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Changes in Cell Wall Composition in Andesu Netted Melon (*Cucumis melo* L.) Fruit as Influenced by the Development of Water-core

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Abstract: Andesu netted melon plants (*Cucumis melo* L.) were grown under shading conditions during the fruit developing stages. Shading treatment resulted in the accelerated fruit softening and the development of water-core during the ripe to over-ripe stages. Fruits that were grown under shading conditions were also characterized by lowered alcohol insoluble solid content and mol% of galactose among the cell wall neutral sugars, suggesting that the changes in cell wall polymers; i.e., in the disappearance of crude cell wall contents and depolymerization of galactose-rich-side-chains might be result in the development of water-core at the ripe to over-ripe stages.

Key words: *Cucumis melo* L., cell wall, pectin, water-core, water-soaked symptom

INTRODUCTION

A popular netted melon cultivar Andesu (*Cucumis melo* L.) is known to develop a physiological disorder called water-core or water-soaked symptom at the ripe stage (Nakanishi *et al*., 1992; Nishizawa *et al*., 1998, 2000). This physiological disorder is characterized by glassiness of mesocarp tissue, low sugar accumulation, high ethylene evolution and alcoholic fermentation (Nishizawa *et al*., 1998, 2000). Water-cored fruit results in the loss of the commercial value, but it is difficult to diagnose from the appearance.

Although the mechanism of water-core in melon fruit has not been clarified yet, the disorder is often induced by short-term-shading of the plant (Nakanishi *et al*., 1992; Nishizawa *et al*., 1998, 2000). Moreover, the development of water-core in Andesu netted melon fruit is often associated with fruit softening (Nishizawa *et al*., 2000). These results suggest that the formation of water-core might be due to the disintegration of cell wall polymers. However, changes in cell wall contents and compositions of Andesu netted melon fruit as influenced by the development of water-core are yet unclear.

In this study, therefore, we induced the development of water-core in Andesu melon fruit by short-term-shading and then compared the changes in cell wall integration with those in unshaded fruit.

MATERIALS AND METHODS

Plant materials: On 7 April 1997, seeds of Andesu netted melon (*Cucumis melo* L.) plants were sown in plastic cell trays at a greenhouse of the Yamagata Sand Dune Experimental Station (Sakata, Japan). On 6 May, 36 seedlings with two expanded leaves were transplanted in the field of the experimental station at a spacing of 2.7×0.8 m. Female flowers were pollinated by bees and allowed to bear 4 fruits per plant as described previously (Nakanishi *et al*., 1992). For 14 days, from 15 to 28 July (35-48 days after pollination: DAP), 18 plants were shaded by using meshed screens which can shade approximately 80% of daylight (shaded plants), while another 18 plants were remained as unshaded control plants.

Fruit sampling and measurement of flesh firmness: On 37, 43 (developing stage), 49 (ripe stage) and 55 DAP (over-ripe stage), each of six fruits was harvested from shaded and unshaded plants. Two centimeter thick slices of the flesh were taken along the longitudinal axis from the central portion of the fruit and the flesh firmness of a slice was measured on the two opposite sides of the central mesocarp with a rheo-meter (model CR-1.10K, Sun Scientific, Tokyo) equipped with a 5 mm diameter columnar plunger. Another slice was used for the measurement of the water-cored area by the binary-image method (Harker *et al*., 1999) and for the subsequent analysis of cell walls.

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Preparation of alcohol-insoluble solids: Inner-mesocarp tissues (approximately 100 g) were taken from the central mesocarp, frozen at -20°C, lyophilized at -50°C and then powdered in a mortar. The rind, placenta and seeds were eliminated from the experiment. A 10 g powdered sample was put into a polyethylene nonwoven fabric bag and then extracted 80°C for 2 h in 500 mL of 80% EtOH. This extraction was repeated 5 times in total. EtOH insoluble solids were extracted at room temperature for 15 h in 100 mL of acetone and then dried at 40°C to obtain Alcohol Insoluble Solids (AIS).

