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Research Article

# Phenotypic response of wheat under application of combinations of herbicides and nitrogen

*Resposta fenotípica do trigo sob aplicação de combinações de herbicidas e nitrogênio*

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## ABSTRACT

The objective of this work was to evaluate the phenotypic response of wheat plants to combinations of herbicides and nitrogen fertilization. The study was carried in the municipality of Augusto Pestana - RS. The experimental design used was randomized blocks, with 19 treatments being evaluated, in 16 replications. The analysis of variance was carried out at 5% probability using the *F* Test. Afterwards, the *Student-Newman-Keuls* mean comparison test was used at the 5% probability level. The main components analysis was carried out using *Biplot*. *Pearson's linear correlation* coefficients were calculated between pairs of characters, with significance imposed by the *Student's* t test at 5% probability. The average Euclidean distance was calculated and grouping was performed using the *UPGMA* method. To express the best assertiveness in wheat yield, regression tree was used, defining grain yield as the dependent variable and the other variables as independent. The application of 1500 ml 2,4-D + 250 ml Clodinafop-propargyl + 100 kg urea reduces spike viability, spike harvest index and spikelet arrangement index in the wheat spike. In wheat, the number of grains in the wheat spike is not influenced by the interaction between herbicides and nitrogen.

**KEYWORDS**: 2,4-D. Grain yield; hormonal. Pearson's linear correlation.

## RESUMO

O objetivo deste trabalho foi avaliar a resposta fenotípica de plantas de trigo às combinações de herbicidas e adubação nitrogenada. O estudo foi realizado no município de Augusto Pestana – RS. O delineamento experimental utilizado foi blocos casualizados, sendo avaliados 19 tratamentos, em 16 repetições. A análise de variância foi realizada a 5% de probabilidade por meio do Teste F. Posteriormente, foi utilizado o teste de comparação de médias de Student-Newman-Keuls ao nível de 5% de probabilidade. A análise dos componentes principais foi realizada utilizando Biplot. Foram calculados os coeficientes de correlação linear de Pearson entre pares de caracteres, com significância imposta pelo teste t de Student a 5% de probabilidade. A distância euclidiana média foi calculada e o agrupamento foi realizado pelo método UPGMA. Para expressar a melhor assertividade na produtividade do trigo, foi utilizada árvore de regressão, definindo a produtividade de grãos como variável dependente e as demais variáveis como independentes. A aplicação de 1500 ml de 2,4-D + 250 ml de Clodinafop-propargil + 100 kg de uréia reduz a viabilidade da espiga, o índice de colheita da espiga e o índice de arranjo de espiguetas na espiga de trigo. No trigo, o número de grãos na espiga de trigo não é influenciado pela interação entre herbicidas e nitrogênio.

**PALAVRAS-CHAVE:** 2,4-D. Produtividade de grãos, hormonal. Correlação linear de pearson.

## INTRODUCTION

In agricultural crops during the periods between summer harvests, several weeds compete with crops for space, water, light and nutrients, consequently reducing the productive potential and grain quality of crops (PEREIRA et al. 2017). For wheat (*Tirticum aestivum*) cultivation, it is one of the most important restrictions in the production system, as competition becomes severe and the accumulation of matter in the cultivated plant is reduced, limiting tillering potential, requiring the use of alternative and effective management to control weeds.

The main way to control weeds in wheat is through the application of post-emergence herbicides. The control of dicotyledonous plants can be carried out using the herbicides 2,4-D and metsulfuron-methyl, and for the control of monocotyledons the herbicides diclofop-methyl, clodinafop-propargyl and iodosulfuron-methyl can be used (KARPINSKI et al. 2018). However, the combined use of herbicides can promote efficient results in weed control, but negative effects on the crop of interest (SHEORAN et al. 2015).

The combination of clodinafop - propargyl and 2,4-D herbicides provides greater weed control, due to their broad spectrum. The herbicide clodinafop – propargyl, inhibits the enzyme acetyl-coA carboxylase (ACCase), and is used to control grasses (*Lolium multiflorum*) in wheat. The 2,4-D herbicide is a mimic of the auxin hormone, also translocated through the phloem and controls dicotyledonous weeds, acts by altering synthesized RNAS and the functioning of respiratory enzymes (PRETTO et al. 2020). KOROTKOVA et al. (2021) observed an increase in wheat grain productivity with the application of herbicides. In wheat, SHEORAN et al. (2015) observed that the application of 60 and 90 g of ai $ha^{-1}$ of clodinafop-propargyl significantly reduced the absorption of NPK by grains and straw, promoting a reduction in agronomic performance.

