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Effects of Plant Growth Regulator on Endogenous Hormone Levels during the Period of the Red Globe Growth

Juan He
College of Agriculture, Shihezi University
Shihezi 832003, China
E-mail: hjmc111@126.com

Songlin Yu (corresponding author)
College of Agriculture, Shihezi University
Shihezi 832003, China
E-mail: songliny@263.net
Chao Ma
College of Agriculture, Shihezi University
Shihezi 832003, China
E-mail:mchj111@126.com

Abstract

Objective. The present study was aimed to elucidate the dynamic changes of endogenous hormone levels during the fruit growth and the changing regulation of endogenous hormone levels between normal and abnormal fruits.

Methods. After using exogenous GA_3 and GA_3+6-BA, endogenous hormone levels such as indoleacetic acid(IAA), cytokinin(ZR), abscisic acid(ABA) and gibberellin(GA_3) in normal fruits and rigid abnormal ones were determined by enzyme-linked immunosorbent assays(ELISA), respectively.

Results. The results indicated that the treatments of 20mg/kg GA_3 and the combination of 20mg/kg GA_3+10 mg/kg 6-BA had no remarkable effects on increasing average weight of berries where the vertical diameter of grape fruits was longer than the transverse diameter. Exogenous hormone GA_3 and GA_3+6-BA mixture also affected the endogenous hormone levels. The content of IAA, ABA and ZR was lower in the rigid abnormal fruits than that in the normal ones, respectively, and those levels during the rapid growth period were nearly in accordance with those during the slow growth period.

Conclusion. The fruit growth was closely related to the endogenous hormone levels.

Keywords: Plant growth regulator, Grape, Fruit growth, Endogenous hormone

1. Introduction

Applications of plant growth regulators have been focused by many researches in areas of plant physiology and nutriology(Pan, 1999, PP. 1-2, Amarjit, 2000, PP. 1-16). The regulators have been intensively and extensively applied for agriculture production, and played a vital role in the growth and development of plants. Along with the development of intensive cultivation of fruits, applications of regulators for controlling the growth of fruits have been progressively paid more attention (Ma, 1998, PP. 27-36).

Phytohormone, used as trace signal molecule in plants, has very important significance in regulating all kinds of growth processes and environmental responses, and meanwhile , made great contribution to the agricultural chemical control of crops, fruits and vegetables (Xu, 2006, PP. 433-442). Phytohormone mainly regulates the the plant physiological processes such as growth and development, organ formation and so on. The dynamic changes of endogenous hormone levels during the period of fruit growth have been investigated by many researchers. Meng et al(2005, PP. 6-10) studied

that the relationship between endogenous phytohormones and preharvest fruit drop of apples and found that a higher level of ABA/IAA caused fruit drop seriously. Xia et al (2000, PP. 6-10) found that the fruit growth was regulated by GA and IAA at earlier stage, while ABA did at later stage. Therefore, it would be of great significance for elucidating the time control of fruit growth by endogenous hormones and giving reasonable insight into production practice that better ordered control was undertaken to coordinate the growth and development.

Effects of exogenous plant growth regulators have been investigated more on seedless varieties but relatively less on seedy varieties. Several results showed that GA could promote the increment of grape fruit grains (Kim, 1991, PP. 199-205, Retamales, 1993, PP. 89-94), but some other results showed no effects on seedy fruits(Nosukchal, 1984, PP. 52-59). Though exogenous plant growth regulators could regulate the growth and development of fruits effectively, they could also cause some problems in practice such as rigid and abnormal fruit. Taken together, in the present paper, through the treatment of exogenous GA_3 or GA_3+6-BA mixture applied to red globe grape, 4 hormone levels were investigated in order to observe their effects on the growth and development of fruits and the internal cause of the rigid and abnormal fruits in hormone level.

2. Materials and methods

2.1 Plant material and treatment

Six-year-old trees of the Red Globe (*V.vinifera* L. *Cv.*) growing at the million mu production base of the red globe grape, 83 Corps, N 5 Division, Xinjiang, were used in this experiment. Grape trees in the garden with strong tree vigor and complete structure have no diseases and pests and similar growth. Tree stands are all small frame with the planting spacing of 1×4m. Supplemental irrigation was provided and standard fertilization practices were followed. Grape trees of similar age and vigor were randomly selected, 4 trees as a block, 3 times replicate, 12 tress for trial altogether. Fruit clusters were processed with micro-jet of GA_3(A) 20 mg/kg and the combination of 20 mg/kg GA_3 +10 mg/kg 6-BA(B) 5 days before (22nd May) and 3 days after(2nd June) blossom respectively, water treatment used as control in the whole trial.

