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Foliar application of prohexadione-calcium improves lodging resistance and yield by regulating stem traits in late-sown wheat under high seeding rates

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17 **Abstract**

18 **Background** Increasing the seeding rate can compensate for the yield losses caused by
19 delayed sowing dates in wheat; however, it also increases the lodging risk of late-sown wheat.
20 Foliar application of prohexadione-calcium (Pro-Ca) at the jointing stage can effectively reduce
21 lodging risk and maintain a stable yield. However, the comprehensive effects and underlying
22 mechanisms of these two measures on wheat production remain unclear.

23 **Results** A two-year field experiment was conducted using three high seeding rates, M1 (360
24 kg ha^{-1}), M2 (450 kg ha^{-1}), and M3 (540 kg ha^{-1}), and four foliar application rates of Pro-Ca:
25 C0 (0 g ha^{-1}), C1 (600 g ha^{-1}), C2 (1200 g ha^{-1}), and C3 (1800 g ha^{-1}). The morphological
26 characteristics of the stems, internode microstructure, biochemical substance synthesis, stem
27 strength, lodging resistance indicators, field lodging performance, and grain yield parameters
28 were systematically analyzed for the different treatments. The objective was to identify the
29 optimal combination of seeding rate and Pro-Ca concentration for late-sown wheat. The goal
30 was to improve stem traits and yield components, enhance lodging resistance, and increase the
31 grain yield. The results demonstrated that increasing the seeding rate in late-sown wheat
32 effectively increased the spike number but significantly reduced the number of grains per spike
33 and 1000-grain weight. The highest yield was recorded for treatment M2. In contrast,
34 increasing the seeding rate caused elongation of the basal internodes, decreased internode
35 diameter and wall thickness, and reduced tissue thickness and number of vascular bundles. It
36 also reduced lignin and cellulose accumulation, lowered stem breaking strength and lodging
37 resistance index (LRI), and increased the field lodging rate and lodging index. Foliar
38 application of Pro-Ca effectively reversed the adverse structural weakening of stems caused by
39 high seeding rates. It enhanced stem breaking strength and LRI and significantly reduced field
40 lodging, with treatment C2 exhibiting the most pronounced effects. In addition, based on the

optimal spike number (M2), C2 increased the number of grains per spike and 1000-grain weight, thereby improving yield.

Conclusion In summary, the combination of a moderately increased seeding rate (450 kg ha^{-1}) and an optimized Pro-Ca rate (1200 g ha^{-1}) improved stem structure, optimized yield components, and synergistically enhanced lodging resistance and yield potential of late-sown wheat. This study provides a theoretical basis and practical guidance for achieving high and stable yields in high-density planting systems for late-sown wheat in the Xinjiang region.

Keywords: Pro-Ca; late-sown wheat; stem traits; lodging resistance; yield

1. Introduction

Wheat (*Triticum aestivum* L.) is a major global food crop, and maintaining a high and stable yield is crucial for ensuring food security [1]. However, in recent years, global extreme weather events have caused widespread delays in wheat sowing dates owing to heavy rainfall during the planting season [2, 3]. In addition, Xinjiang, China, is a typical cropping region suitable for slightly more than one harvest per year but insufficient for two harvests. Currently, planting patterns such as the "wheat–maize" and "wheat/cotton" systems are being actively promoted to improve land use and solar energy use efficiency. This has created conflicts in the growth succession between wheat and other crops, further delaying the wheat sowing dates [4]. Moreover, delayed wheat sowing offers advantages such as reducing the need for pre-winter irrigation and alleviating conflicts in labor and machinery allocation. These advantages have contributed to the expansion of the late-sown wheat planting area [5].

Delayed sowing of wheat adversely affects seed germination, resulting in insufficient temperature accumulation before winter, increased overwinter mortality, and exposure of post-spring growth to high-temperature stress. This shortens the vegetative growth period. Combined with the subsequent impact of hot, dry winds, it impedes grain filling. Consequently, the formation of spike number, grains per spike, and 1000-grain weight is adversely affected, ultimately reducing the yield [3, 6]. "Compensating for late sowing by increasing density" is a conventional strategy for maintaining the yield of late sown wheat. This involves increasing the seeding rate to increase the number of spikes per main stem, thereby compensating for yield losses caused by reductions in grains per spike and 1000-grain weight, and improving the overall wheat yield [5, 7]. However, excessively high seeding rates intensify intraspecific competition, leading to the death of some plants and further reductions in grains per spike and 1000-grain weight, ultimately decreasing yield [5, 8]. In addition, higher seeding rates tend to reduce stem quality and mechanical strength, increase lodging risk, and limit yield

improvement [9, 10]. Recent studies conducted in major wheat-producing regions of China, such as Shandong and Henan provinces, have provided substantial evidence regarding the effects of planting density on wheat yield and lodging resistance [11, 12]. These studies indicate that moderately increasing planting density can enhance the population spike number, optimize the canopy structure, and promote yield formation. However, excessive density intensifies competition among individual plants, reduces the robustness of basal internodes, weakens stem mechanical strength and lodging resistance, and ultimately restricts further yield improvement. Therefore, rationally increasing the seeding rate while simultaneously minimizing lodging risk is essential for achieving high yields in late-sown wheat.

Wheat lodging is primarily classified into stem and root lodging [13, 14], and the frequency of stem lodging is significantly associated with the wheat seeding rate [15]. Therefore, this study focused on improving wheat resistance to lodging. Generally, the morphological and structural characteristics, anatomical traits, and mechanical strength of plant stems are closely related to lodging resistance [16-18]. Lodging resistance can be significantly improved by reducing plant height (PH), basal internode length (IL), and center-of-gravity height (CGH), or by increasing internode diameter (ID) and stem wall thickness [11, 12]. In addition, stem tissue structure and vascular bundle number, which are key anatomical traits, are positively correlated with stem strength and lodging resistance [16]. Lignin and cellulose, the main components of the cell wall, contribute to stem mechanical strength, thereby reducing the risk of lodging [17, 19]. Moreover, the structural traits and mechanical properties of the second basal internode are considered the most critical determinants of overall lodging resistance in wheat [9]. Therefore, enhancing the structure and composition of basal internodes is essential for increasing wheat lodging resistance.

Prohexadione-calcium (Pro-Ca) is a novel cyclohexanecarboxylic acid plant growth regulator. Its mode of action involves the inhibition of gibberellin biosynthesis, which reduces

PH and consequently decreases the incidence of lodging [20, 21]. A meta-analysis of 16 growth regulators demonstrated that exogenous application of Pro-Ca significantly shortened the lengths of basal internodes in rice, thereby reducing PH. Its effect on lodging resistance was superior to that of paclobutrazol and uniconazole [22]. In addition, Pro-Ca prolongs the duration of vegetative growth, promotes reproductive development [20] and enhances stress resistance. These effects contribute to improved spikelet development and grain set in late-sown wheat, thereby alleviating the conflict between excessive seeding rates and population quality [23], while compensating for reductions in grains per spike and 1000-grain weight. Therefore, the foliar application of Pro-Ca represents an ideal field strategy for balancing the trade-off between lodging resistance and yield in densely planted late-sown wheat, thereby supporting higher yields. However, current research on Pro-Ca has primarily focused on horticultural crops, such as pepper [24], cabbage [25], and rapeseed [26]. Its application in high-density late-sown wheat has received limited attention. Specifically, the optimal application rate of Pro-Ca remains unclear, and the mechanisms by which it simultaneously enhances lodging resistance and yield through the regulation of stem structural traits require further investigation.

Most of the aforementioned studies have focused on conventional sowing dates or major wheat-growing regions, such as the Huang-Huai-Hai Plain, and studies on the synergistic regulation of yield and lodging resistance in high-seeding-rate wheat under late-sowing conditions in Xinjiang remain limited. Accordingly, a two-year field experiment was conducted using three high seeding rates and four application rates of Pro-Ca. The effects of different combinations of seeding rate and Pro-Ca concentration on wheat stem morphology, anatomical traits, lodging resistance parameters, and yield components were systematically evaluated. The aim of this study was to identify the optimal seeding rate and foliar Pro-Ca concentration under

late-sowing conditions to synergistically enhance lodging resistance and yield in late-sown wheat.

2. Materials and methods

2.1. Experimental site

The experiment was conducted over two consecutive wheat growing seasons, 2023–2024 and 2024–2025, at the Tacheng Prefecture Institute of Agricultural Sciences (82°57′ E, 46°43′ N; altitude 415 m above sea level). The experimental site was located in the northern Xinjiang winter wheat region, where the cropping system is predominantly single-cropping. However, in recent years, multiple cropping systems, such as wheat–maize and wheat/cotton cropping systems, have been increasingly promoted to improve land-use efficiency and the utilization of light and thermal resources. This has resulted in the delayed harvest of the preceding crops. Moreover, because autumn sowing is heavily dependent on precipitation to provide adequate soil moisture, the area of late-sown wheat has increased in recent years.

According to the Köppen climate classification, this region is classified as BSk (semi-arid steppe), with an annual mean temperature of 6.2℃, annual sunshine duration of 2,832 hours, and mean annual precipitation of 270 mm. The daily temperature and rainfall during the wheat growing season are shown in Fig. 1. The experiment was conducted in the same field in both years, where the preceding crop was spring wheat. The soil type was loam, and the physicochemical properties of the 0–20 cm soil layer were as follows: organic matter content 23.64 g kg^{-1}, total nitrogen 0.31 g kg^{-1}, pH 8.2, alkali-hydrolyzable nitrogen 47 mg kg^{-1}, available phosphorus 9.9 mg kg^{-1}, and available potassium 141 mg kg^{-1}.

2.2. Experimental materials and design

In this study, October 25 was designated as the late sowing date, which was more than one month later than the local conventional sowing date and represented a typical late sowing date

for this region. The experimental cultivar was Xindong 18, a widely planted local winter wheat variety with a whole growth period of approximately 275 days, a plant height of about 90 cm, a compact plant architecture, moderate tillering ability, strong overwintering tolerance, and good yield stability, which makes it well suited for late-sowing conditions. All wheat seeds were manually sown in drill rows with a 20 cm row spacing and sowing depth of approximately 4 cm. Each plot measured 10.0 m^2 (2.0 m × 5.0 m), and the experiment included four biological replicates. No irrigation was applied before winter. In spring, drip irrigation belts were installed, with one tube serving three rows and an interval of 60 cm between the capillary tubes. The spray reagent was a 15% prohexadione-calcium suspension concentrate (CAS No. 127277-53-6), with calcium 3,5-dioxo-4-propionylcyclohexanecarboxylate as the main active ingredient, provided by Zhejiang Shijia Technology Co. Ltd. (China). Uniform foliar spraying was performed 5–7 days before the jointing stage. The spraying criterion was defined as follows: more than 50% of the wheat plants in the field exhibited the third spring leaf on the main stems, expanding from tip emergence to full expansion, with a leaf age of approximately 3.5. Simultaneously, the first basal internode of the main stems showed no distinct elongation and measured less than 0.5 cm in length. All other field management practices followed the local high-standard farmland management protocols. The experimental layout is shown in Fig. 2.

A two-factor split-plot design was used in this study. The main plots consisted of three seeding rate treatments: M1 (360 kg ha^{-1}, approximately 0.75 × 10^7 seeds ha^{-1}), M2 (450 kg ha^{-1}, approximately 1.00 × 10^7 seeds ha^{-1}), and M3 (540 kg ha^{-1}, approximately 1.25 × 10^7 seeds ha^{-1}). The subplots included four foliar application rates of Pro-Ca: C0 (0 g ha^{-1}, water control), C1 (600 g ha^{-1}), C2 (1200 g ha^{-1}), and C3 (1800 g ha^{-1}).

