The effects of foliar application of alginate oligosaccharide at different stage on wheat yield components

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Abstract

In the near and future climate change scenarios, agricultural meteorological disasters, such as drought, cold damage and high temperature, will occur frequently, which will seriously affect agricultural production yield. Oligosaccharides can improve crop stress resistance and protect crop growth. Alginate oligosaccharides (AOS) were prepared by enzymatic degradation of alginate extracted from brown algae. In order to study the growth promotion behavior of Alginate oligosaccharide on wheat (*Triticum aestivum* L.), 25 mg/L, 50 mg/L, 100mg/L aqueous solution of AOS were applied through foliar spraying at the greening stage, jointing stage and booting stage of wheat. The effects of different treatments on wheat yield and yield components were studied in Yongshou county dry farming experiment station of northwest A&F University located in the Weibei Arid Plateau of Shaanxi province. Foliar application of alginate oligosaccharides had a significant effect on the yield of tested three varieties. The effect of leaf spraying of alginate oligosaccharides on the yield of dryland wheat was greatly related to the spraying period, alginate oligosaccharide concentration and variety. The yield of wheat was significantly increased when wheat was treated with 25 mg/L and 50 mg/L, and the yield increase range was 3.21-17.51% on CH1and TM6 variety, but the yield of ZM 175 was not significantly affected The yield of wheat was reduced when wheat was treated with 100 mg/L. Spraying 25 mg/L and 50 mg/L alginate oligosaccharide at the greening stage, jointing stage and heading stage of wheat increased the number of grains per spike and the 1000-grain weight of wheat. The spike number, the number of grains per spike and the 1000-grain weight of wheat were the main factors that formed wheat yield. Alginate oligosaccharides increased the levels of these three factors, thus increasing wheat yield.

Introduction

Alginate consists of a variable amount of β-D-mannuronic acid -L-glucuronic acid, or both heterogeneous linked by β-1,4-glycosidic bonds. Alginate produces 30,000 metric tons per year, which is an abundant organic substance in nature (Donati et al., 2009; Szekalska et al, 2016). As the most abundant marine biomass and low-cost material, alginate has been extensively used in the food, medical industries, agriculture and other fields. However, the applications of alginate have been greatly limited due to its high molecular weight and low bioavailability (Xing et al., 2020). The degradation of large molecular weight alginate into low molecular weight alginate oligosaccharides can not only solve the problem of bioavailability but also broaden the functions of alginate oligosaccharides. Alginate oligosaccharides (AOS), oligomers containing 2 to 25 monomers, can be obtained via hydrolysis of glycosidic bonds, organic synthesis, or through biosynthesis. Generally, AOS have shorter chain lengths and thus improved water solubility when compared with higher molecular weight alginates of the same monomers. It has been reported that alginate oligosaccharides (AOS) possess antioxidant, antibacterial, anti-tumor, anti-oxidative, neuroprotective, immunoregulatory, anti-inflammatory, antihypertensive, and hypoglycemic properties, as well as the ability to suppress obesity and promote cell proliferation and regulate plant growth. Especially, it has a remarkable ability to promote crop growth.

Regarding AOS stimulating plant growth, there were many reports. AOS of 1.8 kDa obtained by depolymerization with a bacterial alginate lyase, promoted germination of rice seeds and the differentiation of tobacco callus cells into plants (Yonemoto et al., 1993). AOS fractions prepared by acid hydrolysis of molecular mass 32,000, 42,000, and 64,000 g mol^{-1} stimulated growth of *Eucomis autumnalis*, and the effect was the strongest for the fraction of 32,000 g mol^{-1} (Salachna et al., 2018). AOS obtained by radiation depolymerized alginate was used to treat tomato, spinach, mung dal, jute, pumpkin, egg plant, green chilli, red chilli, cabbage and cauliflower plants. AOS acted as a growth promoter, disease control activity which resulted in elongation of plant root and shoot growth, increase in plant productivity and an improvement in physiological parameters compared with the untreated plants (Aktar et al., 2017). Application of AOS from irradiated sodium alginate as foliar spray enhanced growth features, enzymatic, yield and quality attributes of coriander plants. Out of seven selected concentrations, 100 ppm proved to be the significantly optimum for all studied parameters. AOS applied as leaf-sprays might improve growth, photosynthesis, physiological activities, and alkaloid production in *Catharanthus roseus* L. significantly. Of the various AOS concentrations, 80 ppm proved to be the best one compared to other concentrations applied (Idrees et al., 2016; 2011). AOS promotes the growth of roots of lettuce, carrots and rice. AOS may be used as a new type of plant growth regulator. This application would represent a novel and very significant utilization of marine resources in agriculture (Xing et al., 2020; Liu, 2019). Alginate oligosaccharides also improve plant quality. The application of AOS (irradiated sodium alginate) resulted in significant improvement in growth attributes, physiological and biochemical parameters in addition to enhancing the citronellal and yield of essential oil in eucalyptus, with 120 mg L^{-1} proving the best AOS concentration (Ali et al., 2014). The growth promoting effect of alginate oligosaccharides is related to molecular weights and Mannuronate/Guluronate ratio. AOS with low MW (500–3000 Da) and higher M/G ratio (> 1) had better promotion on barley (*Hordeum vulgare L.*). The promotion effects of AOS on seedlings might be caused by the stimulation on photosynthesis and the roots growth were promoted probably due to the enhanced absorption activity (Yang et al., 2021).

Alginate Oligosaccharides not only promote plant growth, but also improve drought resistance of plants. Alginate-derived oligosaccharides improve the diameter, fresh weight, photosynthetic rate, transpiration rate, stomatal conductance, maximum quantum yield of photosystem II (Fv/Fm) and chlorophyll degradation, reversing the effects of drought stress. Alginate-derived oligosaccharides induced the expression of some antioxidant enzyme synthetic genes involved in the ABA signaling pathway by stimulating ABA synthesis to improve the drought resistance capacity in cucumber (Li et al., 2018). In our lab, previous work found that AOS can improving *Triticum aestivum* L. resistant ability to drought stress. Seedling and root length, fresh weight and relative water content of wheat treated with AOS were increased under dehydration status compared with that of PEG group. Moreover, the antioxidative enzymes activities were obviously enhanced and malondialdehyde (MDA) content was reduced treated by AOS, the drought resistant related genes involved in ABA signal pathway were up-regulated by AOS (Liu et al., 2013). The results showed that alginate oligosaccharides could improve crop drought resistance and promote crop growth mostly in laboratory experiments, and the effects of alginate oligosaccharides on crop growth and yield in the field were not clear.

