Australian Journal of Crop Science AJCS

AJCS 11(12):1527-1533 (2017) ISSN:1835-2707
doi: 10.21475/ajcs.17.11.12.pne565

Chemical composition and physiological quality of wheat seeds with the application of trinexapac-ethyl, a plant growth regulator

Felipe Koch[1*], Gustavo Zimmer[1], Manoela Andrade Monteiro[1], Angelita Celente Martins[2], Dominique dos Santos Delias[2], Cristian Troyjack[1], Vinicius Jardel Szareski[1], Eduardo Gonçalves Borges[1], Tiago Pedó[1], Luciano do Amarante[2], Francisco Amaral Villela[1], Tiago Zanatta Aumonde[1]

[1]Universidade Federal de Pelotas, Faculdade de Agronomia Eliseu Maciel, Departamento de Fitotecnia, Caixa Postal 354, Cep 96010-900, Pelotas, RS, Brazil
[2]Universidade Federal de Pelotas, Instituto de Biologia, Departamento de Botânica, Caixa Postal 354, Cep 96010-900, Pelotas, RS, Brazil

***Corresponding author: felipe.koch@hotmail.com**

Abstract

This study evaluated the effect of the plant growth regulator trinexapac-ethyl on chemical composition and physiological quality of wheat seeds produced under fertilization for high yield potential. The experiment was performed under completely randomized design with five treatments (0; 200; 400; 600; 800 mL ha^{-1} of the commercial product Moddus®) and four repetitions. The plant growth regulator was applied at elongation via foliar spraying. Seeds were harvested at the end of crop cycle. Variables evaluated were 1000 seeds weight, germination and first count of germination, speed of germination index, seedling dry matter, electrical conductivity, accelerated ageing and the contents of total soluble sugars, starch, amino acids and proteins. The first count of germination increased until the higher dose. Electrical conductivity after three hours of imbibition reduced at the doses of 600 mL ha^{-1} and 800 mL ha^{-1}, indicating greater seed physiological quality. Starch showed a trend of increase until the dose of 448 mL ha^{-1}; amino acids until the dose of 243 mL ha^{-1} and protein until the dose of 417 mL ha^{-1}. The content of total soluble sugars decreased with the increasing dose of the plant growth regulator. Therefore, wheat seeds produced from plants in which trinexapac-ethyl is applied present higher vigor and greater contents of starch, protein and amino acids, but lower content of total soluble sugars. Our results demonstrate that trinexapac-ethyl can be used for wheat management in seed production fields in the recommended dose (400 mL ha^{-1}) with benefits in seed quality and composition.

Key words: *Triticum aestivum* L., trinexapac-ethyl, starch, proteins, vigor.

Introduction

Wheat (*Triticum aestivum* L.) belongs to the *Poaceae* family and represents one of the most cultivated cereals in the world. The cultivation of this crop in Brazil concentrates in the Southern Region and reached almost seven million Mg in the 2016 growing season, yet, the production does not meet the current demand which is almost eleven million Mg (Conab, 2017). The mean yield in southern Brazil is approximately 3.2 Mg ha^{-1} (Conab, 2017), however, the cultivation of modern cultivars allows yields as high as 6 Mg ha^{-1} (Cunha and Pires, 2005). The use of these cultivars associated with intensive management has been stimulated and recommended with the purpose of maximizing yield (Rodrigues et al., 2003). In order to obtain higher yields, it is necessary the joined adoption of proper cultivation techniques, as adequate sowing date, cultivar selection, row spacing and seeding rate, the increase of soil fertility rates and the application of synthetic fertilizers associated with proper control of lodging in cereals (Rodrigues and Teixeira, 2003).

The excessive availability of nutrients, specially nitrogen, results in exacerbated plant growth which, in turn, favors plant lodging and places wheat spikes near the soil surface, exposed to more humid environmental conditions. When lodging occurs during seed filling, photosynthesis and the partition or assimilation of photoassimilates are impaired, reducing yield (Zagonel et al., 2002), hectoliter weight and germination and increasing harvesting issues (Zagonel and Fernandes, 2007).

Plant growth regulators are employed to minimize the effect of plant lodging, in order to increase the resistance to this physiological disorder (Matysiak, 2006). These synthetic substances act in a similar manner to hormones (Chorbadjian et al., 2011; Souza et al., 2013) and operate promoting, inhibiting or modifying physiological and morphological processes of plants (Raven et al., 2007; Taiz and Zeiger, 2013).

Trinexapac-ethyl constitutes a plant growth regulator that interferes in the biosynthesis of gibberellic acid by inhibiting the enzyme 3β-hydroxylase and blocking the biosynthesis of GA_1 (Nakayama et al., 1990). The decrease of gibberellin levels leads to the reduction of plant growth, as it is responsible for cell division and elongation (Taiz and Zeiger, 2013). In addition to height reduction, it increases culm diameter, modifies leaf architecture and increases yield (Zagonel and Fernandes, 2007; Penckowski and Fernandes, 2010).