2.3. Measured indicators

2.3.1. Stem traits

At anthesis, the milk stage, and the wax ripening stage, ten wheat plants with uniform growth

were selected from each of the four replicate plots of each treatment. The underground parts were removed, and the PH and IL of the main stem were measured using a ruler [27]. The plants were then placed on a fixed fulcrum to determine the balance point, and the distance from the stem base to the balance point, defined as the center of gravity height, was measured [27]. Finally, each internode was transversely cut at its midpoint, and the ID and wall thickness were measured using a digital Vernier caliper.

2.3.2. Internode microstructure

At anthesis, the milk stage, and the wax ripening stage, five uniformly grown wheat plants were selected from each of the four replicate plots of each treatment. The second basal internode of the main stem was excised, and thin sections were prepared from the middle portion of the internode using a blade. The sections were then stained with safranin for microscopic observations. The microstructural characteristics were analyzed using an optical microscope (AC 85-256, Shanghai Optical Instrument Factory, China) equipped with the Mvlmage software (v4.1.1, Beijing Micronet Technology Co., Ltd., China).

2.3.3. Lignin content (LC) and cellulose content (CC)

At the milk stage, five wheat plants with uniform growth were selected from each of the four replicate plots of each treatment. The second basal internodes of the main stems were excised and immediately stored at −80°C. LC and CC levels were determined using a double-antibody sandwich ELISA kit according to the manufacturer's instructions (Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China). Absorbance was measured at 450 nm using a microplate reader. A standard curve was established using a series of standard concentrations, and a linear regression equation ($Y = aX + b$) was fitted. The concentration of each sample was calculated by substituting its absorbance value into the regression equation, and the final LC and CC values were obtained by multiplying the calculated concentrations by the appropriate dilution factor.

2.3.4. Stem mechanical traits and lodging resistance index (LRI)

At anthesis, the milk stage, and the wax ripening stage, 20 uniformly grown wheat plants were selected from each of the four replicate plots of each treatment. The leaf sheaths of the second basal internodes were removed, and the breaking strength was measured using a stem strength tester (YYD-1A; Zhejiang Top Instrument Co., Ltd., China). During the measurement, the middle portion of the second internode was placed perpendicularly on the support, and the handle was rotated to apply pressure gradually and uniformly until the maximum breaking strength was recorded. The LRI was then calculated as the ratio of the breaking strength to CGH, according to the following formula [15].

$$\text{LRI} = \text{Breaking strength}/\text{CGH}$$

2.3.5. Lodging occurrence

The time, severity, and location of wheat lodging were recorded during the growing season [28]. Lodging severity was scored on a five-grade scale: grade 1, no lodging; grade 2, slight lodging, with a plant inclination angle of $\leqslant 30^\circ$; grade 3, moderate lodging, with an angle of $>30^\circ$–45°; grade 4, heavy lodging, with an angle of $>45^\circ$–60°; and grade 5, severe lodging, with an angle of $>60^\circ$.The lodging rate was defined as the percentage of the actual lodged area relative to the total plot area. The lodging index (LI) was calculated as the product of the lodging grade and lodging rate using the following equations:

$$\text{Lodging rate (\%)} = (\text{Lodged area} / \text{Total plot area}) \times 100\%$$

$$\text{LI} = \text{Lodging grade} \times \text{Lodging rate}$$

2.3.6. Yield and yield components

On 15 July, 30 wheat spikes were randomly selected from each of the four replicate plots of each treatment to determine the number of grains per spike. In addition, three 2-m-long rows (excluding the border rows) were randomly selected from each plot to determine the spike

number. These samples were manually harvested and threshed to determine the 1000-grain weight and yield.

2.4. Statistical analysis

Data were processed using Microsoft Excel 2021 (Microsoft Corp., Redmond, WA, USA). Statistical analyses were performed using SPSS 29.0 (IBM Corp., Chicago, IL, USA). For stem morphological, anatomical, and mechanical traits measured at anthesis, the milk stage, and the wax-ripening stage, we first examined the temporal response patterns of the treatments across the three sampling stages. Because the treatment responses were generally consistent in direction across the three stages and no stage-by-treatment interaction was observed that altered the main conclusions, the measurements from the three stages were treated as repeated temporal observations and averaged to characterize the overall treatment response during the post-anthesis period. Stage averages were calculated at the plot level and subsequently used for comparisons among treatments. Multiple plants or repeated measurements sampled within each plot were first averaged at the plot level and were not treated as independent experimental replicates. Differences among treatments were assessed using Duncan’s multiple range test. For the Mantel test, Pearson correlation analysis, and PLS-PM, treatment means were used as observations. Specifically, for each treatment combination (seeding rate × Pro-Ca rate), values were first averaged across the four replicate plots, the three post-anthesis sampling stages, and the two experimental years, yielding 12 treatment-level observations (3 seeding rates × 4 Pro-Ca rates). Multiple plants per plot, repeated measurements across stages, and year-specific values were not treated as independent replicates, thereby avoiding pseudoreplication. Mantel and Pearson correlation analyses, together with structural equation modeling (partial least squares path modeling [PLS-PM]), were conducted using RStudio (Posit Software, PBC, Boston, MA, USA). The PLS-PM was evaluated using the goodness-of-

fit statistic (GoF = 0.81) and R^2 values for endogenous variables, and the significance of path coefficients was assessed using the bootstrap procedure.

3. Results and Analysis

3.1. Macroscopic stem structure

3.1.1. IL, PH, and CGH

After anthesis, the CGH of wheat plants increased continuously, whereas the other stem traits remained relatively stable (Fig. 3). Based on the mean values across the two years and three growth stages, increasing the seeding rate significantly increased PH, the lengths of the first to third internodes, and CGH in wheat ($P < 0.05$). At the wax ripening stage, the PH under M2 and M3 increased by 2.62% and 3.73%, respectively, compared with that under M1. Similarly, the lengths of the first to third internodes increased by 5.05% and 9.07%, respectively, whereas the CGH increased by 2.00% and 2.84%, respectively. Foliar application of Pro-Ca significantly reduced PH, the lengths of the first to third internodes, and CGH in wheat. Compared with C0, PH under C1, C2, and C3 decreased by 3.10%, 5.39%, and 9.79%, respectively. Similarly, the lengths of the first to third internodes decreased by 5.44%, 9.87%, and 12.87%, respectively, whereas the CGH decreased by 2.17%, 3.73%, and 5.09%, respectively.

3.1.2. ID and wall thickness

After anthesis, the wheat stem diameter and wall thickness remained stable (Fig. 4). With increasing seeding rate, the mean diameter and wall thickness of the second to fourth internodes followed the order M1 > M2 > M3. In contrast, with increasing Pro-Ca concentration, the average diameter and wall thickness of the first to fourth internodes first increased and then decreased, reaching their maximum values under C2.

3.2. Internode microstructure

3.2.1. Tissue composition

The number of cell layers and tissue thickness in the second wheat internode decreased with increasing seeding rates (Fig. 5A). Pro-Ca treatment increased the number of cell layers and tissue thickness. However, this promotive effect weakened at the highest Pro-Ca concentrations. After anthesis, the number of cell layers remained relatively stable, whereas tissue thickness increased gradually, particularly from the milk stage to the wax ripening stage (Fig. 5B). These results indicate that higher seeding rates significantly reduced the number of cell layers and thickness of both mechanical and parenchymal tissues in the second internode of wheat. Compared with M1, the number of cell layers and the thickness of the mechanical tissue under M2 and M3 decreased by 2.31% and 4.90%, and by 5.57% and 12.08%, respectively (based on the two-year average across the three stages). Similarly, the number of cell layers and thickness of the parenchymatous tissue decreased by 5.63% and 7.64%, and by 6.48% and 16.86%, respectively (based on the two-year average across the three stages). Under Pro-Ca treatment, the number of cell layers and the thickness of both mechanical and parenchymal tissues increased significantly, following the pattern C1 > C2 > C3, although no significant difference was observed between C1 and C2. Moreover, the regulatory effect of Pro-Ca diminished as the seeding rate increased.

3.2.2 Vascular bundle number

After anthesis, the number of large and small vascular bundles in wheat remained relatively stable, whereas both the seeding rate and Pro-Ca treatment significantly affected the vascular bundle number (Fig. 6). Compared with M1, the numbers of large and small vascular bundles under M2 decreased by 4.24% and 7.40%, respectively, whereas those under M3 decreased by 13.18% and 23.34%, respectively (based on the two-year average across the three stages). After Pro-Ca treatment, the number of vascular bundles first increased and then decreased with

293 increasing application concentration, with the highest values observed for C1. Compared with
294 C1, the numbers of large and small vascular bundles under C0, C2, and C3 decreased by 8.51%
295 and 18.00%, 1.31% and 7.03%, and 10.77% and 15.73%, respectively (based on the two-year
296 average across the three stages).

297 **3.3. LC and CC of internodes**

298 To further clarify the effects of increasing seeding rates and Pro-Ca application on the
299 compound contents in the basal internodes of late-sown wheat, samples were collected at the
300 milk stage during the two experimental years for further analysis. The results are shown in Fig.
301 7. As the seeding rate increased, the LC and CC in the internodes of late-sown wheat decreased
302 significantly. Compared with M1, the LC under M2 and M3 decreased by 7.69% and 17.69%,
303 respectively, whereas the CC decreased by 5.68% and 13.52%, respectively (based on a two-
304 year average). Pro-Ca application significantly increased LC and CC in wheat internodes. Both
305 compounds first increased and then decreased with increasing Pro-Ca concentrations. LC
306 reached a maximum under treatment C2, whereas CC peaked under C1.

307 **3.4. Stem mechanical traits, lodging resistance, and actual lodged area**

308 *3.4.1. Breaking strength*

309 After anthesis, the stem-breaking strength of wheat gradually decreased as growth progressed
310 (Fig. 8). The results showed that both seeding rate and Pro-Ca application significantly affected
311 stem-breaking strength, although their interaction effect was not significant. Increasing the
312 seeding rate significantly reduced the stem-breaking strength. Compared with M1, the stem-
313 breaking strength under M2 and M3 decreased by 14.99% and 28.83%, respectively (based on
314 the two-year average across the three stages). Pro-Ca application significantly affected stem-
315 breaking strength in late-sown wheat, with an initial increase followed by a decrease with
316 increasing application concentration. Compared with C1, stem-breaking strength under C0, C2,

and C3 decreased by 23.22%, 8.42%, and 31.06%, respectively (based on the two-year average across all three stages).

3.4.2. LRI

After anthesis, the LRI of wheat progressively declined as the plants developed (Fig. 9). The results showed that both seeding rate and Pro-Ca application significantly influenced LRI, although their interaction effect was not significant. Compared with M1, the LRI under M2 and M3 decreased by 15.69% and 31.43%, respectively (based on the two-year average across the three growth stages). Under Pro-Ca treatment, the LRI of late-sown wheat followed the trend C1 > C2 > C0 > C3, with no significant difference between C1 and C2 ($P > 0.05$).

3.4.3. Field lodging performance

During the two-year experiment, lodging occurred twice in 2023-2024, whereas no lodging was observed in 2024-2025 (Table 1). With increasing seeding rate, the lodging rate, lodging score, and LI increased. In contrast, Pro-Ca application reduced both the frequency and severity of lodging (Fig. 10). As the Pro-Ca application concentration increased, the lodging rate, score, and LI decreased.

