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Salicylic acid: effect on the physiological quality of *Cichorium endivia* L. seeds

Ácido salicílico: efecto sobre la calidad fisiológica de semillas de *Cichorium endivia* L.

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Escarole plantation.

Foto: D.C. Fontana

ABSTRACT

Salicylic acid (SA) is known to be involved in a series of physiological processes in the improvement of seed germination and vigor. The aim of this study was to evaluate the effect of SA concentrations on the physiological quality of escarole (*Cichorium endivia* L.) seeds, Escarola Lisa® cultivar. Escarole seeds were soaked for 24 h at concentrations 0, 0.05, 0.1, 0.15, and 0.2 mM of a similar to salicylic acid (Bion®) and afterwards submitted to germination and vigor tests (laboratory and greenhouse). The tested seed lots differed in their physiological quality. The use of salicylic acid does not present profits for the physiological quality of escarole seeds. However, it is suggested that new studies be carried out by testing this product under stress conditions or applied in stages after seed germination.

Additional key words: germination, vigor, plant growth regulator, escarole.

RESUMEN

El ácido salicílico (AS) es conocido por estar involucrado en una serie de procesos fisiológicos que mejoran las características de germinación y vigor. El objetivo de este trabajo fue evaluar el efecto de concentraciones de AS sobre la calidad fisiológica de semillas de escarola (*Cichorium endivia* L.) cv. Escarola Lisa. Las semillas de escarola fueron embebidas durante 24 horas en concentraciones 0; 0,05; 0,1; 0,15 y 0,2 mM de Bion® (análogo al ácido salicílico) y después sometidas a pruebas de germinación y vigor (laboratorio e invernadero). Los lotes

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de semillas evaluadas diferían en cuanto a su calidad fisiológica. El uso de ácido salicílico no presenta beneficios a la calidad fisiológica de semillas de escarola. Sin embargo, se sugiere que nuevos trabajos sean realizados probando este producto en condiciones de estrés o aplicado en estadios posteriores a la germinación de las semillas.

Palabras clave adicionales: germinación, vigor, regulador de crecimiento vegetal, escarola.

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INTRODUCTION

Escarole (*Cichorium endivia* L.) belongs to the Asteraceae family and is increasing in cultivation in Brazil because of the great productive potential. In addition to environmental factors that interfere with the development of seedlings, such as temperature, air humidity, solar radiation and water availability, seeds with low germination and vigor interfere with the ideal establishment of seedlings.

The seed quality used by farmers is directly related to the physiological potential, represented by germination and vigor, expressing the ability to generate normal seedlings (Pereira *et al*., 2011). We have tested treatment methods for seeds that can potentiate their vigor.

Absicis acid is a promising alternative for seed treatments since it acts as an inducer of the secondary metabolism, promoting mechanisms of resistance in plants against pests and diseases. In addition, this acid helps to improve seed quality and productivity (Tavares *et al*., 2014). It is a compound of natural origin that acts in physiological and biochemical processes (Shi and Zhu, 2008). Klessig *et al.* (2009) reported that this acid has the ability to contribute to the control of stomatal opening, cell growth, gene expression and can also affect seed germination, as well as senescence and fruit production. Negative responses in germination and vigor were found in soybean and rice when using this acid; however, an improvement in plant growth was evident (Costa Maia *et al*., 2000; Silveira *et al*., 2000).

Pre-treatment with acid may inhibited the activity of the catalase enzyme in rice seeds, increasing hydrogen peroxide levels and increasing plant resistance to stress conditions (Guo *et al*., 2009). Its application to calendula seeds, Asteraceae family, contributed positively to the germination rate and speed under conditions of thermal stress (Carvalho *et al*., 2007).

Some studies have been carried out in order to know the role of salicylic acid in seed quality; however, there is a need for information on its effect on the physiological quality of vegetable crop seeds. In this sense, this study aimed to evaluate the effect of salicylic acid concentrations on the physiological quality of escarole seeds.

MATERIALS AND METHODS

The study was conducted in the Laboratory of Seed Production and Technology of the Federal University of Santa Maria, Frederico Westphalen Campus (Brasil). This experiment was conducted in a randomized block design with a 3×5 factorial design (lots × concentrations). The treatments consisted of three lots of escarole (*Cichorium endivia* L.) seeds, Escarola Lisa cultivar, and five salicylic acid (SA) concentrations (Bion®) (0, 0.05, 0.1, 0.15 and 0.2 mM).

