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STUDIES ON THE CHANGES OF PROTEIN SECONDARY STRUCTURE AND CARBOHYDRATE CONTENTS IN SEEDLING-STAGE OF *ABRUS CANTONIENSIS* HANCE IN DROUGHT STRESS BASED ON FTIR AND CHEMOMETRICS

FANG LAN[1], DEXIN KONG[3], YANQUN LI[4] AND RONGSHAO HUANG[2]*

[1]*Postgraduate Department, Guangxi University, Nanning Guangxi 530004*
[2]*College of Agriculture, Guangxi University, Nanning Guangxi, 530004*
[3]*Guangxi Institute of Botany, Chinese Academy of Sciences, Guilin, 541006, China*
[4]*Guangdong Key Laboratory for Innovative Development and Utilization of Forest Plant Germplasm, College of Forestry, South China Agricultural University, Guangzhou, 510642, China*
**Corresponding author's e-mail: hrshao802@163.com; Tel: +86-3235612*

Abstract

To study the response mechanism of seedling-stage *Abrus cantoniensis* to drought stress from the level of infiltration conditions, this research uses Fourier infrared spectroscopy based on such mathematical approaches as Fourier self-deconvolution and Gaussian curve-fitting to analyze the broad characteristic absorption bands of the protein secondary structure and carbohydrates in the root, stem and leaf of seedling-stage under four drought stress treatments. The results show that the relative absorption of every type of secondary structure (namely, α-helix, β-sheet and β-turn), the α-helix/β-sheet ratio and the relative contents of starch, soluble sugars and cellulose show significant differences in the different parts of seedling-stage *A. cantoniensis* under different drought treatments. These differences provide a theoretical basis for investigating the response mechanisms of seedling-stage *A. cantoniensis* to drought stress and for advancing rational cultivation measures for this species. A new method that is fast, efficient and sensitive is presented for the study of the response mechanism of seedling-stage *A. cantoniensis* to drought stress.

Key words: *Abrus cantoniensis,* Protein secondary structure, Carbohydrates, FTIR, Chemometrics, Drought stress

Introduction

Proteins and carbohydrates are important osmoregulation substances and play an important role in plant physiological metabolism and physiological function regulation. At the same time, proteins and carbohydrates, as signals of nutrient supply, adjust the expression of some genes based on the variation of abiotic stress to regulate the molecular structure and physiological function of the plants to allow them to better adapt to the changes in the environment (Osuna *et al*., 2007). The processes of protein expression,synthesis and accumulation in plants respond to changes in the environment of drought stress (Chen & Tabaeizadeh, 1992; Cheng *et al*., 1993). Droughts promote the decomposition of protein and polysaccharide carbohydrates (Hu & Schmidhalter 1998). In most cases, it appears to increase the soluble sugar content to maintain cell osmotic balance to prevent dehydration of cell membranes and proteins, which reduce the loss of water from the blade. Protein and carbohydrates under drought stress have been qualitatively and quantitatively analyzed (Riccardi *et al*., 1998; Crowe *et al*., 1990; Sawhney & Singh, 2002; Arabzadeh, 2012). However, the analytical methods used in these studies are time-consuming and expensive. To obtain accurate results, these methods require a large number of samples and sophisticated operations to minimize all types of errors during the experimental process. Otherwise, the test results are usually insufficient to fully reveal the physiological change mechanisms of plant response to different environmental stresses. Infrared spectroscopy based on multivariate data analysis is a sensitive, rapid, efficient method with the ability to provide rapid screening and composition identification for large samples and various analyte masses (Yang *et al*., 2005; Naumann *et al*., 2010). The spectra can provide a great deal of useful information regarding chemical composition, especially the bands at approximately 1800-800 cm^{-1}, which contain abundant useful spectral information for the analysis of the protein secondary structure and the polysaccharide compositions of biological samples with multiple components (Haris *et al*., 1999; Kačuráková *et al*., 2000). Yang & Yen (2002) reported that early NaCl treatment can influence the change of the chemical composition in the leaves and flowers of the ice plant and *Arabidopsis thaliana*. The infrared spectrum obtained after deconvolution and curve-fitting suggests that the leaves of ice plant can produce advanced protein conformation in response to adversity under saline-alkali stress. Infrared spectroscopy has been successfully applied to determine the changes in polysaccharide pectin, cellulose and hemicellulose in plants under different adversities (Kačuráková *et al*., 2000; Kačuráková & Wilson, 2001). It has been applied particularly widely in combination with chemometrics (Johnson *et al*., 2003; Martín *et al*., 2005; Oliveira *et al*., 2009).

