

Article

Responses of Cell Wall Components to Low Nitrogen in Rapeseed Roots

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Abstract: Rapeseed (*Brassica napus* L.) is a major oil crop in China, with the world's largest planted area and total yield. Rapeseed has a high demand for nitrogen (N), and nitrogen deficiency in soil is an important limiting factor for rapeseed production. However, rapeseed responds to N deprivation by regulating its own morphology, structure, and physiology. We carried out the current experiment by utilizing low N (LN: 0.3 mM NO₃⁻) and normal N (CK: 6.0 mM NO₃⁻) treatments using *Brassica napus* as the experimental material. The study results showed that low N induced root elongation in rapeseed, and the root length of LN treatment was 2.37 times that of HN treatment. The dry matter of roots also significantly increased due to low N treatment. Meanwhile, low N treatment decreased photosynthetic pigment (including chlorophyll a, chlorophyll b, and carotenoids) contents and dry mass accumulation of leaves. A higher root/shoot ratio and N physiological efficiency were observed under low N treatment. The changes in cell wall components (pectin, cellulose, hemicellulose, and lignin), related enzymes, and genes' transcription levels in roots were determined and the results suggested that low N promoted the demethylation of ion-bound pectin (ISP) and covalently bound pectin (CSP), the content of CSP and cellulose. The promoted pectin methylesterase (PME) activity, inhibited pectin and cellulose degradation enzymes, and up/downregulation of related genes also confirming the results of cell wall components. The low N-increased demethylation degree of pectin and content of pectin and cellulose in cell walls was conducive to cell wall loosening and cell wall synthesis during cell division and elongation, ultimately promoting root-adaptive elongation. The study revealed a possible mechanism in which the alteration of cell wall component content and structure participates in cell elongation and expansion, which directly induces root elongation under N deficiency. The successful implementation of this research may be conducive to facilitating the development of rapeseed cultivars with high N use efficiency through root-based genetic improvements and improving plant adaptability to low N.



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Keywords: low N; rapeseed; root; cell wall; pectin

1. Introduction

As an important oil crop with the highest oil production, rapeseed (*Brassica napus* L.) plays an important role in the supply of edible oil in China. Nitrogen (N), also known as “bioelement”, is an essential macroelement for plant growth and development. Nitrogen participates in many important physiological metabolic processes in plants [1]. In agricultural production, rapeseed has a large demand for N fertilizer, and the N deficiency in soil is an important limiting factor for rapeseed production. The N use efficiency of rapeseed is also at a low level [2,3]. Therefore, farmers often increase the application of N fertilizer to obtain a high yield in actual production, while the application of N fertilizer not only promotes agricultural production but also causes many problems for the environment and crops. Excessive N fertilizer causes soil acidification, hardening, and water eutrophication

through surface runoff and leaching loss. At the same time, excess N induces crop overgrowth, aggravates crop diseases and lodging, and reduces grain yield and quality. Plant adaptation to N deficiency is crucial for improving crop N efficiency and reducing chemical N fertilization.

Under low N stress, plant roots make morphological, structural, and physiological adaptation responses, which could be a compensatory effect of plants, hunting for N sources as much as feasible. Nitrogen deficiency-induced changes in root architecture are very important for enhancing the N acquisition of plants [4,5]. A lot of previous studies have focused on the adaptive response of plant roots to low N and found that low N increases root dry weight, total root length, and the change of root structure [6], and significantly increases root/shoot ratio [7–9]. The morphological and physiological responses of roots to changes in N application have been widely reported [10,11]. The length of a root is determined by the combination of cell division, cell differentiation, and the extent of expansion and elongation of cells [12,13]. The plant cell wall is a highly dynamic, complex network structure and is involved in various cell metabolic activities, including maintaining cell adhesion, cell elongation, and expansion [14]. Under biotic and abiotic stresses, cell wall is differently modified in structure and composition by the activation of a wide range of enzymes [15–17], and the modification in cell wall structure and composition is closely related to cell wall loosening, which is a direct cause of cells expansion and plant growth [18]. A lot of studies have focused on revealing the mechanism of roots with low N in many plants, with known responses of auxin transporter and auxin signaling, cell division and expansion, and the abundance of proteins involved in cell differentiation, cell wall modification, and phenylpropanoid biosynthesis [19–22]. However, how low N affects the structure and components of cell walls in rapeseed roots remains largely unknown.

