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The Development of Chiehqua Flowers

Houcheng Liu* • Riyuan Chen • Guangwen Sun

College of Horticulture, South China Agricultural University, Guangzhou 510642, China

Corresponding author: * liuhch@scau.edu.cn

ABSTRACT

Chiehqua (*Benincasa hispida* Cogn. var. *chieh-qua* How.) is one of the main vegetable gourds widely grown in South China. Chiehqua begin flower primordia differentiation at the 6th node in seedlings with only two expanded leaves. There is a positive linear relationship between the nodes of expanded leaves and the nodes of flower bud differentiation and also between the nodes of sexually differentiated flowers. The monoecious sexual type is most prevalent in chiehqua. The flower primordia differentiation and female flowers differentiation in chiehqua seedlings are enhanced by low temperature and short days, and also by lower plant intensity and N fertilizer level. Higher IAA and GA_3 contents, a lower ratio of ABA/IAA, ZT/IAA and higher GA_3/ZT in the stem-tip favor female flower formation in chiehqua. Higher Put, Spd and total polyamine contents and lower Cad content, higher ratio of Spd/Pas and lower ratio of (Put+Cad)/(Spd+Spm) in stem tips favor female flower formation in chiehqua. Exogenous GA_3 application increases the number of male flowers, but silver thiosulphate decrease their presence. The antioxidant enzymes (POD, CAT, SOD, PPO) might influence flower sex expression by influencing ethylene production.

Keywords: antioxidant enzyme, flower primordia differentiation, flower sexually differentiation, nitrogen fertilizer, phytohormone, plant density, polyamine, temperature

Abbreviations: ABA, abscisic acid; **Cad**, cadaverine; **CAT**, catalase; **DPFF**, differentiation proportion of female flower; **GA**, gibberellin; **IAA**, indole acetic acid; **PA**, polyamine; **PGR**, plant growth regulator; **POD**, peroxidase; **PPO**, polyphenoloxidase; **Put**, putrescine; **SOD**, superoxide dismutas; **Spd**, spermidine; **Spm**, spermine; **ZT**, zeatin

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INTRODUCTION

Chiehqua (*Benincasa hispida* Cogn. var. *chieh-qua* How.; Cucurbitaceae) has long been grown in Guangzhou, China. It was mentioned in old literature 300 years ago. It is one of the main vegetables widely grown in Guangzhou where it can be cultivated outdoors in spring, summer and autumn. Chiehqua is a climbing annual gourd vegetable with a strong root system and has a vine stem with 5-7 ridges and many lateral stems, which are coated with hairs. Chiequa's palmate leaf with 5-7 lobes and serrate margin, leaf surface and petiole are covered with stiff hairs. Chiequa has a solitary, yellow flower. Chiehqua's green, cylindrical pepo is coated densely with hairs, with or without waxy powders at maturity. Both young and matured fruit can be cooked, but the former is better. In chiehqua, monoecy is the most prealent, and other sexual types such as gynoecy have been developed (Guan 1994). The regulation of sexual development in chiehqua is under genetic, environmental and hormonal control.

This review serves to introduce regulation of flower differentiation in chiehqua, and how it is influenced by temperature, plant density, nitrogen fertilizer, exogenous GA_3 and silver thiosulphate application, the relationship between flower differentiation and contents of endogenous phytohormones, polyamines, and activities of antioxidant enzymes.

FLOWER DIFFERENTIATION IN CHIEHQUA

Flowers are formed from axillary buds early in the seedling stage in chiehqua, and can be found on lower main stem nodes. Morphological studies have found that male and female flowers arise from anatomically similar 'presexual' primordial, from which the male or female flowers subsequently develop fully (Guan 1994). There are differentiated flower primordia at the 6th node in seedlings with only 2 expended leaves (Song 2004). Flower buds differentiate at the 4th to 5th node from the terminal apex, and male and female flowers differentiate at the 11th to 12th node from the terminal apex. For example, there are 10-11 nodes with differentiated flower buds and 3-4 nodes with sexually differentiated flowers in chiehqua plants with a total of 15 nodes. There is a strong positive linear relationship between the nodes of expanded leaves and those of differentiated flower buds and those of sexually differentiated flowers.

Monoecy is the most prevalent sexual type in chiehqua, in which male and female flowers are found on the same plant. But cultivars differ in the ratio of male to female

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flowers, and in the 1st node bearing female flowers. In chiehqua, some cultivars with gynoecious flowering have been developed. Homozygous gynoecious lines usually produce only female flowers, but in the heterozygous form, male flowers can frequently be found, especially under environmental conditions favoring male flower expression. Other sexual types have been developed in chiehqua, such as androecious (male flowers).

