2,4-D Treatment Could Maintain the Development of Siliques from Emasculated Flowers of an Auxin-resistant Mutant *axr1-12*

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Abstract An auxin-resistant mutant *axr1-12* was discovered twenty years ago, the mechanism of its abnormal silique development related to auxin signaling is still unclear. The effects of auxin (2,4-D), gibberellin (GA_3) and cytokinin (BAP) on emasculated flowers of *axr1-12* and its wild type plant Columbia were comparatively studied. Silique development from emasculated flower of *axr1-12* could be significantly stimulated with 1 μl of 2,4-D (0.1 nmol/pistil) while that of Columbia was severely inhibited. Additionally and interestingly, 2,4-D could also promote *axr1-12* ovule developing into pseudo-seed. The silique elongation of emasculated flower in both *axr1-12* and Columbia could be greatly stimulated with 1 μl of GA_3 (0.1–10.0 nmol/pistil). BAP only presented a very weak stimulation on Columbia' silique development. The application of 2,4-D (0.1 nmol/pistil) along with GA_3 (1.0 nmol/pistil) demonstrated an additive effect on the elongation of emasculated pistil in *axr1-12*. Taken together, our results suggest that 2,4-D obviously stimulated silique elongation through parthenogenesis in the mutant *axr1-12* and GA_3 was also important in this process.

Key words parthenocarpy; silique development; *axr1-12*; auxin; gibberellin

In the majority of flowering plants, fruit set is dependent on successful completion of pollination, fertilization and the following seed development. The fertilized ovules or developing seeds synthesize high levels of auxin and other hormones, stimulate cell division, and lead to fruit set and growth [1–3].

Abundant evidences showed that auxin is a major determinant of fruit development [4,5]. During early stages of floral and fruit development the increment of auxin within the ovules might be a crucial factor to support fruit setting and growth. This was inferred by parthenocarpic fruit development in transgenic tobacco and eggplants expressing *DefH9-iaaM* gene, which codes for tryptophan monoxygenase and is able to increase auxin biosynthesis in transgenic plant cells and organs [4,5]. Fruit growth seems also to depend on auxin produced in developing seeds. Ozga *et al.* [6] reported that seed removal resulted in rapid decreases in pericarp growth of young pea (*Pisum sativum*), while auxin (4-chloroindole-3-acetic acid) or GA application could maintain the growth in deseeded pericarp similarly to that in the control by obtaining the same size of mesocarp cells. Vivian-Smith *et al.* [7] also proved that the application of IAA or NAA, and GA_3 could stimulate silique development of *Arabidopsis*. Despite 2,4-D is known to promote IAA biosynthesis and cell division in normal ovary, there is no evidence concerning that this chemical could stimulate silique development from emasculated flower in an auxin-resistant *Arabidopsis* mutant however.

It was reported that parthenocarpic fruit set and development was induced by exogenous application of gibberellins in mandarins, tomato, and some other fruits [8,9]. A recessive parthenocarpy tomato mutant *pat* could synthesize 160 times higher levels of GA_{20} than normal [10,11]. Elevated levels of endogenous GAs had

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also been observed in the fruits of mandarins plants exhibiting naturally occurring parthenocarpy [8,12]. In these examples gibberellins seems to be a pivotal determinant of fruit development. Thus the cross-talking of different plant hormones, especially auxin and gibberellin, would contribute to regulation of parthenocarpy and fruit development.

axr1-12 is an auxin-resistant mutant. It displays reduced hypocotyl elongation and lack of apical hook formation in the dark. It presents a reduction in apical dominance, and defects in leaf, inflorescence, and flower morphology. It produces significantly less pollen, and fails to elongate the filaments, and as a result, it exhibits greatly reduced fertility and does not produce siliques normally [13,14]. It carries a mutation in AXR1 gene [13,15]. This gene expresses in the shoot, root, and floral meristems, especially in the dividing and elongating cells [16]. It encodes a protein related to the ubiquitin-activating enzyme E1 and has a role in protein degradation related to auxin signaling [15,16]. To explore the influence of 2,4-D on the auxin signaling in the abnormal siliques growth related to AXR1 and to understand the hormonal background concerning silique development, the effects of 2,4-D , GA_3 and BAP with different amount on emasculated ovary of *axr1-12* were studied.

1 Materials and Methods

1.1 Plant materials and growth conditions

axr1-12 is an auxin-resistant mutant, derived from Columbia ecotype [14]. *axr1-12* and its wild type Columbia (Col) were grown in a growth chamber with a 14-h day length, a light intensity of 120 μmol /s·m^2, at 22 ℃, 65% relative humidity (day) / 18 ℃, 75% relative humidity (night).