The seeds were soaked for 24 h in solutions with different SA concentrations. Then, the following tests were carried out:

Germination test

Consisted of 400 seeds per treatment, which were subdivided into eight replicates of 50 seeds, placed in transparent plastic boxes with a lid (Gerbox), 11×11×3 cm, using a paper germitest substrate, washed with distilled water at a ratio of 2.5 times its dry weight. The boxes were kept in a B.O.D. germinating chamber at a constant temperature of 25°C (recommended temperature of 20-30°C). Seed dormancy breaking was performed with light, using a 12-hour photoperiod. Counting was performed five and fourteen days after test installation. The results were expressed as a percentage of germination (G), abnormal seedlings (AN), hard seeds (HS) and dead seeds (DS) (Brazil, 2009).

Accelerated aging test (AA)

This test was conducted with 200 seeds for each treatment. A transparent plastic box (gerbox) was used, adapted with a mini camera, into which 40 mL of distilled water were added. Above the water, a canvas was placed and on it the seeds were arranged. The boxes were incubated at 41°C for 72 h. After this period, the seeds were arranged for germination as described in the germination test, and the normal seedlings were counted on the fifth day after the test installation. The results were expressed as a percentage.

Root length (RL) and shoot length (SL) of seedlings

The length of 10 normal seedlings of each replicate for all treatments was measured on seedlings from the germination test. The lengths were measured with a digital caliper, and the results were expressed in millimeters.

Dry matter of seedlings (DM)

The seedlings used to measure RL and SL were dried in an oven with forced air circulation at 60°C until reaching constant weight. Afterwards, each replicate was weighed on a precision balance. The results were expressed in g/seedling (average from 10 seedlings).

Germination speed index (GSI)

The GSI was calculated with the number of seeds germinated each day, divided by the number of days between test installation and germination, according to Maguire (1962):

$$GSI\colon \left(\frac{G1}{N1}\right) + \left(\frac{G2}{N2}\right) + \ldots + \left(\frac{Gn}{Nn}\right) \quad (1)$$

where: GSI = G_1, G_2, ..., Gn = number of germinated plants in the first, second, third, last count; N_1, N_2, ..., Nn = number of days from sowing to the first, second, third, and last count.

Field evaluations

In field evaluations 200 seeds were used for each treatment. The seeds were arranged for germination in expanded polystyrene trays, with commercial substrate Carolina®, and conditioned on benches in a protected environment. The following variables were determined:

Emergence speed index (ESI)

The ESI was calculated with the number of seedlings emerged each day, divided by the number of days between sowing and emergence, according to Maguire (1962).

Shoot length of seedlings in the field (SLF)

Ten seedlings were measured per replicate, using a digital caliper, and the results were expressed in millimeters (mm).

Dry matter of seedlings in the field (DMF)

The same methodology to quantify the DM was used.

The data were submitted to statistical analysis using the statistical program Genes (Cruz, 2013), in which the interaction between lot × concentration of AS was tested, at a 5% probability of error. The variables that showed interaction were submitted to complementary analysis.

RESULTS AND DISCUSSION

The analysis of variance expressed a significant interaction for lot x SA concentration for germination (G), abnormal seedlings (AN), hard seeds (HS), dead seeds (DS), root length of seedlings (RL), field shoot length of seedlings (SLF), emergence speed index (ESI), dry matter of seedlings (DM) and field dry matter of seedling (DMF). The variables shoot length (SL), accelerated aging (AA) and germination speed index (GSI) were only significant for lot.

The escarole seed lots presented a difference in physiological potential, with the best lots being 1 and 3, which presented a high percentage of G, low percentage of AN, HS and DS (Tab. 1). Lot 1 was higher than the other lots for the variables RL, DM, ESI, SLF and DMF (Tab. 2), besides being superior in SL, AA and GSI (Tab. 3). Lot 2 presented a lower germination and vigor, independent of the SA treatment.