2.2 Methods

2.2.1 Sampling and pretreatment

During the period from 23 days after blossom(19th June, 2007) to fruit maturation(28th August, 2007), normal and rigid abnormal fruits appearing after treatment was randomly sampled once 7 days, 20 fruit grains altogether collected from the upper, central and lower part of the fruit clusters. The samples were baged with silver paper, quick-frozen by liquid nitrogen, and then stored at -80℃ for further investigation.

2.2.2 Separation, purification and determination of endogenous hormone

Accurate 1g sample was grinded into homogenates under ice bath and weak light. Resultant extraction was centrifuged. The supernatant was separated by C-18 solid phase extraction column, transferred into 5ml plastic centrifuge tube, dried by nitrogen gas, eliminated methanol from extractions and then diluted into a constant volume by sample diluents. The endogenous hormone levels in fruits were determined by ELISA as described by He et al (1993, PP. 60-68).

ELISA kit was kindly provided by China Agriculture University as a present. Discarded peels and cores, IAA, ZR, ABA and GA_3 levels were investigated in the fleshes of grapes.

2.2.3 Effects of plant growth regulators on the growth of red globe grape

18 fruit grains were marked, 3 ones from the upper, central and lower part of fruit cluster each treatment. The longitudinal and transect diameter of fruits was measured by vernier caliper every 7 days from 19th June on, and accurate to 0.1 mm.

2.2.4 Effects of plant growth regulators on the quality of red globe grape

18 fruit grains were randomly selected to measure fresh weight and soluble solids. Fresh weight was determined by a balance with the precision of 0.1g while soluble solids by a portable sugar analyzer with the precision of 0.2%.

3. Results and analysis

3.1 Effects of plant growth regulators on the growth dynamics of red globe grape

The growth dynamic changes of grape berry was depicted in Figure 1 and 2 and appeared a double S-type curve, after enlargement treatment which could not affect the growth of fruits. The longitudinal diameters of normal fruits were all higher than transect diameter while the difference of longitudinal diameter between B and CK was significantly higher A and CK after both A and B treatment as the same period. During the whole growth process, the longitudinal diameter in A and B was all higher than CK. In mature period, the difference of longitudinal diameter in A and CK was gradually decreased while transect diameter kept stable, and the growth of fruits became slower; the difference of longitudinal diameter in B and CK was significant while that of transect diameter was not significant. The average transects and

longitudinal diameter increased by 0.11cm and 0.10cm in A and decreased by 0.07cm and increased by 0.10cm compared to the CK in the whole period, respectively. The average transect and longitudinal diameter increased by 0.12cm and 0.11cm in B and increased by 0.01cm and 0.20cm compared to the CK in the whole period, respectively. Results showed that fruits were characterized by elongation of grains, oval shape and increment of fruit shape indices when a treatment was involved. As for the rigid abnormal fruits, there was no significance between the transect and longitudinal diameter and the two diameters had almost no increment and were significantly lower than CK (Figure 1 and 2).

3.2 Effects of plant growth regulators on the quality of red globe grape

The increment was significant for the longitudinal diameter while not obvious for transect after the treatment of Plant Growth Regulator. Fresh weight of single and the biggest grain and soluble solid content increased compared to CK. Through analysis of variance with mean separation by Duncan's multiplerange test, there was no great significance in fresh weight of single grain or soluble solid content, but great significance in fresh weight of the biggest grains between B and CK (Table 1).

3.3 Effects of plant growth regulators on endogenous hormone levels in fruits

IAA level was higher in flesh in early growth period. During the whole growth process of normal fruits, IAA level was higher in A than that in B and CK, attained a peak value 23 days after blossom with the content of 1548.90 ng/g FW and then decreased rapidly to a stable level. IAA level was higher in B than that in CK 23 days after blossom, decreased gradually, attained a valley value on 26th July, and then increased gradually to a stable level. IAA content of rigid abnormal fruit was all in an extremely low level in both A and B, where fruits were in a rigid, stagnant stage all the time during the whole growth process (Figure 3).