The use of hormonal herbicides in crops depends entirely on the stage of development the plant is in and generally precedes the application of nitrogen. The application of herbicides at advanced stages of the crop and the interaction with the application of nitrogen can cause morphological changes in plant development (GAZOLA et al. 2021). Furthermore, the use of combined herbicides can cause reflexes that are capable of directly affecting the growth and development of the plant's reproductive structures. Therefore, understanding the dynamics of the association of herbicides and the use of urea provides the development of efficient strategies to maximize weed control and increase the productivity of the crop of interest. In this sense, the objective of this work was to evaluate the phenotypic response of wheat plants to combinations of herbicides and nitrogen fertilization.

## MATERIALS AND METHODS

The study was carried out at the Regional Institute for Rural Development (IRDeR) belonging to the Regional University of the Northwest of the State of Rio Grande do Sul (UNIJUÍ), located in the municipality of Augusto Pestana - RS. The experimental area is geographically located at 28º 2' 30" South latitude and 54º 00' 58" West longitude, at an altitude of approximately 390 meters. The region's climate is classified as humid subtropical Cfa, according to the Köppen-Geiger climate classification.

The experimental design used was randomized blocks, with 19 treatments being evaluated (combinations of herbicides and nutrition) (Table 1), in 16 replications. The experimental units consisted of five sowing rows spaced 0.18 meters and 3 meters in length, totaling one experimental area. Sowing took place out in the first half of June of 2022, with the wheat cultivar TBIO Audaz being sown at a density of 60 seeds per linear meter. Fertilization of 250 kg per hectare of NPK chemical fertilizer formula 5-20-20 was used.

Table 1. Description of treatments applied to wheat crops.

| Treat | Herbicide | Dose* | Herbicide | Dose* | Fertilization | Dose* |
|---|---|---|---|---|---|---|
| 1 | 2,4-D | 500 ml | - | - | - | - |
| 2 | 2,4-D | 500 ml | Metsulfuron-methyl | 8g | - | - |
| 3 | 2,4-D | 500 ml | Clodinafop-propargyl | 250 ml | - | - |
| 4 | 2,4-D | 500 ml | - | - | Urea | 100 kg |
| 5 | 2,4-D | 500 ml | Metsulfuron-methyl | 8g | Urea | 100 kg |
| 6 | 2,4-D | 500 ml | Clodinafop-propargyl | 250 ml | Urea | 100 kg |
| 7 | Absence | - | - | - | - | - |
| 8 | 2,4-D | 1000 ml | - | - | - | - |
| 9 | 2,4-D | 1000 ml | Metsulfuron-methyl | 8g | - | - |
| 10 | 2,4-D | 1000 ml | Clodinafop-propargyl | 250 ml | - | - |
| 11 | 2,4-D | 1000 ml | - | - | Urea | 100 kg |
| 12 | 2,4-D | 1000 ml | Metsulfuron-methyl | 8g | Urea | 100 kg |
| 13 | 2,4-D | 1000 ml | Clodinafop-propargyl | 250 ml | Urea | 100 kg |
| 14 | 2,4-D | 1500 ml | - | - | - | - |
| 15 | 2,4-D | 1500 ml | Metsulfuron-methyl | 8g | - | - |
| 16 | 2,4-D | 1500 ml | Clodinafop-propargyl | 250 ml | - | - |
| 17 | 2,4-D | 1500 ml | - | - | Urea | 100 kg |
| 18 | 2,4-D | 1500 ml | Metsulfuron-methyl | 8g | Urea | 100 kg |
| 19 | 2,4-D | 1500 ml | Clodinafop-propargyl | 250 ml | Urea | 100 kg |

Applications were carried out on July 18, 2022 using a backpack sprayer with a four-nozzle bar, spaced 0.375 centimeters apart, totaling an application range of 1.5 meters. Applications were made at a single time on all cultivars, after the initial stretching, where the first node was visible and the second was noticeable.

The following characters were measured: spike length (SL, in cm); spike weight (SW, in g per plant); grain weight per spike (GWS, in g per plant); number of spikelets per spike (NSS, in number per plant); number of grains per spike (NGS, in number per plant); spike viability (SV), determined through the number of grains per spike under the number of spikelets per spike; spike harvest index (SHI, in g per plant), determined through the grain weight per ear under the spike weight; and spikelet arrangement index in the spike (SAIS, in number per plant), determined by the number of spikelets in the spike along the spike length. Assessments were made by collecting 10 plants per experimental unit.

