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VARIATION IN PHYSIOLOGICAL AND CHEMICAL CHARACTERISTICS AT DEVELOPMENTAL STAGE IN DIFFERENT DISEASE-RESISTANT VARIETIES OF *CAMELLIA OLEIFERA*

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Abstract

Camellia oleifera Abel. is an important edible oil tree species from Southern China. Anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.), is responsible for more than 50% of *C. oleifera* production loss, and *C. oleifera* varieties differ in their resistance to anthracnose. The aim of this study was to assess resistance mechanisms by monitoring physiological and biochemical parameters of differentially resistant cultivars during the development of *C. oleifera*. *C. oleifera* fruit coats were analyzed between May and September for tannins, anthocyanins, soluble sugar content, pH, buffer capacity, activity of three enzyme s(Phenylalanine ammonia lyase; polyphenol oxidase; peroxidase) and free radical scavenging capacity. Anthocyanins, soluble contents and free radical scavenging capacity were related to anthracnose resistance, with anthocyanins and soluble sugar contents of the resistant varieties nearly twofold higher than those of susceptible varieties. The results of free radical scavenging capacity showed that extracts from highly resistant varieties of *C. oleifera* fruit coats performed more efficiently in the scavenging of free radicals than those from susceptible varieties. The three enzyme activities of highly resistant varieties rose rapidly and continuously, while those of medium resistant and highly susceptible varieties increased initially and then decreased. Tannins, pH and buffer capacity showed no significant differences between different cultivars. This study broadens the understanding of disease resistance mechanisms in *C. oleifera*.

Introduction

Camellia oleifera, a member of the family Theaceae, is an evergreen shrub and an important oil-producing plant in Southern China. The main product of *C. oleifera* is oil obtained from seeds. It contains unsaturated fatty acids of up to 85%-90%, which is higher than the 75-90% found in olive oil. The vitamin E contents are twice as high as in olive oil. This has led to the camellia oil being called "Oriental olive oil" (Shu & Zhang, 2009), and it has been used in cosmetics, medicine and healthcare. By-products like tea seed cakes and fruit shells are used as raw material in industry and agriculture. In addition, *C. oleifera* is a deep-rooted tree which plays an important role in soil and water conservation. Hence, *C. oleifera* is of significant economic, social and ecological value. The development of the camellia industry has broad prospects in China. However, anthracnose, caused by *Colletotrichum gloeosporioides* and known as cancer of this species, is a main disease of *C. oleifera,* and is widespread in its distribution area. The disease can cause not only decay of fruits, buds and leaves, but also shoots to wither, canker of branches and trunk, and even whole plant death. This results in economic losses by anthracnose, such as the reduction of fruits by 30%-50%, occasionally even up to 100% (Cao *et al*., 2011). In the last 10-year survey, the extent of disease in different cultivars at different time points was significantly different in the same *C. oleifera* orchard. For example, the incidence rate of some varieties reached 100%, while adjacent varieties were rarely infected.

Plant pathogens have developed various independent and elaborate mechanisms to penetrate and access plant cell contents. Stopping the penetration of pathogens during plant infection depends on the accurate time-course perception of the pathogen by the plant host cells. This leads to the activation of networking systems, resulting in the induction of secondary metabolites, reactive oxygen species (ROS), expression of defense genes (Palmer & Paulson, 1997; Mittler *et al*., 2004), pathogenesis-related proteins and hypersensitive responses (Leister, 2004; Takakura *et al*., 2008). They are often working in combination to mount an adequate defense mechanism against the pathogen infection (Bolwell *et al*., 2001). These mechanisms include modifications of the plant cell wall, deposition of callose containing papillae and production of hydroxyproline-rich glycoproteins and phenolic compounds (Mazau & Esquerré-Tugayé, 1986; Aist & Bushnell, 1991). In recent years, the study of plant resistance mechanisms have been carried out by determining biochemical indicators, such as various enzymes (Azevedo-Neto *et al*., 1991; Sudhakar *et al*., 2001; Harinasut *et al*., 2003; Sekmen *et al*., 2007; Yang *et al*., 2008; Ahmad *et al*., 2010). The research of *C. oleifera* resistance mechanisms has focused on color, epidermal structure and related enzyme activities of fruits (Zhuang *et al*., 1992; Yang *et al*., 2004; Duan *et al*., 2005; Zhang *et al*., 2012). However, no study has been reported yet to combine different disease-resistant varieties at different development stages. Therefore, the main objective of this study was to identify optimal physiology and biochemistry parameters related to anthracnose resistance at the developmental stage through the analysis of tannins, anthocyanins, soluble sugar contents, enzyme activities, DPPH free radical scavenging activity, pH and buffer capacity of fruit coats. We also wanted to provide a scientific basis for breeding elite varieties and establishing resistance mechanisms of this disease.

