ABRATES

Journal of Seed Science
ISSN 2317-1545
www.abrates.org.br/revista

ARTICLE

Nitrogen fertilization and sowing density on yield and physiological quality of wheat seeds

Journal of Seed Science, v.44, e202244013, 2022

http://dx.doi.org/10.1590/2317-1545v44254717

Jéssica de Lucena Marinho[1], Sérgio Ricardo Silva[2]*, Inês Cristina de Batista Fonseca[1], Claudemir Zucareli[1]

ABSTRACT: The optimal combination between sowing density and nitrogen (N) fertilization rate is a challenge for the production of high-quality seeds, considering the diversity of genotypes and environments. This study aimed to evaluate the yield and physiological quality of wheat seeds as a function of sowing density and N rate, under different edaphoclimatic conditions. The experiment was carried out in three environments (Londrina, Cascavel, and Ponta Grossa) in a randomized block design with four replications, considering a 2 × 3 × 4 factorial arrangement composed of two genotypes (BRS Anambé and WT 15-025), three sowing densities (250, 350, and 450 seeds.m^{-2}), and four N rates (0, 40, 80, and 120 kg.ha^{-1}). The following seed features were evaluated: yield, first germination count, final seed germination, seedling emergence, emergence speed index, seedling length, and seedling dry matter. Seed yield varied as a function of environment and genotype. BRS Anambé produced seeds with higher physiological quality. The optimal combination between sowing density and N rate that provided the more suitable economic yield, without affecting the physiological quality of wheat seeds, varied with the environment and was estimated in 250 seeds.m^{-2} × 0 kg.ha^{-1} N for Londrina and Cascavel, and 350 seeds.m^{-2} × 18 kg.ha^{-1} N for Ponta Grossa.

Index terms: plant stand, seed germination, seed vigor, *Triticum aestivum* L.

RESUMO: A combinação ótima entre densidade de semeadura e dose de nitrogênio (N) na adubação é um desafio para a produção de sementes de alta qualidade, considerando a diversidade de genótipos e ambientes. O objetivo deste trabalho foi avaliar o rendimento e a qualidade fisiológica de sementes de trigo em função da densidade de semeadura e dose de N, em diferentes condições edafoclimáticas. O experimento foi conduzido em três ambientes (Londrina, Cascavel e Ponta Grossa) em delineamento em blocos casualizados com quatro repetições, considerando um arranjo fatorial 2 × 3 × 4 composto por dois genótipos (BRS Anambé e WT 15-025), três densidades de semeadura (250, 350 e 450 sementes.m^{-2}) e quatro doses de N (0, 40, 80 e 120 kg.ha^{-1}). Foram avaliadas as seguintes características de sementes: rendimento, primeira contagem de germinação, germinação final de sementes, emergência de plântulas, índice de velocidade de emergência, comprimento de plântula e massa seca de plântula. O rendimento de sementes variou em função do ambiente e do genótipo. BRS Anambé produziu sementes com maior qualidade fisiológica. A combinação ótima entre densidade de semeadura e dose de N que proporcionou o rendimento econômico mais adequado, sem afetar a qualidade fisiológica de sementes de trigo, variou com o ambiente e foi estimada em 250 sementes.m^{-2} × 0 kg.ha^{-1} N para Londrina e Cascavel, e 350 sementes.m^{-2} × 18 kg.ha^{-1} N para Ponta Grossa.

Termos para indexação: população de plantas, germinação de sementes, vigor de sementes, *Triticum aestivum* L.

***Corresponding author**
E-mail: sergio.ricardo@embrapa.br

Received: 07/28/2021.
Accepted: 03/23/2022.

[1]Universidade Estadual de Londrina, Departamento de Agronomia, PR 445, Km 380, 86057-970 - Londrina, Paraná, Brasil.

[2]Empresa Brasileira de Pesquisa Agropecuária, Embrapa Trigo, Caixa Postal 3081, 99050-970 - Passo Fundo, Rio Grande do Sul, Brasil.

INTRODUCTION

Wheat is the second most produced and consumed cereal worldwide and represents the main winter crop in Brazil, with significant increases in area, yield and production of grains in recent growing seasons (CONAB, 2021). Adopting suitable and specific agronomic techniques for each genotype, which might favor the production of a larger quantity of high-quality seeds, is need for sustaining the expansion of cultivated areas, as well as to explore the wheat yield potential.

Nitrogen (N) fertilization stands out among the agronomic management techniques because N is one of the most important nutrients for seed formation and it is an essential constituent of biomolecules that participate in biochemical and metabolic processes for plant growth and development (Ohyama, 2010). In addition, the amount of N taken up by wheat plant throughout the crop cycle has a positive correlation with seed protein concentration (Brzezinski et al., 2014; Lollato et al., 2021), which is essential for the initial establishment of the seedling during and after germination. Seed proteins are hydrolyzed during the germination process to meet the nutritional demand of the embryo and of the seedling during its early development stages (Caliskan and Makineci, 2015). However, excessive N rates can cause cell toxicity (Elhanafi et al., 2019), increase the severity of plant diseases such as wheat blast (*Magnaporthe oryzae* Triticum) (Silva et al., 2019), and, consequently, reduce wheat quality and yield. According to Souza et al. (2021), unsuitable N supply impairs the physiological quality of wheat seeds, and the fertilization with 40 $kg.ha^{-1}$ N at sowing (alone or combined with 40 $kg.ha^{-1}$ N as topdressing fertilization at the tillering stage) resulted in the production of seeds with better physiological quality.

Sowing density management is another relevant agronomic practice that influences wheat seed quality and yield. The density of established seedlings per unit of area influences the tillering rate and the intensity of intraspecific and interspecific competition between plants (Abati et al., 2018). Cardoso et al. (2021) showed that, despite increasing seed yield, increasing sowing density can be detrimental to some physiological quality attributes of the produced seeds, such as first germination count, seedling root dry matter, and seedling shoot length.

The recommendation of the best sowing density and N rate, individually for each wheat genotype, has been a challenge for the production of seeds with high physiological quality, considering the diversity of edaphoclimatic environments. For instance, increasing sowing density provides a higher number of spikes per square meter, as a result of the higher number of plants per unit area, but this increase favors intraspecific competition between plants and negatively affects seed formation, and, therefore, a higher N rate may be necessary to offset this adverse effect (Tavares et al., 2014).

Despite the availability of studies on the effects of sowing density (Cardoso et al., 2021; Marinho et al., 2021) or N rate (Souza et al., 2021; Bazzo et al., 2021) on the physiological quality of wheat seeds, there are no scientific studies on the interaction between these two factors influencing the quality of wheat seeds under subtropical conditions in Brazil. Thus, the authors hypothesized that there is an optimal combination between sowing density and N rate for each wheat genotype in each edaphoclimatic environment.