Wheat is the most widely planted and productive food crop in the world. The planting area of the world is about 2.1 million km^2 and with over 600 million tons harvested annually (Su et al., 2020). It is contributing to about the 20% of the total dietary calories and proteins worldwide (Zampieri et al., 2017).

And wheat is one of the most important cereals as a food source for > 50% of the world's population. As a consequence of burgeoning population, its inclining demand is expected to reach up to 40% (Ahmed et al., 2020). The fast-growing global population and the resultant growth in food consumption is increasing demand for wheat production. The food security caused by this has drawn wide attention of the international community.

China is the world's largest producer and consumer of wheat, with an area of 23,987.5 million hectares, and the amount of wheat consumption is also more than a quarter of the total grain (Gao et al., 2018). Winter wheat (*Triticum aestivum* L.) feeds approximately 40% of the population of China (Liu et al., 2020). Its production is of great significance to national food security and national economic development. However, with global climate change, the stability and productivity of wheat are affected by various biotic and abiotic stresses. Drought is the most serious factor affecting global wheat production (Liu et al., 2015). To ensure wheat yield, irrigation, planting drought-resistant or drought-tolerant varieties and improving drought resistance are optional techniques. Irrigation has a positive influence in increasing yield, but Irrigation is limited by water resources, many countries have inadequate water supplies to meet agricultural demands (Elliott et al., 2014). Notably, irrigation has also induced a series of environmental issues, for example, intensive use of irrigation water has led to cessation of surface water flows and overexploitation of groundwater resources (Zhao et al., 2015). Drought-resistant or tolerant varieties are relatively scarce in production, because domestication and selective breeding has limited the genetic diversity of wheat, leading to cultivars adapted to artificial environments which has resulted in reduced resistance to drought stress (Budak et al., 2013).

Numerous reports confirm that using plant growth regulator especially plant hormones can improve the drought resistance of crops and increase the yield of crops, such as ABA, as a kind of drought signal substance, plays an important role in the physiological process of drought resistance in plants (Ma & Qin, 2014; Zhang et al., 2006). Under drought stress, ABA can improve drought resistance of plants exogenous treatment led to increase in levels of ascorbate, ascorbate to dehydroascorbate ratio, antioxidant enzymes and decreases in levels of dehydroascorbate, malondialdehyde. LEA genes were induced by ABA (Kaur et al., 2014). it is of great significance to improve the drought resistance of wheat by adopting appropriate control measures to ensure food security. However, the use of the regulator technology is very strict, if the use of improper, not only can not increase the output, but will cause reduced production, and even cause agricultural products on the drug residues and other adverse side effects. It is of great significance to study new natural environmentally friendly and harmless stress resistance agents.

In this paper, the effects of alginate oligosaccharides on wheat growth and yield were studied in Weibei Arid Plateau of Shaanxi province in northwest China, where wheat is the staple food for humans, to evaluate the feasibility of using AOS in wheat production.

Materials And Methods

Experimental site and wheat cultivars

The experiment was conducted from 2016 to 2018 in Yongshou county Dry Farming Experiment Station of Northwest A&F University located in the Weibei Arid Plateau of Shaanxi province (Between longitude 107°56´-108°21´and north latitude 34°29´-34°85´). The experimental region climate is warm temperate continental climate with long and dry winter and low temperature, short and mild summer, with an average frost-free period of 210 d, a total annual sunshine duration of 2166.2 h, a total annual radiation amount of 114.77 kcal /cm^2, an altitude of 1095 m, and an annual precipitation of 570 mm. Drought was the most natural disaster in Yongshou County, followed by hail and frost. It is a typical dry farming county. The previous crop in the experimental field was wheat. The soil in the plough layer (0–20 cm) had 15.0 g/kg of organic matter, 57.01 mg/kg of available nitrogen, 3.36 mg/kg of available phosphorus, 221.0 mg/kg of available potassium, and soil pH 7.98.

To evaluate the effects of AOS on different cultivars, three wheat cultivars, cv. Changhang No.1 (CH1, provided by Shaanxi Jiufeng Seed Industry Co., LTD.), cv Tongmai No.6 (TM6, provided by Shaanxi Datang Seed Industry Co., LTD.) and cv. Zhongmai No.175 (ZM175, provided by The Institute of Crop Science, Chinese Academy of Agricultural Sciences) were used in the experiments. Sowing was performed in around the 20th of September in 2016 and 2017, respectively. Seeding rate was 172.5kg/ha. These winter wheats were grown following conventional management practices during the study. The precipitation of 2016, 2017 and 2018 was 437.1 mm, 672.8 mm and 559.4 mm, respectively. The evaporation of 2016, 2017 and 2018 was 1034.5 mm, 892.2 mm and 899.9 mm, respectively. The experimental area is shown in Fig. 1.

Treatments and experimental design

Alginate oligosaccharides (AOS) powder was obtained from Alginic acid sodium salt by enzymatic degradation in our laboratory, AOS have 2–10 degree of polymerization. The relative molecular weight ranges from 352.24 to 1761.2Da. The ESI-MS spectra of the Alginate oligosaccharides is shown in Fig. 2.

Four treatments were set for the experiments. (1) concentration of AOS was 25 mg/L; (2) concentration of AOS was 50 mg/L; (3) concentration of AOS was 100 mg/L; (4) the control (CK).

All experiments were conducted in a randomized complete block design with three replicates at a seed rate of 172.5 kg ha^{-1}. The plot length was 8 m, the plot width was 5 m, and the row spacing was 25 cm. The oligosaccharides for treatments were applied to the foliage at three different growth stages, Tf1: at returning green stage, Tf2: jointing stage, and Tf3: booting stage. The foliar spraying dosage was 450L.ha^{-1} at each spray.

The yield components and individual quality measurements

The individual quality indexes were measured before the harvest. The measurement indexes and methods are as follows: (1) The spike number of wheat was measured before the harvest. Wheat with uniform growth of 1 m^2 was selected in each plot and the number of panicles was counted. Three replicates were counted for each treatment and the average value of the three replicates was finally obtained. (2) The grains per spike was measured before the harvest. 20 panicles were randomly selected from each treatment, and the total grain number of 20 ears was investigated and recorded. 3 replicates were measured for each treatment, and the average number of grains per panicle was obtained by dividing the average number of grains per panicle by 20. (3) Grains of 3m^2 was harvested in each plot to determine the actual yield and 1000-grain weight. Harvested wheat was threshed in a threshing machine, cleaned of impurities, dried in natural conditions and packaged. The mass of the grain yield was then recorded. The recorded yield data is converted to yield per mu. The 1000-grain weight was determined by the following ways, 200 intact grains were randomly taken out from each plot, weighed (g) by electronic balance, and repeated 5 times. Finally, the average of the 5 values was taken and converted into 1000-grain weight (g).

