Changes of Stem Characteristics, Senescence Indexes and Yield and Quality of Wintering Rye under Different Populations

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Abstract: In response to the production crisis caused by a winter feed shortage due to the rapid development of the animal husbandry industry, winter rye 001 was selected to study differences in stalk and senescence characteristics in yield formation in cold regions. Five density treatments were established in a randomized design as 225 × 10^4 plant hm^−2 (D_1), 275 × 10^4 plant hm^−2 (D_2), 325 × 10^4 plant hm^−2 (D_3), 375 × 10^4 plant hm^−2 (D_4), and 425 × 10^4 plant hm^−2 (D_5). Stem characteristics, SOD activity, POD activity, MDA content, and differences in yield and feeding quality under different population densities were analyzed. The plant height, center of gravity, and stem basal internode length showed an increasing trend with an increase in planting density. The stem wall thickness, diameter, strength, and lodging resistance indices decreased. At 275 × 10^4 plants hm^−2, the rye crude protein content was the highest while neutral washing fiber and acid washing fiber were the lowest, and feed quality was the best. With an increase in density, spike number, grain number per spike, and thousand-grain weight first increased and then decreased. We concluded that the yield and feeding quality were best when the basic seedling was at 275 × 10^4 plants hm^−2.

Keywords: aging indexes; output; planting density; stem characteristics; winter rye

1. Introduction

Rye (Secale cereale), an annual gramineous herbaceous plant, is particularly resistant to environmental stress, which manifests in cold resistance, drought resistance, lodging resistance, and disease resistance [1,2]. Rye is mainly distributed in Germany, Poland, and France and China’s Heilongjiang, Inner Mongolia, Qinghai, and Tibet regions. Rye can be used for both grain and forage, and its grain nutrients are abundant, often being made into rye beer, rye bread, and rye tea [3]. The plant grows easily, with wide leaves and a high grass yield, which can be used as a livestock concentrate feed. Winter rye exhibits high nutritional value and planting it may effectively alleviate the production crisis caused by the shortage of feed in winter due to the current rapid development of the animal husbandry industry. In order to safely overwinter, wintering rye must be sown around September each year, which effectively improves the loss of topsoil and increases the coverage of ground vegetation in winter pastoral areas and has good applications for scientific research [4].

Population structure is an important factor for ensuring crop production. Reasonable planting density can alleviate the stresses between individuals and the group and promote the coordinated development of spike number per unit area, grain number per spike, and thousand-grain weight [5]. Keles, et al. [6–9] showed that with an increase in planting density, rye yield first increases and then decreases, while the total tiller number, plant height, and stem leaf ratio increase gradually with an increase in planting density.

Goncharenko et al. [10,11] found that with an increase in wheat planting density, the ability for light transmission and ventilation between groups gradually weakens, stems
become thinner, and lodging resistance decreases. Therefore, it was concluded that high-density planting was the direct cause of wheat lodging. Reducing the planting density of wheat enhances lodging resistance but reduces the number of effective panicles and grain yield. Many studies have found that the planting density of wheat is negatively correlated with the lodging resistance index; stems are gradually refined, and the length of the basal internodes increases [12]. The overall length of the stems reduces, and thus, the mechanical strength of the stems decreases [13]. Studies have shown that the planting density of winter wheat is closely related to leaf senescence and grain weight. The higher the planting density of winter wheat after flowering, the lower the content of chlorophyll and SOD activity, while the content of soluble protein and MDA increases [14]. Photosynthates are the main source of dry matter in wheat grains after anthesis. After wheat flowering, photosynthetic products, which are the main source of dry matter in the grain, can effectively delay leaf senescence, enhance the physiological function of the leaves, and promote grain weight [15]. Yu et al. [16] stated that reducing the planting density of rye is an important way to optimize its population structure. Further, low-density treatment effectively prolongs the functional period of flag leaves, alleviates the senescence speed of flag leaves, increases the accumulation of dry matter in the late growth stage, and plays a positive role in the formation of yield. Research has indicated that the senescence of rice leaves is related to a decrease in leaf chlorophyll content and an increase in free proline content, indicating that the senescence process of leaves is related to proline content [17]. Wintering rye 001 was bred by the Laboratory of Wheat Breeding, Northeast Agricultural University and has been shown to overwinter safely in the Heilongjiang Province, which is located at high latitudes with cold weather. Due to a short frost-free period, it is often planted only once a year by those with insufficient experience in the cultivation of overwintering crops, which often results in a weaker cultivation of winter rye. Determining the optimum planting density and planting schedule of winter rye requires an in-depth study [18]. To establish the best planting plan for overwintering rye in high-latitude areas, and to explore the physiological basis of yield formation under different planting densities, the physiological characteristics, stem morphological characters, aging characteristics, and feeding quality of winter rye under different planting densities were studied [5]. This study provides a practical reference for establishing the production mode of winter rye feeding and for resolving the quality problems of rye cultivation in early spring cold forages by establishing the optimal planting density of early spring rye and winter forage in cold regions. Further, we clarify the cultivation basis for optimum rye yields.

2. Materials and Methods
2.1. Experimental Materials and Design
2.1.1. Experimental Materials
Winter rye 001, the study material, was provided by the Laboratory of Wheat Breeding, Northeast Agricultural University.

2.1.2. Experimental Design
The experiment was carried out at the experimental base of the Northeast Agricultural University, Harbin, China (126°18’ longitude and 46°25’ latitude) between 2017 and 2018, with a temperate continental climate and an annual precipitation between 500 mm and 550 mm. The test site was characterized by black calcareous soil and the soil fertility profiles are shown in Table 1. The experimental design was randomized with five planting densities being established as follows: $225 \times 10^4$ plants hm$^{-2}$ ($D_1$), $275 \times 10^4$ plants hm$^{-2}$ ($D_2$), $325 \times 10^4$ plants hm$^{-2}$ ($D_3$), $375 \times 10^4$ plants hm$^{-2}$ ($D_4$), and $425 \times 10^4$ plants hm$^{-2}$ ($D_5$). Each plot consisted of eight rows, with each plot area measuring 4.8 m$^2$ (length, 3.2 m; width, 1.5 m). The strip sowing method was employed with a row spacing of 0.2 m. Each treatment was repeated three times. The same amount of fertilizer was used for all five planting densities, with 95 kg·hm$^{-2}$ urea, 150 kg·hm$^{-2}$ diammonium phosphate, and
75 kg·hm\(^{-2}\) potassium sulfate. The experiment was initiated on the 14th of September 2017. All other treatment measures were the same as those used in conventional sowing fields.

