ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 14 (5): 241-247, 2015
ISSN 1682-296X

RESEARCH ARTICLE OPEN ACCESS

DOI: 10.3923/biotech.2015.241.247

Biological Regulation of Growth Regulator on Jujube Fruit Development

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ARTICLE INFO
Article History:
Received: July 03, 2015
Accepted: August 06, 2015

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ABSTRACT
In order to research the influence of different plant growth regulators on the cell development and quality of red jujube fruits, we used the composite spraying method of three growth regulators including GA3, CPPU and ALA. After spraying growth regulators, the red jujube flesh subject to T5 (5 mg L^{-1} 5-ALA+2 mg L^{-1} Na_2SO_4+10 mg L^{-1} GA3+10 mg L^{-1}+5 mg L^{-1} CPPU) and CK (20 mg L^{-1} GA3) treatments would have maximum intercellular space on the whole. As time went by, the intercellular space of pericarp subject to CK treatment would further enlarge. However, the intercellular space of pericarp subject to T1 (20 mg L^{-1} 5-ALA+2 mg L^{-1} Na_2SO_4) treatment decreased gradually. When high-concentration gibberellin was sprayed to red jujubes separately, the secretory cavity of flesh cells was the largest. Moreover, it would be absorbed along with the growth. However, for red jujubes subject to other treatments, the changes to secretory cavities were not significant. The sizes of the flesh cells of red jujubes subject to each treatment were all larger than that of the contrast group. Compound of 5-ALA and CPPU had more significant effect on enlarging the flesh cells of red jujubes in comparison with GA3. Compound of 10 mg L^{-1} CPPU and low-concentration 5-ALA or spraying high-concentration 5-ALA separately could achieve satisfactory effect. The Vc content in the fruits could effectively increase and sugar-acid ratio could be improved by using T1 treatment. The total nitrogen and total phosphorus contents in jujubes could effectively increase by using T5 treatment, thus increasing its nutritional value. Cooperation of CPPU and 5-ALA can prevent adverse symptoms of high concentration gibberellin on red jujubes and T1 treatment can effectively increase cell size and diminish the cell gap of red jujube fruit and then it may improve the quality of jujube.

Key words: Biological regulation, growth regulators, jujube, fruit development

INTRODUCTION

Jujube (*Ziziphus jujuba* Mill.) is the ziziphus plant of rhamnaceae. It is a special fruit tree resource and characteristic advantageous fruit-tree species in our country. It is also the first dried fruit in our country (Fu *et al*., 2008). Red jujube has become the pillar industry of characteristic forest fruits in Xinjiang. Till the end of 2014, planting area of red jujubes in Xinjiang has reached up to 5.3×10^5 m^2, occupying about 30% of red jujube area all over the country and 40% of the forest fruit area in Xinjiang. Product output has occupied 20% of total red jujube output all over the country. As an extremely dry area in the country, Tarim Basin has become a nationwide main producing area for red jujube planting.

Gibberellin (GA3) can promote germination and stem and leaf growth of plant seeds, induce flower bud formation and

promote parthenocarpy and fruit setting. For many years, it is common to spray the growth regulator-gibberellin during local planting. However, after spraying too much gibberellin only in order to increase fruit setting rate, red jujube fruit stems become long and thin, jujube shedding shoot and vain growth appear, fruits fall off prematurely, dehiscent fruits and black head disease can take place easily and "big but less-flesh" jujubes, as well as other phenomena can easily happen, all of which will finally influence quality of red jujubes and healthy development of the industry. As researched, plant growth regulators have been extensively applied in field crops, economic crops, fruit trees, forests, vegetables, flowers because of their prominent and efficient adjusting effect (Zhao *et al*., 2009; Zheng *et al*., 2011; Liu *et al*., 2012). For example, cytokinin can promote cell division and enlargement, induce plant flower bud differentiation and postpone leaf senescence (Ou *et al*., 2006). Ethylene can induce expression of relevant genes and can also realize accumulation of relevant proteases so as to accelerate senescence of separated flowers (Kuroda *et al*., 2004). By applying the auxin inhibitor TIBA from outside, the content of cell cytokinin can be increased so as to bring up quantity of flower buds (Ito *et al*., 2001). The CPPU can prolong the phase of cell division, promote cell growth in the division phase and promote fruit expansion of fruit trees (Castelfranco and Beale, 1983) and can effectively relieve cell wall dissolution and cytomembrane structure changing of kiwi fruit (Zhao *et al*., 2015). As the key precursor generated from biosynthesis of porphyrin compounds (Jao *et al*., 1991), 5-ALA can bring beneficial influences to antioxidant enzyme activities and antioxidant content in the AsA-GSH circulation system of grape leaves under salt stress (Li *et al*., 2015). It is found in Emongor's research that by spraying gibberellin, plant height of cowpea seedlings can be increased and resistance of plants can be reduced at the same time (Emongor, 2007).