Several studies were performed using trinexapac-ethyl with the aim of reducing lodging and to evaluate yield in rice (Buzetti et al., 2006; Alvarez et al., 2007; Nascimento et al.,

2009), soybean (Linzmeyer Junior et al., 2008), corn (Zagonel and Ferreira, 2013) and sunn hemp (Kappes et al., 2011). However, even with its widespread use in wheat, there is a paucity of data of the effects on the chemical composition and physiological quality of wheat seeds produced under fertilization for high yield potential and different concentrations of this plant growth regulator.

Thereby, this work aimed to evaluate the effect of the application of the plant growth regulator trinexapac-ethyl in the chemical composition and physiological quality of wheat seeds produced under fertilization for high yield potential.

Results

Analysis of variance

There was no significant difference for the speed of germination index, shoot and root dry matter contents, accelerated aging and electrical conductivity for the periods of 6 and 24 hours, as it can be verified in Tables 1 and 2.

Effect of the application trinexapac-ethyl in seed physical and physiological quality

1000 seeds weight adjusted to a quadratic model, reaching an estimated point of maximum response at the dose of 260 mL ha^{-1} (Fig 1d). At doses higher than the estimated maximum there was a trend of decrease of the seed weight until the dose of 800 mL ha^{-1}. The decrease in the values of seed weight at the doses of 600 and 800 mL ha^{-1} plant growth regulator was of 1.98 and 4.65%, when compared to seeds from plants which were not subjected to the product.

A linear model for germination was obtained with a high coefficient of determination ($R^2 = 0.92$) which rose with the increase of the dose of trinexapac-ethyl applied in wheat plants (Fig 1a). It is possible to observe an increment in relation with the treatment without application of the plant growth regulator of 1.2; 2.3; 5.8 and 6.9% for the doses of 200, 400, 600 and 800 mL ha^{-1}, respectively.

Values for first count of germination adjusted to a linear model with a tendency of increase for the greater doses of the plant growth regulator (Fig 1b). First count of germination reached increments of 1.2; 1.2; 4.7; and 5.8% for seeds originated from plants subjected to doses of 200; 400; 600 and 800 mL ha^{-1} of the plant growth regulator, respectively, in relation to the dose of 0 mL ha^{-1}.

Electrical conductivity at three hours of imbibition, adjusted to a quadratic model, reaching an estimated point of maximum response at the dose of 299 mL ha^{-1} (Fig 1c). The most pronounced effect occurred for the dose of 800 mL ha^{-1}, which resulted in a decrease of 12.8% in electrical conductivity comparatively to the dose of 0 mL ha^{-1}.

Effect of the application trinexapac-ethyl in seed composition

The content of total soluble sugars adjusted to a quadratic model with a tendency of decrease until a minimum at the estimated dose of 470.6 mL ha^{-1} (Fig 2a). There was, however, a tendency of increase in total soluble sugars from the point of minimum until the higher dose of the plant growth regulator. An increment of 20.6% in the quantity of these compounds is observed when the content of the point of minimal response (470.6 mL ha^{-1}) and the content in seeds produced under the influence of the higher dose (800 mL ha^{-1}) are compared.

The starch content of wheat seeds adjusted to a quadratic model with a high coefficient of determination ($R^2 = 0.94$) (Fig 2b). The values for starch reached a point of maximum estimated content at the dose of 448 mL ha^{-1} of the plant growth regulator, reaching a value of 11.36 mg g^{-1} with a posterior tendency of decrease. In seeds under the influence of this dose, a theoretical increment of 27.6% of starch content was observed comparatively to seeds from plants which were not subjected to the plant growth regulator. Even with the tendency of decrease in starch content at higher doses, wheat seeds from plants subjected to 800 mL ha^{-1} of the commercial product presented an increase of about 11.9% when compared with seeds from plants at the dose of 0 mL ha^{-1}.

The content of amino acids reached a maximum estimated at the dose of 243.7 mL ha^{-1} with the amount of 4.88 µmol g^{-1} (Fig 2c). With doses higher than the estimated maximum there was a decrease in the content of amino acids up to the dose of 800 mL ha^{-1}. The decrease of the content of amino acids in seeds produced under the doses of 600 and 800 mL ha^{-1} of trinexapac-ethyl was 22.1 and 26.9% in comparison to the control (0 mL ha^{-1}).