Determination of cell wall polysaccharides in each cell wall fraction: Cell wall polymers were fractionated and determined as described by Nishizawa *et al.* (2002a). A 100-mg AIS sample was extracted in 8 mL of distilled water at 20°C for 6 h with continuous stirring. The homogenate was centrifuged at 3,000 g for 15 min and the supernatant was recovered as the water-soluble fraction. The water-insoluble residue was suspended in 8 mL of 50 mM CDTA (pH 4.5) containing 50 mM sodium acetate and extracted twice at 20°C for 6 h with continuous stirring. The homogenates were then centrifuged at 3,000 g for 15 min and the supernatant was combined as the CDTA-soluble fraction. The CDTA-insoluble residue was extracted in 8 mL of 50 mM Na_2CO_3 containing 26 mM $NaBH_4$ at 20°C for 6 h with continuous stirring. The homogenate was then centrifuged at 3,000 g for 15 min and the supernatant was neutralized with acetic acid as the Na_2CO_3-soluble fraction. The Na_2CO_3-insoluble residue was extracted in 8 mL of 4 M KOH containing 100 mM $NaBH_4$ at 20°C for 8 h with continuous stirring. The homogenates were then centrifuged at 3,000 g for 15 min and the supernatant was neutralized with glacial acetic acid as the KOH-soluble fraction. The KOH-insoluble residue was washed with 8 mL of 80% EtOH and then with 8 mL of pure acetone and then dried at 40°C. The residue was regarded as cellulose (Siddiqui *et al.*, 1996).

Samples of each fraction were dialyzed exhaustively using spectra pore membranes (6,000-8,000 MW cut-off, Spectram Medical, CL, USA) against distilled water at 5°C for 48 h. Uronic acid and neutral sugar concentration in each fraction was determined by the *m*-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen, 1973) and phenol-H_2SO_4 method (Dubois *et al.*, 1956), respectively, as described previously (Yakushiji *et al.*, 2001).

Analysis of neutral sugar compositions: The dialyzed fractions (8 mL) were dried at 50°C *in vacuo*. The pellets were hydrolyzed with 2 M TFA for 1 h at 121°C and converted into alditol acetates for gas chromatographic analysis (Albersheim *et al.*, 1967; Blakeney *et al.*, 1983). A 1 μL sample of converted sugar was injected into a gas chromatograph (GC-18A, Shimadu, Kyoto) equipped with an SP-2330 fused silica capillary column (30×0.32 mm, Supelco, PA, USA) and FID detector. Each injection was repeated 2 times. Sugars were identified by comparison with authentic standards and quantified using *myo*-inositol as an internal standard (Nishizawa *et al.*, 2002b).

RESULTS AND DISCUSSION

Effect of short-term-shading on fruit firmness and development of water-core: Flesh firmness of the central mesocarp decreased rapidly from 43 to 55 DAP, decreasing to below one-third of the initial value at the over-ripe stage (55 DAP), irrespective of shading treatment (Fig. 1). A significant difference of the flesh firmness between the shaded and unshaded plants was observed after 49 DAP and the value of the former was 34-47% lower than the latter (0.29-0.51 and 0.10-0.24 kg for the unshaded and shaded plants, respectively).

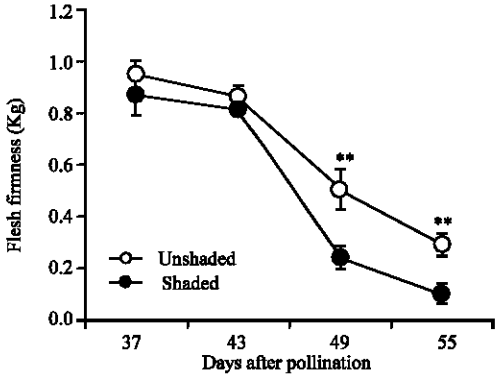

Fig. 1: Changes in flesh firmness of Andesu netted melon fruit as affected by short-term-shading. ** Significant at $p<0.01$, using students t-test

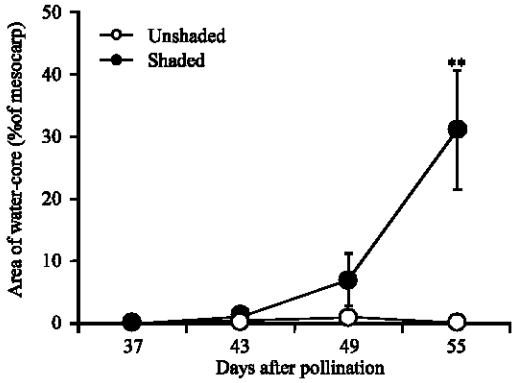

Fig. 2: Effect of short-term-shading on the development of water-core in Andesu netted melon fruit. ** Significant at $p<0.01$, using students t-test

