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Influence of bio-regulators on the seed germination and seedling growth of onion cultivars

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ABSTRACT. Application of bio-regulators improves the productivity of cash crops. Current experiment evaluates the effects of different doses of a bio-regulator on the seed quality of three onion cultivars ('Baia Periforme', 'Bola Precoce' and 'Híbrida Superex'). Six doses of bio-regulator at 0, 2.5, 5.0, 10.0, 20.0 and 30.0 mL kg^{-1} were applied and results were compared to those of three controls. The experimental design was entirely randomized under the factorial arrangement 3 x 6 + 3, with four replications. Data were subjected to analysis of variance ($p < 0.05$), and all seed treatments were compared with controls by Dunnett's test. The analysis of regression described the effects of the increasing doses of bio-regulator on germination and vigor responses which were evaluated by the first count of the germination test, the percentage of germination and the length of the roots and shoots of the normal seedlings. The application of the bio-regulator did not improve the percentage of seed germination and no positive response of the seedlings was reported from the cultivars 'Baia Periforme' and 'Bola Precoce'. However, seedling vigor of 'Híbrida Superex' was positively affected by the application of the bio-regulator.

Keywords: *Allium cepa* L., bio-regulator, seed quality.

Influência de biorregulador na germinação de sementes e desempenho das plântulas de cultivares de cebola

RESUMO. A aplicação de biorreguladores tem sido empregada com a finalidade de aumentar o desempenho de culturas de interesse econômico. Com o objetivo de avaliar o efeito da aplicação de diferentes doses de biorregulador via tratamentos de sementes em cultivares de cebola, foi conduzido um experimento com três cultivares de cebola ('Baia Periforme', 'Bola Precoce' e 'Híbrida Superex') e seis doses de biorregulador (0; 2,5; 5,0; 10,0; 20,0 e 30,0 mL kg^{-1} de sementes), acrescido de três testemunhas. O delineamento foi o inteiramente casualizado, com quatro repetições e os tratamentos arranjados em esquema fatorial (3 x 6 + 3). Os dados foram submetidos à análise de variância ($p < 0,05$) e, posteriormente, aplicou-se o teste de Dunnett para comparar os tratamentos de sementes com as testemunhas, assim como a análise de regressão, com o intuito de obter curvas de resposta, em função do acréscimo nas doses do biorregulador. As sementes foram avaliadas pela primeira contagem e contagem final do teste de germinação e comprimento da raiz primária e da parte aérea das plântulas. A aplicação do biorregulador não foi satisfatória ao processo de germinação das sementes e desempenho das plântulas das cultivares 'Baia Periforme' e 'Bola Precoce'. Entretanto, para a 'Híbrida Superex', o biorregulador estimulou o vigor inicial das plântulas.

Palavras-chave: *Allium cepa* L., biorregulador, qualidade de sementes.

Introduction

Onion (*Allium cepa* L.) is a condimental plant cultivated under different weather conditions since ancient times. It is the third horticultural product worldwide and the second source of net income in agriculture, with China currently ranked as the biggest onion producer (BOITEUX; MELO, 2004; CHEMELLO, 2005).

In Brazil, tomato, potato and onion are the third most important economic produces with the southern, southwestern and northeastern regions featuring the highest acreages in onion planting. Onion growers in all these regions are farmer families whose members participate in the entire production system, aiming at supplying the Brazilian market with this fresh produce (BOEING, 2002; RESENDE; COSTA, 2005; OLIVEIRA; BOITEUX, 2004). However, the constraints in onion cultivation require new approaches so that the usual levels of crop productivity may be exceeded.

In plant growth and development, the seed germination, vegetative growth, flowering, fruit growth and maturation are influenced by several endogenous and exogenous factors. Plant development is regulated by six types of hormones: auxin, gibberellin, cytokinin, ethylene, abscisic acid and brassinosteroid. When applied to plants at low concentration (10^{-4} M), phytohormones promote, inhibit or modify physiological and morphological processes (CASTRO; VIEIRA, 2001). Gibberellins are important to promote seed germination because they induce the synthesis of enzymes through genetic mechanisms that support the breakdown and the mobilization of seed reserves from the endosperm into the embryo axis. The cytokinins interacting with the auxins promote cell division due to their role in cell cycle and participation in the mechanisms of cell lengthen and differentiation. Further, auxins affect cell length through ATPase activation which is necessary to promote the cell wall acidity and therefore make possible the action of gibberellin-synthesized hydrolytic enzymes (CASTRO; VIEIRA, 2001; RAVEN et al., 2007; TAIZ; ZEIGER, 2009).