The data processing

The entire experiment was carried out according to completely randomized design with three replications. Microsoft Excel software was used to process the original data. Data were calculated as mean ± SD (n = 3). The data were analyzed by using SPSS 18.0 for calculating ANOVA and the standard deviation (SD). The level of significance difference was established at $P < 0.05$ or $P < 0.01$.

Results

Effects of AOS on the spike number of different wheat cultivars

The effects of different AOS treatments on spike number of wheat were shown in Table 1. The experimental results showed that alginate oligosaccharides had certain influence on the number of panicles in wheat, and the effect was related to the time, concentration and wheat variety of alginate oligosaccharides.

In the returning greening stage the effects of spraying alginate oligosaccharide on the leaf surface of the three wheat varieties were different in the three concentrations. At AOS 25 mg/L treatment, the number of spikes increase rates of the three cultivars ranged from 0.58% to 6.69%, and the number of spikes increase rates of TM6 in two years were 6.69% and 2.83%, respectively. AOS 50 mg/L and 100 mg/L treatments had irregular effects on the number of spikes of the three varieties, including increasing and decreasing the number of spikes. Through variance analysis, the effect of AOS 50 mg/L and 100 mg/L treatments on the number of spikes of the three varieties was not significantly different from that of the control.

The effect of AOS treatment at jointing stage on the number of ears was similar to that at returning greening stage. Under the treatment of AOS 25 mg/L, the increase rate of the number of ears of the three varieties ranged from -1.58% to 8.08%. AOS 50 mg/L and 100 mg/L had irregular effects on the number of spikes of the three cultivars. Through variance analysis, the effect of AOS 50 mg/L and 100 mg/L on the number of spikes of the three cultivars was not significantly different from that of the control.

In the booting stage, 25mg/L alginate oligosaccharide treatment increased the number of panicles, the increase rate of the number of ears of the three varieties ranged from 0.22% to 7.36%. The effects was not significant at P 0.01. AOS 50 mg/L and 100 mg/L had irregular effects on the number of spikes of the three cultivars.

Overall results showed that 25mg/L alginate oligosaccharide treatment could increase the number of panicles in the returning green and booting stages, the effects was not significant at P 0.01 in most treatments. _

Table 1

Effects of AOS on the spike number of different wheat cultivars

- abbreviations IRCC- The increasingrate compared with the control
- Note: Lower case letters in the table indicate significant at p<0.05, upper case letters indicate significant at p<0.01.

Effects of AOS on the grains per spike of different wheat cultivars

The effect of alginate oligosaccharides on grain number per spike of different varieties was shown in Table 2. The results showed that alginate oligosaccharides had certain effect on grain number per spike of the three varieties tested, but the application effect was closely related to the AOS concentration, application period and variety.

In the returning greening stage, 25 mg/LAOS treatment increased the number of grains per spike of the three cultivars in the range of 4.48%-16.05%. Among them, treatment to TM6 had the most obvious effect on the increase of grain number per panicle, with the rate of increase of grain number per panicle being 16.05% and 15.45%, respectively. The second was to ZM175, with an increase rate of 7.71% and 12.07%, respectively. The grain number per

stage	Treatments	CH1. spike number (Ten thousand/ha)				TM6. spike number (Ten thousand/ha)				ZM175. spike number (Ten thousand/ha)			
		2017	IRCC ±%	2018	IRCC ±%	2017	IRCC ±%	2018	IRCC ±%	2017	IRCC ±%	2018	IRCC ±%
Tf1	1	505.95 bA	5.51	467.70 aA	1.54	612.45 bA	6.69	526.95 aA	2.83	568.05 aA	5.11	523.50 aA	0.58
	2	502.05 bB	4.69	449.70 aA	-2.63	641.55 aA	11.76	526.50 aA	2.73	571.05 aA	5.66	512.55 aA	-1.54
	3	513.45 aA	7.07	445.5 aA	-3.29	612.00 bA	6.61	503.55 aA	-1.76	566.55aA	4.80	513.00 aA	-1.44
	4	479.55 cB		460.65 aA		574.05 cB		512.55 aA		540.45 bA		520.50 aA	
Tf2	1	500.55 aA	1.64	465.75 aA	1.12	536.55aA	7.51	548.55 aA	8.08	531.45bA	-1.58	523.50aA	0.67
	2	490.50 aA	-0.40	461.70 aA	0.24	499.05 bA	0.00	522.45bB	2.96	556.05aA	2.97	520.05 aA	0.0
	3	458.55 bA	-6.88	449.55 aA	-2.41	507.00 aA	1.59	496.05 bB	-2.27	556.05 aA	2.97	519.00 aA	-0.19
	4	492.45 aA		460.65 aA		499.05bA		507.45bB		540.00 bA		520.05aA	
Tf3	1	503.55 aA	7.36	456.60 aA	0.22	523.95 aA	4.80	544.05 aA	6.15	573.45aA	5.99	524.55aA	3.96
	2	508.95 aA	8.51	443.10 aA	-2.73	516.00 aA	3.21	506.55 bB	-1.17	546.00 aA	0.92	516.45 aA	2.38
	3	492.45 aA	4.99	437.85 aA	-3.90	514.95 aA	3.00	502.95 bB	-1.85	564.45 aA	4.32	516.00aA	2.28
	4	469.05 bA		455.55 aA		499.95aA		512.55 bB		541.05 aA		504.45 aA	

panicle of CH1 was increased, but the difference was not obvious. The grain number per panicle of three varieties treated with 50mg/L AOS showed an increasing trend, but the effect was different for different varieties. The grain number per panicle of TM6 treated with 50mg/L AOS showed the most obvious increase, and the increase rates of grain number per panicle in two years were 11.32% and 14.88%, respectively. The second was CH1, with the increase rates of 4.85% and 9.11%, respectively. In the treatment of ZM175 with AOS 50mg/L, the number of grains per panicle decreased in one year, but there was no significant difference in the number of grains per panicle between treatment and control. In the other year, the number of grains per panicle of ZM175 increased significantly, with an increase rate of 11.87%. AOS 100mg/L treatment to the three tested varieties, the effect on the number of grains per panicle is not consistent, one year result showed that the effect of AOS 100mg/ml to tested varieties is not significantly different in reducing grain number per spike compared with the control. Another year effect significantly increased the number of grains per panicle, The grain number per spike increased by 3.46%, 9.87% and 9.10% for CH1, TM6 and ZM175, respectively.

