Research Article

Effect of Exogenous Melatonin on the Quality of Soybean and Natto Products under Drought Stress

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Melatonin can directly or indirectly eliminate reactive oxygen species, regulate hormone levels, and improve drought-stressed soybean crop resistance, yield, and quality. The nutrient contents of soybeans grown under normal conditions (WW), drought stress (D), and drought stress with exogenous melatonin (D + M) ($p < 0.05$) were compared. The differences in the quality of natto from the three groups of soybeans were also analyzed. The results showed that compared with soybeans under normal conditions, those grown under drought stress had reduced yield and carbohydrate, protein, essential amino acid, soybean isoflavone, and other nutrient contents. Besides, natto presented low nattokinase levels (674 U/mL), natto drawing ability was weak, the surface was dull, the taste was poor, and the sensory score was 12 points. Exogenous melatonin increased the carbohydrate content (starch, sucrose, glucose, and fructose) and improved the yield and quality of soybeans under drought. The natto produced by soybeans under drought stress with exogenous melatonin had high nattokinase content (756 U/mL) and long wire drawing. The surface of the product was rich in mucus and had a natto aroma. Its comprehensive sensory score was 20 points. Natto from soybeans under drought stress that were treated with exogenous melatonin showed significantly higher yield, nutrient content, and quality, than those under drought stress without treatment. This study provides theoretical data that can facilitate the development of new methods to improve the quality of soybeans grown under drought conditions.

1. Introduction

Drought reduces the water content of soybean leaves and the integrity of cell membrane, which limits the orderly progress of physiological [1] and biochemical reactions, such as carbon and nitrogen assimilation, transfer, and distribution during soybean growth, and thereby leads to slow crop growth and development [2, 3]. Drought also reduces soybean yield [4, 5] and nutrient accumulation, affecting soybean quality [6–10] and food security [11]. Studies have shown that melatonin (N-acetyl-5-methoxytryptamine) can effectively increase the activity of antioxidant [12] and glutathione-related enzymes and reduce the damage caused by ROS (reactive oxygen species) to cell membrane [13, 14]. Melatonin also regulates the levels of growth hormones and improves the activities of carbon and nitrogen metabolism-

related enzymes and photosynthetic rate [15, 16], thus promoting carbon and nitrogen metabolism and improving yield and quality of soybean. Concomitantly, the expression and regulation of genes related to “amino acid metabolism” and “starch-sucrose metabolism” pathways are upregulated, and the contents of L-asparagine and 6-phosphate glucose metabolites are increased, thus playing a role in drought resistance [17, 18].

Natto is a fermented soybean product, and its minerals and isoflavones are not easily lost after processing. Nattokinase, one of the most important active substances in natto, dissolves thrombus, reduces blood viscosity, improves blood circulation, and softens and increases blood-vessel elasticity. It can also reduce the risk of death due to cardiovascular diseases in both men and women. Its surface is covered with white viscous material, which can form mucus after stirring.

The silky sticky mucus is an important criterion in determining the quality of natto.

Because of a major imbalance between the supply and demand of soybean in China, the soybean yield in Heilongjiang has been declining annually, and it has been difficult for the soybean quality to meet the production standards and requirements. Compared with other methods for conferring drought resistance, the application of melatonin is a simple, effective method that requires low technical skill to improve drought resistance and promote crop growth and yield. Although there are studies on melatonin application and the improvement of antioxidant and drought resistance properties of crops, there is a lack of data on the influence of melatonin treatment on the nutritional composition and quality of soybean and its products. In this study, we explored whether the application of melatonin would improve drought resistance and quality of soybean and its products (such as natto) under drought stress, while aiming at utilizing the findings to improve the quality of soybean products in the future.

2. Materials and Methods

2.1. Test Materials. The drought-sensitive soybean variety Suinong 26 was provided by the National Engineering Technology Research Center for Coarse Cereals, China; *Bacillus subtilis* var. natto strains were purified from commercially available natto starter cultures (MG) bought from Miyagino natto Production Research Institute, Japan; beef extract peptone (NA) agar medium, daidzin, glycerin, genistin, genistein, urokinase standards, fibrin tablets, and daidzein were purchased from Beijing Obosing Biotechnology Co., Ltd., China.

2.2. Instruments and Equipment. The PH104A constant-temperature incubator was purchased from Shanghai Yiheng Technology Co., Ltd., Shanghai, China; the 4802UV/VIS ultraviolet-visible spectrophotometer was purchased from Unocal Instrument Co., Ltd.; the G154DW autoclave steam sterilizer was purchased from Zhiwei Instrument Co., Ltd.; and the A300 advanced amino acid automatic analyzer was purchased from MembraPure Instrument Co., Ltd., Germany.