In this context, Grimes et al. (2019) worked with chia (*Salvia hispanica* L.) and observed that the crop fertilization with 20 to 40 $kg.ha^{-1}$ N combined with the sowing density of 1.5 $kg.ha^{-1}$ seeds improved the yield and quality of the harvested seeds. This result indicates that the definition of the best combination between N rate and sowing density is important to guide the seed farmers – specialized in producing commercial seeds – to obtain a product of better quality and yield.

This study aimed to evaluate the yield and physiological quality of wheat seeds as a function of sowing density and N rate, intending to achieve the optimal combination of these agronomic practices for two wheat genotypes in three edaphoclimatic conditions in southern Brazil.

MATERIAL AND METHODS

The experiment was carried out in the 2018 growing season in three edaphoclimatic environments in the state of Paraná, southern Brazil: Londrina, Cascavel, and Ponta Grossa, located in the Homogeneous Regions of Adaptation of Wheat Cultivars (*Regiões Homogêneas de Adaptação de Cultivares de Trigo* – RHACT) III, II, and I, respectively, according to the Brazilian Commission of Wheat and Triticale Research [*Comissão Brasileira de Pesquisa de Trigo e Triticale* (CBPTT, 2018)].

In Londrina, the experiment was set up at the experimental farm of *Embrapa Soja* (23°11'34.6" S and 51°10'39.0" W; altitude of 598 m). The landscape is slightly undulated, and the soil of the experimental area is a clayey Rhodic Eutrudox, according to the USDA Soil Taxonomy (Soil Survey Staff, 2014), or *Latossolo Vermelho eutroférrico* (according to the Brazilian Soil Classification System). The regional climate, according to the Köppen classification (Köppen, 1931), is humid subtropical (Cfa), with warm and rainy summer, average temperature of 21.2 °C, average annual rainfall of 1438 mm, infrequent frosts, and no defined drought season.

In Cascavel, the experiment was carried out in the experimental area of the *Centro Universitário Fundação Assis Gurgacz* (24°56'23.6" S and 53°30'44.2" W; altitude of 695 m). The landscape is slightly undulated, and the soil of the experimental area is a clayey Rhodic Hapludox or *Latossolo Vermelho distroférrico*. The regional climate is humid subtropical (Cfa), with an average temperature of 18.2 °C and average annual rainfall of 1822 mm.

In Ponta Grossa, the experiment was established on another experimental farm of *Embrapa Soja* (25°08'53.2" S and 50°04'40.4" W; altitude of 884 m). The landscape is slightly undulated, and the soil of the experimental area is a clayey Rhodic Hapludox or *Latossolo Vermelho distroférrico*. The regional climate is mesothermal humid subtropical (Cfb), with an average temperature of 17.5 °C and average annual rainfall of 1500 mm, with well-distributed rainfall and frequent frosts.

Figure 1 shows the meteorological data recorded by weather stations (located less than 800 m from the experimental plots) during the wheat crop cycle. Soil samples from the experimental areas were collected in the 0–10 and 10–20 cm soil layers, before the establishment of the experiment, for chemical and physical analyses (Table 1).

The trials were carried out in a randomized block design, with four replications, in a 2 × 3 × 4 factorial arrangement composed of two wheat genotypes (BRS Anambé and WT 15-025), three sowing densities (250, 350, and 450 seeds.m^{-2}), and four N rates (0, 40, 80, and 120 kg.ha^{-1}) applied in topdressing at the beginning of the plant tillering stage. Sowing densities were based on technical recommendations for wheat crops in the state of Paraná (Foloni et al., 2016), and the number of viable seeds per square meter was calculated considering the laboratory analysis of seed germination (Londrina: BRS Anambé = 97% and WT 15-025 = 98%; Cascavel: BRS Anambé = 96% and WT 15-025 = 97%; Ponta Grossa:

Table 1. Chemical and physical characterization of the soils (layers of 0–10 and 10–20 cm) from the experimental areas of Londrina, Cascavel, and Ponta Grossa.

Layer	pH(1)	SOC(2)	P(3)	K(3)	Al(4)	Ca(4)	Mg(4)	H+Al(5)	CEC(6)	V(7)	Dsoil(8)	Clay	Silt	Sand
cm		g.dm^{-3}	-- mg.dm^{-3} --		---------------- cmol$_c$.dm^{-3} ----------------					%	g.cm^{-3}	--------- g.kg^{-1} ---------		
							Londrina							
0–10	5.2	15.3	36.2	227	0.00	3.65	2.22	4.82	11.27	57.2	1.32	732	161	107
10–20	5.2	15.0	31.3	257	0.01	3.78	2.28	4.98	11.70	57.3	1.31	762	146	92
							Cascavel							
0–10	4.8	30.5	24.7	124	0.19	5.21	1.46	5.66	12.49	54.7	1.12	655	220	125
10–20	4.6	27.4	16.6	94	0.32	4.17	1.18	5.90	11.48	48.7	1.10	690	204	106
							Ponta Grossa							
0–10	4.6	23.9	11.2	121	0.16	2.84	0.99	5.72	9.85	42.0	1.29	526	77	397
10–20	4.8	22.8	4.7	133	0.06	3.03	1.12	5.59	10.09	45.0	1.10	570	65	365

(1)pH in 0.01 mol.L^{-1} $CaCl_2$ (soil:solution ratio = 1:2.5). (2)Soil organic carbon (wet combustion – Walkley-Black). (3)Available phosphorus and exchangeable potassium (Mehlich-1 extractant). (4)Exchangeable aluminum, calcium, and magnesium (1 mol.L^{-1} KCl extractant). (5) Potential acidity (0.5 mol.L^{-1} calcium acetate extractant at pH 7.0). (6)Cation exchange capacity. (7)Base saturation. (8) Soil bulk density.

BRS Anambé = 94% and WT 15-025 = 95%), which was performed in the week before sowing in each environment. The seeds were provided by the wheat breeder, presenting a high degree of genetic purity, coming from the same production area and stored in a cold chamber (7–10 °C) until sowing. Nitrogen rates were based on technical recommendations for wheat crops in the state of Paraná, which consider the results of soil chemical analyses and the expected grain yield for different scenarios of environmental conditions (Foloni et al., 2016; CBPTT, 2018).

The genotype BRS Anambé has a medium cycle (from seed emergence to grain ripening) estimated at 120, 126, and 135 days in the RHACT III, II, and I, respectively, an estimated average height of 74 cm, and stands out as a wheat cultivar with high resistance to pre-harvest sprouting (unpublished data). In turn, WT 15-025 also has a medium cycle, estimated at 127, 134, and 140 days in RHACTs III, II, and I, respectively, an estimated average height of 76 cm, and its grains are classified as 'soft wheat', which are suitable for the production of cookies (unpublished data).