The results obtained were subjected to the normality of residuals and homogeneity of variances test. The analysis of variance was carried out at 5% probability using the F Test. Afterwards, the *Student-Newman-Keuls* mean comparison test was used at the 5% probability level. The main components analysis was carried out using *Biplot*, which aims to determine which management association behaves best considering all the variables analyzed. Pearson's linear correlation coefficients were calculated between pairs of characters, with significance imposed by the Student's t test at 5% probability. The average Euclidean distance was calculated and grouping was performed using the UPGMA method. To express the best assertiveness in wheat yield, an artificial intelligence method with unsupervised learning called regression tree was used, defining grain yield as the dependent variable and the other variables as independent. The analyzes were carried out using the R software (R CORE TEAM 2023) using the agricultural (MENDIBURU 2021), metan (OLIVOTO & LUCIO 2020) and ggplot2 (WICKHAM 2016) packages.

## RESULTS AND DISCUSSION

There was a significant effect of the treatment factor on the traits SL, NGS, SV, SHI and SAIS (Table 2). There was no significant effect of treatments for SW, GWS and NSS, that is, the application of herbicides combined with nitrogen did not influence the expression of these characters. KOROTKOVA et al. (2021) observed an increase in wheat grain productivity with the application of herbicides.

**Table 2.** Summary of the analysis of variance for possible combinations of herbicide treatments associated with nutritional management and quantitative variables.

| FV[2] | DF[3] | MS[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | SL[4] | SW[5] | GWS[6] | NSS[7] | NGS[8] | SV[9] | SHI[10] | SAIS[11] |
| Traeatment | 18 | 1.046* | 0.131 | 0.085 | 11 | 75* | 0.020* | 0.005* | 0.736* |
| Block | 15 | 2.624* | 0.489* | 0.310* | 106* | 172* | 0.025* | 0.002 | 0.560* |
| Residual | 270 | 0.637 | 0.144 | 0.092 | 17 | 45 | 0.010 | 0.001 | 0.254 |
| Total | 303 | | | | | | | | |
| CV% | | 9.47% | 19.38% | 20.91% | 7.81% | 16.22% | 12.77% | 4.72% | 7.96 |

Mean square (MS[1]), Factor of Variation (FV[2]), Degree of Freedom (DF[3]), spike length (SL[4]), spike weight (SW[5]), grain weight per spike (GWS[6]), number of spikelet per spike (NSS[7]), number of grains per spike (NGS[8]), spike viability (SV[9]), spike harvest index (SHI[10]) and spikelet arrangement index per spike (SAIS[11]). *significant to Test F.

The CV ranged from 4.72% (SHI) to 20.91% (GWS), revealing good experimental precision (PIMENTEL-GOMES 2009). The highest mean SL (8.99 cm) was observed in the treatment with 1,500 ml ha$^{-1}$ 2,4-D + 250 ml ha$^{-1}$ clodinafop-propargyl + 100 kg ha$^{-1}$ of urea (T19), while the lower averages were observed in treatments with 500 ml ha$^{-1}$ 2,4-D (T1) (7.89 cm) and 500 ml ha$^{-1}$ 2,4-D + 8 grams ha$^{-1}$ of metsulfurom-methyl (T2) (7.98 cm) (Table 3 and Figure 1).

There was no significant difference between the NGS means for the treatments used. This indicates that the combination of post-emergent herbicides and urea application does not significantly influence NG expression in wheat. The highest SV averages were observed when the treatments 1000 ml $ha^{-1}$ 2,4-D + 8g $ha^{-1}$ metsulfurom-methyl (T9) and 1000 ml $ha^{-1}$ 2,4-D + 250 ml $ha^{-1}$ clodinafop-propargyl + 100 kg $ha^{-1}$ urea (T13). The application of 1,500 ml $ha^{-1}$ 2,4-D + 250 ml $ha^{-1}$ clodinafop-propargyl + 100 kg $ha^{-1}$ of urea (T19) promoted the lowest averages of SV, SHI and SAIS (0.707, 0.690 and 5. 82, respectively) demonstrating a negative effect on reproductive viability in wheat. In wheat, SHEORAN et al. (2015) observed that the application of 60 and 90 g ai $ha^{-1}$ of clodinafop-propargyl significantly reduced the absorption of NPK by grains and straw, promoting a reduction in agronomic performance.