Materials and Methods

Experimental material: In the growing season of *C. oleifera* during 2008-2010, variety Dabieshan No.1, Shuchengxiaohong and Shuchengxiaoqing were investigated by periodically observing fixed plants as experimental material.

Resistance test: *C. oleifera* anthracnose resistance was conducted on the three experimental materials by detached fruit inoculation method (Kanchana-Udomkan *et al.*, 2004; Montri *et al.*, 2009). The inoculated fruit was incubated in artificial climate chest at 25°C, under a 12 h daylight photoperiod, and 95% relative humidity, at Anhui Agricultural University, Hefei, China. Anthracnose symptoms at the inoculation sites on fruit were evaluated at 7 days after inoculation, using disease scores as described in Table 1. The fruit disease scale was developed by Montri *et al.*, (2009).

Healthy and disease-free fruits were collected every mid-month during the growing season. The fruits were rinsed with wet gauze, put on ice and immediately stored at -20ºC.

Determination of tannin and anthocyanin content of fruit coats from different cultivars of *C. oleifera*: Healthy fruit coats were taken from disease-free tea oil plants and rinsed with distilled water. Samples of 1.0 g were weighed, ground to a homogenate with quartz sand and filtered. The filtered volume was filled up to 25 mL in a volumetric flask, shaken, and set aside. To 5.0 mL filtrate, 5.0mL isatin and 20 mL distilled water were added and the mixture was placed in a 100 mL triangular flask. Following this, 0.01 M potassium permanganate were quickly titrated to yellow-green and then slowly titrated to bright gold (Yuan & Yang, 1983).

One gram of fresh fruit coat was weighed and cut into small pieces, placed in a 50 mL triangular flask and filled up to the mark with hydrochloric acid methanol (pH 3.0), shaken at regular intervals, and set side in the dark for 18-24 h (until the fruit coat whitened) to obtain the extract. Using hydrochloric acid methanol as blank liquid, the extract was carried out on a 756 MC spectrophotometer and the OD value was determined (Gross, 1999).

Determination of pH and buffer capacity of fruit coat in different cultivars of *C. oleifera*: Weighed fruit coats (1.2g each) were ground into a homogenate with 7 mL distilled water. Then, the mixture was centrifuged at 6,000 *g* for 15 min to obtain the supernatant. Determination of the supernatant pH was carried out with a PHS-25 C pH tester. Buffer capacity was determined by titrating the supernatant with sodium hydrate. The ability to resist pH value change was regarded as a measurement of stability in the process of titration, which represented the buffer ability. Buffer capacity was expressed as the pH value variation when 2mL 0.1 M NaOH was added to 5mL supernatant (Yuan & Yang, 1983).

Determination of POD, PPO and PAL activity: Fruit coats (0.3g each) were ground into a homogenate with 3mL distilled water. One mL of mixture was centrifuged at 14,000 *g* and 4ºC for 15 min to obtain the supernatant, which contained the crude enzyme extracts. Then, 3 mL PBS reagent, 0.05 mL methyl catechol reagent and 0.01 mL hydrogen peroxide were added to 0.1 mL of the supernatant (Mbouobda *et al.*, 2010).

Five grams of leaves were ground into a homogenate with quartz sand in low temperature. Then, 15 mL phosphate buffer (pH 7.2) was added and the mixture was centrifuged at 8,000 *g* and 4ºC for 10 min to obtain the crude enzyme extract in the supernatant (Zhang *et al.*, 2012).

Weighed leaves (5 g) were ground into a homogenate with 0.5 g polyvinylpyrrolidone, quartz sand and ice. Then, 15mL borate buffer (pH 8.8) was added and the mixture was centrifuged at 8,000 *g* and 4ºC for 10 min to obtain the supernatant, which contained the crude enzyme extracts (Zhang *et al.*, 2012). Enzyme activity was expressed as U/(min·g fresh weight). The determination of POD, PPO and PAL activity was repeated three times.

Determination of soluble sugar of fruit coat: A total amount of 0.5 g fresh fruit coat was weighed and cut into small pieces, placed in a 50 mL triangular flask containing 25 mL distilled water, and heated at 60ºC for 30 min. The extract was then filtered and rinsed with hot water. Soluble sugars were assayed following the anthrone method (Plummer, 1987).

