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Foliar application of plant growth regulators changes the physiological quality of crambe seeds

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ABSTRACT. The application of plant growth regulators can manipulate the development of cultivated plants, and their physiological effects may be reflected in the physiological quality of the seeds. This study evaluated the effects of plant growth regulators (PGR) on the physiological quality of crambe seeds produced in the 2014 and 2015 harvests. During crop development, we applied two foliar sprayings of the following treatments: 1) control treatment with distilled water; 2) indole-3-acetic acid 100 mg L^{-1} (IAA); 3) 3-gibberellic acid P.A. 100 mg L^{-1} (GA_3); 4) commercial PGR Stimulate® 6 mL L^{-1}. The seeds were harvested and evaluated for water content, germination percentage and germination speed index (GSI), electrical conductivity and concentrations of leached Ca, Mg and K ions. Means were compared by Tukey´s and Dunnett tests ($p<0.05$). Applying Stimulate® increased the germination percentage and the germination speed index, while IAA application showed an opposite effect, increasing the electrical conductivity of crambe seeds. Calcium leaching decreased with IAA application, and all regulators reduced K leaching. We therefore infer that the application of plant growth regulators influences the physiological quality of crambe seeds.

Keywords: *Crambe abyssinica* Hochst. ex Fries; growth promoters; germination; electrical conductivity; ion leaching.

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Introduction

Innovative technologies are essential to obtain the maximum productivity potential of a plant and maximum yield quality (Albrecht, Braccini, Scapim, Ávila, & Albrecht, 2012). One of the most efficient and least expensive ways to manipulate agricultural production is to use plant growth regulators, which are analogues of endogenous hormones produced by plants and can be applied to manage plant development (Dantas, Queiroz, Vieira, & Almeida, 2012).

These chemical substances play an important role in different physiological processes related to plant growth and development (Kaur et al., 2018). For example, auxin, gibberellin and cytokinin are growth promoters and directly act on the plant apical dominance, stimulating cell division and differentiation as well as cell stretching and bud formation and preventing chlorophyll degradation, inducing flowering and controlling seed formation (Giannakoula, Ilias, Maksimovic, Maksimovic, & Zivanovic, 2012; Kaur et al., 2018). Exogenous application may reflect on the endogenous levels, influencing the biosynthesis, catabolism and conjugation of these substances (Talaat, Khattab, & Ahmed, 2014).

Plant growth regulators can be applied by foliar spraying during cultivation, with the purpose of improving vegetative characteristics such as the physicochemical quality of the grain, grain mass, yield and total dry biomass (Abrantes et al., 2011; Carvalho, Viecelli, & Almeida, 2013; Silva, Santos, Oliveira, Soares, & Sanos, 2016). In seeds, plant growth regulators commonly used directly to improve germinative performance, prolong vigour and overcome physiological dormancy (Prado Neto, Dantas, Vieira, & Almeida, 2007; Moterle et al., 2011; Nonogaki, 2014).

Thus, the foliar application of plant growth regulators alters the physiological functioning, positively impacting plant development. Seed quality, in turn, depends on three main factors: genetic constitution, post-harvest storage and cultivation conditions during production (Marcos Filho, 2015). In this context, the physiological quality of seeds produced by plants grown with plant growth regulators can be altered. Kuchlan, Kuchlan, and Husain (2017) observed these changes when evaluating the germination and electrical conductivity of soybean seeds produced under the application of plant growth regulators.

The physiological quality of seeds is defined by the capacity of the seeds to germinate and form normal seedlings and by the longevity and vigour. Of these qualities, seed vigour concerns all seed properties that determine the seed performance potential after sowing (Bewley, Bradford, Hilhorst, & Nonogaki, 2012).

The germination test is the most widely used test to evaluate seed germination capacity under controlled optimal conditions (Carvalho & Nakagawa, 2012). Regarding seed vigour, the electrical conductivity test reflects the loss of organization and the selective permeability of the cell membranes of the seeds through the conductivity generated by the leached and diluted solutes in the immersion water of the seed (Marcos Filho, 2015).