**Table 3.** Average of the variables analyzed from the different treatments by the SNK test (*Student Newman Keuls*).

| Treatments | SL[1] | NGS[2] | SV[3] | SHI[4] | SAIS[5] |
|---|---|---|---|---|---|
| T1 | 7.89b | 38.40a | 0.729ab | 0.752ab | 6.73a |
| T2 | 7.98b | 39.39a | 0.755ab | 0.748ab | 6.59abc |
| T3 | 8.49ab | 39.90a | 0.757ab | 0.723abc | 6.29abc |
| T4 | 8.58ab | 41.80a | 0.791ab | 0.718bc | 6.19abc |
| T5 | 8.29ab | 40.21a | 0.786ab | 0.735ab | 6.18ab |
| T6 | 8.51ab | 42.55a | 0.794ab | 0.724abc | 6.34abc |
| AUS | 8.59ab | 40.93a | 0.758ab | 0.723abc | 6.36abc |
| T8 | 8.18ab | 41.42a | 0.774ab | 0.750ab | 6.60ab |
| T9 | 8.50ab | 44.61a | 0.839a | 0.746ab | 6.29abc |
| T10 | 8.52ab | 43.16a | 0.801ab | 0.739ab | 6.28abc |
| T11 | 8.43ab | 44.50a | 0.761ab | 0.746ab | 6.26abc |
| T12 | 8.61ab | 39.99a | 0.798ab | 0.731ab | 6.13abc |
| T13 | 8.31ab | 41.77a | 0.839a | 0.756ab | 6.34abc |
| T14 | 8.54ab | 44.08a | 0.771ab | 0.743ab | 6.37abc |
| T15 | 8.14ab | 41.51a | 0.758ab | 0.748ab | 6.57ab |
| T16 | 8.33ab | 40.25a | 0.827ab | 0.761ab | 6.57ab |
| T17 | 8.56ab | 44.43a | 0.821ab | 0.764a | 6.38abc |
| T18 | 8.62ab | 40.31a | 0.774ab | 0.734ab | 6.09bc |
| T19 | 8.99a | 36.79a | 0.707b | 0.690c | 5.82c |

Spike length (SL[1]), number of grain per spike (NGS[2]), spike viability (SV[3]), spike harvest index (SHI[4]) and spikelet arrangement index per spike (SAIS[5]).

The average Euclidean distance analysis aims to show the dissimilarity between the 19 treatments, which include associations and different doses of herbicide and nutritional products (Figure 2). Three large groups and seven subgroups were formed. Subgroup I was formed only by treatment with 1,500 ml $ha^{-1}$ of 2,4-D + 250 ml $ha^{-1}$ of clodinafop-propargyl + 100 kg $ha^{-1}$ of urea (T19) due to the higher SL and NGS values. Subgroup II was composed of treatments with 500 ml $ha^{-1}$ of 2,4-D + 250 ml $ha^{-1}$ of clodinafop-propargyl (T3), absence (T7), 1,500 ml $ha^{-1}$ of 2,4-D + 8 grams $ha^{-1}$ of metsulfurom-methyl (T15) and 1,500 ml $ha^{-1}$ of 2,4-D + 8 grams $ha^{-1}$ of metsulfurom-methyl + 100 kg $ha^{-1}$ of urea (T18), due to similarity in expression from SL, NGS, SV and SHI. Subgroup III was composed only of treatment with 0.500 ml $ha^{-1}$ of 2,4-D (T1).

