

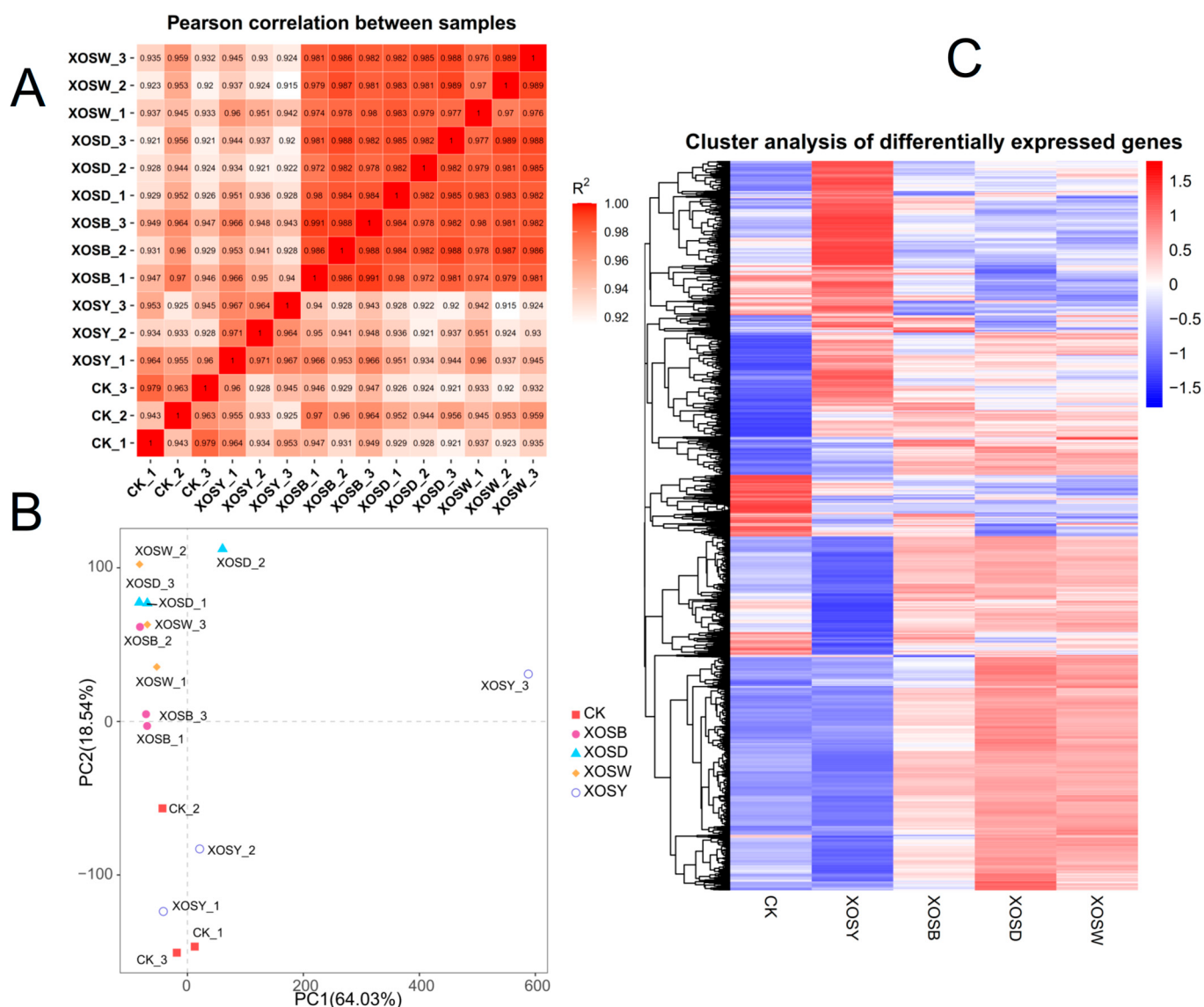


Figure S1. Comprehensive assessment of RNA-seq data accuracy. (A) Correlation analysis demonstrating the consistency and reliability of biological replicates based on pairwise Pearson correlation coefficients. (B) Principal component analysis (PCA) visualizing the variance and grouping of samples with and without xylooligosaccharides (DP 2–5) treatment. (C) Heatmap of clustered differentially expressed genes (DEGs), showcasing distinct expression patterns and clustering of samples under different treatment conditions.