For the qualitative factor lots, when evaluating the interference from SA on the germination, expressed by the percentage of G, it was observed that SA negatively interfered, reducing the germination of seeds; total inhibition was observed at the highest concentrations (0.15 mM and 0.20 mM) in lot 3 (Tab. 1). As for the quantitative factor, the concentrations of SA, we observed a quadratic equation for lot 1 since the increase in the AS concentration resulted in a decrease of the trait (Fig. 1A).

The highest percentage of germination of the escarole seeds was verified with the 0.0 mM SA concentration, with 83.25 and 84.75% G, corresponding to lots 1 and 3, respectively (Tab. 1). However, there was no positive contribution of SA to AN, and the seedlings with the best quality lots had an increase in abnormality as the SA concentrations increased. For the lower quality lots, it was observed that an increase in concentration tended to reduce the AN, but there was no effect on the percentage of normal seedlings (Fig. 1B). Although the increase in the AS concentrations reduced the number of abnormal seedlings, it is believed that this reduction is linked to the fact that it inhibits germination, that is, a phytotoxic effect was caused in the seeds, altering the biochemical and physiological processes.

Because of the physiological difference between the seed lots, it is believed that the values of the abnormal seedling percentages are linked to production, processing and storage of the same since the conditions are not known.

Similar results were found by Silveira *et al*. (2000) when evaluating rice seeds treated with SA, observing germination and vigor at concentrations of 10 and 20 mM that were phytotoxic, possibly causing the death of cells. Since SA is a phenolic compound normally seen in plants (Raskin, 1992), it is possible to exercise allelopathy in some species, and, in this way, it could negatively influence the germination of some seeds and the growth of some plants, as in escarole seeds.

Table 1. Percentages of germination (G), abnormal seedlings (AN), percentage of hard seeds (HS) and dead seeds (DS) of the three lots of escarole seeds submitted to different salicylic acid concentrations.

Concentration (mM)	G			AN		
	Lot			Lot		
	1	2	3	1	2	3
0.00	83 a	2.0 b	85.0 a	6 b	53 a	6 b
0.05	67 a	0.5 b	3.0 b	16 c	42 b	84 a
0.10	37 a	0.3 b	0.3 b	47 b	40 b	89 a
0.15	44 a	0.3 b	0.0 b	39 b	32 b	86 a
0.20	70 a	0.0 b	0.0 b	17 B	26 b	86 a
CV (%)	24.090			15.715		
Concentration (mM)	**HS**			**DS**		
	Lot			Lot		
	1	2	3	1	2	3
0.00	6 b	21 a	6 b	4 b	24 a	3 b
0.05	9 a	14 a	8 a	7 b	43 a	5 b
0.10	9 b	34 a	7 b	7 b	26 a	4 b
0.15	10 b	32 a	9 b	6 b	35 a	5 b
0.20	7 b	17 a	6 b	6 b	48 a	7 b
CV (%)	47.981			43.177		

Means followed by the same letter in line do not differ significantly according to the Tukey test ($P \leq 0.05$).

Table 2. Root length (RL), dry matter of seedlings (DM), emergence speed index (ESI), field shoot length of seedlings (SLF) and field dry matter of seedlings (DMF) of the three lots of escarole seeds submitted to different salicylic acid concentrations.

Concentration (mM)	RL			DM		
	Lot			Lot		
	1	2	3	1	2	3
0.00	33.140 a	6.339 b	13.086 b	0.013 a	0.004 b	0.014 a
0.05	29.851 a	4.809 b	15.368 b	0.014 a	0.000 c	0.008 b
0.10	37.093 a	0.000 b	2.586 b	0.011 a	0.004 b	0.000 b
0.15	33.785 a	0.000 b	0.000 b	0.011 a	0.004 b	0.000 b
0.20	33.406 a	1.188 b	7.144 b	0.013 a	0.000 b	0.000 b
CV (%)	30.592			65.541		
Concentration (mM)	ESI			SLF		
	Lot			Lot		
	1	2	3	1	2	3
0.00	6.017 a	0.51 b	5.715 a	17.23 a	10.73 c	14.59 b
0.05	6.987 a	0.287 c	5.599 b	15.52 a	0.000 b	15.91 a
0.10	7.341 a	0.155 c	4.56 b	15.63 a	0.000 b	14.69 a
0.15	5.916 a	0.207 c	4.687 b	16.37 a	0.000 c	14.48 b
0.20	6.907 a	0.171 c	4.104 b	15.59 a	0.000 c	14.40 b
CV (%)	14.802			6.583		
Concentration (mM)	DMF					
	Lot					
	1	2	3			
0.00	0.086 a	0.015 c	0.062 b			
0.05	0.091 a	0.000 c	0.063 b			
0.10	0.088 a	0.000 b	0.074 b			
0.15	0.089 a	0.000 c	0.067 b			
0.20	0.088 a	0.000 c	0.070 b			
CV (%)	11.573					

