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Organic acid effects on *Brassica napus* L. var. *oleifera* seed germination and seedling growth

Efeitos de ácidos orgânicos na germinação e crescimento inicial de Brassica napus L. var. oleifera

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ABSTRACT

Canola (*Brassica napus* L. var. *oleifera*) is cultivated in the Southern Brazil as an alternative winter crop in the rotation system. No-tillage and hydromorphic soils favor the formation of aliphatic organic acids with short chain and low molecular weight, as acetic, butyric, and propionic acids. These acids may cause negative effects on the canola production. Thus, this study aimed to evaluate the response of five canola hybrid genotypes under the effect of acetic, butyric and propionic acids on the germination and initial phase of seedling growth. The results showed the characters evaluated respond differently to treatments applied within the same genotype. Therefore, the selection of a genotype with a resilient performance for the characters evaluated under stress is complex. Nevertheless, a sensitive profile response was observed in Hyola 433 genotype under different organic acids. Furthermore, acetic acid showed no negative impact on germination and initial growth of the genotypes.

KEYWORDS: acetic acid, butyric acid, propionic acid, canola.

RESUMO

A Canola (*Brassica napus* L. var. *oleifera)* é cultivada no Sul do Brasil como uma alternativa para a estação fria no sistema de rotação de culturas. O sistema de plantio direto e a ocorrência de solos hidromórficos favorece a formação de ácidos orgânicos alifáticos com cadeia curta e baixo peso molecular, como os ácidos acético, butírico e propiônico. Estes ácidos podem causar efeitos negativos na produção de canola. Assim, este estudo teve por objetivo avaliar a resposta de cinco genótipos híbridos de canola sob o efeito dos ácidos acético, butírico e propiônico na germinação e na fase inicial do crescimento da plântula. Os resultados demonstraram que os caracteres avaliados respondem de forma diferente em relação aos tratamentos aplicados dentro do mesmo genótipo. Dessa forma, a seleção de um genótipo que manteve um comportamento padrão nos caracteres avaliados sob estresse é dificultado. Entretanto, em uma visão geral, o genótipo Hyola 433 manteve respostas características de sensibilidade nos caracteres avaliados em relação aos ácidos utilizados. Ainda, foi detectado que o ácido acético teve menor efeito na germinação e na fase inicial de crescimento dos genótipos estudados.

PALAVRAS-CHAVE: ácido acético, ácido butírico, ácido propiônico, canola.

INTRODUCTION

Canola (*Brassica napus* L.) is a plant of the Brassicaceae family, and nowadays it is the most cultivated oleaginous plant in the world. Its origin is from an interspecific hybridization between *Brassica oleracea* (CC, 2n=18) and *Brassica rapa* (AA, 2n=20) (CHALHOUB et al. 2014, LOGANES et al. 2016), resulting in an allotetraploid species (AACC, 2n=38) (MISRA 2016). Due to its erucic acid and glucosinate levels, canola oil is neither recommended for human nor animal consumption (JOHNSON 2013). This scenario led to the need of a new genotype with low level of these compounds in the oil, reached by plant breeding efforts as Canola (short for Canadian Oilseed Low Acid).

Canola production is widespread worldwide and its cultivation extends to the European Union, Canada, United States, Australia, China and India, where winter varieties are used (LOGANES et al. 2016). In Brazil, since the winters are not so harsh, vernalization is not required, and only spring varieties with less daylength requirements are cultivated. Southern Brazil concentrates the cultivation of canola and the extracted oil is used as a raw material for manufacturing products for humans and for biodiesel, and the bran is used as feed supplement (PEREIRA et al. 2016). Also, it is a good alternative for winter oil production and crop rotation (DE MARCO et al. 2014).