As for normal fruits, ZR content in A, B and CK was at a higher level which was 1962.87 ng/g FW, 4735.98 ng/g FW and 1283.19 ng/g FW, respectively, in early growth period, and then decreased gradually to a lower level, almost undetectable; As for abnormal fruits, ZA level in A and B was significantly lower than that in CK, and since 23 days after blossom, there was no great difference in ZA level (Figure 4).

ABA level of normal fruits in A, B and CK all attained a peak value 23 days after blossom which was 218.86 ng/g FW, 1079.77 ng/g FW and 890.96 ng/g FW, respectively. Subsequently, ABA level attained a peak value again 58 days after blossom. ABA level increased gradually in late growth period (79 days after blossom). As for abnormal fruits, ABA level was lower during the period of 23 days after blossom, and then increased gradually. ABA level attained a peak value of 926.99 ng/g FW in B on 9th July while 638.84 ng/g FW in A on 25th July, a little later compared to B. Subsequently, ABA level of abnormal and normal in A, B and CK tended to be basically consistent (Figure 5).

GA_3 levels in normal fruits attained peak values of 1424.16 ng/g FW and 1382.96 ng/g FW on 8th and 15th August in A and B during the growth process, respectively, which were higher than that in CK. As for abnormal fruits, GA_3 level was lower in the whole process, and attained peak values of 559.88 ng/g FW and 921.48 ng/g FW 23 days after blossom in A and later in B, respectively. GA_3 level was higher in B than that in A, but attained the peak value later. Subsequently, GA_3 level of tended to be basically consistent with normal fruits (Figure 6).

4. Discussions

Plant growth regulators applied in production promoted growth through boosting cell division and increasing cell volume, which ascribed to comprehensive effects of many hormones. In the present paper, results showed that after treatment of grape swelling agents, the transect and longitudinal diameter of normal fruits increased rapidly, and longitudinal diameter was larger than transect one. Therefore, indices of fruit shape increased which indicated that swelling agents could significantly enhance the longitudinal growth of grape flesh cell, and was not in accordance with the results of Zhao et al (1998, PP. 28-29) and Dai et al (2002) while in line with Wan et al (2004, PP. 13-14) and Wang et al (2004). Meanwhile, as seen from the growth of abnormal fruits, fruits was under stagnation state during the first rapid swelling period and had no changes with no significant difference between the longitudinal and transect diameter in the early stage of growth. IAA, ABA and ZR levels were higher in normal fruits than abnormal ones possibly due to their effects on the regulation of fruits in early growth stage, which was similar to the study of Tao et al (1994, PP. 35-40) on kiwifruit.

Seeds of development was the source of plant growth hormones for normal growth of fruits(Cui, 2006). Through the treatment of A and B, IAA level was higher than CK when GA_3 was involved which indicated that GA could enhance the synthesis of IAA and conformed to the previous studies(Fan, 2004, PP. 728-733, Uilger, 2004, PP. 89-95, Yu, 2003, PP. 125-129). Gong speculated that the volume growth and embryo sac development of some fruits were strongly inhibited, and fruits were under a stage of young fruit in a long term with no edible values, called small green grains, namely rigid abnormal fruits in the present paper. IAA level was lower in abnormal fruits that that in normal ones. Huang et al (1994, PP. 125-129) also speculated that fruit growth depended on the seeds of normal growth, and the outdision of all hormones in seeds stimulated the growth of flesh tissues all around. Therefore, treatments of exogenous

hormones played a vital role in the growth and development of fruits. On the other hands, ovaries grew rapidly once after pollination and fertilization, or otherwise deteriorated, which was due to that embryo and endosperm became the centre of producing IAA after fertilization.

In the present paper, both A and B have promotion effects on ZR, especially in the early stage of fruit growth. ZA level in B was far higher than that in A and CK which conformed that cytokinins mainly produced young fruits. This might be related to the synergistic effects of IAA, for cytokinins could coordinate fruit sink to distribute nutrients in the presence of IAA. As for rigid abnormal fruits, ZA level was consistent and lower compared to CK in the whole process of growth, which indicated that cytokinins was highly associated with the promotion of cell division and extension and the growth and development of fruits, similar to the report of Zhou et al who found that exuberant cell division was closely related to CTK level in the early stage of seedless Litchi fruit growth (Zhou, 1998, PP. 236-240).

