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ORIGINAL ARTICLE

Residual Effect of Light Intensity on Physio-Biochemical Development, Mineral and Genomic Characterization of Date Fruits

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ABSTRACT
The current study was carried out to evaluate the residual effect of light intensity on the dates fruit growth, quality development, biochemical contents, mineral (NO_3, K, Ca, Na) and DNA characterization at two consecutive seasons 2013 and 2014. In all treatments, lower bud initiation, fruit per bunch, fruit length and weight were found higher in the 1st season (2013) than 2nd season (2014. Early maturity was observed in all treated fruits in the 1st season compared to the 2nd season. Besides, glucose, inverted sugar, and fructose content were found higher in full sunlight treated fruit than those treated by light sunlight and shadow in both seasons. However, that mineral content like nitrate was found higher 1st season than the 2nd season. Moreover, potassium, calcium and sodium were higher in full sunlight treated fruit compared to light shadow and deep shadow treated fruits in both seasons. Moreover, DNA quantification was the highest in full sunlight treated fruit and decreasing trend from 1st season to 2nd season. DNA band (segment) was wider in full sunlight treated fruit compared to light shadow and deep shadow treated fruits and it was found to be decreased from 1st season to 2nd season. Therefore, such results conclude that the residual effect of sunlight (high, medium and low light intensity) on fruit was found to be decreased in 2nd season.
Keywords: *date fruit, fructose and glucose, minerals, DNA band*

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INTRODUCTION

Date fruits are very rich in fiber, fat and proteins. They have a form of sugar that bears the body high level of mobility, heat energy and can be easily broken down in the body. Date fruits contain many vitamins (vitamins A, beta-carotene, B1, B2, B3 and B6) and minerals. They also contain sodium, potassium, calcium, magnesium, iron, sulphur, phosphorus and chlorine [1].

Sunlight is considered to have a significant role for fruit quality development. [2] It was stated that significant differences were found between fruit of sunlight area during ripening and harvest stages. They also reported that avocado fruits in expose sunlight were found to have higher dry matter and higher levels of potassium, calcium and magnesium. In addition, they also described fruits in exposed sunlight were found to be ripened earlier than shade fruits and firmer than the unexposed ones. Moreover, the fruits in exposed sunlight were found to have more pigments than the unexposed sunlight and shade ones. Also [3] it was reported that nutrient content was affected by environmental factors like water stress and temperature in Kiwi fruit.

It was studied by [4] that the effect of light levels by the use of shade cloth and aluminum foil. Peach trees having a randomly chosen half of the canopy covered with 73% shade cloth, had fruit with lower levels of red color, soluble solids concentration (SSC), specific leaf weight, and average photosynthetic photon flux (PPF) than did non-shaded trees. Fruits were larger, less firm, and had lower SSC in foil-covered peach than in non-covered. [4] It was reported that covered fruit developed less yellow color than the uncovered fruit. [5] it was stated that solar radiation was the key factor in apple fruit quality. Fruit size,

firmness, soluble solids, anthocyanin, starch content, pH and acidity were affected by light in apple and peach [6]. Pruning cut increased light interception in apple fruit and leaves, which increased the fruit dry matter [7, 8]. [9] it was suggested that pruning cut was a powerful factor in controlling peach fruit quality by exposing more sunlight to the trees. They found that pruning cut optimized photosynthetic efficiency and decreased the adverse effects of shading on carbon partitioning by increasing light interception through greater exposure of leaves within the canopy. It had been shown that light induced the expression of carotenogenic genes during leaf and flower development and during fruit ripening [10]. It was stated [11] that temperature, water and light in stress condition were affected the fig fruit sugar quality. They also recommended that these environmental factors can affect any fruit quality and development. It was reported [12] that the residual effects on sugar, glucose, anthocyanin and antioxidant by branch ring cut were found during three consecutive seasons in water apple fruit. It was Hossain et al reported [13,14] that the residual effects of the inter-stock cut on sugar, titratable acidity and total soluble solids in peach fruit trees were found to be decreased along three consecutive seasons. It was reported [15] that the residual effect of branch ring cut of peach fruit on starch and sugar content was observed during three consecutive seasons. However, although a few literatures were found related to the residual effects of light intensity on the fruits, no literature of such effect was found on date fruits.
The current study was conducted to investigate the following objectives

1. To find out the residual effect of light intensity (expose sunlight, light shadow and shadow) on date fruit growth and quality development in two consecutive seasons (2013 and 2014).
2. To investigate the residual effect of light intensity on biochemical content like sugar, fructose, nutrient content and DNA isolation and quantification of date fruit in two consecutive seasons.