At elongation stage, alginate oligosaccharide treatment of 25 mg/L and 50 mg/L increased the number of grains per panicle of the three cultivars to a certain extent, and 25 mg/L treatment increased the number of grains per panicle of the three cultivars in the range of 3.56-28.22%. The effect of increasing the number of grains per panicle of CH1 and TM6 was obvious, and the number of grains per panicle of ZM175 was increased, but the difference was not significant. The effect of AOS50 mg/L treatment on the number of grains per ear of the three varieties was similar to that of AOS 25 mg/L treatment, and the increase range of the number of grains per ear of the three varieties was 2.92% to 22.17%. AOS 100 mg/L significantly increased the number of grains per panicle of CH1 and TM6 cultivars, and the increase rate ranged from 5.09 to 11.17%, while the decrease of grain per panicle of ZM175 was not significant.

Table 2

Effects of AOS on the grains per spike of different wheat cultivars

stage	Treatments	CH1. grains per spike				TM6. grains per spike				ZM175. grains per spike			
		2017	IRCC ± %	2018	IRCC ± %	2017	IRCC ± %	2018	IRCC ± %	2017	IRCC ±%	2018	IRCC ±%
Tf1	1	33.15aA	4.48	37.40 aA	13.57	32.82aA	16.05	34.00 aA	15.45	33.52aA	7.71	37.77 aA	12.07
	2	33.27aA	4.85	35.93 aA	9.11	31.48bA	11.32	33.83 aA	14.88	31.02 bB	-0.32	37.70 aA	11.87
	3	31.30aA	-1.36	34.07bA	3.46	31.07bB	9.87	29.30 bB	-0.51	30.70 bB	-1.35	36.77 bB	9.10
	4	31.73aA		32.93 bA		28.28 cC		29.45 bB		31.12 bB		33.70 cC	
Tf2	1	32.67 aA	28.22	38.60 aA	16.15	29.63aA	7.08	32.82 aA	15.15	33.73aA	3.56	34.85 aB	4.72
	2	31.13 bA	22.17	35.13 bA	5.72	29.18aA	5.46	30.33 bB	6.43	33.52aA	2.92	36.67 aA	10.18
	3	28.18 cC	10.60	35.33 bA	6.32	29.08aA	5.09	31.68 aA	11.17	31.90bA	-2.06	33.05 bB	-0.69
	4	25.48dD		33.23 bB		27.67bB		28.50 cB		32.57aA		33.28 bB	
Tf3	1	37.25aA	6.89	36.43 aA	9.50	32.77aA	10.15	34.08 aA	15.54	33.67aA	9.60	39.17 aA	16.05
	2	32.77bA	-5.97	36.20aA	8.81	27.42bB	-7.83	33.88 aA	14.86	32.67aA	6.35	38.05 aA	12.74
	3	33.78bA	-3.07	36.40 aA	9.41	28.10bB	-5.55	31.27 bB	5.99	32.17aA	4.72	34.90 bB	3.41
	4	34.85bA		33.27 bB		29.75bB		29.50 bB		30.72bA		33.75bB	

abbreviations IRCC- The increasing rate compared with the control.

Note: Lower case letters in the table indicate significant at p<0.05, upper case letters indicate significant at p<0.01.

At booting stage, 25 mg/L alginate oligosaccharide treatment significantly increased the number of grains per spike of the three varieties tested, and the increase range was 6.89%-16.05%. The effects of 50 mg/L alginate oligosaccharides on the number of grains per spike of CH1 and TM6 were not consistent in two years. In one year, the number of grains per spike was decreased, but the difference was not obvious. In the other year, the number of grains per spike was increased significantly. 50 mg/L alginate oligosaccharide treatment of wheat ZM175 significantly increased the number of grains per spike. The treatment of 100 mg/L alginate oligosaccharide to ZM175 increased the number of grains per spike of wheat. For CH1 and TM6, the number of grains per spike was inconsistent in two years. One year resulted in a decrease in grain number per spike, but the difference was not significant, and the other year resulted in a significant increase in number of grains per spike. The results showed that AOS 25mg/L, 50 mg/L alginate oligosaccharides could significantly increase the number of grains per spike in wheat at the returning greening stage, jointing stage and booting stage.

Effects of AOS on the 1000-grain weight of different wheat cultivars

The effects of different AOS treatments on the 1000-grain weight of wheat were shown in Table 3. The results showed that 25mg/L and 50mg/L AOS applied on the leaves of the CH1 and TM6 cultivars in the returning greening stage could improve the grain weight of the two cultivars. But 25mg/L AOS applied on ZM175, 1000-grain weight was inconsistent in two years. One year resulted in a decrease in 1000-grain weight, but the difference was not significant, and the other year resulted in a significant increase in 1000-grain weight. The effect of 50mg/L alginate oligosaccharides to 1000-grain weight of ZM175 was not significant. 100 mg/L alginate oligosaccharides could reduce the 1000-grain weight of the three cultivars, but the difference was not significant.

Leaf spraying of alginate oligosaccharide at jointing stage could significantly increase the 1000-grain weight of wheat. The 1000-grain weight of wheat was increased by 6.32% to 12.94% by 25mg/L treatment, and the 1000-kernel weight of ZM175 had the most obvious effect, and the 1000-kernel weight increased by 12.94% and 10.26% in two years, respectively. The 1000-kernel weight of the three cultivars was increased from 1.92% to 8.40% by 50mg/L treatment, and the 1000-kernel weight of TM6 was increased by 5.63% and 8.40%, respectively. The effect of 100mg/L treatment on 1000-grain weight of three wheat varieties was different. The 1000-kernel weight of wheat was significantly increased by 4.58% and 8.33% for TM6 bienniums. The results of two years for CH1 and ZM175 were inconsistent. One year, 1000 grain weight was decreased and one-year 1000 grain weight was increased insignificantly.

