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RESEARCH ARTICLE

Nitrogen and trinexapac-ethyl effects on wheat grain yield, lodging and seed physiological quality in southern Brazil

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Summary

Nitrogen (N) fertilization affects wheat yield and grain protein concentration; however, its mismanagement can increase plant lodging. While the use of plant growth regulators such as trinexapac-ethyl (TE) can mitigate plant lodging, their effects on seed physiological quality are not well known. The aim of this study was to evaluate the effects of N fertilization and TE on wheat yield, lodging and seed quality of spring wheat varieties. It was carried out in the 2018 growing season in the environments of Londrina and Ponta Grossa, Brazil. A randomized complete block design was used with a 2 × 3 × 3 factorial arrangement to evaluate two wheat genotypes (WT 15008 and WT 15025), three top-dressing N rates (0, 40 and 120 kg ha^{-1}), and three TE rates (0, 50 and 100 g ha^{-1}). Agronomic characteristics related to wheat productivity (hectolitre weight, thousand-grain weight, density of fertile spikes, plant height, lodging and grain yield) and seed physiological quality (seed germination and vigour; length and dry matter of normal seedlings) were evaluated. Increasing N rates up to 120 kg ha^{-1} increased plant lodging up to 26.4 percentage points for WT 15025 in Londrina. TE impaired some traits of seed physiological quality. Spraying 100 g ha^{-1} TE on the plants reduced seedling length by 9.4% in the seeds of WT 15008 harvested in Ponta Grossa compared to the TE control (0 g ha^{-1}). The dry matter of the seedlings from the seeds harvested in Londrina declined by 7.2% due to the application of 100 g ha^{-1} TE, compared to the control. However, a lower rate of TE (50 g ha^{-1}) might be enough to minimize plant lodging without impairing the physiological quality of the seeds, depending on the rate of N fertilization. This study is the first step in providing empirical evidence for the detrimental effects of TE in combination with N on wheat seed quality, suggesting that seed producers should exercise caution in managing TE and N fertilization.

Keywords: *Triticum aestivum* L.; plant growth regulator; synthetic plant hormone; seed germination; seed vigour

Introduction

Wheat (*Triticum aestivum* L.) is the main winter crop in Brazil, with approximately 90% of production coming from the southern region of the country, i.e., from the states of Paraná, Santa Catarina and Rio Grande do Sul (CONAB, 2020). Considering that the cultivated wheat area has remained relatively stable in the past decades (an average of 2.2 million hectares from the 2001 to the 2020 crop seasons), the increase observed in grain production from 3.2 to 5.4 million tons in the same period was a result of higher yield. Increases in grain yield are likely a consequence of well-structured plant breeding programmes, as well as improved agronomic management practices, such as the use of seeds with better physiological quality (Hasan *et al.*, 2013;

Pinto *et al.*, 2019), suitable fertilization strategies (Corassa *et al.*, 2018; Lollato *et al.*, 2019), improved pest management (Mehta, 2014) and the use of chemical products that regulate plant growth (Peake *et al.*, 2020).

Although nitrogen (N) management is amongst the most important tools used to increase wheat grain yield (Duncan *et al.*, 2018; Lollato *et al.*, 2019), closing important knowledge gaps may further improve N fertilization management in southern Brazil. These gaps in definition of optimal N fertilization practices (i.e. the integration of rate, timing, source and placement) are brought about by the complex dynamic of N in the soil–plant–atmosphere system (Vieira, 2017). In addition, wheat genotypes exhibit significant differences in N uptake capacity and N use efficiency (Beche *et al.*, 2014; Silva *et al.*, 2014; Lollato *et al.*, 2021). Thus, selecting the wheat genotype most adapted to each environment is another key condition to maximize crop yield (Munaro *et al.*, 2020; Rodrigues *et al.*, 2003). This selection interacts with adequate plant nutrition practices, particularly application of N.

An additional challenge when determining optimal N rates is that high amounts of N fertilizer can cause plant lodging, particularly when combined with abundant and frequent rainfall after the anthesis stage (Peake *et al.*, 2016). Plant lodging causes problems in mechanized harvest and can decrease the milling and baking quality of wheat grain (Pumphrey and Rubenthaler, 1983). Plant lodging often reduces crop yield because it limits the translocation of photoassimilates to grain development, increases the risk of foliar diseases and grain sprouting and causes harvest delay (Berry *et al.*, 2004; Penckowski *et al.*, 2009). The use of plant growth regulators is a practice widely adopted in wheat farm operations in the hope of reducing plant lodging that usually reduces grain quality and yield (Peake *et al.*, 2020; Qin *et al.*, 2020). Plant growth regulators are synthetic hormonal substances sprayed on the crop to reduce plant height, aiming to decrease the risk of lodging (Fagerness and Penner, 1998; Matysiak, 2006; Qin *et al.*, 2020) by decreasing the leverage applied to the base of the stem during wind events (Berry *et al.*, 2003).

The main plant growth regulator used in Brazil is trinexapac-ethyl (TE), which is effective in reducing plant height and lodging (Berry *et al.*, 2004; Espindula *et al.*, 2009). This substance is used in the vegetative phase of the plant. In that phase, it modulates the balance of gibberellins, inhibiting cell division and elongation by reducing gibberellic acid (GA_3) biosynthesis, a reduction promoted by inhibition of the 3-hydroxylase enzyme (Heckman *et al.*, 2002; Taiz and Zeiger, 2010). Therefore, the activity of TE is analogous to some plant hormones, i.e. it can change or inhibit some morphological and physiological processes in the plant. Due to its potential to reduce plant lodging, the application of TE on wheat plants can enable a greater supply of N, allowing the crop to better achieve its yield potential (Rodrigues *et al.*, 2003; Peake *et al.*, 2020).

Although the agronomic effects of TE have been widely investigated, there is a knowledge gap about the potential outcomes on the physiological quality of wheat seeds harvested from plants sprayed with this plant growth regulator. In this context, anecdotal evidence from Brazilian seed producers in the state of Paraná suggests that TE has a negative effect on the seed vigour of some wheat genotypes. This supposed damage to seed vigour can also reduce seed germination, plant stand and plant tillering, which then usually reduces grain yield. The adverse action of TE on modern wheat genotypes is currently under study in temperate regions such as North America (Subedi *et al.*, 2021). However, we are not aware of previous studies evaluating TE damage to spring wheat grown in Brazil or in other tropical countries. Considering the last three crop seasons (i.e. 2019, 2020 and 2021), the seeds traded in Paraná represented approximately 9% of the total production cost of wheat crops (CONAB, 2021). Therefore, the alternative of increasing the sowing rate (kg ha^{-1} seeds) to compensate for lower seed quality may reduce the wheat profit margin, which has been of low quite limited in Brazil, hampering the expansion of national wheat production (USDA, 2018). Brazilian wheat farmers have thus called for improvements in crop management practices to maintain profitability in the wheat sector.

The demand for high quality seeds, combined with better management practices adopted in seed fields (in contrast with grain production fields), is a crucial factor for generation of accurate technical recommendations for TE application on wheat crops. Thus, the aim of this study was to evaluate the effects of TE and N rates on the grain yield, plant lodging and seed physiological quality of different spring wheat genotypes. We hypothesized that spraying TE on wheat plants may reduce seed physiological quality (such as germination and vigour), and that N fertilization may affect the magnitude of these effects.

