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Nitrogen-induced Structural Adaptations in Wheat Inter Nodes for Lodging Resistance

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ABSTRACT

To explore the differences in traits like canopy architecture, culm diameter and stem strength in relation to anatomical and biochemical parameters under varying nitrogen application practices, a field study was conducted at D. B. G. Govt. College in Panipat, Haryana. This research focused on anatomical traits, including cell wall composition, lignin deposition in vascular tissues and the size of vascular bundles, as well as biochemical traits such as Acid Detergent Fiber (ADF), cellulose and Acid Detergent Lignin (ADL). The study also aimed at establishing a standardized methodology for assessing lodging under different nitrogen application practices. Findings revealed that moderate nitrogen application produced superior results compared to both double and zero applications. The standard application of nitrogen improved plant strength and enhanced morphological and physiological traits, making the plants less prone to lodging than those subjected to double or zero applications. The results indicated that moderate nitrogen fertilizer doses were most effective in promoting lignin production and the activity of lignin-synthesizing enzymes, thereby increasing lodging resistance. Correlation analysis confirmed the positive impact of regular nitrogen applications.

Key words: Wheat, nitrogen application, lodging, acid detergent lignin, cellulose, vascular bundles

INTRODUCTION

Wheat is a crucial crop for human consumption; however, the quality of its grain is often constrained by the availability of both micro and macro nutrients in the soil. These nutrients play a vital role in influencing yield by promoting the growth and development of the plants. Taller wheat varieties tend to be more resilient, capable of supporting greater weight, yielding more grains per spike, and ultimately achieving higher overall crop yields compared to shorter, sturdier stem varieties. The enhancement of these morphological, physiological and biochemical characteristics can be achieved through various fertilizer applications, including nitrogen, phosphorus and zinc. Among these, nitrogen management is particularly significant for wheat growth (Chaudhary *et al*., 2021). When applied in appropriate amounts, nitrogen fertilizers can substantially boost crop yields (Chandio *et al*., 2022). Implementing effective nitrogen fertilization strategies include recommended rates and application methods, as most essential for sustaining plant quality and soil health (Shah *et al*., 2019). Insufficient fertilizer use, especially nitrogenous fertilizers, adversely affects crop growth and yield.

Nitrogen influences the mechanical strength of wheat plants at each inter node by improving the anatomical and biochemical robustness of the straw. Applying nitrogen during the heading stage has been shown to enhance the growth of the first and second inter nodes more effectively than application during the vegetative stage (Zhang *et al*., 2017). Different nitrogen application rates increase the area and size of vascular bundles as well as the inner core area of inter nodes. With higher nitrogen fertilizer usage, there is a notable increase in plant height, basal inter node length and the height of the center of gravity, while the density of the basal second inter node and levels of cell wall components, which are inversely related to the lodging index, significantly decrease (Li *et al*., 2022). Research findings indicated that lodging resistance could be evaluated through plant morphological and stem anatomical characteristics (Khobra *et al*., 2019). Nitrogen

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management practices are crucial for influencing the height ratio of wheat plants, which is directly associated with the occurrence of stem lodging. Enhanced crop lodging can be achieved through agricultural practices that promote vigorous plant growth, such as the application of high doses of nitrogenous fertilizers and increased seed rates (Zhang *et al*., 2017). Stem lodging disrupts the upright growth of cereal crops, leading to a collapse of the crop canopy and diminishing the light interception necessary for effective photosynthesis and other vital plant functions (Li *et al*., 2022). Severe lodging can impair the absorption and distribution of water, nutrients and carbon compounds, resulting in a notable reduction in yield (Yang *et al*., 2022). Yield losses of 18 and 61% have been reported for lodging angles of 45° and 90°, respectively. Research indicates that under conditions of extreme lodging, yield can decrease by as much as 80% (Muhammad *et al*., 2020). Therefore, the application of fertilizers is essential for improving crop lodging resistance (Dahiya *et al*., 2018). While various mechanical traits have been identified to select plant varieties with lodging tolerance, breaking strength remains the most significant criterion for variety selection. Numerous studies have emphasized the importance of straw strength in lodging resistance, with plant metabolites such as lignin, cellulose and silica playing critical roles in enhancing this strength (Shah *et al*., 2019). However, due to the diversity of varieties, significant variability among genotypes, and the interactions between genotypes and environmental factors have been noted in relation to lodging (Nguyen *et al*., 2016; Muhammad *et al*., 2020). The inconsistent performance of cultivars across different growing seasons has particularly diminished the heritability of essential traits, complicating the assessment of lodging events.

