World Journal of Agriculture and Forestry Sciences
ISSN: 2960-0227
DOI: 10.61784/wjafsv2n228

EFFECTS OF XYLO-OLIGOSACCHARIDES ON SOYBEAN GROWTH AND MOSAIC VIRUS DISEASE RESISTANCE

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Abstract: Soybeans were treated with xylo-oligosaccharides at different concentrations to study the effects of xylo-oligosaccharides on soybean growth and mosaic virus (SMV) resistance. Greenhouse experimental results. It shows that: compared with the control after treating soybean seeds with xylo-oligosaccharide solution for 18 days, 50 mg·L-1 xylo-oligosaccharide has a significant promoting effect on the plant height, fresh weight and main root length of soybean seedlings. Soybean leaves sprayed with xylo-oligosaccharides were inoculated with SMV 18 hours later, which significantly reduced the SMV disease index compared with the control. Quantitative RT-PCR was used to detect the expression of defense-related genes in soybean leaves treated with xylo-oligosaccharides. Compared with the control, the defense genes PR2, PR10, PR12 and LOX2 had the highest expression levels when treated with xylo-oligosaccharides for 12 h. The relative expression level; PR3 had the highest relative expression level when treated with xylo-oligosaccharide for 18 h; the expression of PR1, PAL, PPO and CHS was significantly up-regulated when treated with xylo-oligosaccharide for 24 h. Studies have shown that xylo-oligosaccharides activate the soybean SA signaling pathway and JA signaling pathway, thereby improving soybean resistance to SMV.
Keywords: Xylo-oligosaccharides; Soybean; Growth promotion; SMV resistance; Uantitative RT-PCR

1 MATERIALS AND METHODS

Soybean mosaic virus (SMV) can infect some leguminous plants, especially soybeans. The virus mainly spreads and re-infects in the field through aphids as a vector, which can lead to poor plant growth and reduced yield and quality [1]. The main prevention and control methods of soybean mosaic virus disease are disease-resistant variety selection and aphid control. However, conventional breeding methods have shortcomings such as long breeding cycles, and the use of chemical pesticides will pollute the ecological environment. Therefore, there is an urgent need to seek new methods. Safe and cost-effective control measures for soybean mosaic virus disease. In recent years, it has become a new research direction to use the plant's own defense system to activate plant disease resistance genes through elicitors and inducers to produce systemic disease resistance in plants.
Xylo-oligosaccharides, also known as xylo-oligosaccharides, are functional polymeric sugars composed of 2 to 7 xylose molecules bound by β-1,4 glycosidic bonds. OK
It is obtained by treating agricultural and sideline products with relatively high xylan content such as corn cobs, rice husks, bagasse, bran, etc. by peracid hydrolysis, hot water extraction, microwave degradation or enzymatic hydrolysis. Xylo-oligosaccharides can make tomato stems and leaves grow robustly, increase tomato's absorption of N and K nutrients in the soil, and increase soil microbial activity [2]. Treating poplar tomentosa callus with xylo-oligosaccharide can reduce its susceptibility index to poplar canker bacteria [3]. At present, chitosan, glucan oligosaccharide and oligogalacturonic acid in oligosaccharide elicitors have achieved good disease prevention effects in tobacco, soybeans, corn and other crops [4], but regarding oligomeric wood There are few reports on sugar promoting soybean growth and preventing and controlling soybean diseases. This article uses xylo-oligosaccharides to treat soybeans to study the effects of xylo-oligosaccharides on soybean growth and soybean mosaic virus disease resistance. At the same time, Quantitative RT-PCR is used to detect the relative expression of defense-related genes in soybean leaves and preliminarily analyze The soybean disease resistance signaling pathway induced by xylo-oligosaccharides lays the foundation for the application of xylo-oligosaccharides.

1.1 Materials

The propagation host of soybean virus is Nannong 1138-2 and soybean mosaic virus strain SC7 (a gift from Professor Zhi Haijian of the National Soybean Improvement Center of Nanjing Agricultural University). The soybeans tested were Jifeng No. 1 (a gift from Jilin Agricultural University) and xylo-oligosaccharide (produced by Shandong Longli Science and Technology Biological Co., Ltd.).