Materials and Methods

Environmental description of the experimental sites

The study was carried out during the 2018 growing season in two edaphoclimatic environments on experimental farms at the Brazilian Agricultural Research Corporation (*Empresa Brasileira de Pesquisa Agropecuária* – Embrapa) in Paraná, Brazil: the first farm near Londrina (23°11'37.1"S, 51°10'37.4"W and 598 m above sea level) and the second near Ponta Grossa (25°08'53.2"S, 50° 04'40.4"W and 884 m above sea level). The experiments were conducted using no-tillage practices, and wheat was sown after the soybean [*Glycine max* (L.) Merrill] crop.

The soil in the experimental field in Londrina is a basaltic Rhodic Eutrudox according to the USDA Soil Taxonomy (Soil Survey Staff, 2010) or Rhodic Ferralsol according to the WRB Soil Taxonomy (IUSS Working Group WRB, 2015) [*Latossolo Vermelho eutroférrico* according to the Brazilian Soil Classification System (Santos *et al.*, 2013)]. It is a predominantly clayey soil (732 g kg^{-1} clay and 107 g kg^{-1} sand) in the 0–10 cm soil layer. The landscape in the study site is slightly rolling with mild slopes (~15%). The regional climate is humid subtropical (Cfa) (Köppen, 1931), with a warm and rainy summer, sparse frosts, no defined dry season, mean temperature of 21.2 °C and annual rainfall of 1,438 mm (Sibaldelli and Farias, 2019).

The landscape of Ponta Grossa is also slightly rolling with mild slopes (~8%), and the soil in the experimental field is a Rhodic Hapludox or Rhodic Ferralsol (*Latossolo Vermelho distroférrico*), a sandy-clayey soil (526 g kg^{-1} clay and 397 g kg^{-1} sand) in the 0–10 cm soil layer. The regional climate is mesothermal humid subtropical (Cfb), with well-distributed rainfall, frequent frosts (from April to September), mean temperature of 17.5 °C and annual rainfall of 1,500 mm (Nitsche *et al.*, 2019).

Soil physical and chemical characterization was performed in November 2016 in soil samples collected in the 0–10 and 10–20 cm soil layers (Supplementary Table S1). Soil mineral N (NO_3^- and NH_4^+ in the 0–10 cm soil layer) was estimated later based on studies performed by Yokoyama *et al.* (2019) and Fagotti *et al.* (2012) in areas with the same soil classification located next to the experimental sites of Londrina and Ponta Grossa, respectively. Estimates were 11.4 ± 5.5 mg kg^{-1} N–NO_3^- and 7.5 ± 5.3 mg kg^{-1} N–NH_4^+ (mean ± standard deviation) for Londrina, and 2.0 ± 0.8 mg kg^{-1} N–NO_3^- and 1.7 ± 0.7 mg kg^{-1} N–NH_4^+ for Ponta Grossa. Meteorological variables [rainfall, temperature and relative humidity (RH)] were recorded throughout the wheat growing season in meteorological stations located less than 1,300 m from the experimental areas (Figure 1). Water balance was calculated according to the Thornthwaite and Mather (1955) method, a procedure that allows estimation of actual evapotranspiration, soil water deficit and water surplus. The method uses air temperature as an index of the energy available for the evapotranspiration process. To initiate the calculation procedure, soil water storage for the root zone is assumed to be at field capacity at the end of the last month of the wet season. After that, when precipitation exceeds potential evapotranspiration (PE), there is a net gain in soil moisture for that period. If the soil is at its saturation limit (i.e. at field capacity), then the difference between excess precipitation and PE is considered water runoff. As long as the soil remains at field capacity, evapotranspiration will continue at its potential rate. For the periods in which the PE is in excess of precipitation (i.e. the soil is drying out), the accumulated potential water loss is increased by the difference of PE and precipitation. In the current study, water deficit and water surplus were presented in 10-day intervals. The accumulated water deficit considered the sum of the 10-day

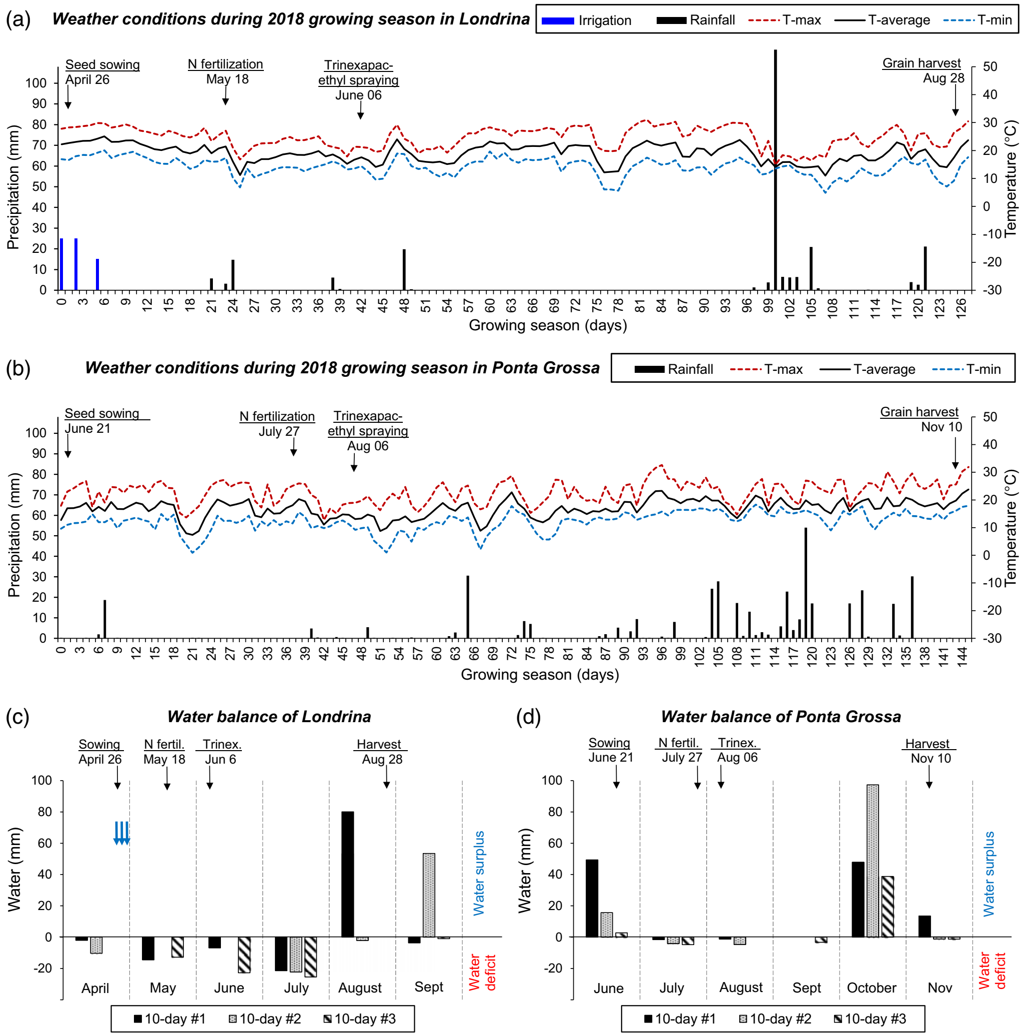

Figure 1. Precipitation (rainfall and irrigation); maximum (T-max), average (T-average) and minimum (T-min) temperatures; and 10-day water balance during the wheat growing season in Londrina (April 26 to August 28 = 125 days; panels 'a' and 'c') and Ponta Grossa (June 21 to November 10 = 143 days; panels 'b' and 'd'). Note: in Londrina, irrigation was applied on April 25 (25 mm), April 27 (25 mm), and April 30 (15 mm) to favour initial establishment of the wheat crop. The blue bars or blue arrows in panels 'a' and 'c' indicate these irrigation applications. Dates of the major field operations are also provided in the panels.

