Vol. 13(45), pp. 2563-2569, 8 November, 2018
DOI: 10.5897/AJAR2018.13456
Article Number: 05314F959194
ISSN: 1991-637X

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African Journal of Agricultural Research

Full Length Research Paper

Water excess in different phenological stages of canola cultivars

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Received 11 August, 2018; Accepted 5 September, 2018

The objective of this study was to determine the stage of development with greater sensitivity to water excess and the period of time required to compromise the emergence and grain yield components of canola. The experiments were performed in a greenhouse at the Federal University of Santa Maria and at the Farroupilha Federal Institute, Campus of São Vicente do Sul, RS during the 2015 agricultural year. The completely randomized experimental design was utilized to investigate phenological stages and periods of continuous water excess in the soil. Also, factors like percentage of emergence, emergence speed index, grain yield, number of siliques per plant, one hundred grains weight, dry matter of aerial part, silique length, number of grains per silique, and weight of 20 siliques were determined. The stages of rosette leaf formation and beginning of anthesis are the most sensitive to water excess in the soil. Water excess for 24 h is enough to reduce the emergence speed index. However, the percentage of emergence is not compromised by water excess up to 192 continuous hours. 24 h of water excess reduces the number of siliques per plant, dry matter of aerial part and grain yield of canola.

Key words: *Brassica napus*, grain yield, lowland cultivation, waterlogging.

INTRODUCTION

In Brazil, Rio Grande do Sul is the largest canola producing state, with potential for expansion due to the difference of six million hectares between the area cultivated with summer crops and winter crops (CONAB, 2015). However, most of these fields cultivated in the summer present edaphic problems such as low natural drainage of the soil and elevated water table, originating areas with frequent water excess, which can limit the development of certain crops.

Water excess is an abiotic stress that compromises plant growth by reducing the oxygen diffusion into the soil and hindering root cellular respiration (Bailey-Serres and Voesenek, 2008). It is caused mainly by excessive rainfall, irrigation, superficial water table, and poor natural drainage of the soils (Sairam et al., 2008), which jointly with the low atmospheric demand during the winter cause excessive accumulation of water in the soil.

The inadequate oxygen supply to the roots is considered the main problem for plants not adapted to water excess, mainly for inhibiting root respiration and

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energy production as ATP (Taiz et al., 2017). It compromises the growth of roots and aerial part (Liao and Lin, 2001) and the absorption of nutrients and water, which causes plant wilt (Ahmed et al., 2013; Loose et al., 2017), hormonal imbalance, early leaf senescence, and subsequent death of plants unadapted to these conditions (Rodríguez-Gamir et al., 2011).

Despite canola (*Brassica napus*) being considered a crop sensible to soil water excess (Zhou and Lin, 1995; Gutierrez Boem et al., 1996; Ku et al., 2009; Xu et al., 2015), recent studies in China have painted this crop as an alternative for cultivation in areas with water excess such as on the banks of the Yangtze River, which irrigated rice is the main crop. Cultivars tolerant to water excess have been developed such as ZS9 (Cheng et al., 2010; Zou et al., 2013, 2014), which presents a low reduction in yield when cultivated under these conditions (Zou et al., 2014).

Determining the phenological stage and period of tolerance of canola to water excess is required for the adoption of techniques that avoid water excess in stages of greater sensitivity. In winter canola, Zhou and Lin (1995) verified that rosette leaf formation stage is the most susceptible to prolonged water excess in the soil, followed by appearance of the floral bud and silique formation. Moreover, Ku et al. (2009) observed that although water excess in the vegetative phase reduces stomatal conductance and photosynthetic rate, the greatest negative effects are observed when the stress is applied in the reproductive phase. In addition to the reduction of stomatal conductance and photosynthetic rate, it causes reduction in shoot growth and later plant death after two weeks of exposure to water excess.

The duration of water excess is also of great importance, since only 3 days under these conditions, canola presents a reduction in grain yield (Gutierrez Boem et al., 1996). Hereupon, determining the most sensitive stage and period of time of water excess tolerated by canola cultivars is important, since better planning of the sowing date can be performed and even increasing the areas suitable for cultivation through agricultural zoning, allowing, in the future, the agricultural financing in areas formerly considered unsuitable for canola cultivation.

Given the aforementioned, the objective of this study was to determine the stage of development with greater sensitivity to water excess and the period of time required to compromise the emergence and grain yield components of canola.