Means followed by same letter in the line do not differ significantly according to the Tukey test ($P \leq 0.05$).

Table 3. Means shoot length of seedlings (SL), accelerated aging (AA) and germination speed index (GSI) for the three lots of escarole seeds.

Lot	SL (mm)	AA (%)	GSI
1	8.623 a	7.200 a	64.505 a
2	1.828 b	0.000 b	10.866 c
3	2.240 b	0.000 b	33.850 b
CV (%)	64.891	71.6	22.233

Means followed by same letter in the line do not differ significantly according to the Tukey test ($P \leq 0.05$).

For HS and DS (Tab. 1), lot 2 presented the highest percentages, complementing the low results found for G (Tab. 1). For these traits, the AS concentrations did not influence the vigor of the escarole seeds (Fig. 1C-D).

RL showed that the SA concentration did not interfere with the seed vigor of the lots with the highest physiological potential (lot 1). However, a negative effect was observed for the lower quality seeds, presenting a negative linear effect and reducing the germination potential (Tab. 2; Fig. 1E). The data agreed with Pacheco *et al.* (2007), who worked with

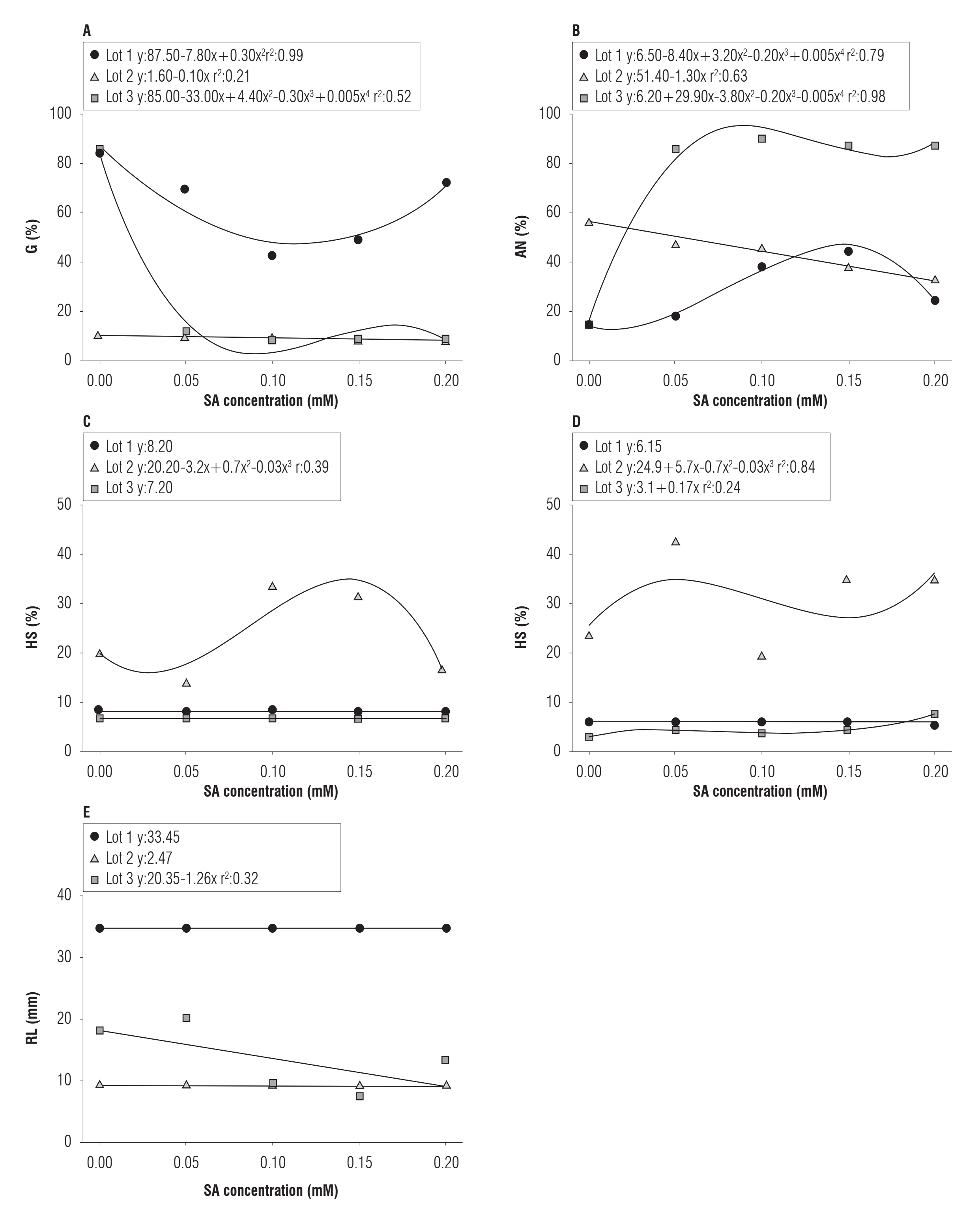

Figure 1. A. Percentage of germination (G); B. Abnormal seedlings (NA); C. Hard seeds (HS); D. Dead seeds (DS); E. Root length of seedlings (RL) of the three lots of escarole, Escarola Lisa cultivar, as a function of the salicylic acid (SA) concentrations.