Table 1. The soil fertility condition of the 0–20 cm soil.

| Organic Matter (g kg\(^{-1}\)) | Total Nitrogen (g kg\(^{-1}\)) | Alkaline Hydrolytic Nitrogen (mg kg\(^{-1}\)) | Available Phosphorus (mg kg\(^{-1}\)) | Available Potassium (mg kg\(^{-1}\)) | pH |
|---------------------------------|---------------------------------|-----------------------------------------------|---------------------------------------|--------------------------------------|----|
| 22.25                           | 1.7                             | 118.21                                        | 65.4                                  | 179.35                               | 6.86|

Rainfall and average temperature in the growing season of triticale in 2017 and 2018.

| Year | Month | Average Temperature (°C) | Precipitation (mm) | Sunshine Hours (h) | Average Wind Speed (m·s\(^{-1}\)) |
|------|-------|--------------------------|--------------------|-------------------|-----------------------------------|
| 2017 | May   | 16.7                     | 37                 | 261               | 3.17                              |
|      | June  | 19.9                     | 215.2              | 218               | 1.8                               |
|      | July  | 24.7                     | 50.2               | 252               | 2.07                              |
|      | August| 22.1                     | 92.2               | 168               | 2.13                              |
|      | September | 15.8                  | 51.8               | 200               | 2.13                              |
| 2018 | May   | 16.6                     | 3.3                | 257               | 2.45                              |
|      | June  | 21.2                     | 55.2               | 202               | 1.85                              |
|      | July  | 24.9                     | 173.5              | 159               | 1.72                              |
|      | August| 21.4                     | 180.1              | 184               | 1.32                              |
|      | September | 15.3                 | 141.7              | 178               | 2.36                              |

2.2. Determination Items and Methods

2.2.1. Stem Characteristics

During the rye flowering period, 10 rye plants that displayed the same flowering time and similar growth were selected from each plot. These sample plants were labeled on a single selected stem. The sampled plants were selected at the milky stage of the rye. The plant height, center of gravity, internode length, stem diameter, and stem wall thickness of the stem were measured, and the stem lodging index was calculated using the fulcrum method [18].

2.2.2. Flag Leaf Senescence Index

The flag leaves from the marked stems were removed every seven days from the beginning of the flowering period and frozen immediately in liquid nitrogen. The flag leaves were then brought back to the laboratory and stored in a refrigerator at \(-80\) °C until analyses. POD activity was measured using the guaiacol method [18], SOD activity was measured using the NBT method [19], and MDA content was measured using the thiobarbituric acid method [20].

2.2.3. Determination of Feed Quality

After harvesting at the milky stage of the rye plants, the aboveground parts were dried and crushed, and impurities were removed using an 80-mesh sieve. The crude protein content of the rye was determined using the Kjeldahl method [21], and cellulose content was determined using the paradigm method [22].

2.2.4. Yield and Yield Components

During the ripening period, two rows of crops were harvested from each plot, and the aboveground weight was measured to calculate the fresh grass yield of each plot. The fresh grass was removed after 30 min in an oven at 105 °C and dried to a constant weight in an oven at 80 °C. The final weight was measured to calculate the hay yield of rye in each plot and repeated three times.

In each plot, 20 rye plants were randomly selected for seed testing. Seed yield was determined by measuring the grain number per spike and the thousand-grain weight. From each plot, a 1 m\(^2\) area was randomly selected during the maturity stage of the rye, and the effective panicle number was counted, followed by manual harvesting with three replicates per treatment.
2.3. Data Analysis

SPSS (IBM Inc., Chicago, IL, USA) and Microsoft Excel 2013 software (San Jose, CA, USA) were used to analyze the experimental data for each plot, and the One-Way ANOVA and Two-Way ANOVA methods were used for data analysis of variance and significance tests for each treatment.

3. Results and Analysis

3.1. Differences in Plant Height, Barycenter Height, and Internode Length of Rye under Different Density Treatments

The plant height, center of gravity, panel length, and the ratio from the base (1 + 2) to total intersection length (lrb%) showed an upward trend with an increase in planting density (Table 2) and reached their maximum values in the D5 density treatment. There was no significant difference in plant height between the plants in the D5 and D4 density treatments; however, the difference was significant compared with the other density treatments (p < 0.05). There were significant differences in the height of the center of gravity between the plants in the different density treatments (p < 0.05). The center of gravity reached the minimum value under the D1 density treatment and was not significantly different under the D1 and D2 density treatments. The center of gravity heights significantly differed between the D5 density treatment and the other densities (p < 0.05). The length of each internode also showed an increasing trend with an increase in planting density which was similar to the change trend in the plant height and the center of gravity height, which reached a peak in the D5 treatment. The difference in these variables between each density treatment was significant (p < 0.05). The lodging resistance of the plants was closely related to the length of the first internode, the length of the second internode, and its proportion to total internode length. As shown in Table 2, the length of the first internode and the length of the second internode at the base of the rye plants increased with an increase in planting density. It can be concluded that the stem characteristics of rye were directly affected by planting density. The plant height, center of gravity height, and internode length of rye under different density treatments.