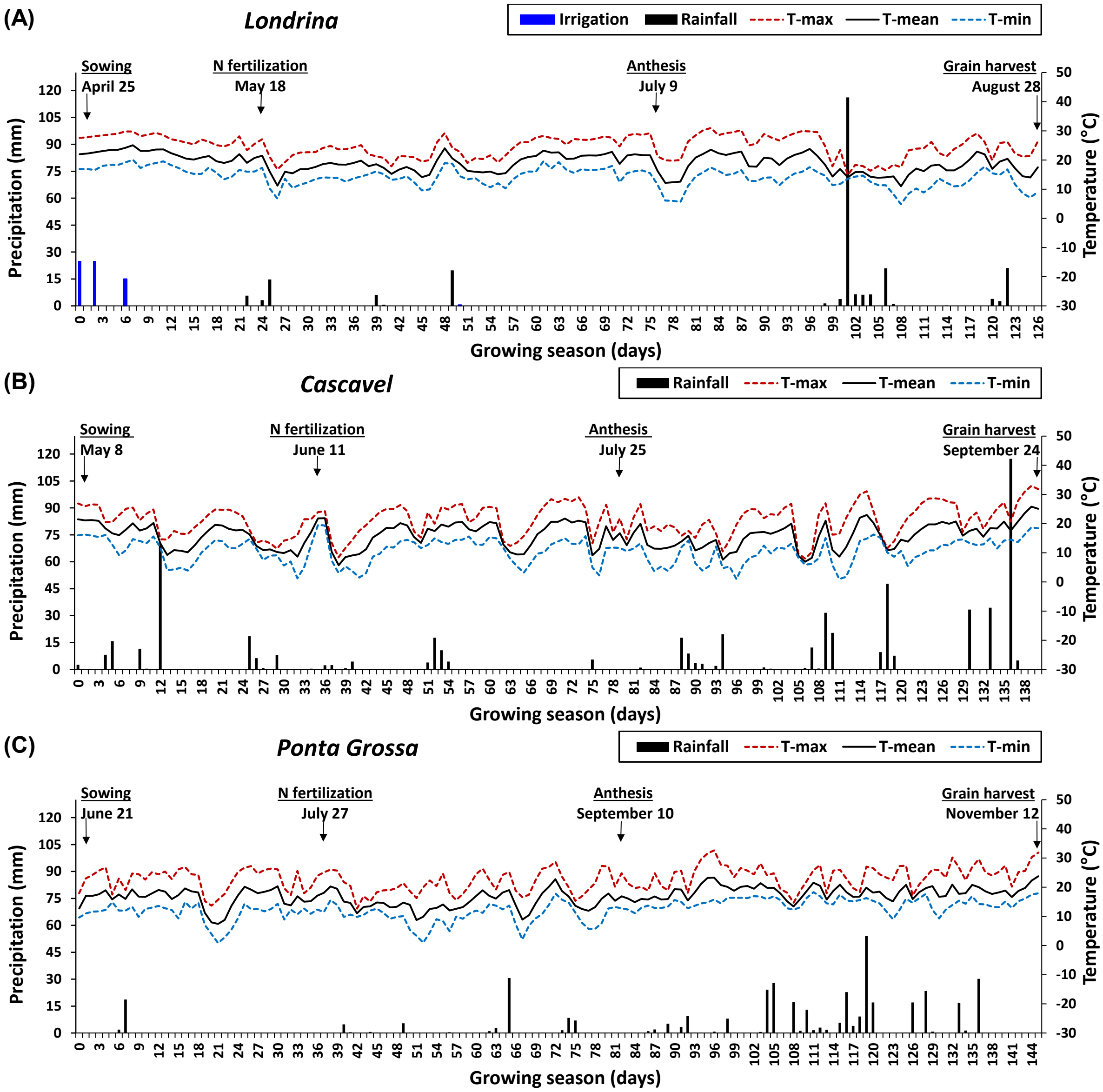


Figure 1. Daily averages of rainfall and maximum (T-max), mean (T-mean), and minimum (T-min) temperatures during the 2018 wheat growing season in the experimental areas of Londrina (A), Cascavel (B), and Ponta Grossa (C) environments. The vertical blue bars indicate irrigations performed on the eve of sowing (25 mm), and on day 2 (25 mm) and day 6 (15 mm) of the growing season in Londrina (chart 'A').

The experimental plot consisted of nine 6-m long sowing rows, with an inter-row spacing of 0.178 m, totaling 9.6 m^2. Wheat was sown on soybean (*Glycine max* L.) straw (Londrina = April 25, Cascavel = May 8, and Ponta Grossa = June 21) in furrows of ± 4 cm depth, using a no-tillage system. The base fertilization applied in the sowing furrow consisted of 100 $kg.ha^{-1}$ of the formulation 00–20–30 ($N–P_2O_5–K_2O$), which is enough to supply the wheat plants with phosphorus (P) and potassium (K) in the three environments, according to indications of the CBPTT (2018). Nitrogen was not added in the base fertilization because the wheat sowing took place in an area covered with soybean straw (with reasonable N content), which has been able to provide N to the wheat plants during their initial growth stages in the three environments. The treatments with N fertilization were carried out in topdressing by applying ammonium nitrate at the beginning of the plant tillering, which occurred approximately 24, 35, and 37 days after wheat sowing in Londrina (May 18), Cascavel (June 11), and Ponta Grossa (July 27), respectively.

In the experiment of Londrina, water supplementation was performed through a self-propelled sprinkler irrigation system, with three irrigations: one before wheat sowing, with 25 mm, and two applications during the seedling establishment, the first with 25 mm and the second with 15 mm (Figure 1A). The phytosanitary management and other cultural practices were based on technical recommendations for wheat crops (CBPTT, 2018).

The experimental plots (7 central rows × 6 m in length, totaling 7.5 m^2) were harvested at the ripening stage to determine seed yield (Londrina = August 28, Cascavel = September 24, and Ponta Grossa = November 12). The harvest was performed using a self-propelled combine developed for small plots of cereal crops (Wintersteiger®). Seed moisture content was evaluated, recorded, and adjusted to 13% for yield calculation. For this purpose, a grain moisture analyzer was used, and the following values (mean ± standard deviation) were obtained: Londrina – BRS Anambé = 15.15 ± 0.42, and WT 15-025 = 15.15 ± 0.55; Cascavel – BRS Anambé = 16.09 ± 0.39, and WT 15-025 = 15.80 ± 0.39; and Ponta Grossa – BRS Anambé = 13.05 ± 0.49, and WT 15-025 = 12.99 ± 0.46.

Immediately after harvesting each experimental plot, 2 kg of seeds were sampled for further analyses of seed physiological quality. The samples were maintained in a cold chamber (7–10 °C) for approximately three months. After that, the following laboratory analyses were performed:

First germination count and final seed germination: this test was performed with eight subsamples of 50 seeds per treatment, distributed on germination paper moistened with distilled water at a proportion of 2.5 times the dry matter of the substrate. The paper rolls with the seeds were maintained in a germinator at a constant temperature of 20 °C. The evaluation consisted of two counts, the first at four days (first count) and the second at eight days after sowing, counting the percentage of normal seedlings (Brasil, 2009).