3.5. Yield and its components

The seeding rate and Pro-Ca application significantly affected the yield and yield components of late-sown wheat. However, their interaction significantly influenced only the yield (Table 2). Under late-sowing conditions, increasing the seeding rate significantly increased the spike number, whereas the grain number per spike and 1000-grain weight decreased accordingly. The yield under M2 was significantly higher than that under M1 and M3 by 3.57% and 8.90%, respectively. At the same seeding rate, the spike number, grain number per spike, 1000-grain weight, and yield of late-sown wheat first increased and then decreased with increasing Pro-Ca concentration, with all variables peaking at C2. Overall, considering both the seeding rate

and Pro-Ca concentration, the M2C2 treatment produced the most balanced performance with respect to the yield and yield components in late-sown wheat.

3.6. Relationships between lodging-related and yield parameters

Based on Mantel tests and Pearson correlation analyses, this study systematically examined the statistical and spatial association patterns between 13 key agronomic traits of late-sown wheat and the LRI, lodging rate, and yield (Fig. 11). The LI was strongly negatively correlated with plant transverse traits (ID and internode wall thickness [IWT]), anatomical structural traits (number of mechanical tissue layers [NMTLs], number of parenchyma cell layers [NPCLs], mechanical tissue thickness [MTT], parenchyma tissue thickness [PTT], number of small vascular bundles [NSVBs], and number of large vascular bundles [NLVBs]), and chemical composition traits (LC and CC) (Mantel's $r \geq 0.6$, $P < 0.01$), indicating that thicker stems, denser cellular tissues, and higher compound content were associated with stronger lodging resistance. IL, PH, and CGH were all significantly positively correlated with LI (Mantel's $r \geq 0.6$, $P < 0.05$), suggesting that greater longitudinal elongation increases the lodging risk. Yield was significantly positively correlated with PH, and LC (Mantel's $r \geq 0.4$, $P < 0.05$), implying that within a certain range, greater PH and LC may enhance yield but simultaneously increase the lodging risk.

In addition, significant positive correlations were observed among plant transverse traits, anatomical structural traits, and chemical composition traits (Pearson's $r > 0.5$, $P < 0.05$), whereas highly significant positive correlations were found among plant longitudinal traits (Pearson's $r > 0.1$, $P < 0.01$). In contrast, NMTLs, NVBs, and LC were negatively correlated with IL, PH, and CGH (Pearson's $r < 0$), indicating a close link between plant morphological and structural traits. Therefore, enhancing plant transverse traits, appropriately increasing longitudinal growth, and increasing internode chemical content may be effective strategies for optimizing stem anatomical structures and improving lodging resistance. These traits were also

positively associated, to some extent, with yield-related traits and collectively provide a structural basis for the coordinated improvement of high yield and lodging resistance in wheat.

3.7. Path relationships between lodging-related and yield parameters

PLS-PM described associations among seeding rate, Pro-Ca, plant morphology, internode structure, lodging, and yield (Fig. 12). Increasing the seeding rate primarily elevated lodging risk (total effect = 0.342) by significantly promoting the longitudinal growth of wheat ($\beta = 0.42$, $P < 0.001$), while exerting a relatively small positive effect on the yield (total effect = 0.077). In contrast, Pro-Ca enhanced lodging resistance and increased yield through the coordinated optimization of plant architecture and biomass production. It significantly reduced longitudinal stem traits ($\beta = -0.53$, $P < 0.001$, $R^2 = 0.58$), while promoting transverse stem traits ($\beta = 0.25$, $P = 0.002$, $R^2 = 0.62$), microstructural reinforcement ($\beta = 0.31$, $P < 0.001$, $R^2 = 0.68$), and internode compound accumulation ($\beta = 0.38$, $P < 0.001$, $R^2 = 0.55$), thereby significantly reducing lodging risk (total effect = -0.600) and increasing yield (total effect = 0.270). Internode compound content was identified as the most important protective factor against lodging ($\beta = -0.31$, $P < 0.001$, $R^2 = 0.68$), whereas longitudinal plant traits were the primary risk factors for lodging, exhibiting the strongest direct effect on LI ($\beta = 0.68$, $P < 0.001$, $R^2 = 0.71$). In addition, the yield pathway ($R^2 = 0.78$) indicated that improving lodging resistance is an important pathway for increasing the grain yield. Therefore, under an appropriate seeding rate, the foliar application of Pro-Ca can synergistically enhance wheat lodging resistance and yield.

4. Discussion

4.1. Increasing seeding rate can achieve high yields in late-sown wheat, but it is accompanied by lodging risk

Owing to an ecological niche mismatch, late-sown wheat experiences misalignment between its growth stages and prevailing climatic conditions. This is evidenced by a low tiller-to-spike conversion rate, severe degeneration of young spikes, and impaired grain filling, ultimately resulting in reduced spike number, grain number per spike, and grain weight, thereby causing yield loss [5, 29]. Compared with other yield-improvement strategies, such as film mulching or soil warming to protect seedlings, biochar-based pelletized seed treatment to improve seedling growth and cold resistance, early nitrogen application to promote tillering, and increased irrigation to enhance tiller spike formation, increasing the seeding rate to improve the productive spike number is a simpler and more reliable approach for compensating for spike number loss under late-sowing conditions and maintaining high grain yields [7, 30]. In this study, the yield of late-sown wheat during the 2023–2024 growing season was significantly higher than that of the 2024–2025, which was likely attributable to less favorable climatic conditions in the latter season (Fig. 1). Specifically, the 2024–2025 growing season was characterized by relatively low overall precipitation, an early onset of low temperatures before winter, and insufficient snow cover, which increased overwinter mortality. In addition, the rapid rise in spring temperatures inhibited tillering and young spike differentiation, thereby contributing to the lower yield (Table 2). Nevertheless, data from both growing seasons consistently demonstrated that increasing the seeding rate increased the number of spikes per unit area, resulting in improved grain yield, with the highest yield observed under the M2 treatment in both years. However, an inherent trade-off exists between late sowing and increased seeding rates in terms of lodging resistance. Late-sown wheat generally exhibits greater lodging resistance owing to a shortened growth period, reduced vegetative plant size, lower plant height, and shortened internode length [27]. However, the yield loss caused by late sowing is commonly compensated for by increasing the seeding rate, a high-density planting strategy that intensifies interplant competition for light, water, and nutrients (Tian *et al.* 2025).

This competition promotes excessive elongation of the basal internodes while reducing their diameter and wall thickness, thereby weakening stem quality and increasing lodging risk [10, 31, 32]. Moreover, previous studies have demonstrated that excessively high seeding rates result in excessive population density, intensifying interplant competition and inhibiting plant development, thereby reducing key yield components, such as grain number per spike and 1000-grain weight, ultimately limiting yield [33]. Consistent with these findings, this study revealed that when the seeding rate increased to the M3 level, the spike number increased significantly; however, the balance among spike number, grain number per spike, and 1000-grain weight was disrupted, resulting in a lower rather than higher yield (Table 2). In addition, under the experimental conditions, a comprehensive analysis of the relevant data indicated that the positive effect of seeding rate on yield was relatively limited (Fig. 11). This may have resulted from the relatively high seeding rates evaluated in this experiment, which restricted the realization of yield potential in late-sown wheat. Therefore, increasing the seeding rate can effectively compensate for the reduction in spike numbers caused by late sowing. However, excessive seeding rates reduce grain number per spike and 1000-grain weight while increasing lodging risk. Overall, M2 treatment (450 kg ha^{-1}) effectively balanced the trade-off between spike formation and individual plant development, representing an optimal cultivation strategy for achieving high and stable yields in late-sown wheat.

4.2. Spraying Pro-Ca restores the quality of individual plants in densely planted wheat and improves lodging resistance

The rational application of plant growth regulators can not only optimize stem structural traits and enhance lodging resistance in wheat under high seeding rates but also improve yield components, thereby synergistically increasing the crop yield [34, 35]. Previous studies have confirmed that uniconazole strengthens stem mechanical strength and alleviates lodging stress by reducing PH and ear height while increasing ID and stem fullness [36]. Furthermore,

spraying a Pro-Ca–uniconazole composite regulator increased LC and CC in rice stems, thereby enhancing lodging resistance [37].In this study, Pro-Ca application was associated with improved stem-breaking strength (Fig. 8), lodging-related traits (Table 1, Fig. 9), stem morphology (Figs. 3 and 4), and structural compound contents (Fig. 7). Similar to the effects of chlormequat and gibberellin [15], Pro-Ca can increase the number of grains per spike and 1000-grain weight in wheat, thereby improving yield [38]. Consistent with these findings, this study showed that spraying Pro-Ca synergistically increased the spike number, grain number per spike, and 1000-grain weight, thereby improving the wheat yield (Table 2). Notably, owing to climatic differences (Fig. 1), the baseline population size in the 2023–2024 growing season was significantly higher than that in the 2024–2025, as reflected by the harvested spike number (Table 2). Unfortunately, lodging occurred only during the 2023–2024 growing season, resulting in yield loss. The larger population size, combined with frequent strong winds and heavy rainfall after anthesis (Fig. 1), was likely the primary cause of lodging in that season. Nevertheless, yields across all treatments in 2023–2024 remained higher than those in 2024–2025, suggesting that Pro-Ca application may exert a more pronounced yield-enhancing effect under larger wheat populations. However, excessive application of plant growth regulators can interfere with normal growth, disrupt the balance of yield components, and ultimately reduce yield [36, 39]. For example, Wang et al. (2015) demonstrated that high concentrations of uniconazole negatively affected yield formation in alfalfa, resulting in a reduced yield. In this study, when the Pro-Ca application rate increased to C3, it inhibited the development of stem tissues (Fig. 5) and vascular bundles (Fig. 6), suppressed the synthesis of LC and CC (Fig. 7), reduced the LRI (Fig. 9), and negatively affected wheat yield components, ultimately leading to reduced yield. Therefore, an appropriate Pro-Ca application rate can effectively restore stem structural quality in high-density wheat populations, thereby establishing a structural basis for improved lodging resistance. In addition, the observed improvements in yield components may

be related to these changes in plant development and stem traits. This study indicates that the C2 concentration of Pro-Ca (1200 g ha^{-1}) provided a favorable balance between lodging-related traits and yield under the tested conditions.

4.3. Rational matching of increased seeding rate and Pro-Ca can optimize stem anatomical structure and synergistically improve lodging resistance and yield in late-sown wheat

Stem tissue thickness and the number of vascular bundles are key factors in enhancing lodging resistance and yield formation [37]. This study confirmed that increasing the seeding rate significantly reduced both MTT and the number of vascular bundles (Figs. 5 and 6), which may explain why high seeding rates increased lodging risk and reduced yield in late-sown wheat. In contrast, Pro-Ca application was associated with a reversal of this trend, possibly through improvements in stem structure, including increased tissue thickness and vascular bundle number (Figs. 6 and 6). The underlying mechanism may be that well-developed tissue thickness and a dense vascular bundle system provide a strong mechanical support framework, thereby enhancing lodging resistance [40]. In addition, an improved vascular network facilitates nutrient transport and the distribution of photosynthates, thereby increasing the number of grains per spike and 1000-grain weight, ultimately supporting yield improvement [41, 42]. In this study, increasing the seeding rate represented population-level regulation, whereby yield loss in late-sown wheat was compensated by increasing population density. In contrast, Pro-Ca application regulated at the individual-plant level improved stem morphology to enhance lodging resistance and nutrient translocation efficiency. This, in turn, promoted a higher grain number per spike and 1000-grain weight per plant. Although the interaction between seeding rate and Pro-Ca treatment was not significant, their coordinated and complementary effects were evident because they acted through different regulatory pathways. A comprehensive analysis showed that, under the conditions of this experiment, M2C2 (450 kg ha^{-1} seeding rate

and 1200 g ha^{-1} Pro-Ca) combined high yield with improved lodging-related traits in this experiment. This combination warrants further evaluation as a management option for late-sown wheat in Xinjiang. It is noteworthy that the wheat in this study was sown exceptionally late, reaching only a one-leaf stage before winter. Combined with the severe winter cold and rapid spring warming in northern Xinjiang, this results in high overwinter mortality and a substantial reduction in tiller-derived spikes. Under these circumstances, adopting a relatively high seeding rate effectively compensated for the insufficient number of harvested spikes by increasing the number of main stem spikes. In contrast, in major wheat-producing regions, such as the Huang-Huai-Hai area, where winters are milder and springs are more humid, late-sown wheat typically experiences low overwinter mortality, greater tiller survival, and spike formation. Therefore, density regulation strategies in these regions should be designed cautiously. Although the seeding rate may be moderately increased, excessively high rates should be avoided.