Subgroup IV was composed of the treatments 0.500 L $ha^{-1}$ of 2,4-D + 100 kg $ha^{-1}$ of Urea (T4) and 0.500 ml $ha^{-1}$ of 2,4-D + 250 ml $ha^{-1}$ of clodinafop- propargyle + 100 kg $ha^{-1}$ of urea (T6), which showed similarity for the variables SL, NGS, SV and SAIS. Subgroup V was composed of treatments with 0.500 ml $ha^{-1}$ of 2,4-D + 8 grams $ha^{-1}$ of metsulfurom-methyl (T2), 0.500 L $ha^{-1}$ of 2,4-D + 8 grams $ha^{-1}$ of metsulfurom-methyl + 100 kg $ha^{-1}$ of urea (T5), 1,000 ml $ha^{-1}$ of 2,4-D (T8) and 1,000 ml $ha^{-1}$ of 2,4-D + 100 kg $ha^{-1}$ of urea (T11), which showed similarity for the variables NGS, SV and SHI. Subgroup VI was composed of the treatments 1,000 ml $ha^{-1}$ of 2,4-D + 250 ml $ha^{-1}$ of clodinafop-propargyl (T10), 1,000 ml $ha^{-1}$ of 2,4-D + 250 ml $ha^{-1}$ of clodinafop-propargyl + 100 kg $ha^{-1}$ of urea (T13) and 1,000 ml $ha^{-1}$ of 2,4-D + 8 grams $ha^{-1}$ of metsulfurom-methyl (T9) which showed similarity with the variables SL, NGS, SHI and SAIS. Group VII was composed of the treatments 1,500 ml $ha^{-1}$ of 2,4-D + 100 kg ha-1 of urea (T17), 1,500 ml $ha^{-1}$ of 2,4-D + 250 ml $ha^{-1}$ of clodinafop-propargyl (T16), 1,000 ml $ha^{-1}$ of 2,4-D + 8 grams $ha^{-1}$ metsulfurom-methyl + 100 kg $ha^{-1}$ of urea (T12) and 1,500 ml $ha^{-1}$ L of 2,4-D (T14) due to the similarity of the values of the characters SL, NGS and SV. According to PIASECKI et al. (2017), the association between 2,4-D (670 or 1,005 g a.i. ha-1) with metsulfuron-methyl (4.2 g a.i. ha-1) was selective to wheat, and did not influence its yield and pH.