While various mechanical characteristics have been identified for screening plant varieties that exhibit tolerance to lodging, breaking strength remains the most critical factor in varietal selection Studies have highlighted the significance of straw strength in relation to lodging resistance, with plant metabolites such as lignin, cellulose and silica playing essential roles in contributing to this strength (Shah *et al*., 2019). Nonetheless, varietal heterogeneity has led to substantial genotypic variability and genotype-environment interactions concerning lodging (Nguyen *et al*., 2016; Muhammad *et al*., 2020). The inconsistent performance of cultivars across different seasons has notably reduced the heritability of key traits, complicating the analysis of lodging occurrences. Consequently, our primary approach to meet the growing demands for both the quantity and quality of food for an expanding global population is to enhance wheat productivity. The prevalent use of high-input agricultural practices, such as excessive fertilizer application in major wheat-producing regions, combined with shifting climatic conditions, has brought lodging issues to the forefront. Therefore, it is crucial to examine the morphological, physiological and anatomical features at the inter node level to address lodging challenges while managing optimal fertilizer application in the field. To understand and differentiate the effects of various nitrogen fertilizer dosages on wheat straw at each inter node, an experiment was conducted. Further research is needed to develop stronger stem structures, as plant height is a critical factor in improving lodging resistance in wheat, and breeders are hesitant to further reduce plant height (Shah *et al*., 2019). This experiment represents one of the initial attempts to simulate anatomical, biochemical and morphological growth patterns at each inter node level while applying three different rates of nitrogen fertilizers. The aim of this study was to enhance our understanding for suitable management of nitrogen fertilizer influencing the growth of wheat straw in relation to lodging behaviour across various wheat genotypes.

MATERIALS AND METHODS

The research was carried out in a natural setting at the D. B. G. Govt. College Campus in Panipat (29º422 N, 77º22 E) over two consecutive **rabi** seasons during 2022-23 and 2023-24. The seed materials were obtained from the Germplasm Unit of the ICAR-Indian Institute of Wheat and Barley Research in Karnal, which serves as a central organization supplying seeds to breeders, researchers and farmers. In both the years of the study, five wheat genotypes were cultivated across three blocks arranged according to a randomized block design (RBD).

Three plots were established for wheat sowing, each receiving a different dosage of nitrogen fertilizers. The first plot received no nitrogen application, the second plot was treated with a moderate amount of nitrogen as recommended by ICAR-IIWBR for farmers (120 kg/acre), and the third plot received double the prescribed dosage (240 kg/acre). Lodging treatments occurred naturally, as the entire setup was conducted under natural conditions.

Daily seasonal data for various weather variables, including minimum relative humidity (%), maximum relative humidity (%), minimum temperature (°C), maximum temperature (°C), wind speed (km/h) and rainfall (mm) were gathered for the growing seasons 2022-23 to 2023-24 (Fig. 1).

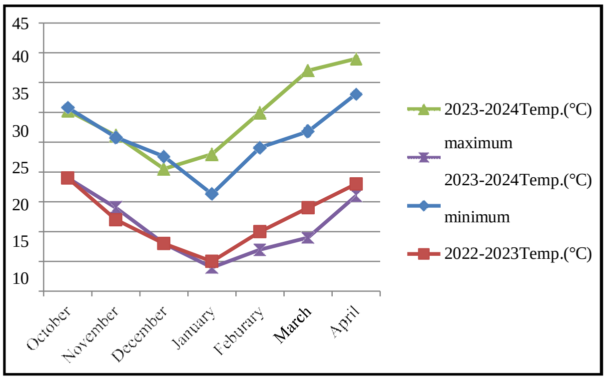

Fig. 1. Seasonal temperatures per month for the years 2022-23 and 2023-24.

Data collection throughout different phases of crop development was performed following Zadok's scale. A variety of traits were evaluated, which included: (a) phenological traits such as days to heading (DH), days to anthesis (DA), days to maturity (DM) and grain-filling duration (GFD); (b) morphological traits comprising plant height (PHT in cm), lengths of the basal inter node, second basal inter node, and first inter node (INTL in cm), inter node width (INTWDT in mm), inter node inner diameter (INTHW in mm), as well as peduncle length (PEDL in cm) and width (PEDWDT in mm); c) resource allocation traits such as biomass (BM in kg), shoot weight (SHTW in g), inter node weight (INTW in g) and spike weight (SPKWT in g); (d) yield-related traits including grain yield (GY in g) and 1000-grain weight (TGW in g); (e) anatomical traits like parenchyma, sclerenchyma, chlorenchyma, pith and the number of vascular bundles and (f) biochemical traits encompassing cellulose (µg/ml), lignin quantified as acid detergent fiber (ADF in % of dry matter) and acid detergent lignin (ADL in % of dry matter). The anatomical characteristics, included parenchyma, sclerenchyma, chlorenchyma, pith and vascular bundle features, along with the biochemical attributes of each inter node, such as cellulose (µg/ml), ADL in dry matter and lignin for ADF in dry matter, for accurately determining the best nitrogen application strategies.