Abrus cantoniensis Hance has an important use in Chinese traditional medicine. It has good curative effects for the liver, healing both hepatitis and cirrhosis of the liver (Zhang *et al*., 2012). *A. cantoniensis* is widely cultivated in Guangdong and Guangxi provinces of China. However, with the aggravation of ecological

conditions and increasingly severe seasonal droughts, the production and quality of *A. cantoniensis* has decreased markedly. Research on the response mechanisms of *A. cantoniensis* to drought stress is necessary to boost the production and quality of this medicinal material. Few studies have investigated the physiological adaptation mechanism of *A. cantoniensis* under adversity stress using Fourier transform infrared spectroscopy combined with chemometrics, such as deconvolution and curve-fitting. In this study, this method is employed to detect the change features of the protein secondary structure and carbohydrate contents in the root, stem and leaf at seedling-stage *A. cantoniensis* under different drought stresses. The vibration characteristics of the functional groups are then used to investigate the adaptation mechanisms of seedling-stage *A. cantoniensis* to drought stress as a function of penetration condition.

Materials and Methods

Materials: The seeds used to grow the *Abrus cantoniensis* plants used in this study were purchased from the medicine market in the city of Yulin and was identified by Professor Rong-shao Huang of the Agricultural College of Guangxi University.

Experimental design: The pot experiment was conducted in an automated greenhouse located in the Agricultural College of Guangxi University in early April 2008. The soil is a loamy soil with moderate fertility. The soil was sieved before planting to remove the impurities. The soil field capacity is 26.8%. In the experiment, a plastic pot 25 cm in height with a diameter of 25 cm was used. Four soil water treatments were used: 80% of field capacity (normal water treatment, N), 60% of field capacity (mild drought treatment, LD), 45% of field capacity (moderate drought treatment, MD) and 35% of field capacity (severe drought treatment, SD). Each treatment consists of nine pots. The dry weight of the soil in each pot is 3.3 kg. According to the design of the soil moisture content, the weight of each pot can be calculated as a standard weight of each treatment pot. Mature and plump seeds were selected for sowing. After seedling emergence, 20 strains with generally consistent growth were retained in each pot. The weighing method was adopted to control the water application after the seedlings reached the appropriate height. Various indices are used to determine when the seedlings have been subjected to the drought stress treatment for 14 d.

Three pots per treatment in the experiment plot were selected as the samples. The samples were brought into the lab to flush the dirt from plant. The root, stem and leaf were stored in separate envelopes. The root, stem and leaf were placed into the oven together and dried to constant weight at a constant temperature of 55ºC after enzyme deactivation at 105ºC for 30 min. The samples were taken out from the oven, ground, and sieved at 200 mesh for the filtration of the final samples. A Fourier infrared spectrometer was used to analyze the samples.

Fourier infrared spectroscopy: The samples were prepared for FTIR measurements as follows. First, 1.0 mg of the ground sample and 200 mg of KBr powder were separately and accurately weighed. The sample and KBr were then mixed with one another and fully and uniformly ground. Finally, the mixture was pressed them into a transparent pellet with a thickness of approximately 1 mm. The pellets were then placed into the infrared spectrometer sample slot for measurement. The testing conditions were set as follows. The cumulative scanning frequency is 64 min^{-1}, and the contributions of CO_2 and H_2O were subtracted. The scanned region was set to 4000 to 400 cm^{-1}, with a spectral resolution of 4 cm^{-1}.

Reagents and instruments: Instrument: Nicolet FTIR 5700, DTGS detector. Software: Nicolet OMNIC 7.3. Pharmaceuticals: KBr spectral purity, produced by the GuangFu Fine Chemical Research Institute in Tianjin.