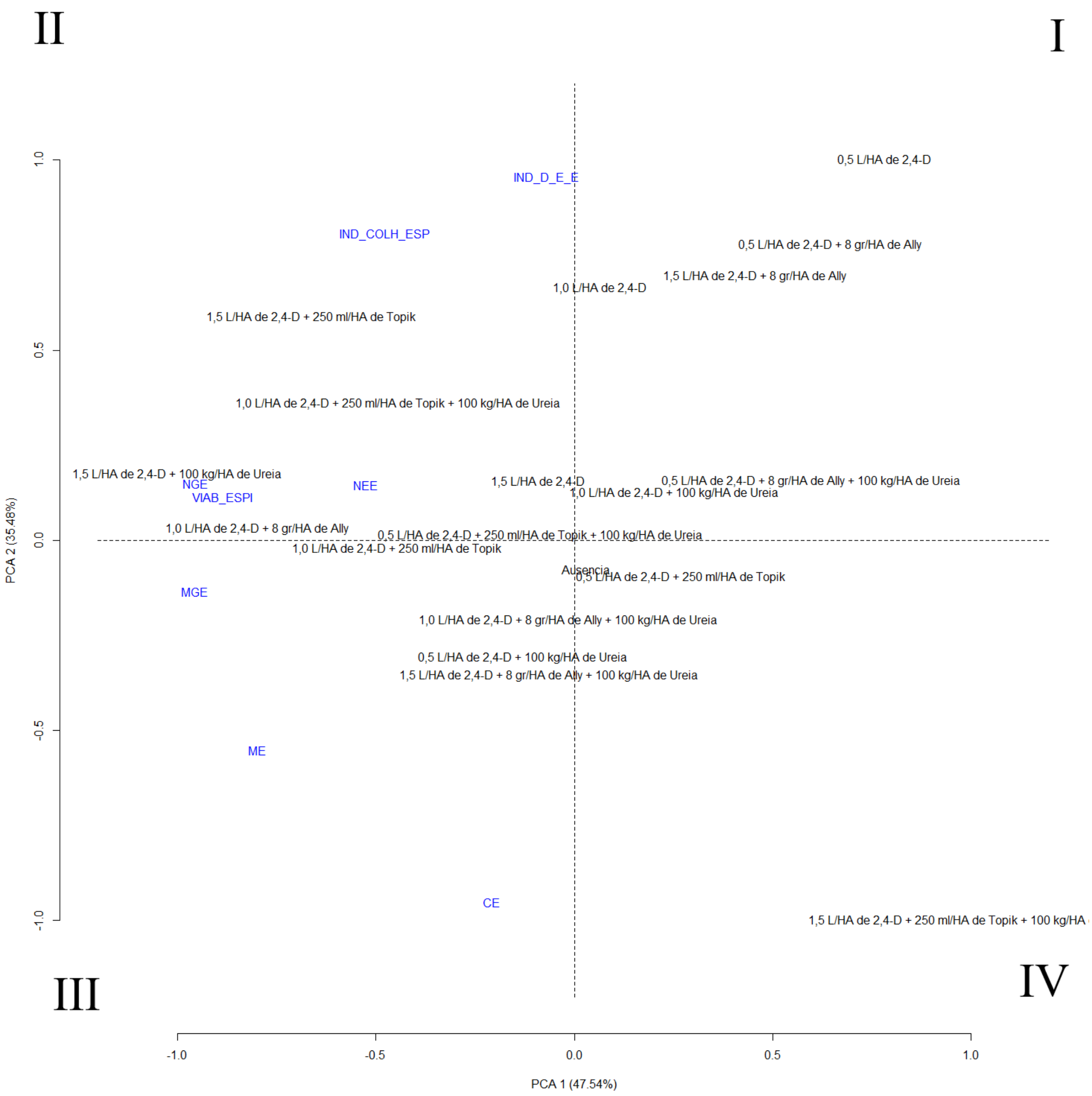


**Figure 1.** Representation of data variability in two main components *Biplot* of characters spike length (SL, in cm); spike weight (SW, in g per plant); grain weight per spike (GWS, in g per plant); number of spikelets per spike (NSS, in number per plant); number of grains per spike (NGS, in number per plant); spike viability (SV); spike harvest index (SHI, in g per plant); and spikelet arrangement index in the spike (SAIS, in number per plant).

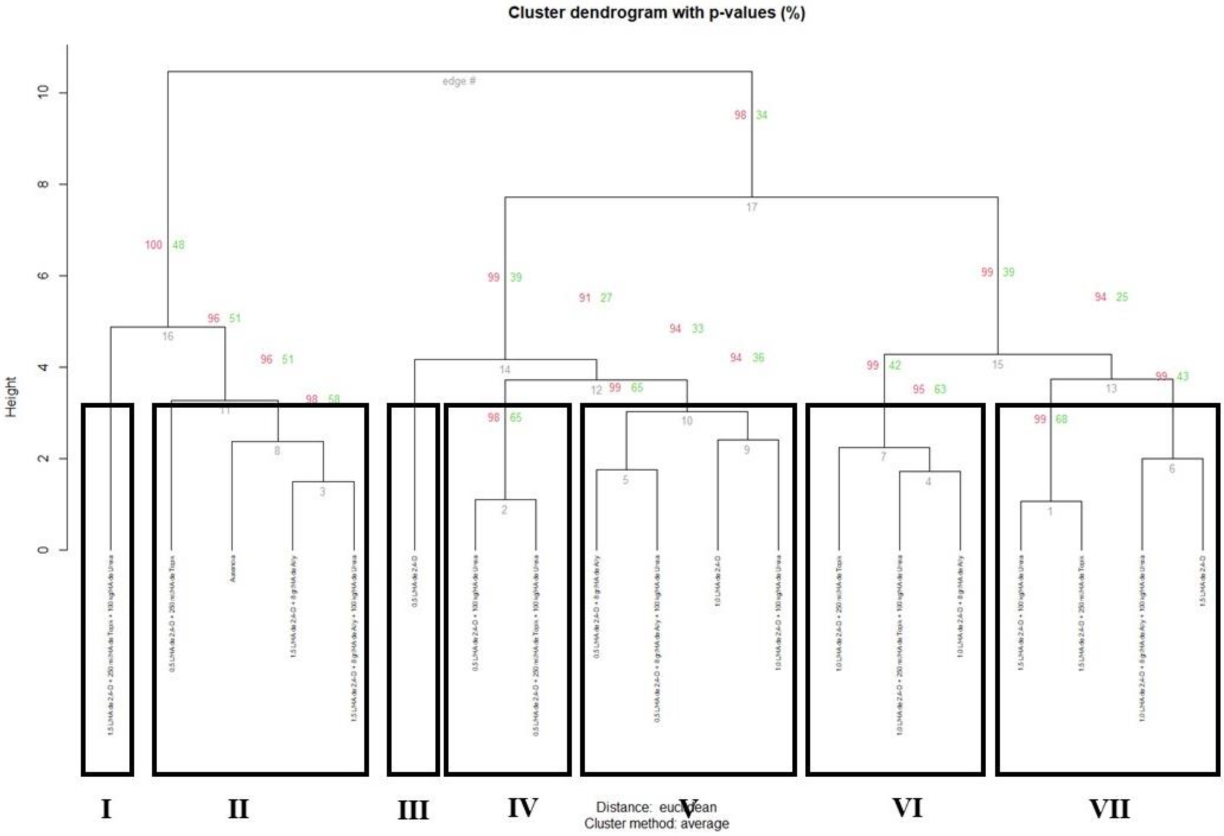


**Figure 2.** Dissimilarity dendrogram of the 19 treatments applied to wheat based on the following characters: spike length (SL, in cm); spike weight (SW, in g per plant); grain weight per spike (GWS, in g per plant); number of spikelets per spike (NSS, in number per plant); number of grains per spike (NGS, in number per plant); spike viability (SV); spike harvest index (SHI, in g per plant); and spikelet arrangement index in the spike (SAIS, in number per plant).