intervals with negative water balance throughout the growing season. The calculation procedure used spreadsheets in Excel™ developed by Rolim *et al.* (1998).

Experimental design and treatments

A randomized complete block design consisting of a 2 × 3 × 3 factorial arrangement with four replications was composed of two wheat genotypes (WT 15008 and WT 15025), three top-dressing N rates (0, 40 and 120 kg ha^{-1}) and three TE rates (0, 50 and 100 g ha^{-1}). The TE rates were based on the manufacturer's technical recommendations for wheat (100–125 g ha^{-1} of active ingredient, i.e. 400–500 mL ha^{-1} of the commercial product) and on previous evaluations, which indicated good efficacy of this plant growth regulator at a lower rate (50 g ha^{-1}) (Foloni *et al.*, 2016). Nitrogen rates were based on technical recommendations for wheat crops in Paraná (Foloni *et al.*, 2016). The calculation procedure for defining N rates considers a decision matrix developed for wheat genotypes from the Embrapa breeding programme for Paraná, specifically for rainfed conditions and a no-tillage system. This decision-making tool takes the following factors into account: i) expected grain yield for each genotype and environmental condition (i.e. macro-region of wheat crop adaptation), ii) previous crop [soybean or maize (*Zea mays* L.)], iii) responsiveness of each genotype to N fertilization (high or low response to N rates in terms of grain yield increase).

The genotypes WT 15008 and WT 15025 are classified as medium maturation with respect to phenology, with average cycles from seedling emergence to grain maturity of around 120 and 128 days, respectively. The genotypes have a similar period from seedling emergence to booting (with an average difference of three days) and similar average height (74 and 76 cm, respectively), but they differ in lodging resistance – WT 15008 is considered resistant, whereas WT 15025 has below-average straw strength (unpublished data). Nitrogen top-dressing fertilization was performed at the beginning of wheat tillering [growth stage GS21 of Zadoks' scale (Zadoks *et al.*, 1974)] using ammonium nitrate (NH_4NO_3) as an N source. TE was applied at the beginning of stem elongation (stage GS32, when the first node of the main stem was visible and the second was detectable) using a backpack sprayer with a compressed CO_2 tank and a 1.5-m hand boom equipped with four Teejet XR 110–020 flat fan nozzles. TE treatments were applied at a constant pressure of 2.11 kgf cm^{-2} (30 lb in^{-2}) and a spray volume of 200 L ha^{-1}.

The experimental unit was 6 m long by 1.6 m wide (9.6 m^2), consisting of nine rows at a spacing of 0.18 m; the space between the edges of different plots had a length of 1.0 m and width of 0.5 m. Wheat was sown on April 26, 2018, in Londrina, and on June 21, 2018, in Ponta Grossa. Sowing density was established according to the breeder's recommendation (350 viable seeds m^{-2} for both wheat genotypes; personal communication), and seeds were sown at a soil depth of 4 cm. Prior to wheat sowing, there were approximately 4,600 and 5,900 kg ha^{-1} of soybean straw residue (based on dry matter) on the surface of the experimental areas of Londrina and Ponta Grossa, respectively. These values were estimated based on data from the 2016 crop season in the same experimental areas (Ferreira *et al.*, 2021).

Base fertilization was performed on the same day as wheat sowing, with 200 kg ha^{-1} of 10–15–15 (N–P_2O_5–K_2O) formulated fertilizer, which was calculated according to soil chemical analysis (based on soil organic carbon, phosphorus and potassium concentrations; Table S1), the previous crop (soybean or maize), and expected grain yield (Foloni *et al.* 2016; CBPTT, 2017).

Commercial pesticides were used as needed to control weeds, insects and fungal diseases, according to technical recommendations for wheat production (CBPTT, 2017).

Agronomic characteristics and grain yield

Plant height, density of fertile spikes (spikes m^{-2}) and plant lodging were evaluated the day before wheat harvest. A visual scoring system for plant lodging evaluation (adapted from Embrapa, 2009) was used, which consists of scores given at maturity (pre-harvest) and recorded on a scale from 0 to 10 (where 0 is upright and 10 is completely lodged); the scores were converted to lodging percentages (Supplementary Table S2).

Wheat grain yield was calculated at maturity (GS92 of Zadoks' scale) by harvesting the seven central rows of 6 m length (7.5 m^2) using a self-propelled combine developed for small plots of cereal grains. The grain moisture content was recorded and adjusted to 13% for yield calculation. The hectolitre weight and the thousand-grain weight were evaluated for the harvested grain. Immediately after harvesting, 500 g of grain were sampled from each experimental plot and placed in cold storage at 12 °C for further analysis of seed physiological quality.

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Seed physiological quality

Seed physiological quality was evaluated in the Seed and Grain Technology Centre at the National Soybean Research Centre (*Embrapa Soja*) using the following analyses:

Germination: performed with four replications of 50 seeds in Germitest® paper towel moistened with distilled water at the rate of 2.5 times the weight of the dry paper. The rolls of paper towel were kept in a seed germinator at a temperature of 20 °C. The count of the germinated seeds was performed 8 days after setting up the test, with subsequent calculation of the percentage of normal seedlings (BRASIL, 2009). Normal seedlings were those that had a well-developed root system with a vigorous set of primary and secondary roots, as well as healthy cotyledon, hypocotyl and epicotyl. An abnormal seedling was characterized by the absence of one or more of its essential structures, such as the root, the shoot or the terminal bud.

Seedling length: evaluated by sowing 20 seeds on the upper third of a sheet of Germitest® paper moistened with distilled water at the rate of 2.5 times the weight of the dry paper. Four replications were performed. The rolls of the paper containing the seeds remained for 5 days in a seed germinator at a temperature of 20 °C, at which time the length of normal seedlings was measured using a millimetre ruler (Nakagawa, 1999). The results were expressed in centimetres per seedling (shoot + root).

Seedling dry matter: the normal seedlings coming from the test of seedling length were placed in paper bags and then in a forced air circulation laboratory oven regulated to a temperature of 80 °C over a 24-hour period (Nakagawa, 1999). The dry matter was weighed on a precision balance with an accuracy of 0.001 g, and the results were expressed in milligrams per seedling.