Seedling emergence in the sand: this test was conducted using four replications of 50 seeds, which were sown at approximately 4 cm depth in boxes containing sand. The number of seedlings that emerged with normal shoot development was counted after 15 days of sowing, and the result was expressed as a percentage.

Emergence speed index (ESI): this test was carried out together with the seedling emergence test, counting the emerged seedlings daily, without discarding them, and obtaining a cumulative value. Thus, the number of emerged seedlings regarding each counting allowed the calculation of the ESI according to Maguire (1962).

Seedling length: obtained by sowing four replications of 20 seeds per treatment on the upper third of the germination paper, which was moistened with distilled water at a proportion of 2.5 times the dry matter of the substrate. The paper rolls were placed in a germinator for five days at 20 °C in the dark. At the end of this period, the length of each normal seedling was measured using a graduated ruler. The results were expressed in $cm.seedling^{-1}$.

Seedling dry matter: the normal seedlings, obtained in the previous test of seedling length, were dried in an oven with forced air circulation at 80 °C for 48 h. Then, the dry matter was measured using an analytical balance with a precision of 0.001 g, and the results were expressed in $mg.seedling^{-1}$.

The experimental data were analyzed individually for each environment, using the R software (R Core Team, 2020). The data were subjected to the tests of normality (Shapiro-Wilk) and homogeneity of variance (Bartlett), and then to the analysis of variance (ANOVA). The means of the 'genotypes' were compared by the F-test ($p \leq 0.05$), the 'sowing densities' were compared by the Tukey's test ($p \leq 0.05$), and the means of the quantitative factor 'N rates' were subjected to linear regression analysis to adjust the linear, quadratic, and quadratic base of square root models that biologically best fit the experimental results.

RESULTS AND DISCUSSION

Weather conditions influenced wheat yield in the three environments (Figure 1 and Table 2). In Londrina, the crop cycle lasted 126 days, with an average temperature of 19 °C and accumulated precipitation (rainfall + irrigation) of 282 mm, without the occurrence of frost (i.e. temperature <3 °C inside the weather shelter) (Figure 1A). The grain filling stage (51 days from anthesis to harvest) received 190 mm of rainfall, with 27.7 mm (i.e. three consecutive rainfalls of 3.9, 2.7, and 21.1 mm) in the last 15 days of the cycle. In Cascavel, the 140-day cycle had an average temperature of

Table 2. Summary of the analysis of variance (ANOVA) and means of seed yield of two wheat genotypes grown in three environments (Londrina, Cascavel, and Ponta Grossa) with three sowing densities and four nitrogen (N) fertilization rates.

Source of variation	DF	Mean square		
		Seed yield		
		Londrina	Cascavel	Ponta Grossa
Block	3	2064163	1236391	918911
Genotype (G)	1	1811^{ns}	536936*	554691*
Sowing density (D)	2	895677***	7749^{ns}	734052**
N rate (N)	3	208521^{ns}	118789^{ns}	424599*
G × D	2	439186**	485802**	220571^{ns}
G × N	3	204819^{ns}	224315^{ns}	163717^{ns}
D × N	6	41106^{ns}	84756^{ns}	117543^{ns}
G × D × N	6	163788^{ns}	89594^{ns}	142215^{ns}
Error	69	86907	84642	110763
CV (%)		12.9	6.3	16.2
Factor	Treatment	Seed yield (kg.ha^{-1})		
		Londrina	Cascavel	Ponta Grossa
Genotype	BRS Anambé	2298	4709	1981 b
	WT 15-025	2289	4559	2134 a
Sowing density	250 seeds.m^{-2}	2112	4625	1916 b
	350 seeds.m^{-2}	2330	4653	2039 ab
	450 seeds.m^{-2}	2441	4625	2217 a
N rate	0 kg.ha^{-1}	2287	4647	1877
	40 kg.ha^{-1}	2309	4716	2040
	80 kg.ha^{-1}	2403	4545	2137
	120 kg.ha^{-1}	2176	4628	2176

DF = degrees of freedom. CV = coefficient of variation. ns, *, **, and *** = not significant, and significant at the $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively, by the F-test. Means followed by different lowercase letters in the column for the factors 'genotype' or 'sowing density' differ from each other by the F-test (according to the ANOVA) or Tukey's test ($p \leq 0.05$), respectively. Note: when significant, the effect of 'N rate' was assessed by regression analysis in Figure 2. Comparisons with significant interaction between the factors 'genotype' and 'sowing density' have additional interpretations in Table 3.

15.9 °C, accumulated rainfall of 573 mm and the occurrence of five frosts (without damage to the wheat plants), and the grain filling stage (62 days of duration) received 379 mm of rainfall concentrated in four events (33.4, 34.4, 117.4, and 5 mm) in the last 15 days of the cycle (Figure 1B). Finally, the wheat crop in Ponta Grossa had a cycle of 145 days, with an average temperature of 17 °C, accumulated rainfall of 409 mm and four frosts (until the 67th day of the cycle, without damage to the plants), and the grain filling stage (64 days of duration) received 324 mm of rainfall, of which only two rainfalls (16.8 and 30.2 mm) in the last 15 days of the cycle (Figure 1C).

The seed moisture at its physiological maturation is around 35%, when the seed becomes progressively susceptible to germination in the spike (i.e. pre-harvest sprouting) due to the activation of the α-amylase enzyme (around 24% moisture), which depends on the tolerance of each wheat genetic material. Considering that this 'seed maturation moisture' takes place a few days or weeks before the 'seed harvest moisture' (i.e. < 20%; ideal in the range of 13–16%), the rainfall at the end of the crop cycle may have affected in a distinct manner the seed quality of the wheat genotypes in the three environments, mainly in Cascavel (with higher intensity and number of rainfalls in the last 15 days of the cycle). However, no visual signal of germination was identified in the harvested seeds, which allows us to infer that these rainfalls at the end of the crop cycle likely did not significantly affect the wheat seed quality. According to Cunha et al. (2001), based on a study that supports the current Agricultural Zoning of Climate Risk for the wheat crop, the risk of damage to wheat grains due to excessive pre-harvest rainfall is defined considering the following weather conditions (singly or in combination) actually performed in the last 15 days of the crop cycle: i) accumulated rainfall of 75–150 mm combined with at least 10 days of rainfall, and ii) accumulated rainfall higher than 150 mm combined with a minimum of five days with rainfall. This information reinforces that Cascavel was the environment most likely to have caused damage to the wheat seeds. However, the seeds of BRS Anambé were likely not affected by the rainfall, as this cultivar has high genetic resistance to pre-harvest sprouting. This fact partially explains the results obtained in the present study that demonstrated the better physiological quality of the BRS Anambé seeds compared to WT 15-025.