DPPH free-radical scavenging assay: The DPPH free-radical scavenging activity was determined by the methods described by Kim *et al*., (2002) and Liu *et al*., (2008) with modifications. Two grams of fruit coats were weighed and cut into small pieces, placed in 50mL triangular flask with 20 mL 95% ethanol and shaken for 48 h to obtain the extract in the supernatant. The supernatant was diluted into 2.0 mg/mL, 1.0 mg/mL, 0.5 mg/mL, and 0.25 mg/mL. Two hundred and fifty microliters of sample solution in EtOH solution (5 mg/mL) was added to 250μL of 5.07×10^{-4} M DPPH EtOH solution. The reaction mixture was incubated in the dark at room temperature, and the absorbance was measured at 517 nm after 1 h. EtOH was used as control and BHA was used as a standard reference. DPPH radical scavenging activity was calculated by using the following equation:

$$\text{Scavenging activity (\%)} = \frac{\text{1-absorbance of essential oil}}{\text{absorbance of control}} \times 100\%$$

Statistical analysis: Data from this study are presented in the form of means ± SE, for at least three independent experiments were performed. Analysis of variance (ANOVA) and Duncan's test were used to compare the variations of physiological and biochemical parameters in different disease-resistant varieties, using SPSS 12 version for windows. *P*-values less than 0.05 were considered significant.

Results

Variation of tannin and anthocyanin contents in different cultivars of *C. oleifera* at developmental stages: Results for tannin and anthocyanin contents of fruit coats are presented in Table 2. Tannin contents generally declined at the developmental stage (highly resistant 0.50-0.32, medium resistant 0.46-0.24, highly susceptible 0.48-0.45), whereas no significant difference was found in tannin levels between differentially disease-resistant cultivars ($p>0.05$).

The growth rate of highly resistant varieties was 12.1% higher than of susceptible ones in May. During developmental stages, the anthocyanin contents of fruit coats in highly resistant, medium resistant and highly susceptible varieties grew by 77.3, 69.4 and 51.5%, respectively, and the synthesis rate of anthocyanins changed mildly after August. The anthocyanin contents differed significantly among different cultivars ($p<0.01$).

Table 1. Anthracnose severity scores and the symptom description on *C. oleifera* fruit in different varieties.

Scores	Fruit symptom descriptions	Type	Varieties
0	No infection	Immune	
1	1-2% of the fruit area shows necrotic lesion or a larger water soaked lesion surrounding the infection site	Highly resistant	Dabieshan No.1
3	>2-5% of the fruit area shows necrotic lesion, acervuli may be present/or water soaked lesion up to 5% of the fruit surface	Medium resistant	Shucheng-xiaohong
5	>5-15% of the fruit area shows necrotic lesion, acervuli present/or water soaked lesion up to 25% of the fruit surface	Resistant	
7	>15-25% of the fruit area shows necrotic lesion with acervuli	Susceptible	
9	>25% of the fruit area shows necrosis, lesion often encircling the fruit, abundant acervuli	Highly susceptible	Shucheng-xiaoqing

Table 2. Average contents[a] of tannins and anthocyanins (%) of fruit coat in different varieties during development.

Varieties	Tannins					Anthocyanins				
	April 15	June 15	July 15	August 15	Sept. 15	April 15	June 15	July 15	August 15	Sept. 15
Highly resistant	0.50aA	0.52aA	0.53aA	0.30aA	0.32aA	10.1aA	13.1aA	14.6aA	18.0aA	20.5aA
Medium resistant	0.46bB	0.52aA	0.43bB	0.24bB	0.32aA	8.5bB	9.7bB	11.3bB	14.0bB	14.4bB
Highly susceptible	0.48bB	0.63bB	0.50cC	0.40cC	0.30bB	6.8cC	7.3cC	8.6 cC	9.7cC	10.3cC

[a]Values are means (n = 3). Means followed by the same capital letter in the columns and by the same small letter in the rows did not share significant difference at 5% probability by Duncan's test

Variations of pH and buffer capacity in different cultivars of *C. oleifera* at developmental stage: There was no significant difference in pH levels between the disease-resistant varieties and the susceptible ones at the developmental stage ($p<0.05$), as shown in Table 3. In July, fruit coats in susceptible varieties reached a pH value of 5, while resistant varieties had a pH of 4-5.

The buffer capacity of different cultivars generally rose with increasing maturation (highly resistant 6.2-7.43, medium resistant 5.33-7.46, highly susceptible 5.45-7.12). In addition, the buffer capacity of different cultivars increased rapidly from June to August. Buffer capacity did not differ significantly between different cultivars ($p>0.05$).

Enzyme activity in different cultivars of *C. oleifera* at developmental stage: PPO, POD and PAL activity in the fruit coats of highly resistant, medium resistant and susceptible strains were determined. The results shown in Fig. 1 demonstrate that activity of the three enzymes generally rose with increasing maturity.