Protein content reached the point of maximum response at the estimated dose of 416.8 mL ha^{-1} (Fig 2d). There was an increment of 17.4% in the protein content when considering seeds under the influence of the dose of 416.8 mL ha^{-1}, comparatively to those where the plant growth regulator was absent. Even with the tendency of decrease in the protein content by continuously increasing the dose beyond 416.8 mL ha^{-1}, there was an increment of 8.5% between seeds produced at the dose of 800 mL ha^{-1} and those from the control treatment (0 mL ha^{-1}).

Discussion

The results indicate that the application of trinexapac-ethyl in wheat plants did not present any residual effect of inhibition in the resumption of growth of the embryo during the process of germination in wheat seeds. This could occur due to the effect of the plant growth regulator trinexapac-ethyl on the biosynthesis of gibberellic acid, inhibiting the enzyme 3 β-hydroxylase and obstructing the biosynthesis of GA_1 (Nakayama et al., 1990). Gibberellin is required for various species, during the process of germination, to activate the growth of the embryo (Taiz and Zeiger, 2013).

The increase of seed germination with greater doses of trinexapac-ethyl might be a consequence of amelioration in plant architecture, favoring the interception of solar radiation (Zagonel and Fernandes, 2007). Further, higher seed germination might be related to a shorter distance between sink and source (Fioreze and Rodriguez, 2014).

However, it is worth mentioning that harvest was performed at the same time for all treatments. Smaller values of germination obtained in this experiment, for seeds produced under the influence of the three smaller doses of the plant growth regulator, might be related to the fact that such seeds reached physiological maturity beforehand when compared to those from the doses of 600 and 800 mL ha^{-1}, thus, remaining longer periods of time stored in the cultivation environment after physiological maturity. Harvest must be performed as close as possible to the point of physiological maturity to prevent harvest delays from leading to loss of seed quality due to deterioration processes (Peske et al., 2012).

Greater values for the first count of germination are related to the increase of seed vigor expression and constitute an indication of higher efficiency in reorganizing the cell

Table 1. Summary of the analysis of variance presenting the mean squares of the first count of germination (FCG), germination (G) speed of germination index (SGI), shoot dry matter (W_{pa}), root dry matter (W_r), accelerated ageing (AA) and electrical conductivity (EC) after 3, 6 and 24 hours of wheat seeds produced by plants under the effect of fertilization for high yields and different doses of plant growth regulator.

SV[1]	DF[2]	Mean Square								
		FCG	G	SGI	W_{pa}	W_r	AA	EC 3 H	EC 6 H	EC 24 H
Doses[1]	4	21.9*	20.5*	0.5^{ns}	0.0001^{ns}	0.0001^{ns}	32.14*	4.9*	1.9^{ns}	5.87^{ns}
Residue	15	4.71	6.06	0.29	0.00004	0.00004	12.04	1.08	1.7	2.89
Total	19									
CV%	-	2.47	2.78	2.39	8.78	8.22	4.16	6.86	7.16	5.75

*Significant at the 5% probability level by the F-test. ^{ns}: not significant. [1]Source of variation. [2]Degrees of freedom. [3]Doses of the plant growth regulator trinexapac-ethyl.

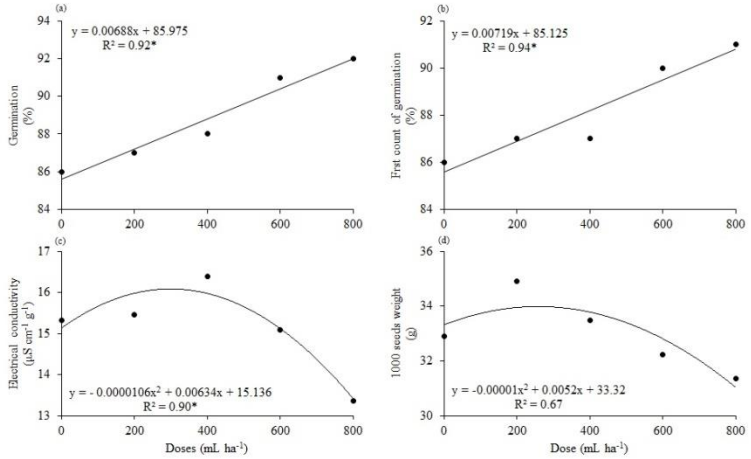

Fig 1. Germination (a), first count of germination (b) and electrical conductivity after three hours of imbibition (c) and 1000 seeds weight (d) of wheat seeds produced by plants under the effect of fertilization for high yields and the application of plant growth regulator. *Significant at the 5% probability level by the F-test.

Table 2. Summary of the analysis of variance presenting the mean squares of 1000 seeds weight (TSW), total soluble sugars (TSS), amino acid (AA), starch (S) protein (P) of wheat seeds produced by plants under the effect of fertilization for high yields and different doses of plant growth regulator.