In Southern Brazil, the most used agricultural practice is no tillage, whose main feature is to keep the soil always covered with plant residues. As the decomposition of these residues occurs, the formation of organic acids increases (SILVEIRA et al. 2014). The main acids produced at this condition are the short chain aliphatic acids with low molecular weight, among them acetic, butyric and propionic acids, which occur in a proportion of 6:3:1, respectively (ANGELES et al. 2005, BOHNEN et al. 2005).

Besides no tillage practices, Southern Brazil has areas with hydromorphic soils characterized by low natural drainage (KOPP et al. 2009). In these soils, due to their low level of oxygen, the decomposition of organic matter is mostly anaerobic, leading to reductions that produce phytotoxic substances such as acetic, butyric and propionic acids (TUNES et al. 2013).

The presence of organic acids in the soil can negatively affect plant biological processes such as germination, shoot and root growth (KOPP et al. 2012a, SILVEIRA et al. 2014), as well as interfere in the processes of energy formation through mitochondria functioning impairment (BORTOLON et al. 2009).

Regarding the possibility of using Canola as an alternative in the Southern Brazil, there is a new request related to the behaviour of this crop at the local climatic conditions, especially with the presence of organic acids. In this sense, this study aimed to evaluate the effects of acetic, butyric and propionic acids on the germination and the initial phase of seedling growth of different canola genotypes.

MATERIAL AND METHODS

The experiments were performed in parallel using three organic acids: acetic, butyric and propionic, which are normally found in soils with O_2 deficiency in the Southern Brazil, and five canola hybrid genotypes (*Brassica napus* L. var. *oleifera*) Hyola 50, Hyola 61, Hyola 433, Hyola 571CL and Hyola 575CL usually grown in Brazil. The genotypes were exposed to the stress caused by the organic acids during germination and initial development (until the 14th day).

The experimental design used was completely randomized in a factorial scheme 5 x 3 x 3 - five canola hybrids, three acids and three doses of each acid: 0 (control), 4 mM and 8 mM (NEVES & MORAES 2005) - with three replicates.

The experiment was conducted in a growth room under controlled conditions with 85% ± 5% RH, 23 °C ± 3 °C, according to the Rules of Seed Analysis (RAS) specific for the species (MAPA 2009), and a photoperiod of eight hours of light and 16 hours of darkness. Fifty seeds were put to germinate in rolls of two and a half sheets of germination paper (germitest), and it was moistened with each specific solution at a rate of 2.5 times its weight, each replicate of each treatment consists of one roll.

To evaluate the effect of the stress caused by organic acids on the germination and initial phase of development, the following variables were measured: germination at seven and 14 days, shoot length, root length, shoot dry weight and root dry weight. The germination results were indicated in percentage.

For the shoot length and root length, seedlings were placed with the radicles to the bottom side. Measurements were made at the 14th day after sowing, and only normal seedlings were evaluated (MAPA 2009). The results were presented in millimetres (mm).

For shoot dry weight, embryonic axes of each replicate were placed in paper bags and oven dried with forced air set at 80 °C for 72 hours. These conditions were also used for root dry weight. Then, the samples were weighted on an analytical balance, with a 0.001 g precision (KRZYZANOWSKI et al. 1999). The results were presented in milligrams (mg).

For the comparison of means, the relative performance (RP) of the seeds germinated at 7 (RPgerm.7) and 14 days (RPgerm.14), shoot (RPsl) and root length (RPrl), shoot (RPsdw) and root dry weight (RPrdw) between the stress and the control conditions were evaluated. For the relative performance calculation, a 100% of the absolute value of the control was used, according to the following formula (MONZÓN 2015):

RPvariable = ($\bar{\bar{x}}$ variable at stress condition / $\bar{\bar{x}}$ variable at control condition)*100

To obtain normality of the residual error and homogeneity of the variance, a transformation $X_{trans} = \sqrt{x}$ was performed using the SAS program (SAS 2002).

Relative performance data were analyzed according to a factorial model. When interaction between treatments and genotypes was detected, a partitioning of the treatment effects on each genotype was performed. For the qualitative and quantitative factors, the comparison of means was performed using GENES (CRUZ 2013).

