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Research Progress of Flower Bud Differentiation of Apple

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Abstract Flowering is a prerequisite for apple fruiting, and apple flower buds are mixed buds, that is, the vegetative organs and flower structure exist in the same terminal bud simultaneously, which are formed in the year before flowering and fruiting, mainly including spur terminal buds and axillary buds. The infrequent formation of flower buds during its growth and biennial bearing are closely related to flower bud differentiation. Therefore, this paper reviews the research progress of flower bud differentiation of apple from the morphological differentiation, plant hormones and flowering-related genes, in order to provide a theoretical reference for efficient cultivation and stable yield of apple.

Key words Apple, Flower bud differentiation, Hormone, Gene

1 Introduction

Apple (*Malus domestica* Borkh.) is an important fruit tree resource in the world, and the planting area and yield of apple in China rank first in the world[1]. The flower buds of apple are mixed buds, that is, the vegetative organs and flower structure exist in the same terminal bud simultaneously, which are formed in the year before flowering and fruiting, mainly including spur terminal buds and axillary buds[2]. The number of flower bud formation, the quality of flower buds and the time of flowering seriously affect the economic benefits of apple. For apple, there are some problems in the cultivation and production, such as difficulties in flower bud formation, biennial bearing and long juvenile period, which are closely related to flower bud differentiation. Therefore, this paper reviews the research progress of flower bud differentiation of apple from the morphological differentiation, plant hormones and flowering-related genes, in order to provide a theoretical reference for efficient cultivation and stable yield of apple.

2 Flower bud morphological differentiation of apple

Flower bud differentiation is completed through three stages of flower induction (flowering determination), flower bud initiation and flower bud development. Flower induction stage, also known as physiological differentiation stage of flower buds, is a critical period for regulating flower bud differentiation. Leaf picking (or ring stripping plus leaf picking) and fruit picking are the main research methods[3-4]. This period is closely related to the time when branches stop growing, and apple trees enter physiological differentiation at that time. The time required for physiological differentiation of the same variety in the same region is relatively stable in different years. In Shunping area of Baoding, it takes about 40 d for 'Tianhong 2' apple from growth cessation of short branches to the end of physiological differentiation[5], and there are significant differences in time from growth cessation of short branches to the end of physiological differentiation of different varieties in the same region. In Yangling area, it takes 56 d for 'Changfu 2' and 49 d for 'Yanfu 6'. Morphological differentiation of axillary flower buds begins in late July[6]. Further studies have shown that the flower bud differentiation of autumn shoots of early maturing apple 'Liaofu' is in succession, and the flower bud morphological differentiation in the middle part starts at 3 - 4 weeks post growth cessation of corresponding leaves[7], while the time required for physiological differentiation of axillary flower buds of different varieties needs to be further studied. The stage of flower bud initiation is an irreversible process[8]. Foster *et al.*[9] took the widening of apex growth point as a sign of the beginning of this period. The stage of flower bud development is the process that various primordia of flower organs successively differentiate and grow, and further develop into a complete flower organ. Predecessors dedicated many efforts to the morphological anatomy of flower buds. In recent years, the process of flower bud morphological differentiation has also attracted much attention. The conversion stage (flower initiation stage), flower bud differentiation stage, inflorescence primordial stage, calyx primordial stage, petal primordial stage, stamen primordial stage and pistil primordial stage occur successively among different buds and overlap mutually. There are differences in the start time and end time of terminal bud morphological differentiation between 'Gala' and 'Fuji' spurs in the same region[4,10]. The occurrence time and duration of flower bud differentiation of 'Tianhong 2' in the same region are affected by rootstock and year, but the concentrated differentiation stage is not affected[5]. Axillary flower bud differentiation of autumn shoots occurs successively in apple[7], and the process of morphological differentiation of each flower bud has not been elaborated in detail.