Results and Discussion

Spectral analysis of the effect of drought stress on various parts of seedling-stage *Abrus cantoniensis*: Samples were obtained from the root, stem and leaf of seedling-stage *A. cantoniensis* under four soil moisture treatments. The pellet comprised of a mixture of KBr and the sample under high pressure was subjected to Fourier transform infrared spectrometry. A typical infrared spectrum for these samples is shown in figure 1. The absorption bands located at approximately 3,420-3300 cm^{-1} correspond to O-H and N-H stretching vibrations, which are mainly from proteins and carbohydrates. The bands at approximately 2,923 cm^{-1} represent C-H stretching vibrations that mainly result from lipid and carbohydrates. Absorption bands at approximately 1,740 cm^{-1} correspond to isolated carbonyl groups (COOR), indicating ester-containing compounds commonly found in membrane lipids and cell wall pectin. The C=O, $-NH_2$ and C-N bonding of the amide linkage absorb radiation in the 1,800 to 1,200 cm^{-1} region. The three absorption bands located at approximately 1,680-1,630 (C=O), 1,535 (N-H) and 1,250 (C-N) cm^{-1} are assigned as amide I, II, and III bands, respectively. The absorption band of the amide I stretching vibrations of the amide group depends on the nature of hydrogen bonding between the amide I and amide II moieties and is particularly useful for determining the protein secondary structure (for a review, see Surewicz *et al*., 1988). Carbohydrates are the major constituents that contribute to these absorption bands, located at approximately 1,100 cm^{-1}, which include contributions from several modes, such as C-H bending or C-O or C-C stretching. Many chemical components overlap to form broad characteristic absorption bands in the original spectra; the characteristic absorption bands of the amide I and carbohydrates are notably broad. To further study the changes in the protein secondary structure and carbohydrates, mathematical approaches such as Fourier self-deconvolution and Gaussian curve-fitting are applied to extract information from the raw IR spectra to resolve the overlapping band components in the broad characteristic absorption bands of amide I and carbohydrates.

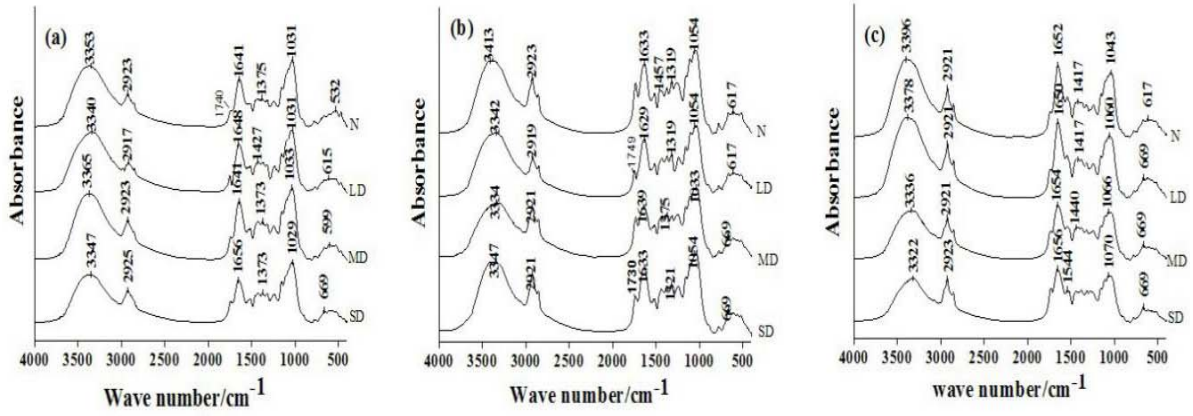

Fig. 1. Absorption FT-IR spectra in the 4,000 to 1,000 cm^{-1} region for the different parts of seedling-stage *A. cantoniensis* under different drought stresses: (a) root, (b) stem and (c) leaf. N: normal water conditions (80%); LD: light drought stress (60%); MD: moderate drought stress (45%); SD: severe drought stress (35%)