5. Conclusion

Under the tested conditions, the combined treatment of a seeding rate of 450 kg ha^{-1} and foliar Pro-Ca application at 1200 g ha^{-1} provided a favorable balance between yield and lodging resistance. This combination improved stem mechanical strength, increased grain number per spike and 1000-grain weight, and enhanced both lodging resistance and wheat yield. Furthermore, the findings support further evaluation of this combination in late-sown wheat, with validation across different sites and cultivars required before wider application.

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Author contributions

Wenqiang Tian: Investigation, Writing-Original draft preparation; **Shan Yu:** Writing-Review and editing; **Jiayu Wang:** Writing-Review and editing; **Qiangbin Zhang:** Investigation, Resources; **Fan Cheng:** Investigation, Resources; **Zhilin Zhang：** Visualization; **Guangzhou Chen:** Software; **Weijun Yang:** Conceptualization; **Shubing Shi:** Conceptualization, Resources; **Jinshan Zhang:** Conceptualization, Supervision; **Jianhua Wang:** Supervision.

Conflict of interest

The authors have no competing interests to declare that are relevant to the content of this article.

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Data availability

Data will be made available on request.

References

1. Dong J, Lu H, Wang Y, Ye T, Yuan W. Estimating winter wheat yield based on a light use efficiency model and wheat variety data. ISPRS journal of photogrammetry and remote sensing. 2020;160:18-32.

2. Liu K, Harrison MT, Wang B, Yang R, Yan H, Zou J, et al. Designing high-yielding wheat crops under late sowing: a case study in southern China: Designing high yielding wheat crops under late sowing: a case study in southern China. Agronomy for Sustainable Development. 2022;42:29.
3. Rezaie B, Hosseinpanahi F, Siosemardeh A, Darand M, Bannayan M. Shifting the sowing date of winter wheat as a strategy for adaptation to climate change in a Mediterranean-type environment. International Journal of Plant Production. 2022;16:595-610.
4. Tang K. Agricultural adaptation to the environmental and social consequences of climate change in mixed farming systems: Evidence from North Xinjiang, China. Agricultural Systems. 2024;217:103913.
5. Tian W, Chen G, Zhang Q, Zhang Z, Zhang J, Yu S, et al. Can Increased Density Compensate for Extremely Late-Sown Wheat Yield? Agronomy. 2025;15:607.
6. Yin L, Dai X, He M. Delayed sowing improves nitrogen utilization efficiency in winter wheat without impacting yield. Field crops research. 2018;221:90-97.
7. Li C, Li W, Luo Y, Jin M, Chang Y, Cui H, et al. Mixed cropping increases grain yield and lodging resistance by improving the canopy light environment of wheat populations. European Journal of Agronomy. 2023;147:126849.
8. Zheng F, Chu J, Guo X, Zhang X, Ma J, He M, et al. Combining wide seedling strip planting with a higher plant density results in greater yield gains in winter wheat. Annals of Agricultural Sciences. 2024;69:1-10.
9. Berry PM, Sterling M, Spink JH, Baker CJ, Sylvester-Bradley R, Mooney SJ, et al. Understanding and reducing lodging in cereals. Advances in agronomy. 2004;84:215-269.

10. Easson DL, White EM, Pickles SJ. The effects of weather, seed rate and cultivar on lodging and yield in winter wheat. The Journal of Agricultural Science. 1993;121:145-156.
11. Shah AN, Tanveer M, Rehman AU, Anjum SA, Iqbal J, Ahmad R. Lodging stress in cereal—effects and management: an overview. Environmental Science and Pollution Research. 2017;24:5222-5237.
12. Zhang H-J, Teng LI, Li Y, Yang Z. Genetic progress in stem lodging resistance of the dominant wheat cultivars adapted to Yellow-Huai River Valleys Winter Wheat Zone in China since 1964. Journal of Integrative Agriculture. 2020;19:438-448.
13. Rashid MAR, Zhao Y, Azeem F, Zhao Y, Ahmed HGM-D, Atif RM, et al. Unveiling the genetic architecture for lodging resistance in rice (Oryza sativa. L) by genome-wide association analyses. Frontiers in Genetics. 2022;13:960007.
14. Wang C, Ruan R, Yuan X, Hu D, Yang H, Li Y, et al. Relationship between lignin metabolism and lodging resistance of culm in buckwheat. Journal of Agricultural Science. 2014;6:29.
15. Zhang JC, Zhang YQ, Xu QJ, Lei JJ, Chen CX, Li J, et al. Optimising the Morphology and Quality of Basal Stem Nodes to Improve Wheat Lodging Resistance and Yield. Journal of Agronomy and Crop Science. 2025;211:e70118.
16. Jiang AN, Yan JQ, Lu HB, Zhao HC, Huang ZH. Response of stem microstructure of different spring maize varieties to bending strength. J Maize Sci. 2020;28:53-59.
17. Lv R, Zhang W, Xie X, Wang Q, Gao K, Zeng Y, et al. Foliar application uniconazole enhanced lodging resistance of high-quality indica rice (Oryza sativa L.) by altering anatomical traits, cell structure and endogenous hormones. Field Crops Research. 2022;277:108425.

582 18. Shah L, Yahya M, Shah SMA, Nadeem M, Ali A, Ali A, et al. Improving lodging
583 resistance: using wheat and rice as classical examples. International journal of
584 molecular sciences. 2019;20:4211.
585 19. Chen L, Yi Y, Wang W, Zeng Y, Tan X, Wu Z, et al. Innovative furrow ridging
586 fertilization under a mechanical direct seeding system improves the grain yield and
587 lodging resistance of early indica rice in South China. Field Crops Research.
588 2021;270:108184.
589 20. Li D, Yang J, Dai Z, Chen Y, Shao Z, Wang C, et al. Prohexadione-calcium improves
590 grape quality by regulating endogenous hormones, sugar and acid metabolism and
591 related enzyme activities in grape berries. BMC Plant Biology. 2024;24:122.
592 21. Stachecki S, Praczyk T, Adamczewski K. Adjuvant effects on plant growth regulators
593 in winter wheat. Journal of Plant Protection Research. 2004;44.
594 22. Liao P, Bell SM, Chen L, Huang S, Wang H, Miao J, et al. Improving rice grain yield
595 and reducing lodging risk simultaneously: A meta-analysis. European Journal of
596 Agronomy. 2023;143:126709.
597 23. Zhang R, Zheng D, Feng N, Qiu Q-S, Zhou H, Liu M, et al. Prohexadione calcium
598 enhances rice growth and tillering under NaCl stress. PeerJ. 2023;11:e14804.
599 24. Xia K, Hu P, Wu G, He X, Li Y, Li Y, et al. Prohexadione calcium boosts pepper stem
600 rigidity: coordinated regulation of hormonal crosstalk and lignin deposition drives
601 xylem reinforcement. BMC Plant Biology. 2025;25:1225.
602 25. Hamano M, Yamato Y, Yamazaki H, Miura H. Endogenous gibberellins and their effects
603 on flowering and stem elongation in cabbage (Brassica oleracea var. capitata). The
604 Journal of Horticultural Science and Biotechnology. 2002;77:220-225.
605 26. Thomidis T, Zioziou E, Koundouras S, Navrozidis I, Nikolaou N. Effect of
606 prohexadione-Ca on leaf chlorophyll content, gas exchange, berry size and composition,

607 wine quality and disease susceptibility in Vitis vinifera L. cv Xinomavro. Scientia
608 Horticulturae. 2018;238:369-374.
609 27. Cui J, Cui Z, Lu Y, Lv X, Cao Q, Hou Y, et al. Maize grain yield enhancement in modern
610 hybrids associated with greater stalk lodging resistance at a high planting density: a
611 case study in northeast China. Scientific Reports. 2022;12:14647.
612 28. MARA(Ministry of Agriculture and Rural Affairs of China). Technical Regulations for
613 Regional Trials of Crop Varieties: Wheat. Beijing, China: Ministry of Agriculture of the
614 People's Republic of China; 2007.
615 29. Dreccer MF, Chapman SC, Rattey AR, Neal J, Song Y, Christopher JT, et al.
616 Developmental and growth controls of tillering and water-soluble carbohydrate
617 accumulation in contrasting wheat (Triticum aestivum L.) genotypes: can we dissect
618 them? Journal of experimental botany. 2013;64:143-160.
619 30. Lollato RP, Ruiz Diaz DA, DeWolf E, Knapp M, Peterson DE, Fritz AK. Agronomic
620 practices for reducing wheat yield gaps: a quantitative appraisal of progressive
621 producers. Crop Science. 2019;59:333-350.
622 31. Wang X, Zhang J, Wang X, Hu Y, Ren X, Jia Z, et al. Non-uniform wheat population
623 distribution enhances wheat yield and lodging resistance synchronously. European
624 Journal of Agronomy. 2024;152:127033.
625 32. Zheng M, Chen J, Shi Y, Li Y, Yin Y, Yang D, et al. Manipulation of lignin metabolism
626 by plant densities and its relationship with lodging resistance in wheat. Scientific
627 Reports. 2017;7:41805.
628 33. Bastos LM, Carciochi W, Lollato RP, Jaenisch BR, Rezende CR, Schwalbert R, et al.
629 Winter wheat yield response to plant density as a function of yield environment and
630 tillering potential: A review and field studies. Frontiers in plant science. 2020;11:54.

631 34. Kamran M, Ahmad I, Wu X, Liu T, Ding R, Han Q. Application of paclobutrazol: a
632 strategy for inducing lodging resistance of wheat through mediation of plant height,
633 stem physical strength, and lignin biosynthesis. Environmental Science and Pollution
634 Research. 2018;25:29366-29378.
635 35. Voronov SI, Vlasova OI, Shtyrkhunov VD, Govorkova SB, Savinov EV. The effect of
636 growth regulators with retardant properties on the growth and development of winter
637 wheat. 1 ed: IOP Publishing; 2021. p. 012022.
638 36. Ahmad I, Kamran M, Ali S, Bilegjargal B, Cai T, Ahmad S, et al. Uniconazole
639 application strategies to improve lignin biosynthesis, lodging resistance and production
640 of maize in semiarid regions. Field Crops Research. 2018;222:66-77.
641 37. Ma D, Ding Y, Liu Y, Du L, Mo Y, Dai A, et al. Optimizing application strategies of
642 prohexadione-calcium· uniconazole composite regulator to improve lodging resistance
643 and grain yield in rice cultivation in East China. Field Crops Research.
644 2025;328:109931.
645 38. Peng C, Guo Y, Huang G, Liu Y, Li Z, Zhou Y, et al. Effects of a novel plant growth
646 regulator B2 on stalk quality and grain yield of winter wheat in North China. Plant and
647 Soil. 2023;487:437-453.
648 39. Wang C, Hu D, Liu X, She H, Ruan R, Yang H, et al. Effects of uniconazole on the
649 lignin metabolism and lodging resistance of culm in common buckwheat (Fagopyrum
650 esculentum M.). Field Crops Research. 2015;180:46-53.
651 40. Yang Y, Mu J, Hao X, Yang K, Cao Z, Feng J, et al. Identification and analysis of the
652 mechanism of stem mechanical strength enhancement for maize inbred lines QY1.
653 International Journal of Molecular Sciences. 2024;25:8195.
654 41. Housley TL, Peterson DM. Oat Stem Vascular Size in Relation to Kernel Number and
655 Weight. I. Controlled Environment 1. Crop Science. 1982;22:259-263.