Soil was another environmental factor that influenced seed yield. Considering the availability of nutrients in the soil (Table 1) as well as those provided by the base fertilization [100 $kg.ha^{-1}$ of 00–20–30 ($N–P_2O_5–K_2O$)], it is possible to infer that there was a suitable supply of P, K, calcium (Ca), and magnesium (Mg) for wheat plant nutrition in all environments. On the other hand, soil acidity may have influenced the nutrient availability and plant root growth. Soil pH was between 4.6 and 5.2 (medium acidity level) in the three environments and at both soil depths, minimizing N losses by volatilization, which is higher in the pH range from neutral to alkaline (Ferreira et al., 2021). The concentrations of Al^{3+} in the soil were low (< 0.32 $cmol_c.dm^{-3}$), and the potential acidity (H+Al) was medium (between 2.5 and 5.0 $cmol_c.dm^{-3}$), not representing a significant hindering for root growth of the two wheat genotypes, which were selected by a genetic improvement program that considers the plant tolerance to soil acidity as one of the main selection criteria (M.C. Bassoi, personal communication).

Seed yield was influenced by the 'genotype × sowing density' interaction in Londrina and Cascavel (Table 2). On the other hand, the three studied factors (genotype, sowing density, and N rate) individually influenced seed yield in Ponta Grossa.

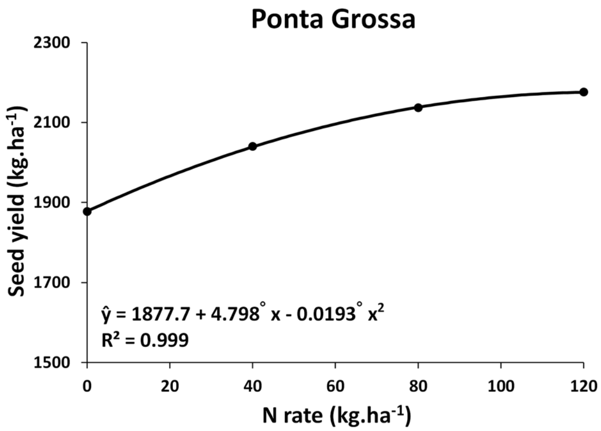
In Londrina, the seed yield of BRS Anambé was 9.4% higher than that of WT 15-025 within the treatment with 350 $seeds.m^{-2}$ (Table 3). On the other hand, WT 15-025 yielded 10.8% more than the other genotype within the sowing density of 450 $seeds.m^{-2}$. The isolated effect of 'sowing density' for the WT 15-025 genotype provided an average increase of 19.2% in seed yield using 450 $seeds.m^{-2}$ as compared with the other two sowing densities. Cardoso et al. (2021) also found an increase in wheat seed yield as a function of increasing sowing density until approximately 450 $seeds.m^{-2}$.

In Cascavel, seed yield was influenced by the 'genotype' factor at the lowest and the highest sowing densities (i.e. 250 and 450 $seeds.m^{-2}$) in which BRS Anambé yielded 6.4% more seeds than WT 15-025 (Table 3). Regarding the effect of 'sowing density', the seed yield of WT 15-025 was 5.9% higher in the treatment with 350 $seeds.m^{-2}$ compared to 450 $seeds.m^{-2}$.

Table 3. Decomposing of the analysis of variance (ANOVA) for the significant interaction between the factors 'sowing density' (250, 350, and 450 seeds.m^{-2}) and 'genotype' (BRS Anambé and WT 15-025) and comparisons between treatment means for wheat seed yield in Londrina and Cascavel environments.

Sowing density	Seed yield (kg.ha^{-1})			
	Londrina		Cascavel	
	BRS Anambé	WT 15-025	BRS Anambé	WT 15-025
250 seeds.m^{-2}	2145 bA	2078 bA	4741 aA	4509 abB
350 seeds.m^{-2}	2434 aA	2225 bB	4589 aA	4716 aA
450 seeds.m^{-2}	2316 abB	2565 aA	4797 aA	4453 bB

Means followed by different lowercase letters in the column and uppercase letters in the row (individually for each environment) differ from each other by the Tukey's test ($p \leq 0.05$) or F-test ($p \leq 0.05$), respectively.



° = significance of the regression model coefficients by the F-test at the $p \leq 0.10$.

Figure 2. Wheat seed yield as affected by nitrogen (N) fertilization rates in Ponta Grossa environment.

In Ponta Grossa, the seed yield of WT 15-025 was 7.7% higher than that of BRS Anambé (Table 2). Furthermore, the sowing of 450 seeds.m^{-2} increased seed yield by 15.7% when compared to the lowest sowing density (250 seeds.m^{-2}). Finally, a quadratic seed yield increase was observed by increasing the N rate, achieving an average rate of 2.48 kg.kg^{-1} (seeds/N) and a maximum yield of 2176 kg.ha^{-1} (at the estimated N rate of 124 kg.ha^{-1}) (Figure 2). Considering that the maximum economic efficiency is generally obtained by farmers with agronomic practices that provide 90% of the maximum seed yield, it is estimated that 18 kg.ha^{-1} N would be needed to achieve it. These results reinforce that suitable N availability is essential to increase seed yield and, therefore, N fertilization must be properly managed for wheat crop feasibility (Bazzo et al., 2016; Bazzo et al., 2021; Ferreira et al, 2021).

Alves et al. (2017) studied N fertilization in wheat crop and observed that N rates up to 120 kg.ha^{-1} promoted an increase in grain yield, corroborating the current results achieved in Ponta Grossa. On the other hand, wheat crops did not change grain yield in response to N fertilization in the environments of Londrina and Cascavel. This lack of response to N rates in these two environments was not expected and can be partially attributed to the higher temperatures experienced during the crop season as compared to that of Ponta Grossa. The microbial activity increases as the temperature rises (up to a certain limit), which promotes higher soil organic matter mineralization and soybean straw decomposition, providing higher amounts of mineral N (nitrate and ammonium) for plant uptake throughout the wheat crop cycle (Ferreira et al., 2021; Souza et al., 2021). In this context, in addition to seed yield, other aspects such as seed quality must be considered to define the best N fertilization rate, aiming to achieve maximum economic efficiency and a suitable N use efficiency by the wheat plants, avoiding N losses and environmental contamination caused by excess nitrate in aquifers (Bouchet et al., 2016; Mahmud et al., 2021).

The first germination count was influenced by the ‘genotype’ factor in all environments (Table 4). In Cascavel, there was also an effect of ‘sowing density’. In all evaluated environments, BRS Anambé produced seeds with higher germination in the first count, with an average increase of 6.6 percentage points compared to WT 15-025. In Cascavel, the increase in sowing density resulted in the production of seeds with higher germination in the first count, with an increase of 4 percentage points from 250 to 450 seeds.m^{-2}.

Table 4. Summary of the analysis of variance (ANOVA) and means of first germination count and final seed germination of two wheat genotypes grown in three environments (Londrina, Cascavel, and Ponta Grossa) with three sowing densities and four nitrogen (N) fertilization rates.