High level of ABA in the early stage of grape fruit growth might have stimulatory effect on cell division and meristem activities. ABA attained a second peak level about 58 days after blossom, which was probably associated with transport of substancesin cell, sugar accumulation and initiation of fruit afterripening in grape berries. IAA and GA_3 level was low and stable while ABA increased again about 79 days after blossom. This increment of ABA played a more important role in the declining of GA_3/ABA than that of GA_3, which showed that a new balance mainly attributed to ABA among endogenous hormones was confirmed and determined the physiological state and storage characteristics of grapes postharvest. Beruter found that ABA level was higher in the young fruits growing fast and lower in the fruits growing slowly (1983, PP. 737-743). It was obvious that increment of ABA was beneficial to the promotion of assimilates absorption of metabolic pool cells in line with that ABA level was lower than CK in the early stage of rigid abnormal fruits. Moreover, ABA level was high in grape fruits at high temperature in summer, might due to the functions of ABA on promotion of stomatal closure, reduction of transpiration rate, enhancement of dormancy and high temperature and drought resistance.

In A and B, GA_3 level in normal fruits was obviously higher than that in CK. GA_3 level in abnormal fruits was all higher than that in normal ones 23 days after blossom, decreased subsequently during the period of fruit growth and then became lower than that in normal fruits, which indicated that GA_3 was closely associated with the growth and development of fruits. Ma et al(2007) speculated that effects of GA on the fruit growth was exerted through enhancing the synthesis of IAA, promoting fibrovascular growth and nutrient distribution in combination with IAA, and boosting the enlargement of flesh cells. Hu et al (1997, PP. 36-38) considered that exogenous GA treatment could significantly enhanced the allocation of ^{14}C photosynthetic product to fruit cluster. Exogenous GA could enhance amylase activities of many plants, resulting in degradation of storage starch in order to provide abundant energy substrates and bond structure, and thus promoted the growth and development of fruits. Exogenous GA could also cause parthenocarpy instead of seeds. It was reported that GA level in ovaries of parthenocarpical grapes and persimmon after blossom was higher than that in seeded fruits (Ma, 1998, PP. 27-36), and was in accordance with our research, which was maybe one causative factor of rigid abnormal fruits.

5. Conclusions

Investigation indicated that the involvement of GA_3 could result in rigid abnormal fruits. Both exogenous GA_3 and GA_3+6-BA mixture could enhance endogenous hormone levels such as IAA, ZA, ABA and GA_3 in normal fruits but had no significant effects on rigid abnormal ones. The causative factors for rigid abnormal fruits in our observation could be listed as follows;

(1) Maybe associate with pollination and fertilization.

(2) As seen from hormone level, IAA, ABA and ZR level which promoted cell division and enlargement in rigid abnormal fruits was lower than that in CK, and was in a stable and lag state which was inadequate for normal growth of fruits in the whole process of growth. Herein, rigid abnormal fruits emerged chronically in the stage of young fruit and did not fall.

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Table 1. Effect s of plant growth regulators on the quality of Red Globe berry

	longitudinal diameter	Transect diameter	Single grain weight	Biggest grain weight	TSS
GA_3	2.69ABb	2.37Aa	10.11Aa	16.23Aa	17.94Aa
GA_3+6-BA	2.84Aa	2.50Aa	9.86Aa	18.33Ab	18.29 Aa
CK	2.57Bb	2.46Aa	9.76Aa	15.77 Ab	17.67 Aa

Note: Capital and small letter represent significant at $p \leq 0.01$ and $p \leq 0.05$,respectively