GWS showed a positive correlation with SV, NGS, SW, SHI, NSS and SL ($0.47 \leq r \leq 0.97$) (Figure 3). The highest correlation magnitudes were observed between GWS and MG ($r = 0.97$) and GWS and NGS ($r = 0.91$). Therefore, it can be understood that the highest SW and the highest NGS promote the highest GWS. This indicates that SW and NGS can be used to indirectly select wheat plants with higher GWS. Using SW in indirect selection facilitates the process, since it is not necessary to thresh the spike to count or weigh the grains, that is, with a simple weighing of the spike it is possible to identify the plants with the highest productive performance. Similar correlation coefficient between SW and GWS ($r = 0.90$) was also reported by LORO et al. (2021) in wheat. The positive relationship between spike weight and wheat grain yield was also reported by PANSERA et al. (2022).

NGS was the main division node for GWS prediction (Figure 4). The highest productive performance (GWS = 2.08 grams per plant) was observed in plants with NGS ≥ 49.40 grains with spikes longer than 9.10 cm. The lowest productive performance was observed in plants with NGS < 30.40 grains (GWS = 0.912 grams). Therefore, it can be observed that NG was decisive for the expression of GWS, making it possible to select plants with greater productive performance through NGS.

However, SW should also be considered for indirect selection, due to the ease of measurement.