Seed vigour by the accelerated ageing test: performed with four replications of 240 seeds aged in a seed germinator at a temperature of 41 °C for 48 hours. The seeds were then tested for germination (at a temperature of 20 °C), and the normal seedlings were counted 4 days after sowing (Maia *et al.*, 2007).

Seed vigour by the cold test: conducted through the method of rolled paper towel without soil. Four replications of 50 seeds were sown on Germitest® paper moistened with distilled water at the rate of 2.5 times the weight of the dry paper. The rolls were kept in a cold chamber at a temperature of 10 °C for 7 days, and after that, in a seed germinator at 20 °C for 4 days, at which time the normal seedlings were counted (Barros *et al.*, 1999).

Statistical analysis

The experimental data for each site were analysed using the GENES® statistical package (Cruz, 2013). Lilliefors' test was used to evaluate the assumptions of the model regarding the normality of residuals, and Bartlett's test was used regarding the homogeneity of variance. The skewness and kurtosis coefficients were also evaluated. According to these tests, no data transformation was needed. Since all the assumptions required for a valid analysis of variance (ANOVA) were met, the F-test was performed. When the three-way ANOVA resulted in a significant p-value ($p \leq 0.05$), the means of the treatments were compared by Tukey's test ($p < 0.05$). When the subject factors (i.e. the main effects) were significant but the interaction was not, multiple comparisons among the treatment means were performed within each factor. However, when the three-way interaction between factors was significant, comparisons among the treatment means for one factor were performed individually within each level of the other factor (Wei *et al.*, 2012).

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Results

Weather conditions

In Londrina, the wheat growing season had a cycle of 125 days, with mean values of average, maximum and minimum temperature of 19 ± 3.2, 24.7 ± 3.8 and 14.1 ± 3.2 °C (mean ± standard deviation), respectively (Figure 1a). The mean RH was 78% (data not shown). The wheat crop received a total of 307 mm of water, including 65 mm from three irrigation applications that were performed in the first 6 days of the crop cycle (to allow proper crop establishment), and 242 mm from rainfall (with 116 mm on day 100 of the growing season). There was a 50-day drought period (from days 49 to 98) between the end of the stem elongation stage (GS39 of Zadoks' scale) and the late milk-grain development stage (GS77). In this period, the RH decreased substantially, reaching values close to 50% (data not shown). The accumulated water deficit throughout the wheat cycle was 128 mm (Figure 1c). There were no frost events during the growing season, and the minimum temperature was 4.9 °C (Figure 1a).

In Ponta Grossa, the wheat growing season lasted 143 days, with mean values of average, maximum and minimum temperatures of 16.8 ± 3.1, 22.8 ± 4.0 and 12.2 ± 3.6 °C, mean RH of 77% (data not shown), and total rainfall of 410 mm (Figure 1b). There was a drought period in the initial phases of crop development, mainly between day 8 (post-emergence of seedlings, GS11 of Zadoks' scale) and day 39 (beginning of stem elongation, GS30). Apart from that, 372 mm of rainfall was well distributed from the beginning of the booting phenological stage (GS41, on day 65) until the grain ripening stage (GS91, on day 136). This combination of weather conditions resulted in an accumulated water deficit of 19.7 mm over the growing season (Figure 1d). The RH had a smaller range of variation throughout the growing season, remaining predominantly between 70% and 85% (data not shown). There were two light frost events during the growing season: on day 21 (before the beginning of tillering, GS15) and day 52 (in the stem elongation stage, GS33), when the minimum daily temperatures reached 0.9 and 1.0 °C, respectively (Figure 1b). These frosts did not affect the wheat crop because they did not occur during the phenological stage most susceptible to cold damage (i.e. anthesis).

Crop agronomic characteristics and wheat yield

Among the factors studied, 'genotype' had the greatest effect on the crop agronomic characteristics evaluated in the two environments, followed by 'TE rate' and 'N rate', with few cases of interaction between these factors (Table 1).

The application of TE (50 and 100 g ha^{-1}) reduced plant height on an average of 3.4% in Londrina and 8.5% in Ponta Grossa, compared to the TE control treatment (0 g ha^{-1} TE) (Table 2). However, the effect of this plant growth regulator on plant lodging was only observed in Ponta Grossa, where lodging was reduced up to 4.8 percentage points with 100 g ha^{-1} TE (Table 2). Furthermore, TE did not affect the density of fertile spikes, thousand-grain weight and hectolitre weight in either environment (Londrina and Ponta Grossa), and it did not affect wheat grain yield in Londrina.

In Ponta Grossa, there was significant interaction between the 'N rate' and 'TE rate' factors in their effect on wheat grain yield (Table 1). In the absence of TE, the application of 120 kg ha^{-1} N increased grain yield by an average of 28.1% compared to the N control treatment (0 kg ha^{-1} N) (Figure 2a). However, when the plants received 50 or 100 g ha^{-1} TE, N rates did not affect grain yield. This meant that within the N control treatment, grain yield increased by an average of 25.6% compared to the control (0 g ha^{-1} TE) due to the application of 50 or 100 g ha^{-1} TE.

Nitrogen fertilization affected plant lodging in both environments (Table 1). In Ponta Grossa, the application of 120 kg ha^{-1} N increased the plant lodging scores by 4.4 percentage points (from 7.9 to 12.3%) compared to the N control treatment (Table 2). Significant interaction was observed between 'N rate' and 'genotype' in Londrina, where WT 15008 had almost no lodging regardless of

Table 1. Significance (*p*-value) of the analysis of variance of the main effects (genotype, top-dressing nitrogen rate, and trinexapac-ethyl rate) and their interactions regarding plant height, lodging, density of fertile spikes (DFS), thousand-grain weight (TGW), hectolitre weight (HW) and grain yield

Source of variation	Df[1]	Plant height	Lodging	DFS	TGW	HW	Grain yield
Londrina							
Block	3	0.022	ns[3]	ns	<0.001	ns	ns
Genotype (G)	1	0.018	<0.001	ns	<0.001	ns	ns
Nitrogen (N)	2	ns	<0.001	ns	ns	ns	ns
Trinexapac-ethyl (TE)	2	0.050	ns	ns	ns	ns	ns
G × N	2	ns	<0.001	ns	ns	ns	ns
G × TE	2	ns	ns	ns	ns	ns	ns
N × TE	4	ns	ns	ns	ns	ns	ns
G × N × TE	4	ns	ns	ns	ns	ns	ns
CV (%)[2]		5.8	148	15.1	4.3	1.83	12.7
Ponta Grossa							
Block	3	ns	<0,001	<0.001	<0.001	<0.001	<0.001
Genotype (G)	1	<0.001	<0.001	0.050	ns	<0.001	<0.001
Nitrogen (N)	2	ns	0.038	ns	ns	ns	ns
Trinexapac-ethyl (TE)	2	<0.001	0.025	ns	ns	ns	0.047
G × N	2	ns	ns	ns	ns	ns	ns
G × TE	2	ns	ns	ns	ns	ns	ns
N × TE	4	ns	ns	ns	ns	ns	0.023
G × N × TE	4	ns	ns	ns	ns	ns	ns
CV (%)		4.7	62.6	14.7	7.8	3.4	13.8

[1]Df = degrees of freedom (note: df from error = 51).
[2]CV = coefficient of variation.
[3]ns = not significant by the F test ($p > 0.05$).