1.2 Pistil emasculation and controlled pollination

Flowers were emasculated approximately 1 to 2 days pre-anthesis. Controlled pollination was performed 1 to 2 days after emasculation by dusting a freshly dehisced anther over the extended stigmatic papillae until pollen was seen adhering to the stigmatic surface.

1.3 Application of plant growth regulators (PGRs)

2,4-D, GA_3, and BAP (Duchefa, Haarlem,The Netherlands) was dissolved in minimum volume of 2 mol/L NaOH, ethanol, and 2 mol/L HCl, respectively. The dissolved hormones were then buffered in 0.1 mmol/L MES. The pH was adjusted to 6.5. 0.04% (*V/V*) Triton X-100 was added as a surfactant.

1 μl of the respective PGRs solution was applied to each pistil 2 days after the emasculation. Controls were made by the corresponding solutions without growth regulators.

1.4 Determination of the silique growth

Final silique length was measured at 5 days after PGRs treatment. Growth data for each treatment were collected by the examination of 5 to 8 individual pistils. For observing the seed development, siliques were opened 10 days after PGRs treatments and photographs were taken.

2 Results

2.1 Effects of GA_3, 2,4-D and BAP on silique growth from unpollinated pistil of *axr1-12* mutant and its wild type, Columbia

Photoghaphs in Fig.1 and data in Fig.2 showed both length and width of pollinated silique in *axr1-12* mutant were much lower than those in its wild type, Columbia, at seventh day in experiment. This phenomenon implied that some hormones or their signaling related to cell division or elongation would be deficient or suppressed due to the mutation in AXR1, and therefore 2,4-D, GA_3 and BAP were applied respectively.

As shown in Fig.2, silique length from unpollinated pistil of *axr1-12* could be stimulated with the application of 1 μl 2,4-D at levels below 1.0 nmol/pistil. 0.1 nmol/pistil of 2,4-D caused the silique a double elongation and a thickened growth significantly. While silique length from unpollinated pistil of Columbia was inhibited at any tested amount of 2,4-D. This fact indicated that *axr1-12* mutant really possessed a higher patience to 2,4-D while silique growth of Columbia would only need much lower amount of 2,4-D which will be tested in further work.

Silique growth of unpollinated ovary in both *axr1-12* and Columbia could be greatly stimulated with 1 μl of GA_3 (0.1–10.0 nmol/pistil). The extent of pistil elongation was dependent on the dosage applied. GA_3

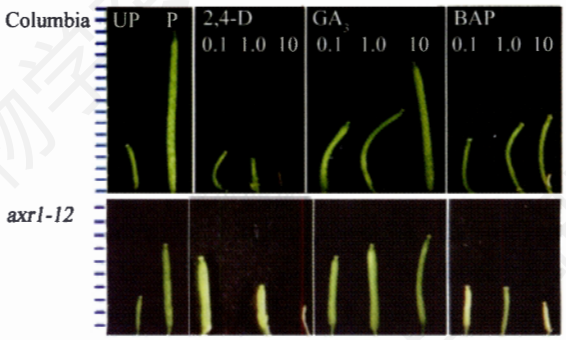

Fig.1 Siliques of wild type Columbia (top) and the *axr1-12* (bottom) after different PGRs treatments

1 μl of GA_3, 2,4-D or BAP was applied at different levels as indicated in each panel (nmol/pistil) to the pre-emasculated pistils. Silique growth was assayed seven days after the treatments. Scale on the left: 1 mm. UP: unpollination, P: pollination.

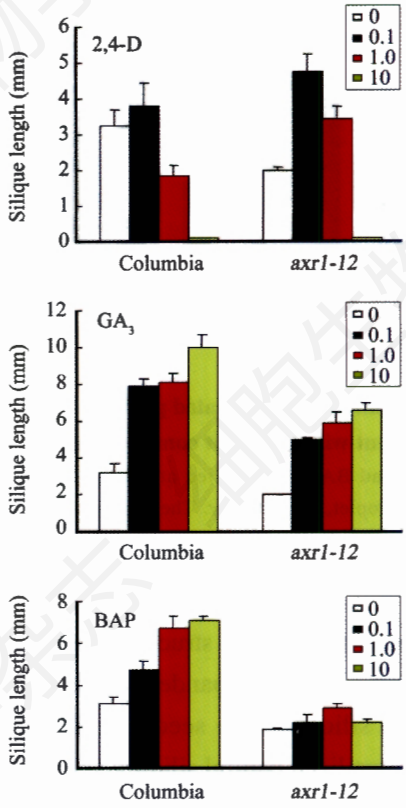

Fig.2 Silique length of Columbia and *axr1-12* seven days after the treament of emasculated pistils with different PGRs