MATERIALS AND METHODS

Plant materials

Dates palm trees were selected from dates palm field, Nugra Hail, Saudi Arabia during 2013 (1st season) and 2014 (2nd season).

First season (2013) treatment

A total of 12 date trees were selected from the field that was located in Hail, KSA. Four trees were subjected to each treatment. Treatments were as follows: exposed sunlight (Full sunlight), shadow sunlight (light sunlight) and deep or dark shadow inside trees (Control). For exposed sunlight 50% branches were removed by pruning cut from the trees as clock wise round shape in 2013 (1st season). For light sunlight (shadow), 25% branches were removed by pruning cut in 2013 (1st season) and for control no pruning cut or branches were removed in 2013 (1st season) (Figure 1). In 2014 (2nd season) no branches were removed for all treatments and kept as it was until the end of the season.

Full sunlight Light shadow Deep shadow (Control)

Figure 1. Photos show the date palm trees at different treatments

Methods of data Collection

First of all flower bud was recorded per panicle of each bunch. Then visual observation was done regularly and fruit were harvested from all of the experimental trees and tagged and finally they were brought to the Laboratory for measurement. Fruit per bunch was recorded as well as fruit length and fruit weight. Then fruit were ground and then juice was extracted by cheese cloth net which was kept for short time in the freezer for further analysis.

Biochemical, nutritional and genomic determination in 2013 (1st season)

Fructose content determination

Fructose was determined by using fructose refractometer, Atago-Japan. Three drops of juice sample were put on the disc of the meter and data were displayed and recorded (Fig. 1b).

Glucose content determination

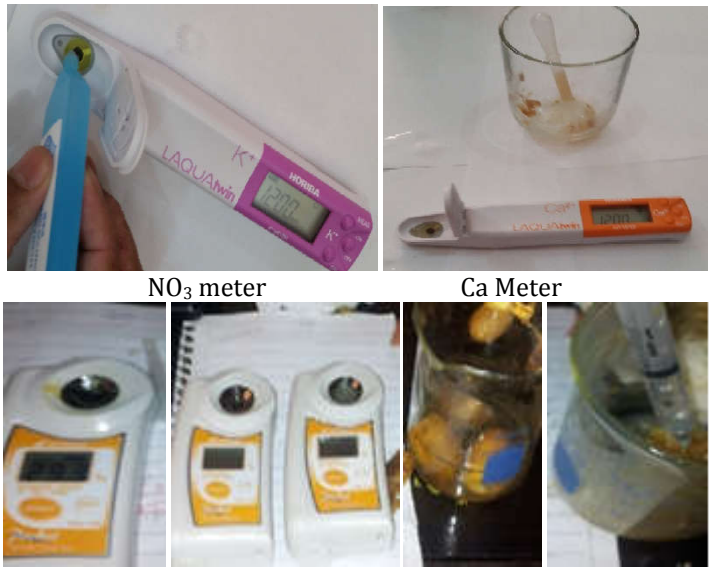
Glucose was determined by using glucose refractometer, Atago-Japan. Three drops of juice sample were put on the disc of the meter and data were displayed and recorded (Fig. 1b).

Inverted sugar determination

Inverted sugar (combination of glucose, fructose and sucrose) was determined by using inverted sugar refractometer, Atago-Japan. Three drops of juice sample were put on the disc of the meter using small syringe dropper and data were displayed and recorded (Fig. 2).

Percent Maturity determination

Full mature (full ripen) fruit was scored of 5 (100% ripe fruit) by visual observation. Mixed ripe and green (75% ripe + 25% green) was scored of 4. Mixed ripe and green (50% ripe + 50% green) was scored of 3 and mixed ripe and green (25% ripe + 75% green) was scored of 2 and less.



NO_3 meter Ca Meter

Glucose**,** Inverted sugar and fructose meter with dates samples

Figure 2.. Photographs show the different steps of biochemical and nutrient test in the laboratory.