Table S1. Root morphology characteristics of lettuce plants treated with xylooligosaccharides (DP 2 – 5).

Treatments	Total root length (cm)	SurfArea (cm ²)	AvgDiam (mm)	RootVolume (cm ³)	Lateral root length (cm) 0<.L.≤0.25	Adventitious root length (cm) 0.25<.L.
CK	4261.05±548.12b	303.08±42.58b	0.23±0.00ab	1.72±0.26b	1005.55±219.26b	3170.14±217.58b
XOSY	4466.73±308.35ab	318.13±15.50b	0.23±0.00b	1.87±0.10ab	1139.73±116.65b	3374.93±243.57ab
XOSB	4676.98±403.86ab	317.15±20.80ab	0.23±0.00ab	1.80±0.11ab	1091.44±67.55b	3280.46±300.10b
XOSD	5060.64±437.54a	361.12±32.62a	0.24±0.00a	2.10±0.16a	1395.92±104.47a	3838.44±350.78a
XOSW	4627.98±300.73ab	337.95±21.16ab	0.24±0.01a	1.97±0.12ab	1203.73±69.34ab	3423.84±233.09ab

Note: Means ± SD (n=6) followed by the same letter within a column are not significantly different at the 0.05 probability level according to the least significant difference (LSD) test.

Table S2. Sequencing data quality and alignment results with the reference genome.

Sample	Raw Reads	Clean Reads	Clean Base (G)	Error Rat (%)	Q30 (%)	GC Content (%)
CK_1	47879030	45906234	6.89G	0.0003	0.93	0.47
CK_2	44248664	43336030	6.50G	0.0003	0.94	0.47
CK_3	46726616	45382788	6.81G	0.0003	0.94	0.47
XOSY_1	45216960	43740650	6.56G	0.0003	0.94	0.46
XOSY_2	52820254	49889950	7.48G	0.0003	0.93	0.46
XOSY_3	38907234	36385590	5.46G	0.0003	0.93	0.49
XOSB_1	45636912	44686692	6.70G	0.0003	0.93	0.46
XOSB_2	46551434	45748678	6.86G	0.0003	0.93	0.46
XOSB_3	45001846	43840756	6.58G	0.0003	0.93	0.46
XOSD_1	39036270	38125434	5.72G	0.0003	0.93	0.46
XOSD_2	55290836	53501390	8.03G	0.0003	0.94	0.47
XOSD_3	39555362	38806908	5.82G	0.0003	0.93	0.46
XOSW_1	45147652	43954154	6.59G	0.0003	0.93	0.46
XOSW_2	43784662	43122258	6.47G	0.0003	0.93	0.46
XOSW_3	41556596	40738546	6.11G	0.0003	0.94	0.46

Table S3. Key metabolic pathways associated with lettuce growth under xylooligosaccharide (DP 2 - 5) treatment.