42. Zhang G, Cui K, Li G, Pan J, Huang J, Peng S. Stem small vascular bundles have greater accumulation and translocation of non-structural carbohydrates than large vascular bundles in rice. Physiologia plantarum. 2022;174:e13695.

Tables

Table 1 Effects of seeding rate and Pro-Ca concentration on wheat lodging

Seeding rate	Pro-Ca	2023-2024 Anthesis			2023-2024 Milk stage		
		Lodging rate	Lodging score	LI	Lodging rate	Lodging score	LI
M1	C0	4.88%	3	0.1464	42.13%	4	1.6852
	C1	3.60%	2	0.0720	19.73%	4	0.7892
	C2	0.23%	2	0.0046	3.00%	2	0.0600
	C3	0%	1	0	0%	1	0
M2	C0	15.13%	4	0.6052	54.58%	4	2.1828
	C1	7.90%	4	0.3160	45.30%	4	1.8120
	C2	1.10%	3	0.0330	11.30%	3	0.3390
	C3	0%	1	0	4.45%	2	0.0890
M3	C0	30.43%	5	1.5215	65.13%	5	3.2565
	C1	18.38%	5	0.9190	58.90%	5	2.9450
	C2	7.18%	3	0.2154	33.53%	4	1.3412
	C3	3.70%	2	0.0740	20.78%	3	0.6234

Note: M1, M2, and M3 represent seeding rates of 360, 450, and 540 kg ha^{-1}, respectively. C0, C1, C2, and C3 represent Pro-Ca application rates of 0, 600, 1200, and 1800 g ha^{-1}, respectively.

Table 2 Effects of seeding rate and Pro-Ca concentration on wheat yield and yield components

Seeding rate	Pro-Ca	2023-2024				2024-2025			
		Spikes number (10^4 ha^{-1})	Grain number per spike	1000-grain weight（g）	Yield (kg ha^{-1})	Spikes number (10^4 ha^{-1})	Grain number per spike	1000-grain weight（g）	Yield (kg ha^{-1})
M1	C0	693.67^{c}	39.42^{b}	39.67^{b}	7280.29^{c}	676.50^{b}	30.63^{b}	46.13^{b}	5956.15^{b}
	C1	755.83^{a}	39.89^{b}	40.30^{a}	7848.44^{b}	739.75^{a}	35.58^{a}	46.38^{b}	6727.21^{ab}
	C2	758.61^{a}	40.19^{a}	40.78^{a}	8712.75^{a}	746.00^{a}	35.65^{a}	46.64^{a}	6848.73^{a}
	C3	733.33^{b}	39.47^{b}	39.90^{b}	8150.00^{b}	715.50^{a}	34.70^{a}	46.25^{b}	6529.67^{ab}
Average		735.36^{c}	39.74^{a}	40.16^{a}	7997.87^{b}	719.44^{c}	34.14^{a}	46.35^{a}	6515.44^{b}
M2	C0	814.79^{b}	36.36^{b}	38.24^{b}	7268.10^{c}	793.50^{a}	28.45^{b}	45.14^{b}	6042.46^{c}
	C1	849.17^{a}	37.58^{ab}	39.18^{ab}	8823.13^{a}	820.25^{a}	31.08^{ab}	45.86^{ab}	7058.65^{a}
	C2	850.28^{a}	38.78^{a}	39.90^{a}	8870.50^{a}	824.25^{a}	31.70^{a}	46.06^{a}	7115.00^{a}
	C3	833.61^{ab}	37.92^{ab}	39.10^{ab}	8134.33^{b}	813.50^{a}	29.95^{ab}	45.58^{ab}	6890.13^{b}
Average		836.96^{b}	37.66^{b}	39.10^{b}	8274.02^{a}	812.88^{b}	30.29^{b}	45.66^{b}	6776.56^{a}
M3	C0	916.17^{ab}	32.97^{b}	37.91^{b}	6962.73^{b}	877.50^{ab}	24.60^{a}	44.46^{b}	5862.15^{c}
	C1	919.04^{ab}	34.61^{a}	38.32^{a}	7048.19^{b}	896.75^{a}	26.93^{a}	45.18^{ab}	6121.56^{b}
	C2	939.93^{a}	35.31^{a}	39.07^{a}	8388.13^{a}	904.00^{a}	27.30^{a}	45.73^{a}	6526.10^{a}
	C3	898.96^{b}	34.17^{ab}	38.17^{a}	7837.56^{a}	864.75^{b}	25.28^{a}	45.10^{ab}	6535.10^{a}

Average		918.52^{a}	34.26^{c}	38.37^{c}	7559.15^{c}	885.75^{a}	26.03^{c}	45.12^{c}	6261.23^{c}
	M	46.26**	110.67**	24.08**	12.41**	26.65**	74.24**	19.12**	8.94**
F	C	7.45**	6.23**	3.86**	13.69**	10.72**	9.21**	5.26**	16.83**
	M×C	0.29^{ns}	0.68^{ns}	0.12^{ns}	0.31*	1.72^{ns}	0.68^{ns}	0.37^{ns}	0.32*

Note: Different lowercase letters indicate significant differences at $P < 0.05$. M1, M2, and M3 represent seeding rates of 360, 450, and 540 kg ha^{-1}, respectively. C0, C1, C2, and C3 represent Pro-Ca application rates of 0, 600, 1200, and 1800 g ha^{-1}, respective.

Figure legends

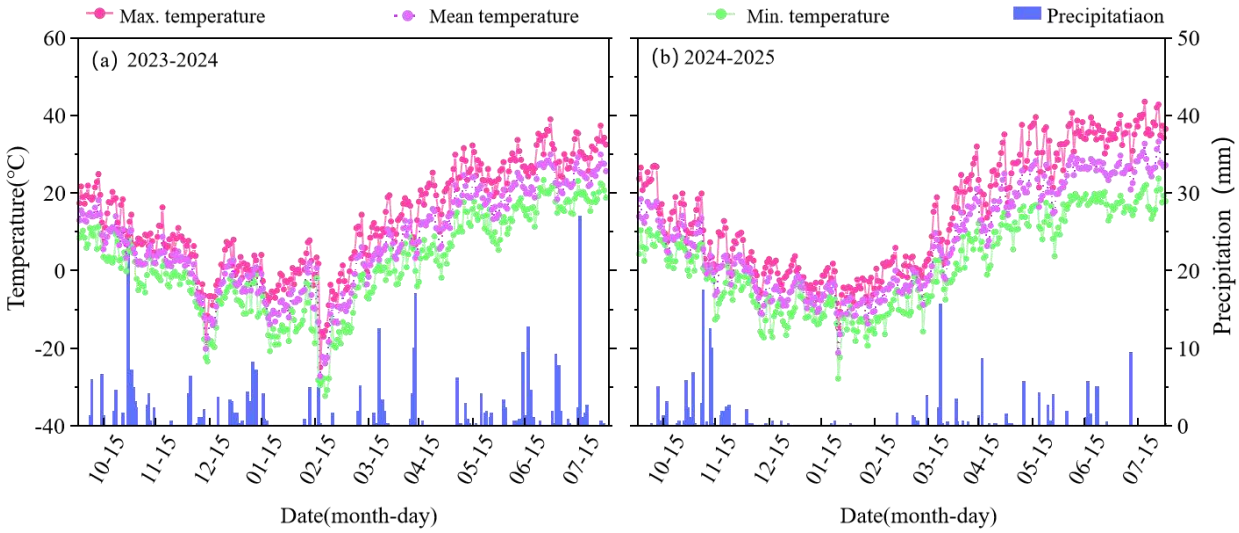


Fig. 1 Meteorological variables during the wheat growing season at the experimental site from 2023 to 2025