At booting stage, 25 mg/L and 50 mg/L AOS treatment significantly increased 1000-grain weight of wheat of the three tested varieties. The increase ranges were 3.61%-14.22% with 25 mg/L AOS, and 2.21%-8.73% with 50mg/L AOS. AOS 100mg/L treatment to the CH1 and TM6 varieties, the effect on the 1000-grain weight is not consistent, one year result showed reducing 1000-grain weight compared with the control. Another year effect significantly increased 1000-grain weight, AOS 100mg/L treatment to the ZM175, it reduced 1000-grain weight compared with the control for two years.

The results showed that the 1000-grain weight of wheat could be increased by AOS treatment of 25 mg/L and 50 mg/L.

Table 3

Effects of AOS on the 1000-grain weight of different wheat cultivars

stage	treatments	CH1. 1000-grain weight (g)				TM6.1000-grain weight (g)				ZM175. 1000-grain weight (g)			
		2017	IRCC ± %	2018	IRCC ± %	2017	IRCC ± %	2018	IRCC ± %	2017	IRCC ±%	2018	IRCC ±%
Tf1	1	38.26aA	0.63	39.78 aA	7.52	41.99aA	2.46	43.30 aA	6.99	42.13 cB	-1.66	41.40 aA	8.00
	2	38.54aA	1.37	39.10 aA	5.68	42.29aA	3.20	43.73 aA	8.08	43.52aA	1.59	38.20 bB	-0.35
	3	36.63bB	-3.66	36.18 bA	-2.21	40.15bB	-2.03	43.02 aA	6.30	42.07 cB	-1.80	38.02 bB	-0.83
	4	38.02aA		37.00 bA		40.98aA		40.47 bB		42.84bA		38.33 bB	
Tf2	1	40.49aA	11.19	39.37aA	6.32	40.86aA	10.08	43.85 aA	8.90	39.98aA	12.94	42.27 aA	10.26
	2	37.12 bB	1.92	38.70 aA	4.59	39.21bB	5.63	43.26 aA	8.40	37.04bB	4.63	39.97 bB	4.26
	3	37.08 bB	1.81	36.90 aA	-0.27	38.82bB	4.58	43.22 aA	7.33	36.16 cB	2.15	37.63 cC	-1.8
	4	36.41 cC		37.00 aA		37.12 cC		40.27 bB		35.40 cC		38.33 cC	
Tf3	1	36.99aA	3.61	44.97aA	11.07	42.07 aA	8.90	43.97aA	7.20	43.26aA	11.44	43.78 aA	14.22
	2	36.49aA	2.21	43.80 aA	8.19	40.88 bB	5.82	42.93 bA	4.67	42.21bA	8.73	40.75 bA	6.05
	3	36.94aA	3.47	38.00 cC	-6.13	38.49 cC	-0.36	43.06 aA	4.97	35.86 cC	-7.62	35.75 cC	-6.73
	4	35.70bA		40.48 bB		38.63cC		41.02 bB		38.82 bB		38.33 bB	

abbreviations IRCC- The increasing rate compared with the control.

Note: Lower case letters in the table indicate significant at $p<0.05$, upper case letters indicate significant at $p<0.01$.

Effects of AOS on the grain yield of different wheat cultivars

The results of the effect of alginate oligosaccharide on wheat yield were shown in Table 4. The results showed that the effect of leaf spraying of alginate oligosaccharides on the yield of dryland wheat was greatly related to the spraying period and alginate oligosaccharide concentration, and also to some extent related to the variety. In the returning greening stage, alginate oligosaccharides treated with 2 5 mg/L and 50 mg/L had obvious effect on the yield increase of the three tested varieties in two years. Compared with the control, 25 mg/L alginate oligosaccharide treatment significantly increased the yield of the three varieties. AOS 25 mg/L treatment increased the yield of wheat by 3.21%-17.51%, and the performance of different varieties was different. The yield increase effect of TM6 was good and stable, and the yield increase was more than 10% in two years. The yield increases were 17.51% and 12.08%, respectively. Under AOS 50mg/L treatment, the yield of the three varieties increased by 2.85%-10.97%, among which TM6 and CH1 showed relatively stable performance, with the two-year increase effect of 9.49% and 11.00% on CH1, 10.97% and 15.94% on TM6, respectively. AOS 100mg/L treatment had not obviously increase effect for only one year, or there was a certain reduction, with a reduction range of 2.36%-7.99%.

At the shooting stage, alginate oligosaccharides treated with 25mg/L and 50mg/L for two years all increased the yield of the three tested cultivars. The effect of 25 mg/L treatment on the yield increase of the three varieties was significantly higher than that of the control, and the yield increase range was 6.17-13.88%, among which TM6 had a larger yield increase, which was 11.69% and 13.88% in two years, respectively. The yield increase rate of 50 mg/L treatment was 2.08-10.10%, and the yield increase effect of the three varieties was different. The yield increase of CH1 in two years was 4.37% and 9.21%, TM 6 was 10.10% and 9.57%, and ZM175 was 6.42% and 2.08%, respectively. The 100 mg/L treatment could increase the yield of CH1 and TM6. The two-year increase effect of CH1 was 4.74% and 6.17%, TM6 was 2.04% and 4.84%, respectively. The two-year decrease rate of ZM175 was 6.4% and 2.96%, respectively. However, the statistical analysis of 100 mg/L treatment on ZM175 showed no significant difference compared with the control yield.

At booting stage, alginate oligosaccharides treated with 25 mg/L and 50 mg/L could increase the yield of three tested varieties, and the yield increase effect was different with different cultivars. The increase rate range was 6.74%-16.14 of 25 mg/L treatment. and 3.35%-15.68% of 50 mg/L treatment. The effect of AOS 100 mg/L treatment on the yield of the three tested cultivars was inconsistent. The most treatment with 100 mg/L AOS decreased the yield. There was no significant difference in the statistical analysis of the effect of AOS 100mg/L on the yield of variety with control.

Table 4

Effects of AOS on the grain yield of different wheat cultivars

abbreviations IRCC- The increasing rate compared with the control

Note: Lower case letters in the table indicate significant at $p<0.05$, upper case letters indicate significant at $p<0.01$.