SV[1]	DF[2]	Mean Square				
		TSW	TSS	AA	S	P
Doses[3]	4	7.11*	25.77*	3.23*	6.57*	136.83*
Residue	15	0.28	0.97	0.12	0.91	23.71
Total	19					
CV%	-	1.60	6.94	8.06	9.46	8.49

*Significant at the 5% probability level by the F-test. ^{ns}: not significant. [1]Source of variation. [2]Degrees of freedom. [3]Doses of the plant growth regulator trinexapac-ethyl.

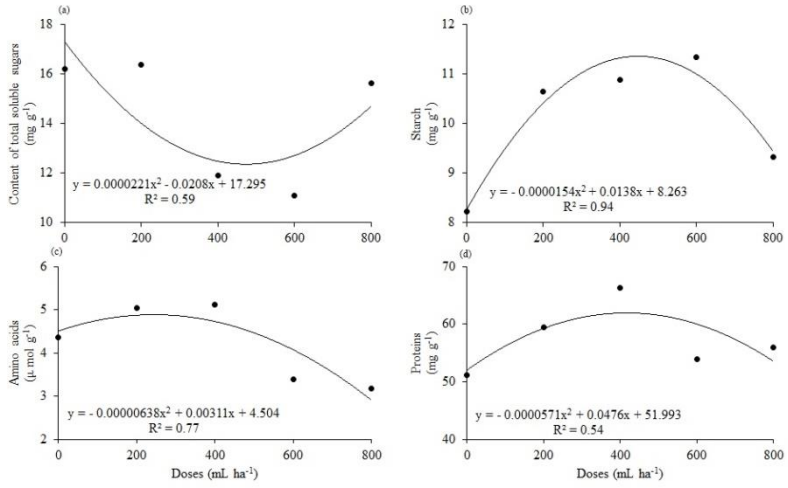

Fig 2. Content of total soluble sugars (a), starch (b) amino acids (c) and proteins (d) in wheat seeds produced under the influence of fertilization for high yields and the application of plant growth regulator. *Significant at the 5% probability level by the F-test.

membrane system, hydrolysis and in the allocation of reserves in the seedling (Peske et al., 2012).

Seeds produced by plants subjected to the dose of 800 mL ha^{-1} of the plant growth regulator presented higher ability of reorganizing cell membranes in the initial period of imbibition, resulting in less leaching of electrolytes to the solution, such fact can be verified by the lower values observed in the electrical conductivity test performed after three hours of seed inhibition. The greater release of electrolytes to the solution is related to damages or to the decrease in the ability of the membrane system to reestablish its integrity, leading to loss of selective capacity, which indicates lower vigor (Vieira and Krzyzanowski, 1999).

The increase of starch content may be explained by the amelioration of leaf architecture of plants subjected to the application of the plant growth regulator (Zagonel and Fernandes, 2007), enabling greater efficiency in capturing and absorbing solar radiation, which resulted in a higher rate of assimilate production. Similar levels of starch were quantified while studying starch content of rice seeds (Neves et al., 2007) and the chemical composition of wheat seeds (Silva et al., 2008).

During the process of assimilate allocation, when sugars are deposited at sink cells and may be stored as such or transformed into other compounds. In storage sinks, carbohydrates may accumulate as sucrose or hexose in the vesicle or as starch at the amyloplasts (Taiz and Zeiger, 2013). The use of imported sugars is maximized when the supply is abundant (Koch, 1996), thus, the decrease observed in total soluble sugars may be explained by the increase of starch content.

The increase of amino acid and protein contents is related to morphological modifications of the plant provoked by the plant growth regulator. Such modifications can favor the utilization of environmental resources, mainly solar radiation (Zagonel and Fernandes, 2007). Environmental factors, such as irrigation, temperature, fertility, nutrient mobility in the plant and availability of nitrogen in the soil, may also affect protein content (Cazetta et al., 2008).

In conditions of low availability of nitrogen to the plant, the synthesis of protein in seeds is decreased, increasing the starch content in the other hand (Rosa Filho, 2010). Accordingly, the increase of protein content was verified, and might be related to a better utilization of environmental resources by plants subjected to the application of trinexapac-ethyl, especially the better use of the nitrogen fertilizer which was provided in high amounts for plants of all treatments. Moreover, a better capture of solar radiation may have occurred due to an improved plant architecture which was induced by potential physiological and morphological alterations caused by the application of trinexapac-ethyl. Similar results for protein content were observed in sorghum seeds produced under the effect of the same product (Li et al., 2011).