Source of variation	DF	Mean square					
		First germination count			Final seed germination		
		Londrina	Cascavel	Ponta Grossa	Londrina	Cascavel	Ponta Grossa
Block	3	62.9	5.71	83.6	18.0	12.8	9.34
Genotype (G)	1	2420***	651***	368***	689***	360***	173***
Sowing density (D)	2	31.8^{ns}	75.3*	66.8^{ns}	5.93^{ns}	18.3^{ns}	83.2**
N rate (N)	3	4.60^{ns}	31.6^{ns}	24.5^{ns}	11.4^{ns}	18.2^{ns}	5.57^{ns}
G × D	2	47.5^{ns}	7.29^{ns}	14.0^{ns}	22.6^{ns}	3.88^{ns}	46.2*
G × N	3	65.2^{ns}	36.2^{ns}	9.61^{ns}	1.26^{ns}	17.5^{ns}	5.51^{ns}
D × N	6	28.0^{ns}	18.2^{ns}	12.8^{ns}	21.4^{ns}	4.07^{ns}	16.8^{ns}
G × D × N	6	39.7^{ns}	37.7^{ns}	29.5^{ns}	11.9^{ns}	10.7^{ns}	18.7^{ns}
Error	69	42.2	20.2	28.7	23.1	9.83	13.2
CV (%)		7.5	5.0	7.3	5.2	3.3	4.0

Factor	Treatment	First germination count (%)			Final seed germination (%)		
		Londrina	Cascavel	Ponta Grossa	Londrina	Cascavel	Ponta Grossa
Genotype	BRS Anambé	91 a	93 a	75 a	95 a	96 a	92
	WT 15-025	81 b	87 b	71 b	89 b	92 b	89
Sowing density	250 seeds.m^{-2}	86	88 b	72	92	94	91
	350 seeds.m^{-2}	87	90 ab	73	92	94	89
	450 seeds.m^{-2}	85	92 a	75	92	95	92
N rate	0 kg.ha^{-1}	86	90	73	92	95	90
	40 kg.ha^{-1}	87	92	74	93	95	91
	80 kg.ha^{-1}	86	89	74	92	93	90
	120 kg.ha^{-1}	87	90	72	91	94	91

DF = degrees of freedom. CV = coefficient of variation. ns, *, **, and *** = not significant, and significant at the $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively, by the F-test. Means followed by different lowercase letters in the column for the factors ‘genotype’ or ‘sowing density’ differ from each other by the F-test (according to the ANOVA) or Tukey’s test ($p \leq 0.05$), respectively. Comparisons with significant interaction between the factors ‘genotype’ and ‘sowing density’ have additional interpretations in Table 5.

The final germination of the seeds produced in Ponta Grossa was influenced by the ‘genotype × sowing density’ interaction (Table 4). In this environment, a significant difference was observed between genotypes only within the densities of 250 and 350 seeds.m^{-2}, in which the mean germination of BRS Anambé seeds was 4.5 percentage points higher than that of WT 15-025 (Table 5). Comparing sowing densities, WT 15-025 produced seeds with higher germination (92%) when sown with 450 seeds.m^{-2} compared to the other two densities (average of 87.5%) (Table 5). The germination of the BRS Anambé seeds harvested in Londrina and Cascavel was on average 5 percentage points higher than that of WT 15-025 (Table 4). It is important to highlight that the seeds produced in all treatments showed a high germination rate, i.e. germination above 80%, which is the minimum value established for wheat seed trade in Brazil (Brasil, 2013), reinforcing the suitable adaptation of these two genotypes to the three RHACTs.

Seedling emergence in the sand was influenced by ‘genotype’ in all environments (Table 6). In Cascavel, there was also an effect of ‘N rate’. Thus, BRS Anambé seeds had higher seedling emergence than WT 15-025, with an average increase of 6.3 percentage points (Table 6). In Cascavel, seedling emergence increased in response to the N rate increase, with a maximum value (94.8% of emergence) at the estimated N rate of 21 kg.ha^{-1} (Figure 3A).

Table 5. Decomposing of the analysis of variance (ANOVA) for the significant interaction between the factors ‘sowing density’ (250, 350, and 450 seeds.m^{-2}) and ‘genotype’ (BRS Anambé and WT 15-025) and comparisons between treatment means for the final germination of wheat seeds harvested in Ponta Grossa environment.

Sowing density	Final seed germination (%)	
	BRS Anambé	WT 15-025
250 seeds.m^{-2}	93 aA	88 bB
350 seeds.m^{-2}	91 aA	87 bB
450 seeds.m^{-2}	92 aA	92 aA

Means followed by different lowercase letters in the column and uppercase letters in the row differ from each other by the Tukey’s test (p ≤ 0.05) or F-test (p ≤ 0.05), respectively.

Table 6. Summary of the analysis of variance (ANOVA) and means of seedling emergence and emergence speed index of seedlings from wheat seeds of two genotypes grown in three environments (Londrina, Cascavel, and Ponta Grossa) with three sowing densities and four nitrogen (N) fertilization rates.

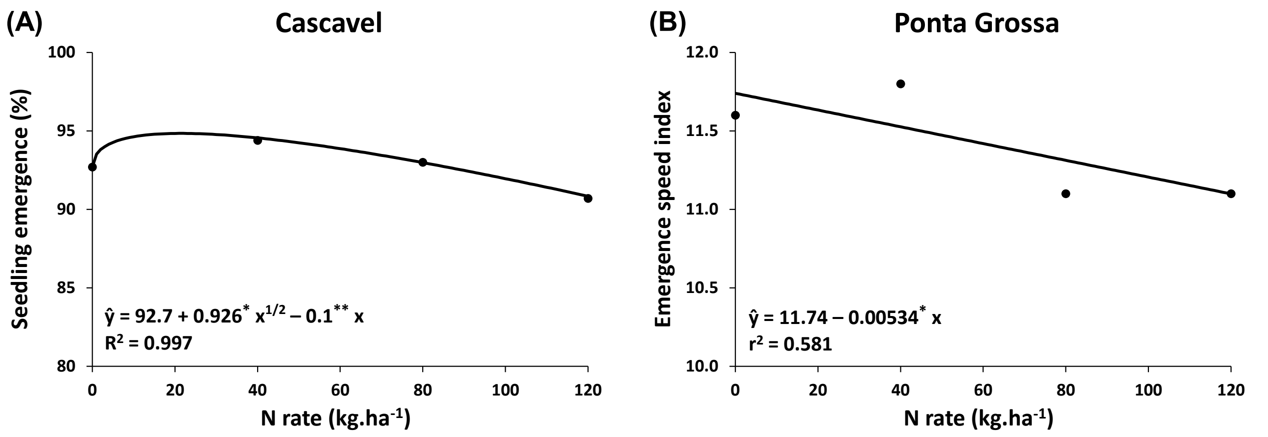
Source of variation	DF	Mean square					
		Seedling emergence			Emergence speed index		
		Londrina	Cascavel	Ponta Grossa	Londrina	Cascavel	Ponta Grossa
Block	3	15.6	81.5	144	2.49	0.89	5.27
Genotype (G)	1	2709***	513***	124*	105***	25.3***	19.2***
Sowing density (D)	2	7.95^{ns}	27.1^{ns}	2.09^{ns}	0.58^{ns}	0.48^{ns}	0.40^{ns}
N rate (N)	3	13.0^{ns}	57.4*	54.3^{ns}	1.02^{ns}	1.45^{ns}	3.14*
G × D	2	8.09^{ns}	12.1^{ns}	29.4^{ns}	0.59^{ns}	0.33^{ns}	2.59^{ns}
G × N	3	47.1^{ns}	29.2^{ns}	3.93^{ns}	0.43^{ns}	0.95^{ns}	0.13^{ns}
D × N	6	37.6^{ns}	29.3^{ns}	26.9^{ns}	0.68^{ns}	0.99^{ns}	1.26^{ns}
G × D × N	6	9.47^{ns}	14.4^{ns}	29.4^{ns}	0.53^{ns}	0.46^{ns}	0.78^{ns}
Error	69	27.6	17.7	24.7	1.26	0.87	1.07
CV (%)		6.2	4.6	5.6	10.1	8.3	9.0