Till now, there are rarely researches about side effects of gibberellin which is deemed as the good fruit setting drug for red jujubes and its improvement of red jujube cell development and fruit quality through combination with its growth regulators. In this research, three growth regulators GA3, CPPU and ALA are compounded in order to research their influences on cell development and quality of red jujube fruits; good measures of improving red jujube quality by regulation of growth regulators were explored in order to provide technological basis for high-quality and efficient production of Xinjiang red jujube industry.

MATERIALS AND METHODS

Contents and method of test

Observatory analysis of pericarp and fruit stem structures: Since, the 15th day after the second time of spraying, fruits were picked every 5 days for totally 5 times (5 stages). The FAA stationary liquid (prepared by 90 mL of 70% alcohol+5 mL of glacial acetic acid+5 mL of formalin) was used to fix and store the samples. As for the treated red jujubes, the epidermis and a 5 mm^3 section under it were extracted from the pericarp; a 3 mm long section in the fruit stem middle was extracted from the fruit stem. Through paraffin section, safranin-fast green counter dyeing, microscope photography and result observation, characteristics of anatomical structures inside pericarp and fruit stem of red jujubes under different treatments at 5 different development stages after spraying (July 6, 12, 18, 24 and 30) were analyzed, and size of the central cell in fruit flesh was measured.

Profile of experimental field: The experiment was carried out in Block 731 Jun Jujube Garden, Company 12, Arael Farm, First Division, Xinjiang Production and Construction Corps from 2013-2014. The jujube garden was live broadcasted in 2010. Grafting was implemented in 2011 with planting spacing of 1×1.5 m, growth situations of jujube trees in this jujube garden are ordered and consistent, so that the jujube garden is representative in this district. The experimental point was located in Arael Farm of First Division of Xinjiang Production and Construction Corps Located in the North of Xinjiang Takla Makan Desert and Akesu Prefecture of Tarim Riverside. It belongs to the typical continental extreme drought desert, inland middle-latitude warm temperature zone desert and semi-desert continental arid climate type. The experimental point has altitude of 1012.6 m and average annual precipitation of 42.4 mm. Jujube garden soil is mainly the sandy soil, with soil alkali-hydrolysable nitrogen content of 56 mg kg^{-1}, rapid available phosphorus content of 8 mg kg^{-1}, rapid available potassium content of 61mg kg^{-1}, pH = 7.8 and salt content of 1.47%.

Experiment design: Randomized block design was used in this research with block area of 30×40 m^2. Six formulas were compounded for treatment. The GA3, which is now applied commonly in jujube garden was taken as the contrast (Table 1). Each treatment was repeated for 3 times and then the mean value was obtained. When blossom rate of jujube

Table 1: Table of experimental treatments

Experimental treatment	Drug name	Concentration (mg L^{-1})	Drug name	Concentration (mg L^{-1})	Drug name	Concentration (mg L^{-1})	Drug name	Concentration (mg L^{-1})
T1	5-ALA	20	Na_2SO_4	2				
T2	5-ALA	10	Na_2SO_4	2	GA3	5		
T3	5-ALA	10	Na_2SO_4	2	GA3	10		
T4	5-ALA	10	Na_2SO_4	2	GA3	15		
T5	5-ALA	5	Na_2SO_4	2	GA3	10	CPPU	5
T6	5-ALA	5	Na_2SO_4	2	GA3	10	CPPU	15
CK					GA3	20		

trees reached about 40%, jujube flowers were dense and disk became bright, the GA3 would be sprayed after 18 O'clock in sunny and windless afternoons. It was sprayed for the second time 8 days later.