Metabolism, morphogenesis and plant growth depend on the signalling transduction pathway for plant hormones. These signalling phytohormones are important to maintain the regulatory mechanisms during the entire plant development (TAIZ; ZEIGER, 2009). In fact, the pathways transmit information on the development and physiological status of plant cells and tissues (CASTRO; VIEIRA, 2001). The origin, biosynthesis, chemical structure, mechanisms of transport, action and physiological effects of these substances should be currently known to enhance the plants' physiological responses to these chemicals or their counterparts (VIEIRA; CASTRO, 2004). Thus, plant hormones are of paramount importance in controlling the mechanisms of plant productivity.

The application of bio-regulators has been recently recommended to increase the productivity of several crops, such as soybean, beans, maize and rice (KLAHOLD et al., 2006; ALBRECHT et al., 2009, 2010). Bio-regulators are natural or synthetic substances applied to seeds and other parts of plant to produce the plant's hormone effects. In general, they increase plant productivity although they have not been applied to agriculture systems in which plants are not propped by high technology (VIEIRA; CASTRO, 2001; VIEIRA; SANTOS, 2005). Since several benefits have been obtained through the use of these bio-regulators, the mixture of one or more of these substances or their mixture with amino acids, nutrients and vitamins is usually called bio-stimulants (CASTRO; VIEIRA, 2001). Bio-regulators have increased the percentage of seed germination and plant height of *Strelitzia reginae* (GARCIA et al., 2006), the percentage of seed germination and the germination rate of *Annona cherimola* Mill. x *Annona squamosa* L. cv. Gefrer, the percentage of emergence and plant development of *Passiflora edulis* Sims. f. *flavicarpa* Deg. (FERREIRA et al., 2007), and the development and crop yield of *Phaseolus vulgaris* (ALLEONI et al., 2000). The dry weight of the seedling at the three trifoliate stages and the dry weight of 1,000 grain were increased.

Bio-regulators with a wide range of concentrations were applied on the seeds or sprayed on the leaves of soybean and common bean crops (ALLEONI et al., 2000; MOTERLE et al., 2008) or only on the seeds of *Passiflora edulis* Sims. f. *flavicarpa* Deg. and *Strelitzia reginae* (FERREIRA et al., 2007; GARCIA et al., 2006). Whereas bio-regulator at 250 mL ha^{-1} was applied on the seeds and 750 mL ha^{-1} was sprayed on the leaves of common beans (ALLEONI et al., 2000), 12 and 16 mL kg^{-1} were applied on the seeds of *Passiflora edulis* Sims. f. *flavicarpa* Deg. (FERREIRA et al., 2007), and 4.07 mL kg^{-1} increased by 14.2% the percentage of normal seedlings of *Annona cherimola* Mill. x *Annona squamosa* L. cv. Gefrer. On the other hand, high doses of bio-stimulants applied on cotton seeds affected negatively seedling emergence (VIEIRA; SANTOS, 2005). No difference either in plant height or in pod numbers per plant was detected in soybean (MOTERLE et al., 2008).

The presence of inconsistent reports in the literature may be due to the few species investigated, environmental conditions and crop management. Furthermore, industrialized phytohormones hardly ever work alone because the target tissue has endogenous hormones that may influence responses (VIEIRA; CASTRO, 2004). Thus, the efficacy of bio-regulators coupled to different concentrations should be determined (VIEIRA; CASTRO, 2004). The products' composition, concentration and proportion in the chemical solution may increase the plants' growth and development by stimulating the cell length and division and the differentiation of plant tissues, with the consequent increase in the absorption nutrient and efficient use of water by the plants (STOLLER DO BRASIL, 1998).