KEGG Terms		Up-regulated DEGs				Down-regulated DEGs			
		XOSY	XOSB	XOSD	XOSW	XOSY	XOSB	XOSD	XOSW
Carbohydrate metabolism	Amino sugar and nucleotide sugar metabolism	—	5	11	6	4	2	7	3
	Ascorbate and aldarate metabolism	—	2	5	3	—	3	10	7
	Butanoate metabolism	—	1	2	—	2	2	5	2
	C5-Branched dibasic acid metabolism	—	1	2	—	2	—	4	—
	Citrate cycle (TCA cycle)	—	2	2	2	3	4	10	3
	Fructose and mannose metabolism	—	2	3	2	5	4	11	5
	Galactose metabolism	—	1	8	6	2	2	5	3
	Glycolysis / Gluconeogenesis	—	8	9	7	8	8	23	11
	Glyoxylate and dicarboxylate metabolism	—	2	2	3	8	15	23	16
	Inositol phosphate metabolism	—	3	9	2	2	3	5	—
	Pentose and glucuronate interconversions	—	5	13	7	2	5	7	4
	Pentose phosphate pathway	—	1	—	—	6	8	16	11
	Propanoate metabolism	—	1	5	3	2	—	2	—
	Pyruvate metabolism	1	4	7	5	6	6	16	7
	Starch and sucrose metabolism	4	7	25	17	8	5	11	7
Energy metabolism	Carbon fixation in photosynthetic organisms	—	—	—	—	9	19	31	22
	Nitrogen metabolism	—	—	1	—	5	7	8	7
	Photosynthesis	—	—	—	1	1	17	37	30
	Photosynthesis-antenna proteins	—	—	—	—	2	15	22	23
	Oxidative phosphorylation	—	—	1	—	2	4	19	6

Table S4: KEGG pathway enrichment of downregulated genes in lettuce leaves treated with xylooligosaccharides (DP 2–5).

XOSY				
#Term	ID	Input number	P-Value	Corrected P-Value
Purine metabolism	lsv00230	9	0.00321256	0.091926937
Biosynthesis of amino acids	lsv01230	15	0.003649608	0.091926937
Carbon fixation in photosynthetic organisms	lsv00710	8	0.003655434	0.091926937
Carbon metabolism	lsv01200	16	0.00483826	0.091926937
Glyoxylate and dicarboxylate metabolism	lsv00630	7	0.009067608	0.137827637
RNA transport	lsv03013	10	0.018392129	0.215554942
Pentose phosphate pathway	lsv00030	5	0.019853745	0.215554942
Nitrogen metabolism	lsv00910	4	0.026492338	0.251677211
Alanine, aspartate and glutamate metabolism	lsv00250	4	0.043250315	0.365224886
Porphyrin and chlorophyll metabolism	lsv00860	4	0.064782401	0.460114653
Vitamin B6 metabolism	lsv00750	2	0.066595542	0.460114653
Glycine, serine and threonine metabolism	lsv00260	4	0.087419364	0.534956401
Pyruvate metabolism	lsv00620	5	0.0915057	0.534956401
Selenocompound metabolism	lsv00450	2	0.102044233	0.553954407
Glycolysis / Gluconeogenesis	lsv00010	7	0.127456199	0.608456449
XOSB				
#Term	ID	Input number	P-Value	Corrected P-Value
Photosynthesis - antenna proteins	lsv00196	14	5.98E-11	4.66E-09
Photosynthesis	lsv00195	18	4.21E-09	1.64E-07
Carbon fixation in photosynthetic organisms	lsv00710	18	5.56E-08	1.45E-06
Carbon metabolism	lsv01200	29	3.75E-06	7.30E-05
Glyoxylate and dicarboxylate metabolism	lsv00630	14	1.17E-05	0.000182098
Porphyrin and chlorophyll metabolism	lsv00860	10	0.00010604	0.001378523
Nitrogen metabolism	lsv00910	6	0.006250472	0.069648117
Pentose phosphate pathway	lsv00030	7	0.007549625	0.073608847
Terpenoid backbone biosynthesis	lsv00900	8	0.012703721	0.110098917
Vitamin B6 metabolism	lsv00750	3	0.024300728	0.189545675
Biosynthesis of amino acids	lsv01230	16	0.034438168	0.229980792
Glycine, serine and threonine metabolism	lsv00260	6	0.03538166	0.229980792
Biosynthesis of secondary metabolites	lsv01110	59	0.058970266	0.353821597
Thiamine metabolism	lsv00730	3	0.104300336	0.559213326
Metabolic pathways	lsv01100	107	0.12201639	0.559213326
XOSD				
#Term	ID	Input number	P-Value	Corrected P-Value
Photosynthesis - antenna proteins	lsv00196	24	1.76E-14	1.78E-12
Photosynthesis	lsv00195	34	2.47E-13	1.25E-11
Carbon fixation in photosynthetic organisms	lsv00710	30	3.23E-09	1.09E-07
Carbon metabolism	lsv01200	57	1.09E-08	2.74E-07
Glyoxylate and dicarboxylate metabolism	lsv00630	22	1.22E-05	0.000246922
Pentose phosphate pathway	lsv00030	15	0.000222723	0.003749175
Porphyrin and chlorophyll metabolism	lsv00860	14	0.000561698	0.008104493
Glycine, serine and threonine metabolism	lsv00260	13	0.003634936	0.045891069