Measurement of fruit quality: After fruit ripening and harvest, relevant indexes were measured. Titrimetry was used to measure organic acid content of fruits; Fehling reagent method (Li, 2003) was used to measure content of soluble sugar, Vc content was measured according to GB/T 5009·86-2003, Kjeldahl method was used to measure total N content, vanadium molybdenum yellow colorimetry was used to measure total P content and flame photometer method was used to measure total K content (Bao, 2000).

RESULTS

Influences of different growth regulators to red jujube anatomical structure comparison of pericarp anatomical structures of red jujube under 7 different treatments at 5 development stages: Red jujube pericarp is mainly consisted of epidermis, collenchyma close to the epidermis elementary tissue and vascular tissue.. The epidermis is consisted of one cell layer and covered by a thick cuticle. During pericarp development, thicknesses of epidermis cells and the cuticle gradually increased. Collenchyma is constituted of one to five cell layers, namely the structural layers, which are dyed into red and close to the epidermis as observed by the microscope. Distribution of collenchyma is not uniform. Under different treatments and different parts of the same treatment, the layers will vary to a certain extent. During earlier development, the collenchyma only has one to two cell layers. During later development, it is constituted of 3-5 cell layers. The elementary tissue is mainly constituted of multiple layers of parenchymal cells and is located between the thick tissue and the red jujube kernel. At the first development stages, parenchymal cells are small and arranged closely; during later development, the cells gradually increase and big intercellular space is differentiated from the parenchymal tissue. Later development leads to more obvious intercellular space. In addition, a few of vascular bundles are also distributed in the elementary tissue. In generally, the vascular bundles in the center of pericarp are big and those on two sides gradually decrease. A part of red jujube can be observed from the paraffin section level and there are only a few of distributed vascular bundles, so that similarities and differences of vascular bundles under 7 different treatments cannot be compared (Fig. 1 and 2).

As for the anatomical structure, T5 obtained the biggest intercellular space (refer to 4 or 10 multiples of magnification) at the first stage (July 6), T4 obtained the secondly biggest intercellular space and the intercellular space of T6, T1, T3, T2 and T7 gradually decreased. In particular, the secretory cavity was big in the red jujube flesh treated by T7. As for flesh cell size, the average sizes of cells from T1-T7 were 33.03, 29.92, 28.58, 30.47, 28.78, 32.75 and 25.87 μm, respectively.

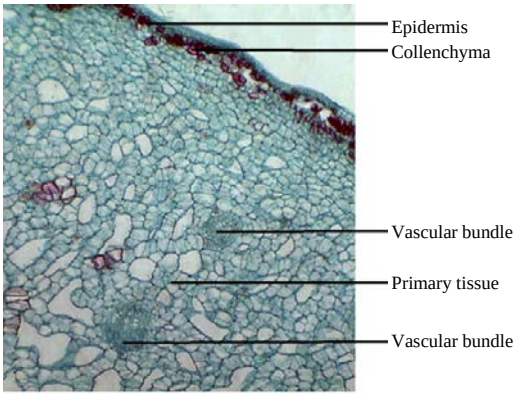

Fig. 1: Red jujube flesh structure (10×10 multiples)

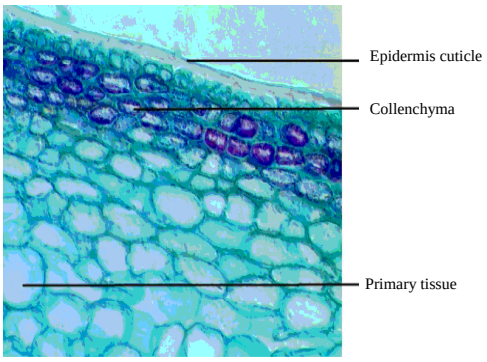

Fig. 2: Red jujube pericarp structure (10×40 multiples)