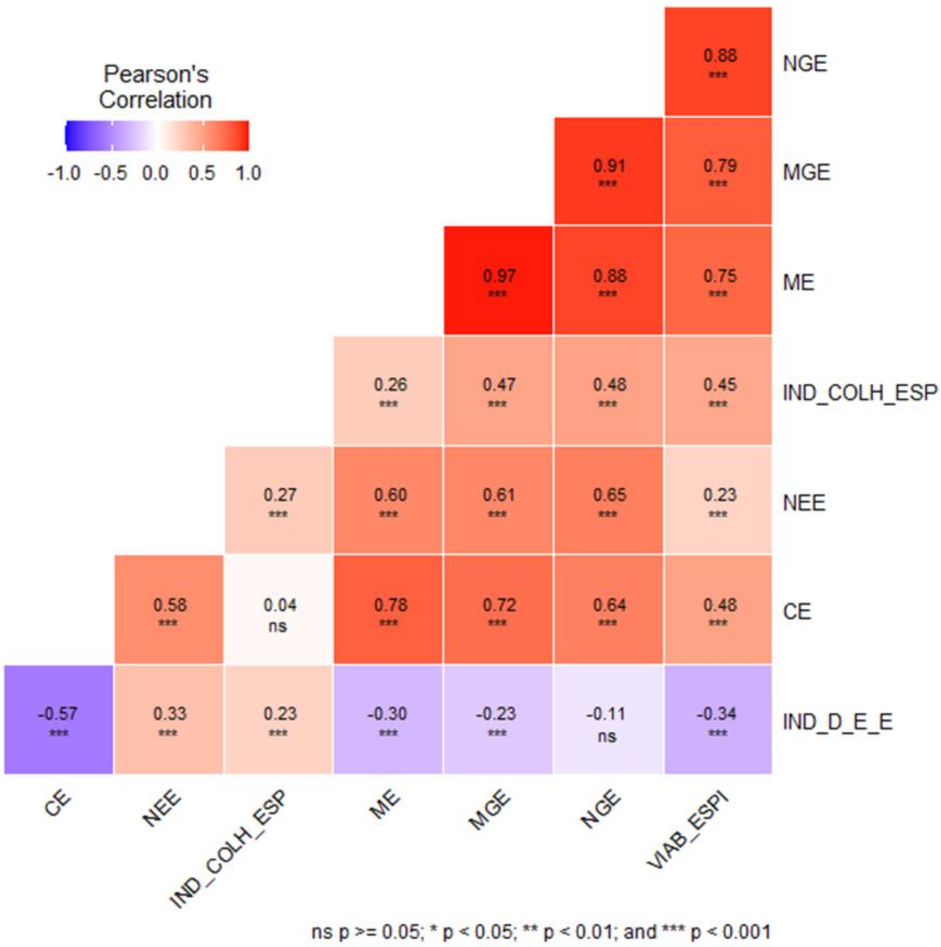


**Figure 3.** Pearson linear correlation coefficient matrix between spike length characters (SL, in cm); spike weight (SW, in g per plant); grain weight per spike (GWS, in g per plant); number of spikelets per spike (NSS, in number per plant); number of grains per spike (NGS, in number per plant); spike viability (SV); spike harvest index (SHI, in g per plant); and spikelet arrangement index in the spike (SAIS, in number per plant).

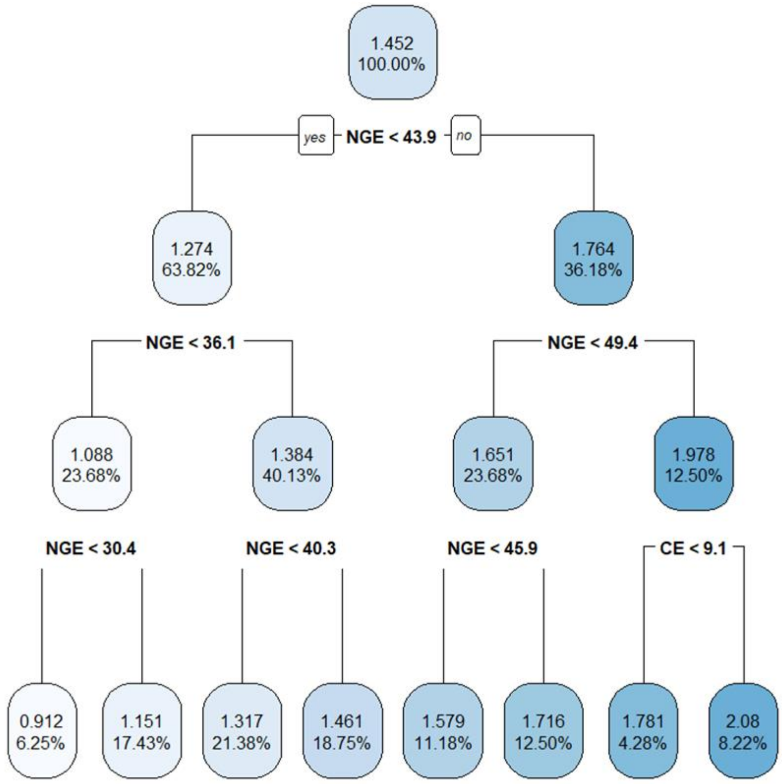


**Figure 4.** Regression tree for predicting grain weight per spike (GWS, in g per plant). Characters: spike length (SL, in cm); grain weight per spike (GWS, in g per plant); number of spikelets per spike (NSS, in number per plant); number of grains per spike (NGS, in number per plant); spike viability (SV); spike harvest index (SHI, in g per plant); and spikelet arrangement index in the spike (SAIS, in number per plant).

## CONCLUSION

The association of 1500 ml of 2,4-D + 250 ml of Clodinafop-propargil + 100 kg of urea, applied at the same time, reduced ear viability, ear harvest index and spikelet arrangement index in the ear. wheat,

The associated application of the herbicides 2,4-D, Metsulfuron-methyl and Clodinafop-propargyl did not influence the grain mass of the ear.

## AUTHOR CONTRIBUTIONS

Conceptualization, methodology, and formal analysis**, Matheus Silva Cazarotto and Ivan Ricardo Carvalho;** software and validation**, Ivan Ricardo Carvalho, Murilo Vieira Loro and Leonardo Cesar Pradebon;** investigation**, Gabriel Mathias Weimer Bruinsma, João Pedro Dalla Roza and Leonir Terezinha Uhde;** resources and data curation**,** writing-original draft preparation, editing, visualization**,** supervision, funding acquisition, **Ivan Ricardo Carvalho**. All authors have read and agreed to the published version of the manuscript.

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**INSTITUTIONAL REVIEW BOARD STATEMENT**
Not applicable for studies not involving humans or animals.

**INFORMED CONSENT STATEMENT**
Not applicable because this study did not involve humans.

**DATA AVAILABILITY STATEMENT**
The data can be made available under request.

**CONFLICTS OF INTEREST**
The authors declare no conflict of interest.

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