For the measurement of plant height (PHT), three representative plants were randomly selected from each replication of every block, with height recorded from the ground to the top of the spike, excluding the awns. A venire caliper was utilized to measure the peduncle length (PED) and width (WDT) in millimeters, while a measuring scale in centimeters was employed to assess the peduncle length from the base of the spike to the first node of the mother tiller (PEDL). A ruler in centimeters was used to manually document the inter node length (INTL). Additionally, three spikes were randomly collected in each replication to measure spike length (SPKL in cm) and spike weight (SPKWT in g). Regarding yield-related traits, plants were harvested at physiological maturity, and the spikes were threshed manually. The final grain yield (GY) was determined by weighing the total threshed grains for each genotype using a digital balance in grams. To calculate the 1000-grain weight (TGW), grains were counted manually and weighed on an electronic balance. For crop lodging assessment, the percentage of the crop area that was lodged within a plot was measured, along with the angle of inclination of the stem from the vertical position.

The lodging score was calculated using the formula:

Lodging score = (% of plot area lodged) x (Angle of lodging from the vertical)/90.

Lodging percentage was determined using the formula:

Lodging (%) = (Lodging area in plot/Total plot area) x 100.

This investigation utilized sampling methods to explore the changes in anatomical traits resulting from various nitrogen applications. The middle segments of the first, second and third basal inter nodes of the plant were isolated and preserved in a solution of formalin, acetic acid and ethanol. The stem tissue was then sectioned into slices of 10-20 ìm thickness using a Lyzer Semiautomatic Microtome. Following this, the sections were double-stained with 1% safranin O and 1% light green, and subsequently examined using a light microscope (Olympus, BH2 REC, Tokyo, Japan) equipped with a digital camera and an image analysis system (Moticam 2, Japan).

A sample of plant material weighing between 0 and 5 g was first combined with three ml of an acetic/nitric reagent. This combination was then cooled and subjected to centrifugation for 15 to 20 min following a 30-min heating period at 100°C in a water bath. After the supernatant was removed and the remaining residue was rinsed with distilled water, 10 ml of 67% sulfuric acid was added, and the mixture was allowed to sit for one hour. Subsequently, the solution was diluted to a total volume of 100 ml, and 10 ml of anthrone reagent were added to 1 ml of the diluted solution to ensure thorough mixing. The resulting mixture was heated in a boiling water bath for 10 min, after which the absorbance was measured at 630 nm.

Lignin, along with cellulose and complex polyphenolic compounds, imparts rigidity and stiffness to the inter nodes. Therefore, a quantitative analysis was performed utilizing the method, in which lignin was measured as Acid Detergent Lignin (ADL) and Acid Detergent Fiber (ADF).

A round-bottom flask received 100 ml of an acid detergent solution, which was then mixed with one g of dried and powdered wheat culm. The mixture was heated to boil for duration of 5 to 10 min. Once boiling commenced, the heat was reduced to minimize foaming. Following this, the mixture was refluxed for one hour. After swirling the contents and rinsing the flask twice with hot water, the mixture was filtered by suction through a pre-weighed sintered glass crucible (G0-2). After a second wash with acetone, any lumps were disintegrated, and the filtrate was purified repeatedly until it became colourless. The final product was cooled in a desiccator and weighed after being dried overnight at 100°C.

ADF was calculated as:

$$\text{ADF}(\%) = W / S \times 100$$

where, W was the weight of the fibre and S was the weight of the sample.

Following the transfer of ADF to a 100 ml beaker, 25-50 ml of 72% sulfuric acid and 1 g of asbestos were introduced. The mixture was stirred occasionally with a glass rod and allowed to stand for three hours. Distilled water was then employed to dilute the acid, and filtration was performed using Whatman filter paper. The glass rod and remaining residue were thoroughly cleaned to eliminate any acid. After cooling in a desiccator, the filter paper was weighed and subsequently dried at 100°C. The dried filter paper was then placed in a pre-weighed silica crucible and subjected to ashing in a muffle furnace for approximately three hours at 550°C. After cooling in a desiccator, the crucible was weighed again, and the amount of ash was calculated. The 1 g of asbestos served as the blank, to which 72% sulfuric acid was added, and all steps outlined were executed.