Discussion

Effects of spraying alginate oligosaccharides at different growth stages on wheat yield

The effect of plant growth regulator is different in different periods. Four different levels of plant growth regulator (NAA) i.e. 0, 60, 90 and 120 ml ha^{-1} were applied at the time of tillering stage (S1), panicle initiation stage (S2) and grain formation stage(S3). The results showed that application of plant growth regulator (naphthalene acetic acid at the rate of 90 ml ha^{-1}) at panicle initiation stage enhanced yield and yield attributing characters of coarse rice (IR-6). It is also evident from the results that panicle initiation stage is a critical stage, where application of growth regulator showed better performance compared to other two stages i.e. tillering and grain formation stage (Bakhsh et al.,2011). To evaluate the effects of differing times of application of Tianda 2116 plant growth regulator on the growth and yield of wheat (variety SC Sekuru), a trial was conducted at Gwebi Agricultural College Farm in Mashonaland West Province of Zimbabwe, during the 2012 winter wheat season (May to August). The results showed that application of Tianda 2116 at 2 and 3 WAP delayed flowering and physiological maturity. Early application of Tianda 2116 increased the number of spikelets per spike, thousand grain weight and grain yield. Conclusively, Tianda 2116 applied 2 or 3 WAP is effective in reducing growth while enhancing yield parameters in wheat production (Manenjiet al.,2016). This experiment results showed that spraying 25 mg/L to 50 mg /L of alginate oligosaccharide at three different stages of wheat could improve wheat yield, but the effect of increasing yield was different. The overall results showed that spraying 25 mg/L to 50 mg /L of alginate oligosaccharide at the stage of elongation and booting had stable yield increase, which may be closely related to increasing grain number per spike and 1000-grain weight of wheat. It may also be related to the different physiological reactions of wheat in the non-growth stage and the different regulatory mechanisms of alginate oligosaccharides, but further research is needed.

Effects of spraying alginate oligosaccharides on wheat yield of different varieties

The application effect of growth regulators often varies with the sensitivity of different plant species and varieties to growth regulators. plant growth regulators (PGR, thidiazuron, paclobutrazol, and ascorbic acid) was used to study the effects on physiological traits of wheat genotypes under water surplus and deficit conditions. Study revealed that relative water content, membrane stability index, chlorophyll content, photosynthetic rate (PN), and maximal quantum yield of PSII improved with PGRs application across the genotypes both under irrigation and water stress. The response of HD 2733

stage	Treatments	CH1. grain yield (kg/ha)				TM6. grain yield (kg/ha)				ZM175. grain yield (kg/ha)			
		2017	IRCC ±%	2018	IRCC ±%	2017	IRCC ±%	2018	IRCC ±%	2017	IRCC ±%	2018	IRCC ±%
Tf1	1	6933.30 aA	3.21	6047.85 aA	12.17	7207.65 aA	17.51	6056.25 aA	12.08	7183.95 aA	2.63	6205.05 aA	3.87
	2	7355.10 aA	9.49	5983.20 aA	10.97	7111.05 aA	15.94	5971.95 aA	10.52	7169.85 aA	2.43	6144.30 aA	2.85
	3	6229.95 cB	-7.33	5509.65 bA	2.19	6285.45 bB	2.62	5628.90 bA	4.17	6772.20 aA	-3.25	5496.45 cC	-7.99
	4	6717.30 bA		5391.60 bA	_	6133.65bB		5403.60 bA		6999.75 aA		5974.05 bA	
Tf2	1	6599.40 aA	6.17	6032.70 aA	12.59	6687.45 aA	11.69	6125.40 aA	13.88	6704.70 aA	2.90	6279.40 aA	5.55
	2	6487.65 bB	4.37	5851.50 aA	9.21	6592.05 aA	10.10	5893.65 aA	9.57	6577.20 aA	0.94	6072.75 bA	2.08
	3	6510.30 bB	4.74	5688.30 bA	6.17	6109.50 bB	2.04	5639.25 bB	4.84	5841.45 bB	-10.35	5797.50 bB	-2.55
	4	6216.00 bB		5357.85 bB		5987.40 bB		5378.70 bB		6515.85 aA		5949.15 bB	
Tf3	1	5928.90 aA	16.14	5878.95 aA	9.04	6559.80 aA	6.74	6035.85 aA	11.18	7977.00 aA	12.14	6609.75 aA	10.18
	2	5905.20 aA	15.68	5572.05 bA	3.35	6635.4 aA	7.97	5893.80 aA	8.57	7295.55 bB	2.56	6359.55 aA	6.01
	3	4970.10 bB	-2.64	5457.45 bA	1.22	5918.85 bB	-3.69	5892.30 aA	8.54	5872.20 cC	-17.45	5797.65 bB	-3.36
	4	5104.80 bB		5391.60 bA	-	6145.65 bB		5428.80 bB		6113.20 bB		5998.95 bA	

genotype was more positive toward PGRs treatment as compared to other genotypes under water stress (Dwivedi et al.,2018). Effects of spraying a blended plant growth regulator on the tillering growth cold-resistance and yield of different winter wheat varieties (Xinong 979 Xinong 889 Zhengzhou 831 and Xiaoyan 22) were compared in a field experiment The results showed that the application of the blended plant growth regulator significantly improved the tillering in the winter and increased above-ground biomass of Xinong 979 Xi-nong 889 and Zhengzhou 831 while only Xiaoyan22's tiller number and biomass were not significantly affected (Peng et al.,2019) The results showed that spraying 25 mg/ L-50 mg/L alginate oligosaccharide on three wheat varieties increased the yield of the three wheat varieties, but the effect of increasing yield was different among different varieties. The response of TM6 was more positive toward AOS treatment as compared to other varieties. This may be related to the characteristics of wheat varieties, TM6 has the characteristics of high 1000-grain weight and high yield potential, CH1 is a large-grain variety with good stress resistance, and ZM175 is a multi-panicle type variety with small grains. Alginate may increase the number of panicles, grain number per panicle and 1000-grain weight of TM6, and thus increase the yield of TM6.

AOS improves disease and stress resistance of wheat

Wheat (*Triticum aestivum* L.) is most widely grown in arid and semiarid areas. Drought is one of the most important environmental factors limiting growth and yield of wheat, and improving wheat yield under drought stress is an essential target (Cattivelli et al., 2008). Improving drought resistance of wheat has positive effect on increasing wheat yield. Alginate oligosaccharides have the activity of improving crop stress resistance (Moenne et al., 2021; Zhang et al., 2020), including the improvement of crop disease resistance (Zhang et al., 2019), drought resistance (Liu et al., 2013), cold resistance (Yang et al., 2020; Liu et al., 2009) and salt resistance (Tang et al., 2011). Previous laboratory studies have shown that alginate oligosaccharides can improve drought resistance in wheat. The experimental results showed that AOS application, seedling and root length, fresh weight and relative water content of wheat were increased by 18%, 26%, 43% and 33% under dehydration status compared with that of PEG group, respectively. Moreover, the antioxidative enzymes activities were obviously enhanced and MDA content was reduced by 37.9% in samples treated by AOS. Additionally, the drought resistant related genes involved in ABA signal pathway, such as late embryogenesis abundant protein 1 gene (*LEA1*), *PsbA* gene, Sucrose nonfermenting 1-related protein kinase 2 gene (*SnRK2*) and Pyrroline-5-Carboxylate Synthetase gene (*P5CS*) were up-regulated by AOS. AOS might regulate ABA-dependent signal pathway to enhance drought stress resistance of wheat during growth period (Liu et al., 2013). Spraying alginate oligosaccharides alleviated the growth inhibition of three wheat varieties caused by drought stress in varying degrees which reflected in that the seedling length root length and biomass significantly increased the contents of MDA and proline significantly decreased, and chlorophyll contents (except Zhengmai 101) and part of antioxidant enzymes activities elevated (Zhang et al., 2016).