The 1st male flower usually blossoms at the 3rd-5th node at the base of the main vine, and the 1st female flower usually blossoms at 5th-8th node (Guan 1994). The node bearing 1st male or female flower differ according to cultivar and environmental condition. In the main vine, female flowers blossom at intervals of 4-5 male flowers, and the 2nd female flower usually blossoms at 9th-12th node, the 3rd female flower at the 13th-17th node, the 4th female flower at the 17th-20th node, and the 5th female flower at the 20th-24th node. In lateral vines, in contrast, the 1st female flower blossoms at the 1st-2nd node, and female flowers also blossom at intervals of 4-5 male flowers, as in the main vine.

INFLUENCE OF ENVIRONMENTAL FACTORS

The regulation of sexual development is under genetic, environmental and hormonal control. Temperature and photoperiod have an important influence on flower primordia differentiation and sex expression in chiehqua. Under 10-30°C and a 9-13 h photoperiod, flower primordia differentiation and sex expression begin at the seedling stage, and cool and short-day conditions favour flower primordia differentiation and the production of female flowers (Guan 1994). In January and February of Guangzhou, China, the mean temperature is about 13-14°C, and photoperiod is about 9~10 hrs. Under this condition, the nodes of flower primordia differentiate, female flower blossoms decrease and sometimes appear earlier than male flowers.

A lower temperature treatment favors male and female flower differentiation in chiehqua (Huang 2005). There are significant differences in the node of 1st male flower blossoms between higher temperature treatment (30°C/25°C, day/night) and lower temperature treatment (15°C/10°C); significant differences in the node of 1st female flower bud appearance between 3 temperature treatments. However, there is no significant difference in node of 1st female flower blossom and days from sowing to 1st female flower blossom, and lower temperature treatment increases the days from sowing to 1st male flower blossom. This may be due to that the seedling growth decrease under lower temperature, and some female flower abortion after differentiation.

The influence by temperature may occur during flower primordia differentiation, or during the development of the flower to blossom. Low temperature may inhibit male flower development after differentiation, leading to precocious female flowering. The number of male and female flowers reaching blossom might be influenced by temperature in a cultivar-dependent manner (Guan 1994).

High light conditions generally favor female flower production in chiehqua, while shading, or low incident radiation delays the onset of female flowering. Under lower plant population density, the node of the 1st female flower and days from sowing to the 1st female flower blossom decrease (Wei *et al*. 2001). High plant population density or close spacing within rows increases male flowering and may operate through reduced light levels available to individual plants in crowded stands.

The nitrogen status of the plant also influences sex expression in chiehqua, with high ($N_{24}K_{18}$) nitrogen fertilizer levels delaying the production of female flowers (Guan 1994). The number of female flowers and ratio of female to male flowers also decreases at high nitrogen fertilizer levels.

In summary, the environmental conditions which reduce carbohydrate build-up such as high temperature, low light conditions, high nitrogen level and close spacing increase the tendency for male flower production in chiehqua.

INFLUENCE OF PHYTOHORMONES

Evidence both from studies in which endogenous levels of the growth hormones are related to sex expression and from the effect of applying compounds exogenously to the plant show that plant growth regulators (PGRs) play a key role in determining flower gender in chiehqua. Use of PGRs to manipulate sex expression has facilitated the development of hybrid cucurbit cultivars.

It has been known since the early days of plant hormone research that auxin is involved in sex expression of cucurbits; almost 30 years ago its exact role was uncertain (Rudich 1990). Lower temperature treatment (15°C/10°C) at the seedling stage, which fosters female flower formation, increases the endogenous auxin level in stem tips (Huang 2005). A clear role for auxin is difficult to determine, in part because higher levels of auxin cause the liberation of ethylene in tissues. In addition, ethylene has been shown to inhibit auxin translocation, and to contribute to the inactivation of auxin through decarboxylation (Beyer and Morgan 1969). Finally the reliable determination of endogenous auxin levels in plants has been difficult and has contributed to the uncertainty of the role of this hormone in sex expression of cucurbits.