| Treatment | Plant Height (cm) | Height of Center of Gravity (cm) | Panel Length (cm) | Ratio of Base (1 + 2) to Total Intersection Length (%) |
|-----------|------------------|---------------------------------|-----------------|-----------------------------------------------|
| D1        | 119.7 ± 4.3 c    | 69.4 ± 1.40 d                  | 7.6 ± 0.9 c     | 17.5 ± 0.7 c                                | 17.9 ± 1.3 d | 24.1 ± 1.0 d | 34.6 ± 1.7 d | 23.7 ± 0.9 c |
| D2        | 123.7 ± 3.6 c    | 71.53 ± 1.25 d                 | 8.0 ± 0.8 c     | 17.3 ± 1.0 c                                | 20.5 ± 0.6 c | 26.2 ± 0.7 d | 37.4 ± 0.9 d | 23.1 ± 0.6 c |
| D3        | 131.3 ± 2.6 b    | 76.60 ± 1.18 c                 | 9.4 ± 0.4 b     | 19.5 ± 1.6 b                                | 21.5 ± 0.6 b | 28.6 ± 0.6 b | 39.0 ± 0.9 b | 24.4 ± 0.3 b |
| D4        | 141.1 ± 2.1 a    | 82.13 ± 2.12 b                 | 11.0 ± 0.6 b    | 20.4 ± 0.8 b                                | 22.7 ± 1.1 b | 30.4 ± 2.0 b | 42.3 ± 1.6 b | 24.8 ± 0.2 ab |
| D5        | 144.7 ± 3.1 a    | 88.20 ± 1.82 b                 | 12.1 ± 0.4 b    | 22.6 ± 0.9 b                                | 25.2 ± 0.9 b | 33.8 ± 2.6 b | 45.1 ± 3.4 b | 25.0 ± 0.6 a  |

Note: lowercase letters (a, b, c, etc.) indicate the significant difference between the same number of days and different treatments (p < 0.05).

3.2. Difference between Stem Wall Thickness and Diameter, Lodging Resistance Index, and Mechanical Strength between Wintering Rye Internodes under Different Planting Densities

As shown in Table 3, for each density treatment, the thickness of the culm wall was greater in the first internode than in the second internode. At each density, the basal diameter between the first and second internodes showed an opposing trend to that of the thickness of the culm wall; that is, the diameter between the second internode was larger than that between the first internode. With an increase in planting density, the stem wall thickness between the first and second internodes showed a downward trend, and the trend of the basal internode diameter was similar. The thickness of the stem wall of the first and second internodes at the base was significantly greater in the D1 treatment than in the other density treatments. It was concluded that the stem quality of rye plants was higher, and the lodging resistance was stronger, under D1 planting density conditions.
Table 3. Differences in thickness and diameter, lodging resistance index and mechanical strength of stalk wall at the base of rye internodes under different density treatments.

| Treatment | Thickness of Culm Wall between Base Internodes (mm) | Base Internode Diameter (mm) | Mechanical Strength of Base Stem (N) | Culm Lodging-Resistance Index (N cm⁻¹) |
|-----------|-----------------------------------------------|----------------------------|------------------------------------|--------------------------------------|
|           | Section 1 The Second Internode | Section 1 The Second Internode | Section 1 The Second Internode | Section 1 The Second Internode |
| D1        | 2.11 ± 0.07 a | 1.95 ± 0.07 a | 6.50 ± 0.25 a | 7.06 ± 0.08 a | 22.50 ± 1.00 a | 19.40 ± 0.70 a | 0.34 ± 0.01 a | 0.28 ± 0.01 a |
| D2        | 1.93 ± 0.07 b | 1.82 ± 0.04 a | 6.08 ± 0.26 b | 6.17 ± 0.29 a | 20.27 ± 0.91 c | 18.70 ± 0.46 b | 0.32 ± 0.01 b | 0.27 ± 0.01 a |
| D3        | 1.89 ± 0.03 b | 1.73 ± 0.03 b | 5.70 ± 0.21 b | 5.83 ± 0.33 b | 18.23 ± 0.72 c | 17.13 ± 0.43 b | 0.31 ± 0.01 b | 0.24 ± 0.01 b |
| D4        | 1.80 ± 0.04 c | 1.68 ± 0.04 c | 4.61 ± 0.51 c | 5.77 ± 0.35 b | 17.20 ± 0.85 c | 15.50 ± 0.66 c | 0.29 ± 0.01 c | 0.23 ± 0.01 b |
| D5        | 1.74 ± 0.02 c | 1.54 ± 0.11 d | 3.59 ± 0.57 d | 5.49 ± 0.34 d | 15.40 ± 0.95 d | 13.90 ± 0.30 d | 0.27 ± 0.02 d | 0.21 ± 0.01 c |

Note: lowercase letters (a, b, c, etc.) indicate the significance of the difference between the same internode and different treatments (p < 0.05).

The mechanical strength of the basal stem and the lodging resistance index of the rye plants both showed a decreasing trend with an increase in planting density. There was no significant difference between the mechanical strength of the basal stem of the first internode and the lodging resistance index of the stem of the second internode under the D3 and D4 density treatments, but the difference was significant compared with the other density treatments. There were no significant differences in the mechanical strength and the lodging resistance index of the basal stem under D1 and D2 density treatments, but the differences were extremely significant compared with the other density treatments. Across the different density treatments, the mechanical strength and the lodging resistance index of the stem between the second internode were smaller than those between the first internode. It was concluded that the first internode contributed greatly to the lodging resistance of the stem in the rye plants.

3.3. Correlation Analysis between Basal Stem Characteristics and Lodging Resistance Index of Rye Plants under Different Planting Densities

As shown in Table 4, there was a significant difference between the length and lodging resistance index of the first internode and the stem diameter and lodging resistance index of the second internode, as well as significant correlations for the other relationships. In Sections 1 and 2, internode length was negatively correlated with lodging resistance index, which was particularly significant in Section 2. The correlation coefficient between the stem traits and the lodging resistance index of Section 1 was generally larger than that of Section 2, indicating that Section 1 contributed more to lodging resistance. Combined with the above correlation characteristics, it can be concluded that the thicker the stem wall, the shorter the internode length, and the thicker the stem, the stronger the mechanical strength of the stem and the stronger the lodging resistance.