PPO activity in highly resistant plants increased more rapidly during May-August and increased slowly after September, especially in May. PPO activity was 18 $U \cdot g^{-1}min^{-1}$ higher than in highly susceptible plants. PPO activity of medium resistant and highly susceptible cultivars rose slowly in the first three months, and then declined in August and September. PPO activity of highly susceptible varieties decreased by 14 $U \cdot g^{-1}min^{-1}$ from July to September. PPO activity differed significantly between disease-resistant and susceptible varieties ($p<0.01$).

POD activity of disease-resistant varieties was higher than of susceptible ones, and the increase rate of highly resistant varieties was 6.2% higher than in highly susceptible varieties in May. POD activities of medium resistant and highly susceptible varieties declined after August by $10U \cdot g^{-1}min^{-1}$ and $17U \cdot g^{-1}min^{-1}$, respectively. There was significant difference in PPO activity between disease-resistant and susceptible varieties ($p<0.01$).

In May, PAL activity of high resistant cultivars was higher than that of medium resistant and high susceptible by 17% and 30%, respectively. PAL activity was significantly different between disease-resistant and susceptible cultivars ($p<0.01$).

Soluble sugars in different cultivars of *C. oleifera* at development stages: Soluble sugar contents of fruit coats gradually increased from the fruitlet period to maturity period, and the results are presented in Fig. 2. From May to September, the soluble sugar contents in highly resistant, medium resistant and highly susceptible varieties rose by 60.9, 58.6 and 40%, respectively. Soluble sugar contents of highly resistant varieties were 45% higher than of highly susceptible ones. Soluble sugar contents differed significantly between disease-resistant and susceptible varieties ($p<0.05$).

DPPH free radical scavenging activity in different cultivars: The free radical scavenging capacities of fruit coat extracts from highly resistant, medium resistant and highly susceptible varieties of *C. oleifera* were determined. The results shown in Table 4 demonstrate that fruit coat extracts with a concentration of 2.0 mg/mL had strong free radical-scavenging capacity in different incidence cultivars, and that fruit coat extracts from highly resistant varieties had the highest scavenging capacity with a value of 91.46% ± 0.32%. The free radical scavenging capacity decreased with declining extract concentration. The free radical scavenging capacity of fruit coat extracts differed significantly between different cultivars ($p<0.05$).

Discussion

C. oleifera forests in Shu-cheng County, China, were investigated during 2008-2010. It was found that the disease incidence of highly resistant varieties was 2.8%, while that of highly susceptible varieties was 100%. Therefore, in the production and management of *C. oleifera*, the first and most important measure should involve selecting disease-resistant and elite varieties for plantations to achieve high quality and a high and stable yield.