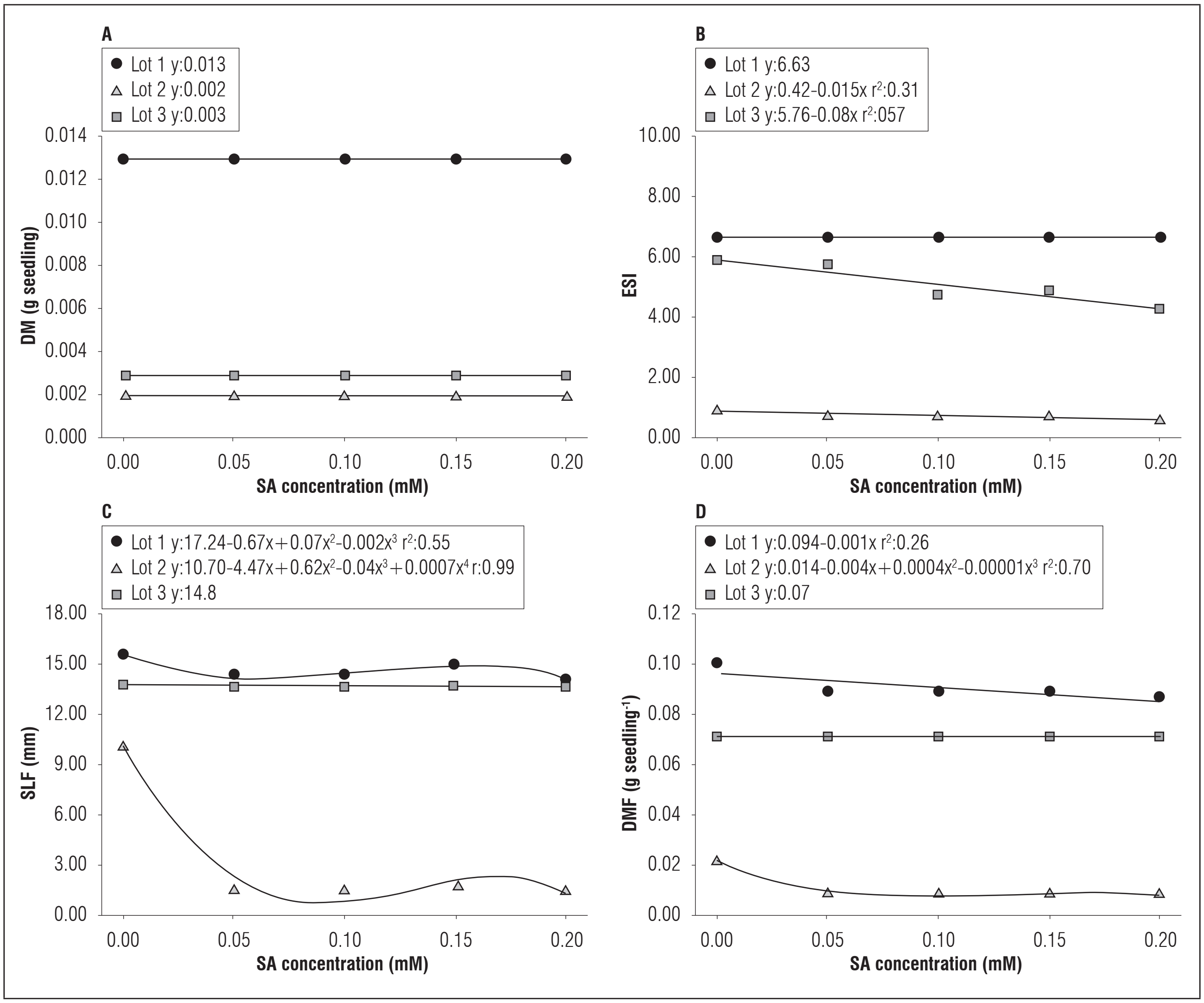

Figure 2. A. Dry matter of seedlings (DM); B. Emergence speed index (ESI); C. Field shoot length of seedlings (SLF); D. Field dry matter of seedlings at field (DMF) of the three lots of escarole, Escarola Lisa cultivar, as a function of the salicylic acid (SA) concentrations.

camomile seeds, revealing that concentrations equal to or greater than 0.20 mM SA caused a reduction in vigor, presenting a phytotoxic effect.

The concentration equal to or greater than 10 μM SA had an inhibitory effect on the vigor (first count) for the germination of rice seeds (Silveira *et al*., 2000). These results agreed with Kerbauy (2008) who verified that salicylic acid is a phenolic compound with different functions, such as germination inhibitor.

This phytotoxic effect exerted by SA as observed in the high concentrations was evident since, at this stage, the seed is using its reserves to start the germination process, and phytoalexins, produced for defense, are not yet fully formed, causing germination losses. A SA application after seedling germination and growth probably provides greater benefits to plant establishment and defense to abiotic factors than to germination.

The DM and ESI were not influenced by the SA concentrations for the lot with greater physiological vigor, with the highest values observed in the dose of 0.015 mM AS (Tab. 2). For the quantitative effects (Fig. 2A-B), no influence from the AS concentrations on the increase of DM and ESI was observed. The results found by Brunes *et al*. (2015), corroborating the data obtained in the present study, while studying the effects of AS concentrations on sunflower seeds showed that there were no interference in field emergence tests.