In comparison with the first stage, cell size and intercellular space did not change much at the second stage (July 12). As for the anatomical structure, T5 and T6 had big intercellular space (refer to 4 or 10 multiples of magnification), T1 obtained the secondly biggest intercellular space and the intercellular space of T3, T4, T7 and T2 gradually decreased. At this stage, the secretory cavity in flesh of red jujube treated by T7 did not vary in comparison with the previous stage. As for cell size of fruit flesh, average cell sizes from T1-T7 were 31.72, 29.54, 29.38, 30.16, 29.87, 35.30 and 26.81 μm, respectively.

In comparison with the previous two stages, except for T6 treatment, cell size and intercellular space varied greatly at the third stage (July 18). As for the anatomical structure, the intercellular space of T1 and T5 was very big (refer to 4 or 10 multiples of magnification), T2 obtained the secondly largest intercellular space and the intercellular space of T4, T7, T3 and Y6 gradually decreased. At this stage, the intercellular space obviously increased under the T7 treatment and the secretory cavity in flesh had been absorbed. As for the fruit flesh cell size, the average cell sizes from T1-T7 were 38.52, 38.22, 40.74, 34.03, 38.77, 32.48 and 31.33 μm, respectively.

In comparison with the third stage, intercellular space differentiation at the fourth stage (July 24) tended to be

mature. Therein, T5 obtained the largest intercellular space (refer to 4-10 multiples of magnification) and the intercellular space of T1, T2, T3, T4, T7 and T6 gradually decreased. As for the fruit flesh cell size, the fruit flesh cells of T7 were obviously much bigger than those at the third stage, wherein the cells increased by 17.94 μm, cells of T1 and T6 increased by about 10 μm and cell sizes in other treatments did not vary much. Average cell sizes from T1-T7 are 49.02, 38.07, 38.19, 35.42, 38.51, 42.58 and 49.27 μm, respectively.

At the fifth stage (July 30), fruit flesh of red jujube had tended to be mature. As for the anatomical structure, the central space of flesh treated by T4, T5, T6 and T7 was highly developed, T3 and T2 obtained the secondly developed space and T1 obtained the smallest space. As for cell size of fruit flesh, the flesh cells treated by T1 and T7 were obviously smaller than those at the fourth stage. Cells treated by T3 and T5 obviously grew up a lot, respectively by 13.06 and 14.42 μm. Cell sizes of T1, T2, T4 and T6 increased to a small extent. Average cell sizes from T1-T7 were 43.11, 42.23, 51.25, 43.99, 52.93, 45.68 and 44.72 μm, respectively.

Comparison of anatomical structures of red jujube fruit stem under 7 different treatments at 5 development stages: During red jujube development and different from the red jujube fruit, the fruit stem of it does not vary very obviously, while its size and anatomical structure only change a little. The anatomical structure of red jujube fruit stem comprises epidermis, cortex and vascular column. Therein, the epidermis is constituted of one cell layer and covered by thick cuticle, the cortex is constituted of collenchyma and parenchymal tissue, the vascular column comprises xylem, phloem, cambium and pith (Fig. 3-6). Among the anatomical structures of fruit stems of red jujubes under 7 different treatments and at different development stages, the vascular column was always a dominant component. It is located in the center of stem and has no obvious boundary with the cortex. The fruit stem changes very little during jujube fruit development, so that the anatomical structure of red jujubes under different treatments have no obvious difference.