Water-core in the shaded plants was first observed at 43 DAP (Fig. 2). The symptom increased thereafter, attaining a value of 31% at 55 DAP, while no visible symptom was observed in the unshaded plants throughout the growing period. Thus, the formation of water-core in Andesu netted melon fruit at the ripe to over-ripe stages (49-55 DAP) was induced by short-term-shading during the developmental stage (35-48 DAP) and was correlated with rapid flesh softening (Fig. 1), as Nishizawa *et al.* (2000) found previously.

Effect of short-term-shading on cell wall polymers: AIS contents are regarded as crude cell walls (Rose *et al.*, 1998). In present study, the change in AIS content during the fruit development was less than 10%, irrespective of shading treatment (Fig. 3) as some other researchers reported previously (Rose *et al.*, 1998; Simandjuntak *et al.*, 1996). The AIS content of the shaded plants, however, was significantly lower than that of the unshaded plants, especially during 43-49 DAP, suggesting that lower AIS content during the rapid flesh softening stages might result in the development of water-core. Yamaki *et al.* (1976) also reported that AIS content in Japanese pears with water-core was lower than that without the disorder.

Fruit softening is often associated with the changes in the cell wall polymers, particularly through the depolymerization of pectic polysaccharides (Ridgwell *et al.*, 1997). Among cell wall polymers, polymers in the water-soluble fraction contribute little to the firmness because they consist of polymeric materials that have been solubilized from the cell wall (Rose *et al.*, 1998). In ripening melon fruit, polymers in the water-soluble fraction increase as the flesh softens (Rose *et al.*, 1998). In our study, however, uronic acid in the water-soluble fraction decreased after 43 DAP, irrespective of shading treatment and no significant difference between the treatments was observed throughout the growing periods (Table 1). Although neutral sugars in the water-soluble fraction increased slightly, no significant difference was found between the treatments throughout the growing period. These results suggest that acceleration of flesh softening by shading treatment is not due to increased uronic acid and/or neutral sugar contents in this fraction. The neutral sugar to uronic acid (NS/UA) ratio increased during 43-55 DAP (Table 1), suggesting that the side-chain-rich polymers in the cell wall might be partially depolymerized and then transferred into the water-soluble fraction. However, this result also does not explain the lower flesh firmness of shaded plants, because there was no significant difference between the treatments.

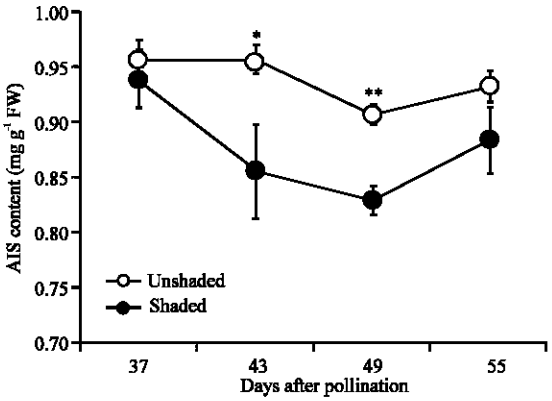

Fig. 3: Changes in Alcohol Insoluble Solid (AIS) content of Andesu netted melon fruit as affected by short-term-shading. *,** Significant at $p<0.05$ or 0.01 respectively, using students t-test

Polymers in the CDTA-soluble fraction are considered to be ionically bound cell walls with adjacent polymers cross-linked by Ca ions (Fry, 1986). Water-core in melon fruit also develops under low Ca conditions (Bernadac *et al.*, 1996; Du Chatenet *et al.*, 2000; Jean-Baptiste *et al.*, 1999). Du Chatenet *et al.* (2000) hypothesized that the formation of water-core in melon fruit might be due to the enhanced loss of inter-cellular spaces due to the lack of Ca ions. In this study, however, both the uronic acid and neutral sugar contents in the CDTA-soluble fraction were relatively lower than those in other fractions and no significant difference was observed between shaded and unshaded plants except for a significant increase of uronic acid in the shaded plants at 49 DAP. Therefore, water-core which is induced by shading treatment may not be due to the result of the lack of ionically cross-linking Ca ions.