The calculation for ADL (%) was as follows:

ADL (%) = [Weight of 72% H_2SO_4 washed fiber (Test - Asbestos blank) - Ash (Test - Asbestos Blank)] × 10.

The analysis of variance (ANOVA) was conducted utilizing the Proc GLM procedures in SAS version 9.3 for all measured traits. Statistical significance was established at P < 0.05 (*), P < 0.001 (**), or P < 0.0001 (***).

RESULTS AND DISCUSSION

Individual analysis of different nitrogen application rate management demonstrated significant effects on most of the traits recorded. Furthermore, the interaction effects were also significant (P d” 0.0001) for traits such as lodging (LODGE), degree of lodging (LODGED) and peduncle length (PEDL), while plant height (PHT) and days to anthesis (DA) were significant at P d” 0.01. Significant variation (P d” 0.0001) was also noted at the genotypic level for several traits, including PHT, SPKWT, BM, TGW, PEDL, LODGE and LODGED. The ANOVA results indicated significant interactions between genotypes and treatments across both the years regarding various nitrogen application practices and their influence on plant traits. Result findings illustrated the effects of three different rates of nitrogen fertilizer application on the genotypes over the two years.

The morphological characteristics of plants, particularly plant height (PHT), spike weight

(SPKWT) and inter node width (INTWDT), were significantly affected by fertilizer availability across the genotypes studied. The PHT exhibited considerable variation, with measurements ranging from 44 cm in genotype DM7 to 125 cm in C306 among the five genotypes analyzed (Table 1). Under all experimental conditions, moderate nitrogen application was found to improve tolerance and stability, thereby promoting consistent resistance to lodging. However, the maximum plant height varied according to the treatment applied. A significant positive correlation was observed between PHT and fertilizer application methods (Table 2). A similar trend was noted for inter node length (INTL), where each genotype reached its maximum height in blocks that received double doses of fertilizer, while the lowest INTL was found in the block without nitrogen application. For culm width (CW), notable differences were observed across various conditions.

The highest INTWDT was recorded for the dwarf genotype DM7 under field conditions N_2 and N_1, measuring 4.60 mm, while the lowest INTWDT was noted in DBW303 (N_2–3.62 mm; N_0–3.14 mm). Under N_2 conditions, DBW303 and HD2967 displayed the thickest culms at 4.3 and 4.28 mm, respectively, whereas DBW303 had the least thickness at 3.62 mm. Significant differences ($P \leq 0.01$) were observed for PHT at both the treatment and genotypic levels. Furthermore, traits such as INTL, INTWDT, peduncle length (PEDL) and the inter node ratio exhibited positive correlations with PHT, lodging (LODGE) and lodged plants (LODGED) under field conditions.

The analysis revealed significant treatment effects on yield traits, including grain yield and 1000 grain weight, with a notable correlation between 1000-grain weight and genotypic variability. The wheat genotype HD2967 was identified as the leading yielder in control and artificial environments, producing yields of 245.2 and 191.6 g, respectively. In the N_2 field condition, DBW303 achieved the highest grain yield of 306.4 g, while the dwarf genotype DM7 recorded the lowest yield at 173.1 g. Under N_0 conditions, the genotype DPW621-50 displayed the least grain yield. The maximum 1000 grain weight was found in DBW 303, measuring 46.68 and 45.50 g in N_2 and N_1 natural field conditions, respectively. In contrast, HD2967 had the highest grain weight of 39.01 g under

