REVIEW

Phytohormones in Shoots and Fruits of Tomato; Apoplast Solution and Seedless Fruit

Kiyohide KOJIMA*

Faculty of Agriculture, Graduate School of Science and Technology, Niigata University
(Ikarashi, Niigata 950–2181, Japan)

Abstract

Indole-3-acetic acid (IAA) endogenous levels were determined by liquid chromatography-mass spectrometry, diffusible levels were determined by fluorometric detection and polar transport activity was determined by radioactive IAA in tomato plants. IAA concentration in the apoplast (AP) solution was higher than those in the symplast (SP) solution in both upper and lower parts of stems. It is suggested that the amount of polar IAA transport might be only 19% of the amount of endogenous IAA in stems. In fruits, concentrations of abscisic acid (ABA) were high in the pericarp, axis and the locule tissue in the early growth stages. In seedless fruits of parthenocarpic tomato, the levels of IAA, gibberellic acid$_1$ (GA$_1$), trans-zeatin (Z) and trans-zeatin riboside (ZR) were analyzed. IAA concentrations of the pericarps and partitions were higher in fruits of 8 cm in diameter (8-cm-fruit) than in 6-cm-fruit. In the partitions, IAA concentrations of SP were higher than those of AP solution. The SP of the partitions in 6-cm-fruit had the highest concentration of Z and was 2.7 times greater than the AP. The ZR concentration in locule tissues in 6-cm-fruit was the highest. These results suggest that the sites of synthesis may be the SP of partitions for IAA and Z, and locules for ZR.

Introduction

Tomato plants have been cultivated and investigated as a major commercial crop all over the world. Additionally, tomato plants have been used as a model system to research fruit growth[1,15,24]. Thus, a series of investigations using tomato plants have been carried out because tomato plants are suitable for basic physiological researches and the ripple effect is large.

Indole-3-acetic acid (IAA) might exert its physiological effect on a cell from outside the plasma membrane[32]. In etiolated seedlings, hypocotyl growth might be related to the higher concentration of IAA in the apoplast (AP) solution (sap outside a cell) rather than the lower one in the symplast (SP) solution[29].

The polar transport of IAA from a shoot apex to a root serves as a correlative signal involved in physiological phenomena. IAA transport has been studied extensively using seedlings[10,11], while there are few investigations of IAA balance at the whole plant level using adult plants.

Fruit growth is controlled by various phytohormones such as IAA, gibberellins (GAs) and cytokinins (CKs)[8]. The growth of pollinated fruits is promoted by hormones synthesized mainly in seeds[26,31], while in seedless fruits the fruit growth may be the result of elevated hormone levels in the ovary/fruit[5,8], but the origins of hormones are not clear.

The present review shows endogenous levels in the AP and SP solutions, the diffusible levels and polar transport activity of IAA for whole tomato plants, and the endogenous levels of IAA, GA$_1$ and CKs in seedless fruits of parthenocarpic tomatoes.

Levels and transport of IAA in vegetative organs[20]

1. Endogenous levels in stems and roots

AP solution was collected by the centrifugation method[28] with some modifications. There was almost no difference between upper and lower plant parts for the

(1) Definition of parts [a]	(2) Contents of IAA analyses	(3) Cross area (cm²)	(4) Weight (g/stem)	(5) Endogenous [b] Amount (pmol/stem)	Conc. (pmol/gFW)	(6) Diffusible [c] Stem (pmol/stem/h)	Leaf (pmol/stem/h) [pmol/gFW/h]	(7) Transport activity [d] (pmol/stem/h)
Apex part [10 cm]	Diffusible		15.9			2.6 ± 0.6		
Upper part [39 cm] 2.5 cm→	Endogenous Transport activity	0.78 ± 0.12	27.8	(AP) 1.1 → 170 (SP) 17.1 → 1,300	150 75		1.82 [0.15]	4.6 ± 0.9
Middle part [39 cm] 20 cm→	Discard Diffusible Discard		34.8			7.7 ± 1.3	0.23 [0.01]	
Lower part [39 cm] 2.5 cm→	Endogenous Transport activity	0.96 ± 0.18	29.9	(AP) 1.2 → 360 (SP) 15.4 → 1,100	300 72		0.10 [0.01]	2.9 ± 0.5
[10 cm]	Discard							
[Root] Lateral root		5.0		600	120			
Main root		10.2		560	55			