Onion seeds, which need more time to germinate than other kitchen garden species, constitute a constraint in onion production. Seedling growth is also slow even when the photoperiod and temperature are satisfactory for the plant's development and the bulbs' formation (OLIVEIRA; BOITEUX, 2004; OLIVEIRA et al., 2004). Moreover, intrinsic factors may also influence the other stages of the crop's development (OLIVEIRA; BOITEUX, 2004). Current experiment evaluates the percentage of seed germination and the growth of seedlings from three cultivars of onion (*Allium cepa* L.) after the application of several concentrations of the bio-regulator on the seeds.

Material and methods

The experiment was conducted in the Laboratory of Seed Technology at the Applied Agriculture Research Nucleus (NUPAGRI), Center of Agrarian Sciences of the State University of Maringá (UEM), Maringá, Paraná State, Brazil, during the growth season 2009-2010.

Cultivars, treatments and experimental design

Current experiment comprised three onion genotypes ('Baia Periforme', 'Bola Precoce' and 'Híbrida Superex'), six doses of bio-regulator at 0, 2.5, 5.0, 10, 20 and 30 mL kg^{-1} of seeds and three controls. The bio-regulator used in this experiment was the Stimulate®, a liquid product with 50 mg L^{-1} (0.005%) indole-3-butyric acid (auxin), 50 mg L^{-1} (0.005%) gibberellin acid, and 90 mg L^{-1} (0.009%) kinetin (cytokinin) (STOLLER DO BRASIL, 1998). Treatments were completely randomized under the factorial arrangements of 3 onion genotypes and six doses of bio-regulator, with four replications. The treatments were compared with three controls (without seed imbibition in distilled water or bio-regulator), one for each genotype (3 x 6) + 3.

All the laboratory equipments used in this experiment were cleansed with alcohol 70% and commercial sodium hypochloride. Laboratory instruments were treated under ultraviolet light for one hour; the water and the blotting paper were disinfected with alcohol 70%. The seeds were weighed with an analytical scale with a 0.001 g readability of. Micro-pipettes were used to apply the solution on the seeds in graduate 50 mL beakers. The container was then stirred for 1 min. so that the solution could be distributed on all the seeds.

Seed quality

Seed germination

Four replications or sub-samples with 25 seeds for each treatment were used. The seeds were sown in plastic gerboxes on three sheets of towel paper moistened with distilled water, the amount of which was approximately 2.5 times the mass of dry paper. After the treatments, the gerboxes were transferred to a B.O.D. germination chamber, regulated to maintain a constant temperature of 20°C during 12 days, in the absence of light. The first germinated (normal seedlings) seed count occurred on the 6th day and the final count on the 12th day. Seedlings with primary root longer than or equal to 2 mm were considered germinated (HADAS, 1976). Results were expressed as a percentage of seed germination (BRASIL, 2009; MARCOS FILHO et al., 1987).

First count of seed germination

Seed germination was determined by normal seedling percentage obtained in the first count of the germination test, or rather, those that germinated faster. The test was based on the principle that the high percentage of samples that produced normal seedlings in the germination test's first count was more vigorous and thus determined the relative vigor of the seed lot (BRASIL, 2009; MARCOS FILHO et al., 1987; NAKAGAWA, 1999).

Seedlings root and shoot length

Procedures were undertaken following Nakagawa (1999), in which onion seedling lengths was measured. Samples that demonstrate the highest average values were considered the most vigorous.

Four replications of 10 seeds sown in plastic gerboxes on three sheets of paper towel on hanging distilled-water moistened towels were used. The amount of water was equivalent to 2.5 times the mass of the dry paper. Two lines were drawn lengthwise in the upper third of the germination paper where the seeds were placed, at an interleaved manner, between the papers. The plastic gerboxes were positioned at an angle of 45° in a B.O.D germination chamber for 12 days at 20°C, in the dark. At the end of this period, the sections of the germinated normal seedlings (primary root and shoot) were measured by a ruler and average results given in centimetres.