In contrast to the findings for the CDTA-soluble fraction, the uronic acid and neutral sugar contents in the Na_2CO_3-soluble fraction were influenced by the ripening stage and shading treatment. In the unshaded plants, uronic acid content in the Na_2CO_3-soluble fraction decreased from 48.0 to 23.7 mg g^{-1} AIS during 37-49 DAP (Table 1). In the shaded plants, however, the uronic acid content increased after 43 DAP, reaching approximately two-fold that in the unshaded plants during 49-55 DAP (23.7-30.1 vs. 42.1-64.6 mg g^{-1} AIS for the unshaded and shaded plants, respectively), when a remarkable water-core was observed (Fig. 2). Significantly higher neutral sugar contents in the shaded than in the unshaded plants were also found at 55 DAP. Pectin polymers in the Na_2CO_3-soluble fraction often act as a key factor for regulating cell wall integrity because they covalently bind to adjacent cell wall polymers and the softening of some

Table 1: Changes in sugar contents and composition of cell wall polymers in Andesu netted melon fruit as affected by short-term-shading

Treatments	Uronic acid (mg g^{-1} AIS)	Neutral sugar (mg g^{-1} AIS)	NS/UA [a]	Non-cellulosic neutral sugar (mol%)						
				Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose
Water-soluble fraction										
37 DAP										
Unshaded	70.1[b]	42.1	0.61	7.95	1.15	21.68	17.02	4.57	31.31	16.32
Shaded	75.3	46.4	0.63	7.23	0.97	19.93	16.46	4.23	31.09	20.10
Significance[c]	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
43 DAP										
Unshaded	75.3	52.5	0.71	7.21	1.16	21.46	15.31	3.35	25.36	26.14
Shaded	73.0	55.6	0.77	8.21	1.30	24.71	18.13	3.72	28.61	15.32
Significance	ns	ns	ns	*	ns	*	*	ns	*	**
49 DAP										
Unshaded	62.2	66.7	1.09	7.30	1.46	23.24	15.17	2.46	26.41	23.96
Shaded	52.2	56.6	1.09	9.11	1.32	31.50	15.93	3.10	26.38	12.65
Significance	ns	ns	ns	**	ns	**	ns	ns	ns	**
55 DAP										
Unshaded	53.2	60.9	1.17	7.54	1.60	27.40	16.51	2.10	27.57	17.29
Shaded	44.6	53.2	1.24	9.16	1.39	29.54	17.10	1.94	28.02	12.86
Significance	ns	ns	ns	*	ns	ns	ns	ns	ns	*
CDTA-soluble fraction										
37 DAP										
Unshaded	18.9	11.7	0.60	9.60	0.29	25.69	10.09	2.61	33.73	18.00
Shaded	19.2	12.0	0.62	9.56	0.28	28.26	8.08	1.12	35.60	17.10
Significance	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
43 DAP										
Unshaded	18.5	13.3	0.76	9.03	0.42	26.14	8.53	1.35	29.32	25.21
Shaded	17.3	12.3	0.71	10.88	0.80	31.01	10.58	1.10	32.29	13.34
Significance	ns	ns	ns	*	ns	*	ns	ns	ns	**
49 DAP										
Unshaded	16.6	13.4	0.81	9.25	0.89	27.29	10.95	0.69	29.79	21.14
Shaded	25.8	15.5	0.60	13.20	0.98	37.66	9.77	0.82	24.83	12.74
Significance	**	ns	ns	**	ns	**	ns	ns	**	**
55 DAP										
Unshaded	21.1	14.8	0.70	9.70	1.30	28.88	12.05	1.06	28.32	18.70
Shaded	18.5	12.5	0.70	11.67	0.87	36.63	12.46	0.45	25.99	11.94
Significance	ns	ns	ns	*	ns	**	ns	ns	ns	*
Na_2CO_3-soluble fraction										
37 DAP										
Unshaded	48.0	34.9	0.73	11.10	0.22	36.46	2.42	0.00	47.43	2.37
Shaded	40.2	33.6	0.84	11.10	0.57	34.30	2.30	0.00	48.86	2.87
Significance	ns	ns	**	ns	ns	ns	ns	ns	ns	ns
43 DAP										
Unshaded	27.2	24.0	0.91	11.47	0.17	38.49	3.74	0.00	39.99	6.14
Shaded	32.0	24.8	0.78	12.35	0.16	41.23	5.44	0.14	38.19	2.48
Significance	ns	ns	ns	ns	ns	ns	ns	ns	ns	*
49 DAP										
Unshaded	23.7	17.8	0.77	12.06	0.36	37.97	7.02	0.00	34.45	8.14
Shaded	42.1	18.7	0.45	15.59	0.31	47.06	6.12	0.00	26.20	4.72
Significance	**	ns	**	*	ns	**	ns	ns	**	ns
55 DAP										
Unshaded	30.1	16.6	0.65	13.27	0.52	44.83	5.84	0.00	32.24	3.30
Shaded	64.6	25.0	0.41	18.51	0.51	49.84	7.30	0.00	20.99	2.85
Significance	*	*	*	**	ns	ns	ns	ns	**	ns
KOH-soluble fraction										
37 DAP										
Unshaded	21.6	75.8	3.51	1.58	2.75	6.61	32.45	7.17	17.09	32.34
Shaded	23.0	76.9	3.36	1.39	2.67	6.52	31.38	7.05	18.22	32.77
Significance	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
43 DAP										
Unshaded	22.0	82.6	3.77	1.06	2.33	5.44	26.97	6.07	13.83	44.28
Shaded	20.4	77.3	3.79	1.13	2.89	5.92	32.47	7.61	14.90	35.09
Significance	ns	ns	ns	ns	*	ns	**	*	ns	**
49 DAP										
Unshaded	19.7	69.6	3.53	1.08	2.60	5.64	26.98	5.13	13.77	44.80
Shaded	20.1	74.9	3.73	0.78	2.73	4.87	33.83	5.98	11.94	39.87
Significance	ns	ns	ns	*	ns	ns	**	ns	*	*
55 DAP										
Unshaded	19.7	75.2	3.82	1.07	2.78	6.48	31.09	5.44	13.76	39.37
Shaded	18.5	72.3	3.91	0.92	3.16	5.31	34.97	5.52	11.93	38.19
Significance	ns	ns	ns	ns	*	**	*	ns	*	ns