When immersed in distilled water, the seed leaches electrolytes, thereby changing the electrical resistance of the water (Krzyzanowski, Vieira, & França Neto, 1999). The quantification of the leached ions is also important, because inorganic ions are the first compounds to be leached when the seed is in contact with water (Costa, Vahl, & Villela, 2007). Studies performed by Binotti et al. (2008) and Zucareli et al. (2013) quantified potassium, calcium and magnesium as the main leached ions in order to develop new parameters for seed vigour analyses of different crops.

Crambe (*Crambe abyssinica* Hochst. ex Fries), an oilseed belonging to the cruciferous family (Souza, Favaro, Ítavo, & Roscoe, 2009), is a relatively new crop in the agricultural scenario. Information on management techniques during plant cultivation and development are scarce (Bassegio et al., 2016). Due to its origins, crambe tolerates drought and cold; it is therefore suitable for autumn/winter cultivation in Brazil and is an excellent alternative for the late growing season and crop rotation (Carlsson, 2009). The main industrial interest in this agricultural product is its oil, which contains 50 to 60% erucic acid, a component used in the production of plastic films, plasticizers, nylon, adhesive glue and electrical insulation material (Li, Ahlman, Lindgren, & Zhu, 2011). In addition, crambe oil has a high oxidative stability, which is interesting for the storage and production of biofuels (Lalas, Gortzi, Athanasiadis, Dourtoglou, & Dourtoglou, 2012).

Seeds with a high physiological potential are essential to obtain satisfactory results in the production of economically viable expression cultures (Binotti et al., 2008). To develop suitable technologies for obtainment and seed production, it is essential to understand seed physiology and germination. Therefore, laboratory studies incorporating the physiological knowledge of crambe are important for making this crop available to farmers.

This work evaluated the physiological quality of crambe seeds produced with foliar application of the plant growth regulators indole-3-acetic acid, gibberellic acid and Stimulate® in the 2014 and 2015 harvests.

Material and methods

Crop characteristics and field experiment

Crambe FMS Brilhante was cultivated in two harvests, from April 20 to August 30, 2014, and from April 4 to September 5, 2015, in the area for agricultural experimentation of the *Centro Universitário Fundação Assis Gurgacz*. The *Sistema Meteorológico do Paraná* (Simepar) provided the relative humidity and precipitation variations for the study periods.

Prior to cultivation, the soil was prepared with 3,000 kg ha^{-1} avian bed. Sowing was performed at a depth of 4 cm, with a line spacing of 0.25 m, resulting in a planting density of 20 kg ha^{-1}. Cultivation was performed in a completely randomised block design with four repetitions. Each plot had a size of 6 x 6 m (36 m^2), with a spacing of 2 m between plots, totalling an experimental area of 936 m^2. The edges of each plot (0.5 m) were not considered during harvesting.

Plant growth regulators were applied manually by foliar spraying with a volume of 2 L per plot when the plants were in transition from the vegetative stage to the beginning of flowering, about 60 days after sowing in the 2014 harvest and 90 days after planting in the 2015 harvest. A second application took place after 15 days (Ouzounidou, Ilias, Giannakoula, & Papadopoulou, 2010; Giannakoula et al., 2012).

To prepare the solution, we used 10 mL ethyl alcohol P.A. as diluent and corresponded to 1) control treatment with distilled water; 2) indole-3-acetic acid 100 mg L^{-1} (IAA); 3) 3-gibberellic acid P.A. 100 mg L^{-1} (GA_3) and 4) commercial plant growth regulator Stimulate® 6 mL L^{-1}.

Stimulate® is a commercial plant growth regulator consisting of 0.009% kinetin + 0.005% gibberellic acid + 0.005% 4-indol-3-ylbutyric acid + 99.98% incipients, and we used the concentration recommended by the manufacturer, proportional to the portion area (Stoller do Brasil, 1998).

Laboratory bioassays

Seeds were harvested, taken to the Laboratory of Quality Control of Agricultural Products (Unioeste campus Cascavel), selected and sanitized. Humidity was determined by oven-drying at 105 ± 3°C for 24 hours, as indicated by Brasil (2009), and the results are expressed as percentages.