1 μl of PGRs was applied at different levels (0, 0.1, 1.0, 10 nmol/pistil) to the pre-emasculated pistils. Silique growth was assayed seven days after the treatments. The data is presented as the average (±SD) of 10 siliques.

treatment gave 2–3 folds of silique length as that without PGRs application. Pistils of Col treated with 10 nmol of GA_3 were the most similar to pollinated siliques. The length of silique in *axr1-12* treated with 10 nmol of GA_3 could be even comparable to that observed in fertilized pistils.

BAP only presented a very weak stimulation on Columbia' silique development while no positive effect of BAP could be found in *axr1-12* mutant.

The above results showed that the mutation in AXR1 alters the sensitivity of silique development in perceiving 2,4-D.

2.2 Exo-2,4-D induced the pseudo-seed forms

Parthenocarpic siliques were stimulated in *axr1-12* by application of suitable amount of 2,4-D and GA_3 respectively. For comparing the internal structure of different treated pistils, the natural pistils, the unpollinated pistils treated with water, and the elongated siliques following PGR treatment were simply opened for

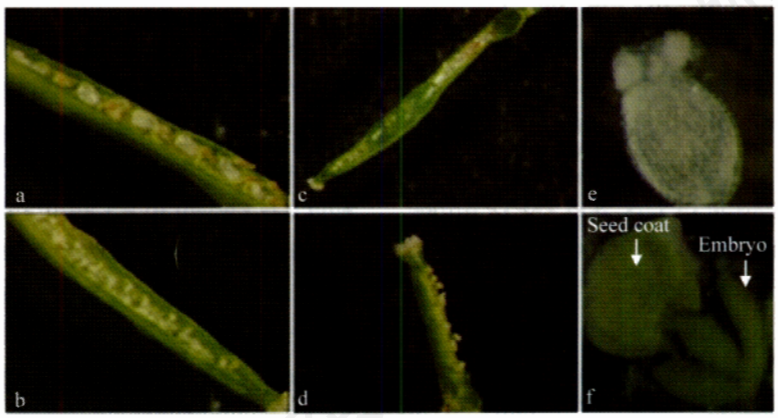

Fig.3 Seed and silique development in *axr1-12* ten days after application of PGR

It shows the siliques treated with (a) 2,4-D (0.1 nmol/pistil), or (b) GA_3 (0.1 nmol/pistil). The middle panel shows the control siliques grew (c) in nature, and (d) the emasculated pistil treated with H_2O. (e) and (f) are the slightly pressed pseu-seed of (a) and a nature seed of *axr1-12*.

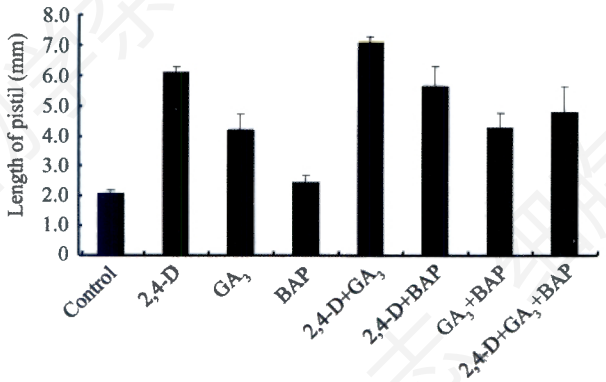

Fig.4 Elongation of emasculated pistils of *axr1-12* seven days after treatment with different combinations of PGRs

2,4-D, GA_3, and BAP was applied at 0.1, 1.0 and 1.0 nmol/pistil with 1 μl of droplet, respectively. The corresponding solution without growth regulators was used as the control. The data is presented as the average (±SD) of 10 siliques.

observation of the internal structure. Fig.3a showed that the ovule was slightly expanded in auxin-induced parthenocarpic siliques. Few seeds could be observed in the partial-fertilized natural siliques (Fig.3c) and no developed ovule was observed in the unpollinated pistils treated with water or GA_3 (Fig.3b, Fig.3d). Further observation revealed that the expanded ovule in the 2,4-D treatment carried no embryo, and developed into pseudo-seed consisting of seed coat and endosperm (Fig.3e). These seed-like structures are definitely different from seeds developed in nature (Fig.3f). Results implied that 2,4-D application stimulated the ovule growth and then the developed pseudo-seeds boosted silique development.