Table 2. Multiple comparisons between treatment averages for each main effect (genotype or nitrogen rate or trinexapac-ethyl rate) regarding plant height, lodging, density of fertile spikes (DFS), thousand-grain weight (TGW), hectolitre weight (HW) and grain yield

Factor	Treatment	Plant height (cm)	Lodging (%)	DFS (spikes m^{-2})	TGW (g)	HW (kg hL^{-1})	Grain yield (kg ha^{-1})
Londrina							
Genotype	WT 15008	67.1 b	1.0*	346	30.8 a	79.4	2647
	WT 15025	69.4 a	15.6	340	28.3 b	79.4	2769
Nitrogen (kg ha^{-1})	0	67.9	1.8*	351	29.6	79.4	2776
	40	68.6	5.7	343	29.6	79.8	2767
	120	68.1	17.3	336	29.5	79.0	2581
Trinexapac-ethyl (g ha^{-1})	0	69.8 a	11.8	343	29.7	79.1	2751
	50	67.6 b	7.6	348	29.5	79.5	2740
	100	67.2 b	5.4	339	29.5	79.7	2633
Ponta Grossa							
Genotype	WT 15008	68.8 b	4.9 b	513 a	30.0	69.3 b	2005 b
	WT 15025	75.2 a	14.6 a	479 b	30.5	72.8 a	2381 a
Nitrogen (kg ha^{-1})	0	72.3	7.9 b	482	30.4	71.2	2170*
	40	71.5	9.0 ab	491	30.4	71.0	2118
	120	72.1	12.3 a	514	30.0	70.9	2291
Trinexapac-ethyl (g ha^{-1})	0	76.3 a	11.9 a	507	30.8	71.5	2073*
	50	72.8 b	10.2 ab	475	30.1	71.2	2294
	100	66.8 c	7.1 b	505	29.9	70.4	2213

*Comparisons with significant interaction between main effects that have further interpretations in Figure 2. Individually for each factor, averages in the column followed by different lowercase letters differ from each other by Tukey's test ($p \geq 0.05$).

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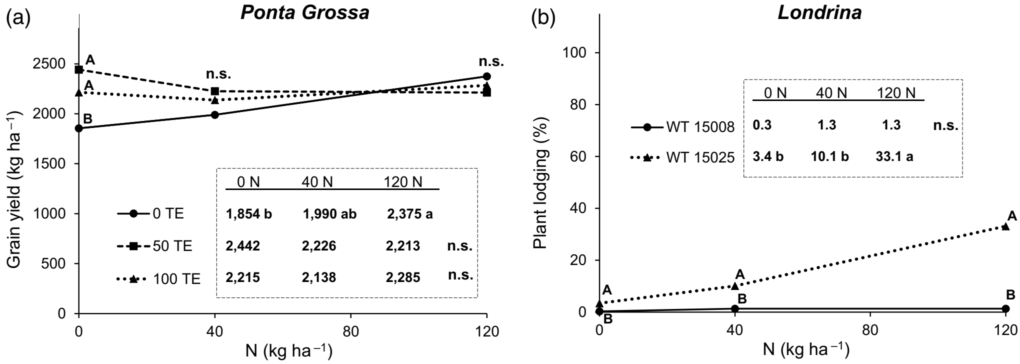

Figure 2. Comparisons between treatment means when the three-way interaction between the main factors was significant, i.e. 'trinexapac-ethyl (TE) × nitrogen (N)' for grain yield in Ponta Grossa (panel 'a') and 'genotype × N' for plant lodging in Londrina (panel 'b'). Individually for each TE rate (0, 50 and 100 g ha^{-1}) or each genotype (WT 15008 and WT 15025), values within the box comparing N rates (0, 40 and 120 kg ha^{-1}) followed by different lowercase letters differ from each other by Tukey's test ($p \geq 0.05$). Individually for each N rate, symbols (circle, square and triangle) comparing TE rates or genotypes followed by different capital letters differ from each other by Tukey's test ($p \geq 0.05$). Note: n.s. = not significant.

N rate, but WT 15025 had increased lodging (average increase of 26.4 percentage points) as the N rate increased from 0 and 40 kg ha^{-1} N to 120 kg ha^{-1} N (Figure 2b).

The effect of 'genotype' was significant for most of the crop characteristics (Table 1). In Ponta Grossa, WT 15025 showed a lower density of fertile spikes (6.6%) and greater plant height (9.3%), plant lodging (9.7 percentage points), hectolitre weight (5.1%) and grain yield (18.8%) compared to the other genotype (Table 2). Meanwhile, in Londrina, WT 15008 had a greater thousand-grain weight (8.8%), shorter plant height (3.3%) (Table 2) and lower plant lodging (14.6 percentage points) (Figure 2b) compared to WT 15025.

Seed physiological quality

The effect of the 'genotype' factor was significant for most seed physiological traits, except for seed vigour evaluated by the cold test (Table 3). In contrast, the 'N rate' factor did not affect the seed physiological parameters in either environment. However, in Londrina, the 'N rate' interacted with the 'TE rate' and 'genotype' factors, affecting seed germination and seed vigour (evaluated by the accelerated ageing test), respectively. TE applied on wheat plants affected normal seedling dry matter of the seeds harvested in Londrina, and seed germination of the seeds harvested in Ponta Grossa. In addition, 'TE rate' interacted with 'genotype', affecting the length of normal seedlings from seeds produced in Ponta Grossa.

Regarding the seeds harvested in Londrina, the normal seedling dry matter declined by 7.2% compared to the control (0 g ha^{-1} TE) due to the spraying of 100 g ha^{-1} TE on the plants (Table 4). For the seeds harvested in Ponta Grossa in the treatments sprayed with 50 g ha^{-1} TE, the germination rate increased by 2.9 percentage points (from 75.1 to 78%) compared to the TE control.

Significant interaction was observed between the 'TE rate' and 'genotype' factors in Ponta Grossa, where spraying 100 g ha^{-1} TE on the plants reduced the normal seedling length of WT 15008 by 9.4% compared to the TE control; however, WT 15025 was not affected by TE rates (Figure 3a). As a result, the normal seedling length of WT 15025 was 12.8% greater than that of WT 15008 within the treatments that received 100 g ha^{-1} TE.