Therefore, in the present experiment, *Brassica napus* (L.) was used as the research material, and we aimed to suggest the compositional response of rapeseed root cell walls to low N and reveal the relationship of cell wall components and root elongation under N deficiency. The results provide scientific and theoretical guidance for breeding rapeseed varieties with high N use efficiency and benefits to realizing “zero growth” of chemical fertilizer.

2. Results

2.1. Effects of Low N on the Growth and Photosynthetic Pigment Content in Rapeseed

As shown in Figure 1, after 10 days of treatments with low N (0.3 mM NO_3^-) or normal N (0.6 mM NO_3^-), the roots and leaves of rapeseed displayed significantly N-deficient symptoms. The leaves of N-starved rapeseed were significantly smaller than those treated with high N, and low N induced large amounts of anthocyanin accumulation in leaves. The results in Figure 2 show that low N significantly decreased the content of photosynthetic pigments (chlorophyll a, b, and carotenoids) in leaves, while the roots showed adaptive elongation in rapeseed under low N treatment.

2.2. Effects of Low N on N Content, Root Shoot/Ratio, and N Physiological Efficiency of Rapeseed

Dry matter accumulation of plants reflects the growth status of plants to a certain extent. Low N stress remarkably reduced the dry matter accumulation of rapeseed leaves, while it significantly promoted the dry matter accumulation of roots (Table 1), which echoed the results of root morphology. The root/shoot ratio can be used to characterize the distribution characteristics of photosynthetic products in plants. Under N starvation conditions, plants distribute more photosynthetic products to roots to promote the preferential growth of roots. As shown in Table 1, the root/shoot ratio of rapeseed treated with low N was significantly higher than that of rapeseed treated with normal N. In the meantime, low N decreased the total N content and N accumulation in rapeseed leaves and roots at a significant level. Nitrogen physiological use efficiency is an index reflecting the N use efficiency of plants, which represents the production of dry matter per unit of N accumulation. Low N treatment of rapeseed has a higher N physiological efficiency, which

indicates that N-deficient rapeseed can produce more dry matter with less N accumulation, and this phenomenon is also a low N adaptive response of plants.



Figure 1. The growth of rapeseed under different N treatment. (A) The growth of the whole seedlings; (B) leaf size and anthocyanin accumulation in leaves; (C) the root morphology reflected by root scanner.

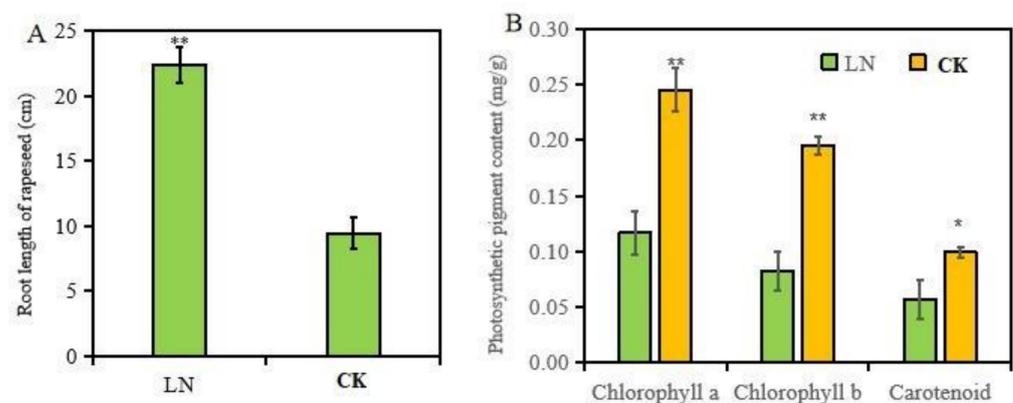


Figure 2. The root length (A) and photosynthetic pigment content (B) in rapeseed under different N treatment. The * and ** represent the statistical significances at the levels of $p < 0.05$ and $p < 0.01$, respectively.

Table 1. The difference of dry mass, N content, root/shoot ratio, and N physiological efficiency between rapeseed in low and normal N levels.