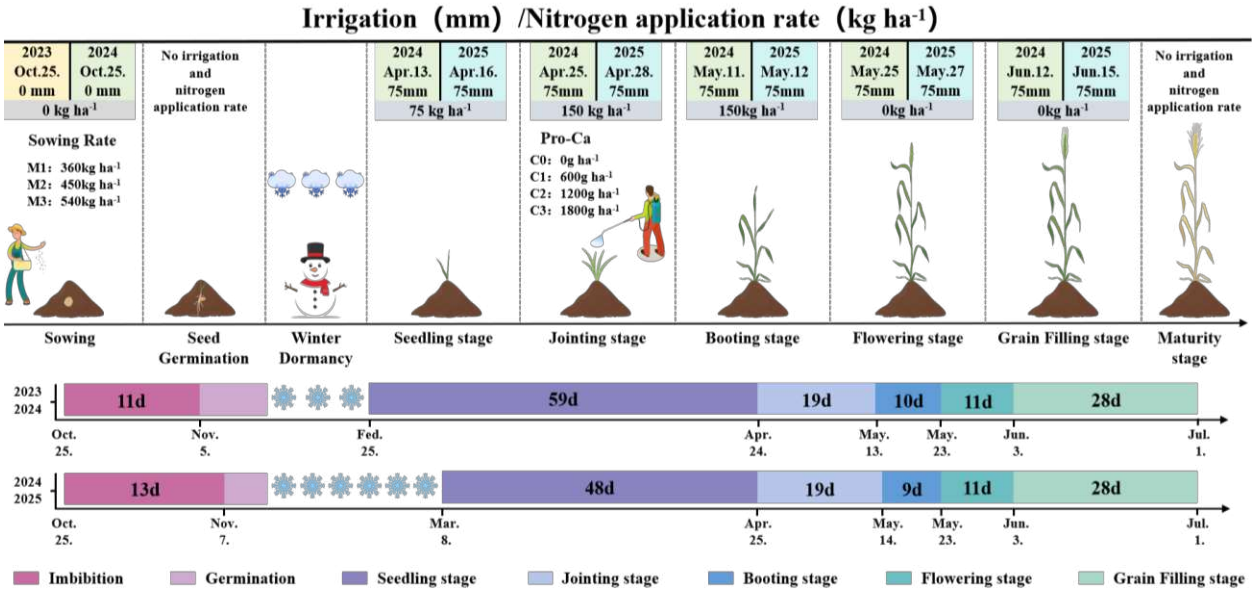


Fig. 2 Experimental layout

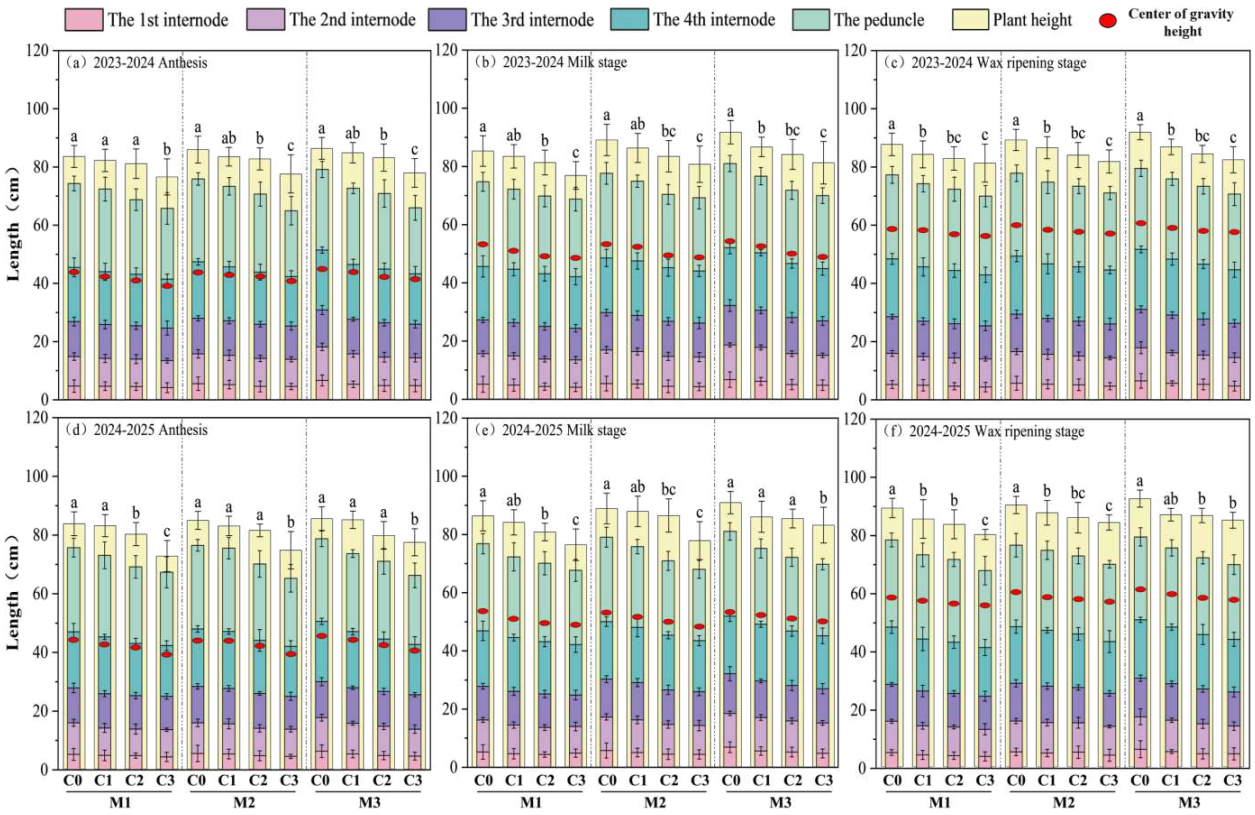


Fig. 3 Effects of seeding rate and Pro-Ca concentration on wheat IL, PH, and CGH

Note: Different lowercase letters indicate significant differences between treatments at $P < 0.05$, and vertical bars represent the standard error of the mean. M1, M2, and M3 represent seeding rates of 360, 450, and 540 kg ha^{-1}, respectively. C0, C1, C2, and C3 represent Pro-Ca application rates of 0, 600, 1200, and 1800 g ha^{-1}, respectively

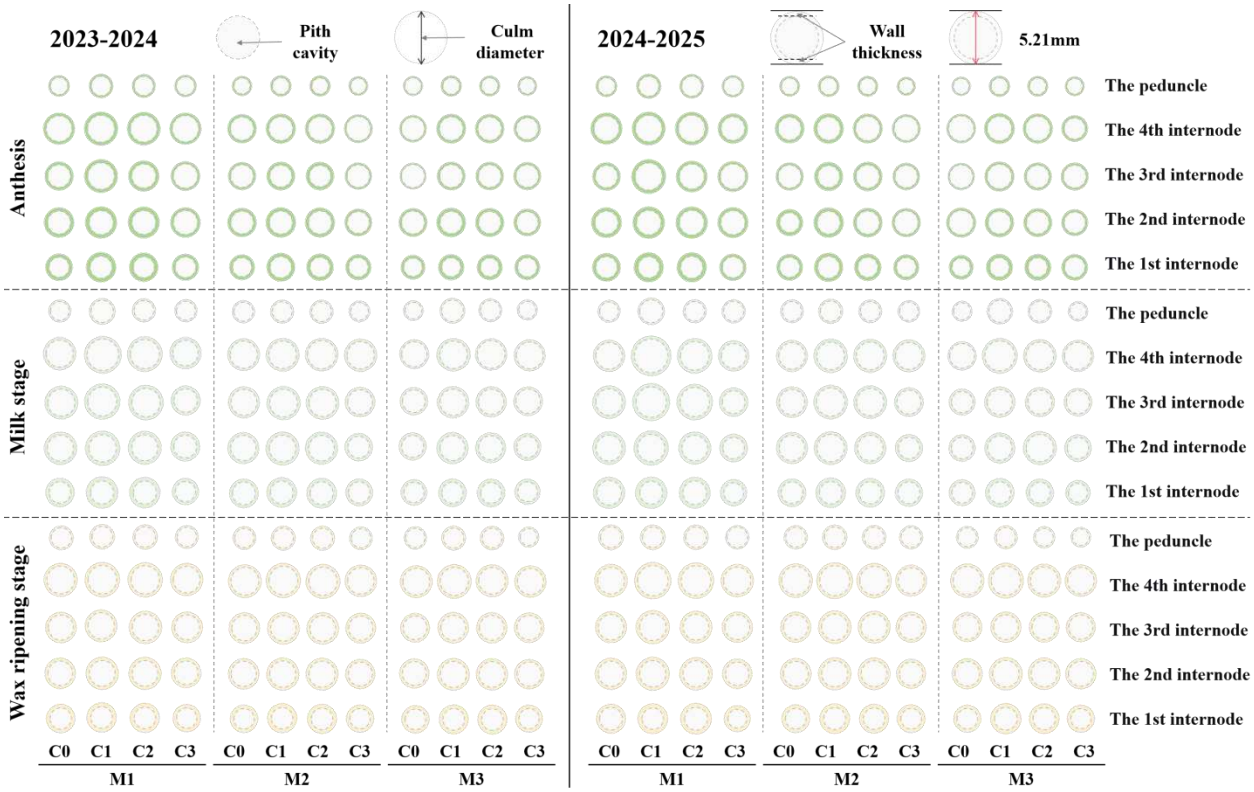


Fig. 4 Effects of seeding rate and Pro-Ca concentration on wheat ID and wall thickness

Note: ID and wall thickness are expressed in millimeters (mm), respectively. This figure was drawn using proportional scaling based on the actual measured values of wheat stem ID and wall thickness to reflect typical cross-sectional structural characteristics. M1, M2, and M3 represent seeding rates of 360, 450, and 540 kg ha^{-1}, respectively. C0, C1, C2, and C3 represent Pro-Ca application rates of 0, 600, 1200, and 1800 g ha^{-1}, respectively

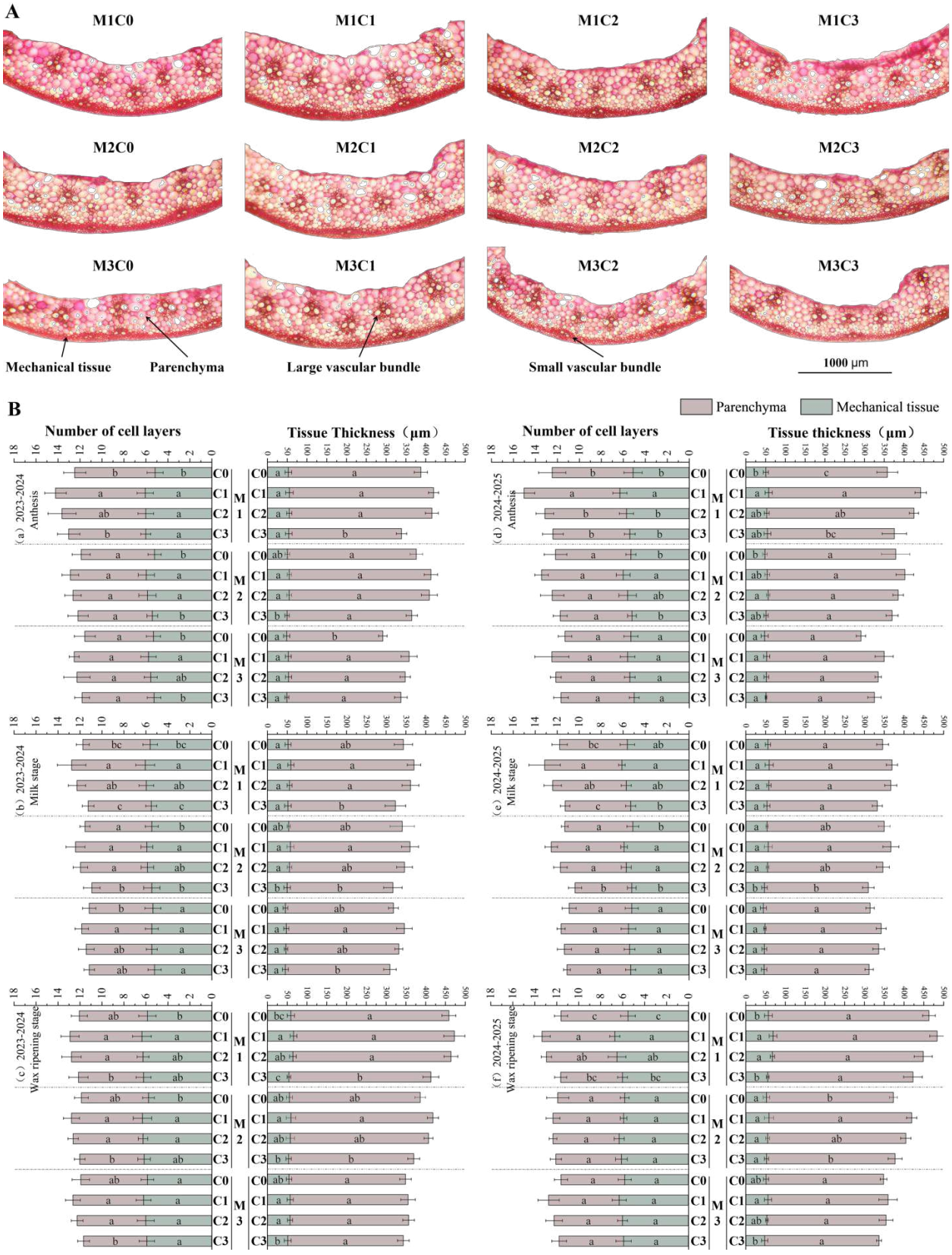


Fig. 5 Effects of seeding rate and Pro-Ca concentration on tissue composition in wheat internode cross-sections

Note: Panel A shows an anatomical micrograph of the second internode at the grain-filling stage in the 2023–2024 wheat growing season. In Panel B, tissue-related traits were measured in the second basal internode of the wheat. Different lowercase letters indicate significant differences at $P < 0.05$, and horizontal bars represent the standard error of the mean. M1, M2, and M3 represent seeding rates of 360, 450, and 540 kg ha^{-1}, respectively. C0, C1, C2, and C3 represent Pro-Ca application rates of 0, 600, 1200, and 1800 g ha^{-1}, respectively