Table 4. Correlation analysis of stalk characteristics and lodging resistance index of rye base.

| Culm Mechanical Strength | Stem Wall Thickness | Length of a Knot | Stem Diameter |
|--------------------------|---------------------|------------------|--------------|
| Section I lodging resistance index | 0.97 ** | 0.96 ** | −0.91 * | 0.99 ** |
| Section II lodging resistance index | 0.99 ** | 0.95 ** | −0.94 ** | 0.87 * |

Note: * and ** indicate significant differences at 0.05 and 0.01 levels, respectively.

3.4. Difference in Senescence Indices among Rye Grown under Different Density Treatments

The activities of SOD and POD showed an initial increasing trend, which then decreased (Table 5). SOD activity reached its peak on the 14th day, while POD activity reached its peak on the 21st day. With an increase in planting density, SOD activity and POD activity gradually decreased significantly, indicating that high-density planting accelerated the aging process of the rye leaves. POD activity under D4 and D5 densities was significantly lower than that under D1 and D2 densities. POD activity under the D5 density treatment decreased by 65.45% from the 21st day to the 28th day following anthesis. Table 6 shows
that the MDA content increased with increasing planting density on the same days following anthesis. MDA content increased slowly with an increase in density from the initial flowering day to the 14th day following anthesis; however, the accumulation rate of MDA increased significantly from the 14th day to the 28th day. The MDA content of the rye plants across the different density treatments reached a maximum value between the 21st day and the 28th day.

### Table 5. Differences in rye SOD and POD activity under different density treatments.

| Treatment | Days after Flowering (Days) | SOD Activity (µ g⁻¹FW) | POD Activity (±470 g⁻¹FW min⁻¹) |
|-----------|-----------------------------|-------------------------|----------------------------------|
|           | 0 d | 7 d | 14 d | 21 d | 28 d | 0 d | 7 d | 14 d | 21 d | 28 d |
| D1        | 252.03 ± 2.20 a | 271.00 ± 3.53 b | 305.47 ± 4.40 c | 273.57 ± 4.40 c | 231.03 ± 6.40 c | 35.33 ± 1.34 a | 42.03 ± 0.74 a | 57.37 ± 1.71 a | 69.43 ± 1.18 a | 34.53 ± 2.17 a |
| D2        | 246.03 ± 1.73 b | 260.90 ± 3.53 b | 293.40 ± 2.14 b | 265.07 ± 2.14 b | 225.70 ± 2.69 a | 33.57 ± 1.59 b | 39.07 ± 1.08 b | 53.10 ± 0.67 b | 63.53 ± 1.76 a | 34.67 ± 1.19 a |
| D3        | 244.20 ± 1.85 b | 247.20 ± 2.56 c | 283.10 ± 2.03 c | 251.90 ± 2.03 c | 214.43 ± 1.16 b | 31.13 ± 0.46 b | 34.43 ± 1.15 b | 51.10 ± 1.04 b | 61.57 ± 0.61 b | 29.27 ± 1.10 b |
| D4        | 234.33 ± 3.15 c | 241.90 ± 2.27 b | 272.07 ± 2.38 b | 248.03 ± 1.71 c | 196.70 ± 1.71 c | 25.70 ± 1.07 b | 29.57 ± 1.63 c | 47.73 ± 0.76 c | 59.20 ± 1.85 c | 24.80 ± 1.04 c |
| D5        | 223.57 ± 2.19 a | 233.82 ± 2.02 a | 259.73 ± 3.11 a | 237.00 ± 3.37 b | 185.83 ± 3.27 a | 223.57 ± 2.19 a | 29.00 ± 1.22 c | 47.33 ± 1.05 c | 55.93 ± 1.25 d | 19.27 ± 0.52 d |

Source of variation:
- **: p < 0.05
- *: p < 0.01

Note: lowercase letters (a, b, c, etc.) indicate the significant difference between the same number of days and different treatments (p < 0.05). * and ** indicate significant differences at 0.05 and 0.01 levels, respectively. D: time; T: treatment.

### Table 6. Differences in MDA content of rye under different density treatments.

| Treatment | Days after Flowering (Days) | MDA Content (µmol g⁻¹) |
|-----------|-----------------------------|------------------------|
|           | 0 d | 7 d | 14 d | 21 d | 28 d |
| D1        | 11.73 ± 1.04 e | 16.90 ± 1.04 e | 17.5 ± 0.52 e | 26.50 ± 0.50 d | 39.50 ± 1.03 d |
| D2        | 13.43 ± 0.64 cd | 17.60 ± 0.59 bc | 24.60 ± 1.63 b | 29.40 ± 0.35 ed | 41.30 ± 1.19 d |
| D3        | 14.20 ± 1.19 bc | 20.00 ± 0.71 b | 27.60 ± 0.70 ab | 32.50 ± 1.20 c | 47.80 ± 1.25 c |
| D4        | 17.57 ± 0.62 ab | 25.40 ± 1.09 a | 27.50 ± 0.38 ab | 35.70 ± 1.85 b | 51.50 ± 0.61 b |
| D5        | 18.10 ± 0.76 a | 28.30 ± 1.24 a | 30.30 ± 1.64 a | 41.40 ± 1.00 a | 61.60 ± 1.13 a |

Source of variation:
- **: p < 0.05
- *: p < 0.01

Note: lowercase letters (a, b, c, etc.) indicate the significant difference between the same number of days and different treatments (p < 0.05). * and ** indicate significant differences at 0.05 and 0.01 levels, respectively. D: time; T: treatment.