Treatment	Organ	Dry Mass (g)	Total N Content (mg/g)	N Accumulation (mg/Plant)	Root/Shoot Ratio (%)	N Physiological Efficiency (%)
LN	Leaf	0.200 ± 0.02	22.19 ± 0.73	4.19 ± 0.28	19.66 * ± 0.92	47.83 ** ± 2.49
	Root	0.044 * ± 0.01	15.62 ± 0.14	0.59 ± 0.02		
CK	Leaf	0.295 * ± 0.01	42.18 ** ± 1.30	10.25 ** ± 0.73	13.43 ± 0.85	24.36 ± 1.09
	Root	0.037 ± 0.01	39.35 * ± 1.08	1.05 * ± 0.09		

Note: The * and ** represent the statistical significances at the levels of $p < 0.05$ and $p < 0.01$, respectively.

2.3. Differences in Cell Wall Components of Rapeseed Roots under Different N Treatments

The extraction rate of root cell walls was significantly increased by low N treatment, which was 30.58% higher than that of CK treatment (Figure 3A). TEM images were used to show the difference of cell wall and our result suggested no obvious change in cell wall thickness and morphological structure (Figure 3B).

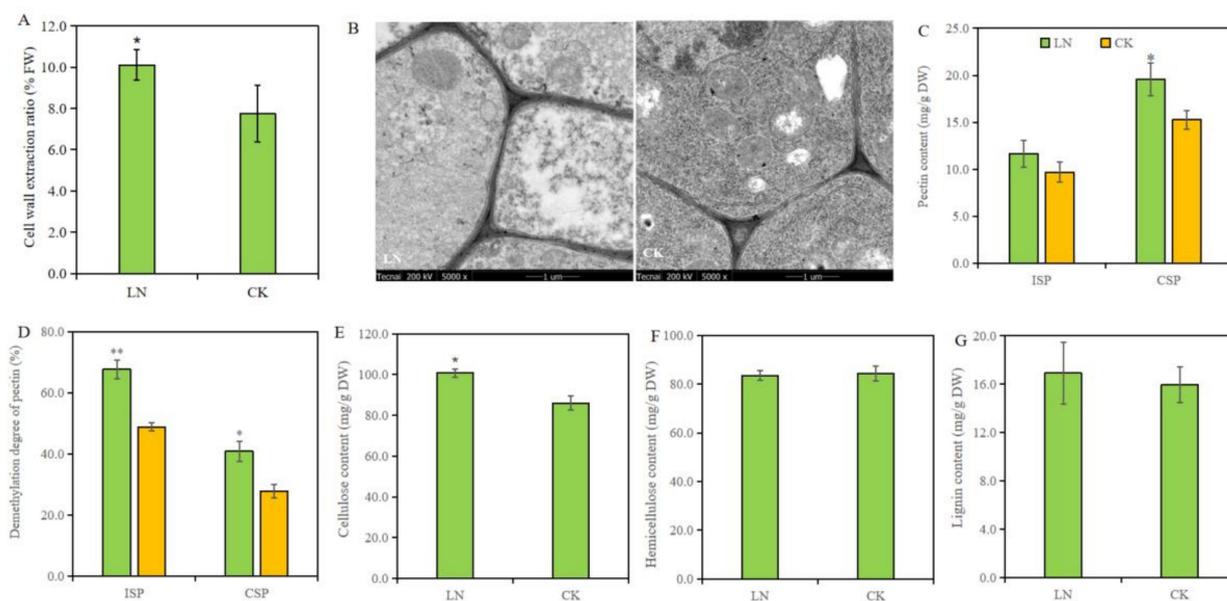


Figure 3. The responses of cell wall structure and compositions to different N treatment. (A) Cell wall extraction ratio; (B) TEM images of roots; (C,D) content and demethylation degree of ionic soluble pectin (ISP) and chelated soluble pectin (CSP); (E–G) concentrations of cellulose, hemicellulose, and lignin in root cell walls. The * and ** represent the statistical significances at the levels of $p < 0.05$ and $p < 0.01$, respectively.

In order to more accurately study the responses of main chemical components in root cell walls to low N treatments, we quantitatively measured the content of different types of pectin, cellulose, hemicellulose, and lignin in cell walls. Compared to normal N treatment, low N stress significantly increased the content of CSP and cellulose in root cell walls by 17.81% and 17.44%, respectively, while having no obvious effect on ISP, hemicellulose, and lignin content (Figure 3C,E–G). Furthermore, the pectin demethylation was analyzed and the results showed that low N increased the demethylation degree of ISP and CPS (Figure 3D), which was beneficial to cell wall loosening.