Statistical analysis

After testing data normality, the analysis of variance was calculated to determine initial treatment effects on seed quality as measured by each test. Significant effects were described by analysis of regression. Dunnett's test compared treatments and control at level of significance $p < 0.05$ by statistical package SISVAR (BANZATO; KRONKA, 2008).

Results and discussion

The application of the product showed significant difference on cultivar responses ($p < 0.05$). The first count of normal seedlings, for example, was significantly different only for seeds of the cultivar 'Híbrida Superex' (Figure 1). The cubic model showed the highest percentage of normal seedlings (77%) at dose 24.18 mL kg^{-1} (Figure 1) unlike the application of dose 8.31 mL kg^{-1} (66%). Whereas the 'Baia Periforme' had 6.8% of normal seedlings, the 'Bola Precoce' had 5.8%.

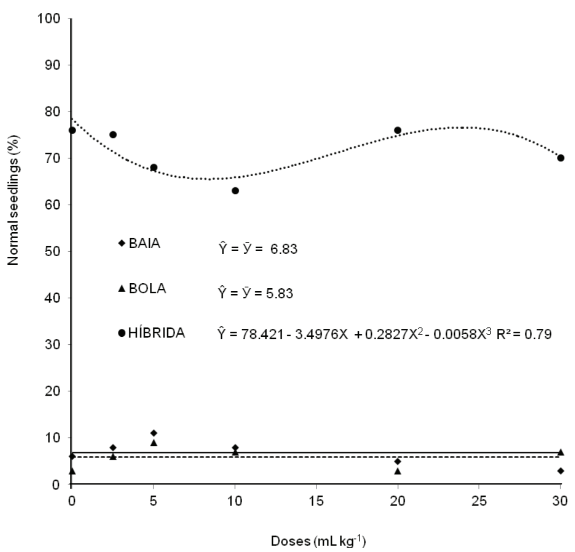

Figure 1. Polynomial curve shows the effects of doses of bio-regulator on the percentage of normal seedlings evaluated for different onion cultivars using the first count of the germination test (Maringá, Paraná State, Brazil, 2009).

Dose increase of the bio-regulator applied on the seeds of 'Baia Periforme' caused a significant fall on seed germination percentage ($p < 0.05$). In fact, the estimated control (dose 0) had 22% of normal seedlings, whereas dose 30 mL kg^{-1} had only 7% (Figure 2). Thus, decrease in the percentage of normal seedlings was 0.49% for every 1 mL of bio-regulator kg^{-1} of seed. No significant differences ($p > 0.05$) occurred with the other two cultivars. Average normal seedlings was 21% for 'Bola Precoce' and 77% for 'Híbrida Superex'. Further, quadratic responses were reported for other species and indicated losses in the percentage of normal seedlings at the highest doses of bio-regulators (ALBRECHT et al., 2010; ÁVILA et al., 2008). High physiological quality of seeds from the cultivar 'Híbrida Superex' enhanced the importance of the genotype in maintaining seed vigour and the germination of horticulture plants (MARCOS FILHO, 2005; PRETE; GUERRA, 1999).

Only the 'Híbrida Superex' had significant responses to treatments. Length of primary roots was significantly different from that in 'Híbrida Superex'. The quadratic model showed 0.73 cm as the highest root length at 18 mL kg^{-1} (Figure 3). On average, the other cultivars showed 0.26 cm and indicated that seed treatment had no influence on root length.

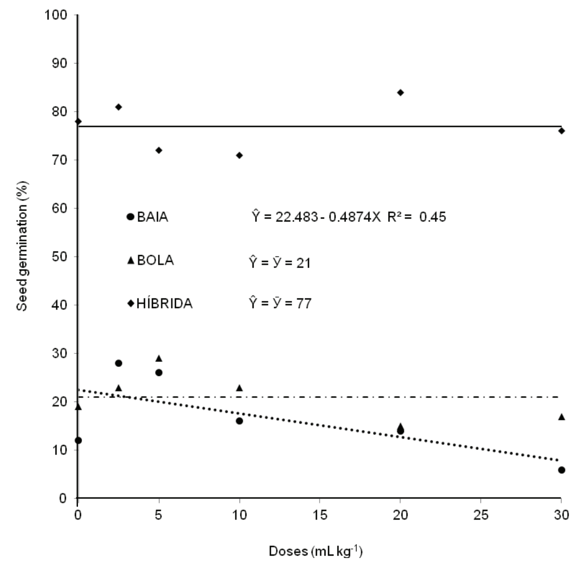

Figure 2. Linear curves describe the effects of bio-regulator doses on the percentage of normal seedlings evaluated for different onion cultivars using the germination test (Maringá, Paraná State, 2009).