Fig. 1. Endogenous IAA levels, diffusible IAA levels, and transport activity of IAA in vegetative organs of tomato

Plants 3 month after seeding were sampled. Mean height of main stem was 137 ± 6 cm (n = 8).

a): The sections (36.5 cm long on the average) of upper and lower parts of tomato stems were sampled.

b): The AP and SP solutions were collected by the centrifugation method. Endogenous IAA levels were determined by LC-MS using $^{13}C_6$-IAA[19].

c): Diffusible IAA levels in leaves of each part of the plant were estimated on the basis of fresh weight of total leaves. The water including diffusible IAA was collected after incubation for 3 h (stem) and 24 h (leaf). Diffusible IAA levels were analyzed by an HPLC system with a fluorometric detector using IPA. Data are means ± SE (n = 8).

d): Amount of radioactive IAA transported to basipetal end (1 cm long) of stem segments (2.5 cm long) was measured after incubation at 25°C for 24 h. Data are means ± SE (n = 8). The data are expressed in pmol by changing dpm using the value of specific activity. Data from published report[20].

calculated IAA concentrations in the SP solution (Fig. 1 (5)). On the other hand, IAA concentration in the AP solution of upper plant parts was half of that of lower parts. In adult plants, the presence of endogenous IAA in the AP solution was demonstrated for the first time in the report of Kojima et al.[20]. In seedlings, IAA concentration in the AP solution was higher than that in the SP solution[29]. In the tomato stem, IAA concentrations in the AP solution were also higher than those in the SP solution of both upper and lower plant parts (Fig. 1 (5)).

In main roots, the endogenous IAA concentration was twice of that in lateral roots (Fig. 1). Citrus seedlings of three cultivars ranged from 6 to 21 pmol/g fresh weight[18] in the IAA level of roots. IAA levels of tomato roots are higher than those of citrus roots.

2. The relationship between transport and endogenous IAA levels in shoots

The level of polar IAA transport might join IAA from the apex part (2.6 pmol/h, Fig. 1 (6)), the leaves in

the upper part (1.82 pmol/h) and the leaves in most of the middle part (0.23 pmol/h), which might correspond to the level of diffusible IAA from the middle plant parts (7.7 pmol/h). The difference in the level may be transported from the flower trusses.

The actual diffusible level (corresponds to intensity[14]) of IAA from the middle part of the stem was 7.7 pmol/h (Fig. 1 (6)). On the other hand, the amount of endogenous IAA per stem (36.5 cm long) was 1,500 pmol in both upper and lower parts (Fig. 1 (5)). If all IAA is mobile and transport velocity is 1 cm/h in the tomato stem, the intensity of IAA transport is calculated to be 41 pmol/h (1,500 pmol/36.5 cm), suggesting that the amount of mobile IAA might be only 19% (7.7/41 × 100) of the amount of endogenous IAA present in the stem.