2.4. Differences in Degradation of Pectin and Cellulose in Roots under Different N Treatments

Our analyses of cell wall components in rapeseed roots suggested that low N not only increased CSP and cellulose content but also promoted the demethylation of ISP and CSP. To further confirm this, we determined the activity of enzymes involved in the pectin demethylation, and degradation of pectin and cellulose in root cell walls. Using the chemical colorimetry method, we determined the activity of the PME and three pectinases, pectic lyase, polygalacturonase, and β -galactosidase, in the root cell walls. We found that the activity of β -galactosidase and polygalacturonase was remarkably inhibited by N deficiency (Figure 4B,D). Thus, the lower β -galactosidase and polygalacturonase activity may be involved in inhibited pectin degradation in roots under low N condition. Low N increased the PME activity of ISP and CSP from 0.36 to 0.41 U/g and 0.33 to 0.42 U/g, respectively (Figure 4E), which corresponding to the result of pectin demethylation degree (Figure 3D).

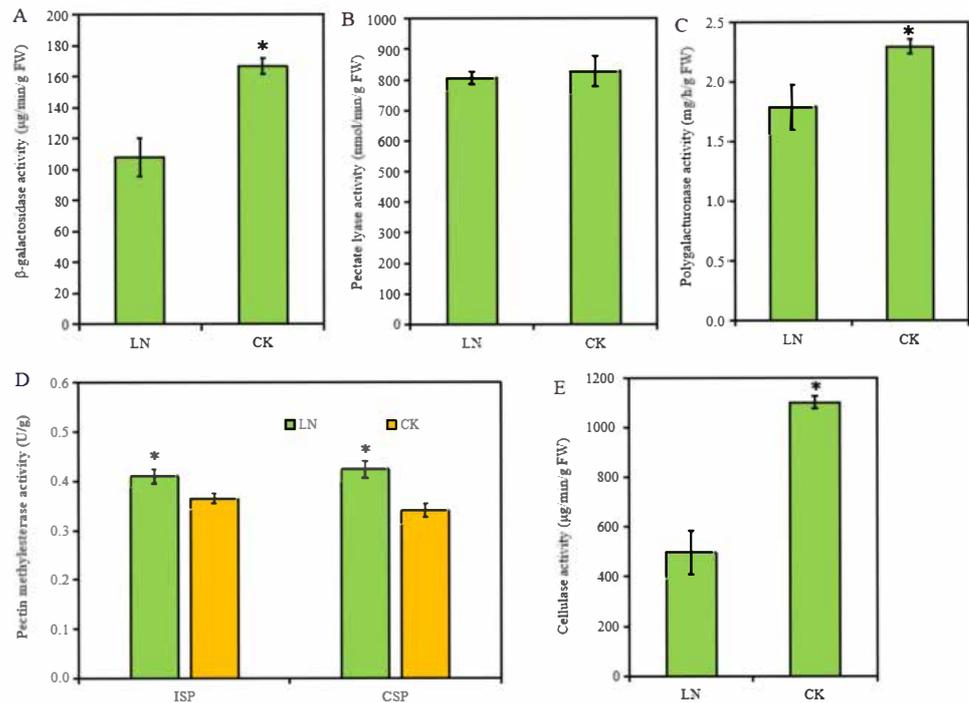


Figure 4. The enzyme activity related to pectin and cellulose in rapeseed roots under different N treatment. (A–E) Activities of β -galactosidase, pectate lyase, polygalacturonase, pectin methylesterase, and cellulase in rapeseed roots. The * represents the statistical significance at the levels of $p < 0.05$.

2.5. Responses of Genes Involved in Pectin Demethylation and Degradation of Pectin and Cellulose in Roots to Low N Treatment

Our physiological analyses indicated that low N promoted pectin demethylation and increased ISP and cellulose content in root cell walls. The transcriptional profiling of the pectinases and cellulase further verified the above results. The comparison of low N and CK treatments identified five DEGs that were involved in pectate lyase and were both downregulated by N deficiency; two DEGs involved in polygalacturonase were significantly downregulated; 5/7 DEGs involved in β -galactosidase were significantly downregulated; and 7/9 DEGs related to cellulose degradation were significantly downregulated (Table 2). In the meantime, between low N and CK treatment, we identified 15 and 2 DEGs of PME family and PME inhibitor (PMEI) family, respectively; the transcription level of 12 DEGs related to PME was improved, and 2 PMEI genes all were down-expressed by low N.