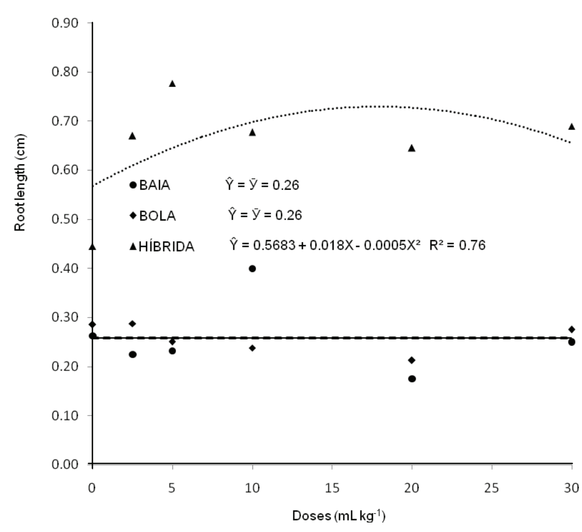

Figure 3. Polynomial curves describe the effects of bio-regulator doses on seedling root length of different onion cultivars (Maringá, Paraná State, 2009).

Responses from the shoot length were similar to the root length (Figure 4), but the maximum shoot length 2.34 cm was obtained at 15.24 mL kg^{-1}. Cultivars 'Baia Periforme' and 'Bola Precoce' were 0.10 and 0.17 cm long, respectively. Responses from cultivar 'Híbrida Superex' indicate that the product had an effect similar to auxin application. High root length in jenipapo was found after applying bio-regulator at 10 mL L^{-1} (PRADO NETO et al., 2007). This treatment was 84.31% higher than that of control. In all the cases, the dose to improve root growth was lower than that applied to enhance shoot growth. High doses inhibit the root and cause shoot growth (RAVEN et al., 2007; TAIZ; ZAIGER, 2009). Similar to what was reported for 'Híbrida Superex', positive effects have been reported for other species as, for example, increases in the physiological and sanitary quality of soybean (*Glycine max*) seeds (ALBRECHT et al., 2010; ÁVILA et al., 2008) and increases in soybean yield (MOTERLE et al., 2008). In fact, the application of bio-regulator has improved the seed quality of some kitchen garden plants (PRADO NETO et al., 2007).

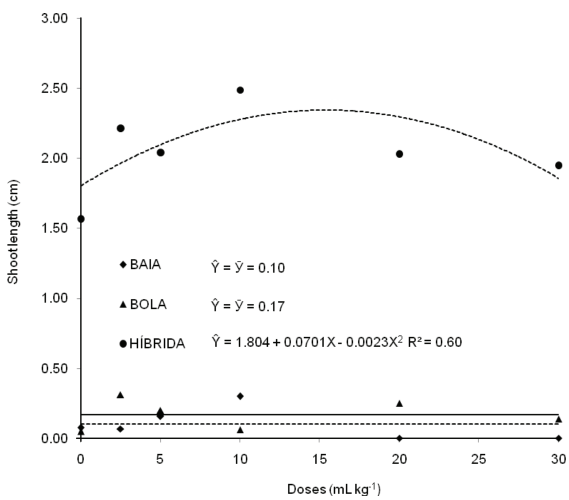

Figure 4. Polynomial curves describe the effects of bio-regulator doses on seedling shoot length of different onion cultivars (Maringá, Paraná State, 2009).