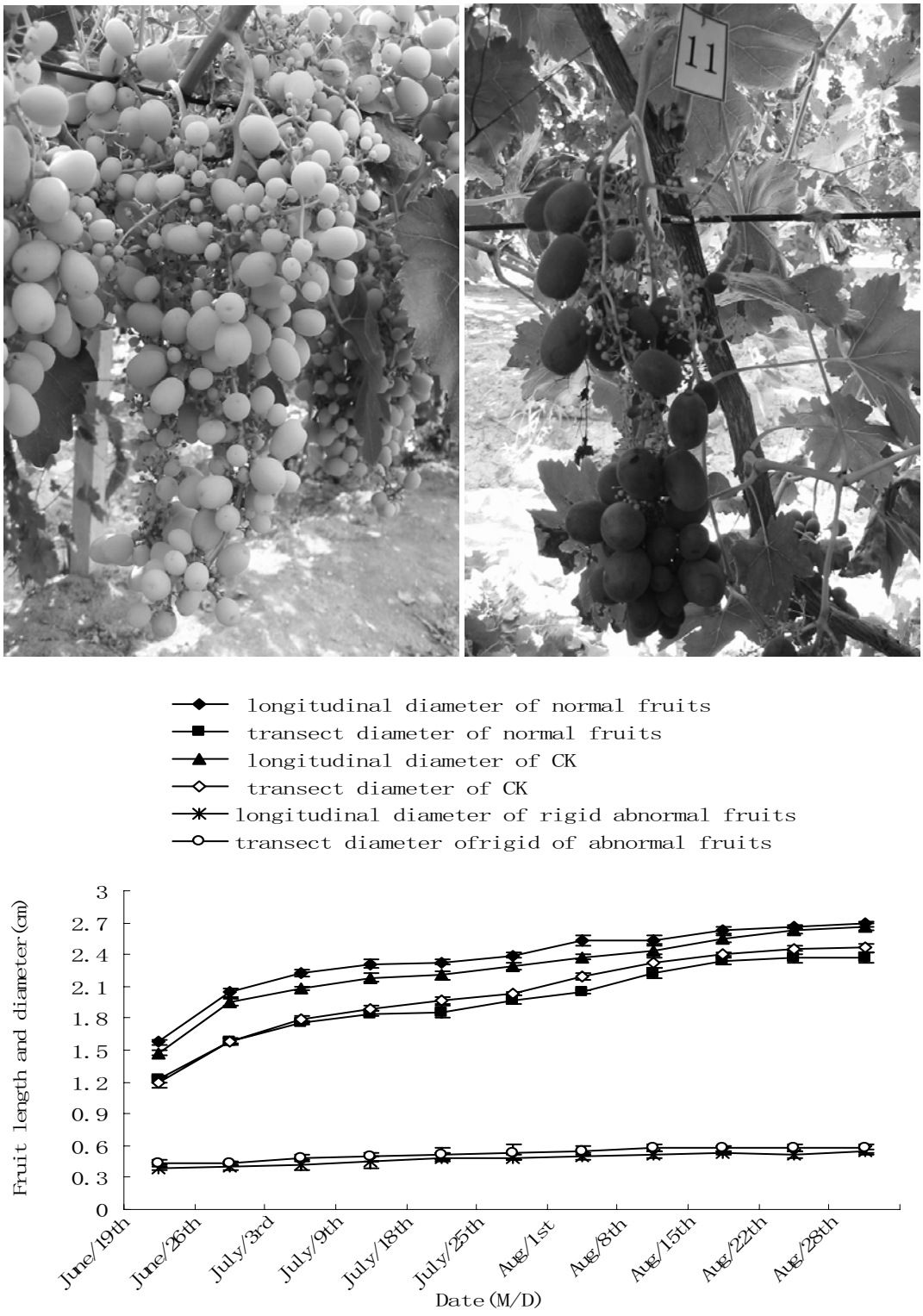

Figure 1. Effects of GA3 treatment on the transect and longitudinal diameter of normal fruit and abnormal ones