The application of TE did not affect the germination of the seeds produced in Londrina, considering both genotypes (Table 3 and Figure 3b). Nevertheless, there was a significant interaction between 'N rate' and 'TE rate' in this environment. TE had a significant effect on seed germination

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Table 3. Significance (*p*-value) of the analysis of variance of the main effects (genotype, top-dressing nitrogen rate and trinexapac-ethyl rate) and their interactions regarding seedling length (SL), seedling dry matter (SDM), germination by the germination test (G-GT), seed vigour by the accelerated ageing test (SV-AAT) and seed vigour by the cold test (SV-CT)

Source of variation	Df[(1)]	SL	SDM	G-GT	SV-AAT	SV-CT
Londrina						
Block	3	0.015	<0.001	0.006	0.002	ns
Genotype (G)	1	<0.001	0.014	<0.001	0.002	ns
Nitrogen (N)	2	ns[(3)]	ns	ns	ns	ns
Trinexapac-ethyl (TE)	2	ns	0.011	ns	ns	ns
G × N	2	ns	ns	ns	0.030	ns
G × TE	2	ns	ns	0.030	ns	ns
N × TE	4	ns	ns	0.029	ns	ns
G × N × TE	4	ns	ns	ns	ns	ns
CV (%)[(2)]		6.4	8.8	3.7	7.3	3.1
Ponta Grossa						
Block	3	<0.001	ns	<0.001	ns	ns
Genotype (G)	1	0.001	0.029	<0.001	<0.001	ns
Nitrogen (N)	2	ns	ns	ns	ns	ns
Trinexapac-ethyl (TE)	2	ns	ns	0.032	ns	ns
G × N	2	ns	ns	ns	ns	ns
G × TE	2	0.016	ns	ns	ns	ns
N × TE	4	ns	ns	ns	ns	ns
G × N × TE	4	ns	ns	ns	ns	ns
CV (%)		6.6	14.4	5.6	9.7	5.8

(1)Df = degrees of freedom (note: df from error = 51).
(2)CV = coefficient of variation.
(3)ns = not significant by the F test ($p > 0.05$).

Table 4. Multiple comparisons between treatment averages for each main effect (genotype or top-dressing nitrogen rates or trinexapac-ethyl rates) regarding normal seedling length (SL), seedling dry matter (SDM), germination by the germination test (G-GT), seed vigour by the accelerated ageing test (SV-AAT) and seed vigour by the cold test (SV-CT)

Factor	Treatment	SL (cm)	SDM (g)	G-GT (%)	SV-AAT (%)	SV-CT (%)
Londrina						
Genotype	WT 15008	23.2 a	8.2 a	89.5*	76.2*	90.4
	WT 15025	22.0 b	7.8 b	84.4	71.9	90.2
Nitrogen (kg ha^{-1})	0	22.3	8.1	86.7*	75.2*	90.2
	40	22.7	8.0	87.5	74.5	90.7
	120	22.7	7.8	86.7	72.5	89.9
Trinexapac-ethyl (g ha^{-1})	0	22.8	8.3 a	87.1*	74.3	90.1
	50	22.8	8.0 ab	86.4	74.0	90.5
	100	22.1	7.7 b	87.4	73.9	90.3
Ponta Grossa						
Genotype	WT 15008	21.4*	7.6 b	72.0 b	69.9 b	83.1
	WT 15025	22.6	8.2 a	80.3 a	83.6 a	81.9
Nitrogen (kg ha^{-1})	0	22.0	8.2	76.5	77.8	83.6
	40	22.1	7.8	76.8	77.4	81.9
	120	22.0	7.8	75.1	75.0	82.1
Trinexapac-ethyl (g ha^{-1})	0	22.6*	7.9	75.1 b	76.9	81.9
	50	21.8	8.2	78.0 a	76.6	83.9
	100	21.6	7.6	75.2 b	76.7	81.8

*Comparisons with significant interaction between main effects that have further interpretations in Figure 3. Individually for each factor, averages in the column followed by different lowercase letters differ from each other by Tukey's test ($p \geq 0.05$).

only within the treatment with 40 kg ha^{-1} N, in which the application of 100 g ha^{-1} TE on the plants increased seed germination by 4.7 percentage points compared to the TE control (Figure 3c). In contrast, N rates did not affect the germination of the seeds harvested in Londrina, even considering an independent analysis within each TE rate (Figure 3c).

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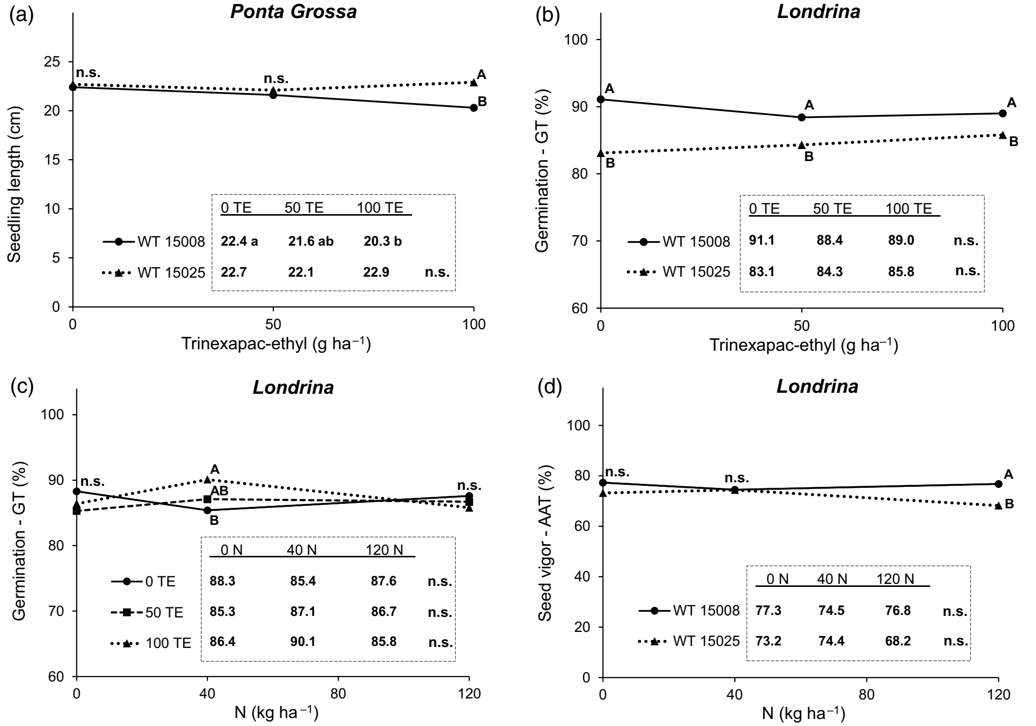

Figure 3. Comparisons between treatment means when the three-way interaction between the main factors was significant, i.e. 'genotype × trinexapac-ethyl (TE)' for normal seedling length (panel 'a') and for germination test (GT) (panel 'b'); 'TE × nitrogen (N)' for GT (panel 'c'); and 'genotype × N' for seed vigour by the accelerated ageing test (AAT; panel 'd'), in Londrina and Ponta Grossa. Individually for each genotype (WT 15008 and WT 15025) or each TE rate (0, 50 and 100 g ha^{-1}), values within the box comparing TE rates or N rates (0, 40 and 120 kg ha^{-1}) followed by different lowercase letters differ from each other by Tukey's test ($p \geq 0.05$). Individually for each TE rate or each N rate, symbols (circle, square and triangle) comparing genotypes or TE rates followed by different capital letters differ from each other by Tukey's test ($p \geq 0.05$). Note: n.s. = not significant.

In Londrina, there was a significant interaction between the 'genotype' and 'N rate' factors, where seed vigour (evaluated by the accelerated ageing test) of the seeds of WT 15025 was 8.6 percentage points lower than the seeds of WT 15008 when combined with 120 kg ha^{-1} N, but there was no genotype effect within the other N rates (Figure 3d). In those treatments, N rates did not affect the seed germination of either genotype.