2.3. Experimental Methods

2.3.1. Sample Processing. Sui Nong 26 was used as the test variety. The pot cultivation barrel diameter was 30.0 cm, and the height was 33.0 cm. Six seeds were sown in each pot, and three of the seedlings with moderate growth vigor were selected for the experiment. The beginning period of grain filling was recorded as the first day of the experiment, and the field capacity was controlled at 80%. The vermiculite, perlite, and chernozem were mixed in the ratio of 1:3:12 (v:v:v), and the physicochemical properties (alkali hydrolyzable nitrogen 72.1 mg kg⁻¹, available phosphorus 14.1 mg kg⁻¹, available potassium 173.0 mg kg⁻¹, pH 7.1, and organic matter 3.9 mg kg⁻¹) were used as substrates. The amount of

fertilizer was 0.3 g DAP, 0.075 g urea, and 0.075 g potassium sulfate per kg of soil. Each treatment pot weighed 0.5 kg and contained 16.5 kg soil. Each treatment consisted of 100 pots, and it was performed in triplicate. The specific treatment settings are as follows.

In normal water supply treatment (WW), the soil moisture content was maintained at 80% water holding capacity (WHC). In drought-stress treatment (D), the soil WHC reached 50% on the 10th day to reach the drought standard; from 11th to 27th day, the drought standard of 50% WHC was maintained by replenishing water. To ascertain normal plant growth, 80% of the WHC of the soil was adopted at 28 d until harvest at maturity. Drought-stress plants were sprayed with melatonin (D + M), when reaching the drought standard on the 10th day, 20 mL of 100 μmol/L melatonin was sprayed on soybean leaves at 21:00 on 11th, 12th, and 13th day, at the same time keeping the WHC reached 50%. After that, WHC is kept at 80% at 28th day until harvest at maturity.

2.3.2. Nutrient Composition Testing. The starch content was determined by the GB/T 5009.9 method [19]; the sucrose, fructose, and glucose contents were determined according to the method described by Zhang et al. [20]; soybean protein and amino acid contents were determined according to the GB/T 14489.2–2008 method [21]; and isoflavone content was determined according to the method described by Ye et al. [22].

2.3.3. Natto Fermentation Process. Soybean grains were screened, washed, and soaked in water for 20 h. The grains were placed in an oven at 121°C for 35 min and then cooled. 4% of natto starter cultures (MG) was inoculated in soybean after cooking. Grains were fermented at 37°C and 90% RH for 24 h. Natto was obtained after maintaining the grains at 4°C for 24 h.

2.3.4. Determination of Nattokinase Activity. Nattokinase activity was measured according to the method described by Xiong et al. [23]. Fibrin plate method was used to determine the nattokinase activity in the crude natto enzyme solution. Two grams of the prepared natto were added to 6 mL of sterile physiological saline, and the solution was left at 4°C for 24 h or 30°C for 30 min with shaking. It was centrifuged at 4°C, 10,000 × g for 10 min, and 2 μL of the supernatant crude enzyme was used. The liquid was distributed in the wells of the fibrin plate by making three parallel streaks for each sample; the plates were incubated at 37°C for 8 h. The diameter of the dissolution circle was measured with a Vernier caliper, and the average area was calculated. The nattokinase activity of the crude natto enzyme solution produced by the strain was analyzed according to the urokinase standard curve.

2.3.5. Sensory Evaluation of Natto. A 20-point system of four indicators was used for scoring (Table 1). A sensory evaluation team was composed of 20 people, and a blind

TABLE 1: Criteria for sensory evaluation.

Score	Appearance	Brushed	Smell	Taste
0–3	Beans are distinct, and the surface is dark brown	Almost no mucus, 0–10 cm	Strong ammonia smell	Not soft, no mucus, dry, astringent, bitter
4–7	The beans are loose and brown on the surface	There is less mucus, 10–20 cm	Heavy ammonia smell	Soft, waxy, dry, astringent, not bitter
8–11	The bean grains are not easy to separate, and the surface is dull	The mucus is normal, and the viscosity is strong, 20–30 cm	Ammonia smell	Soft, waxy, moist, not astringent, not bitter
12–15	Grains are compact, the color is dull, there is white powder attached to the surface, and the surface is shiny	There is more mucus and strong viscosity, 30–40 cm	Minor ammonia smell	Soft, waxy, moist, not astringent, not bitter
16–20	Bean grains have strong adhesiveness, bright color, white powdery attachments, and shiny surface	A lot of mucus and strong viscosity, 40–50 cm	Natto aroma	Soft, waxy, slippery, not astringent, not bitter

evaluation model was adopted. The highest and lowest scores were removed. The experiment designer did not participate in the scoring, and the average of the 18 scores was taken. The drawing score of the natto is to stir the natto evenly for 2 min, observe the amount of mucus, and then pick up with chopsticks to measure the drawing length [24].