Continue...

Table 6. Continuation.

Factor	Treatment	Seedling emergence (%)			Emergence speed index		
		Londrina	Cascavel	Ponta Grossa	Londrina	Cascavel	Ponta Grossa
Genotype	BRS Anambé	91 a	95 a	90 a	12.2 a	11.8 a	11.9 a
	WT 15-025	80 b	90 b	87 b	10.1 b	10.8 b	11.0 b
Sowing density	250 seeds.m^{-2}	85	92	89	11.2	11.2	11.5
	350 seeds.m^{-2}	86	93	88	11.2	11.4	11.4
	450 seeds.m^{-2}	85	93	88	11.0	11.4	11.3
N rate	0 kg.ha^{-1}	85	93	89	11.2	11.3	11.6
	40 kg.ha^{-1}	86	94	90	11.1	11.6	11.8
	80 kg.ha^{-1}	85	93	87	10.9	11.4	11.1
	120 kg.ha^{-1}	85	91	87	11.4	11.0	11.1

DF = degrees of freedom. CV = coefficient of variation. ns, *, **, and *** = not significant, and significant at the $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively, by the F test. Means followed by different lowercase letters in the column for the factors ‘genotype’ or ‘sowing density’ differ from each other by the F-test (according to the ANOVA) or Tukey’s test ($p \leq 0.05$), respectively. Note: when significant, the effect of ‘N rate’ was assessed by regression analysis in Figure 3.



* and ** = significance of the regression model coefficients by the F-test at the $p \leq 0.05$ and $p \leq 0.01$, respectively.

Figure 3. Seedling emergence (A) and emergence speed index of seedlings (B) from wheat seeds harvested in Cascavel and Ponta Grossa environments, respectively, as affected by nitrogen (N) fertilization rates.

The ESI was influenced by the ‘genotype’ factor in all environments, thus the BRS Anambé seeds had an average ESI increase of 12.6% compared to WT 15-025 (Table 6). Ponta Grossa also showed an effect of ‘N rate’, with a linear decrease in ESI by increasing the N fertilization rate in the wheat crop, but the differences were of low magnitude (Figure 3B).

Seedling length was influenced by ‘genotype’ in all environments, so the BRS Anambé seedlings were on average 10% larger than those of WT 15-025 (Table 7). A quadratic seedling length increase was observed by increasing the N rate in Londrina, achieving the maximum length of 17.3 cm at the estimated N rate of 60 kg.ha^{-1} (Figure 4A).

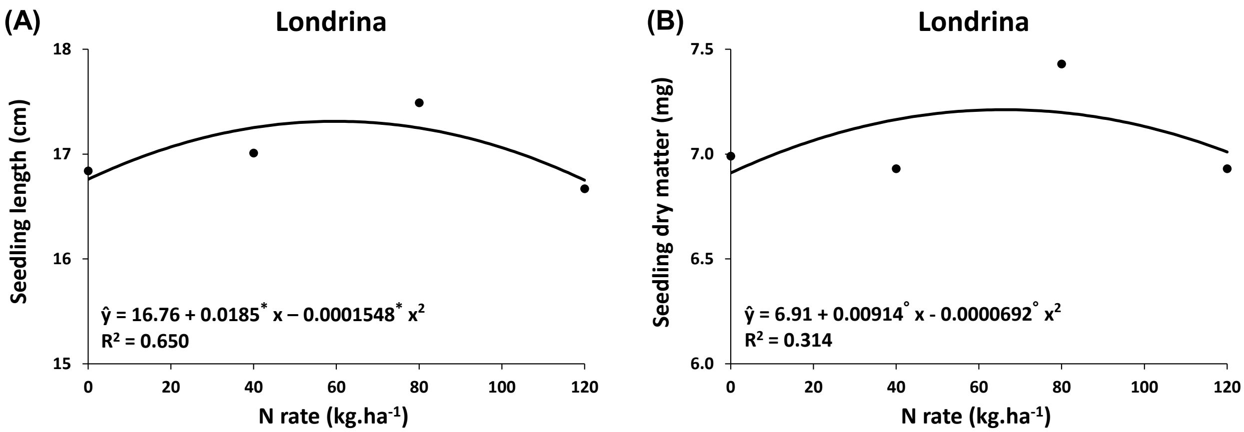
Seedling dry matter was influenced by ‘genotype’ and ‘N rate’ factors in Londrina, and only by ‘genotype’ in Cascavel (Table 7). In these two environments, the seedling dry matter of BRS Anambé was on average 18.4% higher than that of WT 15-025. In Londrina, the seedling dry matter increased by increasing the N rate, with the maximum value of 7.2 mg obtained with 66 kg.ha^{-1} N (Figure 4B). In Ponta Grossa, the seedling dry matter was influenced by the ‘genotype × sowing density' interaction, thus the BRS Anambé seedlings had an average dry matter increase of 19% within the densities of 250 and 450 seeds.m^{-2} compared to WT 15-025 (Table 8). Furthermore, the seedling dry matter of BRS Anambé was 12.1% higher in the treatment with 450 seeds.m^{-2} as compared to the average of the other sowing densities.

The previous results demonstrate that the seeds produced by BRS Anambé showed better physiological quality when compared to WT 15-025. Thus, BRS Anambé was more effective in producing commercial seeds in the three environments, showing its better adaptability to different environmental conditions. According to Gonçalves et al. (2020), the choice of genotypes with higher adaptability to each environment is important to mitigate the possible negative effects resulting from the genotype × environment interaction, thus providing the production of better quality seeds combined with a higher yield.