[a] Ratio of neutral sugar to uronic acid, [b] The average of six replicates, [c] ns, *, ** Nonsignificant or significant at $p<0.05$ or 0.01, respectively, using students t-test

fruits such as tomatoes (Carrington *et al*., 1996), peaches (Hegde and Maness, 1996), persimmons (Cutillas-Iturrald *et al*., 1993) and melons (Rose *et al*., 1998; Simandjuntak *et al*., 1996) correlates with the decrease in this fraction. In present study, the findings for the fruit of unshaded plants were consistent with these results. The uronic acid content in the Na_2CO_3-soluble fraction of the shaded plants, however, was significantly higher than that of the unshaded plants during 49-55 DAP, regardless of their softer flesh firmness (Fig. 1). Although the reason for the higher uronic acid content in the shaded fruit is not clear, this higher content does not contribute to the flesh firmness, as indicated by the softer flesh of the shaded fruit (Fig. 1). This was also true of the neutral sugars: the value in the unshaded plants at 55 DAP was significantly higher than that in the unshaded plants, regardless of the lower flesh firmness of the former (Fig. 1).

The significantly lower NS/UA ratio in the shaded plants than in the unshaded plants after 49 DAP was found only in the Na_2CO_3-soluble fraction, suggesting that the pectic backbone with poor side-chains was increased by the shading treatment. Therefore, the lower flesh firmness in the shaded plants than in the unshaded plants might be due to the balance between main-and side-chains in the covalently bound pectin fraction rather than to the content of the AIS.

Although the neutral sugar contents were highest in the KOH-soluble fraction among the four fractions, the contents did not significantly differ between the treatments throughout the growing periods (Table 1). The cell wall components in the KOH-soluble fraction are mainly thought to be hemicelluloses (Rose *et al*., 1998). Rose *et al*. (1998) showed that hemicellulosic polymers in melon fruit partially depolymerized only during the ripe to over-ripe stages. In our study, however, the neutral sugar contents in the KOH-soluble fraction did not decrease even at the over-ripe stage (55 DAP), irrespective of shading treatment, suggesting that the volume of hemicellulosic polymers affects neither the flesh firmness nor the development of water-core of Andesu netted-melon fruit.