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3 Plant hormones and flower bud differentiation of apple

Flower bud differentiation is closely related to various hormone levels in the body, and requires the coordination of various hormones. Therefore, hormone levels and hormone balance at different stages are crucial. Endogenous hormones play an important regulatory role in all stages of flower bud development[11]. Kondo *et al.*[12] suggested that IAA content might be related to flower bud differentiation of apple. Some scholars held that high IAA content could promote the activation of flower bud initiation of apple[13], while some scholars proposed that high IAA content might have an inhibitory effect on apple flowering[14]. Cytokinin promotes the formation of flower buds[15]. Exogenous application of 6-BA induces the increased expression of *MdTFL*1 and promotes apple flowering[16-17]. Sanyal *et al.*[18] showed that the decrease of ZR content increased the proportion of flower buds. Wang *et al.*[19] put forward that the decrease of ZR content was conducive to floral induction. GA3 inhibits the flowering of woody plants[20], and low GA3 content is conducive to floral induction[21-22]. Spraying GA3 would break the balance of ZR/GAs, and inhibit the expression of *MdSPLs*, thus inhibiting the flower bud formation of apple[23]. Chang *et al.*[24] found that GA3 might promote flowering. High content of ABA promotes floral induction of apple, and spraying uniconazole increases ABA content in terminal bud and accelerates the process of flower bud differentiation[25]. In addition, the balance of endogenous hormones is considered to be a key factor in flower bud differentiation[26-27]. Hence, there are different views about the effect of endogenous hormones on flower formation of apple.

4 Flowering genes and flower bud differentiation of apple

There have been reports on molecular biology related to flower bud differentiation of apple. In apple, in addition to the two homologues *AFL*1 and *AFL*2, there are other homologues of *LFY* gene, but *AFL*1 and *AFL*2 have low homology with other homologous genes. Taking 'Jonathan' apple as the test material, Kotoda *et al.*[28] and Masato *et al.*[29] pointed out that *AFL*1 was only expressed during the transformation process from vegetative buds to floral buds, while *AFL*2 was expressed at the tip of vegetative shoots, floral buds, floral organs and roots. Cao *et al.*[30] also studied *LFY* homologous genes in 'Jonathan' apple; the results showed that *AFL*1 and *AFL*2 were homologous genes of *LFY*, and their base sequences had 90% homology in the coding region, but only about 60% homology in the 3' non-coding region; *AFL*2 was expressed in sepals, ovaries, roots, stems, leaves and terminal buds, while *AFL*1 was only expressed in terminal buds; *AFL*1 was clearly expressed in the terminal buds at different developmental stages after the terminal buds stopped vegetative growth and turned to reproductive growth, while *AFL*2 was expressed from June to October, consistent with previous studies. Zhang[31] took three main cultivars 'Starkrimson', 'Gala' and 'Fuji' as materials, and found that *AFL*1 expression lasted from the beginning to winter dormancy, while *AFL*2 also lasted from the beginning of expression, which was somewhat different from previous reports.

Two AP1 homologous genes, named *MdAP*1 and *MdMADS*2, have been isolated from apple. *MdAP*1 is detected only during the period of sepal differentiation in flower meristem, so it does not directly cause the initiation of flower, but it is associated with sepal and fruit development. Kotoda *et al.*[28] concluded that *MdAP*1 was only expressed in sepals of 'Jonathan' apple, nearly 2 months later than *AFL*. Kotoda *et al.*[32] also studied *MdMADS*5 from 'Jonathan' apple; overexpression of *MdMADS*5 gene in *Arabidopsis thaliana* resulted in earlier flowering, shorter inflorescence, and fewer rosette leaves, which was considered to be the same product as *MdAP*1. There were only a small part of base differences in the 3' non-coding region, and the expression pattern was consistent. *MdMADS*2, another homologous gene of *AP*1, was expressed at all stages of flower bud differentiation and in four flower organs. Transgenic tobacco expressing the *MdMADS*2 gene showed early flowering and shorter bolts, but did not show any homeotic changes in the floral organs, indicating that *MdMADS*2 plays an important role during early stages of flower development[33].

*TFL*1 gene is a flowering inhibitory gene related to flowering induction signal, and it is a functional gene maintaining the development of inflorescence meristem. Kotoda *et al.*[34] studied the expression pattern of *MdTFL*1 (homolog of *TFL*1) from 'Jonathan' apple and the performance of transforming *A. thaliana*. The study showed that the gene was expressed in vegetative tissues such as vegetative buds, stems and roots of apples, and the expression became weak about two weeks before flower bud differentiation. The transgenic *A. thaliana* blossomed later than the wild type, and its phenotype was similar to that of transgenic *A. thaliana* overexpressing *TFL*1.