There is considerable uncertainty about the role of abscisic acid (ABA) in sex expression of cucurbits (Rudich 1990). Higher temperature treatment (30°C/25°C) and lower temperature treatment (15°C/10°C) at the seedling stage both increases the endogenous ABA level in stem tips (Huang 2005), while the ratio of ABA/indole acetic acid (IAA) in stem tips under a lower temperature treatment (15°C/10°C) decreases. Regression analysis shows that there is a significant linear regression relationship between the number of female flowers in the 6th-15th node in the main vine and the ratio of ABA/IAA in chiehqua stem tips ($y = -4.5952x + 3.4801$, $R^2 = 0.9701$). Thus the ratio of ABA/IAA in stem tips might play an important role in chiehqua flower sex differentiation, while a lower level of ABA/IAA may favor female flower formation. ABA might not play a direct role in sex expression, but might have a secondary involvement in response to the more important action of other hormones such as IAA in sex expression.

There were differences in the differentiation proportion of female flowers (DPFF) in 4 chiehqua cultivars (lines) (Liu *et al*. 2006). Differences between the contents of IAA, gibberellic acid (GA_3), zeatin and ABA in stem tips of chiehqua plants were found at two sampling stages: in the 1st stage, plants with 10 expanded leaves showed flower sex differentiation at the 14-20th node; in the 2nd stage, plants with 19 expanded leaves showed flower sex differentiation at the 23-35th node. The IAA contents in stem tips of cvs. '14-2', 'Yueke No. 2', 'Texuan No. 4' and 'Huangmao' decreased in succession at both sampling stages, and there was a significant positive correlation between IAA contents in stem tips and DPFF of corresponding nodes at both sampling stages (r = 0.732, 0.949, respectively) (P<0.01). There were significant differences between GA_3 content in stem tips of 4 cultivars (lines) at both the 1st and 2nd sampling stages (P<0.05), The GA_3 contents in stem tips of cvs. '14-2', 'Yueke No. 2', 'Texuan No. 4' and 'Huangmao' decreased in succession at both sampling stages, which is consistent to the DPFF of corresponding nodes. Moreover, there was a significant positive correlation between GA_3 content in stem tips and DPFF of corresponding nodes at the 2nd sampling stage (r = 0.949) (P<0.01) indicating that higher GA_3 content in stem tips favors female flower differentiation in chiehqua plants.

There were significant differences between zeatin content in stem tips of the 4 cultivars at the 1st sampling stage (P<0.05), but no significant differences at the 2nd sampling stage; moreover, the zeatin contents were lower than those of the 1st stage in all 4 cultivars (lines). There were significant negative correlations between zeatin content in stem tips at the 1st sampling stage and DPFF at corresponding nodes (P<0.05), but small correlations at the 2nd sampling stage. The ABA content in stem tips of cvs. '14-2', 'Yueke No. 2', 'Texuan No. 4' and 'Huangmao' decreased in succession at the 1st sampling stages, and there was a significant positive correlation between ABA content in stem tips and DPFF of corresponding nodes ($r = 0.950$) ($P<0.01$), but a negative correlation at the 2nd sampling stage ($r = 0.253$).

A significant positive correlation between IAA content in stem tips and DPFF of corresponding nodes and a positive correlation between GA_3 content in stem-tips and DPFF of corresponding nodes in chiehqua was found at both sampling stages. These results indicate that higher IAA and GA_3 content in stem-tips favored female flower differentiation in chiehqua. However the correlation between zeatin or ABA contents in stem-tips and DPFF of corresponding nodes are ambivalent at both sampling stages in chiehqua. The biological effects of phytohormones do not just rely on the absolute content of a certain hormone, but usually their relative contents. The ratios of GA_3/ZT in the stem-tip of cvs. '14-2', 'Yueke No. 2', 'Texuan No. 4' and 'Huangmao' decreased in succession at both sampling stages, and there was a significantly positive correlation between GA_3/ZT in stem-tips and DPFF of the corresponding nodes both at both sampled stages (r = 0.819, 0.984, respectively) ($P<0.01$). However the ratios of ZT/IAA in stem-tips of cvs. '14-2', 'Yueke No. 2', 'Texuan No. 4' and 'Huangmao' increased in succession at both sampling stages, and there was a significantly negative correlation between ZT/IAA in stem-tips and DPFF of corresponding nodes both at both sampling stages ($r = -0.860, -0.838$, respectively) ($P<0.01$). There was no correlation between GA_3/ABA in stem-tips at the 1st sampling stage and DPFF of corresponding nodes, but there was a significantly positive correlation at the 2nd sampling stage ($r = 0.782$) ($P<0.01$).

A significantly positive correlation between GA_3/ZT in stem-tips and DPFF of corresponding nodes and a significantly negative correlation between ZT/IAA in stem-tips and DPFF of corresponding nodes was found in chiehqua plants at both sampling stages. These indicated that sex differentiation in chiehqua plants could be prognosed by GA_3/ZT and ZT/IAA in stem tips.

