15.90	16.41

Table 3 Average squares for fresh weight, dry weight and biomass of broccoli seedlings due to the exogenous application of hydrogen peroxide.

Samples of the plant height and stem diameter variables of broccoli seedlings by foliar applications of PH had their highest level of significance at week 3 (sampling 6) when reaching the maximum values with 15.72 cm and 1.41 mm respectively, while That for the stem length the maximum value was observed in sampling 3 and 4 with values of 2.01 and 2.00 cm respectively.

The maximum root length was observed in Samples 2 and 3 with values of 7.41 and 7.04 cm respectively, is possibly to the adaptation process of the seedling.

sample	Height Plant		Height Stem		Root Length		Stem Diameter	
1	9.28	e	1.37	d	5.13	d	1.01	d
2	12.40	d	1.93	a	7.41	a	1.19	c
3	13.39	c	2.00	a	7.04	a	1.27	b
4	13.37	c	2.01	a	6.17	c	1.36	a
5	14.35	b	1.81	b	6.47	c	1.36	a
6	15.72	a	1.55	c	7.71	a	1.41	a

Table 4 Analysis of variance (ANOVA) for the effect of sampling in the variables seedling height, stem length, root length and stem diameter of broccoli seedlings with exogenous applications of hydrogen peroxide.

Table 5 shows the effect of the sampling on fresh weight, dry weight and biomass of broccoli seedlings sprayed with PH, where it is observed that for the fresh weight and accumulated biomass the maximum value was 1.38 and 1.25 g in The sampling 5 for each of the variables, while for dry weight sampling 5 and 6 presented values of 0.13, which were the highest values.

For the seedling height variable, there were no statistically significant differences between the applied concentrations with an average value of 13.15 cm (Table 6), these results differ from those reported by Quesada et al. (2005) who observed significant differences in seedlings of Broccoli germinated in different substrates and with a maximum growth value of the seedling of 5.8 cm. For root length no significant differences were observed between treatments.

According to the Tukey's test ($P \leq 0.05$), there was a significant variation for the variables of stem length, being the treatment with the lowest concentration of 0.6 mM in which the lowest development of the stem with 1.72 cm was observed and statistically behaving Concentrations of 1.4, 1.8 mM and the control with values of 1.74, 1.83 and 1.82 respectively, which were the highest values.

Sample	Fresh weight		Dry weight		Biomass	
1	0.18	e	0.02	e	0.15	e
2	0.74	d	0.05	d	0.69	d
3	0.83	d	0.07	c	0.75	d
4	0.94	c	0.10	b	0.84	c
5	1.38	a	0.13	a	1.25	a
6	1.25	b	0.13	a	1.12	b

Table 5 Analysis of variance (ANOVA) for the effect of sampling in the variables fresh weight, dry weight and biomass of broccoli seedlings with exogenous applications of hydrogen peroxide.

For the stem diameter the treatments with PH exceeded the control with values greater than 1.27 mm, these values are lower than those reported by Quesada et al. (2005) who observed maximum values of 5.3 mm in germinated broccoli seedlings in different substrates.

No Treatment	Concentration PH (mM)	Height Plant	Height Stem	Root Length	Stem Diameter
1	0.6	13.4	1.72	6.73	1.27
2	1.4	12.75	1.74	6.5	1.27
3	1.8	13.27	1.83	6.83	1.28
4	Testigo	13.18	1.82	6.55	1.23

Table 6 Analysis of variance (ANOVA) for the effect of foliar applications of different concentrations of hydrogen peroxide on the variables seedling height, stem length, root length and stem diameter of broccoli seedlings.

Table 7 shows statistical differences ($P \leq 0.005$) between the concentrations of PH applied foliarly in broccoli seedlings in the fresh weight, dry weight and biomass variables.

In the variable of fresh weight of broccoli seedlings the highest value of 0.96 g with the concentration of 1.4 mM is observed, this value differs with that reported by Caserra et al. (2010), which reported a value of 12 g in the Witness of a work done in broccoli with different concentrations of Zn applied to the soil.

The concentration of 1.4 mM of PH applied to broccoli seedlings affected the dry weight and biomass with the highest values of 0.09 and 0.87 g respectively, with the same values being statistically equal to 0.6 and 1.8 with the control.

No Trat	Concent. PH (mM)	Fresh weight		Dry weight		Biomass	
1	0.6	0.85	b	0.08	b	0.77	b
2	1.4	0.96	a	0.09	a	0.87	a
3	1.8	0.88	b	0.08	a	0.8	b
4	Testigo	0.85	b	0.08	a	0.76	b

Table 7 Analysis of variance (ANOVA) for the effect of foliar applications of different concentrations of hydrogen peroxide on the variables fresh weight, dry weight and biomass of broccoli seedlings.

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