Mineral content determination

Mineral content N (as NO_3), K and Ca was determined by using the nitrate, potassium and calcium digital meter, model: Horiba NO3 Meter (USA), Horiba K meter (USA) and Horiba Ca meter (USA) (Fig. 2). Five drops of juice sample were put on the disc sensor of the meter using small dropper and data were displayed and recorded (Fig. 2).

DNA isolation

5ml CTAB was preheated (added 10µl mercaptoethanol to each 5ml CTAB) in a blue-topped 50ml centrifuge tube at 60-65^{o}C. Fruit skin was separated and wrapped with aluminium foil and freezed in liquid nitrogen. A sample of 1.0 g tissue/5ml CTAB was stored after liquid Nitrogen for two days at –20^{0}C. Fruit tissue was crumbled over cold pestle of liquid nitrogen. Frozen fruit tissue was ground with one spatula (a measuring steel material) of fine sand added 0.5 spatula of PVPP powder after grinding scraped powder into dry tube and added pre-heated buffer and mixed gently.

CTAB volume was adjusted to give a slurry-like consistency of the samples. It was incubated for 60 min at 60 ^{o} C. Equal volume of chloroform/iso-amyl alcohol (24:1) was added and mixed for 3mins, then transferred contents to narrow bore centrifuge tubes. It was balanced after that by adding extra chlor/iso. It was spun at 5,000rpm for 10mins and broken off. Supernatant was removed with wide-bore paste (cut off blue tip) to clean tube, repeat chloroform extraction once. Supernatant might be clear, though might be colored. Precipitated DNA with 0.66 vol. of cold isopropanol was left overnight. Spooled out or spin down DNA, 2mins at 2,000rpm. DNA sample was transferred to 5ml wash buffer for 20mins it was dried briefly and re-suspended in 1ml T.E. One µl of 10mg/ml RNAse was added to each 1ml T.E./DNA mixture and incubated for 60min at 37 ^{o} C. It was diluted with 2 volumes TE and added 0.3vol 3M sodium acetate [(pH 8) + 2.5 vol cold 100% ethanol]. Spooled DNA was taken out, air dried and re-suspended in 0.5 to 1ml TE or water and it was freezed until required.

0.8% agarose gel was made with 99.2% 1x TAE and 0.1µl of Ethidium bromide (10mg/ml) per 10ml solution. Loaded samples undiluted and at a 1 in 10 (1+9) dilution with 3µl loading buffer. Incubated overnight at room temperature or 2 hours at 38 0C. It was loaded 1 ul of loading dye into each sample.

Adjusted the micropipet to 11 ul and load the samples in lanes 2-8. In lane one added the Lambda/HindIII digestion (10 ul of 0.1 ug/ul sample for a total of 1 ug of DNA) plus one ul of loading dye (11 ul). It was run at 90-100 volts for one hour. The gels were stained for approximately 5 minutes in ethidium bromide and de-stain in water for 2 minutes. Photograph was taken for gels. DNA molecules are negatively charged due to dissociation of the phosphate backbone. During electrophoresis they migrated towards the positively charged electrode. Small DNA fragments migrated more rapidly in the gel matrix compared to large fragments, resulting in molecule separation based on size.

Methods for Treatment setting in 2nd season (2014)

The same treatments were continued from 2013 to 2014 for the observation of residual effect.

Biochemical, nutritional and genomic determination in 2014 (2nd season)

The same methods of physiological, biochemical, nutritional and genomic analysis were used in 2013 (1st season) and 2014 (2nd season).

Statistical Analysis

Standard error (SE) and Least Significant difference Test (LSDT) were employed in both seasons (2013 and 2014).