Table 1. The morphological characters with mean values

Treatment	PHT	PEDL	SPKL	1ST INTL	2ND INTL	3RD INTL	1ST INTWDT	2ND INTWDT	3RD INTWDT
V_1N_1	104.667±1.528	44.9±0.200	8.333±0.058	2.867±0.058	8.267±0.058	12.833±0.058	8.333±0.058	3.237±0.025	3.24±0.020
V_1N_2	123.11±1.018	53.967±0.153	10.633±0.058	3.633±0.058	10.167±0.058	14.867±0.058	10.633±0.058	4.363±0.006	4.503±0.021
V_1N_3	124.557±0.510	54.94±0.164	10.747±0.040	3.713±0.075	10.247±0.040	15.153±0.040	10.747±0.040	4.333±0.012	4.547±0.025
V_2N_1	81±1.000	23.367±0.451	8.7±0.100	3±0.100	8.067±0.058	9.867±0.058	8.7±0.100	3.257±0.045	3.403±0.015
V_2N_2	99.333±1.58	25.967±0.153	11.833±0.058	4.167±0.058	8.933±0.058	11.033±0.058	11.833±0.058	4.343±0.021	4.633±0.021
V_2N_3	100.667±0.577	26.543±0.075	11.967±0.058	4.013±0.075	9.01±0.101	11.253±0.040	11.967±0.058	4.34±0.026	4.653±0.047
V_3N_1	82.667±1.155	22±0.700	8.367±0.058	2.767±0.058	7.333±0.058	8.733±0.058	8.367±0.058	2.813±0.075	2.62±0.046
V_3N_2	96.333±1.528	30.5±0.361	10.667±0.058	4.2±0.000	8.167±0.058	10.067±0.058	10.667±0.058	3.783±0.015	3.91±0.026
V_3N_3	97.667±0.577	31.433±0.058	10.93±0.011	4.133±0.058	8.287±0.075	10.157±0.075	10.93±0.000	3.747±0.025	3.933±0.031
V_4N_1	82.333±1.528	23.367±0.252	8.167±0.058	3.833±0.058	8.233±0.058	10.833±0.058	8.167±0.058	2.793±0.025	3.06±0.040
V_4N_2	96.667±0.577	32.5±0.300	10.267±0.058	5.967±0.058	9.567±0.058	12.833±0.058	10.267±0.058	3.65±0.010	4.013±0.012
V_4N_3	98.333±0.577	33.033±0.058	10.467±0.058	6.087±0.075	9.747±0.040	13.033±0.058	10.467±0.058	3.627±0.021	4.053±0.031
V_5N_1	44.833±0.764	17.233±0.153	5.533±0.306	1.267±0.058	3.653±0.081	5.767±0.058	5.533±0.306	3.527±0.116	3.92±0.122
V_5N_2	53.24±0.104	18.167±0.058	7.033±0.153	1.733±0.058	4.233±0.153	6.5±0.100	7.033±0.153	4.363±0.045	4.623±0.025
V_5N_3	53.703±0.254	18.233±0.031	7.247±0.050	1.767±0.058	4.337±0.148	6.6±0.100	7.247±0.050	4.39±0.010	4.68±0.036

Where, five wheat genotypes (V_1 to V_5) treated with three doses of nitrogen (N_1 to N_3) showing morphological differences in the traits: PHT–Plant height PEDL– Peduncle length and SPKL–Spike length 1st, 2nd and 3rd inter node length (1st INTL, 2ND INTL and 3RD INTL, respectively) and 1st, 2nd and 3RD inter node width (1st INTWDT, 2ND INTWDT and 3RD INTWDT, respectivly).

Table 2. Lodging score with mean values

Treatment	Mean	Std	r	LCL	UCL	Min.	Max.
V_1N_1	100.000	0.000	3	93.785	106.215	100	100
V_1N_2	90.000	0.000	3	83.785	96.215	90	90
V_1N_3	100.000	0.000	3	93.785	106.215	100	100
V_2N_1	30.000	17.321	3	23.785	36.215	20	50
V_2N_2	20.000	0.000	3	13.785	26.215	20	20
V_2N_3	30.000	0.000	3	23.785	36.215	30	30
V_3N_1	50.000	0.000	3	43.785	56.215	50	50
V_3N_2	30.000	0.000	3	23.785	36.215	30	30
V_3N_3	40.000	0.000	3	33.785	46.215	40	40
V_4N_1	16.667	11.547	3	10.452	22.881	10	30
V_4N_2	10.000	0.000	3	3.785	16.215	10	10
V_4N_3	23.333	5.774	3	17.119	29.548	20	30
V_5N_1	1.333	1.155	3	-4.881	7.548	0	2
V_5N_2	0.000	0.000	3	-6.215	6.215	0	0
V_5N_3	3.333	2.887	3	-2.881	9.548	0	5

Where, V_1 to V_5 indicated five varieties and N_1 to N_3 all three nitrogen treatments.

N_0 conditions. The dwarf genotype DM7 consistently showed the lowest 1000 grain weight, ranging from 29.41 to 32.11 g across all the three environments.