The application of trinexapac-ethyl resulted in the increase of germination and in the values of the first count of germination up to the higher dose tested (800 mL ha^{-1}). The increase in the number of seeds germinated in the first count is related to a greater ability of reorganization of the cellular membrane system, such fact was remarkable in the values of electrical conductivity, only after three hours of inhibition, where a decrease in the quantity of electrolytes leached for the doses of 600 and 800 mL ha^{-1} was observed. Furthermore, the growth regulator resulted in the increase of protein and starch contents, which reached estimated maximums at 417 mL ha^{-1} and 448 mL ha^{-1}, respectively, indicating an increase in the levels of seed reserves.

Material and methods

Location, genotype, soil characteristics and fertilization

The work was performed in a chapel greenhouse, disposed in a North-South orientation, which is coated with polycarbonate and equipped with temperature control. Seed quality tests were carried out at the seed laboratory of the Seed Science and Technology Graduate Program at Universidade Federal de Pelotas, situated at an altitude of 13 meters, 31º 52' South and 52º 21' West.

The cultivar used in the experiment was OR Topázio, indicated for all wheat producing regions of Rio Grande do Sul and Paraná States, with plant heights of 880 mm and semi-erect growth habit. Seeding was performed at 06/27/2014 in polyethylene pots, with 12 liters of capacity, containing as substrate soil of horizon A1 of an Albaqualf (Usda, 1999). The soil had the following physical and chemical characteristics: pH (H_2O): 5.1; P: 121.2 mg dm^{-3}; K: 123 mg dm^{-3}; Ca: 2.7 $cmol_c$ dm^{-3}; Mg: 0.9 $cmol_c$ dm^{-3}; Al: 0.2 $cmol_c$ dm^{-3}; B: 0.3 mg dm^{-3}; Cu: 5.6 mg dm^{-3}; Zn: 2.2 mg dm^{-3}; Mn: 9 mg dm^{-3}; CTC: 8.1 $cmol_c$ dm^{-3}; percent base saturation: 52%; organic matter: 1.8%; clay: 17%.

Soil fertilization was carried out in accordance with the recommendations of the "Fertilization and liming handbook for the states of Rio Grande do Sul and Santa Catarina" (Cqfs, 2004), wherein the soil was amended to meet a yield expectation of 5 Mg ha^{-1}. Phosphorous and potassium fertilizers were incorporated to the soil in pre-sowing, employing 0.023 and 0.015 kg m^3, respectively, using as sources, triple superphosphate (41% P_2O_5) and potassium chloride (58% K_2O). Nitrogen fertilization was accomplished with 0.010 kg m^3 of nitrogen using urea (45% N) as source which was incorporated to the soil at sowing. Thereafter, a broadcast application of 0.050 kg m^3 of nitrogen was performed at the beginning of tillering, 30 days after sowing.

Experimental Design

The experiment was performed under completely randomized design with five treatments and four repetitions. The treatments consisted of five doses of the plant growth regulator trinexapac-ethyl [0 – without application; 200; 400; 600; 800 mL of the commercial product ha^{-1}]. The plant growth regulator used in the experiment was trinexapac-ethyl (Moddus®) "{ethyl 4-cyclopropyl(hydroxy)methylene-3,5-dioxocyclohexanecarboxylate"} applied 47 days after sowing via foliar spraying using a CO_2 backpack sprayer equipped with flat fan nozzles (110-020) and the speed of movement calibrated for a spraying volume of 150 L ha^{-1}. During the application, the pots of each treatment were disposed linearly side by side, then, with the aid of the CO_2 backpack sprayer calibrated as previously described and with the dose for each treatment adjusted, the full extent of each line of pots was roamed performing the application. The product was applied at elongation, between the first and the second detectable node, which corresponds to stage 6 of Feekes scale (Large, 1954).

Seed harvest and processing

Seed harvest was performed manually at October 31th, 2014. Thereafter, seeds were dried using a kiln at the temperature of 41ºC until the obtainment of a water content of 12%. Seed processing was performed manually by employing sieves with slotted holes. After processing, seeds were stored in a cold room.

Evaluation of seed physical and physiological qualities

In order to evaluate the effect of the plant growth regulator in the physical and physiological attributes of wheat seeds the following analysis were performed:

a) 1000 seeds weight was evaluated using eight replicates of 100 seeds per repetition, accordingly to (Brasil, 2009).

b) Germination was evaluated using four replicates of 50 seeds per repetition. The seeds of each replicate were placed between three sheets of germination paper, moistened with 2.5 times the mass of the dry substrate. Paper rolls were placed into an incubator at the temperature of 20ºC and photoperiod of 12 hours. The evaluation (counting) was carried out eight days after sowing and the results were expressed by the percentage of normal seedlings (Brasil, 2009).

c) First count of germination was performed alongside with the germination test, evaluating the percentage of normal seedlings at four days after sowing and expressing the results in percentage.