Influences of different growth regulators to central cell of red jujube fruit flesh: During the third measurement (July 18), except for T6, which had an obvious decrease trend, other treatments showed the increase tendency. Treatment results of T3 were the most obvious and higher than results of other treatments; this stage was exactly the rapid expansion period of red jujube fruit. Due to high concentration of cytokinin in T6 treatment, the cell division was accelerated but water and fertilizer nutrition could not satisfy normal growth of fruits under such concentration. Hence, the cell size obviously decreased. At the last two stages, T1 and T7 realized increase and then the decrease, obvious increase trends appeared in other treatments. Therein, the cell size under T5

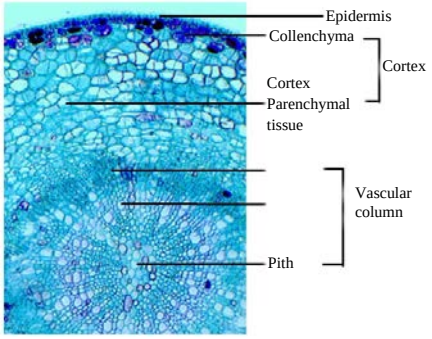

Fig. 3: Structures of red jujube fruit stem (10×40 multiples)

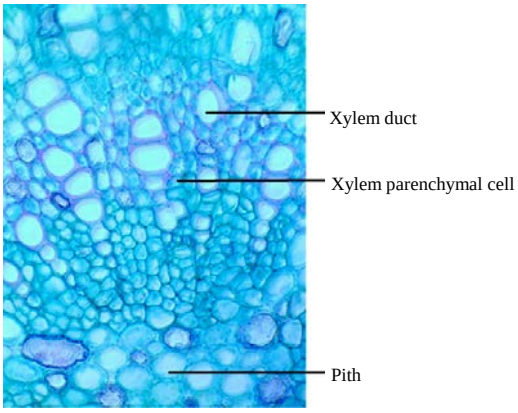

Fig. 4: Xylem structure of red jujube fruit stem (10×40 multiples)

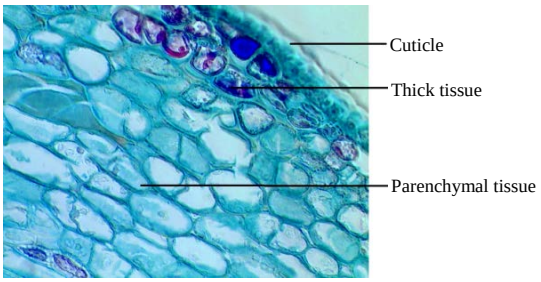

Fig. 5: Epidermis and collenchyma of red jujube fruit stem (10×40 multiples)

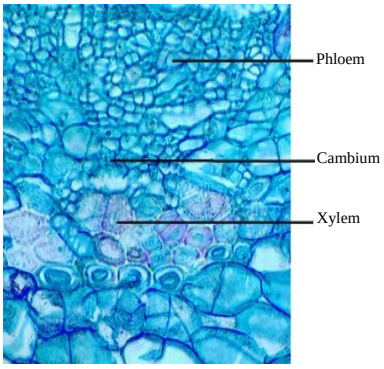

Fig. 6: Red jujube fruit stem phloem (10×40 multiples)

Table 2: Measurement results of red jujube fruit quality under treatments of different growth regulators

Measure index	T1	T2	T3	T4	T5	T6	CK
Total sugar (g kg^{-1})	42.60	47.40	45.70	43.40	50.70	45.90	51.40
Total acid	6.50	7.56	7.62	7.71	8.16	7.02	9.36
Sugar-acid ratio	6.60	6.30	6.00	5.60	6.20	6.50	5.50
Vc (mg/100 g)	18.34	23.12	15.64	8.74	20.92	11.50	16.24
N (g/100 g)	3.42	4.25	4.48	3.72	4.16	3.52	3.79
P (mg/100 g)	76.00	88.50	90.80	75.00	98.50	86.80	97.00
K (mg/100 g)	531.96	607.88	519.09	527.60	608.84	573.00	638.57

treatment changed most obviously, reached 52.93 and increased by 18.35% in comparison with the contrast. As a whole, since the 15th day after the second spraying, red jujube flesh cells under each treatment obviously increased and were larger than those in the contrast. It is shown in the results that combination of 5-ALA and CPPU plays a more obvious role in cell enlargement of red jujube fruit flesh than GA_3. The CPPU of 10 mg L^{-1} and low-concentration 5-ALA could obtain very good effect. From July 12 to July 24, jujube fruits expand rapidly. Without GA3 and CPPU, the high-concentration 5-ALA can prominently promote development of central cell in the red jujube flesh.