Some physiological traits of the seeds showed a genotype × environment interaction. In Londrina, WT 15008 generated seeds with better quality; normal seedling length increased by 5.5% and dry matter by 5.1% in WT 15008 compared to WT 15025 (Table 4). However, in Ponta Grossa, the seeds harvested from WT 15025 had a higher normal seedling dry matter and germination rate and better vigour (evaluated by the accelerated ageing test), with increases of 7.9%, 8.3 percentage points and 13.7 percentage points, respectively, compared to seeds harvested from WT 15008.

Discussion

Our study confirmed our hypothesis and the anecdotal evidence from seed producers in southern Brazil that agronomic management of crops can affect the quality of seeds and hence the

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germination and emergence of subsequent crops. However, the effect of management on seed quality was small and may not translate to significant economic effects.

Effects of TE on wheat agronomic characteristics and grain yield

TE reduced wheat plant height and lodging more effectively in Ponta Grossa than in Londrina, which was associated with the differences in weather conditions between those environments (Figures 1a and 1b). Although the application of TE was performed in the appropriate wheat phenological stage, there was no rainfall in Londrina for about 2 months following TE application. Thus, plant growth was naturally reduced by the water deficit, explaining the limited effect of TE under those environmental conditions. This is also a common concern in other wheat-growing regions where drought is frequent (Jaenisch *et al.*, 2019). In contrast, in Ponta Grossa, the effects of TE on plant growth and lodging were evident. The total in-season rainfall in Ponta Grossa could be considered a suitable water volume for wheat cultivation (Patrignani *et al.*, 2014). The rainfall distribution following the application of TE was even better in Ponta Grossa than in Londrina, occurring mainly in the last quartile of the wheat-growing season, when plants are more susceptible to lodging due to the greater weight of the grains.

In Ponta Grossa, an unexpected result was the increase in wheat grain yield in the treatments sprayed with 50 or 100 g ha^{-1} TE combined with 0 kg ha^{-1} N, compared to the TE control. This result may have occurred because under conditions of soil N deficiency, TE reduced unnecessary shoot biomass production, allowing more photoassimilates and N to be used for grain formation. We also hypothesize that this finding may be attributed in part to the effects of TE on leaf architecture, particularly the angle of the flag leaf, which becomes almost upright, favouring the interception of sunlight and increasing the photosynthetic rate and the accumulation of carbohydrates for grain filling (Penckowski *et al.*, 2010). While wheat is most often sink-limited (i.e. changes in light interception only result in modest changes in grain yield; Borrás *et al.*, 2004), increases in radiation interception during the grain filling stage can lead to greater biomass accumulation and grain weight, increasing grain yield (Cruppe *et al.*, 2021). In addition, plant lodging was lower (−3.1 percentage points) and the density of fertile spikes was higher (6.7%) in the treatments sprayed with TE compared to the TE control. These results were found by evaluating the effects of 'TE rates' within each 'N rate' (data not shown). This may have contributed to higher grain yield in the treatments sprayed with TE because lodged plants generally yield less due to stem strangulation and consequent reduction of photoassimilate translocation to the grain (Penckowski *et al.*, 2009). Furthermore, increasing the density of fertile spikes (spikes m^{-2}) usually increases the grain yield per unit area, since the number of spikes per area is considered a coarse regulator of wheat yield (Slafer *et al.*, 2014). According to Lozano and Leaden (2001), the application of TE on wheat plants at the beginning of stem elongation leads to changes in the density of fertile spikes due to better tiller development, increasing wheat grain yield.

This positive effect of TE on increasing wheat grain yield should be carefully analysed and not extrapolated to other situations in a generalized way due to the environmental specificity of the response. For example, Guerreiro and Oliveira (2012) reported that TE reduced white oat (*Avena sativa* L.) grain yield in an area of Araruna in the northwest region of Paraná, at a distance of 170 km from the current experiment in Londrina. According to those authors, the adverse effect of TE was caused by its combination with intense drought conditions from the beginning of the fourth week after sowing, i.e. in that growing season there was no plant lodging (even in the TE control treatment). Thus, the application of TE was not only not necessary, but it also intensified a decrease in plant height, stem diameter and grains per panicle, resulting in an average grain yield reduction of 25% compared to the TE control. In another experiment with winter wheat, carried out in three regions of Kansas (USA) over two crop seasons, Jaenisch *et al.* (2019) found that application of TE decreased grain yield in the lower yielding season (yield of 2100 kg ha^{-1}) in the Hutchinson environment; but it did not affect grain yield in the other seasons with yield levels from 3200 to 4900 kg ha^{-1}. Knott *et al.* (2016) found similar results in three environments in Kentucky (USA), where TE did not consistently affect grain yield. This was related to the fact that plant height was already significantly reduced due to prolonged periods of extremely cold temperatures. TE is a synthetic hormone that causes several physiological changes in plant metabolism, and the magnitude of its effects depends on the environmental conditions experienced in the season, such as availability of water and nutrients (Qin *et al.*, 2020) and temperature (Knott *et al.*, 2016).

Crop agronomic characteristics and wheat yield affected by nitrogen fertilization and its interaction with TE

Nitrogen fertilization did not increase wheat grain yield in Londrina. This result can be attributed to the low water availability in the initial stages of plant development, which impaired N uptake and N accumulation in shoot biomass at anthesis, the wheat growth stage with peak N accumulation in the plant (Wiethölter, 2011; Lollato *et al.*, 2021). Considering that N is the key nutrient for wheat grain filling and yield, the effectiveness of N fertilization in rainfed environments is dependent on the intensity and distribution of rainfall throughout the growing season, which affects the colimitation of N and water (Cossani and Sadras, 2018).

In Ponta Grossa, N fertilization increased grain yield only in the treatments not sprayed with TE. While this outcome suggests that there was a deficiency of N in this environment, the absence of plant response to N application at different rates combined with TE application was not expected. This finding might be attributable to the lower plant lodging and higher density of fertile spikes in the treatments sprayed with TE, which had the effect of increasing grain yield, offsetting the effects of N rates, as previously discussed. This compensatory effect of TE on yield under conditions of water deficit corroborates the results obtained by Barányiová and Klem (2016). Nonetheless, our study only provides preliminary evidence for this phenomenon, which requires further research.

The absence of and small increase in grain yield due to N fertilization in Londrina and Ponta Grossa, respectively, are also associated with the low yielding crop season in both environments ($\leq$2776 kg ha^{-1}; Table 2), caused mainly by drought conditions, which means that the main limiting factor was water and not N. In this context, we hypothesize that the site of Ponta Grossa had lower soil N availability than Londrina, allowing some response to N rates in that colder environment. This hypothesis is supported by the studies of Yokoyama *et al.* (2019) and Fagotti *et al.* (2012) in areas located near the experimental sites of Londrina and Ponta Grossa, respectively. They found the following levels of soil mineral N: 11.4 ± 5.5 mg kg^{-1} N–NO_3^- and 7.5 ± 5.3 mg kg^{-1} N–NH_4^+ for the same site of Londrina; and 2.0 ± 0.8 mg kg^{-1} N–NO_3^- and 1.7 ± 0.7 mg kg^{-1} N–NH_4^+ for the Irati experimental site located 70 km from Ponta Grossa. Thus, the soil mineral N level in Londrina was about 5.1 times higher than that in Ponta Grossa.