Table 2. The transcription level of genes related to pectinase and cellulase in root of rapeseed under different N treatment.

Gene ID	Transcription Level		Fold Change	\log_2 (Fold Change)	Regulation	Significant	Description
	LN	CK					
BnaAnng22760D	20.49	44.66	0.46	−1.12	down	yes	Pectate lyase
BnaA02g13340D	5.09	17.88	0.28	−1.81	down	yes	Pectate lyase
BnaA07g16550D	3.50	5.30	0.66	−0.60	down	no	Pectate lyase
BnaA09g55050D	5.71	6.13	0.93	−0.10	down	no	Pectate lyase
BnaC08g25770D	3.55	4.66	0.76	−0.39	down	no	Pectate lyase
BnaCnng18640D	0.13	0.87	0.15	−2.76	down	yes	polygalacturonase
BnaA01g10390D	0.27	1.54	0.18	−2.49	down	yes	polygalacturonase
BnaC01g18170D	13.52	6.933	1.95	0.96	up	no	β -galactosidase 12
BnaA01g15330D	12.76	7.23	1.77	0.82	up	no	β -galactosidase 12

Table 2. Cont.

Gene ID	Transcription Level		Fold Change	log ₂ (Fold Change)	Regulation	Significant	Description
	LN	CK					
BnaC02g12030D	3.14	24.36	0.13	−2.96	down	yes	β-galactosidase 12
BnaA03g10550D	6.25	24.71	0.25	−1.98	down	yes	β-galactosidase 12
BnaAnng30540D	4.34	26.93	0.16	−2.63	down	yes	β-galactosidase 12
BnaC03g13250D	7.16	19.73	0.36	−1.46	down	yes	β-galactosidase 12
BnaA02g08480D	8.66	31.33	0.28	−1.86	down	yes	β-galactosidase 12
BnaA06g00690D	46.57	10.92	4.27	2.09	up	yes	PME1
BnaC06g41950D	15.24	4.66	3.27	1.71	up	yes	PME1
BnaA05g14120D	10.78	10.97	0.98	−0.02	down	no	PME1
BnaC06g41940D	9.21	9.70	0.95	−0.07	down	no	PME2
BnaC01g37010D	74.38	20.67	3.60	1.85	up	yes	PME3
BnaA01g29470D	39.47	12.30	3.21	1.68	up	yes	PME3
BnaC05g39340D	79.58	64.15	1.24	0.31	up	no	PME3
BnaA05g25150D	96.59	90.11	1.07	0.10	up	no	PME3
BnaA01g37100D	0.26	0.23	1.12	0.16	up	no	PME3
BnaA06g31060D	8.48	6.88	1.23	0.30	up	no	PME31
BnaC07g25560D	4.54	4.06	1.12	0.16	up	no	PME31
BnaA08g11860D	1.82	1.74	1.05	0.06	up	no	PME44
BnaC03g66720D	4.63	4.54	1.02	0.03	up	no	PME44
BnaC04g23290D	1.26	1.58	0.79	−0.33	down	no	PME61
BnaA09g55410D	0.55	0.34	1.60	0.68	up	no	PME61
BnaA02g21340D	6.40	26.41	0.24	−2.05	down	yes	PMEI1
BnaC02g46740D	23.70	57.45	0.41	−1.28	down	yes	PMEI1
BnaA09g51160D	0.04	0.11	0.33	−1.60	down	yes	cellulase 2
BnaC06g43770D	6.55	23.25	0.28	−1.83	down	yes	cellulase 3
BnaA07g23740D	1.16	4.24	0.27	−1.88	down	yes	cellulase
BnaC05g09040D	0.17	0.36	0.48	−1.07	down	yes	cellulase
BnaC07g23300D	5.17	16.97	0.30	−1.72	down	yes	cellulase
BnaA07g28820D	15.26	10.54	1.45	0.53	up	no	cellulase
BnaC06g24550D	5.79	5.97	0.97	−0.04	down	no	cellulase
BnaA10g05530D	50.15	51.63	0.97	−0.04	down	no	cellulase
BnaC09g26420D	64.25	60.88	1.06	0.08	up	no	cellulase