Most of the flower organ identity genes belong to the MADS-box gene family (such as *AP*1, *AP*3, *PI*, *AG*, *etc.*), whose family members play an important role in the process of flower bud differentiation as transcriptional regulators[35]. At present, a cDNA containing the conserved region of MADS has been isolated from 'Fuji', named *MdMADS*1. This gene is expressed in all young fruits and flower organs, and the expression level is high in the early stage of flower and fruit development, indicating that *MdMADS*1 mainly acts on the initial stage of flower bud differentiation. The expression pattern of *MdMADS*1 is similar to *AGL*2 gene in *A. thaliana*, except that the former is not expressed in leaves[36]. *MdMADS*3 and *MdMADS*4 isolated from 'Fuji' apple have high homology with *AGL*2 and *AGL*4 of *A. thaliana*. *MdMADS*3 is expressed in stamen primordia, petal primordia and carpellary primordia, but not in fruit. *MdMADS*4 is expressed in inflorescence meristem, flower meristem, four flower organs and fruits. *Md-*

*MADS*4 is highly expressed in style vascular bundle and carpel vascular bundle of fruits in the early stage, and is transcriptionally accumulated in developing seed embryos[37]. The *AP*2 homologous gene, *MAP*2, isolated from apple flower buds exists in the genome in low copy and is expressed in vegetative tissues, flower buds and different flower organs, consistent with the *AP*2 gene expression pattern of *A. thaliana*[38].

5 Prospects

Flower bud differentiation of apple is a complex physiological and biochemical process. Signal changes in internal and external factors are transmitted to stem meristem, to initiate a series of physiological and molecular regulatory mechanisms of flower bud differentiation. The research on apple flower bud differentiation mainly focuses on the observation of morphological structure in buds, the changes in related physiological level, the cloning and expression of related genes and functional verification during differentiation of terminal buds. At present, few efforts have been dedicated to the influencing factors of flower bud differentiation of apple axillary buds and the flowering mechanism, which can be explored and studied in the future.

References

[1] CHEN XS, HAN MY, SU GL, *et al.* Discussion on today's world apple industry trends and the suggestions on sustainable and efficient development of apple industry in China[J]. Journal of Fruit Science, 2010, 27(4): 598 – 604. (in Chinese).

[2] BUBAN T, FAUST M. Flower bud induction in apple trees: Internal control and differentiation[M]. London: Palgrave Macmillan UK, 1982.

[3] HUANG H, QIAO XS, CAO SY. Study on physiological differentiation period of apple flower buds[J]. Acta Horticulturae Sinica, 1986, 13(3): 181 – 186. (in Chinese).

[4] XIN MZ, TAO L, FAN S, *et al.* Effect of latitude and altitude on flower bud differentiation of major apple cultivars[J]. Acta Horticulturae Sinica, 2019, 46(4): 761 – 774. (in Chinese).

[5] WANG JX, ZHANG H, SHAO JZ, *et al.* Studies on the dynamic development period of flower bud differentiation of 'Tianhong 2' apple[J]. Journal of Hebei Agricultural University, 2019, 42(6): 45 – 50. (in Chinese).

[6] FU YH, MENG SQ, SU RY, *et al.* Observation on phenological stage of flower bud differentiation of apple[J]. China Fruits, 1980(2): 20 – 23. (in Chinese).

[7] PAN ZG, ZHU DX, SU HR. Observation on the beginning stage of morphological differentiation of axillary flower buds in autumn shoots of apple[J]. Journal of Fruit Science, 1997, 14(3): 172 – 173. (in Chinese).

[8] HANKE MV, FLACHOWSKY H, PEIL A, *et al.* No flower no fruit: Genetic potentials to trigger flowering in fruit trees[J]. Genes Genomes Genomics, 2007, 1(1): 1 – 20.

[9] FOSTER T, JOHNSTON R, SELEZNYOVA A. A morphological and quantitative characterization of early floral development in apple (*Malus* × *domestica* borkh.)[J]. Annals of Botany, 2003, 92(2): 199 – 206.