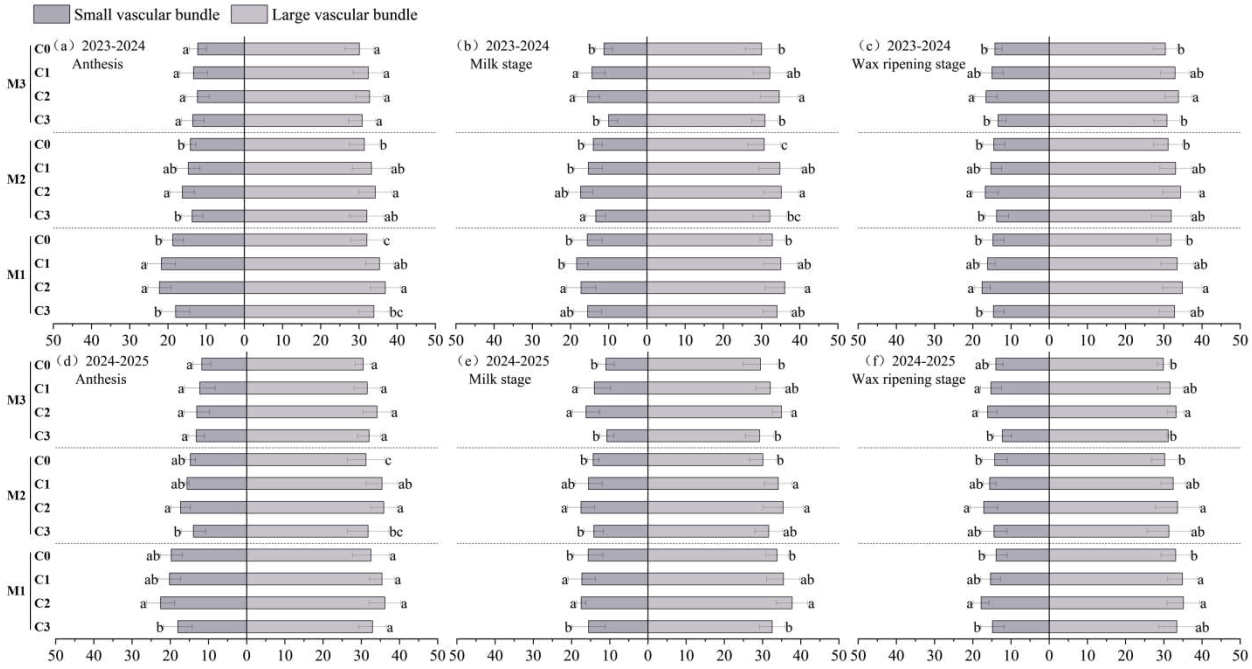


Fig. 6 Effects of seeding rate and Pro-Ca concentration on large and small vascular bundles in wheat internode cross-sections

Note: The number of vascular bundles in the figure was measured in the second basal internode of wheat, and the morphology of the large and small vascular bundles is shown in Fig. 6A. Different lowercase letters indicate significant differences in the number of vascular bundles at $P < 0.05$, and horizontal bars represent the standard

error of the mean. M1, M2, and M3 represent seeding rates of 360, 450, and 540 kg ha^{-1}, respectively. C0, C1, C2, and C3 represent Pro-Ca application rates of 0, 600, 1200, and 1800 g ha^{-1}, respectively

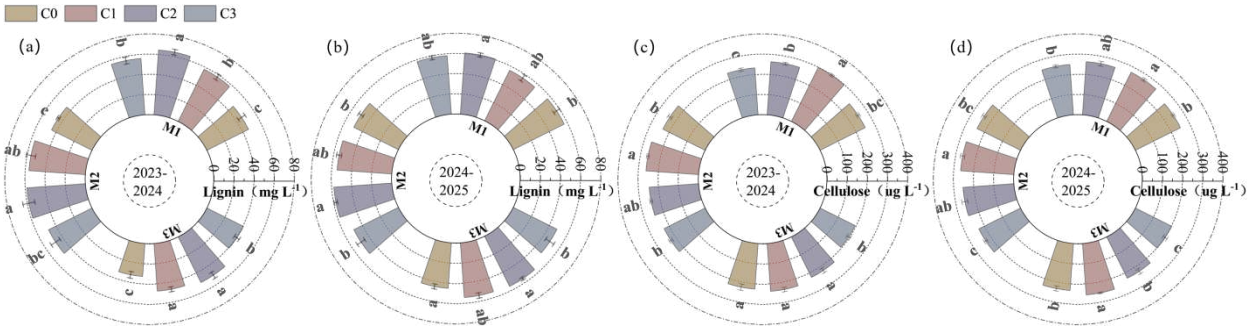


Fig. 7 Effects of seeding rate and Pro-Ca concentration on lignin and cellulose levels in wheat internodes

Note: LC and CC in the figure were determined for the second basal internode of wheat. Different lowercase letters indicate significant differences between treatments at $P < 0.05$, and bars represent the standard error of the mean. M1, M2, and M3 represent seeding rates of 360, 450, and 540 kg ha-1, respectively. C0, C1, C2, and C3 represent Pro-Ca application rates of 0, 600, 1200, and 1800 g ha-1, respectively

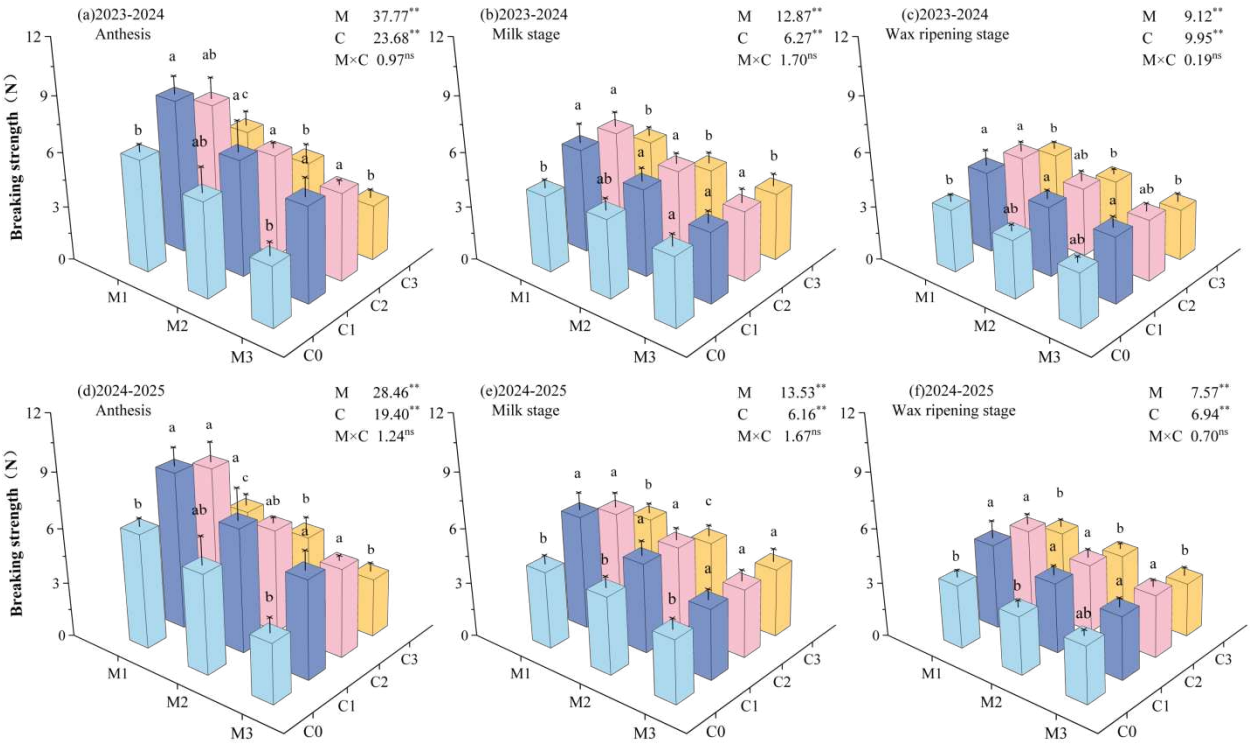


Fig. 8 Effects of seeding rate and Pro-Ca concentration on breaking strength of wheat internodes

Note: Breaking strength shown in the figure was measured at the second basal internode of wheat. Different lowercase letters indicate significant differences between treatments at $P < 0.05$, and vertical bars represent the standard error of the mean. M1, M2, and M3 represent seeding rates of 360, 450, and 540 kg ha^{-1}, respectively. C0, C1, C2, and C3 represent Pro-Ca application rates of 0, 600, 1200, and 1800 g ha^{-1}, respectively

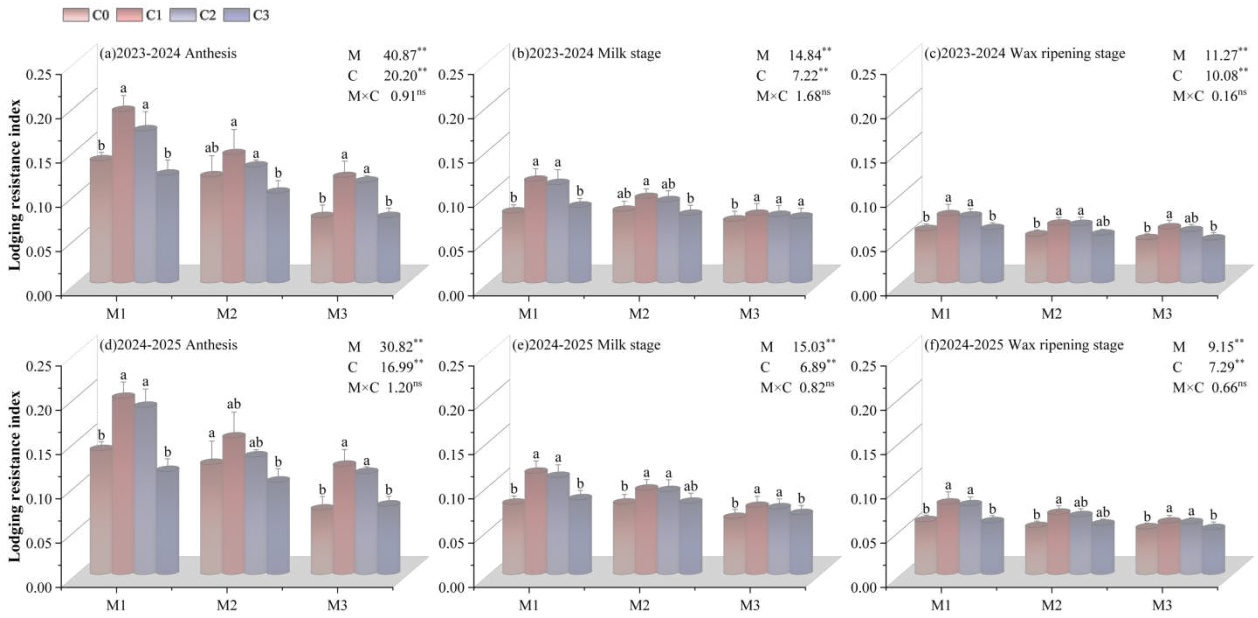


Fig. 9 Effects of seeding rate and Pro-Ca concentration on wheat LRI.