2.3.6. Data Processing and Analysis. Microsoft Excel 2013 was used for data entry and sorting. All experiments were conducted in triplicates ($n = 3$), and results were displayed as mean $\pm$ SD. SPSS 19.0 software (Chicago, IBM SPSS, USA) was used for variance analysis and Origin 9.1 software (Northampton, MA, USA) was used for illustrations; p values < 0.05 were considered statistically significant.

3. Results

3.1. The Effect of Exogenous Melatonin on Soybean Yield under Drought Stress. As shown in Table 2, drought stress reduced the number of pods, grain weight and grain per plant, 100 grain weight, and ultimately yield. There were significant differences in the number of pods and seeds per plant, 100 grain weight, and grain per plant between the WW and D treatments. These were reduced in treatment D by 7.04%, 8.07%, 28.75%, and 27.70%, respectively. Compared with D treatment, the above indicators of D + M treatment increased by 2.02%, 5.77%, 12.91%, and 10.86%, respectively. The differences between D and D + M treatments in grain weight, 100-grain weight, and yield reached significant levels. Based on the production data of 2018 and 2019, spraying exogenous melatonin under drought stress can effectively prevent the decrease in the number of pods per plant, the number of grains per plant, 100 grain weight, and grain weight per plant, and ultimately increase the soybean yield under drought stress.

3.2. The Effect of Exogenous Melatonin on Soybean Carbohydrate Content under Drought Stress. As shown in Figure 1, compared with WW treatment, the soybean starch, sucrose, and glucose content of D treatment decreased by 30.5%, 9.83%, and 9.14%, while the fructose content increased by 70.12%. Compared with D treatment, the soybean starch, sucrose, fructose, and glucose content of D + M treatment

increased by 24.01%, 19.67%, 34.92%, and 19.70%, respectively. These results indicate that the addition of exogenous melatonin can significantly increase the content of sugars in soybean under drought stress.

3.3. The Effect of Exogenous Melatonin on Soybean Protein Content under Drought Stress. Figure 2 shows that the protein content of D soybean was significantly lower than that of the WW and D + M groups. This result was because drought stress decreased the activity of key enzymes in nitrogen metabolism, increased inorganic nitrogen content, decreased amino-acid content, and blocked nitrogen accumulation and transportation. Under drought stress, exogenous melatonin can significantly increase the activities of key enzymes in nitrogen metabolism (NR, GS, GOGAT, and GDH), increase amino acid content, promote nitrogen accumulation in the whole plant, and facilitate nitrogen assimilation and transportation [25].

3.4. The Effect of Exogenous Melatonin on Soybean Essential Amino Acid Content under Drought Stress. Amino acids play a positive role in crop resistance. As shown in Figure 3, compared with the WW group, D group had decreased lysine (Lys), tryptophan (Tyr), methionine (Met), and threonine (Thr), leucine (Leu), isoleucine (Ile), valine (Val), and phenylalanine (Phe) content. Compared with the D group, the Thr, Tyr, Met, Thr, Leu, Ile, Val, and Phe contents of the D + M group had increased, suggesting that spraying exogenous melatonin can increase the essential amino acid content of soybean.

3.5. Effect of Exogenous Melatonin on Soybean Isoflavone Content under Drought Stress. Table 3 shows that drought stress caused a significant decrease in the isoflavone content of mature soybean grains. Compared with WW treatment, daidzin, glycitin, genistin, genistein, and total isoflavones contents were reduced by 9.28%, 15.15%, 14.31%, 20.71%, and 12.62% in D treatment, respectively. Spraying exogenous melatonin increased the contents of the above indicators in D + M treatment, and the above indicators increased by 3.14%, 8.24%, 6.23%, 25.00%, 5.66%, respectively, except for genistein. Besides, there were significant differences in other indicators according to varying

TABLE 2: Effects of melatonin treatment on yield components of soybean under drought stress.