3. Discussions

Nitrogen starvation is a major limiting factor for rapeseed production. The root system is the most important organ for nutrient acquisition from the soil. Growing evidence has indicated that the availability of N can modulate root growth and architecture in plants. However, most previous studies on the response of rapeseed to N starvation have mainly focused on leaves. Our study suggested that low N significantly decreased the photosynthetic pigments content in leaves (Figure 2B), and thereby inhibited the progress of photosynthesis and the accumulation of dry matter in leaves (Table 1). Under low N stress, plant roots make morphological, structural, and physiological adaptive responses, such as increased root/shoot ratio [9,23], promoted root elongation and root weight, and changes in root architecture [6,24,25]. In a recent study, a 3D in situ quantification analysis system was used to reveal the architectural response of rapeseed roots to N deficiency and found that N deficiency not only stimulated the elongation of the main root and lateral root, but also increased the number of root tips [26]. In our study, the root images obtained from the root scanner and root length showed that low N stress changed the root morphology with promoted root elongation and increased root surface area (Figures 1C and 2A), which is conducive to more effective contact with N in soil and improving absorption efficiency in an N-limited environment. Furthermore, low N treatment significantly increased the dry matter accumulation of rapeseed roots (Table 1), which is closely related to root morphology alteration.

A lot of studies have focused on revealing the modulation mechanism of root architecture alteration, and Giehl et al. noted that the most plausible mechanistic view is that once perceived nutrient signals, a set of genes/proteins in the root developmental program were affected in order to modify specific root developmental processes [13]. Root system architectural changes can be triggered by complex signaling mechanisms such as brassinosteroid signaling, auxin transporter, and auxin signaling that are involved in the regulation of root architecture under low N conditions [19,20,27,28]. Root morphogenesis is regulated by cell division and expansion [21]. Cell wall biosynthesis is involved in the processes of cell division, elongation, and differentiation [14], and affects cell size and shape. Cell wall structure and cell wall metabolism are strongly influenced by various abiotic stresses, such as drought, flooding, extreme temperature, salinization, and heavy metals [29]. A previous study found that the amount of several cell-wall-related proteins in rapeseed roots increased under N deficiency, which participates in the adaptive elongation of roots [26]. Nitrogen also has a significant effect on cell wall composition [30]. It has been reported that ammonium could reduce the content of pectin and hemicellulose in cell walls of rice root tips [31]. Cell elongation also promotes cell wall components (such as cellulose, hemicellulose, pectin, etc.) synthesis to maintain cell wall thickness [22]. Xin et al. identified proteins related to cellulose, hemicellulose, and pectin under different N conditions and proposed that the response of rice root morphology to N availability could be achieved through coordination of relevant proteins involved in cell structure, cell wall modification, and cell division [22]. Our results also showed that low N stress changed the cell wall composition of rapeseed roots, accompanied by a significant increase in two different types of pectin and cellulose content, but had no remarkable effect on hemicellulose and lignin content (Figure 3C,E–G). A study on sorghum seedlings also confirmed that N deficiency affected root cell wall components by upregulated expression of cell-wall-related genes, including cellulose synthase-like proteins and pectate lyase [32]. The analysis of pectinase, cellulase, and pectin monosaccharide group, and the expression of related genes, indicated that the increase of pectin and cellulose was mainly attributed to inhibited degradation of pectin and cellulose in root cell walls induced by N deficiency. Pectin demethylation was also promoted by low N treatment (Figure 3D), and the increased PME activity, upregulated PME genes and downregulated PME genes, also proved the results (Figure 4D and Table 2). As the main component of plant cell walls, pectin has many functions in plants, such as promoting cell adhesion, providing structural support, affecting the formation of secondary cell walls, etc. [33,34]. It has been reported that PME activity appeared to create an acidic environment within the cell walls, causing cell wall loosening [35]. Cellulose is part of cell walls, and it performs physiological functions in plants and also plays a skeleton supporting role in plant cell walls and has excellent tensile strength [36]. It is widely known that root morphogenesis is controlled by cell division and expansion, and these are related to the loosening and hardening of cell walls. In this study, low N treatment induced promoting pectin demethylation, more pectin and cellulose in root cell walls, which were conducive to promote cell wall loosening and provide more components for cell wall synthesis, thus promoting root cell division and elongation, ultimately promoting root elongation.