#### 3.5. Feeding Quality of Wintering Rye under Different Planting Densities

As shown in Table 7, crude protein content indicated an initial trend that then decreased with the increase in planting density; crude protein content was highest under the D2 density treatment. The crude protein content in the rye plants under the D1, D2, and D3 densities was significantly higher than at the D5 density. The neutral detergent fiber content of the rye plants showed an initial decreasing trend which then increased as the planting density increased. The neutral detergent fiber content reached the lowest value under the D2 density treatment; however, the difference in neutral detergent fiber among the different density treatments was not significant. Acidic detergent fiber and neutral detergent fiber levels displayed similar trends; the smallest values of acid detergent were found in the rye plants under the D2 density treatment. It was concluded that the fiber content of reygrass was at its lowest level, and the forage quality was at its highest level, under the D2 density treatment.
Table 7. Forage quality of rye under different densities.

| Treatment | Crude Protein \(\pm\) SE | Neutral Detergent Fiber \(\pm\) SE | Acid Detergent Fiber \(\pm\) SE |
|-----------|---------------------------|-----------------------------------|-------------------------------|
| D1        | 9.59 \(\pm\) 0.46 ab     | 72.30 \(\pm\) 4.92 bc            | 59.66 \(\pm\) 1.02 c          |
| D2        | 10.95 \(\pm\) 0.60 a      | 68.53 \(\pm\) 6.98 c             | 51.37 \(\pm\) 2.46 d         |
| D3        | 9.38 \(\pm\) 0.51 b      | 79.86 \(\pm\) 7.91 ab            | 65.49 \(\pm\) 3.65 b         |
| D4        | 8.50 \(\pm\) 1.28 bc     | 81.74 \(\pm\) 5.92 ab            | 65.45 \(\pm\) 1.08 b         |
| D5        | 7.85 \(\pm\) 0.61 c      | 88.69 \(\pm\) 2.76 a             | 71.55 \(\pm\) 3.63 a         |

Note: lowercase letters (a, b, c, etc.) indicate that the same crude protein, medium acid detergent fiber and different treatments have significant differences \((p < 0.05)\).

3.6. Differences in Yield and Yield Components of Rye under Different Planting Densities

The fresh and hay yield of the ryegrass reached its maximum under the D2 density treatment, and its minimum under the D5 density treatment, where it was significantly lower than that of the other density treatments (Table 8). The difference in overall yield between the D2 density treatment and that of the other density treatments was significant, with an overall performance of D2 > D3 > D1 > D4 > D5. Under the D5 density treatment, the spike number was at its lowest level; the number of spikes was highest under the D3 density treatment and significantly higher than that of the other density treatments. The overall trend of spike numbers in the ryegrass first increased and then decreased. The grain number per spike and thousand-grain weight showed an overall downward trend as the density increased, which reached a significantly high peak under the D1 density treatment compared to the other planting densities. The difference in the thousand-grain weight and the grain number per spike between the ryegrass in the D2 and D3 planting treatments was not significantly different. The grain yield of rye under the D3 density treatment was significantly higher than the other density treatments \((p < 0.05)\).

Table 8. Rye yield factors and their composition under different density treatments.

| Treatment | Fresh Yield (kg hm\(^{-2}\)) | Hay Yield (kg hm\(^{-2}\)) | Grain Yield and Its Composition |
|-----------|-------------------------------|-----------------------------|--------------------------------|
|           |                               |                             | Number of Panicles \(10^4\) hm\(^{-2}\) | Grain Number Per Spike (1000 per spike) | Thousand Seed Weight (g) | Grain Yield (kg hm\(^{-2}\)) |
| D1        | 65,613 c                      | 33,005 c                    | 486 c                          | 35.29 a                        | 40.779 a                  | 6644 b                       |
| D2        | 72,065 a                      | 36,766 a                    | 562 b                          | 34.04 ab                       | 39.157 b                  | 7129 a                       |
| D3        | 67,734 b                      | 35,069 b                    | 626 a                          | 32.00 c                        | 39.100 b                  | 7444 a                       |
| D4        | 41,283 d                      | 21,597 d                    | 501 c                          | 32.96 bc                       | 37.748 c                  | 5923 c                       |
| D5        | 35,154 e                      | 16,593 e                    | 465 d                          | 29.55 d                        | 36.255 d                  | 4735 d                       |

Note: Lowercase letters (a, b, c, etc.) indicate that the same yield, panicle number and grain weight were significantly different among different treatments \((p < 0.05)\).

4. Discussion

Planting density is an important factor affecting the lodging resistance of rye stems. In the later stages of wheat growth and development, with an increase in planting density, the lodging resistance of the stem decreases [23]. The lodging resistance of wheat is related to the basal internode thickness and the wall thickness of the wheat stem. The thicker the stem, the thicker the stem wall, and the stronger the lodging resistance [24]. The lodging resistance of stems is closely related to plant height and stem base traits [25]. Rebetzke et al. [26] found that plant height was the most important factor affecting the lodging resistance of wheat stems. The higher the plant, the greater the probability of lodging. Related studies in wheat have found that within a certain range, the higher the seedling density, the higher the amount of storage substances that are transported from the stem to the ear, and the higher the probability of stem lodging [27]. Increasing sowing density increases population density resulting in a closed population, poor stem quality, and a population that is prone to lodging [28]. The length of the first and second internodes, center of gravity height, and height of the basal internodes of the wheat stem have been shown to be positively correlated with lodging rate [29]. Stem lodging occurs
mostly in the first and second basal internodes; therefore, the shorter the internodes, the better the lodging resistance [30,31]. Zhirov et al. [32] found that the internode thickness and stem diameter between the first and second internodes of the stem are important indexes that affect the lodging resistance of the stem. Miranda et al. [33,34] found that with an increase in planting density, the plant height of wheat and the length of the first and second internodes of the stem base increased, thereby increasing the proportion of the first and second internodes of the base to the total length of the upper internodes. As density increases, the center of gravity increases, and the stem diameter decreases, which reduces the mechanical strength of the stem and the lodging resistance index [34]. The mechanical strength and lodging resistance index of stems are also crucial indicators for determining the lodging resistance of plants [35], and the lodging resistance index of stems can be used to directly reflect the lodging resistance of overall plants. The ANOVA and correlation analyses between stem characteristics and lodging resistance index showed that stem strength, stem wall thickness, and stem diameter were closely related to lodging resistance, and further, contributed greatly to lodging resistance, which is consistent with previous research results [35]. The longer the length of the second section of the stem base, the weaker the lodging resistance of the stem, indicated by a significant negative correlation found in our study. However, the correlation was higher between the length of the first section of the stem base and the lodging resistance, which is consistent with the findings of Zhang. The results of this study showed that plant height, barycenter height, and internode length of the first and second internodes at the stem base of rye increased with an increase in density. Stem wall thickness, diameter, strength, and lodging resistance index decreased gradually with an increase in planting density, which is similar to the results of Miranda. Therefore, at lower planting densities, rye stem lodging resistance is higher, and the stem is stronger, which is conducive to yield formation.