Year	Treatment	Pod number per plant	Grain number per plant	100 grain weight/g	g/plant
2018	WW	21.30 ± 1.25 a	47.10 ± 0.99 a	23.76 ± 2.78 a	10.83 ± 0.70 a
	D	19.80 ± 0.79 b	43.30 ± 1.16 c	17.35 ± 0.97 c	7.83 ± 0.60 c
	D + M	20.20 ± 1.23 ab	45.80 ± 1.03 b	19.59 ± 1.30 b	8.68 ± 0.72 b
2019	WW	21.50 ± 3.27 a	46.53 ± 0.48 ab	23.10 ± 0.36 a	10.85 ± 1.08 a
	D	18.20 ± 1.99 b	43.73 ± 0.33 c	17.83 ± 0.93 c	7.80 ± 0.75 c
	D + M	19.80 ± 1.23 ab	45.47 ± 0.66 b	19.28 ± 1.25 b	8.77 ± 0.92 b

FIGURE 1: Effect of melatonin on carbohydrate contents in soybean under drought stress. Data are represented as average ± SD ($n = 3$). Different letters indicate significant differences ($p < 0.05$). FIGURE 2: Effect of melatonin on protein contents in soybean under drought stress. Data are represented as average ± SD ($n = 3$). Different letters indicate significant differences ($p < 0.05$).

treatments, suggesting that melatonin can promote the increase in isoflavone content of mature soybean grains.

3.6. Nattokinase Activity in Natto. The concentrations of urokinase and natto crude enzyme solution were different; the size of the dissolution circle after the reaction on the fibrin plate was also different. Nattokinase activity was calculated according to the standard curve of urokinase $y = 8.1034 \times -909.13$ ($R^2 = 0.9991$). The results showed that nattokinase activity of the natto products obtained from the

three treatments were 731 U/mL, 674 U/mL, and 756 U/mL, respectively. This result suggests that D + M treatment was more conducive for the growth of natto bacteria, thereby producing more biologically active substances such as nattokinase, which improves the nutrition contents and sales of the natto products.

3.7. Sensory Evaluation of Natto. The appearance, drawing length, smell, and taste of natto are also important factors affecting its commodity value. The higher the yield of

FIGURE 3: Effect of exogenous melatonin on amino acid content in soybean under drought stress. Data are represented as average $\pm$ SD ($n = 3$). Different letters indicate significant differences ($p < 0.05$).

TABLE 3: Effect of exogenous melatonin on the isoflavone content in soybean under drought stress.

Treatment	Daidzin ($\mu\text{g}\cdot\text{g}^{-1}$)	Glycitin ($\mu\text{g}\cdot\text{g}^{-1}$)	Genistin ($\mu\text{g}\cdot\text{g}^{-1}$)	Genistein ($\mu\text{g}\cdot\text{g}^{-1}$)	Total isoflavones ($\mu\text{g}\cdot\text{g}^{-1}$)
WW	168.29 $\pm$ 0.62 a	49.56 $\pm$ 0.26 a	226.17 $\pm$ 8.60 a	5.60 $\pm$ 0.12 a	449.62 $\pm$ 8.23 a
D	152.67 $\pm$ 0.88 c	42.05 $\pm$ 0.37 c	193.72 $\pm$ 6.99 c	4.44 $\pm$ 0.10 b	392.89 $\pm$ 6.59 c
D + M	157.46 $\pm$ 0.11 b	45.55 $\pm$ 0.22 b	206.58 $\pm$ 3.6 b	5.55 $\pm$ 0.06 a	415.14 $\pm$ 3.87 b

γ -polyglutamic acid (γ -PGA), the more will be the mucus and the better will be the drawing effect and taste [26]. As shown in Table 4, the sensory evaluation results revealed that the WW natto group had higher protein content, moist glossy surface, obvious and long drawing, high viscosity, ammonia smell, and a comprehensive sensory score of 15 points. The D natto group had the lowest protein content, poor surface gloss, nonwetting, short drawing, low viscosity, relatively poor taste, and a sensory score of 12 points. In the D + M natto group, the soybean protein content was moderate, with bright color, longer drawing, moderate viscosity, smooth taste, and it had a natto aroma without any peculiar smell, which was in line with the taste of the Chinese people. The comprehensive sensory score was 20 points.