The number of male flowers increases and that of female flowers decreases by application of GA_3 on chiehqua plant with 2 expanded true-leaves (Wei *et al*. 2003). As the concentration of GA_3 increases, more male flowers form while the number of female flowers decreases. The number of nodes bearing the 1st male flower decreases but the number of nodes bearing the 1st female flower increase significantly following the application of GA_3 (Wei *et al*. 2003).

Th enumber of male flowers increase following the application of GA_3 but decrease after silver thiosulphate is applied (Chen *et al*. 1999), and as the concentration of GA_3 increases, so the number of male flowers increase. The effects of application of GA_3 on male flowers depend on the plant growth stage: the number of male flowers increase more by application of 1.5 mmol/L GA_3 at the stage with a 3rd true leaf than at the stage with the 1st true leaf.

On the whole, it can be postulated that auxins and gibberellins are the most important phytohormones for sex differentiation in chiehqua plants, while the biological effects of other phytohormones are mediated by the stimulation and inhibition of their activity.

INFLUENCE OF POLYAMINES

Polyamines (PAs) are ubiquitous nitrogenous compounds that appear to be present in all organisms. Putrescine (Put), spermidine (Spd) and spermine (Spm) are recognized as plant growth regulators that are present in plants (Kumar *et al*. 1997). There are many observations correlating alterations in polyamine titers to many physiological and developmental processes like normal and abnormal growth, cell division, differentiation, embryogenesis, fruit ripening, flower development, and normal or stress-induced senescence (Galston and Kaur-Sawhney 1995; Kakkar *et al*. 2000).

PAs in the stem tips of chiehqua plants are involved in flower bud formation. At both sampling stages (1st stage: plants with 10 expanded leaves in which flowers show sex differentiation at the 14-20th nodes; 2nd stage: plants with 19 expanded leaves in which flowers show sex differentiation at the 23-35th nodes), the contents of Spd were higher than those of Put, cadaverine (Cad) and Spm in stem tips (Liu *et al*. 2007). At the 1st sampling stage, the correlation coefficient between polyamine contents and DPFF are low while at the 2nd sampling stage, there are significant positive relationships between Put, Spd and total polyamine contents and DPFF, and a significant negative relationship between Cad content and DPFF. At both 2 sampling stages, there is a significant positive relationship between the ratio of Spd/PAs and DPFF, and a significant negative relationship between the ratio of (Put+Cad)/(Spd+Spm) and DPFF. Thus the ratio of Spd/PAs and (Put+Cad)/(Spd+Spm) play an important role in female flower formation in chiehqua plants.

INFLUENCE OF ANTIOXIDANT ENZYMES

The activities of antioxidant enzymes in stem tips were closely related to sex differentiation in chiehqua. Lower temperature treatment (15°C/10°C) at the seedling stage, which fosters female flower formation, enhance the activities of peroxidase (POD) and catalase (CAT) and weakens the activities of superoxide dismutase (SOD) in stem tips of chiehqua plants (Huang 2005). These relationships are different from the growth stage (Song 2004). There is a positive relationship between POD activity and DPFF, a negative relationship between CAT activity and DPFF at both sampling stages, while the relationship between the activities of polyphenoloxidase (PPO), SOD and DPFF different at both sampling stages. The antioxidant enzymes play a role in scavenging reactive oxygen species (ROS) in plants, and there is an interplay between ethylene and ROS (Moeder *et al*. 2002), thus the antioxidant enzymes might influence flower sex expression by influencing ethylene production.

CONCLUDING REMARKS

Chiehqua has for a long time been grown as one of the main vegetables in Guangzhou, China. Chiehqua has been introduced for planting in other regions of China and other countries where Chinese reside Yield production of chiehqua is closely related to flower differentiation. Control of gender expression in chiehqua appears to act primarily at the 'presexual' primordia stage that allows either male or female flowers to development, and which begins at a very early seedling stage. Flower primordia and female flower differentiation are enhanced by short days, lower plant intensity and the N fertilizer level. Little is known about the hormonal control of these processes. Higher endogenous GA_3 contents favors female flower formation (Liu *et al*. 2006) but exogenous GA_3 application increases male flowers (Wei *et al*. 2003). The effects of phytohormones and polyamines on flower differentiation are dependent on the growth-stage (Liu *et al*. 2006, 2007). Antioxidant enzymes might influence flower sex expression by influencing ethylene production (Song 2004), although the role of ethylene in flower sex expression is not clear. Further studies are needed to elucidate the role of phytohormones and polyamines in flower gender determination, and to understand the mechanism of flower primordia and female and male flower differentiation.

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