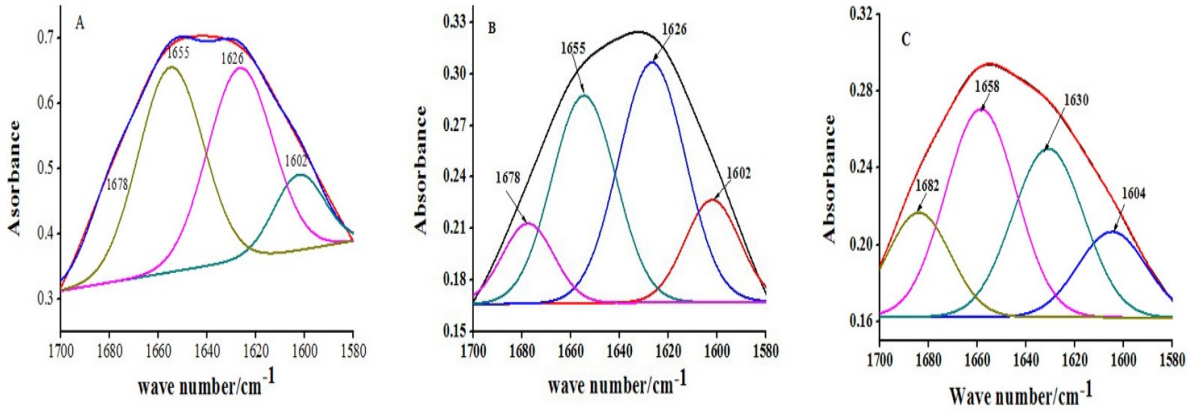

Fig. 2. Comparison of the different parts of seedling-stage *A. cantoniensis* after curve-fitting under the bands at approximately 1700-1580 cm^{-1}. A: root; B: stem; C: leaf. Annotation: application of a Gaussian curve fitting method, bandwidth: 55 cm^{-1}, enhancement factor: 3.0.

Protein secondary structure changes in response to drought stress in seedling-stage *A. cantoniensis*: Drought is the most severe abiotic stress factor limiting plant growth and crop production. When plants are subjected to drought stress, some protease activities are changed. Protein conformation, including secondary structure, is known to influence protease activity (Mafakheri *et al*., 2011). Deconvolution is a band-narrowing technique that can enhance small features buried in an overlapped band (Surewicz & Mantsch., 1988). The curve-fitting technique enables further quantitative analysis of individual bands buried in an overlapping band. A series of Gaussian curve shapes is used to compose a synthesized spectrum. Decomposition of the amide I band by curve fitting into its constituents and the assignment of these components to a protein structure has been successfully applied to predicting the structure of membrane proteins (Arrondo & Govi, 1999). To distinguish the small differences in patterns caused by overlapping bands, deconvolution and curve-fitting techniques are used in this study. The deconvolved spectra of the amide I band between 1700 and 1600 cm^{-1} clearly consist of four sub-bands: 1678 cm^{-1}, assigned to β-turns; 1655 cm^{-1}, assigned to α-helices; and 1626 and 1602 cm^{-1}, assigned to β-sheets. The deconvolved and original spectra can be compared in figure 2. The relative peak area of each four sub-bands can express the relative absorption of one type of secondary structure among the three types, as shown in table 1. From figure 2 and table 1, we can see that the difference in the sub-band position is slight, but significant differences stem from the relative absorption of every sub-band or type of secondary structure in the different parts of seedling-stage *A. cantoniensis*. For example, the relative absorption of the β-sheets in the stem first decreases, then increases and finally decreases again, whereas that in the leaf generally decreases and that in the root shows no obvious difference. The relative absorption of the α-helices increases and then decreases in both the stem and root, whereas it increases gradually in the leaf. The relative absorption of the β-turns increases and then decreases in the root, decreases slowly in the leaf but increases sharply in the stem. These results indicate that the manifestation of each type of protein secondary structure exhibits a notable difference in response to different degrees of drought stress in the different parts of seedling-stage *A. cantoniensis*.

Table 1. Amide I sub-bands, secondary structure assignments and relative absorption of every sub-band or type of secondary structure in different parts of seedling-stage *A. cantoniensis* under different drought treatments.

Position	N			LD			MD			SD			Assignment
	ν/cm^{-1}	Area	RC (%)	ν/cm^{-1}	Area	RC (%)	ν/cm^{-1}	Area	RC (%)	ν/cm^{-1}	Area	RC (%)	
Root	1602	12.9	48.51	1602	14.47	47.8	1602	13.62	47.87	1602	10.75	46.48	β-sheet
	1626			1628			1628			1627			
	1655	10.45	39.30	1656	11.08	36.61	1655	10.98	38.60	1656	9.28	40.12	α-helix
	1678	3.24	12.19	1680	4.72	15.59	1679	3.85	13.53	1679	3.10	13.40	β-turn
Stem	1601	5.28	56.59	1595	7.38	52.26	1593	9.32	58.21	1595	7.01	52.51	β-sheet
	1628			1624			1628			1624			
	1656	2.99	32.05	1654	5.13	34.30	1655	4.91	30.67	1653	3.79	28.39	α-helix
	1680	1.06	11.36	1684	1.61	11.44	1671	1.78	11.12	1682	2.55	19.10	β-turn
Leaf	1613	4.91	45.09	1604	5.14	44.94	1604	4.39	41.61	1604	4.31	41.01	β-sheet
	1631			1625			1628			1631			
	1650	3.58	32.87	1662	3.9	34.39	1656	4.14	39.24	1658	4.45	42.34	α-helix
	1668	2.4	22.04	1683	2.4	20.67	1683	2.02	19.15	1684	1.75	16.65	β-turn

Table 2. α-Helix/β-sheet ratio in different plant parts under different drought treatments.