d) Speed of germination index was obtained through daily counts of germinated seeds with a minimal radicle protrusion of 3 to 4 mm, until stabilization (Nakagawa, 1994).

e) Seedlings dry matter was evaluated eight days after sowing. Four replicates of ten seedlings per repetition were split into shoot and root. Each part was placed separately in Kraft paper envelopes and subjected to kiln drying under forced air circulation, at the temperature of 70 + 2ºC, until constant mass. The dry matter was determined using a precision scale and the results were expressed in mg seedling^{-1}.

f) Accelerated ageing was evaluated using four replicates of 50 seeds per repetition. The seeds were disposed over a wire mesh attached to the interior of germination boxes (gerbox), which contained a blade of 40 mL of saturated saline solution (Pedroso et al., 2010). Saturated saline solution was composed of 110 g NaCl L^{-1} and the germination boxes containing the seeds were maintained at an incubator at 43ºC for 48 hours (Lima et al., 2006). After this period, the seeds were placed to germinate in the conditions stablished for the germination test and the evaluation (counting) was performed four days after sowing (Brasil, 2009).

g) Electrical conductivity was evaluated using four replicates of 25 seeds per repetition, where each replicate had its mass previously recorded. Afterwards, seeds were laid in polyethylene cups containing 80 mL of deionized water, and held in an incubator at the temperature of 20ºC, with a photoperiod of 12 hours. Electrical conductivity was determined after 3; 6 and 24 hours of imbibition, by means of a digital conductivity meter, model Digimed 32. The results were expressed in µS cm^{-1} g^{-1} of seeds (Vieira and Krzyzanowski, 1999).

Evaluation of seed composition

a) Extraction of total soluble sugars, starch and amino acids: the extracts were obtained according to the adapted methodology of Bieleski and Turner (1966). For this purpose, samples of 200 mg of seeds were macerated into a mortar and homogenized with 10 mL of extracting solution "MCW" (methanol: chloroform: water, in the ratios of 12:5:3). After 24 hours, the extracts were centrifuged at 2500 rpm for 30 minutes, the supernatant was recovered and the precipitated was placed into a kiln at the temperature of 30ºC to dry. For each 4 mL of the supernatant, 1.0 mL of chloroform and 1.5 mL of water were added, and the mix centrifuged for phase-separation. The supernatant containing the metabolites was collected and transferred to a bath at 38ºC for a period of 24 hours in order to eliminate chloroform residues and to concentrate the samples for the quantification of total soluble sugars and amino acids content. The precipitate, after dried, was resuspended in 10 mL of perchloric acid (PCA) 30% (*v/v*) and subjected to continuous stirring for 30 minutes in an orbital shaker, for starch digestion (McCready *et al*., 1950). Then, the extract was centrifuged at 2000 rpm for 30 minutes and the supernatant was recovered and used for quantification of the starch content.

b) Total soluble sugars quantification was fulfilled accordingly to the method described by (Graham and Smydzuk, 1965). Foremost, 40 µL of each sample, from a blank sample (distilled water) and from the standard samples (10-150 µg of glucose mL^{-1}) were collected into test tubes and held in an ice bath. Next, 3 mL of cold solution of anthrone (0.15% *p/v* of concentrated sulfuric acid) were added for each tube, which were later covered with glass beads. After 15 minutes, tubes were agitated and then incubated in bath at 90ºC for 20 minutes. Then, test tubes were maintained at darkness until reach room temperature. Finally, the tubes were agitated again and the optical density (D.O.) of the standard samples was determined via spectrophotometer Biospectro, model SP-22 at 620 nm.

c) Amino acid content quantification was performed accordingly to Yemm and Cocking (1955). For this purpose, 50 µL of each sample and a blank sample (distilled water) were collected into test tubes and then 0.5 mL of citrate buffer (0.2 M pH 5.0), 0.2 mL of ninhydrin reagent (5% of methylcellulose) and 1 mL of KCN (2% v/v of methylcellulose) were added. Next, test tubes were agitated, covered with glass beads and placed in a heated bath at 100ºC for 20 minutes. After that, the tubes were transferred to a dark room until reach room temperature. Finally, 1.3 mL of ethanol (60%) were added to each tube, stirred, and the optical density (O. D.) of the standards was measured using a spectrophotometer Biospectro SP-22 at 570 nm.

d) Starch quantification: firstly, 50 µL of each sample were collected into test tubes, held in an ice bath. Next, 3 mL of cold solution of anthrone (0.15% *p/v* of concentrated sulfuric acid) were added in each tube, which were then covered with glass beads. After 15 minutes, tubes were agitated and incubated in a heated bath at the temperature of 90ºC for 20 minutes. Then, the tubes were maintained in the dark until they reached room temperature. Finally, the tubes were agitated and the optical density (OD) of standards was measured via spectrophotometer Biospectro SP-22 at 620 nm.