2.3 Silique growth of *axr1-12* responses to combinations of PGRs

Since various plant hormones may play different roles and cross-talk in the fertilization-independent silique development, 2,4-D (0.1 nmol/pistil), GA_3 (1.0 nmol/pistil) and BAP (1.0 nmol/pistil) were then combined for analyzing their effect on silique development of *axr1-12* (Fig.4). The combination of GA_3 and 2,4-D stimulated silique growth more effectively than the 2,4-D or GA_3 alone, and therefore an additive effect was found between 2,4-D and GA_3 in silique development. The addition of BAP counteracted the effect of 2,4-D or GA_3.

3 Discussion

In general, fruit development is initially stimulated by the fertilization and seed development, and followed by the early fruit development process. Plant hormones play crucial roles in regulating the processes. The absence of fertilization led to *Arabidopsis* pistils producing fruits early arrest of parthenocarpic fruit development (Fig.3d). However, when pistils treated with 2,4-D, GA_3, and BAP, parthenocarpic siliques developed in various extents (Fig.1, Fig.2). 2,4-D could induce the carpel and ovule of *axr1-12* develop. As the result, parthenocarpic siliques containing seeds with embryo-free grew (Fig.3a). GA_3 stimulated carpels develop into siliques. In this condition, ovules did not develop (Fig. 3b). While BAP could give a very weak stimulation to the silque of the wild type Columbia. The ability to induce silique growth with various PGRs indicates that *Arabidopsis* pistils are receptive to a variety of hormonal signals. However, gibberellin, auxin, and cytokinin presented regulation on various aspects in fruit development. It is available to use *Arabidopsis* to investigate the growth of unpollinated pistil caused by various plant hormones.

axr1-12 is an auxin-resistant mutant screened by 2,4-D [14,15]. AXR1 gene encodes a protein related to the ubiquitin-activating enzyme E1 and regulates auxin signaling through ubiquitin degradation pathway [15,16]. The mutation in AXR1 changed the sensitivity of silique development to 2,4-D (Fig.1, Fig.2). However, the increased application levels of 2,4-D could induce silique development much efficiently. It indicates that 2,4-D can be perceived by the silique tissues and lead to the auxin signaling related to AXR1, despite the exo-2,4-D owns a specific transporter not common for the endogenous auxin IAA [17]. The reception of 2,4-D and the following transduction is presumed to promote protein degradation via the ubiquitin pathway related to AXR1 and break the function of negative regulator, e.g. ARF8, which is known to act as an inhibitor for further carpel development in *Arabidopsis* in absence of pollination/fertilization [18,19]. ARF8, together with ARF2 and ARF6 may restrict integument growth in ovule and lead to integuments arrested [20]. In our experiment, 2,4-D application stimulated the ovule of *axr1-12* developing into pseudo-seed. Other research showed that the 2,4-D application could also induce the pseudoembryo

development in tomato [21,23]. Relieve of the inhibition of transcriptional regulators ARFs by auxin, and promoting the ovule development, might be the suitable explanation for these results.

Application of GA_3 was also showed to be able to induce the silique development of *axr1-12* (Fig.1, Fig.2, Fig.3b). Other reports illustrated that the elevation of GA level induced natural parthenocarpy in tomato [10]. GA_3 has been observed to induce mesocarp cell expansion and get larger cells in the internal region of the mesocarp in tomato [22,23]. GA_3 could also stimulate mesocarp and endocarp cell division in *Arabidopsis* [6]. These date evidenced that GA is involved in the induction of fruit development upon pollination. The mechanism for this phenomenon is probably due to the degradation of the nuclear factors DELLA proteins which restrain growth in most reproductive structures [24]. It was reported that GA_s could promote cell growth by inducing the degradation of DELLA protein [25]. In tomato, silencing or depletion of a single endogenous tomato DELLA gene homologue (SlDELLA) caused elongated parthenocarpy fruit [24]. Auxins and ethylene signals are also integrated using the DELLA pathway, either directly affecting DELLA protein stability or indirectly via changes in GA levels [26,27]. Thus the degradation of DELLA through GA signaling is presumed to be responsible for GA_3-induced *Arabidopsis* parthenocarpy.