Seven sugars, namely galactose (Gal), arabinose (Ara), glucose (Glc), xylose (Xyl), rhamnose (Rha), mannose (Man) and fucose (Fuc) were detected in the neutral sugar fractions. In both the water-and CDTA-soluble fractions, the mol% of Rha and Ara in the shaded plants was higher than that in the unshaded plants, while the mol% of Glc was lower, especially after 43 DAP. This was also partially true in the Na_2CO_3-soluble fraction; i.e., higher mol% of Rha and Ara in the shaded plants than that in the unshaded plants was found after 49 DAP, while lower mol% of Glc was detected only at 43 DAP. In contrast to Rha and Ara, Gal was significantly higher in the unshaded plants than in the shaded plants, especially during the ripening to over-ripening stages in the CDTA and/or Na_2CO_3-soluble fractions. Rha is located in the main chains of pectin molecules as rhamno-galacturonan and is connected with side chains such as arabinan, galactan or arabinogalactan, which consist of Ara and Gal (McNeil *et al*., 1984). These side chains also play a substantial role in sustaining cell wall integration and flesh firmness by attaching to other polymers (Rose *et al*., 1998). Gross and Sams (1984) found that the predominant neutral sugars in the cell wall of musk-melon fruit were Gal and Ara and the former decreased markedly while the latter decreased slightly during the ripening stages. Therefore, the higher mol% of Ara and Rha but lower mol% of Gal in the shaded plants as compared with the unshaded plants detected in the CDTA-and Na_2CO_3-soluble fractions during 49-55 DAP suggest that the flesh firmness might be sustained mainly by Gal-rather than Ara-rich side chains.

In the KOH-soluble fraction, Xyl, Gal and Glc were the major neutral sugars and the mol% of Xyl was higher in the shaded plants than in the unshaded plants, while the opposite was true for Gal and Glc, mainly during the ripe to over-ripe stages (Table 1). The KOH-soluble fraction is regarded to consist of hemicelluloses (McCollum *et al*., 1989) and Xyl and Glc are the main constituents of xyloglucan (McNeil *et al*., 1984). Therefore, the accelerated disintegration in the hemicellulosic polymers of Andesu netted melon flesh as the result of shading treatment may be characterized by the depletion of neutral sugar components such as Gal and Glc but not the total content of hemicellulose polymers per AIS, since there was no significant decrease of neutral sugar contents in the KOH-soluble fraction, irrespective of shading treatment (Table 1). McCollum *et al*. (1989) also showed that Gal and Glc were mainly depolymerized from highly integrated hemicellulosic polymers of ripening melon fruit.

Yamaki and Kajiura(1983) found that a significant decrease of cellulose occurred in water-cored Japanese pears. In our study, however, no apparent decrease in the cellulose content occurred during the ripe to over-ripe stages (Table 2) and the values did not significantly differ between the treatments. Some other researchers also

Table 2: Changes in cellulose content (g g^{-1}AIS) in Andesu netted melon fruit as affected by short-term-shading

	Days after pollination (DAP)			
Treatment	37	43	49	55
Unshaded	364.5[a]	321.1	291.8	292.9
Shaded	359.0	303.9	321.7	327.9
Significance[b]	ns	ns	ns	ns

[a] The average of six replicates, [b] ns Nonsignificant, using students t-test.

reported that no depolymerization of cellulose was found in melon fruit even at the over-ripe stage (Lester and Dunlap, 1985; Simandjuntak *et al*., 1996). Therefore, changes in cellulose content do not appear to be associated with the development of water-core in Andesu netted melon fruit.

CONCLUSIONS

Shading treatment during the fruit developing stage of Andesu netted melon plants induces cell wall disintegration especially during the ripe to over-ripe stages. Decreases in the AIS content and mol% of Gal-side-chains in the fruit mesocarp as a result of the shading treatment might induce accelerated flesh softening, leading to the development of water-core at the ripe to over-ripe stages.

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