MATERIALS AND METHODS

The experiments were carried out in greenhouse in two locations at the Crop Science Department of the Federal University of Santa Maria (UFSM), Santa Maria, RS (29°43'23" S; 53º43'15" W; 95) and at the Farroupilha Federal Institute (IFFar), São Vicente do Sul Campus, RS (29°42'21''S; 54°41'39'' W; 129 m), during the 2015 agricultural year. According to the Köppen climate classification, the climate of the region is Cfa, subtropical humid with warm summers and without defined dry season (Heldwein et al., 2009).

In Santa Maria, the experiment was performed in a greenhouse with transparent polyethylene coverage, sides closed by screen with protection against aphids, and oriented in the north-south direction. In São Vicente do Sul, the experiment was carried out in a greenhouse with transparent polyethylene coverage and open sides.

The experimental units were polyethylene vessels externally painted with white paint, filled with soil and placed in a bucket larger than the vessel. Wooden blocks were placed at the bottom of each bucket in order to support the vessels with soil that stays suspended three centimeters from the bottom to enable drainage of water when necessary.

Sixty days before sowing the soil was sieved, homogenized and calcareated. Both the application of limestone and the fertilization (base and cover) were carried out according to the chemical analysis of the soil, following the recommendations of the manual of fertilization and liming for the states of Rio Grande do Sul and Santa Catarina.

The water level during the application of water excess treatment was maintained at a height of 15 cm by a perforation on the lateral border of each bucket (Loose et al., 2017). In this way, the water level was maintained around two centimeters below the seeds. The vessels were randomized and arranged in benches 50 cm high and in two simple rows on each bench.

In the treatments that received excess water, the water was applied daily directly on the soil of the vessels and inside the buckets. The water was removed from the bucket after the period of water excess, allowing drainage of the excess through the holes in the bottom of the vessel. The soil moisture of the other treatments that did not have water excess treatment was kept close to the field capacity.

In Santa Maria, the experimental design used was completely randomized with five replications in a 4 × 5 factorial scheme (phenological stages and periods of water excess), totaling 100 experimental units. In São Vicente do Sul, a completely randomized design with four replications was used in a 5 × 4 + 1 factorial scheme (phenological stages and periods of water excess) with one additional treatment, without water excess, totaling 69 experimental units.

The qualitative factor phenological stage comprised four levels in Santa Maria, being sowing (S), rosette leaf formation (RF), beginning of anthesis (BA), and end of anthesis (EA). In São Vicente do Sul, in addition to those levels mentioned previously, water excess was also applied in the emergence stage (EM), totaling five levels for this factor. The phenological stages were characterized according to Iriarte and Valetti (2008).

The quantitative factor period of water excess was comprised five periods of continuous water excess in the soil: 0, 24, 48, 96 and 192 h applied at each level of the phenological stage, when 50% of the plants were in the desired phenological stage. Water excess was maintained in each vessel for the corresponding period of each treatment. After removal of the excess water, the plants received the same management as the other plants without water excess.

The sowing procedure was carried out with the soil under field capacity conditions on 28 May, 2015 in Santa Maria, with the hybrid Hyola 411. In São Vicente do Sul, sowing was performed on 08 May, 2015 with the hybrid Hyola 433. Both cultivars presented germination percentage of 87% and were treated with fungicide. Water excess treatments were started after sowing in the experimental units in which the water excess treatment was applied at sowing.

Thinning was performed when the plants were with two definitive leaves, leaving one plant per hole and two plants per vessel that were conducted until harvest. The harvest of two plants of each experimental unit was carried out on 10 October, 2015 (129 days after sowing) in Santa Maria and on 09 September, 2015 (121 days after sowing) in São Vicente do Sul.

The following variables were analyzed: percentage of emergence, emergence speed index (Maguire, 1962), dry matter of aerial part, grain yield per vessel, silique length, number of grains per silique, weight of 20 siliques and one hundred grains weight. The grain moisture was corrected to 10%.

Data of each variable were submitted to the Shapiro-Wilk test of normality of errors and Bartlett test of homogeneity of variances of the treatments in the Action software (Equipe Estatcamp, 2014). Data that did not meet these assumptions were transformed by log (x) and utilized for analyses of variance.

The data were submitted to analysis of variance at 5% of probability and when a significant effect was verified; data referring to the period of water excess were submitted to regression analysis while data referring to the phenological stages were compared by the Scott-Knott test at 5% probability using the Sisvar software (Ferreira, 2011).