Influences of different growth regulators to red jujube fruit quality: Influences of different types of plant growth regulators to jujube fruit quality indexes are shown in Table 2. In comparison with other treatments, total sugar content and total acid content of the contrast were the highest. The total sugar content increased by 17.12% in comparison with the T1 treatment with the lowest value. The total acid content increased by 44% in comparison with the lowest T1 treatment. From high values to low values, the sugar-acid ratios were in sequence T1>T6>T2>T5>T3>T4>CK, wherein the sugar-acid ratio of T1 was 19.36% higher than CK. High Vc contents of T2, T5 and T1 treatments, respectively, increased by 42.36, 28.82 and 12.93% in comparison with CK. Elements including; nitrogen, phosphorus and potassium are three basic elements of plants (Table 3) and also the quality elements of plant fruit. High nitrogen element contents of T3, T2 and T5 respectively increased by 18.20, 12.13 and 9.76% in comparison with CK; the highest phosphorus element content of T5 only increased by 1.54% in comparison with the contrast, wherein the lowest contrast ratios of T4 and T1 increased by 29.33 and 27.63% from higher values to lower values, contents of potassium element are in sequence CK>T5>T2>T6>T1>T4>T3, wherein the lowest contrast ratio of T3 treatment increased by 23.01%.

The 5-ALA of different concentrations and Na_2SO_4 of the same concentration were mixed in other treatments. It is thus clear that 5-ALA can effectively improve sugar-acid ratio of red jujube fruit; as for the Vc content, the Vc content in the contrast was close to the median. According to comparison between T1, T2 and T5 treatments with low contents and the contrast, the first two treatments had high concentration of 5-ALA, while 5 mg L^{-1} of Vc was mixed in T5.

Table 3: Different central cell size of fruit under different spraying treatments at different stages

Date	Treatment	Mean value	Significance level 5 (%)	Significance level 1 (%)
July 6	T1	33.03	a	A
	T6	32.75	a	AB
	T4	30.46	ab	AB
	T2	30.03	ab	ABC
	T5	28.78	bc	BC
	T3	28.57	bc	BC
	CK	25.87	c	C
July 12	T6	35.30	a	A
	T1	31.71	b	AB
	T4	30.16	b	BC
	T5	29.87	bc	BC
	T2	29.53	bc	BC
	T3	29.38	bc	BC
	CK	26.80	c	C
July 18	T3	40.74	a	A
	T5	38.77	a	A
	T1	38.51	a	A
	T2	38.22	a	A
	T4	34.02	b	B
	T6	32.47	b	B
	CK	31.33	b	B
July 24	CK	49.26	a	A
	T1	49.01	a	A
	T6	42.58	b	B
	T5	38.50	c	BC
	T3	38.19	c	C
	T2	38.07	c	C
	T4	35.42	c	C
July 30	T5	52.93	a	A
	T3	51.25	a	A
	T6	45.68	b	B
	CK	44.72	bc	B
	T4	43.98	bc	B
	T1	43.11	bc	B
	T2	42.22	c	B

The contrast had the lowest sugar-acid ratio. Through analysis of the cytokinin except for the contrast, these factors might be the reasons for increase in Vc content. In all the treatments, the GA3 concentration was lower than that in the contrast. It is shown in preliminary analysis that gibberellin can reduce Vc content in fruits.

DISCUSSION

Occurrence of plant growth regulators greatly promoted the development of modern agriculture. With progress in science and technology, its update changes with each passing

day. Application of plant growth regulators in fruits and vegetables has obtained good economic benefits. In recent years, gibberellin has been applied most frequently. As an efficient growth irritant, gibberellin can enter plants by leaves, tender shoots, flowers and fruits. By breaking plant dormancy, it can promote germination (Fu *et al*., 2008) and effectively promote growth and development of hyacinth, increase its chlorophyll content, realize blossom in advance and prolong flower season (Zhang and Hou, 2014).