Statistically significant differences were observed in ADF (% of DM), cellulose (µg per ml) and ADL (% of DM) across various treatments. The ADF content peaked at 73.1 in HD2967 and reached 65.58 in DBW303 under the N_2 treatment, although the difference between these two figures was not statistically significant. The lowest ADF concentration, recorded at 55.43, was associated with DPW621-50 under the same treatment. In terms of cellulose, DBW303 had the highest concentration at 56.38 during imposed lodging, followed by HD2967 at 55.6, while DM7 had the lowest at 10. Under the N_0 condition, DPW621-50 exhibited the maximum cellulose level of 28. Regarding ADL, C306 recorded the highest value of 27.7, with DBW303 following at 26 under the N_0 condition. DBW303 also showed the highest ADL value under artificial lodging conditions, while C306 produced the highest ADL concentration of 25.6 under the N_0 treatment (Fig. 2).

Microscopic analysis indicated that the dwarf genotypes (DM7) possessed well-formed solid pith and finely developed multilayered sclerenchyma tissue in blocks subjected to moderate nitrogen application (Fig. 3). Conversely, the tall (C306) and semi-dwarf genotypes exhibited hollow pith, with the extent of hollowness varying among the genotypes (Fig. 4). This variation in hollowness clearly depicted by the area of pith filled with parenchymatous

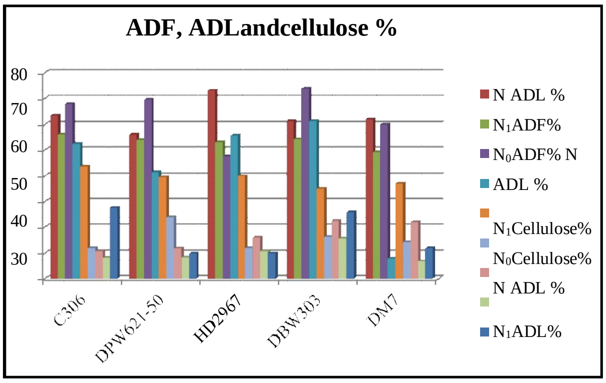

Fig. 2. Five wheat cultivars composition of acid detergent fibre (ADF), acid detergent lignin (ADL) and cellulose percentile under three different rates of nitrogen.

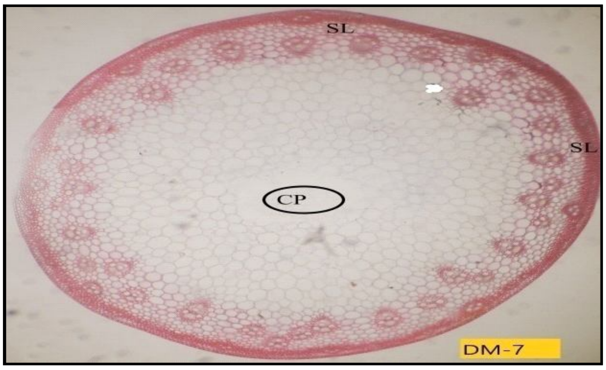

Fig. 3. Central pith (CP) and sclerenchyma layer (SL) in DM7 wheat genotype.

cells. Furthermore, among the semi-dwarf genotypes (HD2967, DPW621-50 and DBW 303), there were differences in the number of vascular bundles and the expansion of sclerenchymatous tissue (Figs. 5 and 6). None the less, the differences in vascular bundle count were not found to be significant.

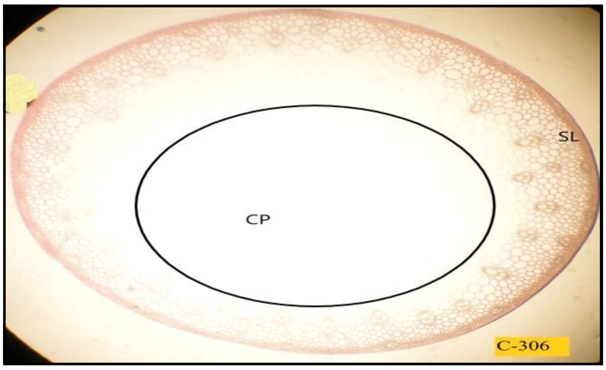

Fig. 4. Central pith (CP) and sclerenchyma layer (SL) in C306 wheat genotype.