In Londrina, N fertilization increased plant lodging only for the genotype WT 15025. Despite the water deficit in the early stages of plant development, the occurrence of lodging indicates that there was a significant uptake of N by plants in the last quartile of the crop cycle, i.e. after day 99 of the growing season, when rainfall was sufficient (Figure 1a). Post-anthesis N uptake may or may not contribute to grain yield, depending on environmental conditions (Lollato *et al.*, 2019; 2021), and additional N in this period can increase plant lodging (Penckowski *et al.*, 2009).

Another outcome of interest was the genetic resistance to plant lodging shown by the genotype WT 15008. This resistance is a relevant aspect that has been considered in wheat breeding programmes (Piñera-Chavez *et al.*, 2016) because adopting genotypes resistant to plant lodging might avoid the need for application of a plant growth regulator. The availability of wheat genotypes resistant to lodging is an alternative for farmers to achieve higher crop yields by increasing N rates without an additional expense on plant growth regulators. However, if other agronomic

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attributes (such as high grain yield potential, milling and baking quality, and resistance to pests and diseases) demand the use of a wheat genotype more prone to lodging, the preventive use of TE can be beneficial, particularly when the genotype is responsive to N fertilization (Zagonel *et al.*, 2002; Zagonel and Fernandes, 2007; Penckowski *et al.*, 2009).

Effects of TE on seed physiological quality

The use of the highest rate of TE impaired the physiological quality of wheat seeds due to lower normal seedling dry matter and seedling length of the seeds harvested in Londrina and Ponta Grossa, respectively. Similar outcomes were obtained by Kaspary *et al.* (2015), who found lower seed physiological quality (i.e. reductions in germination rate and vigour of the seeds and length and dry matter of the normal seedlings) due to TE application on white oat plants. Those authors attributed the lower seed quality to the negative effect of TE on the accumulation of photoassimilates by plants, which resulted in smaller seeds with fewer carbohydrate reserves. According to Espindula *et al.* (2010), TE can reduce the photosynthetic capacity of the plants, which become shorter and have reduced leaf area. That can decrease the accumulation of photoassimilates and, consequently, impair the development of the seeds. Whether this reduced leaf area nullifies the effect of increased light interception resulting from more upright leaves (see discussion above) could be the focus of future research.

TE had inconsistent effects (i.e. varying according to genotype and N rate) on seed germination and seed vigour (measured by the accelerated ageing test). Seed germination is a trait strongly determined by the genetic factor (Martínez-Andújar *et al.*, 2012) and is affected by the availability of N (Wen *et al.*, 2018). Seeds acquire vigour throughout the maturation-drying phase in seed development, which not only involves the water loss process but also is considerably dependent on gene expression (Angelovici *et al.*, 2010). Seed vigour is reflected in seedling establishment after seed germination and requires remobilization of reserves to supply nutrients. In this context, N is the main mineral nutrient that constitutes the seed protein structure, which is part of seed reserve substances (Naegle *et al.*, 2005). Thus, considering the multiple possible interactions among genotype × N rate × TE rate in the current study, TE was likely to have inconsistent effects on seed germination and vigour. To the best of our knowledge, similar outcomes were not reported in the literature for the wheat crop.

Seeds with better physiological quality favour seedling emergence and development, accelerating crop establishment and ground cover, which reduces competition from weeds, achieving the yield potential of the crop (Gustafson *et al.*, 2004). Considering the possibility of negative effects of TE on seed quality, farmers must manage this product carefully in crops exclusively intended for seed production. In this respect, we emphasize that the scale of the effects of TE on the physiological quality of wheat seeds was small, which indicates that the seeds produced in commercial fields that use this sort of plant growth regulator might not have a negative impact on the farmers who buy these seeds. However, further testing may be needed to confirm these results across a wider range of environmental conditions and management systems, particularly considering different wheat genotypes and rates of TE and N.

Seed physiological quality affected by nitrogen fertilization and its interaction with genotype

Nitrogen fertilization did not affect most of the physiological quality traits of wheat seeds harvested in the two environments. Considering that N is part of the seed protein structure, a greater effect of N fertilization on the physiological quality of the seeds was expected. This negligible effect of N can be attributed mainly to the weather conditions experienced throughout the growing season, because the extensive periods of drought reduced N uptake by plants in the period prior to anthesis, a critical phenological period for wheat plants to accumulate N for later grain development (Wiethölter, 2011). Thus, because the accumulation of N in the plant biomass up to anthesis was not largely affected by N rates under the conditions studied, the potential effects of N fertilization on seed physiological quality were reduced. Consequently, within the crop season of lower yield (caused mainly by drought conditions) in both environments, the main limiting factor affecting seed physiological quality was water and not N. Furthermore, as soil mineral N (NO_3^- and NH_4^+) was not measured in the current study, it was difficult to interpret some results (i.e. whether N was limiting or not). Future studies should ensure that soil N analyses are conducted to allow a full understanding of the availability of N for plant uptake, which is essential for estimating the appropriate N rates to be used in the experiment.

There was an interaction between 'N rate' and 'genotype' that affected the physiological quality of the seeds. In Londrina, the highest rate of N led to lower seed vigour for WT 15025 compared to WT 15008. We hypothesize that this outcome can be attributed in part to the higher plant lodging of WT 15025 (33.1%) than of WT 15008 (1.3%) within the treatment fertilized with 120 kg ha^{-1} N (Figure 2b). Indeed, one of the critical consequences of plant lodging is its negative effect on wheat seed quality. Wheat spikes in contact with the soil after lodging are exposed to higher moisture conditions, which favour infection by pathogenic and opportunistic fungi, deteriorating the wheat seeds (Zagonel and Fernandes, 2007). In addition, this higher moisture favour the germination of seeds when they are in the maturation phase in the spikes. Therefore, in commercial fields for seed production, excessive rates of N should be avoided for wheat genotypes susceptible to plant lodging.

Conclusions

Our study validated our hypothesis and the anecdotal evidence from Brazilian seed producers that the application of TE rates combined with N rates can affect the quality of the seeds harvested from different spring wheat genotypes. The application of TE on wheat plants impairs the physiological quality of the harvested seeds by reducing the length and the dry matter of the normal seedlings. However, these effects of TE were small and may not cause significant economic losses for either the seed producers or the farmers who buy the seeds. In several situations, the effects of TE and N rates on seed physiological quality were derived from their effect on plant lodging, which can directly damage the quality of the seeds. In this context, a lower rate of TE might be enough to minimize plant lodging without harming the quality of the seeds, which, in addition, depends on the rate of N fertilization. Thus, in commercial fields destined for seed production, TE and N should be managed carefully to avoid potential negative impacts on seed physiological quality, which depends on the genotype grown and environmental conditions, especially the availability of water throughout the growing season.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0014479722000217

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Conflicts of Interest. The authors declare there is no conflict of interest.

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