Several studies have shown that, in wheat, flag leaves exhibit their highest photosynthetic efficiency at the late growth stage compared to other leaves, which promotes the formation of grain yield, and thus, significantly improves overall yield [36]. A decline in the physiological and biochemical functions of flag leaves in the late growth stage of rye are the main indicators of senescence. Grain filling and leaf senescence in post-anthesis wheat have been characterized extensively [36,37]. In the plant internal defense system, the primary role of antioxidant phosphatase is to effectively remove reactive oxygen species so that the generation and removal of reactive oxygen species achieves a dynamic balance [38,39]. In this study, at the late growth stages of the rye, the SOD and POD activities in the flag leaves first increased after anthesis, and then decreased after reaching a maximum value. The SOD and POD activities decreased mostly from the late flowering stage; SOD activity reached its peak on the 14th day after anthesis. The activities of SOD and POD in leaves gradually decrease with an increase in planting density [40]. The results of this study showed that as time progressed, the leaves gradually senesced, and there was a negative correlation between SOD activity and POD activity and planting density, indicating that increasing planting density would significantly reduce SOD activity and POD activity and aggravate the leaf senescence process. MDA is the product of membrane peroxidation and can be used as an indicator of membrane lipidization [41]. The results showed that MDA content gradually accumulated with the senescence of the flag leaves and increased after the 21st day of flowering. We found a negative correlation between SOD activity and MDA content in the wheat. The decrease in SOD and POD activity in leaves accelerated the senescence of wheat, which directly resulted in a decrease in the function of scavenging reactive oxygen species and the accumulation of MDA content. With an increase in planting density, leaf senescence was aggravated, and MDA content showed an upward trend, a finding which is similar to the results of Zhang et al. [42]. Our results showed that low planting density had a positive effect on optimizing the population structure of rye and could effectively extend the functional period of flag leaves and slow down their senescence, which was conducive to the increase in dry matter accumulation during the late growth stage and an observed improvement in yield.
High planting density has been shown to promote the accumulation of NDF and ADF and reduce the content of crude protein in plants. In this study, the content of crude protein showed a gradual downward trend with planting density; however, neutral and acid detergent fiber content showed an upward trend with the increase in rye planting density, which is consistent with previous research results [43,44]. The crude protein content of rye under the D2 planting density was higher than the other planting densities. Further, the neutral detergent fiber and acid detergent fiber contents under the D2 planting density were lower than other higher planting densities, indicating that, to a certain extent, a higher planting density could improve yield but reduce feed quality.

Adjusting planting density is an important method for farmers to improve the yield and economic coefficients of their crops [43]. Many studies have shown that suitable planting density has a positive effect on crop photosynthesis and intra-population gas exchange, which can improve crop yield [44,45]. Planting density affects the grain yield of wheat, as well as other yield components such as spike number, grain number per spike, and thousand-grain weight. You [46] showed that rye exhibits a higher grain number per spike and thousand-grain weight under a low planting density, resulting in high yields. Ma [47] found a significant effect of wheat spike number on yield. In this study, when rye was planted at a density of 225 × 10^4 plants hm^{-2}, the spike number was significantly lower than in other density treatments. In addition, the thousand-grain weight and grain number per spike fully compensated for the disadvantage of a low spike number, resulting in a much higher grain yield in the high-density treatment. When the density of rye planting was 325 × 10^4 plants hm^{-2}, the spike number of the rye was higher than the other density treatments; in this treatment, grain yield reached its maximum, and the yield of the rye was much higher than the other density treatments, which is consistent with previous research [48]. We concluded that the planting density had a comprehensive effect on the grain yield of winter rye, and a too high or too low planting density had adverse effects on the formation of grain yield.

5. Conclusions

The overwintering type of rye in the Northern cold land has a longer growth period, which promotes the tillering ability and strong lodging resistance, and is suitable for reasonable close planting. It also does not show premature aging and could obtain better feeding quality. From the point of view of the forage harvest, it is not suitable for high density planting, and the yield and quality are highest when the basic seedling is 275 × 10^4 plants hm^{-2}. From the perspective of the seed harvest, it is suitable for medium density planting, and the seed yield is highest when the basic seedling is 325 × 10^4 plants hm^{-2}.

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References

1. Crespo-Herrera, L.A.; Garkava-Gustavsson, L.; Åhman, I. A systematic review of rye (Secale cereale L.) as a source of resistance to pathogens and pests in wheat (Triticum aestivum L.). Hereditas 2017, 154, 14. [CrossRef]

2. Sievers, T.; Cook, R.L. Aboveground and Root Decomposition of Cereal Rye and Hairy Vetch Cover Crops. Soil Sci. Soc. Am. J. 2018, 82, 147–155. [CrossRef]

3. Lachowicz, S.; Świeca, M.; Pejcž, E. Improvement of Health-Promoting Functionality of Rye Bread by Fortification with Free and Microencapsulated Powders from Amelanchier alnifolia Nutt. Antioxidants 2020, 9, 614. [CrossRef]

4. Cummins, D.G.; Newton, J.P.; Craigmiles, J.P. Effect of seeding date on seasonal production and quality of temporary winter grazing crops. Crop. Sci. 2020, 4, 6. [CrossRef]

5. Zhang, Y.-S.; Lee, G.-J.; Joo, J.-H.; Lee, J.-T.; Ahn, J.-H.; Park, C.-S. Effect of Winter Rye Cultivation to Improve Soil Fertility and Crop production in Alpine Upland in Korea. J. Pract. Diagn. Ther. 2007, 26, 300–305. [CrossRef]