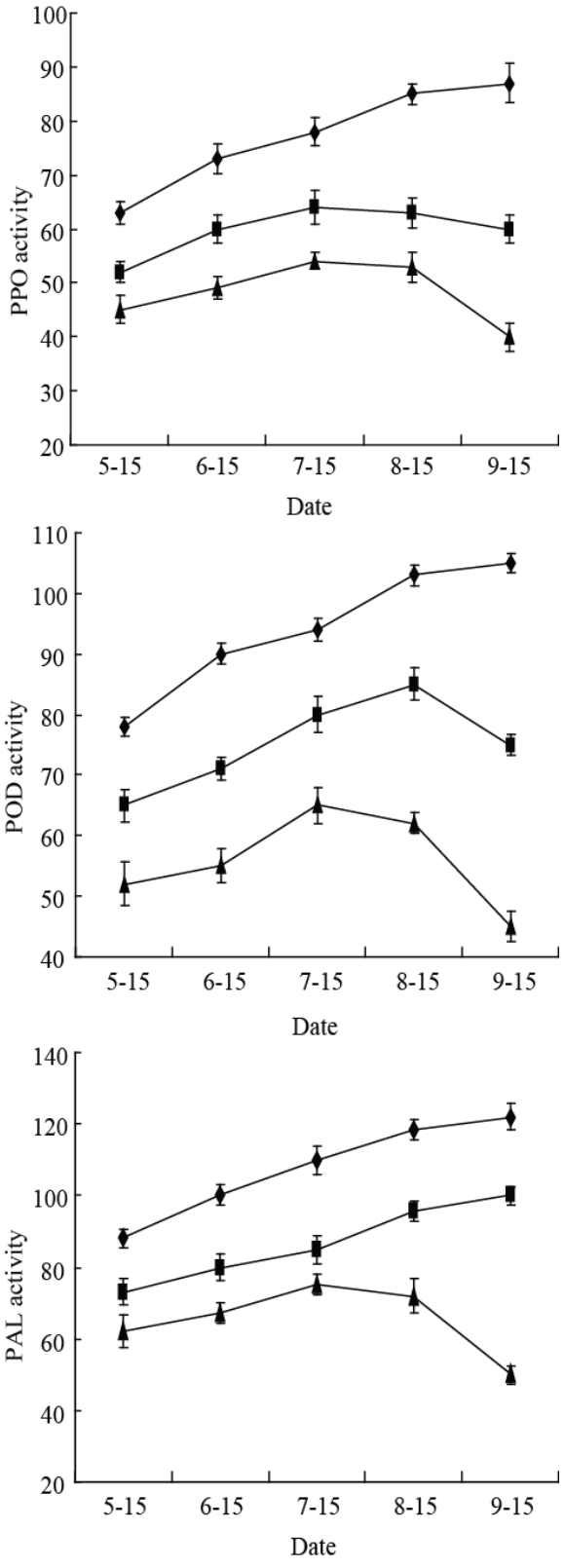

Fig. 1. Average activities of POD, PPO and PAL (U/g·min) of fruit coats from different varieties during development.

Tannin contents of fruit coats in *C. oleifera* generally declined during the developmental stage. The same tendency has been observed in grapes (Kennedy *et al*., 2000). Tannin contents did not significantly differ between cultivars, while a continuous reduction and significant difference in tannin contents is common for other fruits, such as myrtle and persimmon (Del Bubba *et al*., 2009; Fadda & Mulas, 2010). Anthocyanins are phenolic compounds responsible for fruit coloration (Abyari *et al*., 2006), and accumulate in *C. oleifera* fruit coats (highly resistant 20.5%, highly susceptible 10.3%). The twofold rise in the fruit coats (highly resistant 10.1-20.5, highly susceptible 6.8-10.3) is due to the production of cyanidin-3-glucose (Reynertson *et al*., 2008) and petunidin-3-glucose (Montes *et al*., 2005). Other fruits, such as myrtle, American cranberry and pomegranate arils, also exhibit a similarly drastic increase in pigment levels during development (Vvedenskaya & Vorssa, 2004; Kulkarni & Aradhya, 2005; Fadda & Mulas, 2010). Anthocyanin contents seemed to be the most effective parameter to reveal resistance mechanisms of different cultivars. In addition, tannin and anthocyanin contents of resistant varieties were higher than of susceptible ones. Plants accumulated a large number of phenolic compounds that are synthesized by acetic acid or shikimic acid (Balasundram *et al*., 2003; Wong *et al*., 2008). Many of these compounds have antimicrobial properties and can trigger a defense response by the plant. Phenylalanine ammonia lyase is a key enzyme of the shikimate pathway, and its activity and production of phenolic compounds are closely related to PAL and PPO activity, with the amount of polyphenol content increasing synchronously (Perveen *et al*., 2011; Dai *et al*., 2012). Therefore, the activity of these enzymes or phenolic compound reflected in turn the level of plant disease resistance at an early stage (Wei *et al*., 2004; Shang & Zhang, 2008). In this study, the activity of three enzymes were significantly higher in resistant varieties than in susceptible ones, and the increase and synthesis rates in resistant cultivars were faster than that in susceptible ones, indicating that the resistant varieties had a rapid defense response when attacked by the pathogen.

The soluble sugar content of fruit coats in disease-resistant varieties was much higher than in susceptible ones, which continuously increased at the developmental stage. The opposite tendency has been observed in poplar, Jinhua pear (Tang *et al*., 2000; Li *et al*., 2003), while a similarly tendency has been observed in other plants (Li *et al*., 2006; Balal *et al*., 2011). Soluble sugars are respiratory substrates of metabolism, with raised contents indicating better oxidation and accumulation of phenolic compounds. Therefore, soluble sugar can be used as an indicator of disease resistance in *C. oleifera* breeding. The more antifungal substances (such as phenolic compounds) disease-resistant varieties possess, the stronger their DPPH free radical scavenging abilities.

Fruit coat pH and buffering capacity of *C. oleifera* did not significantly differ between different cultivars. In September, highly susceptible varieties had a pH above 5, which is beneficial for reproduction and growth of fungi (McClendon, 1960). The role of skin pH and buffering capacity in resistance to antifungal requires further study.

Table 3. Average values[a] of pH and buffer capacity of fruit coat in different varieties during development.

Varieties	pH					buffer capacity				
	5-15	6-15	7-15	8-15	9-15	5-15	6-15	7-15	8-15	9-15
Highly resistant	4.77 aA	4.69aAB	3.95 aA	4.08 aA	4.26 aA	6.20 aA	5.89 aA	5.85 aA	7.48 aA	7.43 aA
Medium resistant	4.48 aA	4.59aA	4.04 aA	4.04 aA	4.40 aA	5.33 bB	5.33 bB	7.58 bB	7.66 aA	7.46 aA
Highly susceptible	4.74 aA	4.84bB	4.47 bB	5.04 bB	5.07 bB	5.45 bB	5.25 bB	6.92cC	6.59 bB	7.12 bB

[a]Values are means (n = 3). Means followed by the same capital letter in the columns and by the same small letter in the rows did not share significant difference at 5% probability by Duncan's test

Table 4. Average values[a] of scavenging activity of fruit coat in different varieties during development.