RESULTS AND DISCUSSION

The water excess in the soil influenced emergence speed index but did not influence percentage of emergence for both canola cultivars. The emergence speed index decreased exponentially for cultivar Hyola 411 with the increase in the period of water excess, being maximum without water excess (5.67) and minimum with the application of 192 h of water excess (0.85). The greater period of water excess in the soil (192 h) reduced the emergence speed index by 85% when compared to the treatment without the application of water excess (Figure 1) and a period of only 24 h of water excess reduced emergence speed index by 21.3%.

Similar results were obtained by Loose et al. (2017) with sunflower, where the increase in periods of water excess from zero to 240 h exponentially reduced emergence speed index. Moreover, the 48 h period of water excess was enough to compromise and reduce the emergence speed index.

A linear decrease in the emergence speed index with the increase of the period of water excess in the soil occurred for the cultivar Hyola 433, being maximum without water excess (3.33) and minimum with the application of 192 h of water excess (1.46). The greater period of water excess in the soil (192 h) reduced the emergence speed index of canola seedlings by 46.4% when compared to the treatment without water excess (Figure 1) and a period of only 24 h of water excess reduced emergence speed index by 5.8%.

The emergence speed index in soils with water excess reflects seed vigor, that is, the capacity that the seeds have for a rapid emergence and establishment in unfavorable conditions. Thus, water excess in the soil reduces the vigor of canola seeds and this may cause a reduction of plant stand in the field. Furthermore, seedlings may die due to the lack of oxygen in the soil if the water excess persists for many days due to frequent rainfall, low atmospheric demand, poor soil drainage, or groundwater level near the surface.

The reduction of emergence speed index in canola is probably due to the reduction in soil oxygen content, which temporarily inhibits or reduces the physiological activity of the seeds by reducing the respiratory process, reducing germination speed (Marcos Filho, 2005) and consequently the emergence of seedlings. However, oxygen returns to the soil after the stress removal, giving continuity to the germination process.

The emergence speed index under water excess can be used to select canola cultivars tolerant to water excess in an early stage of development (Cheng et al., 2010). Selection of cultivars from germination tests is a relatively inexpensive and effective option and the tolerance to water excess is a trait transmitted during plant ontogeny (Zou et al., 2014).

Although there was a great reduction in emergence speed index, no differences occurred in percentage of emergence. A delay in the emergence time of the plants with longer periods of water excess was noticed during the conduction of the experiment. However the seeds remained viable, since the emergence of the seedlings occurred a few days after removing the water excess.

The capacity of Hyola 411 and Hyola 433 cultivars to maintain an elevated percentage of emergence when submitted to water excess may be related to the melanin content of the seed integument, since these seeds have a darker coloration. Therefore, the greater the melanin content (darker integument), the slower the water absorption and lower the loss of solutes by the seeds (Zhang et al., 2008), explaining the delay in the emergence without the loss of seed viability.

The phenological stage of application of water excess influenced the number of siliques per plant and grain yield in the Hyola 411 cultivar. Water excess influenced dry matter of the aerial part and grain yield. Treatments interacted with and influenced dry matter of the aerial part, number of siliques per plant, and grain yield.

The dry matter of aerial part adjusted to the linear model when the water excess was applied at sowing, increasing the dry matter of aerial part with increased exposure time of the crop to water excess (Figure 2A). When water excess was applied at the beginning of anthesis, the values also adjusted to the linear model but the dry matter of aerial part decreased with increasing exposure time of the crop to the water excess. Water excess of 192 h applied at sowing and at the beginning of anthesis caused respectively an increase of 21.2% and a reduction of 22.9% of the dry matter of aerial part of canola when compared to the control treatment without water excess.

When the water excess was applied at the rosette leaf formation stage and at the end of anthesis, the values did not fit to the mathematical models tested (linear, quadratic, exponential, and power model). Although, there was a tendency (not significant at 5% probability) of dry matter of aerial part reduction with the increase in the exposure time of the crop to water excess wherein the continuous occurrence of water excess for 192 h reduced