For the germination test, we used Petri dishes previously autoclaved for 20 min. at 125°C, with two filter paper sheets. The test was conducted with four replicates containing 25 seeds, moistened with 2.5 times the mass of the dry paper and packed into a B.O.D.-type germination chamber at 20 ± 2°C, with a 12 hours photoperiod. Evaluations were performed daily for 5 days; the results were used in the calculations of total percentage of germinated seeds and germination speed index (GSI), according to the formula proposed by Maguire (1962), according Equation 1:

$$GSI = \sum_{i=1}^{\sim} \frac{Gi}{Ni} \quad (1)$$

where:

GSI = germination speed index;

i – 1, ..., n; Gi = number of seeds germinated on the respective day;

N_i = number of days after the experiment setup.

The electrical conductivity test was performed with 50 seeds per replicate, placed in plastic glasses with 75 mL deionized water (Costa, Resende, Gonçalves, & Souza, 2012) and kept at 20°C in a B.O.D. germinating chamber for 24 hours. After this period, we performed the electrical conductivity reading of the leached crambe seed, using a benchtop conductivity meter; the data are expressed in mS cm^{-1} g^{-1}.

The leached solutions obtained in this test were analysed via direct atomic absorption spectrometry reading, following a methodology proposed by the *Instituto Adolfo Lutz* (2008). We sued a Shimadzu model AA-6300 to determine the ion concentrations of calcium (Ca), magnesium (Mg) and potassium (K); concentrations are expressed in µg mL^{-1}.

Statistical analyses

The laboratory experiments followed a completely randomised experimental design with three replicates for each repetition (four) of field treatments. We performed descriptive data analysis as well as the Anderson-Darling normality test. No dataset needed to be transformed.

Data for the two harvests were submitted separately to the analysis of variance for the treatment factor, using four levels of plant growth regulators: 1) control, 2) IAA, 3) GA_3 and 4) Stimulate®. Factor levels that showed significant effects were compared by Tukey´s test and with the control by the Dunnett test. For all analyses, a significance level of 5% was assumed. Analyses were performed using the software package Minitab® 17.

Results and discussion

Table 1 shows the differences in relative humidity (RH) and cumulative precipitation between the 2014 and 2015 harvests. Relative humidity in 2014 was higher than that in 2015 in April, June and August. In 2014, rainfall was concentrated in May and June. In 2015, June had a lower precipitation index.

The 2015 production cycle was longer than the 2014 cycle. Plant development was not homogeneous. Some plants were flowering when other plants were already in the seed formation phase.

The application of plant growth regulators significantly influenced the germination percentage, germination speed index, electrical conductivity and calcium, magnesium and potassium concentration of crambe seeds in the 2014 crop (Table 2). In relation to the seeds harvested in 2015, the p-value was significant for germination percentage, germination speed index, electrical conductivity and leached potassium concentration.

The difference between the harvests may be a result of the climatic conditions in the two agricultural years. Due to a variety of factors, it is still unclear how the climatic variation between the years influences the quality of the agricultural product. However, small differences in RH and cumulative precipitation are sufficient to influence different components of the agricultural production (Kang, Khan, & Ma, 2009).

Crambe seeds produced with Stimulate® in 2014 showed a higher germination percentage and a higher germination speed index when compared to seeds of the control treatment (Table 3). In the 2015 harvest, the IAA application resulted in a lower germination percentage compared to the control and the Stimulate® treatment. In addition, seeds in this treatment showed delayed germination, with a lower germination speed index.

Amaro et al. (2014) analysed five lots of crambe seeds and obtained an average germination of 42%, indicating that crambe seeds have a low germination percentage, which was also observed in this study. This relates to the fact that crambe seeds are characterised by post-harvest dormancy, resulting in a low germination percentage when compared to other agricultural seeds (Costa et al., 2012; Oliveira et al., 2014). Thus, an increase to 66.67% of germinated seeds can be regarded as significant.

Abiotic factors such as management, mineral nutrition and physiological conditions of the plant during seed production may also influence the dormancy process (Graeber, Nakabayashi, Miatton, Leubner-Metzger, & Soppe, 2012). Seed dormancy may be controlled by hormones, being induced and maintained by high concentrations of abscisic acid (ABA) or reversed by an increase in gibberellin concentrations (Nonogaki, 2014). Interactions between the hormonal classes are common; a substance can often interfere with the enzymatic activity of the biosynthesis or inactivation of another (Kucera, Cohn, & Leubner-Metzger, 2005; Taiz, Zeiger, Moller, & Murphy, 2017).