Biosynthesis of amino acids	lsv01230	33	0.009255371	0.103865827
Glycolysis / Gluconeogenesis	lsv00010	22	0.015930827	0.160901357
Sulfur metabolism	lsv00920	7	0.022788579	0.209240593
Biosynthesis of secondary metabolites	lsv01110	125	0.02487915	0.209399509
Ascorbate and aldarate metabolism	lsv00053	9	0.04044416	0.30271135
Pyruvate metabolism	lsv00620	13	0.041959989	0.30271135
C5-Branched dibasic acid metabolism	lsv00660	3	0.047185709	0.317717104

XOSW

#Term	ID	Input number	P-Value	Corrected P-Value
Photosynthesis - antenna proteins	lsv00196	21	1.44E-16	1.14E-14
Photosynthesis	lsv00195	28	3.68E-15	1.45E-13
Carbon fixation in photosynthetic organisms	lsv00710	21	1.69E-08	4.46E-07
Carbon metabolism	lsv01200	37	1.35E-07	2.66E-06
Glyoxylate and dicarboxylate metabolism	lsv00630	17	2.36E-06	3.72E-05
Pentose phosphate pathway	lsv00030	10	0.000657134	0.008652265
Terpenoid backbone biosynthesis	lsv00900	11	0.002051239	0.020723845
Porphyrin and chlorophyll metabolism	lsv00860	9	0.002098617	0.020723845
Biosynthesis of amino acids	lsv01230	21	0.011039498	0.09690226
Biosynthesis of secondary metabolites	lsv01110	77	0.014971552	0.118275257
Nitrogen metabolism	lsv00910	6	0.016743831	0.12025115
Sulfur metabolism	lsv00920	5	0.02166608	0.142635027
Glycine, serine and threonine metabolism	lsv00260	7	0.033395071	0.202939276
Vitamin B6 metabolism	lsv00750	3	0.041928564	0.236596896

Table S5. Specific primers used for RT-qPCR amplification.

ID Gene id	Gene-description	Primers sequences	RNA-seq (log2FC)	RT-qPCR (Log2FC)
LOC111904911 XOSY vs CK	calcium-binding protein CML38-like	F:TGGTTTCGTTTCGTCTCAGTAT R:GAGTAAAATTCCTTGGTTCCAGAT	2.74	0.77
LOC111917576 XOSD vs CK	probable WRKY transcription factor 26	F:GGTTATAGATGGAGGAAATATGGAC R:TTGTTATTGGTGTTACCGTTGTTAG	2.06	18.51
LOC111876531 XOSY vs CK	pathogenesis-related genes transcriptional activator PTI6-like	F:GCTGCCTCCTTTGGGTTT R:GTGTCTCCTGTTTCTGCTTCG	1.05	1.15
LOC111886653 XOSY vs CK	pathogenesis-related genes transcriptional activator PTI6-like	F:ATCCGAATAGGAGGAAAAGAGT R:TTCAATTAAACGGCAGCATC	2.03	6.22
LOC111881482 XOSD vs CK	protein EDS1-like	F:GGCGACTGTTTGAGCCTTTAG R:GGCGACTGTTTGAGCCTTTAG	2.47	3.57
LOC111881492 XOSD vs CK	protein EDS1-like	F:ACATTTGAAGGGAGATGGGTAT R:GTATTTTATCATCAGAACGCCAC	2.07	7.64