Varieties	Scavenging activity (%)			
	2.0 mg/mL	1.0 mg/mL	0.5 mg/mL	0.25 mg/ml
Highly resistant	91.46 ± 0.32 aA	88.80 ± 0.29 aA	81.48 ± 0.28 aA	62.92 ± 0.53 aA
Medium resistant	90.49 ± 0.08abA	84.73 ± 0.15 bA	61.44 ± 0.14 bB	53.21 ± 0.88 bB
Highly susceptible	89.43 ± 0.34 bA	77.40 ± 0.28 cB	59.39 ± 0.14 bB	51.50 ± 0.45 cC

[a]Values are means ± SE (n = 3). Means followed by the same capital letter in the columns and by the same small letter in the rows did not share significant difference at 5% probability by Duncan's Test

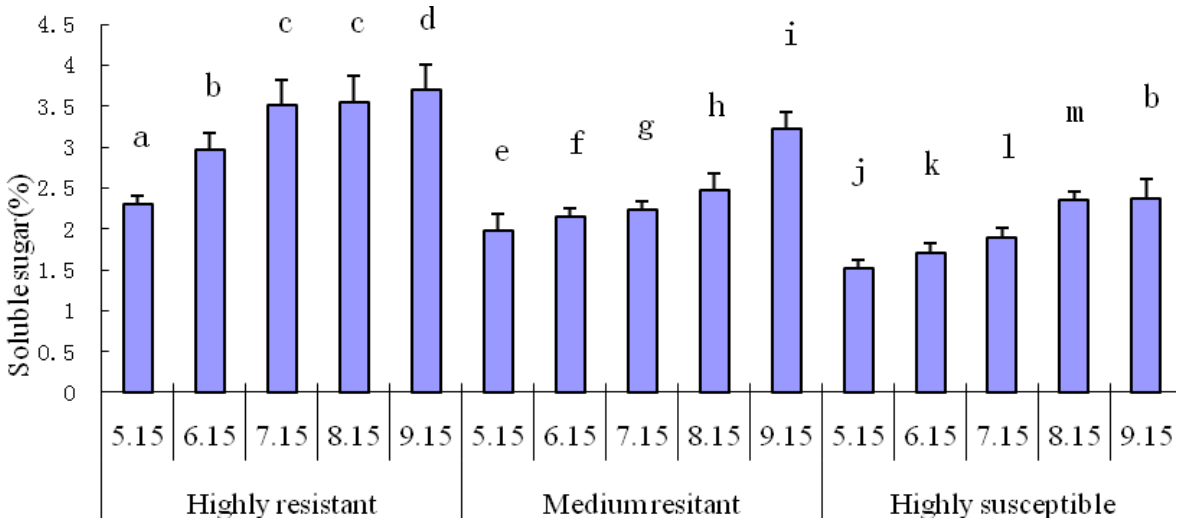

Fig. 2. Average contents of soluble sugars (%) of fruit coats from different varieties during development.

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References

Abyari, M.R., R. Heidari and R. Jamei. 2006. The effects of heating, UV irradiation and pH on stability of siahe sardsaht grape anthocyanin-copigment complex. *J. Biol. Sci.*, 6: 638-645.

Ahmad, P., M.A. Salem and S. Sharma. 2010. Roles of enzymatic and nonenzymatic antioxidants in plants during abiotic stress. *Crit. Rev. Biotechnol.*, 30(3): 161-175.

Aist, J.R. and W.R. Brushnell. 1991. Invasion of plants by powdery mildew fungi, and cellular mechanisms of resistance. In: *The fungal spore and disease interaction in plants and animals*. (Eds.): G.T. Cole and H.C. Hoch. Plenum Press, New York, pp. 321-345.

Azevedo-Neto, A.D., J.T. Prisco, J. Enéas-Filho, C.E. Braga-de-Abreu and E. Gomes-Filho. 2006. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt tolerant and salt sensitive maize genotypes. *Environ. Exp. Bot.*, 56: 87-94.

Balal, R.M., M.Y. Ashraf, M.M. Khan, M.J. Jaskani and M. Ashfaq. 2011. Influence of salt stress on growth and biochemical parameters of citrus rootstocks. *Pak. J. Bot.*, 43(4): 2135-2141.