RESULT

Figure 3 shows the dates flower number initiated per panicle and fruit number per bunch. The highest number of flower bud was found in the sunlight treated trees in 2013 and 2014. However, flower number was the least in 2014 (2nd season). Date fruits yield (fruit/bunch) was measured (Fig. 3). The highest fruit yield was found (10.0kg/bunch) in the full sun treated trees followed by light sun and shadow trees. Fruit weight was 10.0, 7.5 and 5.5g in 2013 (1st season) and 9.0, 7.2, 5.0 in 2014 (2nd season) in full sunlight, light sunlight and dark shadow treated trees respectively. Figure 4 shows the early fruit maturity according to the different treatments. Fruits matured (grade 5, 4 and 3) 15 days earlier (full maturity time of 45 days) in the full sun, shadow and dark shadow treated trees in the 1st season (2013) than in 2nd season (2014) [Table 1]. Fruit length and per fruit weight (Fig. 5) was higher in all treated fruits in the 1st season (2013) than in 2nd season (2014). Maximum fruit length was found 3.1 cm in the 1st season and 2.8 cm in the 2nd season by the treatment of full sunlight. However, decreasing trend was found for all treated fruits from 1st season (2013) to 2nd season (2014).

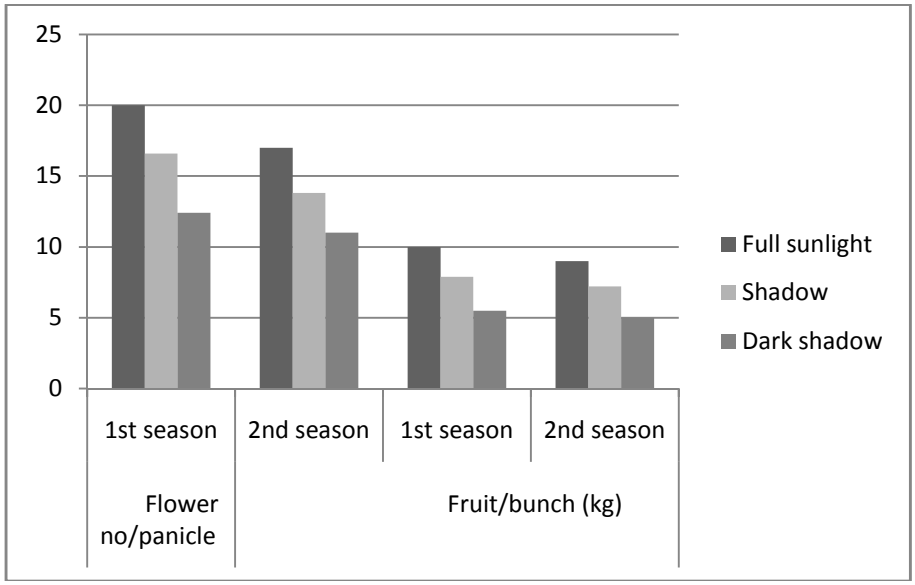
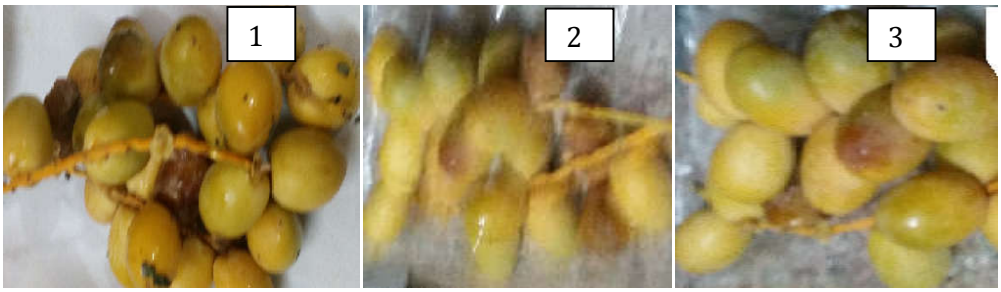


Figure 3. Measurement of flower and fruit at different seasons (years). Mean (n = 4).

The percent of fruit glucose content showed decreasing trend during 2013 and 2014 (Table 2). In both seasons, the highest percentage (34.5 and 29.5) of glucose content was found in full sunlight treated fruit. Table 3 shows the percent of fruit inverted sugar content decreasing trend at different seasons in 2013 and 2014. In both seasons, the percentage of inverted sugar was found 35.0 (2013) and 28.5 (2014), 31.3 (2013) and 26.4 (2014), 12.0 (2013) and 11.4 (2014) in the case of full sunlight, light sunlight and deep shadow treated fruit respectively. It was found the decreasing trend of fructose content in both seasons (2013 and 2014) from exposed sunlight and light sunlight (shadow) treated fruit to the dark shadow

(control) fruit shown in Table 4. Table 5 shows the DNA yield after isolation. DNA yield was found to be decreased from the 1st season to 2nd season in the case of full sunlight, light shadow and deep shadow treated fruit.