1.2 Method

1.2.1 Greenhouse growth promotion test

Select healthy soybean seeds without lesions, disinfect them with 10% NaClO for 3 minutes, wash them with clean water 3 to 4 times, and place them in a ventilated place to dry. Then soybean seeds were soaked in xylo-oligosaccharide solutions with concentrations of 0, 10, 30 and 50 mg·L -1 for 6 h respectively, and then sown in flower pots containing a mixture of sterilized soil and vermiculite, 15 seeds per pot. Soybeans, 4 pots per treatment, 3 replicates. The flowerpots were placed in a greenhouse at (25 ± 5) ℃, and the germination rate, plant height, fresh weight and aboveground weight were investigated 18 days later.

1.2.2 Greenhouse disease resistance test

When the true leaves of soybean plants sown in the greenhouse are spread out, xylo-oligosaccharide solutions with concentrations of 0, 10 and 100 mg·L -1 are evenly sprayed on the surface of the soybean leaves. The spray volume of the leaves was 1 mL, and each treatment had 10 plants. It was repeated three times. After 18 h, the soybean mosaic virus SC7 strain was rubbed and inoculated. After 20 days, the disease index was investigated and the disease prevention effect was calculated. The classification standard of soybean mosaic virus disease refers to the method of Zhi Haijian et al.[5].

1.2.3 Quantitative RT-PCR to detect the expression of defense-related genes

When the true leaves of soybean plants sown in the greenhouse are spread out, select leaves of similar size and evenly spray 1 mL of 100 mg·L -1 xylo-oligosaccharide on each leaf. solution, with clean water treatment as the control, and soybean leaves were taken at 12, 18, and 24 h after spraying. Omiga's plant RNA extraction kit was used to extract total RNA from soybean leaves, and TaKaRa PrimerScript ®Reverse Transcriptase kit was used to reverse-transcribe the mRNA into cDNA. Using an appropriate amount of reverse transcription product as a template, using SYBR Premix ExTaqTM Kit, two-step-qRT-PCR was performed on the ABI PRISM 7500 fluorescence quantitative PCR instrument to perform PR1, PR2, PR3, PR10, PR12, PAL, PPO, CHS and For the determination of LOX2 gene expression, the EF-1a gene was used as the internal reference. The primer sequences are shown in Table 1. The experiments were set up in three replicates, and the plants treated with water were used as controls. The qRT-PCR data were analyzed using the 2-ΔΔCt method [6].

Table 1 Real-time fluorescence quantitative PCR primers

Gene	Primer sequence (5'-3')	target sequence	Annealing temperature	gene length	Gene Description	references
EF-1a	CTGTAACAAAATGGATGCTACTAC AGTCAAGGTTTTGTGGACCT	X56856	61	176	Elongation factor EF-1a	[7]
PR1	TGATGTTGCCTACGCTCAAG AAGCAGCAACCGTATCATCC	AF136636	61	137	PR1a precursor	[8]
PR2	GTCTCCTTCGGTGGTAGTG ACCCTCCTCCTGCTTTCTC	M37753	57	104	Beta 1-3 Endoglucanase	[9]
PR3	GCACTTGGTCTGGATTTG GGCTTGATGGCTTGTTTC	AF202731	53	115	Chitinase class I	[10]
PR10	GCCCAGGAACCATCAAGAAG	AJ289155	58	108	Stree-induced	[11]
PR12	CGCTGTAGCTGTATCCCAAG CATGGACAAGGCACGATTTGG AACCGATGGCTCTTTGACTCAC	BU964598	62	108	ribouclease-like protein Defensin precursor	[12]
PAL	GTGCAAGGGCTGCTTATG CCCAGTCCCTAATTCCTCTC	X52953	57	107	Phenylalanine ammonia-lyase	[13]
PPO	GGGTTGGTGCTGCTGATAAG CGATCCGAGTTCGTGTGATG	EF158428	62	100	Polyphenol oxidase	[14]
CHS	AGGCTGCAACTAAGGCAATC TAATCAGCACCAGGCATGTC	X53958	57	103	Chalcone synthase	[15]
LOX2	TGGTTGCGGGTGTAAATC CAAGGGCATCTGCTGTTATC	D13949	59	117	Lipoxygenase 2	[16]

1.3 Data Analysis

SPSS 16.0 statistical software was used for variance analysis statistics.