Position		Root			Stem			Leaf		
Assignment		α-helix	β-sheet	α-helix/β-sheet	α-helix	β-sheet	α-helix/β-sheet	α-helix	β-sheet	α-helix/β-sheet
N	Area	10.45	12.90	0.81	2.99	5.28	0.57	3.58	4.91	0.73
LD	Area	11.08	14.47	0.77	5.13	7.38	0.70	3.90	5.14	0.76
MD	Area	10.98	13.62	0.81	4.91	9.32	0.53	4.14	4.39	0.94
SD	Area	9.28	10.75	0.86	3.79	7.01	0.54	4.45	4.31	1.03

It had been found that an increase in the α-helix/β-sheet ratio was associated with desiccation tolerance in developing maize embryos (Wolkers *et al.,* 1998, 1999). In this study, we find that the α-helix/β-sheet ratio indicates obvious differences in the different parts of seedling-stage *A. cantoniensis* under different drought treatments, as shown in table 2. This ratio exhibits no remarkable change by drought treatment in the root, whereas, in the stem, the highest ratio is detected under light drought stress. In contrast, this ratio increases gradually with increasing drought stress in the leaf.

Carbohydrate changes in response to drought stress in different parts of seedling-stage *A. cantoniensis*: Carbohydrates found in plants are frequently grouped into two different classes: structural carbohydrates and non-structural carbohydrates. The former, including cellulose and lignin, are mainly used in morphogenesis, and the latter, including glucose, fructose, sucrose, fructan and starch, play important roles in metabolism. In non-structural carbohydrates, the biosynthesis of sugar and starch both originate from the same precursor: triosephosphate (Cao & Song, 1998). Large amounts of soluble carbohydrates have been proven to be involved in the acquisition of desiccation tolerance. Due to their solubility, they may help plants survive periods of osmotic stress induced by drought (Arabzadeh, 2012). The broad absorption band between 1200 and 900 cm^{-1} was deconvolved and fitted by a Gaussian curve to investigate the changes in carbohydrates in response to drought stress in the different parts of seedling-stage *A. cantoniensis*. The deconvolved spectra are composed of six sub-bands, as shown in figure 3. The absorption bands at 1159, 1083, 1027, 1016 and 995 cm^{-1} are the characteristic absorption peak of starch. The characteristic absorption peaks of soluble sugars are located at 1115, 1070, 1053, 1027 and 987 cm^{-1}. Cellulose provides an intense absorption peak in the region between 1060 and 1040 cm^{-1} (Kačuráková *et al*., 2000; Oliveira *et al*., 2009). The area under the characteristic absorption peak of a carbohydrate can express its relative content. Figure 4 shows that the relative contents of carbohydrates have obvious differences in the different parts of seedling-stage *A. cantoniensis* under different drought treatments. Overall, the relative contents of starch and soluble sugars are higher than that of cellulose in the root, stem and leaf. In the root, the relative contents of carbohydrates change in different ways. For example, the relative content of starch is the highest under the LD treatment and then decreases with increasing drought stress, whereas that of soluble sugars does not change significantly under N and LD but increases with further increases in drought stress. Finally, that of cellulose exhibits only small changes. In the stem, the relative content of soluble sugars and cellulose decreases but that of starch increases with the aggravation of drought stress. The leaf is the main site of photosynthesis, and the effect of drought stress is more obvious in the leaf than that in the root and stem due to its large transpiration area. With increasing drought stress, the relative content of starch decreases. The relative content of soluble sugars first increases and then decreases, and that of cellulose experiences no obvious change.