1= Full sunlight, 2 = light shadow/light sunlight 3 = Dark shadow/full shadow (1st season)



1= Full sunlight, 2 = light shadow/light sunlight 3 = Dark shadow/full shadow (2nd seaon)

Figure 4. Photos show the fruit maturity and color by visual observation in the 1st and 2nd season

Table 1. Determination of the maturity percent of date fruits at different seasons.

Treatment	% Maturity			
	1st season		2nd season	
	Grade	Days	Grade	Days
Full sun light	5.0a	45	4.0	60
Shadow (Less sun light)	4.0b	45	3.0b	60
Dark shadow	2.0c	45	1.8c	60

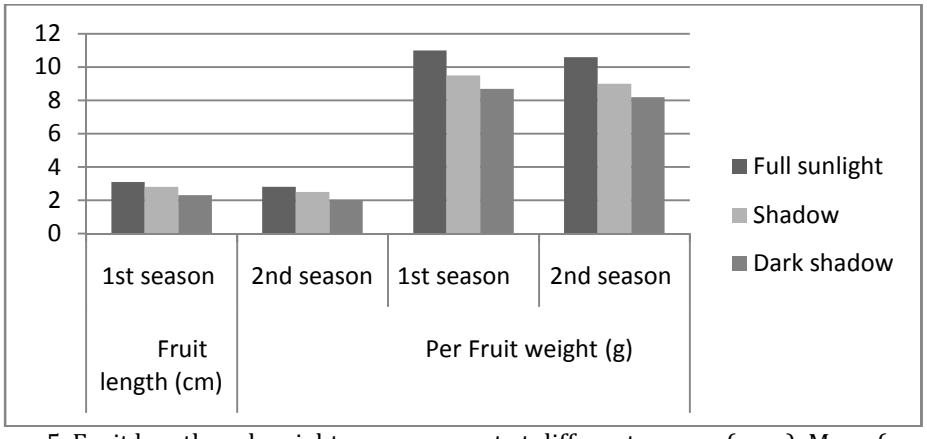


Figure 5. Fruit length and weight measurement at different season (year). Mean (n = 4).

Table 2. Fruit Glucose measurement. Mean followed by the same letters in column are not significantly different at the 5%level by least significant difference test (LSDT). (n = 4).

Treatment	% Glucose	
	1st season	2nd season
Full sun light	34.5a	29.5a
Shadow (Less sun light)	20.3b	17.3b
Dark shadow	12.5c	11.5c

Table 3. Fruit inverted sugar measurement. Mean followed by the same letters in column are not significantly different at the 5%level by least significant difference test (LSDT). (n = 4).

Treatment	% Inverted sugar	
	1st season	2nd season
Full sun light	35.0a	28.5a
Shadow (Less sun light)	31.3b	26.4b
Dark shadow	12.0c	11.4c

Table 4. Fruit Fructose (%) measurement. Mean followed by the same letters in column are not significantly different at the 5%level by least significant difference test (LSDT). (n = 4).

Treatment	% Fructose	
	1st season	2nd season
Full sun light	38.1a	32.5a
Shadow (Less sun light)	32.3b	27.3b
Dark shadow	17.5c	16.3c

Table 5. Measurement of DNA yield. Mean±SE (n = 4).

Treatment	DNA yield µg/g	
	1st season	2nd season
Deep shadow	280±1.1	266±1.2
Light shadow	620±1.3	590±1.1
Full sunlight	810±1.5	780±1.3

Figure 6. Fruit DNA measurement at different treatment. 1 = Dark shadow/full shadow 2 = light shadow/light sunlight, 3= Full sunlight

Table 6. Nitrate, potassium and calcium determination at different seasons.

Treatment	NO3- ppm		K+ ppm		Ca++ ppm	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Full sun light	89±0.3	88±0.1	780±0.5	778±0.4	88±0.3	86±0.2
Shadow (Less sun light)						
Dark shadow	80±0.1	78±0.2	630±0.4	625±0.2	84±0.2	81±0.1
	122±0.4	122.2±0.3	590±0.5	588±0.3	81±0.2	80±0.3

Table 7. Sodium and pH determination at different seasons.