Note: The LRI shown in the figure was calculated using the second basal internode of wheat. Different lowercase letters indicate significant differences between treatments at $P < 0.05$, and vertical bars represent the standard error of the mean. M1, M2, and M3 represent seeding rates of 360, 450, and 540 kg ha^{-1}, respectively. C0, C1, C2, and C3 represent Pro-Ca application rates of 0, 600, 1200, and 1800 g ha^{-1}, respectively

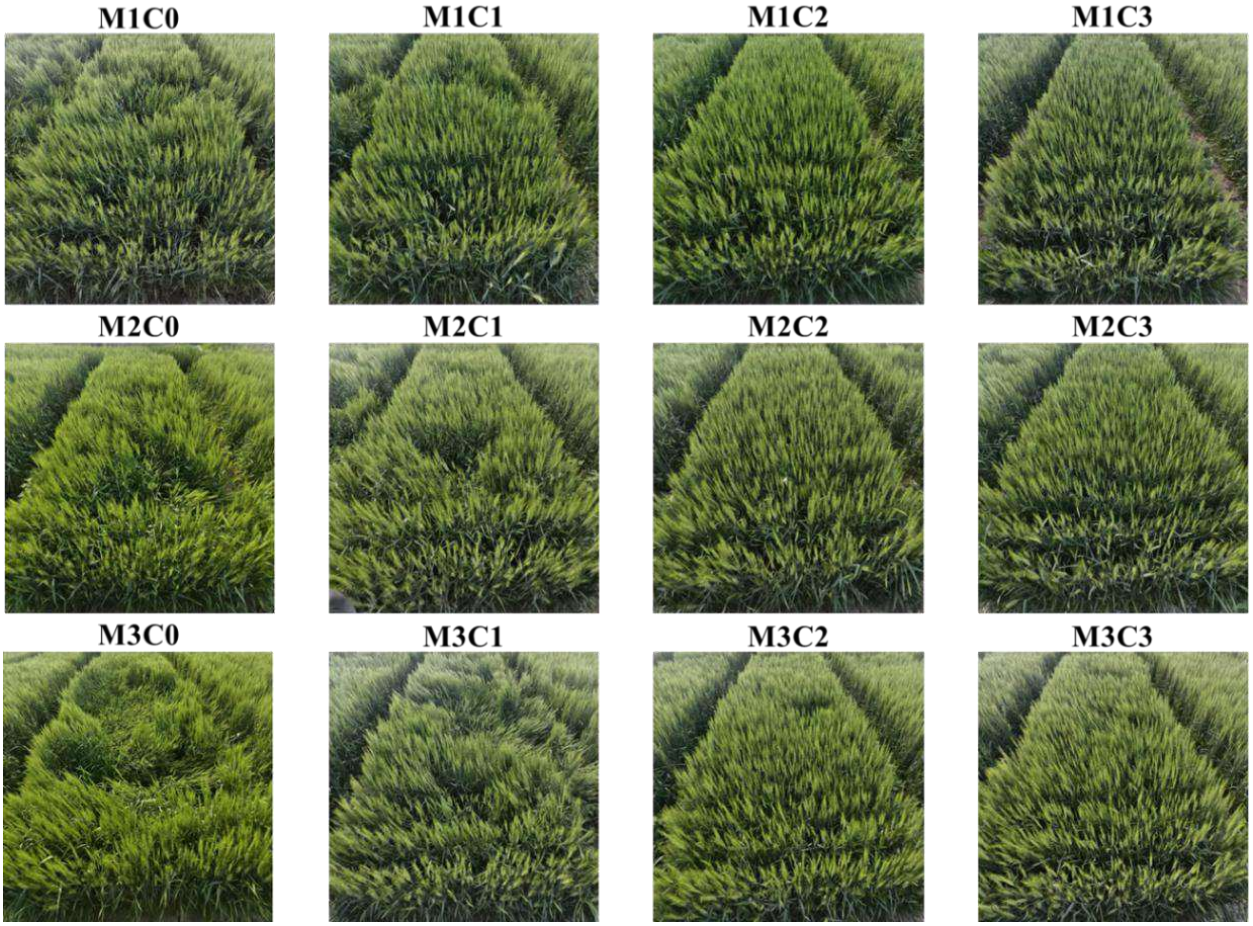


Fig. 10 Effects of seeding rate and Pro-Ca concentration on actual field lodging of wheat.

Note: The field lodging photographs in the figure were taken at anthesis on May 26, 2024. M1, M2, and M3 represent seeding rates of 360, 450, and 540 kg ha^{-1}, respectively. C0, C1, C2, and C3 represent Pro-Ca application rates of 0, 600, 1200, and 1800 g ha^{-1}, respectively

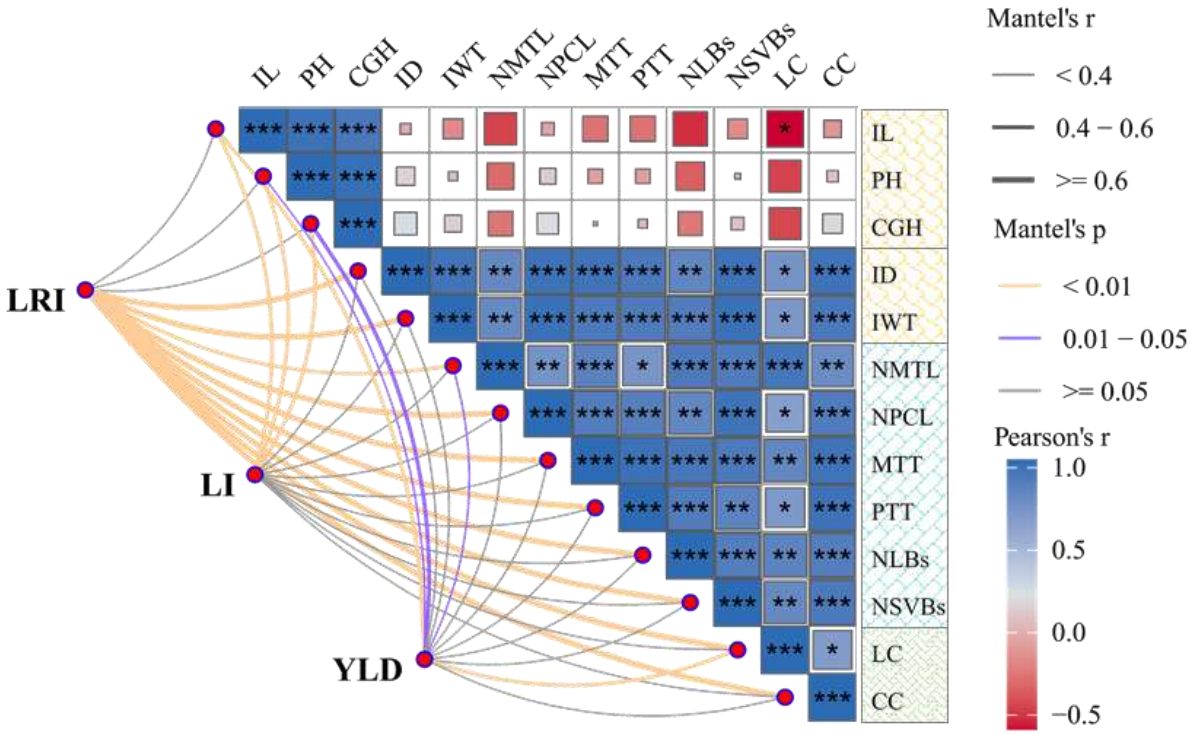


Fig. 11 Heatmap of Mantel and Pearson correlation analyses of wheat yield, lodging-related parameters, plant morphology, internode anatomical structure, and chemical composition traits

Note: The upper half of the figure shows the Mantel correlation coefficients (thin lines indicate correlation coefficients < 0.4, medium lines indicate 0.4–0.6, and thick lines indicate ≥ 0.6) and their significance levels (orange indicates $P < 0.01$, purple indicates $P = 0.01$–0.05, and gray indicates $P \geq 0.05$). The lower half shows Pearson correlation coefficients, with colors ranging from red to blue representing values from -0.5 to 1.0; * indicates $P < 0.05$, ** indicates $P < 0.01$, *** indicates $P < 0.001$, and no * indicates $P > 0.05$. YLD represents yield

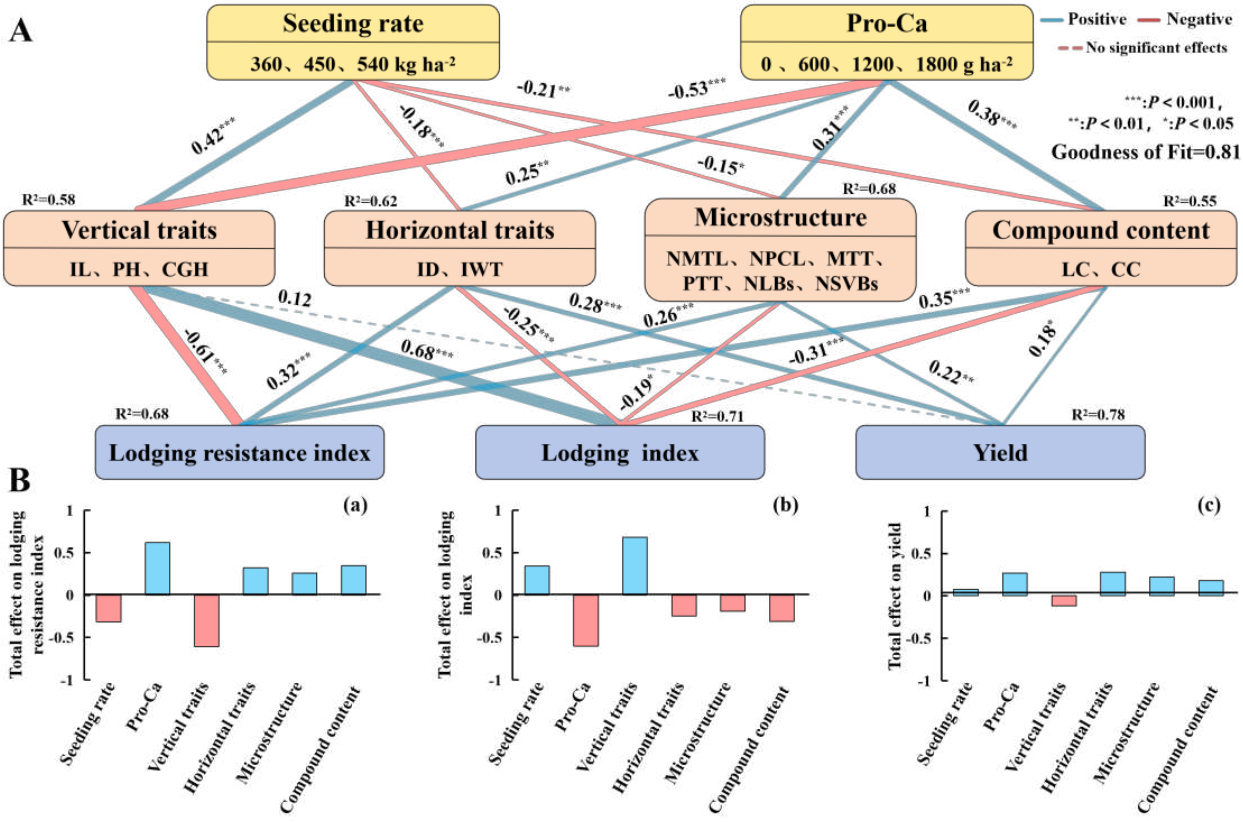


Fig. 12 PLS-PM of seeding rate, Pro-Ca, plant morphology, internode anatomical structure and chemical composition

Note: Panel A shows the positive and negative effects of the relevant indicators in PLS-PM. Panel B shows the standardized total effect of these indicators. Blue arrows indicate positive effects, red dashed arrows indicate negative effects, and R^2 values indicate the variance explained by each endogenous variable. * indicates $P < 0.05$, ** indicates $P < 0.01$, *** indicates $P < 0.001$, and no * indicates $P > 0.05$; blue or red dashed lines indicate non-significant effects. The bar chart shows the standardized total effects, including the direct and indirect contributions

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- GraphicalAbstract.pdf