RESULTS AND DISCUSSION

According to the analysis of variance, for the effect of acetic acid on canola (*Brassica napus l*. var. *oleifera*), significant differences in the genotype factor were observed only for RPrdw (Table 1). The low effect of acetic acid on the seedlings of canola was expected, since it is known that the acetic acid is less phytotoxic, even at higher concentrations. It happens due to the toxicity of the organic acids related to the size of the carbon chain, thus, the longer the carbon chain, the higher its phytotoxicity. Among the three acids used in this study, the acetic acid has the smallest chain of carbons and the butyric acid has the highest (BOHNEN et al. 2005, BORTOLON et al. 2009).

The genotype Hyola 571CL showed the better RPrdw under stress by acetic acid when compared with Hyola 50 and Hyola 433; however, it did not differ significantly from Hyola 61 and Hyola 575CL (Table 1). The root system is adversely affected by the presence of acetic acid and other organic acids, decreasing the roots dry mass accumulation (SOUSA & BORTOLON 2002). Thus, one suggests that the Hyola 571CL, Hyola 61 and Hyola 575CL show less sensitivity to acetic acid.

Table 1. Relative performance (RP) of canola (*Brassica napus* L. var. *oleifera*) genotypes for the variable root dry weight (RPrdw) under stress caused by acetic acid.

Genotype	RPrdw (%)
Hyola 50	53.09 b*
Hyola 61	101.26 ab
Hyola 433	86.52 b
Hyola 571CL	148.65 a
Hyola 575CL	94.48 ab

*Means followed by the same letter do not differ significantly by the Tukey test ($p \leq 0.05$).

The analysis of variance for the effect of butyric acid on canola showed significant differences for RPgerm.7 for both, genotype and doses. Significant differences were also observed for the interaction between the factors RPgerm.14, RPsl and RPrdw (Table 2).

The Hyola 433 genotype showed the lowest RPgerm.7 when under butyric acid stress, while the others showed a higher RPgerm.7 (Table 2). Reduction in seed germination under butyric acid was also observed in wheat (TUNES et al. 2012), maize and barley (KROGMEIER & BREMMER 1990). This behavior suggests most canola genotypes tested were not affected by butyric acid in the initial germination.

Table 2. Relative performance (RP) of canola (*Brassica napus* L. var. *oleifera*) genotypes for the variable germination at 7 days (RPgerm.7) under the stress caused by butyric acid.

Genotype	RPgerm.7(%)
Hyola 50	106.49 a*
Hyola 61	98.79 a
Hyola 433	64.44 b
Hyola 571 CL	97.6 a
Hyola 575 CL	99.72 a

*Means followed by the same letter do not differ significantly by the Tukey test ($p \leq 0.05$).

The RPgerm.7 did not show significant differences between the butyric acid concentrations tested (Table 3).

Table 3. Relative performance (RP) of the butyric acid doses on the canola (*Brassica napus* L. var. *oleifera*) genotypes for the variable at germination 7 days (RPgerm.7).

Doses	RPgerm.7 (%)
Butyric acid (4mM)	99.83 a*
Butyric acid (8mM)	86.98 a

*Means followed by the same letter do not differ significantly by the Tukey test ($p \leq 0.05$).

In different butyric acid concentrations, Hyola 571CL showed a reduction for RPgerm.14 at 8mM, while Hyola 433 increased (Table 4). In oat cultivars, a reduction in the germination with increase in the butyric acid doses was also reported (TUNES et al. 2008). Similarly, the behavior observed in Hyola 433 was also reported in oat genotypes; however, regarding other organic acids, at different concentrations of lactic and malic organic acids, increased percentage of germination up to certain concentrations was verified. This result can be associated with the capability of organic acids to influence the uptake of oxygen by seeds positively (ADKINS et al. 1985, GALLAGHER et al. 2009). On the other hand, Hyola 50, Hyola 61, and Hyola 575CL did not differ at higher butyric acid concentrations. Germination reduction at different doses of butyric acid were not detected for oats (SILVEIRA et al. 2014). Thus, the plant responses under butyric acid stress appear to be variable within and between plant species. Considering the specific case of canola, the Hyola 571CL genotype was negatively affected by butyric acid dose increase.