However, in recent years with the prominent effect brought by gibberellin, more and more people have begun excessively depending on it, leading to excessive use or even abuse of gibberellin, as well as increased gibberellin residue. As for red jujubes, frequent repeated utilization of gibberellin will cause long and thin red jujube fruit stems, shedding shoot and vain growth of jujubes, easy falling of fruits and black head disease of red jujubes, etc., as for the spraying concentration, low concentration can promote growth of leaves and tender shoots of jujube trees and facilitate cell growth of fruits to a certain extent; however, too high concentration will inhibit growth, leading to growth inhibition and then reduction in output and quality. A lot of fruit growers lack knowledge and experience of this aspect. In order to increase the output, they continuously increase dosage and concentration, causing a series of symptoms and output reduction of fruit trees. It is found in research that 5-ALA of suitable concentration can promote advance shading and maturity of peach fruits (Guo *et al*., 2013). The 5-ALA is not only a kind of plant growth promoter (Hotta *et al*., 1997) and is a kind of plants to strengthening agent (Watanabe *et al*., 2000; Nishihara *et al*., 2003). The CPPU treatment may increase the fruit spike length, spike weight and single fruit weight, mean, while, it can promote the fruit setting ratio increased and promote fruit enlargement (Han and Lee, 2004; Costa *et al*., 2004; El-Sabagh and Ahmed, 2004). During growth and development of grape fruits, CPPU can effectively prolong rapid growth period of Xiahei grape fruits. Treatments with different concentrations of CPPU can reduce soluble solid content of grape fruits and increase titratable acid content (Zhang *et al*., 2013).

In this experiment, 5-ALA and CPPU with different concentrations were mixed. The Na_2SO_4 with certain concentration was then added as the stabilizer to replace gibberellin or match with gibberellin spraying to improve cell development and quality of red jujube fruits.

CONCLUSION

After spraying of regulators, the T5 (5 mg L^{-1} 5-ALA+2 mg L^{-1} Na_2SO_4+10 mg L^{-1} GA3+10 mg L^{-1} +5 mg L^{-1} CPPU) obtained the overall largest intercellular space. As time went by, the pericarp intercellular space of CK further increased, while that of T1 (20 mg L^{-1} 5-ALA+2 mg L^{-1} Na_2SO_4) gradually decreased. After spraying of growth regulators, red jujube flesh cells under each treatment would increase obviously as time went by and the sizes were bigger that those in the contrast. Combination of 5-ALA and CPPU could more prominently increase red jujube fruit flesh cells than GA_3. The CPPU of 10 mg L^{-1} and low-concentration 5-ALA could obtain very good effect. During the rapid expansion period of jujube fruits, without effects of GA3 and CPPU, the high-concentration 5-ALA treatment (T1) could prominently promote development of central cell of the red jujube fruit flesh. In other 6 treatments, the sugar-acid ratios of jujube fruit flesh were all higher than that of the contrast, wherein T1 treatment obtained the most prominent effect and the sugar-acid ratio increased by 19.36% in comparison with CK. Under treatments of T2 (5 mg L^{-1} 5-ALA+2 mg L^{-1} Na_2SO_4+5 mg L^{-1} GA3), T5 and T1, Vc contents of fruit flesh respectively increased by 42.36, 28.82 and 12.93% in comparison with the contrast. Meanwhile, it is shown in analysis that T5 treatment could effectively increase whole nitrogen and whole phosphorus contents in fruit flesh of Jun jujubes .

So, during production, 5-ALA and CPPU can be used. 5 mg L^{-1} 5-ALA+5-10 mg L^{-1} CPPU and 5-10 mg L^{-1} GA3 are combined or 20 mg L^{-1} 5-ALA can be used separately to take the place of high-concentration GA3 in order to increase quality and stress resistance of Jun jujube fruits.

ACKNOWLEDGMENTS

The research is funded and supported by open topics of Xinjiang Production and Construction Corps, including Research and Demonstration of High-quality and High Yield Technologies of Jujubes Based on Inflorescence Development Regulation, Major and Special Technological Project of Xinjiang Production and Construction Corps (2013AA001-2) and Guidance Plan of Xinjiang Agricultural Reclamation Academy of Science (84YYD201509).

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