Treatment	Na+ ppm		pH	
	1st season	2nd season	1st season	2nd season
Full sun light	55±0.2	54±0.3	7.70±0.01	7.60±0.02
Shadow (Less sun light)				
Dark shadow	51±0.1	50±0.2	7.61±0.2	7.50±0.01
	38±0.1	36±0.1	7.58±0.1	7.44±0.01

DNA ladder or probe was measured by gel electrophoresis method (Fig 6). It was very remarkable and distinct that DNA band or fragment was found wider and bigger in full sunlight treated trees than in light sunlight and shadow treated fruit. Mineral content like K and Ca was higher in the full sunlight (by pruning cut) treated trees than in light sunlight and shadow trees in the 1st season (2013). However, they were found lower in the 2nd season (2014)[Table 6]. Moreover, NO_3 was found higher in the shadow treated trees than full and light sunlight treated trees in both seasons (2013 and 2014) [Table 6]. Table 7 shows the decreasing trend of sodium and pH in the both seasons in 2013 and 2014 for all treated fruit.

DISCUSSION

Flower initiation, Fruit length and yield, glucose, inverted sugar, fructose content and minerals were higher in full sunlight treated trees than in light sunlight and shadow trees in the 1st season (2013) and then the 2nd season (2014). The residual effect's trend were found to be decreased from 1st season (2013) to 2nd season (2014). This might be due to the sunlight penetration into the fruit trees. By the pruning cut of branches, sunlight penetrated more than untreated trees (control). This resulds could be explained as sunlight enhanced more photosynthesis and therefore different photosynthetic products like sugars were produced more than the unexposed sunlight treated trees. It was suggested [13] that fruit yield, weight, length and diameter were higher in the more sunlight penetrated trees (summer pruning) than (less sunlight penetrated trees in winter (winter pruning). They also reported that it might be due to the longer photo period they received in summer than in winter. Similar results of fruit size, firmness, soluble solids, anthocyanin and starch content, pH, and acidity were affected by light in apple and peach as observed by [1].

[16] It was reported that sunlight (represented by summer pruning cut) affected the carbohydrate and total soluble solids (TSS) of peach fruit. DNA band was different at different treatments. It might be due to the effect of light intensity during the growing season. It has been shown that light induces the expression of carotenogenic genes during leaf and flower development and during fruit ripening [10]. In the current results, it has been shown that the residual effects of light intensity on fruit quality were found to be decreased from 1st season (2013) to 2nd season (2014). It might be due to the effect of exposed sunlight penetration into the fruit trees. In the 1st season, light penetration was excessively more than in the second season. When pruning cut was done in the branches in first season, at the onset light intensity was high which leads to producing high photosynthetic products by occurring a better photosynthesis in the fruit leaves. Obviously, in the second season (2014), after one year, light penetration was less and Photosynthesis could not occur more as well.

It was reported [12] that the residual effects of light intensity on sugar, glucose, anthocyanin and antioxidant by branch ring cut were found until three seasons in water apple fruit. Also [14] stated that the residual effects of light intensity on sugar, titratabale acidity and total soluble solids by pruning cut were decreasingly found in the 2nd season in peach fruit sugar quality development. Moreover, it was reported [17] that the residual effects of the inter-stock cut on sugar, titratabale acidity and total soluble solids in peach fruit trees were found to be decreased during three years. It was reported [15] that the residual effect of branch ring cut of peach fruit trees on starch and sugar content was found until 3 seasons.

Nutrient contents like K and Ca were higher in the full sunlight (by pruning cut) treated trees than in light sunlight and shadow trees. However, NO_3 was higher in the shadow treated trees than full and light sunlight treated trees. It was reported that dates contain carbohydrates, 58%, potassium, 696mg, calcium [1]. .

CONCLUSION

Current results conclude that the residual effects (seasonal variation) of sunlight on dates fruit quality development like (physiological, biochemical, mineral and genetic) are higher in the 1st season (2013) than in the 2nd season having the highest content in the full sunlight treated fruit.

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