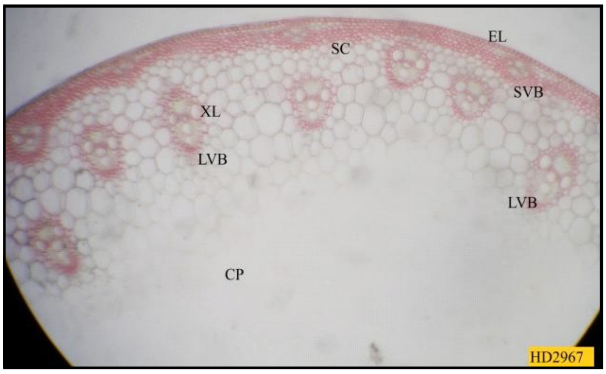

Fig. 5. The number of large and small vascular bundles (LVB and SVB), central core pith (CP), epidermal layer (EL) and sclerenchyma cells (SC) in genotype HD2967.

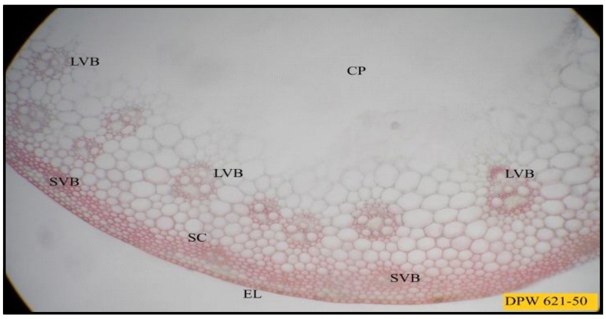

Fig. 6. The number of large and small vascular bundles (LVB and SVB), central core pith (CP), epidermal layer (EL) and sclerenchyma cells (SC) in genotype DPW621-50.

The study revealed that lodging had a statistically significant effect influenced by both treatment and genotype. Different nitrogen application rates led to varying levels of lodging. Natural lodging resulted in significant crop lodging, with percentages ranging from 1% for DM7 to 62.89% for C306 in areas with moderate nitrogen application. Under N_2 and N_0 conditions, lodging was recorded at 6.5-7.35% for DM7 and 70.69-80.35% for C306. Throughout all the three nitrogen treatments, genotype C306 displayed the highest lodging rates, while genotype DM7 showed the lowest. The lodging percentages were recorded as 73-16, 70-5 and 74-16% under N_2, N_1 and N_0 conditions, respectively (Table 2). Among the tall genotypes, C306 and DPW621-50 were the highest performers, while the shorter genotype DM7 ranked the lowest across all nitrogen treatments in natural lodging scenarios.

Managed fertilizers practices generated a wide range of responses among the screened genotypes. In natural conditions with heavy supplying of nitrogen fertilizers significantly increased crop lodging in different genotypes compared with same while growing under optimum practices of nitrogen application. Plant height is the most realistic and easily selectable visual marker to recognize effect of managed practices for fertilizers. It is also well documented that common dose of nitrogen is advantageous over all structural development and shows lodging tolerance (Dahiya *et al*., 2018). Genotype C306 scored maximum height under maximum fertilizer practices. The block having zero nitrogen application was ranked first for LODGE. A strong positive correlation was found between PHT and optimum usage of N_2 among diverse wheat populations (spring and spelt) under artificially created lodging conditions (Langer *et al*., 2017; Wu *et al*., 2023). It has also been documented that genotypic short stature limits photosynthesis and accumulation in the sink, ultimately reducing crop yields. Dwarf genotypes (DM7) produced lower biomass and lower photosynthetic accumulation in all three conditions due to their shorter stature. Though, short stature and lower biomass are positive traits for reducing crop lodging but not enviable by the breeders due to their low yield potential. Different growth stages viz., DH, DA, DM and GFD showed different levels of significance under different N_2 condition. These findings are also supported by the results of grey correlation analysis during lodging stress assimilate partitioning gets diverted to produce more dry matter instead of spike growth and hence decrease the yield. Reduced grain size and 1000-grain weight also indicated the disturbed supply of assimilates to spike in lodged crop (Khobra *et al*., 2019). The maximum reduction in GY was recorded under zero N_2 application; so the results are also statistically significant. GY is a complex trait regulated by plant vigour, photosynthetic efficiency, biomass and many more factors. Under the induced setup, overall structural growth of inter nodes of plant was simulated, at specific stage which causes significant stem weakening and makes it more susceptible for lodging.

In wheat, the second basal inter node is more prone to lodging (Zhang *et al*., 2017), so it was emphasized for each inter node assessing lodging tolerance. Plant height and inter node length were found to be positively correlated while culm diameter was negatively correlated to lodging (Madic *et al*., 2016; Shah *et al*., 2019). The similar trend was observed in the experimental results and a positive correlation was found between the plant height and the inter node length ratio. Higher inter nodes diameter values were recorded for short stature genotypes (DM7) were found more as compared to the semi dwarf and tall genotypes. Lower values of culm architecture traits in some genotypes like C306 increase their rank in grey correlation analysis and make them more prone to lodging. Spike length and spike weight also affect sensitivity of genotype towards lodging (Khobra *et al*., 2019).