The ability of plants to accumulate DM is directly related to seed vigor, but, as stages progress, this influence tends to reduce plant performance, becoming more dependent on genotype and environment relationships. This is explained by the low influence of SA soaking on escarole seeds under DM accumulation and ESI in the initial phase. If the hypothesis that the AS concentrations provided phytotoxicity to the seeds is correct, this application tends not to influence or negatively influence the initial development of seedlings, as verified by the DM, ESI, SLF and DMF (Tab. 2; Fig. 2).

This negative effect on seed germination and vigor was also verified for soybean seeds, concluding that salicylic acid does not present a positive influence on germination. Therefore, α-amylase enzyme activity was stimulated, increasing shoot and root length and fresh matter, but dry matter of shoot and root was reduced in the 50 and 100 mg kg^{-1} concentrations (Costa Maia *et al*., 2000).

However, under effect of water stress, Carvalho *et al*. (2007), studying SA concentrations in marigold seeds, verified a significant increase in the germination percentage and germination speed index (0.025 mM SA). This plant activator probably did not positively influence the physiological quality of escarole seeds because they were under optimal conditions of temperature and humidity, and the physiological quality of the seeds was more influenced by genetic factors in the initial phase.

CONCLUSIONS

The use of salicylic acid did not provide benefits to the physiological quality of the escarole (*Chicorium endivia* L.) seeds; however, new research should be done by testing this product under stress conditions or as applied in stages after germination.

Conflict of interests: the manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts in risk the validity of the presented results.

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