With the exception of 'Hibrida Superex', only the percentage of normal seedlings was different from that of the treatment with bio-regulator and control (Table 1 and 2), discriminated by Dunnett's test ($p < 0.05$). All doses, except doses 2.5 and 5.0 mL kg^{-1}, applied on the seeds of 'Baia Periforme', reduced the percentage of seed germination by 66.7, 55.6, 38.9 and 83.3%, respectively. Seed germination percentage of cultivar 'Bola Precoce' was reduced by 72.72% using bio-regulator at 20 mL kg^{-1} of seeds (Table 1). Comparison between growth and seedling components by Dunnett's test (Table 2) showed no difference between the treatments and control ($p > 0.05$). Current responses corroborate the literature in which few reports have been produced on kitchen garden products such as the onion, for example. Therefore, the treatment of onion seeds with bio-regulator depended on the dose and the plant cultivar. The cultivar 'Híbrida Superex' produced positive responses to the application of this bio-regulator.

Table 1. Percentage means of normal onion seedlings at the time of the first and final counts of the germination test (Maringá, Paraná State, 2009).

Doses (mL kg^{-1})	Normal seedlings[1]					
	First count (%)			Percentage of seed germination (%)		
	Baia	Bola	Híbrida	Baia	Bola	Híbrida
0	6.00 ^{ns}	3.00 ^{ns}	76.00 ^{ns}	12.00 -	19.00 -	78.00 ^{ns}
2.5	8.00 ^{ns}	6.00 ^{ns}	75.00 ^{ns}	28.00 ^{ns}	23.00 -	81.00 ^{ns}
5	11.00 ^{ns}	9.00 ^{ns}	68.00 ^{ns}	26.00 ^{ns}	29.00 -	72.00 ^{ns}
10	8.00 ^{ns}	7.00 ^{ns}	63.00 ^{ns}	16.00 -	23.00 -	71.00 ^{ns}
20	5.00 ^{ns}	3.00 ^{ns}	76.00 ^{ns}	14.00 -	15.00 -	84.00 ^{ns}
30	3.00 ^{ns}	7.00 ^{ns}	70.00 ^{ns}	6.00 -	17.00 -	76.00 ^{ns}
Control	11.00	15.00	81.00	36.00	55.00	82.00

[1]Superior (+), inferior (-) ($p < 0.05$), or non-significant differences (^{ns}) ($p > 0.05$) when compared to control, by Dunnett's test, with every cultivar.

Table 2. Means of primary root and shoot length of onion seedlings (Maringá, Paraná State, 2009).

Doses (mL kg^{-1})	Length (cm)[1]					
	Primary root			Seedling shoot		
	Baia	Bola	Híbrida	Baia	Bola	Híbrida
0	0.2625 ^{ns}	0.2850 ^{ns}	0.4450 ^{ns}	0.0750 ^{ns}	0.0500 ^{ns}	1.5675 ^{ns}
2.5	0.2250 ^{ns}	'0.2875 ^{ns}	0.6700 ^{ns}	0.0675 ^{ns}	0.3125 ^{ns}	2.2150 ^{ns}
5	0.2325 ^{ns}	0.2500 ^{ns}	0.7775 ^{ns}	0.1575 ^{ns}	0.2000 ^{ns}	2.0425 ^{ns}
10	0.4000 ^{ns}	0.2375 ^{ns}	0.6775 ^{ns}	0.3000 ^{ns}	0.0625 ^{ns}	2.4875 ^{ns}
20	0.1750 ^{ns}	0.2125 ^{ns}	0.6450 ^{ns}	0.0000 ^{ns}	0.2500 ^{ns}	2.0325 ^{ns}
30	0.2500 ^{ns}	0.2750 ^{ns}	0.6900 ^{ns}	0.0000 ^{ns}	0.1375 ^{ns}	1.9500 ^{ns}
Control	0.3300	0.2625	0.6525	0.3450	0.0325	1.6275

[1] Superior (+), inferior (-) ($p < 0.05$), or non-significant differences (^{ns}) ($p > 0.05$) when compared to control, by Dunnett's test, with every cultivar.

Conclusion

The application of bio-regulator did not improve the percentage of seed germination and no positive seedling response was observed from cultivars 'Baia Periforme' and 'Bola Precoce'. However, the seedling vigor of 'Híbrida Superex' was improved by the application of the bio-regulator.

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