Lignin and cellulose are the main strength providers to plant architecture to reduce the lodging pressure as lignin content was positively correlated with increased lodging tolerance index. But in many cases, these structural carbohydrates do not necessarily work collectively for lodging tolerance (Li *et al*., 2022). It has been reported that the deposition of more cellulose in rice improves its resistance to acclimatization to strong winds. Soybean varieties, having low stem cellulose content, cause significant reduction in mechanical strength and are more prone to lodging. Similar trend for cellulose contents has been reported in the wheat genotypes. DBW303 and HD2967 represented the maximum accumulation of cellulose under artificial lodging induction and hence showing tolerance to lodging. In barley, Lignin-deficient mutants were unable to stand upright due to collapsed xylem and lignin content and the activity of lignin associated enzymes was correlated significantly with the lodging tolerance in barley (Wang *et al*., 2018). The current study also indicated the lodging prone behaviour of wheat genotypes showing lower concentration of lignin.

Anatomical variation in plants is well documented to be a good indicator of how plants adapt to different stresses. The pattern of organization of sclerenchymatous and parenchymatous cells, their proportion and biochemical composition determine the adaptability of a genotype under variable fertilizers condition and practices and also weather uncertainties. Due to different N_2 practices fully or partially filled stems with higher proportion of mechanical (sclerenchyma) tissues make the genotypes more tolerant to lodging then hollow stemmed wheat. Parenchyma cells have a good shock absorbing capacity that can withstand against the harmful environmental effects without any mechanical damage (Khobra *et al*., 2019; Bisht *et al*., 2022). Moderate nitrogen gave the highest proportion of parenchyma in the dwarf genotypes. as compared to the semi dwarf and tall genotypes. Same significant difference was also identified from all the three blocks of N_2 practices for the number of vascular bundles. Some old findings showed a positive correlation between the number of vascular bundles per mm^2 and bending resistance. Results also found that higher percentage of scelerenchymatous layers was equally correlated to lodging tolerance as plant height. Multiple lignified layers of sclerenchyma containing greater amount of cellulose and lignin were shown by the block having moderate amount of nitrogen fertilizer not only provide better tolerance against lodging but also decrease the respiratory losses. So, a moderate amount of N_2 fertilizer gives a solid stemmed cultivar with thicker sclerenchyma layer and parenchymatous pith (as represented by DM7 followed by semi dwarf wheat varieties i.e. DBW303 and HD2967) being tolerant to lodging. At the anatomical level variations in sclerenchyma tissue, vascular bundles and pith patterns were identified (Fig. 5).

Lodging is highly unpredictable situation, which is governed by multiple factors related to the fertilizer practices and other environment condition as well as the plant architecture. In the reported experiment of various nitrogen practices with lodging treatment, an attempt was made to screen and identify practices better for the enhancement of resistance trait in wheat genotypes for lodging under natural conditions simultaneously. Because of the uncertainty of natural lodging events the wind speed may reach up to 26.5 m/s (Niu *et al*., 2016). Theoretically, the calculated wind speed for lodging induction is 11.6 m/s at the canopy (Erndwein *et al*., 2020), however, multiple factors such as crop type, variety, moisture level and planting density affect the range of wind intensity significantly. Nevertheless, the various dosages of fertilizer practice also explained well the relative difference among the genotypes against lodging tolerance.

CONCLUSION

Mismanaged nitrogen application is a highly multifaceted problem driven by multiple plant attributes, such as, inter node width, inter node length, shoot weight, spike weight, cellulose, lignin and lignin synthesizing enzymes. The findings highlight that biochemical molecules, and the efficacy of structural components can be enhanced by applying managed practices of required fertilizers. The findings also accentuate the plant height was the best correlated trait with lodging. However, the other aforesaid traits also contributed significantly to lodging tolerance. Besides the stability of dwarf genotypes (DM7) against lodging, some of the semi dwarf wheat genotypes such as HD2967, DPW621-50 and DBW303 can also be categorized as moderately tolerant against lodging and can be utilized in wheat breeding programs to improve lodging tolerance. Moreover, the role of biochemical molecules, anatomical and morphological characteristics provided overall structural development to determine the basis of lodging tolerance in wheat.

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