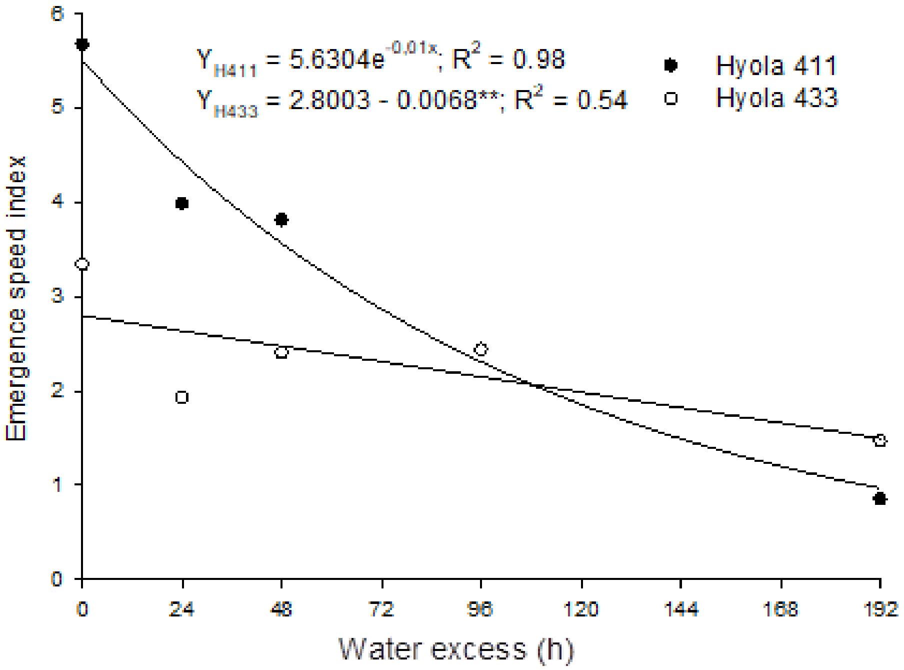

Figure 1. Emergence speed index of the cultivars Hyola 411 and Hyola 433 as a function of the period of water excess in the soil (h).

the canola dry matter of aerial part in 7.6 and 1% when applied respectively at the rosette leaf formation and at the end of anthesis.

These results somewhat disagree from those obtained by Issarakraisila et al. (2007), who studying two species of Brassicas reported a reduction of 81% in the dry matter of aerial part when the plants were submitted to water excess at the rosette leaf formation stage (with four to six leaves). Moreover, Gutierrez Boem et al. (1996) concluded that the rosette leaf formation stage is the most sensitive in relation to the grain filling and that the longer the period of exposure of the crop to water excess, the greater the reduction of dry matter of aerial part. In this study, sowing and early stages of anthesis were more sensitive to water excess for Hyola 411 cultivar regarding the dry matter of aerial part.

The number of siliques per plant increased with increasing exposure time of the crop to water excess applied at sowing (Figure 2B). Water excess of 192 h increased the number of siliques per plant by 46.4%. Although water excess in other stages did not fit to any mathematical model tested, there was a tendency to reduce the number of siliques per plant with increased exposure time of canola to water excess mainly at the rosette leaf formation stage. Water excess of 192 h at the rosette leaf formation stage reduced the number of siliques per plant by 26.5% when compared to the control treatment. Similar results were obtained by Zhou and Lin (1995) and Leul and Zhou (1998) with winter canola, with a reduction in number of siliques per plant respectively of 14.2 and 28.2% when water excess was applied at the rosette leaf formation stage (five leaves).

Smaller reduction in number of siliques per plant occurred when water excess was applied at the end of anthesis, which may be due to siliques being already formed at this stage of development with no occurrence of abortion. This revealed that at this stage the negative effects of the water excess are smaller for the plant than in the rosette leaf formation, when the plant yield is being defined.

Water excess applied for 192 h at the beginning of anthesis reduced the number of siliques per plant compared to the control treatment (without water excess) by 5.2%. This reduction is smaller when compared with that obtained by Ku et al. (2009) and by Xu et al. (2015), in which the water excess in the reproductive phase and at the beginning of anthesis reduced respectively, the number of siliques per plant on average 65.9 and 24.3%.

The grain yield with application of water excess at sowing and at the end of anthesis did not adjust to the mathematical models tested but increasing the period of water excess tended to decrease the grain yield (Figure 2C). When the water excess was applied at the stages of rosette leaf formation and at the beginning of anthesis, the grain yield was reduced linearly with the increase of the exposure time of canola to water excess.