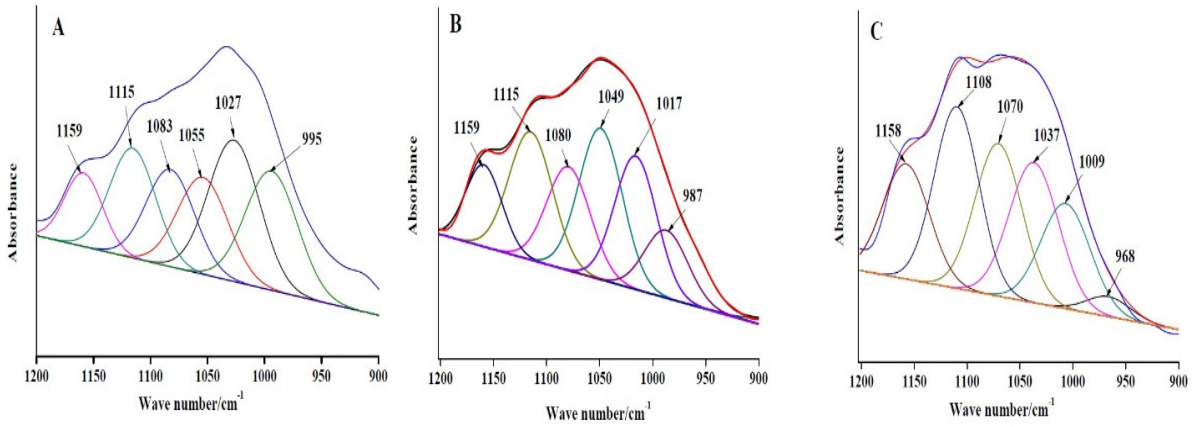

Fig. 3. Curve fitting of the bands at approximately 1200-900 cm^{-1}. A: root; B: stem; C: leaf

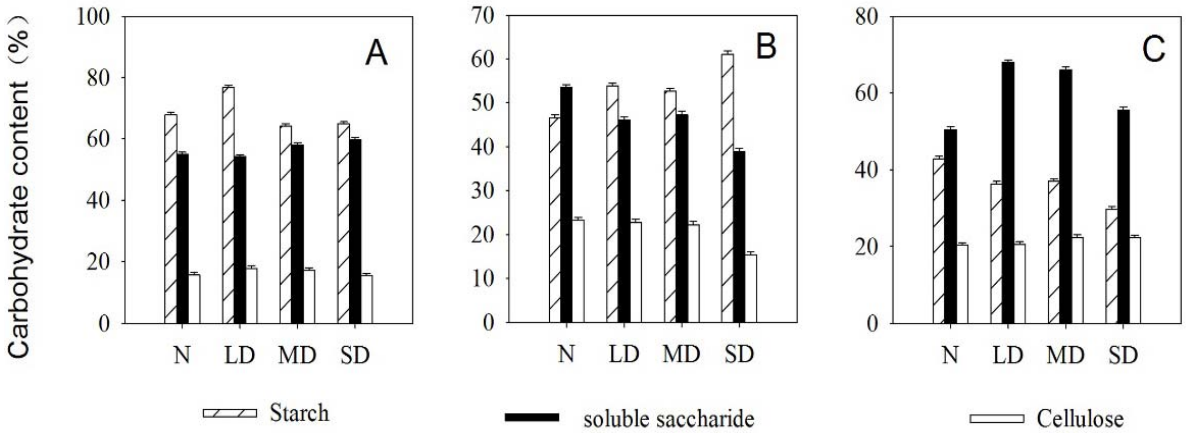

Fig. 4. Relative carbohydrate contents in root (A), stem (B) and leaf (C).

Conclusion

This study uses FTIR to scan different samples from the root, stem and leaf of seedling-stage *A. cantoniensis* under different drought treatments. Characteristic absorption bands are identified between 1800 and 600 cm^{-1}. The broad absorption bands between 1700 and 1600 cm^{-1} are characteristic of protein secondary structure, and those between 1200 and 900 cm^{-1} are characteristic of carbohydrates. Fourier self-deconvolution and Gaussian curve-fitting methods are applied to treat the characteristic absorption bands of protein secondary structure and carbohydrates to detect changes in the protein secondary structure and relative carbohydrate contents in the different parts of seedling-stage *A. cantoniensis* under different drought stress treatments. The result shows that the changes in these characteristics are significant. That is, the manifestation of every type of protein secondary structure and the relative contents of starch, soluble sugars and cellulose show notable differences in response to different degrees of drought stress. Hence, this study provides a theoretical basis for investigating the response mechanism of seedling-stage *A. cantoniensis* to drought stress and for advancing rational cultivation measures for *A. cantoniensis*. Meanwhile, it also provides a new method that is fast, efficient and sensitive for the study of the response mechanisms of seedling-stage *A. cantoniensis* to drought stress.

Acknowledgments

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