2 RESULTS AND ANALYSIS

2.1 Greenhouse Growth Promotion Test

It can be seen from Table 2 that the plant height of soybeans treated with 10, 30 and 50 mg·L -1 xylo-oligosaccharides significantly increased, and the plant height increased by 31.58%, 23.36% and 49.63% respectively compared with the control; 50 mg·L -1 xylo-oligosaccharide had a significant promoting effect on the plant height, fresh weight and main root length of soybean seedlings, which were increased by 49.63%,

29.76% and 20.96% respectively compared with the control; The germination rate of 30 mg·L -1 xylo-oligosaccharide treatment was higher than that of other treatments, and increased by 19.51% compared with the control.

Table 2 Effects of different concentrations of xylo-oligosaccharide solutions on soybean germination and growth

concentration	Germination rate	Plant height	Fresh weight	Taproot long
0	60.00 ± 14.35a	10.70 ± 1.36c	1.68 ± 0.16c	1.24 ± 0.22b
10	66.67 ± 17.97a	14.08 ± 1.22b	1.89 ± 0.20b	1.34 ± 0.15b
30	71.71 ± 16.60a	13.20 ± 1.11b	1.83 ± 0.31bc	1.29 ± 0.20b
50	66.67 ± 19.89a	16.01 ± 1.71a	2.18 ± 0.20a	1.50 ± 0.16a

The data in the table are expressed as mean ± standard deviation. Different lowercase letters after the values in the same column represent significant differences at the 0.05 level, the same as in the table below.
Values are x ± SD, values in a column followed by different lowercase letters are significantly different at 0.05 level, the same below.

2.2 Effect of Xylo-oligosaccharide Treatment on Soybean SMV Resistance

It can be seen from Table 3 that compared with the control, the incidence of soybean virus diseases was reduced by 12.00% and 19.99% respectively under 10 and 100 mg·L^{-1} xylo-oligosaccharide treatments, but neither reached significance. level; the disease index of xylo-oligosaccharide treatment was significantly lower than that of the control; the control effects on soybean virus diseases were 30.43% and 34.59% respectively. Xylo-oligosaccharides significantly induced soybean resistance to SMV.

Table 3 The control effect of different concentrations of xylo-oligosaccharides on soybean virus diseases in the greenhouse

concentration	Incidence	disease index	Prevention effect
0	83.33 ± 15.27a	61.33 ± 6.11c	-
10	73.33 ± 5.77a	42.67 ± 6.11b	30.43
100	66.67 ± 5.77a	40.00 ± 8.00a	34.59

2.3 Effects of Xylo-Oligosaccharide Treatment on the Expression of Defense-Related Genes

In order to detect xylo-oligosaccharide-induced resistance to SMV in soybean for the molecular mechanism, Quantitative RT-PCR was used to detect the expression of defense-related genes in soybean leaves 12, 18 and 24 hours after xylo-oligosaccharide was sprayed (Figure 1). Among the 9 defense-related genes, β-1,3-glucanase gene (PR2), ribonuclease protein control gene (PR10).

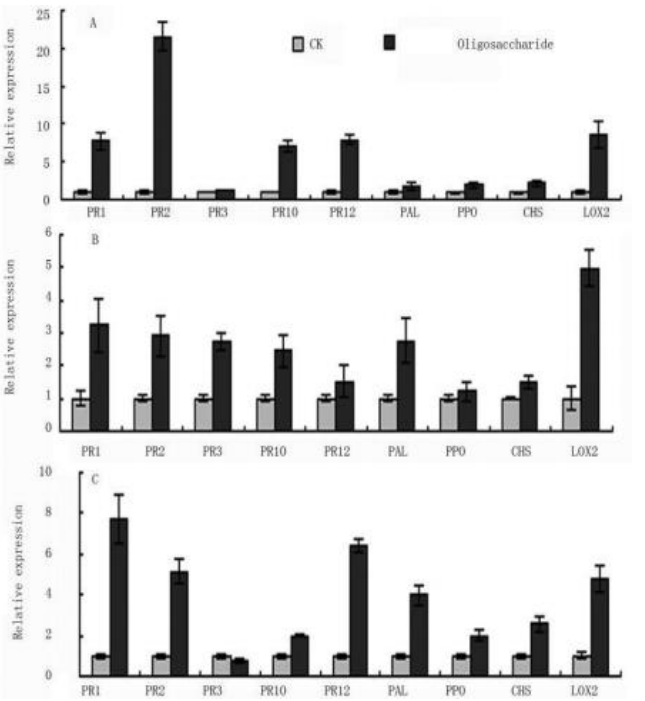

Figure 1 Expression levels of defense-related genes in soybean leaves at different time periods after xylooligosaccharide treatment
Note: A, B, and C indicate xylooligosaccharide treatment for 12, 18, and 24 h respectively.