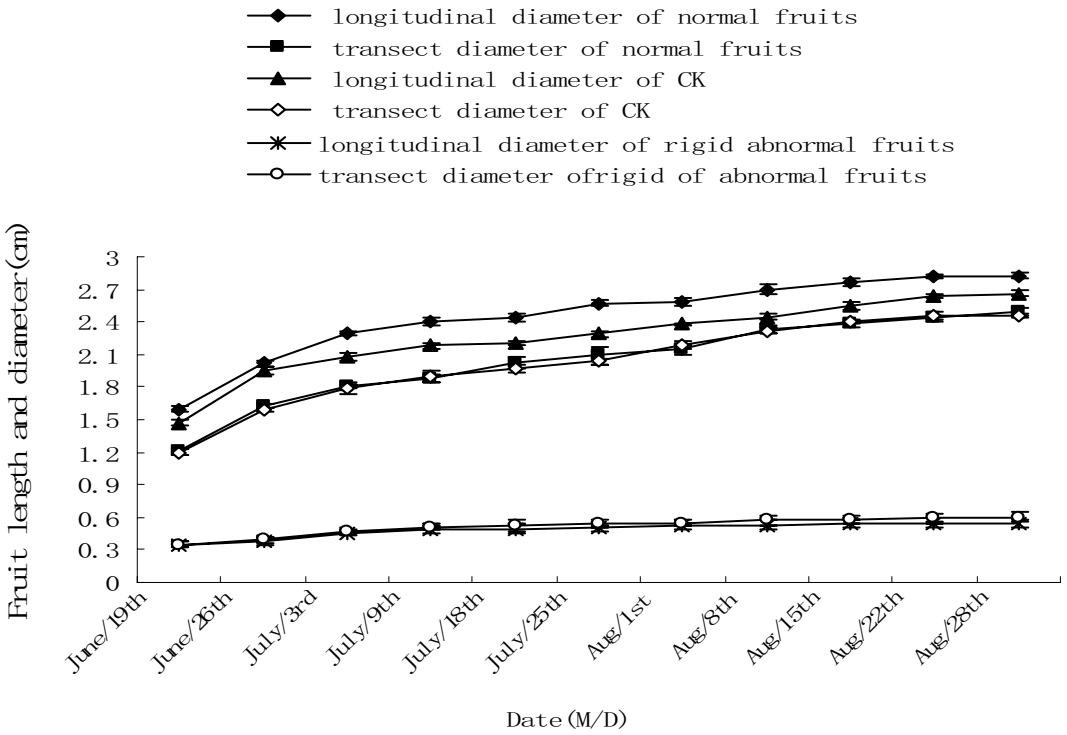

Figure 2. Effects of GA_3+6-BA treatment on the transect and longitudinal diameter of normal fruit and abnormal ones