This experiment was carried out in the Weibei Arid Plain of Shaanxi Province of China, where wheat would be stressed by drought. The results of field experiment showed that the alginate oligosaccharides could improve wheat yield, The results showed that the increase of wheat yield of alginate treatments may be closely related to the improving drought resistance of wheat by alginate oligosaccharide. The results also showed that the increase of yield of alginate was closely related to the application of alginate oligosaccharide concentration. The yield of wheat was significantly increased when wheat was treated with 25 mg/L and 50 mg/L, while the yield of wheat was reduced when wheat was treated with 100 mg/L. Therefore, the concentration of 25 mg/L to 50 mg/L is better to wheat in practical use.

Recently, alginates and the oligosaccharides derived from alginates have become a focus of interest due to their environmentally friendly nature and their ability to stimulate plant defense mechanisms and enhance growth(Riseh et al., 2022). AOS was used to evaluate the Arabidopsis thaliana resistance to *Pseudomonas syringae* pv. *tomato DC3000* (*Pst DC3000*). Resistance was vitally enhanced at 25 mg/L AOS in wild type (WT), showing the decreased disease index and bacteria colonies, burst of ROS and NO, high transcription expression of resistance genes PR1 and increased content of salicylic acid (Zhang et al., 2019). Algino-oligosaccharides could induce the enzyme activities of phenylalanine ammonia lyase, peroxidase and catalase in rice plant cells for protection of the plant against the invading pathogen. With utilization of alginate oligosaccharides, the disease index caused by Magnaporthe grisea could be reduced from 17.74% to 10.81% and the protection efficacy was 39.06%. These findings suggest that alginate oligosaccharides could be applied as a biological agent to control rice blast disease (Zhang et al., 2015). The control effect of AOS on powdery mildew and stripe rust of wheat were investigated in this experiment. The investigation results showed that AOS treatment could induce resistance of wheat to powdery mildew and stripe rust, and had a certain control effect, but there was no significant difference between the treatment and the control because the disease occurred lightly in the field.

Influence of AOS on wheat yield components

Wheat yield is a complex quantitative trait determined by three components, spike number per unit area, 1000-grain weight and grain number per spike (Simmonds et al. 2014). The grain yield of wheat and cereals is influenced by environmental and genetic interactions at all stages of the plant's growth (Slafer, 2003). Adverse environmental conditions and abiotic stresses negatively affect grain yield, causing serious economic losses (Ullah et al., 2021). The experiment was conducted in the Weibei Arid Plateau, Drought is one of the most important environmental factors limiting growth and yield of wheat. The effects of water supply conditions on yield and yield factors were different in different growth stages of winter wheat. The maximum affected spike number period was tillering stage, the maximum affected grain weight period was filling stage-maturity stage, the maximum affected kernels per spike period was jointing-booting stage (Mei et al., 2003). The greening and filling stages were the critical periods of water demand for winter wheat (Yao et al. 2015). The results showed that AOS had a certain effect on effective panicle number, especially on grain number per panicle and 1000-grain weight. These effects may be closely related to the functions of AOS in promoting crop root growth and improving photosynthesis of wheat.

Root systems play a critical role in the uptake of water and nutrients, and often are the first plant organ that senses and responds to various edaphic stresses, such as soil water deficit, salinity, waterlogging and nutrient deficiencies. Several studies are considering roots as central to their efforts to produce crops with a better yield (Chen et al., 2020; Gewin, 2010). In arid and semi-arid areas, natural selection tends to allocate more biomass to roots to absorb more resources (Palta et al., 2011). Growing evidence supports the notion that AOS promote the growth of plant roots. For example, AOS can promote the root growth of lettuce (Iwasaki and Matsubara, 2000), carrots and rice (Xu et al., 2003). In our own lab, we found that AOS can induce root development in wheat (*Triticum aestivum* L.) and rice (Zhang et al. 2016; Zhang et al., 2013; Liu et al., 2013; Zhang et al., 2014). AOS promotes the growth of wheat root system, which is beneficial to the absorption of water and nutrients from deep soil, and to the formation of tillering, effective panicle number, grain number per panicle and grain weight of wheat.

It has been reported that AOS can improve the chlorophyll content and photosynthesis of crops (Zhang et al., 2016; Aftab et al., 2011; Fazili et al., 2017; An, 2018). In cereals, grains are the most active sinks for carbon and N assimilates after anthesis and most carbohydrates are provided by photosynthesis during grain filling. (Dordas, 2012). Post-anthesis dry matter accumulation of wheat is critical for grain yield and contribute 60–90 % of the final grain weight (Masoni et al., 2007; Yang and Zhang, 2006). When moisture conditions are inadequate for plants, post-anthesis dry matter accumulation is generally reduced, and leading to considerable yield penalties (Koutroubas et al., 2012; Wang et al., 2014b). AOS can improve the chlorophyll content and root growth of wheat, which is beneficial to the accumulation of matter after flowering, and the increase of grain number per spike and grain weight.

Numerous studies have implied that the endogenous plant growth regulators are one of the important factors in determining grain set in cereal. Auxins are stimulus in flower induction and fruit development (Aloni et al., 2006). Also, Indole-3-Acetic Acid (IAA) and cytokinins are a factor in stimulating transport of assimilates for seed development (Darussalam et al., 1998). IAA can act on the transportation and accumulation of organic matter in grains, and the content of IAA in grains reaches the maximum value at the peak of grain filling, indicating that IAA may accelerate the accumulation of phosphorus in wheat grains, and has an impact on grain number per spike and grain weight of wheat (Zhu et al., 2005). Foliar Application of IAA can increase wheat yield, grain number per spike and 1000-grain weight (Jalali-Honarmand et al., 2015; Abdoli et al., 2013). In a previous work, we found that AOS could induce IAA generation in tabacco and rice (Guo et al. 2012; Zhang et al., 2014). The results of this study indicated that the effect of alginate oligosaccharides on wheat yield, grain number and 1000-grain weight may be closely related to the induction of IAA in wheat by alginate-oligosaccharides.