4. Discussion

Relevant research shows that the main reasons for drought to reduce crop yield were the drought would destroy the different growth stage of plant water balance in the body; reduce the antioxidant enzymes [27], sucrose phosphate synthase (SPS), sucrose synthase (SS) [28, 29], glutamine synthetase (GS) [30] activity, suppression, and cell division and chloroplast membrane structure was destroyed, resulting in the decrease of leaf area coefficient and photosynthetic rate, crop carbon, and nitrogen assimilation, metastasis; and affect the growth and development of root, stem, and leaf [31, 32], resulting in significant decrease in biomass [2,3]. Drought in different periods had different

effects on yield and quality. Cell division was inhibited, and leaf area was decreased under drought stress at the seedling stage. Dryness in the flowering pod stage causes stunted plants, reduced carbon assimilation, abscisic acid accumulation, and pod shedding [33, 34]. Droughts in the bulge stage mainly affect the grain formation, and the accumulation of nutrients gradually decreases [35], ultimately affecting the yield and quality [13]. Li et al. [32] believed that, with the persistence of drought stress, the contents of soluble sugar and fructose in soybean leaves increased, while the content of sucrose decreased. Zhang et al. [36] believed that drought increased the content of reducing sugar and soluble total sugar in soybean leaves at the bulging stage. Studies have shown that drought stress significantly reduced the relative water content of tomato [37], blueberry [38], wheat [39], and other crops and severely damaged the integrity of cell membranes. Grain development of wheat and sesame [40] was inhibited, eventually leading to a yield reduction of 17%–70% in wheat [4, 5], and the contents of crude fat, oleic acid, and total phenols in sesame decreased significantly. Under drought stress, the grain size and protein and amylose content of fresh maize decreased, leading to a decrease in yield and quality.

Li [41] and Marinobet [42] believed that melatonin could promote nitrogen accumulation, amino acid synthesis, and soybean yield under normal water supply and drought stress. Wang et al. [14], Zou et al. [43], Zheng et al. [44], and other scholars believe that melatonin through the activity of antioxidant enzymes reduces ROS cell membrane injury caused by the effect of soybean drought. Zhang et al. [45] and Liu's

TABLE 4: Sensory evaluation of natto samples.

Sensory evaluation group	Appearance	Brushed	Odor	Taste	Comprehensive score
WW	5 ± 0.5	4 ± 1.0	3 ± 1.0	3 ± 1.0	15 ± 1.0
D	3 ± 1.0	2 ± 0.5	4 ± 0.5	3 ± 1.0	12 ± 0.5
D + M	5 ± 0.5	5 ± 0.5	5 ± 1.0	5 ± 0.5	20 ± 0.5

research [46] suggests that melatonin by increasing the growth of soybean plant hormones IAA, GA, and ZR contents has the effect of drought resistance and promote growth. Therefore, to alleviate the effect of drought stress on soybean growth and development, it was verified that melatonin could increase soybean yield under drought stress, and the melatonin was used to spray soybean leaf surface. The results of this study showed that the growth of soybean was inhibited under drought stress, leading to low yield, poor composition, and quality of soybean, while the addition of melatonin could significantly improve the yield of soybean under drought stress, promote the accumulation of sugar, protein, amino acid, and soybean isoflavones, and thus improve the nutritional value and product quality of soybean.

5. Conclusion

The results of this study showed that melatonin spraying improved drought resistance of soybean. Melatonin can effectively alleviate the low yield of soybean and increase the content of carbohydrate, protein, amino acid, and soybean isoflavones under drought stress. Compared with D treatment, soybean yield of D + W treatment increased by 10.86%, illustrating that the melatonin can effectively alleviate the low yield of soybean caused by drought. The soybean starch, sucrose, fructose, and glucose content in D + W treatment had increased by 24.01%, 19.67%, 34.92%, and 19.70%, protein increased by 2.6%, and daidzein, glycitin, genistin, genistein, and total soybean isoflavone increase by 3.14%, 8.24%, 6.23%, 25.00%, and 5.66%. It indicates that melatonin spraying can effectively increase the nutritional content of soybeans, thus improving the quality of soybeans. In addition, the soybean product of natto has good taste and flavor. This study clarifies the effects of drought stress and addition of exogenous melatonin on soybean nutrient components and natto quality and provides theoretical data that support soybean quality improvement.

Data Availability

The data used to support the results of this study are available from the corresponding author upon request.

Disclosure

Liang Cao and Fang Kou are co-first authors.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Liang Cao and Fang Kou contributed equally to this work. Yuxian Zhang conceptualized the study, did investigation, did funding acquisition, and wrote the original draft; Liang Cao and Fang Kou developed methodology and reviewed and edited the article; Mingcong Zhang did investigation, validated the study, and did formal analysis; Xijun Jin, Chunyuan Ren, and Gaobo Yu developed methodology and reviewed and edited the article; Mengxue Wang conceptualized the study, developed methodology, supervised the study, and reviewed and edited the article. All authors have read and agreed to the published version of the manuscript.

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