6. Li, T.; Deng, G.; Tang, Y.; Su, Y.; Wang, J.; Cheng, J.; Yang, Z.; Qiu, X.; Pu, X.; Zhang, H.; et al. Identification and Validation of a Novel Locus Controlling Spikelet Number in Bread Wheat (Triticum aestivum L.). Front. Plant Sci. 2021, 12, 227. [CrossRef]

7. Cui, K.; Peng, S.; Ying, Y.; Yu, S.; Xu, C. Molecular Dissection of the Relationships among Tiller Number, Plant Height and Heading Date in Rice. Plant Prod. Sci. 2004, 7, 309–318. [CrossRef]

8. Keles, G.; Ates, S.; Coskun, B.; Alatas, M.S.; Isik, S. Forage yields and feeding value of small grain winter cereals for lambs. J. Sci. Food Agric. 2016, 96, 4168–4177. [CrossRef]

9. Yang, G.; Wang, J. Co-fermentation of sewage sludge with ryegrass for enhancing hydrogen production: Performance evaluation and kinetic analysis. J. Biorenewal. Technol. 2017, 243, 1027. [CrossRef]

10. Gontarenko, A.A.; Berkutova, N.S.; Timoshchenko, A.S.; Ermakov, S.A.; Makarov, A.V.; Semenova, T.V.; Tochilin, V.N. Comparative analysis of baking qualities of grain of winter rye varieties with various short-stem types. Russ. Agric. Sci. 2007, 33, 351–355. [CrossRef]

11. Xiao, Y.; Liu, J.; Li, H.; Cao, X.; Xia, X.; Haosheng, L. Lodging resistance and yield potential of winter wheat: Effect of planting density and genotype. Front. Agric. Sci. Eng. 2015, 20, 168. [CrossRef]

12. Zheng, M.; Chen, J.; Shi, Y.; Li, Y.; Yin, Y.; Yang, D.; Luo, Y.; Pang, D.; Xu, X.; Li, W.; et al. Manipulation of lignin metabolism by plant densities and its relationship with lodging resistance in wheat. Sci. Rep. 2017, 7, 481805. [CrossRef] [PubMed]

13. He, J.; Yu, Z.; Shi, Y. Effects of strip rotary tillage with subsoiling on soil enzyme activity, soil fertility, and wheat yield. Plant Soil Environ. 2019, 65, 9. [CrossRef]

14. Yu, Z.; Egli, D.B. Effects of different densities on leaf senescence and grain weight after flowering in winter wheat. J. Acta Agron. Sinica. Sinica. 1995, 45, 412–418.

15. Schultness, A.W.; Wang, Y.; Miedaner, T.; Wilde, P.; Reif, J.C.; Zhao, Y. Multiple-trait- and selection indices-genomic predictions for grain yield and protein content in rye for feeding purposes. Theor. Appl. Genet. 2016, 129, 273–287. [CrossRef] [PubMed]

16. Zeng, L.D.; Zhang, Q.L.; Cai, M.L.; Chow, W.; Peng, C. Changes in photosynthetic pigments and chlorophyll fluorescence parameters in the super-high-yielding rice hybrid Peiai64S/E32 during senescence. Photosynthesis 2020, 58, 862–868. [CrossRef]

17. Hung, K.T.; Kao, C.H. Hydrogen peroxide is necessary for abscisic acid-induced senescence of rice leaves. J. Plant Physiol. 2004, 161, 1347–1357. [CrossRef]

18. Feng, S.-W.; Ru, Z.-G.; Ding, W.-H.; Hu, T.-Z.; Li, G. Study of the relationship between field lodging and stem quality traits of winter wheat in the north China plain. Crop. Pasture Sci. 2019, 70, 772. [CrossRef]

19. Rapacz, M.; Sascal, M.; Gut, M. Chlorophyll Fluorescence-Based Studies of Frost Damage and the Tolerance for Cold-Induced Photoinhibition in Freezing Tolerance Analysis of Triticale (×Triticosecale Wittmack). J. Agron. Crop. Sci. 2011, 197, 378–389. [CrossRef]

20. Jiang, H.; Zhang, J. Experimental Guidance of Plant Physiology; Higher Education Press: Beijing, China, 2019; pp. 12–29.

21. Fu, Q. Effect of Malate-oligosaccharide Solution on Antioxidant Capacity of Endurance Athletes. J. Open Biomed. Eng. 2015, 9, 326–329.

22. Zhou, Z.F.; Li, Z.A. Experimental Guidance of Plant Physiology; Guangxi University Press: Nanning, China, 2005; pp. 88–89.

23. Miranda-Romero, L.A.; Tirado-González, D.N.; Tirado-Estrada, G.; Améndola-Massiotti, R.; Sandoval-González, L.; Ramírez-Valverde, R.; Salem, A.Z. Quantifying non-fibrous carbohydrates, acid detergent fiber and cellulose of forage through an in vitro gas production technique. J. Sci. Food Agric. 2020, 100, 3099–3110. [CrossRef] [PubMed]

24. Khan, A.; Liu, H.H.; Ahmad, A.; Xiang, L.; Ali, W.; Kamran, M.; Ahmad, S.; Li, J. Impact of Nitrogen Regimes and Planting Densities on Stem Physiology, Lignin Biosynthesis and Grain Yield in Relation to Lodging Resistance in Winter Wheat (Triticum aestivum L.). Cereal Res. Commun. 2019, 47, 566–579. [CrossRef]

25. Srikanth, J.; Subramonian, N.; Premachandra, M.N. Advances in Transgenic Research for Insect Resistance in Sugarcane. Trop. Plant Biol. 2011, 4, 52–61. [CrossRef]

26. Rebetzke, G.J.; Ellis, M.H.; Bonnett, D.G.; Mickelson, B.; Condon, A.; Richards, R. Height reduction and agronomic performance for selected gibberellin-responsive dwarfing genes in bread wheat (Triticum aestivum L.). Field Crop. Res. 2012, 126, 87–96. [CrossRef]