The stages of rosette leaf formation and the beginning of anthesis in canola can be considered the most sensitive to water excess in the soil after the plant emergence, since the application of 192 h of water

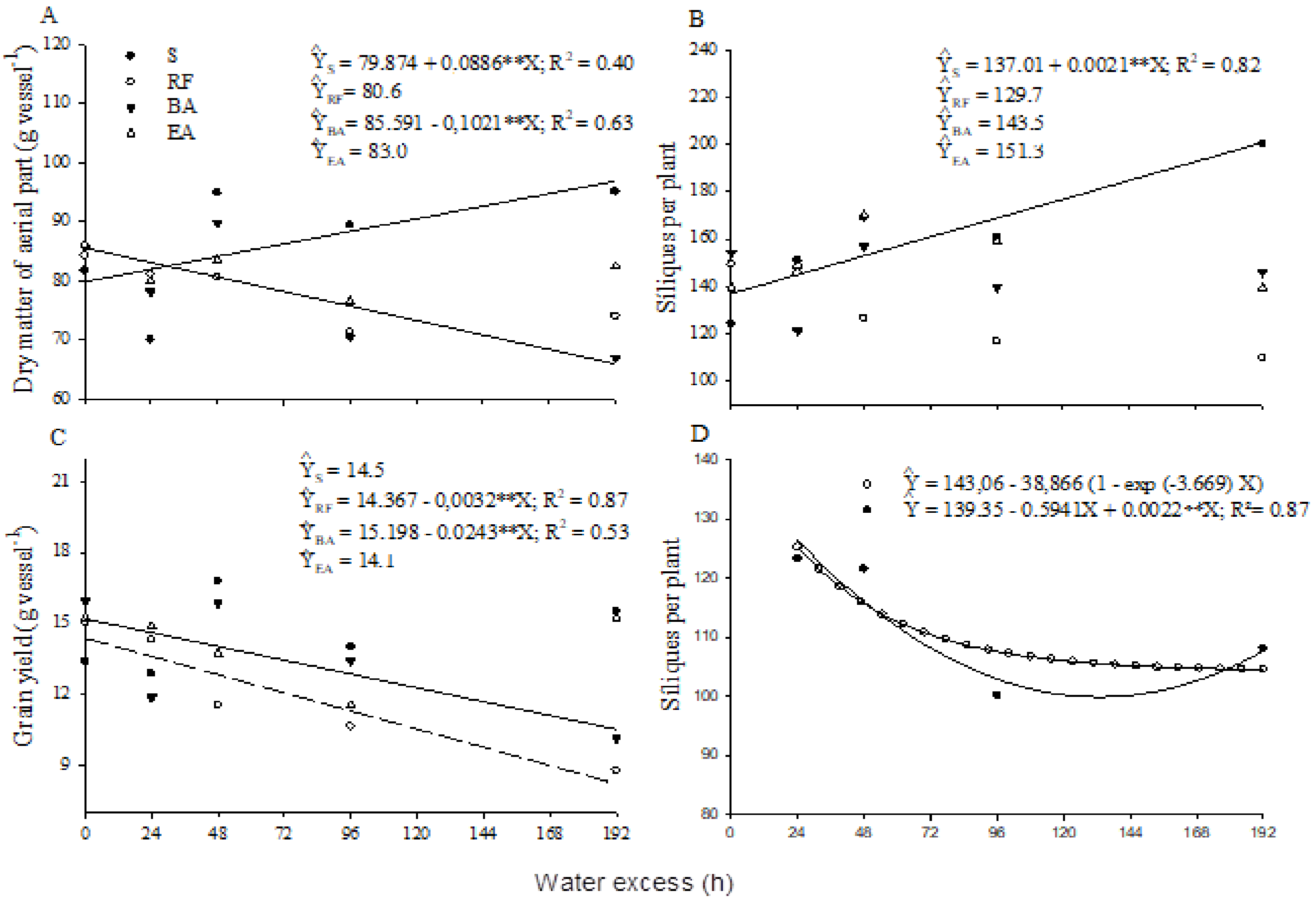

Figure 2. Relation between the dry matter of aerial part (A), number of siliques per plant (B) and grain yield (C) of the canola cultivar Hyola 411; and number of siliques per plant of the canola cultivar Hyola 433 subjected to different periods of water excess at sowing (S) and at the stages of rosette leaf formation (RF), beginning of anthesis (BA), and end of anthesis (EA). Only the data that fit the mathematical models were plotted with a line.

excess in the soil caused respectively a reduction of 42.7 and 30.7% in grain yield when compared to the control treatment (without water excess). Some authors (Zhou and Lin, 1995; Leul and Zhou, 1998; Zou et al., 2014) also found a reduction in grain yield when the water excess was applied at the rosette leaf formation stage, with reduction respectively of 26.2, 21.3, and 50%. At the beginning of anthesis, Xu et al. (2015) found an average reduction of 20% in the grain yield of canola but some cultivars presented a reduction of 30 to 41.9% when compared to the control treatment.