e) Extraction and quantification of protein content: protein content was determined from samples of 200 mg of seeds. Samples were macerated in a mortar, homogenized in 1500 µL of extraction buffer (potassium phosphate 100 mM; pH 7.8; EDTA 0.1 mM; ascorbic acid 1 mM and distilled water). After that, a centrifugation was carried out at 11800 rpm for 10 minutes and 4 ºC and the supernatant was collected. Next, 10 µL of the supernatant and 2.5 mL of Bradford reagent (1976) were added to test tubes. The tubes were agitated and the optical density was measured in a spectrophotometer at 595 nm (Biospectro, model SP-22). A study evaluating the protein content in wheat seeds using the method described above was performed by (Silva et al., 2008).

Statistical analysis

For evaluations where replicates were used the value of each repetition consisted of the mean of the replicates. Data

collected were subjected to analysis of variance and when F-test was significant at 5% level, data were also subjected to regression analysis adjusting the equation accordingly to the degree of the more representative polynomial.

Conclusion

Under the conditions in which the experiment was performed the increase in the dose of the plant growth regulator trinexapac-ethyl results in the increase of the values of the first count of germination and in the decrease of the values of electrical conductivity of wheat seeds after three hours of inhibition, for the doses of 600 and 800 mL ha^{-1}. The application of trinexapac-ethyl in wheat plants promotes the increment of starch content up to the dose of 448 mL ha^{-1}; amino acids to the dose of 243 mL ha^{-1} and protein until the dose of 417 mL ha^{-1}, with doses higher than the estimated maximum there was a decrease up to the higher dose. However, it provokes the decrease of the content of total soluble sugars.

Acknowledgments

This work was carried out with CNPq support, National Council for Scientific and Technological Development – Brazil.

References

Alvarez RCF, Crusciol CAC, Ocheuze PC, Rodrigues JD, Alvarez ACC (2007) Influência do etil-trinexapac no acúmulo, na distribuição de nitrogênio (^{15}N) e na massa de grãos de arroz de terras altas. R Bras Ci Solo. 31(6):1487-1496.

Bieleski RL, Turner NA (1996) Separation and estimation of amino acids in crude plant extracts by thin-layer electrophoresis and chromatography. Anal Biochem. 17(2):278-293.

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem, 72(1):248-254.

Brasil (2009) Ministério da Agricultura, Pecuária e Abastecimento. Regras para análise de sementes, Brasília.

Buzetti S, Bazanini GC, Freitas JG, Andreotti M, Arf O, Sá ME, Meira FA (2006) Resposta de cultivares de arroz a doses de nitrogênio e do regulador de crescimento cloreto de clormequat. Pesq Agropec Bras, 41(12):1731-1737.

Cazetta DA, Fornazieri Filho D, Arf O, Germani R (2008) Qualidade industrial de cultivares de trigo e triticale submetidos à adubação nitrogenada no sistema de plantio direto. Bragantia. 67(3): 741-750.

Chorbadjian RA, Bonello P, Herms DA (2011) Effect of the growth regulator paclobutrazol and fertilization on defensive chemistry and herbivore resistance of Australian pine (Pinus nigra) and Paper birch (*Betula papyrifera*). Arboric & Urban For. 37(6):278-287.

Comissão de Química e Fertilidade do Solo (RS/SC) [Cfqs] (2004) Manual de adubação e calagem para os estados do Rio Grande do Sul e Santa Catarina. Porto Alegre: Sociedade Brasileira de Ciência do Solo/Núcleo Regional Sul, 400p.

Companhia Nacional de Abastecimento [Conab] (2017) Acompanhamento de safra brasileira de grãos, safra 2016/17. Companhia Nacional de Abastecimento. Brasília: Conab.

Cunha GR, Pires JLF (2005) Sistemas de cultivo para rendimentos elevado em trigo e o desafio das correlações indesejadas. Passo Fundo: Embrapa Trigo.

Fioreze SL, Rodrigues JD (2014) Componentes produtivos do trigo afetados pela densidade de semeadura e aplicação de regulador vegetal. Semin: Cien Agrar. 35(1):39-54.

Graham D, Smydzuc, J (1965) Use of anthrone in the quantitative determination of hexose phosphates. Anal Biochem. 11(2):246-255.

Kappes C, Arf O, Arf MV, Gitti DC, Alcalde AM (2011) Uso de reguladores de crescimento no desenvolvimento e produção de crotalária. Pesq Agropec Trop. 41(4):508-518.

Koch KE (1996) Carbohydrate-modulated gene expression in plants. Annu Rev Plant Physiol Plant Mol Biol. 47:509-540.

Large EC (1954) Growth stages in cereals illustration of the Feeks scales. Plant Pathol. 4(1):22-24.