The royal-related protein expression gene (PR12) and lipoxygenase gene (LOX2) had the highest relative expression levels when treated with xylo-oligosaccharides for 12 h, which were 21.56, 7.19, 7.95 and 8 compared to the control, respectively.. 56 times; when the chitinase gene (PR3) was treated with xylo-oligosaccharides for 18 h, the gene expression was 2.73 times that of the control; when soybeans were treated with xylo-oligosaccharides for 24 h, the system acquired the resistance gene (PR1) , polyphenol oxidase gene (PPO), phenylalanine ammonia lyase gene (PAL) and chalcone synthase gene (CHS) expression levels were 7.75, 1.99, 3.98 and 2% of the control, respectively.. 56 times. This indicates that xylo-oligosaccharides may simultaneously activate the SA signaling pathway and the JA signaling pathway in soybeans, inducing resistance to SMV in soybeans.

3 CONCLUSION

As a new type of plant hormones and plant resistance response activators, oligosaccharide biopesticides have broad application and development prospects in crop growth and development and pest and disease control, and have received widespread attention. Xylo-oligosaccharide is a new type of oligosaccharide, which is currently mainly used in the food and feed fields. Its application in agriculture is rarely reported. In this study, we found for the first time that treating soybean with xylo-oligosaccharides can promote soybean growth, activate defense-related genes, and induce SMV resistance in soybean. In addition, because xylo-oligosaccharides are safe, have stable structure, low viscosity and are easily soluble in water, xylo-oligosaccharides can also be used as an ideal carrier for biopesticides.

Oligosaccharides can regulate plant growth, development and reproduction, stimulate plants to undergo a series of defense reactions, produce phytoalexins and other defense substances, synthesize disease progression-related proteins (PR proteins), and induce allergic reactions in plants [17]. There are two main types of signal transduction used by plant extracellular signaling molecules to induce plant disease resistance: one is the SA pathway, and the other is the JA or ET pathway. Genes PR1 and PR2 are marker genes of the SA signaling pathway, and JA induces the expression of PR3, PR10 and PR12 genes; LOX is a key enzyme in the formation process of JA [18-20]. In this study, after xylo-oligosaccharide treatment of soybean leaves, except for PR3, which was not up-regulated at 24 h, the other eight genes were up-regulated at 12, 18 and 24 h, and ultimately showed resistance to SMV in soybeans. Sexual enhancement. Therefore, it is speculated that xylo-oligosaccharides may simultaneously activate the SA signaling pathway and JA signaling pathway in soybeans, causing the plants to develop systemic resistance and enhance their resistance to SMV invasion. In addition, there are reports that the glucan elicitor (WGE) obtained from the cell wall of Phytophthora sojae can induce the expression of PR1, PR2, PR4, PR6 and PR10 proteins in soybeans [21]. Studies have shown that treating tobacco with oligogalacturonic acid in oligosaccharides can induce the accumulation of JA and SA, activate the expression of defense genes, and then produce a defense response [22-23]. Therefore, the signal transduction pathways of xylo-oligosaccharides and oligogalacturonic acid-induced plant defense responses are similar, involving signal pathways such as SA and JA, activating transcription factors to express defense genes, producing phytoalexins and PR proteins, and finally inducing Plants develop resistance.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