Table 4. Relative performance of the variables germination at 14 days (RPgerm.14), shoot length (RPsl) and root dry weight (RPdrw) for each canola (*Brassica napus* L. var. *oleifera*) genotype under the stress caused by butyric acid.

			RPgerm.14 (%)			
Genotype	Butyric acid (4mM)			Butyric acid (8mM)		
Hyola 50	100	A	a*	94	A	a
Hyola 61	106.5	A	a	99.92	A	a
Hyola 433	79.61	B	b	97.91	A	a
Hyola 571CL	101.52	A	a	87.66	B	a
Hyola 575CL	103.58	A	a	98.69	A	a
			RPsl (%)			
Genotype	Butyric acid (4mM)			Butyric acid (8mM)		
Hyola 50	84.61	A	a	69.86	A	ab
Hyola 61	82.02	A	a	86.67	A	a
Hyola 433	102.11	A	a	45.6	B	b
Hyola 571CL	82.27	A	a	61.06	A	ab
Hyola 575CL	66.83	A	a	40.35	A	b
			RPrdw (%)			
Genotype	Butyric acid (4mM)			Butyric acid (8mM)		
Hyola 50	89.63	A	b	88.64	A	b
Hyola 61	195.21	A	a	149.35	B	a
Hyola 433	92.21	A	b	57.1	A	b
Hyola 571CL	51.16	A	b	54.03	A	b
Hyola 575CL	88.13	A	b	60.54	A	b

*Means followed by the same lower case letter in the column and upper case in the row did not differ by the Tukey test ($p \leq 0.05$).

Regarding RPsl, a development reduction was observed for Hyola 433 while the other genotypes were not affected by butyric acid concentration increase (Table 4). A similar result was found in oats, in which one of the cultivars studied was negatively affected with increasing doses of butyric acid (SILVEIRA et al. 2014). These results indicate that the Hyola 433 genotype shows sensitivity to the butyric acid stress. Previous studies showed the presence of organic acids in the soil at higher concentrations impairs the accumulation of nutrients in plants and decreases the nitrogen, potassium, calcium, and magnesium content found in the shoot of rice seedlings (SCHMIDT et al. 2007). Therefore, Hyola 50, Hyola 61, Hyola 571CL, and Hyola 575CL, which did not show reduction in RPsl, could maintain nutrient absorption even with increasing doses of butyric acid.

Differences between the genotypes analyzed was observed at higher butyric acid concentrations (8mM) (Table 4). Hyola 61 showed higher RPsl compared with Hyola 433 and Hyola 575CL, suggesting that these genotypes are sensitive to higher butyric acid concentrations.

The increase in butyric acid concentration causes a significant reduction in RPrdw for Hyola 61 (Table 4). However, among the genotypes studied, the performance of Hyola 61 was different, showing a higher RPrdw in both butyric acid concentrations. This difference is attributed to the Hyola 61 physiology, which developed greater dry mass accumulation even under stress conditions.

Concerning propionic acid, the analysis of variance allowed observing significant difference for DRsl between genotypes. Regarding the concentration factor, a significant difference for RPsl and RPrl was detected. Interactions for RPgerm.7 and RPgerm.14 were observed (Supplementary Table 3).

The genotype Hyola 50 showed higher RPsl compared with Hyola 61, but did not differ significantly from the genotypes Hyola 433, Hyola 571CL, and Hyola 575CL (Table 5). Effects of propionic acid on shoot length was also previously reported in wheat (TUNES et al. 2012), which suggests an effect of this stress over shoot development. However, Hyola 50 was less sensitive to this stress.