Balasundram, N., W. Bubb, K. Sundram and S. Samman. 2003. Antioxidants from palm (Elaeis guineensis) fruit extracts. *Asia Pac. J. Clin. Nutr.*, 12 (Suppl): S37.

Bolwell, P.P., A. Page, M. Pislewska and P. Wojtaszek. 2001. Pathogenic infection and the oxidative defenses in plants apoplast. *Protoplasma*, 217: 20-32.

Cao, Z.H., Q.L. Shu and X. Zhang. 2011. Occurrence and identification of *Camellia oleifera* diseases in Anhui province. *Anhui For. Sci. Technol.* 37: 55-58.

Dai, H.P., C.J. Shan, C. Lu, G.L. Jia, A.Z. Wei, W.Q. Sa and T.X. Yang. 2012. Response of antioxidant enzymes in populus × canescens under cadmium stress. *Pak. J. Bot.*, 44(6): 1943-1949.

Del Bubba, M., E. Giordani, L. Pippucci, A. Cincinelli, L. Checchini and P. Galvan. 2009. Changes in tannins ascorbic acid and sugar content in astringent persimmons during on-tree growth and ripening and in response to different postharvest treatments. *J. Food Compos. Anal.*, 22: 668-677.

Duan, L., G.D. Yang, Q.L. Shu and H.B. Zheng. 2005. Relationship of peel color with resistance to anthracnose in oil tea Camellia. *Nonwood Forest Res.*, 23(2): 9-12.

Fadda, A. and M. Mulas. 2010. Chemical changes during myrtle (*Myrtus communis* L.) fruit development and ripening. *Sci. Hort.*, 125: 477-485.

Gross, G.G. 1999. Biosynthesis, biodegradation, and cellular localization of hydrolyzable tannins: Recent advances in phytochemistry. In: *Phytochemicals in Human Health Protection, Nutrition and Plant Defenses*. (Eds.): N.G. Lewis, J.T. Romeo and G.H.N. Towers. Plenum Press, New York, pp. 185-213.

Harinasut, P., D. Poonsopa, K. Roengmongkoi and R. Charoensataporn. 2003. Salt effects on antioxidant enzymes in mulberry cultivar. *ScienceAsia*, 29: 109-113.

Kanchana-udomkan, C., P.W.J. Taylor and O. Mongkolporn. 2004. Development of a bioassay to study anthracnose infection of chili fruit caused by *Colletotrichum capsici*. *Thai J. Agric. Sci.*, 37: 293-297.

Kennedy, J.A., M.A. Matthews and J.A. Waterhouse. 2000. Changes in grape seed polyphenols during fruit ripening. *Phytochemistry*, 55: 77-85.

Kim, D., K.W. Lee and H.J. Lee. 2002. Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. *J. Agr. Food Chem.*, 50: 3113-3717.

Kulkarni, A.P. and S.M. Aradhya. 2005. Chemical changes and antioxidant activity in pomegranate arils during fruit development. *Food Chem.*, 93: 319-324.

Leister, D., 2004. Tandem and segmental gene duplication and recombination in the evolution of plant disease resistance gene. *Trends Genet.*, 20: 116-122.

Li, C.Y., M.G. Liao, Y.P. Liu and G.E. Xiong. 2003. Study on resistance to disease and frigid of Jinhua pear varied strains. *Agr. Res. Arid Area.*, 21(3): 137-140.

Li, H.Y., T.R. Liu and Y. Zhen. 2006. Study on the resistance to phytophthora blight of pepper and the effect of pro, MAD and dissolubility sugar. *Chinese Agr. Sci*., 22 (11): 315-317.

Liu, X., M. Zhao, J. Wang and B. Yang. 2008. Antioxidant activity of methanolic extract emblica fruit (*Phyllanthus emblica* L.) from six regions in China. *J. Food Compos. Anal.*, 21: 219-228.

Mazau, D. and M.T. Esquerré-Tugayé. 1986. Hydroxyproline rich glycoprotein accumulation in the cell walls of plants infected by various pathogens. *Physiol. Mol. Plant P.*, 29: 147-157.

Mbouobda, H.D., P.F. Fotso-Djocgoue, N.D. Omokolo, I. EI-Hadrami and T. Boudjeko. 2010. Benzo-(1,2,3,)-thiadiazole-7-carbothioic S-methyl ester (BTH) stimulates defense reactions in *Xanthosoma sagittifolium*. *Phytoparasitica*, 38: 71-79.

McClendon, J.H. 1960. The occurrence of variety enzymes hydrolyzing cell wall polysaccarides in apples rotted by *Btryosphaeria ribis*. *Phytopathology*, 50: 258-261.

Mittler, R., S. Vanderauwera, M. Gollery and F. Van Breusegem. 2004. Reactive oxygen gene network of plants. *Trends Plant Sci*., 9: 490-498.