The grain yield reduction with increased exposure time of canola to water excess at the rosette leaf formation stage may be due to the plants being juvenile and fragile (Zou et al., 2014). At this stage, the plants present lower nutrient reserves and smaller root system, reducing the absorption of water and nutrients. In addition to having a small root system, the absence of oxygen in the soil reduces the root growth rate (Vidal, 2011), causing a reduction in ATP production and in the transport of photoassimilates in the aerial part (Wample and Davis, 1983), lower production of dry matter of aerial part (Figure 2A), and lower grain yield. In addition, tissues exhibit greater respiration rates because they require more energy and carbon skeletons for dividing and lengthening cells in the early stages of development such as in the rosette leaf formation (Taiz et al., 2017). Thus, the effects of absence of oxygen are more pronounced and more strongly affect growth and subsequently, productivity.

At the beginning of anthesis, the reduction in grain yield with the increased periods of water excess can be due to the abortion of flowers and siliques by the reduction in the transport of photoassimilates to these structures as the water excess compromises the transport in the phloem (Wample and Davis, 1983), besides reducing the production of photoassimilates (Liao and Lin, 2001). Thus, water excess reduces the production of chemical energy by the plant and its transport and distribution to the sinks, compromising the production and fixation of reproductive structures such as flowers and siliques.

No significant difference was observed for one hundred grains weight and grain yield for the cultivar Hyola 433 when the control treatment (without water excess) was compared with the other treatments (with water excess). However, water excess in the soil reduced number of siliques per plant. Within the water excess treatments, the phenological stage and the interaction of the factors did not influence any of the studied variables, even though the water excess factor influenced the number of siliques per plant (Figure 2D).

The greatest number of siliques per plant for the cultivar Hyola 433 was obtained without the application of water excess in the soil, with a mean of 131 siliques per plant. A second-degree concave curve is one option to represent the decrease in the number of siliques per plant as a function of increased periods of water excess, which would imply an increase in the number of siliques with periods of water excess greater than 192 h. However, this variation is not logical, since longer periods of water excess would cause root respiration problems and from a certain critical value would possibly cause the plants to die.

Thus, another explanation for the number of siliques per plant can be given with an asymptotic curve, which from 96 h of water excess there would be a tendency to stabilize the reduction of the number of siliques per plant near a minimum limit value (104 siliques), which would result from the plant energy expenditure to modify morphological or physiological structures to acclimatize to the condition of water excess. Exposure of canola for 24, 48, 96 and 192 h to water excess caused a reduction in the number of siliques per plant respectively of 9.3, 16.8, 26.3, and 23.6% when compared to the control treatment (without water excess). The results corroborated with those obtained by Zhou and Lin (1995), Leul and Zhou (1998), Xu et al. (2015) and Zou et al. (2014).

The number of siliques per plant is an important component of grain yield in canola (Diepenbrock, 2000; Xu et al., 2015) and plants that produce the greatest number of siliques possibly reach higher yields. However, no difference was obtained in grain yield for the cultivar Hyola 433 in the present study, although there was a difference for number of siliques per plant. Another reason may have been the partial recovery of the plant after the removal of excess water in the soil that allowed a good nutrition of the grains in formation with less competition between sinks in the plant, partially compensating the reduction of number of siliques per plant. Further studies should be developed to confirm the results obtained.

The grain yield components obtained for the Hyola 433 and Hyola 411 cultivars were divergent in the direction of the variables that suffered an effect of the factors studied but the trend remained: lower grain yield with longer period with water excess.

Conclusions

1. For canola, the stages of rosette leaf formation and beginning of anthesis are the most sensitive to water excess in the soil.
2. Water excess for 24 h is enough to reduce the emergence speed index of canola. However, the percentage of emergence is not compromised by water excess up to 192 continuous hours.
3. The tolerance period to water excess varies according to the phenological stage although 24 h of water excess reduces the grain yield components, essentially the number of siliques per plant, dry matter of aerial part, and grain yield of canola.
4. The canola cultivars Hyola 411 and Hyola 433 respond differently when subjected to water excess in the soil, however, both present the tendency of reduction in grain yield with increased period of water excess.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful to the Brazilian Agricultural Research Corporation (Embrapa) of Passo Fundo - RS for seed supply; the Coordination for the Improvement of Higher Education Personnel (CAPES); the National Council for Scientific and Technological Development (CNPq) for the concession respectively of research and productivity grants; the IFFar SVS and the UFSM for the allowance of space and structure to perform the experiments together with the students and employees for carrying out the experiments.

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