[10] ZHU MD, ZHANG R, SHI ZG. Morphological differentiation characteristics of *Malus pumila* Mill. flower bud[J]. Journal of Anhui Agricultural Sciences, 2017, 45(4): 42 – 44. (in Chinese).

[11] CHANDLER JW. The hormonal regulation of flower development[J]. Journal of Plant Growth Regulation, 2011, 30(2): 242 – 254.

[12] KONDO S, HAYATA Y, INOUE K. Relationship between indole-3-acetic acid and flowering in two apple cultivars, Fuji and Ohrin[J]. Journal of the Japanese Society for Horticultural Science, 1999, 68(3): 563 – 565.

[13] LI TH, HUANG WD, MENG SQ. Study on the mechanisms of flower bud induction in apple tree[J]. Journal of Plant Physiology, 1996, 22(3): 251 – 257. (in Chinese).

[14] CAO SY, ZHANG JC, WEI LH. Studies on the changes of endogenous hormones in the differentiation period of flower bud in apple trees[J]. Journal of Fruit Science, 2000, 17(4): 244 – 248. (in Chinese).

[15] HOAD G. Hormonal regulation of fruit-bud formation in fruit trees[J]. Flowering and Fruit Set in Fruit Trees, 1984(149): 13 – 24.

[16] LI YM, ZHANG D, XING LB, *et al.* Effect of exogenous 6-benzylaminopurine (6-BA) on branch type, floral induction and initiation, and related gene expression in 'Fuji' apple (*Malus domestica* Borkh.)[J]. Plant Growth Regulation, 2016(79): 65 – 70.

[17] LI YM, ZHANG D, ZHANG LZ, *et al.* Identification and expression analysis of cytokinin response-regulator genes during floral induction in apple (*Malus domestica* Borkh)[J]. Plant Growth Regulation, 2017(83): 455 – 464.

[18] SANYAL D, BANGERTH F. Stress induced ethylene evolution and its possible relationship to auxin-transport, cytokinin levels, and flower bud induction in shoots of apple seedlings and bearing apple trees[J]. Plant Growth Regulation, 1998, 24(2): 127 – 134.

[19] WANG JX, LUO T, ZHANG H, *et al.* Variation of endogenous hormones during flower and leaf buds development in 'Tianhong 2' apple[J]. HortScience, 2020, 55(11): 1794 – 1798.

[20] PHARIS RP, KING RW. Gibberellins and reproductive development in seed plants[J]. Annual Review of Plant Physiology, 1985, 36(1): 517 – 568.

[21] XING LB, ZHANG D, LI YM, *et al.* Genome-wide identification of vegetative phase transition-associated micro RNAs and target predictions using degradome sequencing in *Malus hupehensis*[J]. BMC Genomics, 2014(15): 1125.

[22] XING LB, ZHANG D, LI YM. Transcription profiles reveal sugar and hormone signaling pathways mediating flower induction in apple (*Malus domestica* Borkh.)[J]. Plant Cell Physiology, 2015, 56(10): 2052 – 2068.

[23] ZHANG SW, ZHANG D, FAN S, *et al.* Effect of exogenous GA3 and its inhibitor paclobutrazol on floral formation, endogenous hormones, and flowering-associated genes in 'Fuji' apple (*Malus domestica* Borkh.)[J]. Plant Physiology and Biochemistry, 2016(107): 178 – 186.

[24] CHANG MZ. HUANG CH. Effects of GA3 on promotion of flowering in *Kalanchoe* spp.[J]. Scientia Horticulturae, 2018(238): 7 – 13.

[25] CAO SY, ZHANG QM, ZHANG JC, *et al.* Effects of uniconazol spray on flower bud differentiation and endogenous hormone level in terminal bud of apple trees[J]. Acta Photophysiologica Sinica, 2003, 29(5): 375 – 379. (in Chinese).

[26] WANG L. Effects of branch bending on endogenous hormones, carbon and nitrogen nutrition for red Fuji/*Malus hupehensis*[D]. Tai'an: Shandong Agricultural University, 2010. (in Chinese).

[27] YANG Y. Effects of branch bending angle on carbon, nitrogen and endogenous hormones contents in buds and leaves of apple trees[D]. Yangling: Northwest A&F University, 2010. (in Chinese).