Table 1. Means of relative humidity (RH) and cumulative precipitation values for the crambe production periods from the harvest (H1) from April 20 to August 30, 2014, and from the harvest (H2) from April 1 to September 5, 2015.

Month	RH (%)		Cumulative Precipitation (mm)		
	H1	H2		H1	H2
April	83.90	78.47		107.00	102.20
May	83.30	84.65		180.80	261.20
June	84.70	80.92		442.40	91.20
July	79.30	87.03		107.60	384.60
August	65.70	63.82		55.00	55.20
September	-	62.93		-	1.40
Means	79,38	76.30	Total	892,80	895,80

Table 2. Values of f and p of the analysis of variance for germination percentage (G, %), germination speed index (GSI, seeds/day), humidity content (U; %), electrical conductivity (EC; mS cm^{-1} g^{-1}) and concentrations (μg mL^{-1}) of calcium (Ca), magnesium (mg) and potassium (K) for the treatments with plant growth regulators.

	G	GSI	U	EC	Ca	Mg	K
				Harvest 2014			
f-value	7.530	5.790	1.890	7.280	5.800	6.870	4.620
p-value	0.004	0.011	0.184	0.005	0.011	0.006	0.023
				Harvest 2015			
f-value	4.900	3.870	3.170	2.830	1.330	1.130	14.20
p-value	0.019	0.038	0.064	0.008	0.311	0.374	0.000

Table 3. Mean values of germination percentage (G, %), germination speed index (GSI, seeds per day), humidity (U; %) electrical conductivity (EC; mS cm^{-1} g^{-1}) and concentrations (μg mL^{-1}) of calcium (Ca), magnesium (Mg) and potassium (K) ions leached from crambe seeds produced with plant growth regulators.

	G	GSI	U	EC	Ca	Mg	K
				Harvest 2014			
Control	47.67 b	4.48 b	6.01^{ns}	94.01 ab	4.56 a	1.33 a	0.103 ab
IAA	55.33 ab	5.05 ab	6.42	84.80 b	3.56 b$^{(+)}$	1.10 b	0.094 b
GA_3	47.33 b	4.25 b	6.15	104.1 a	4.53 a	1.33 a	0.108 ab
Stimulate®	66.67 a$^{(+)}$	6.29 a$^{(+)}$	6.94	99.51 a	4.23 a	1.33 a	0.113 b
				Harvest 2015			
Control	57.00 a	6.14 a	5.82^{ns}	47.51 a	2.60^{ns}	0.86^{ns}	0.097 a
IAAA	35.02 b$^{(+)}$	3.35 b$^{(+)}$	5.77	65.28 a$^{(+)}$	3.27	1.05	0.096 b$^{(+)}$
GA_3	54.50 a	3.99 ab	6.30	56.60 a	2.80	0.96	0.096 b$^{(+)}$
Stimulate®	56.50 a	5.38 ab	6.31	50.70 a	3.04	0.99	0.096 b$^{(+)}$

Values followed by the same letter in the column are not significant by Tukey test (p ≤ 0,05). Values followed by (+) differ statistically from the control treatment by Dunnett test (p ≤ 0,05). Control – distilled water; IAA – indole-3-acetic acid 100 mg L^{-1}; GA_3 – 3-gibberellic acid 100 mg L^{-1}, Stimulate® - commercial plant growth regulator kinetin-AIB-GA_3 6 mL L^{-1}. ns = not significant.

Through this mechanism, the mixture of plant growth promoters in Stimulate® may have altered the endogenous hormonal balance of crambe seeds, increasing germination performance. Direct seed treatment with gibberellin is a common and effective practice to improve the germination performance of several species (Silva, Landgraf, & Machado, 2013; Feitosa et al., 2015).

In addition, the main effect of the cytokinin present as kinetin in the Stimulate® is the establishment of drains, acting on proteins necessary for the apoplast discharge of the phloem and favouring the continuous transport of nutrients to fruits and flowers (Taiz et al., 2017), which may contribute to the production of seeds with better physiological quality.

The positive effect of Stimulate® application on the germination speed has also been observed in soybean seeds (Moterle et al., 2011) and jeripape seeds (Prado Neto et al., 2007). Applying Stimulate® in the production of soybean (Carvalho et al., 2013), bean (Abrantes et al., 2011), cowpea beans (Oliveira et al., 2015) and cotton (Silva et al., 2016) improved the vegetative development of these crops and increased the physicochemical quality of the grain, grain mass, yield and total dry biomass. These morphological aspects result from the physiological changes caused by the application of plant growth regulators and directly reflect the physiological quality of the seeds produced.