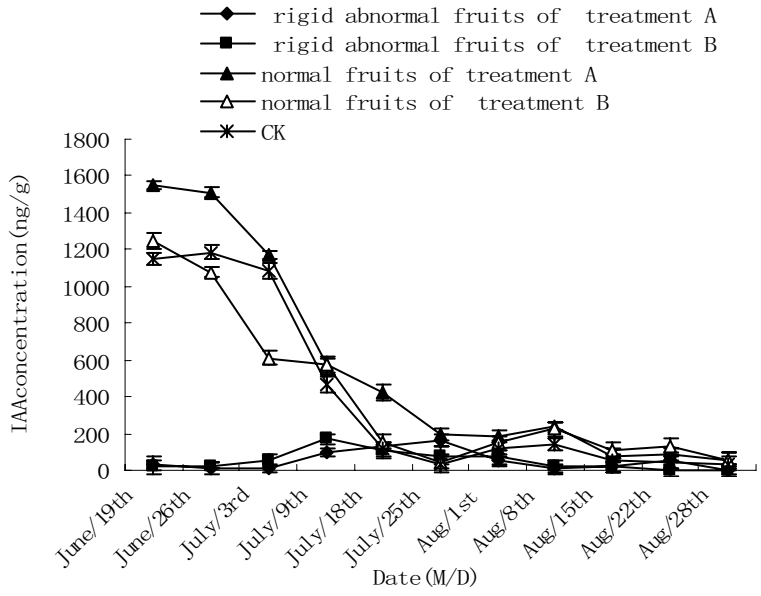

Figure 3. IAA level in fruits after A and B

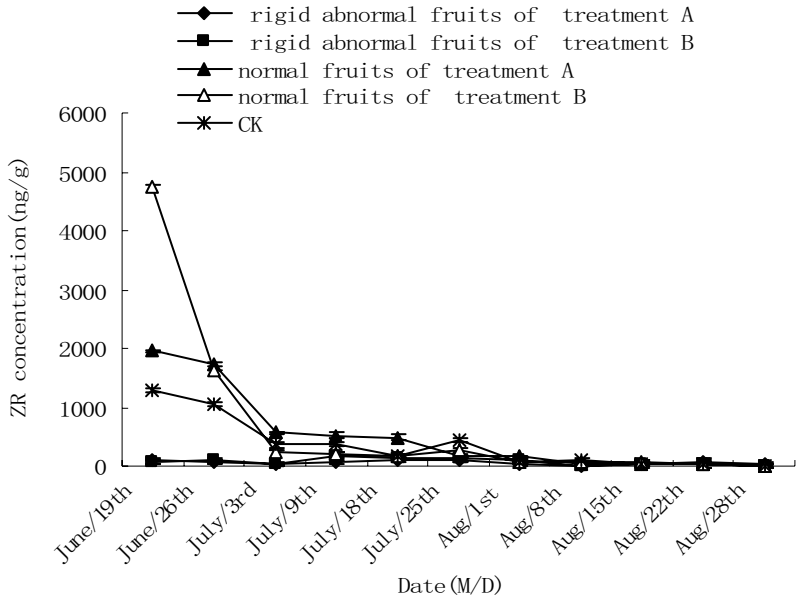

Figure 4. ZR level in the fruit after A and B

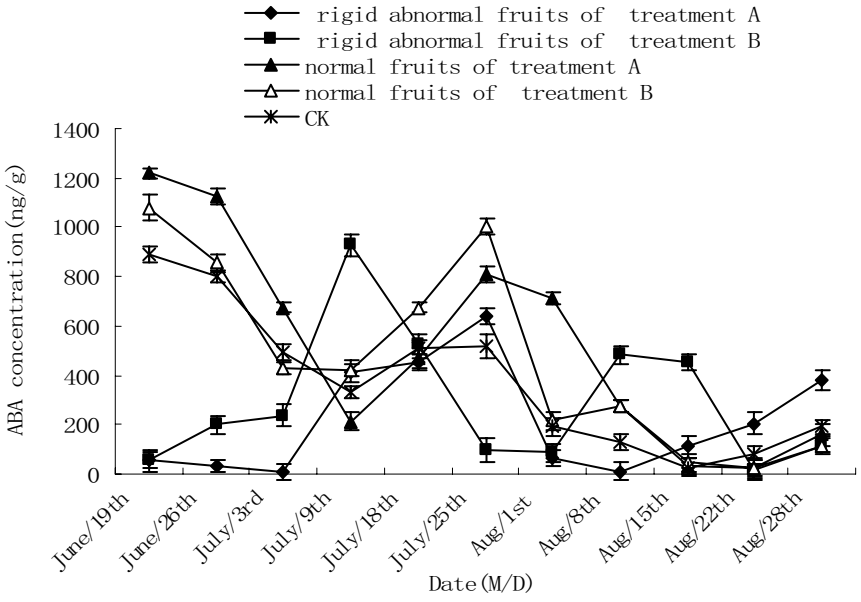

Figure 5. ABA level in fruits after A and B

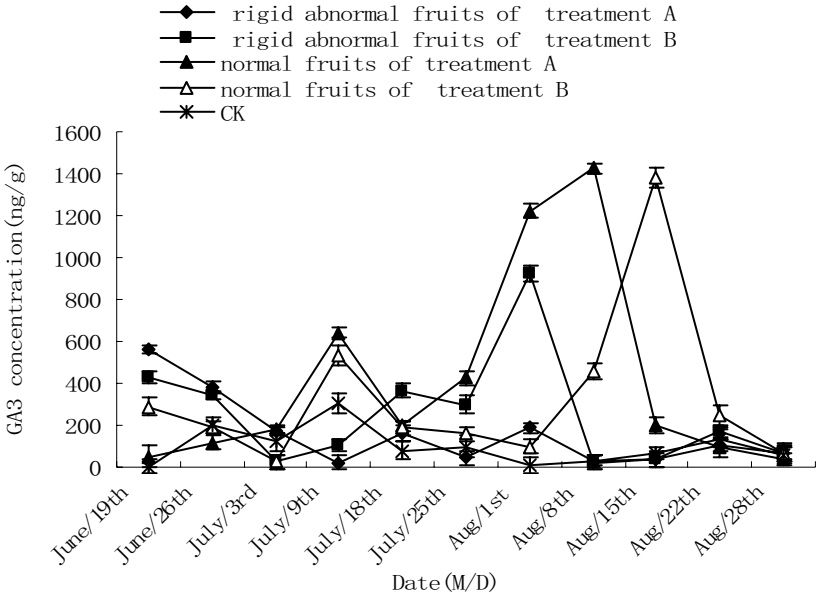

Figure 6. GA_3 level in fruits after A and B