[28] KOTODA N, WADA M, KOMORI S, *et al.* Expression pattern of homologues of floral meristem identity genes *LFY* and *AP*1 during flower development in apple[J]. Journal of the American Society for Horticultural Science, 2000, 125(4): 398 -403.
[29] WADA M, CAO QF, KOTODA N, *et al.* Apple has two orthologues of *FLORICAULA/LEAFY* involved in flowering[J]. Plant Molecular Biology, 2002(49): 567 -577.
[30] CAO QF, WATA MN, MENG YP, *et al.* Cloning and expression analysis of LEAFY homologues genes from apple[J]. Acta Horticulturae Sinica, 2003, 30(3): 267 -271. (in Chinese).
[31] ZHANG LS. Morphological differentiation of apple floral bud and cloning and expression analysis of genes related to flower initiation[D]. Baoding: Agricultural University of Hebei, 2009. (in Chinese).
[32] KOTODA N. Overexpression of *MdMADS*5, an *APETALA*1-like gene of apple, causes early flowering in transgenic Arabidopsis[J]. Plant Science, 2002(162): 679 -687.
[33] SUNG SK, YU GH, AN G. Characterization of *MdMADS*2, a member of the *SQUAMOSA* subfamily of genes, in apple [J]. Plant Physiology, 1999(120): 969 -978.
[34] KOTODA N, WADA M. *MdTFL*1, a *MdTFL*1-like gene of apple, retards the transition from the vegetative to reproductive phase in transgenic *Arabidopsis*[J]. Plant Science, 2005(168): 95 -104.
[35] CUI RF, MENG Z. Functional conservation and diversity of floral homeotic *MADS*-box genes in angiosperms[J]. Chinese Bulletin of Botany, 2007, 24(1): 31 -41. (in Chinese).
[36] SUNG SK, AN G. Molecular cloning and characterization of a *MADS*-Box cDNA clone of the Fuji apple[J]. Plant Cell Physiology, 1997, 38(4): 484 -489.
[37] SUN SK, YU GH, NAM JM. Developmentally regulated expression of two *MADS*-box genes, *MdMADS*3 and *MdMADS*4, in the morphogenesis of flower buds and fruits in apple[J]. Plant, 2000(210): 519 -528.
[38] ZHOU SM, SU HY, WANG L, *et al.* Cloning and transformation of apetala2 homologues genes from apple [J]. Acta Horticulturae Sinica, 2006, 33(2): 239 -243. (in Chinese).

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stant, heat-resistant and evergreen native wild lawns to solve the problem of "no lawn under the forest".

5.3 Strengthening the promotion of mixed planting mode of shade-tolerant lawn and ground cover plants Mixed planting of two or more shade-tolerant lawns or ground cover plants can make up for the defects of single ground cover plant planting and enrich the ornamental effect and seasonal landscape of ground cover plants. According to the ecological habits and ornamental value of shade-tolerant lawn and ground cover plants, mixed planting can make the varieties complement each other, improve the landscape stability and homogeneity of ground cover communities, and prolong the ornamental period.

References

[1] ZHANG LQ, ZHOU Q, XIAO CL. Advances in research of shade tolerance of garden plant[J]. Journal of Anhui Agricultural Sciences, 2006(19): 4851 -4853. (in Chinese).
[2] REN BB, SHANG R, LI F, *et al.* Close-to-nature plant community construction in urban greenspace of Beijing[J]. Chinese Journal of Ecology, 2019, 38(10): 2911 -2917. (in Chinese).
[3] QIAN YY. Analysis of shade-tolerant plants for urban multi-layer plant community construction[J]. Garden, 2013(3): 14 -15. (in Chinese).
[4] DOU J, ZHOU SY, XU ZF. Tropical native groundcover plants resources of southern Yunnan and their sustainable use in landscape[J]. Journal of Zhejiang Forestry College, 2004, 21(1): 54 -60. (in Chinese).
[5] SUN WB. Importance of indigenous plants in their application to the modern urban landscape architecture[J]. Journal of Chinese Landscape Architecture, 2003(7): 63 -65. (in Chinese).
[6] WEI SL, JIANG MH. The distribution characteristics of light intensity and plants application for greening space on ground level roads under elevated highways of diverse structures[J]. Chinese Landscape Architecture, 2016(1): 78 -82. (in Chinese).