Kuchlan et al. (2017) also observed that the foliar application of plant growth regulator during the production improved seed germination and vigour, which indicates that plant growth regulators could be applied in order to increase the physiological quality in seed production.

Humidity values were similar between the treatments and ranged from 5.77 to 6.94% (Table 3), similar to those obtained by Cardoso, Binotti, and Cardoso (2012) and Cardoso et al. (2014). Crambe is a conventional oilseed that can present 5-10% humidity at the end of the physiological maturation process (Oliveira et al., 2014).

The electrical conductivity of the crambe seeds produced in the 2014 harvest with application of GA_3 and Stimulate® was higher than that of the seeds produced with IAA application; however, conductivity values were similar to those of the control treatment (Table 3). In 2015, IAA foliar application had a significant effect on the integrity of the crambe membranes, which was reflected in higher solute leaching than in the control treatment.

Seeds under these conditions require higher nutrient intakes from their reserves to restore deteriorated membranes and reactivate their metabolism, compromising their vigour (Vanzolini & Nakagawa, 2005). The electrical conductivity values in this work are lower than those presented by other authors (Costa et al., 2012; Oliva, Biaggioni, & Cavariani, 2012; Bessa et al., 2015), but similar to those observed by Bezerra, Biaggioni, Silva, Sperotto, and Brandão (2015). Cruz et al. (2013) evaluated five lots of crambe seeds and observed a variation from 58 to 877 mS cm^{-1} g^{-1} at different soaking times.

As parameters of seed quality and vigour, the germination test results corroborate the electrical conductivity results, disagreeing with Cruz et al. (2013), who concluded that in comparison to other methods, the electrical conductivity test may not be the most adequate to evaluate the physiological quality of crambe seeds. Thus, based on the germination percentage and germination speed index values as well as on the electrical conductivity of crambe seeds, the physiological quality of the crambe seeds decreased with the application of IAA during the production.

The increase in the endogenous concentration of a growth promoter can be attributed to the increase of its biosynthesis or the decrease in its degradation and conjugation (Talaat et al., 2014). Studies performed by Liu et al. (2013) show that auxin controls the dormancy process mediated by ABA in the seeds at the gene expression level; this effect was proven for the exogenous auxin application. These authors list two possible mechanisms by which auxin acts in these ABA-mediated processes: it either stimulates ABA biosynthesis or activates the ABA response.

However, the concentrations of calcium and magnesium ions in the leaching of seeds produced with IAA application in the 2014 production were statistically lower when compared to the application of other plant growth regulators; only calcium leaching differed from that in the control treatment. The potassium leaching of crambe produced with IAA, GA3 and Stimulate® was lower and statistically different from that in the control, but was similar between the treatments. Thus, the application of IAA and other plant growth regulators led to the reduction of the leaching of Ca and K ions, respectively, which are important for the embryonic development and the germination process.

Auxin regulates many aspects of plant development and growth (Chapman & Estelle, 2009). Since there is an association between auxin and endogenous ABA production in seeds, intracellular auxin levels are also associated with gibberellin levels (Taiz et al., 2017). In the seeds, the increase of the gibberellin concentration activates enzymes that degrade reserve substances (Linkies & Leubner-Metzger, 2012), while a reduction in gibberellin levels impedes the degradation of starches and other substances. In this situation, fewer ions would leach through the seed coatings.

According to Favorato, Rocha, Espindula, Souza, and Paula (2011), Barbieri, Menezes, Conceição, and Tunes (2012) and Zucareli et al. (2013), ions with the highest concentrations in the leached solution are potassium, magnesium and calcium; they can therefore be used in the vigour test. In this work, calcium was the ion with the highest leaching concentration, diverging from the results obtained by these authors, as already mentioned.

Conclusion

Crambe seeds produced with Stimulate® via foliar application showed an increased germination potential, while IAA application compromised germination and the membrane system quality of crambe seeds. However, a reduction in calcium leaching loss occurred in response to the IAA application, and a reduction in potassium leaching occurred with the application of all the regulators studied in this work.

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