Table 7. Summary of the analysis of variance (ANOVA) and means of seedling length and seedling dry matter from wheat seeds of two genotypes grown in three environments (Londrina, Cascavel, and Ponta Grossa) with three sowing densities and four nitrogen (N) fertilization rates.

Source of variation	DF	Mean square					
		Seedling length			Seedling dry matter		
		Londrina	Cascavel	Ponta Grossa	Londrina	Cascavel	Ponta Grossa
Block	3	1.24	25.61	0.16	0.19	5.31	0.48
Genotype (G)	1	55.1***	83.4**	87.5***	29.5***	54.7***	37.3***
Sowing density (D)	2	0.31^{ns}	5.40^{ns}	0.49^{ns}	0.16^{ns}	0.49^{ns}	2.55^{ns}
N rate (N)	3	3.02*	1.49^{ns}	0.22^{ns}	1.39*	0.49^{ns}	1.20^{ns}
G × D	2	1.78^{ns}	5.69^{ns}	2.94^{ns}	0.42^{ns}	1.09^{ns}	4.79*
G × N	3	1.57^{ns}	0.38^{ns}	0.60^{ns}	0.23^{ns}	0.29^{ns}	0.56^{ns}
D × N	6	1.83^{ns}	0.27^{ns}	2.09^{ns}	0.45^{ns}	0.53^{ns}	1.11^{ns}
G × D × N	6	0.72^{ns}	1.42^{ns}	1.92^{ns}	0.28^{ns}	0.19^{ns}	0.84^{ns}
Error	69	1.07	2.44	1.69	0.36	0.52	1.20
CV (%)		11.8	13.6	11.9	34.7	28.8	29.6
Factor	Treatment	Seedling length (cm)			Seedling dry matter (mg)		
		Londrina	Cascavel	Ponta Grossa	Londrina	Cascavel	Ponta Grossa
Genotype	BRS Anambé	17.76 a	18.93 a	21.57 a	7.62 a	9.27 a	9.56
	WT 15-025	16.24 b	17.07 b	19.66 b	6.51 b	7.76 b	8.31
Sowing density	250 seeds.m^{-2}	16.98	18.16	20.63	7.03	8.44	8.71
	350 seeds.m^{-2}	16.91	18.31	20.48	7.02	8.66	8.86
	450 seeds.m^{-2}	17.11	17.54	20.73	7.15	8.44	9.25
N rate	0 kg.ha^{-1}	16.84	17.94	20.61	6.99	8.66	8.93
	40 kg.ha^{-1}	17.01	17.68	20.61	6.93	8.50	8.69
	80 kg.ha^{-1}	17.49	18.23	20.50	7.43	8.32	9.23
	120 kg.ha^{-1}	16.67	18.16	20.74	6.93	8.56	8.89

DF = degrees of freedom. CV = coefficient of variation. ns, *, **, and *** = not significant, and significant at the $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively, by the F-test. Means followed by different lowercase letters in the column for the factors 'genotype' or 'sowing density' differ from each other by the F-test (according to the ANOVA) or Tukey's test ($p \leq 0.05$), respectively. Note: when significant, the effect of 'N rate' was assessed by regression analysis in Figure 4. Comparisons with significant interaction between the factors 'genotype' and 'sowing density' have additional interpretations in Table 8.



° and * = significance of the regression model coefficients by the F-test at the $p \leq 0.10$ and $p \leq 0.05$, respectively.

Figure 4. Seedling length (A) and seedling dry matter (B) from wheat seeds harvested in Londrina environment, as affected by nitrogen (N) fertilization rates.

Table 8. Decomposing of the analysis of variance (ANOVA) for the significant interaction between the factors 'sowing density' (250, 350, and 450 seeds.m^{-2}) and 'genotype' (BRS Anambé and WT 15-025) and comparisons between treatment means for the seedling dry matter of wheat seeds harvested in Ponta Grossa environment.

Sowing density	Seedling dry matter (mg)	
	BRS Anambé	WT 15-025
250 seeds.m^{-2}	9.22 bA	8.19 aB
350 seeds.m^{-2}	9.16 bA	8.55 aA
450 seeds.m^{-2}	10.30 aA	8.20 aB

Means followed by different lowercase letters in the column and uppercase letters in the row (individually for each environment) differ from each other by the Tukey's test ($p \leq 0.05$) or F-test ($p \leq 0.05$), respectively.

Regarding the 'sowing density' factor, despite the plasticity of wheat crop in compensating failures or excesses of plants in the stand through plant tillering and yield components (such as number of spikes/area, number of grains/spike, and grain weight), increasing sowing density improved the yield and physiological quality of the seeds produced under several environmental conditions, but the improvements were of a low magnitude. The plant population effectively established in a wheat field is usually below the initial planning because germination, seed vigor, soil and weather conditions, and agronomic practices also directly influence the plant establishment in the field (Marinho et al., 2021). In this context, the use of higher sowing density can promote a more suitable plant stand, favoring the best use of natural resources (water, nutrients, and light), and reducing the incidence of weeds (i.e. lower interspecific competition) due to better canopy development and ground cover. On the other hand, the farmer's option for wheat genotypes with high tillering capacity allows the use of lower sowing density that might provide the same beneficial effects mentioned above, in addition to being economically advantageous due to an expense reduction with seed purchase.

Concerning the 'N rate' factor, the results reinforce the importance of N for seed commercial production since the amount of N taken up by the plant during its cycle has a positive correlation with the physiological quality of the produced seeds (Carvalho and Nakagawa, 2012; Bazzo et al., 2021). However, the results also show that high N rates applied to the crop might impair the physiological quality of the produced seeds, as the excess of N may cause several damages to the plant such as cell toxicity (Elhanafi et al., 2019) and increased lodging (Galetto et al., 2017), which impair seed formation. Bazzo et al. (2018) evaluated the effects of N fertilization on white oat (*Avena sativa* L.) seed production and found that increasing the topdressing N rate worsened some attributes of physiological quality of the produced seeds, which was also observed by Zucareli et al. (2012) in sweet corn (*Zea mays* L.), corroborating the results obtained in the present study. Finally, despite favoring seed yield and some attributes of seed physiological quality in some situations, the N fertilization must not exceed the rate of maximum economic efficiency for feasible seed production with lower costs and reduced environmental impacts.

CONCLUSIONS

The BRS Anambé genotype produced seeds with higher physiological quality in the three environments, and the regional edaphoclimatic conditions favored the expression of its genetic potential for seed production.

The optimal combination between sowing density and N rate that provided the more suitable economic yield, without affecting the physiological quality of wheat seeds, varied with the environment and was estimated in 250 seeds.m^{-2} × 0 kg.ha^{-1} N for Londrina and Cascavel, and 350 seeds.m^{-2} × 18 kg.ha^{-1} N for Ponta Grossa.

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