4. Materials and Methods

4.1. Experimental Design

Zhongshuang “11” rapeseed variety was chosen as the study material and cultured in a nutrient solution in an illuminated room. The temperature of the illumination room was set at 22 °C–24 °C, the light cycle was 14 h (light)/10 h (dark), and the light intensity was 300–320 $\mu\text{mol}/\text{m}^2/\text{s}$. The humidity was maintained at 60–75%. The macro and microelements were provided according to Hoagland and Arnon’s nutrient solution formula. In the experiment, two different N treatments were set: low N (LN: 0.3 mmol/L NO_3^-) and normal N (CK: 6.0 mmol/L NO_3^-), and six biological replicates were set for each treatment. The germinated and consistently growing rapeseed plants were chosen and transplanted into the shading culture plates. After being cultured in the normal nutrient

solution for 5 days, the seedlings were transferred into the nutrient solution with different N concentrations for 10 days.

4.2. Determination Indicators and Methods

Growth parameter: during the treatment period, the root and leaf phenotypes of rapeseed were observed and recorded, and the root length was measured after being harvested.

Measurement of photosynthetic pigment content: according to the previously described method [37], the photosynthetic pigment of fresh leaves was extracted by ethanol (95%). The absorbance of chlorophyll a, b, and carotenoid was gained by UV-VIS spectrophotometry (TU-1810, PGENERAL, Beijing, China) at wavelengths of 470, 649, and 665 nm, respectively. The chlorophyll a, chlorophyll b, and carotenoid contents were calculated according to their corresponding formulae: chlorophyll a (mg/L) = $13.95A_{665} - 6.88A_{649}$, chlorophyll b (mg/L) = $24.96A_{649} - 7.32A_{665}$, carotenoid (mg/L) = $\frac{1000A_{470} - 2.05A_{665} - 114.8A_{649}}{245}$.

Transmission electron microscope (TEM) imaging: the TEM slices of roots were prepared using the method of Kong et al. [38]. Ultrathin sections were examined with a TEM (Hitachi 500 electron microscope) at an acceleration voltage of 60 kV, and representative plant images were chosen for each N treatment.

Dry matter accumulation: after harvesting, the roots and leaves of rapeseed seedlings were sampled and then the enzymes in plants were first inactivated at 105 °C for 30 min, and later dried at 75 °C for 3 days, and finally weighed to measure the dry weight.

Determination of total N content: about 0.1 g of the sample was weighed after being crushed and evenly mixed, and then placed into a triangular flask, and 5 mL of concentrated sulfuric acid was added for digestion on an electric heating plate. When the samples were clear, the hydrogen peroxide was added and then transferred to a volumetric flask. After dry filtration and dilution, the AA3 flow analyzer (autoanalyzer 3, seal, Germany) was used to determine the total N content.

Extraction of root cell walls was conducted based on the methods of Weigel and Jager [39] and Rathore [40]: after the fresh root was finely ground in a precooled quartz mortar with a homogenate solution (pH = 7.2) of sucrose, Tris-HCl, and dithiothreitol, the mixed solution was centrifuged at $2000 \times g$ at 4 °C for 10 min, and the precipitate was considered as cell wall material.

Separation of root cell wall components: the cell wall material was further separated into ion-bound pectin (ISP), covalently bound pectin (CSP), cellulose, and hemicellulose, as previously described by Redgwell and Selvendran [41] with slight modification. Firstly, the ground cell wall powder was suspended in 0.05 M sodium acetate buffer (pH 6.5) containing 0.05 M CDTA and stirred for 12 h at 24 °C in a horizontal shaker, and the supernatant was defined as CDTA-soluble pectin (CSP). Then, the CDTA-insoluble pellet was resuspended in 0.05 M Na₂CO₃ and incubated for 12 h at 24 °C. After centrifugation at $5000 \times g$ for 20 min, the supernatant was designated as ion-soluble pectin (ISP). Finally, the remaining residue was added to 20 mL of 4 M KOH solution, extracted by shaking at 24 °C for 3 h, and centrifuged. The supernatant was KOH-soluble component, which was mainly hemicellulose, and the last residue was mainly cellulose.