REFERENCES

[1] Chen Lifeng, Xu Jingyou. Agricultural plant pathology. Beijing: China Agricultural Press, 2001: 220-222.
[2] Ji Xiaoming, Lu Quanjian, Zhao Mingqin. Characteristics and application research progress of xylo-oligosaccharides. Anhui Agricultural Sciences, 2007, 35 (23): 7088-7089.
[3] He Ying. Preparation of several oligosaccharide elicitors and study on their induction of disease resistance in poplar poplar callus. Yangling: Northwest A&F University, 2006: 3034.
[4] Yamaguchi T, Ito Y, Shibuya N. Oligosaccharide elicitors and their receptors for plant defense responses. Trends in Glycoscience and Glycotechnology, 2000, 12(64): 113-120.
[5] Zhi HJ, Gai JY. Performances and germplasm evaluation of quantitative resistance to soybean mosaic virus in soybeans. Agricultural Science in China, 2004, 3(4): 247-253.
[6] Livak K J, Schmittgen T D. Analysis of relative gene expression data using real-time quantitative PCR and the 2 ΔΔCT method. Methods, 2001, 25(4): 402-408.
[7] Aguilar F, Montandon P E, Stutz E. Two genes encoding the soybean translation elongation factor eEF-1 alpha are transcribed in seedling leaves. Plant Molecular Biology, 1991, 17: 351-360.

[8] Kim C S, Yi S Y, Lee Y K. Isolation and differential expression of an acidic PR-1 cDNA gene from soybean hypocotyls infected with Phytophthora sojae f.sp.glycines. The Plant Pathology Journal, 2000, 16: 9-18.
[9] Takeuchi Y, Yoshikawa M, Takeba G. Molecular cloning and ethylene induction of mRNA encoding a phytoalexin elicitor-releasing factor, β -1, 3-endoglucanase, in soybean. Plant Physiology, 1990, 93: 673-682.
[10] Gijzen M, Kuflu K, Qutob D. A class I chitinase from soybean seed coat. Journal of Experimental Botany, 2001, 52:2283-2289.
[11] Ludwig A, Tenhaken R. Defence gene expression in soybean is linked to the status of the cell death program. Plant Molecular Biology, 2000, 44: 209-218.
[12] Thomma B P H J, Cammue B P A, Thevissen K. Plant defensins. Planta, 2002, 216: 193-202.
[13] Frank R L, Vodkin L O. Sequence and structure of a phenylalanine ammonia-lyase gene from Glycine max. Mitochondrial DNA, 1991, 1(5): 335-346.
[14] Robert G U, Martha E R. Defense-related gene expression in soybean leaves and seeds inoculated with Cercospora kikuchii and Diaporthe phaseolorum var. meridionalis. Physiological and Molecular Plant Pathology, 2010, 75: 64-70.
[15] Akada S, Kung S D, Dube S K. The nucleotide sequence of gene 3 of the soybean chalcone synthase multigene family. Nucleic Acids Research, 1990, 18: 5899.
[16] Wang W H, Takano T, Shibata D. Molecular basis of a null mutation in soybean lipoxygenase 2: substitution of glutamine for an iron-ligand histidine. Proceedings of the National Academy of Sciences USA, 1994, 91: 5828-5832.
[17] Francois G, Michael G H. Oligosaccharins: structures and signal transduction. Plant Molecular Biology, 1994, 26: 1379-1411.
[18] Van L C. The nomenclature of pathogenesis-related proteins. Plant Molecular Biology, 1990, 37: 229.
[19] Thomma B.P.H.J., Eggermont K, Penninckx I.A.M.A. Separate jasmonate-dependent and salicylate-dependent defense-response pathways in Arabidopsis are essential for resistance to distinct microbial pathogens. Proceedings of the National Academy of Sciences USA, 1998, 95(25): 15107-15111.
[20] Vick B A, Zimmerman D C. Oxidative systems for modification of fatty acids: the lipoxygenase pathway. The Biochemistry of Plants, 1987, 9: 53-90.
[21] Graham M Y, Weidner J, Wheeler K. Induced expression of pathogenesis-related protein genes in soybean by wounding and the Phytophthora sojae cell wall glucan elicitor. Physiological and Molecular Plant Pathology, 2003, 63: 141-149.
[22] Creelman R A, Mullet J E. Oligosaccharins, brassinolides, and jasmonates: Nontraditional regulators of plant growth, development and gene expression. The Plant Cell, 1997, 9: 1211-1223.
[23] Klarzynski O, Descamps V, Plesse B. Sulfated fucanoligosaccharides elicit defense responses in tobacco and local and systemic resistance against tobacco mosaic virus. Molecular Plant-microbe Interactions, 2003, 16(2): 115-122.