Some evidences showed that auxin is able to stimulate the synthesis of GA. IAA was proposed to promote GA biosynthesis [28–30] at the final activation step, from GA_{20} to GA_1 via GA_3ox in pea, or the step from GA_{19} to GA_{20} via GA_{20}ox in tobacco [31]. Yin *et al.* [32] also proved that IAA was able to regulate stem elongation through promoting GA synthesis in rice. In the fruit development, it is not clear if auxin (2,4-D) stimulates fruit development partly through activating the synthesis of endogenous gibberellins. To elucidate this doubt, further study should be conducted in analysis of the endogenous gibberellins' levels. Nevertheless, a cooperative additive effect of GA_3 and 2,4-D on the fruit development was presented in our experiment (Fig.4). It indicates that parthenocarpy in *Arabidopsis* is able to be achieved by altering both of the auxin and gibberellins signaling pathway.

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References

[1] Gillaspy G *et al. Plant Cell*, 1993, **5**: 1439
[2] Ben-Cheikh W *et al. Plant Physiol*, 1997, **114**: 557
[3] Rodríguez-Concepción M *et al. Plant Mol Biol*, 2001, **46**: 373
[4] Rotino GL *et al. Nat Biotechnol*, 1997, **15**: 1398
[5] Acciarri N *et al. BMC Biotechnol*, 2002, **2**: 4
[6] Ozga JA *et al. Plant Physiol*, 2002, **128**: 1379
[7] Vivian-Smith A *et al. Plant Physiol*, 1999, **121**: 437
[8] Talon M *et al. Plant Physiol*, 1992, **99**: 1575
[9] Gorguet B *et al. Plant Biol*, 2005, **7**: 131
[10] Mazzucato A *et al. Development*, 1998, **125**: 107
[11] Fos M *et al. Plant Physiol*, 2000, **122**: 471
[12] Talon M *et al. Physiol Plant*, 1990, **79**: 400
[13] Estelle MA *et al. Mol Gen Gene*, 1987, **206**: 200
[14] Lincoln C *et al. Plant Cell*, 1990, **2**: 1071
[15] Leyser HM *et al. Nature*, 1993, **364**: 161
[16] del Pozo JC *et al. Plant Cell*, 2002, **14**: 421
[17] Ito H *et al. Plant Physiol*, 2006, **142**: 63
[18] Goetz M *et al. Plant Cell*, 2006,**18**: 1873
[19] Goetz M *et al. Plant Physiol*, 2007, **145**: 351
[20] Wu MF *et al. Development*, 2006, **133**: 4211
[21] Kataoka K *et al. Sci Hort*, 2003, **98**: 9
[22] Serrani JC *et al. J Plant Growth Regul*, 2007, **26**: 211
[23] Bünger-Kibler S *et al. Plant Growth Regu*, 1982, **1**: 143
[24] Martí C *et al. Plant J*, 2007, **52**: 865
[25] Dill A *et al. Proc Natl Acad Sci USA*, 2001, **98**: 14162
[26] Fu X *et al. Nature*, 2003, **421**: 740
[27] Achard P *et al. Plant Cell*, 2003, **15**: 2816
[28] Ross JJ. *J Plant Growth Regu*, 1998, **17**: 141
[29] Wolbang CM *et al. Planta*, 2001, **214**: 153
[30] Wolbang CM *et al. Plant Physiol*, 2004, **134**: 769
[31] Ross JJ *et al. Plant J*, 2000, **21**: 547
[32] Yin C *et al. J Exp Bot*, 2007, **58**: 2441

2,4-D 处理可刺激拟南芥抗生长素突变体(*axr1-12*)去雄后雌蕊的荚果发育

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摘要 *axr1-12* 是 20 年前发现的抗生长素突变体, 其荚果非正常发育的机制尚不明了。分别研究生长素(2,4-D)、赤霉素(GA_3)和细胞分裂素(BAP)对去雄后 *axr1-12* 和野生型(Columbia)雌蕊发育的影响, 结果表明, 1 μl (0.1 nmol/ 花柱)的 2,4-D 处理可促使去雄后的 *axr1-12* 子房发育成荚果, 而对 Columbia 荚果的发育起严重的抑制作用。与此同时, 0.1 nmol 2,4-D 处理还可促进去雄后 *axr1-12* 的胚珠发育成假胚。1 μl (0.1~10.0 nmol/ 花柱)的 GA_3 处理对去雄的 *axr1-12* 和 Columbia 的荚果发育均有促进作用。而 BAP 仅对 Columbia 荚果有微弱的刺激作用。0.1 nmol 2,4-D 和 1.0 nmol GA_3 配合处理去雄 *axr1-12* 雌蕊, 对其荚果的伸长生长表现出了加性效应。由此可见, 2,4-D 可明显促进 *axr1-12* 荚果的单性生殖生长, GA_3 在此过程中也起着重要作用。

关键词 单性生殖; 荚果发育; *axr1-12*; 生长素; 赤霉素

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