Determination of cell wall components: the contents of ISP, CSP, cellulose, and hemicellulose were respectively determined using corresponding kits (Keming Biotechnology Co., Ltd., Suzhou, China).

Determination of cell wall lignin content: lignin content in root samples was determined using the acetyl bromide method [42,43]. Briefly, 10 mg of dry root samples were incubated in 0.6 mL of a 25% acetyl bromide solution (*w/w*, AcBr in glacial acetic acid) in 2 mL Eppendorf tubes at 80 °C for 3 h. After cooling to 25 °C and centrifuging at $12,000 \times g$ for 5 min, 0.1 mL of the above mixture was transferred into a new 2 mL Eppendorf tube. Then, 0.4 mL of 2 M NaOH and 0.07 mL of 0.5 M hydroxylamine hydrochloride were added and the mixture was thoroughly vortexed. Subsequently, 100 µL of the mixture was transferred to a 96-well microplate followed by 200 µL of glacial acetic acid. Finally, the percentage of acetyl bromide soluble lignin was measured by UV-Vis spectrophotometry

(TU-1810, PGENERAL, Beijing, China) at a wavelength of 280 nm. Lignin concentration was expressed as $A_{280} \text{ g}^{-1} \text{ DW}$.

Determinations of pectin methylation degree: by the method of Anthon and Barrett [44], with some modifications, the methylation degrees of ISP and CSP were measured. After the pectin extraction with NaOH (1.5 M) was treated at 25 °C for 30 min, the mixed solution of H_2SO_4 (0.75 M), Tris-HCl (0.2 M, pH 7.5), MBTH (3 mg/mL), and alcohol oxidase (0.01 units/mL) was added. Then, the reaction was terminated by a solution containing ammonium ferric sulfate (5 mg/mL) and sulfamic acid (5 mg/mL). Finally, after incubation at 25 °C for 20 min, the absorbance was measured at 620 nm using a spectrophotometer (TU-1810, PGENERAL, Beijing, China). Pectin demethylation degree = 100 – pectin methylation degree.

The activity of pectinase and cellulose analysis: the activities of the PME, pectate lyase, polygalacturonase, β -galactosidase, and cellulase were measured with the available commercial PME-2-G, PL-1-G, PG-1-G, β -GALB-1-Y, and CL-2-Y kits, respectively, following the manufacturer's guidelines (Suzhou Comin Biotechnology Co., Ltd. Suzhou, China).

High-throughput sequencing data analysis of the transcriptome: the mapped reads of each sample were assembled using StringTie (<http://ccb.jhu.edu/software/stringtie/>, accessed on 1 February 2022). Then, the transcriptomes from the samples were merged to reconstruct a comprehensive transcriptome using perl scripts. After the final transcriptome was generated, StringTie and edgeR (<http://bioconductor.org/packages/release/bioc/html/edgeR.html>, accessed on 1 February 2022) were used to estimate the expression levels of all the transcripts. StringTie was used to examine the expression levels of the mRNAs by calculating the FPKM (fragments per kilobase of transcript sequence per million base pairs sequenced). The differentially expressed genes (DEGs) related to cell wall remodeling were defined as those with p -value < 0.05.

4.3. Statistical Analysis

The data were statistically analyzed by SAS 9.1.3 (SAS Institute, Cary, NC, USA). ANOVA analysis based on the Student's t -test method was used to determine the significance of differences between LN and HN treatments. The * and ** represent the statistical significances at the levels of $p < 0.05$ and $p < 0.01$, respectively.

5. Conclusions

Compared with normal N treatment, low N treatment significantly promoted rape-seed root elongation and improved root dry mass accumulation, root/shoot ratio, and N physiological efficiency, while reducing photosynthetic pigment content and dry matter accumulation in leaves. Analysis of the main chemical components of root cell walls showed that low N increased the demethylation degree of ISP and CSP, the content of CSP, and cellulose in root cell walls, which were closely related to promoted PME activity, and inhibited pectin degradation enzymes, cellulase. The low-N-increased demethylation degree of pectin and content of pectin and cellulose in cell walls was conducive to cell wall loosening and cell wall synthesis during cell division and elongation, ultimately promoting root adaptive elongation.

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