27. Zhang, M.; Wang, H.; Yi, Y.; Ding, J.; Zhu, M.; Li, C.; Guo, W.; Feng, C.; Zhu, X. Effect of nitrogen levels and nitrogen ratios on lodging resistance and yield potential of winter wheat (Triticum aestivum L.). PLoS ONE 2017, 12, e0187543. [CrossRef]
28. Peng, D.; Chen, X.; Yin, Y.; Lu, K.; Yang, W.; Tang, Y.; Wang, Z. Lodging resistance of winter wheat \( (Triticum aestivum L.) \): Lignin accumulation and its related enzymes activities due to the application of paclobutrazol or gibberellin acid. Field Crop. Res. 2014, 157, 1–7. [CrossRef]

29. Berry, P.M.; Spink, J. Predicting yield losses caused by lodging in wheat. Field Crop. Res. 2012, 137, 19–26. [CrossRef]

30. Fujii, Y. Physiological and Morphological Differences in the Roots according to Nodes Succession in Rice and Wheat Plants. Jpn. J. Crop. Sci. 1957, 26, 156–158. [CrossRef]

31. Zhirov, E.G. New amphidiploids from crossing \( Aegilops sharonensis \) Eig. and \( Ae. speltoides \) Tausch with diploid species of wheat. Tr. Prikl. Bot. Genet. Sel. 1960, 68, 364–373.

32. Zhang, H.-J.; Li, T.; Liu, H.-W.; Mai, C.-Y.; Yu, G.-J.; Li, H.-L.; Yu, L.-Q.; Meng, L.-Z.; Jian, D.-W.; Yang, L.; et al. Genetic progress in stem lodging resistance of the dominant wheat cultivars adapted to Yellow-Huai River Valleys Winter Wheat Zone in China since 1964. J. Integr. Agric. 2020, 19, 148–158. [CrossRef]

33. Singh, B.; Ahuja, S.; Yadav, P. Radiotracer and radiation application for rapid measurement of contribution of stem assimilates towards grain filling and for alleviating terminal heat stress in wheat. J. Radioanal. Nucl. Chem. 2019, 321, 255–262. [CrossRef]

34. Giunta, F.; Motzo, R. Sowing rate and cultivar affect total biomass and grain yield of spring triticale \( ( \times Triticosecale Wittmack) \) grown in a Mediterranean-type environment. Field Crop. Res. 2004, 87, 179–193. [CrossRef]

35. Hossain, M.; Hasan, M. Physiological parameters, yield and seed quality of wheat as influenced by irrigation and split application of nitrogen. Fundam. Appl. Agric. 2018, 3, 398–406. [CrossRef]

36. Zhao, F.S.; Guo, S.; Zhang, H.; Zhao, Y. Expression of yeast SOD2 in transgenic rice results in increased salt tolerance. Plant Sci. 2006, 170, 216–224. [CrossRef]

37. Fang, X.; Li, Y.; Nie, J.; Wang, C.; Huang, K.; Zhang, Y.; Zhang, Y.; She, H.; Liu, X.; Ruan, R.; et al. Effects of nitrogen fertilizer and planting density on the leaf photosynthetic characteristics, agronomic traits and grain yield in common buckwheat \( (Fagopyrum esculentum M.) \). Field Crop. Res. 2015, 219, 160–168. [CrossRef]

38. Wang, Z.H.; Jiang, L.N.; Li, C.X.; Zhu, F.; Qiu, Z. Effects of planting density and plant growth regulators on winter wheat grain yield and quality. Acta Agric. Sci. Plant. 2003, 17, 15–17.

39. Zhang, Y.L.; Lan, L.; Li, Y.M.; Xiao, K. The effect of planting density on population growth and grain yield of hybrid wheat C6-38/Py85-1. Acta Tritice Sin. 2008, 28, 113–117.

40. Tan, D.; Fan, Y.; Liu, J.; Zhao, J.; Ma, Y.; Li, Q. Winter Wheat Grain Yield and Quality Response to Straw Mulching and Planting Pattern. Agric. Res. 2019, 8, 548–552. [CrossRef]

41. Kadar, R.; Muntean, L.; Racz, I.; Ona, A.; Cezalan, A.; Hirisǎu, D. The Effect of Genotype, Climatic Conditions and Nitrogen Fertilization on Yield and Grain Protein Content of Spring Wheat \( (Triticum aestivum L.) \). Not. Bot. Horti Agrobot. Cluj-Napoca 2018, 47, 515–521. [CrossRef]

42. Zhang, Y.Q.; Zhang, N.; Wang, N.; Xie, F.T. Effects of planting density on photosynthetic characteristics and yield components of summer soybean. J. Nucl. Agric. 2015, 29, 1386–1391.

43. Yan, Z.; Gao, C.; Ren, Y.; Zong, R.; Ma, Y.; Li, Q. Effects of pre-sowing irrigation and straw mulching on the grain yield and water use efficiency of summer maize in the North China Plain. Agric. Water Manag. 2017, 186, 21–28. [CrossRef]

44. Yan, S.; Wu, Y.; Fan, J.; Zhang, F.; Qiang, S.; Zheng, J.; Xiang, Y.; Guo, J.; Zou, H. Effects of water and fertilizer management on grain filling characteristics, grain weight and productivity of drip-fertigated winter wheat. Agric. Water Manag. 2019, 213, 983–995. [CrossRef]

45. García del Moral, L.F.; Boujena, A.; Yanez, J.A.; Ramos, J.M. Forage Production, Grain Yield, and Protein Content in Dual-Purpose Triticale Grown for Both Grain and Forage. Agron. J. 1995, 87, 902–908. [CrossRef]

46. Fang, X.; She, H.; Wang, C.; Liu, X.; Li, Y.; Nie, J.; Ruan, R.; Wang, T.; Yi, Z. Effects of fertilizer application rate and planting density on photosynthetic characteristics, yield and yield components in waxy wheat. Cereal Res. Commun. 2018, 46, 1–11. [CrossRef]

47. Du, N.X.T. Effects of green manures during fallow on moisture and nutrients of soil and winter wheat yield on the Loss Plateau of China. Emir. J. Food Agric. 2017, 29, 978–987.