Montes, C., I.M. Vicario, M. Raymundo, R. Fett and F.J. Heredia. 2005. Application of tristimulus colorimetry to optimize the extraction of anthoycanins from Jaboticaba (*Myricia Jaboticaba* Berg.). *Food Res. Int.*, 38: 983-988.

Montri, P., P.W.J. Taylor and O. Mongkolporn. 2009. Pathotypes of *Colletotrichum capsici* the causal agent of chili anthracnose in Thailand. *Plant Dis.*, 93: 17-20.

Palmer, H.J. and K.E. Paulson. 1997. Reactive oxygen species and antioxidants in signal transduction and gene expression. *Nutr. Rev.*, 55: 353-361.

Perveen, S., M. Shahbaz and M. Ashraf. 2011. Modulation in activities of antioxidant enzymes in salt stressed and not-stressed wheat (*Triticum aestiwum* L.) plants raised from seed trearted with triacontanol. *Pak. J. Bot.*, 43(5): 2463-2468.

Plummer, D.T. 1987. *Practical Biochemistry*. McGraw-Hill, New York.

Reynertson, K.A., A.M. Wallace, S. Adachi, R.R. Gil and H. Yang. 2006. Bioactive depsides and anthocyanins from jaboticaba (*Myrciaria cauliflora*). *J. Nat. Prod.*, 69: 1228-1230.

Sekmen, A.H., I. Türkan and S. Takio. 2007. Differential responses of antioxidative enzymes and lipid peroxidation to salt stress in salt-tolerant *Plantago maritima* and salt-sensitive *Plantago media*. *Physiol. Plantarum*, 131: 399-411.

Shang, Q.M. and Z.G. Zhang. 2008. Roles of spermidine in induced resistance of cucumber seedlings to *Botrytis cinerea* Pers. *Chinese J. Appl. Ecol.*, 19: 825-830.

Shu, Q.L. and L.F. Zhang. 2009. Chinese *Camellia oleifera*. China Forestry Press, Beijing.

Sudhakar, C., A. Lakshmi and S. Giridarakumar. 2001. Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. *Plant Sci.*, 16: 613-619.

Takakura, Y., F.S. Che, Y. Ishida, F. Tsutsumi, K. Kurotani, S. Usami, A. Isogai and H. Imaseki. 2008. Expression of a bacterial flagellin gene triggers plant immune responses and confers disease resistance in transgenic rice plants. *Mol. Plant Pathol.*, 9: 525-529.

Tang, M., H. Chen and H. Shang. 2000. Mechanism of vesicular-arbuscular mycorrhizal fungi enhanced resistance of poplar to canker. *Sci. Silvae Sinicae*, 36(2): 87-92.

Vvedenskaya, I.O. and N. Vorssa. 2004. Flavonoid composition over fruit development and maturation in American cranberry, *Vaccinium macrocarpon* Ait. *Plant Sci.*, 167: 1043-1054.

Wei, G., Z. Zhe, J. Li and Q. Yao. 2004. Effects of silicon supply and *Sphaerotheca fuliginea* inoculation on resistance of cucumber seedlings against powdery mildew. *Chinese J. Appl. Ecol.*, 15: 2147-2151.

Wong, S.Y., I.R. Grant, M. Friedman, C.T. Elliott and C. Situ. 2008. Antibacterial activities of naturally occurring compounds against *Mycobacterium avium* subsp. paratuberculosis. *Appl. Environ. Microb.*, 74: 5986-5990.

Yang, G.D., Q.L. Shu, L. Duan, C.Y. Chen and H.B. Zheng. 2004. Resistance of main cultivars of oil tea to *Colletotrichum gloeosporioides*. *J. Anhui Agr. Univ.*, 31(4): 480-483.

Yang, Y., C. Han, Q. Liu, B. Lin and J. Wang. 2008. Effect of drought and low light on growth and enzymatic antioxidant system of *Picea asperata* seedlings. *Acta Physiol. Plant.*, 30: 433-440.

Yuan, X.H. and Z.H. Yang. 1983. *Plant physiology and biochemistry assay*. Academic Press, Beijing.

Zhang, X., G.D. Yang, J. Yang and Q.L. Shu. 2012. Physiological mechanism of resistance to anthracnose of different *Camellia* varieties. *Afr. J. Biotechnol.*, 11(8): 2026-2031.

Zhuang, R.L., A.Z. Huang, R.X. Dong, D.B. Wang, Y.Z. Chen, X.Q. Cai, M.M. Su, X.A. Deng, Y.Y. Kuang, Q.N. Zeng and G.R. Jia. 1992. Studies on the breeding of 19 new tea oil varieties with high yield. *Forest Res.*, 5(6): 619-627.

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