Table 5. Relative performance (RP) of the canola (*Brassica napus* L. var. *oleifera*) genotypes for the variable shoot length (RPsl) under propionic acid stress.

Genotype	RPsl (%)
Hyola 50	97.23 a*
Hyola 61	47.70 b
Hyola 433	51.31 ab
Hyola 571CL	88.42 ab
Hyola 575CL	81.97 ab

*Means followed by the same letter do not differ significantly by the Tukey test (p≤0.05).

Considering propionic acid doses, RPsl and RPrl were not different between the concentrations tested (Table 6). The opposite was found in oats, in which the root length was the most negatively affected (TUNES et al. 2008). The increase in the concentration of organic acids leads to reduced root length and dry matter (TUNES et al. 2012). Considering the RPrl results, one suggests the tested canola genotypes in this study show low sensitivity to stress by propionic acid..

Table 6. Relative performance (RP) of the propionic acid doses on the canola (*Brassica napus* L. var. *oleifera*) genotypes for the variables shoot length (RPsl) and root length (RPrl).

Doses	RPsl (%)	RPrl (%)
Propionic acid (4mM)	85.13 a*	81.21 a
Propionic acid (8mM)	61.52 a	59 a

*Means followed by the same letter do not differ significantly among themselves in the column by Tukey test (p≤0.05).

Hyola 61 and Hyola 433 showed a reduction in RPgerm.7 and RPgerm.14 with increased propionic acid concentration (Table 7). Also, these genotypes showed their lowest germination under 8mM propionic acid. Germination at higher doses of propionic acid also decreased in studies performed in oats (TUNES et al. 2008). This reduction may occur because the acid impairs seed viability (CAMARGO et al. 2001, TUNES et al. 2013). The results indicate that the genotypes Hyola 50, Hyola 571CL, and Hyola 575CL are less sensitive to the stress caused by propionic acid during germination.

Overall, the effect of organic acids is extremely dependent on the genotype (KOPP et al. 2012b). Thus, the genotype selection in low natural drainage hydromorphic soils is suggested as a canola breeding strategy for organic acid tolerance.

Reports regarding organic acid effects, as well as different abiotic stresses in canola, are still scarce in the literature. In this sense, this preliminary study reveals initial insights in the understanding of canola responses under stress conditions. Therefore, this study opens up new opportunities for new researches regarding plant management and physiology.

Table 7. Relative performance of the variables germination at 7 (RPgerm.7) and at 14 days (RPgerm.14) for each genotype of canola (*Brassica napus* L. var. *oleifera*) under stress by propionic acid.

	RPgerm.7 (%)					
Genotype	Propionic acid (4mM)			Propionic acid (8mM)		
Hyola 50	112.25	A	a*	106.92	A	a
Hyola 61	116.23	A	a	65.51	B	b
Hyola 433	117.98	A	a	68.61	B	b
Hyola 571CL	109.04	A	a	98.41	A	a
Hyola 575CL	104.29	A	a	104.29	A	a
	RPgerm.14 (%)					
Genotype	Propionic acid (4mM)			Propionic acid (8mM)		
Hyola 50	100	A	a	96	A	ab
Hyola 61	115.25	A	a	81.83	B	b
Hyola 433	114.66	A	a	78.9	B	b
Hyola 571CL	100.92	A	a	101.74	A	a
Hyola 575CL	103.58	A	a	103.58	A	a

*Means followed by the same lower case letter in the column and upper case in the row did not differ by the Tukey test ($p \leq 0.05$).

CONCLUSION

Here, the effect of acetic, butyric, and propionic acids on the germination and initial growth of different canola genotypes was studied. The low acetic acid effect on germination and initial growth was verified, demonstrating that butyric and propionic acids impair germination and initial growth in canola seedlings. The genotype Hyola 433 showed greater sensitivity to the acids tested, being not indicated for area with the presence of organic acids in the soil.

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