Li E, Hasjim J, Dhital S, Godwin ID, Gilbert RG (2011) Effect of a gibberellin-biosynthesis inhibitor treatment on the physicochemical properties of sorghum starch. J of Cereal Sci. 53(3):328-334.

Lima TC, Medina PF, Fanan P (2006) Avaliação do potencial fisiológico de sementes de trigo pelo teste de envelhecimento acelerado. Rev Bras Sementes. 57(2):106-113.

Linzmeyer Junior R, Guimarães VF, Santos D, Bencke MH (2008) Influência de retardante vegetal e densidades de plantas sobre o crescimento, acamamento e produtividade da soja. Acta Sci Agron. 30(3):373-379.

Matysiak, K (2006) Influence of trinexapac-ethyl on growth and development of winter wheat. J Plant Prot Res. 46(2):133-143.

McCready RM, Guggolz J, Silviera V, Owens HS (1950) Determination of starch and amylase in vegetables. Anal Chem. 22(9):1156-1158.

Nakagawa, J (1994) Testes de vigor baseados na avaliação de plântulas. In: Vieira RD, Carvalho M. (ed) Testes de vigor em sementes. Jaboticabal, FUNEP, p. 49-85.

Nakayama K, Kobayashi M, Abe H, Sakurai A (1990) Effects of a plant-growth regulator, prohexadione, on the biosynthesis of gibberellins in cell-free systems derived from imature seeds. Plant Cell Physiol. 31(8):1183-1190.

Nascimento V, Arf O, Silva MG, Binotti FFS, Rodrigues RAF, Alvarez RCF (2009) Uso do regulador de crescimento etil-trinexapac em arroz de terras altas. Bragantia. 68(4):921-929.

Neves LAS, Moraes DM, Lopes NF, Abreu CM (2007) Vigor de sementes e atividade bioquímica em plantas de arroz submetidas a ácidos orgânicos. Pesq Agropec Gau, 1(13):79-80.

Pedroso DC, Tunes LM, Barbieri AP, Barros ACSA, Muniz MFB, Menezes VO (2010) Envelhecimento acelerado em sementes de trigo. Cienc Rural. 40(11):2389-2392.

Penckowski LH, Fernandes EC (2010) Utilizando regulador de crescimento na cultura do trigo: aspectos importantes para garantir bons resultados. Castro. Fundação ABC.

Peske ST, Villela FA, Meneguello GE (2012) Sementes: Fundamentos Científicos e Tecnológicos. 3 ed. Pelotas: Editora Universitária / UFPel.

Raven PH, Evert RF, Eichhorn SE (2007) Biologia vegetal. Rio de Janeiro: Guanabara Koogan.

Rodrigues O, Didonet AD, Teixeira MCC, Roman ES (2003) Redutores de crescimento. Passo Fundo: Embrapa Trigo.

Rodrigues O, Teixeira MCC (2003) Efeito da adubação nitrogenada no arranjo de plantas e redutor de crescimento no acamamento e em características de cevada. Passo Fundo: Embrapa Trigo.

Rosa Filho O (2010) Introdução ao manejo para qualidade industrial em trigo. Informativo Técnico Biotrigo, Passo Fundo.

Silva RN, Duarte GL, Lopes LF, Moraes DM, Pereira ALA (2008) Composição química de sementes de trigo (*Triticum aestivum* L.) submetidas a estresse salino na germinação. Rev Bras Sementes. 30(1):215-220.

Taiz L, Zeiger E. Fisiologia vegetal. 5. ed. Porto Alegre: ArtMed, 2013. 954p.

USDA (1999) United States Department of Agriculture - Soil taxonomy: a basic system of soil classification for making and interpreting soil surveys. USDA, Washington, USA.

Vieira RD, Krzyzanowski FC (1999) Teste de condutividade elétrica. In: Krzyzanowski FC, Vieira RD, França-Neto JBF. (ed) Vigor de sementes: conceitos e testes. Londrina: ABRATES. 218p.

Yemm EW, Cocking EC (1955) The determination of aminoacid with ninhydrin. Analyst, 80 209-213.

Zagonel J, Fernandes EC (2007) Doses e épocas de aplicação de redutor de crescimento afetando cultivares de trigo em duas doses de nitrogênio. Planta Daninha. 25(2):331-339.

Zagonel J, Ferreira C (2013) Doses e épocas de aplicação de regulador de crescimento em híbridos de milho. Planta Daninha. 31(2):395-402.

Zagonel J, Venancio WS, Kunz RP, Tanamat H (2002) Doses de nitrogênio e densidades de plantas com e sem um regulador de crescimento afetando o trigo, cultivar OR-1. Cienc Rural, 32(1):25-29.