The results of this study indicated that the main reasons of the increase of wheat yield by alginate oligosaccharide treatment were the increase of grain number per spike and 1000-grain weight.

Conclusion

Our study indicated that spraying 25-50mg/L alginate oligosaccharide could increase wheat yield in dryland wheat growth stage. The main factors of the increase of wheat yield by alginate oligosaccharide treatment was the increase of grain number per spike and 1000-grain weight.

Declarations

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Authors' contributions

X.Z., R.Z. and H.Y. conceived the idea, designed experiments, and supervised the study. R.Z., W.W., B.H. and S.Z. performed the experiments. X.Z., W.W. and B.H. acquired the funding. X.Z. and R.Z. analyzed data and wrote the initial draft of the manuscript. All authors provided editorial advice for finalizing draft. H.Y. was responsible for the final draft.

Data availability

All data are within the manuscript.

Code availability

Not applicable.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflicts of interest/Competing interests

The authors declare that no competing interests exist.

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Figures

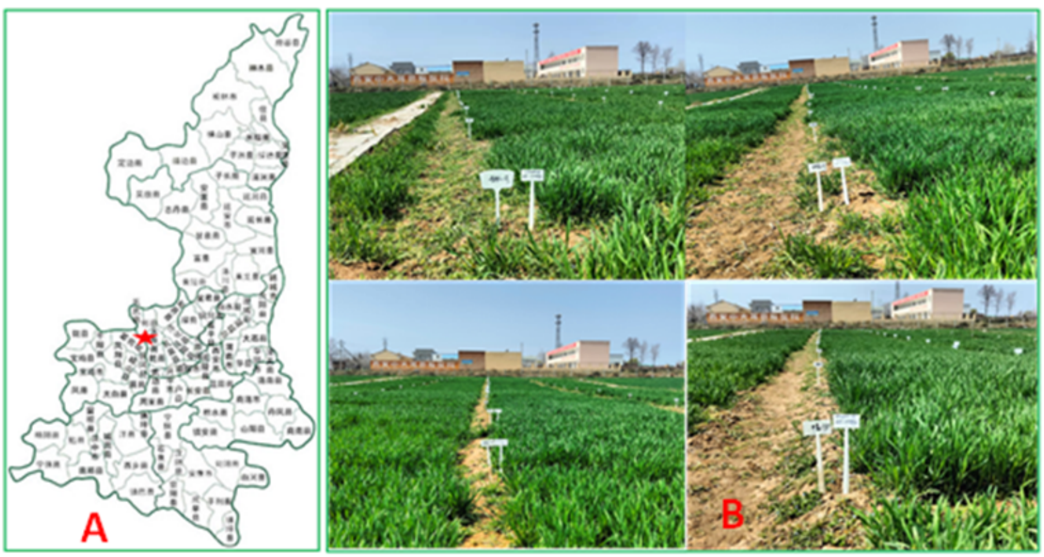

Figure 1

The experimental area and location. A: Map of Shaanxi Province.

The five-pointed star represents the location of Yongshou County in Shaanxi Province. B: Photos of experimental site.

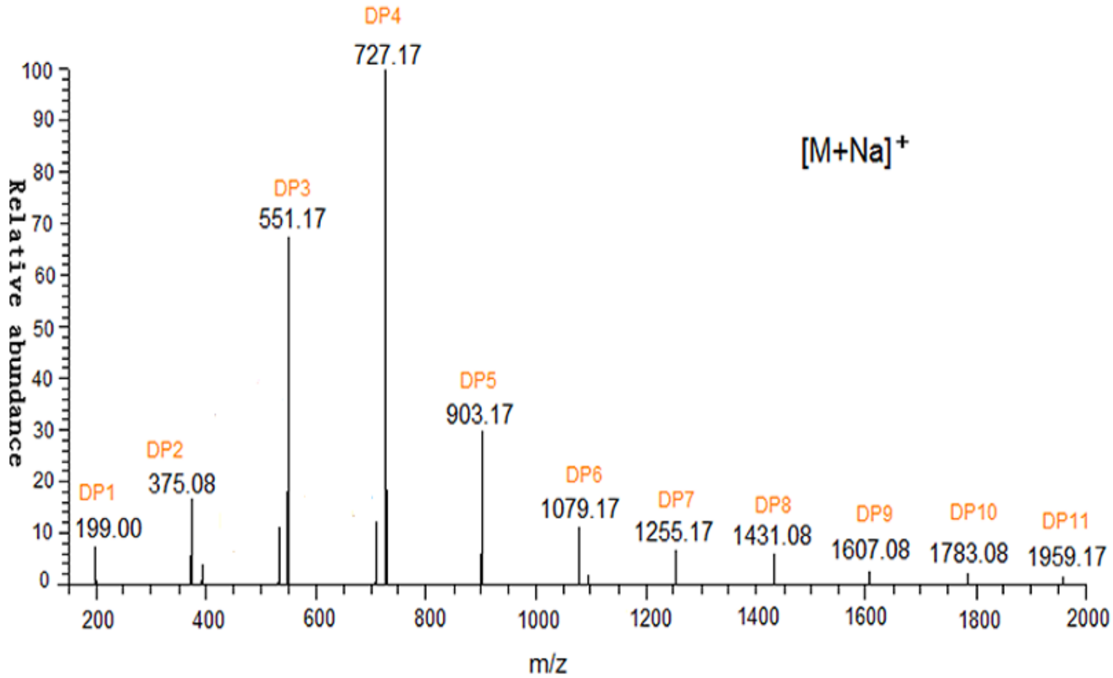

Figure 2

The ESI-MS spectra of the Alginate oligosaccharides

Supplementary Files

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- Table1.EffectsofAOSonthespikenumberofdifferentwheatcultivars.doc
- Table2.EffectsofAOSonthegrainsperspikeofdifferentwheatcultivars.doc
- Table3.EffectsofAOSonthe1000grainweightofdifferentwheatcultivars.doc
- Table4.EffectsofAOSonthegrainyieldofdifferentwheatcultivars.doc