3. Polar transport capacity in stems

A sufficient amount of radioactive IAA was supplied to the samples and then IAA transport activity was
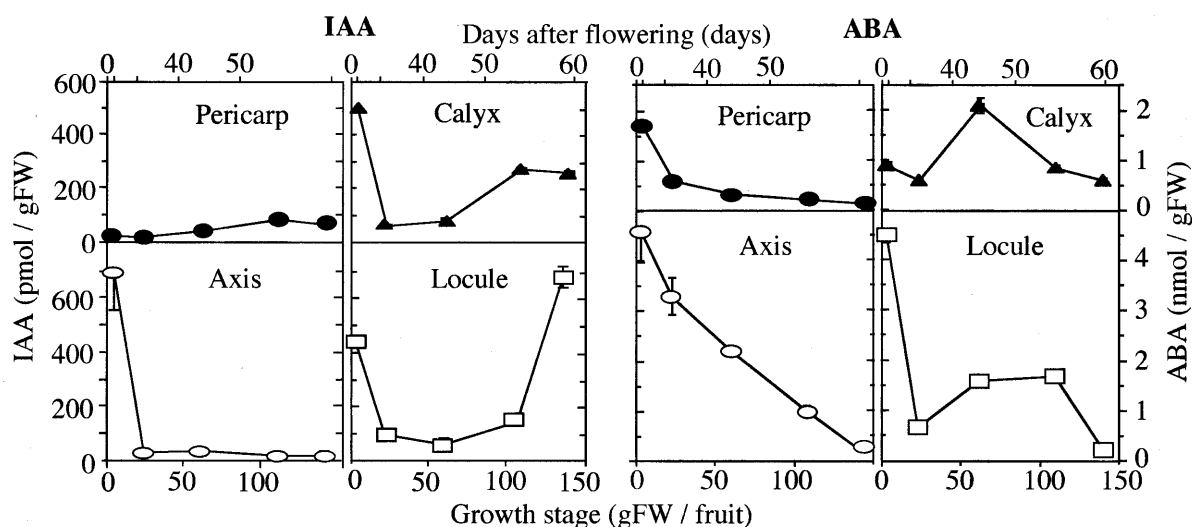
Fig. 2. **Changes in IAA concentration (left) and ABA concentration (right) by fresh weight of tomato fruit parts**
Fruit-set was induced by pollination + 4-CPA treatment. Locule tissue includes the seeds. Means of three determinations and their SE are shown. The population of fruits used in this study reached full size ca. 60 DAF. Data from published reports[15,16].

assayed. We had anticipated that apex parts of stems might not produce enough IAA in early winter season and therefore the amount of endogenous IAA in stems might be lower than the capacity of IAA transport.

IAA transport activity was assayed according to the method of Okada et al.[27] with some modifications. IAA transport activity was calculated to be 4.4 pmol/h (110 pmol/24 h, Fig. 1 (7)). IAA transport activity was assayed during a 24 h period in this study. In sections from carnation stems, the amount of IAA transported from 8 to 24 h was less than 10% of that transported during the first 8 h period[9]. Our estimated value by radioactive IAA may be lower than the true value. Thus, the level of IAA transport activity may be almost the same level as that of diffusible IAA (7.7 pmol/h, Fig. 1 (6)). IAA may be transported at full transport capacity at the time of sampling. This is probably the limiting factor for IAA transport of tomato stems rather than the synthesis in tops of plants.

Normal tomato plants showing weak apical dominance were used in this study. The concentration of auxin-like substances in the apical region of tomato mutants showing strong apical dominance was much higher than that of normal tomatoes[30]. IAA transport capacity might be involved in determining the degree of apical dominance based on the application of radioactive IAA in tomato plants[2].

Distribution of IAA and ABA in flowers

1. Endogenous IAA levels in flowers

IAA concentration of a stamen was lowest in the bud 5 days before flowering (DBF), but highest in the flower 0 days after flowering (DAF)[16]. Within a pollen grain, a macrospore made the first nuclear division between 6 and 3 DBF[12]. The rapid increase of IAA in the stamen may be related to maturation of the pollen.

2. Endogenous ABA levels in flowers

Abscisic acid (ABA) was determined by a GC system equipped with an electron capture detector[15,17]. Pistils and calyxes maintained their ABA concentrations from 5 DBF to the day of flowering[15]. The ABA concentrations of stamens and petals at 5 DBF were higher, but had decreased to about one-sixth on the day of flowering.

Distribution of phytohormones in fruits

1. Changes of endogenous IAA and ABA levels within fruits

(1) Changes of endogenous IAA levels within fruits[16]

Marked high concentrations of IAA in the axis and loculus of 3.4 g fruit (Fig. 2), which almost corresponds to the stage of highest relative growth rate[25], may be related to the onset of fruit growth[13]. The IAA concentrations in the pericarp were low throughout development. These results suggest that the expansion of the pericarp tissue is not directly caused by IAA, but indirectly caused

Table 1. Phytohormone concentrations (pmol / gFW) of apoplast (AP) and symplast (SP) solutions from fruit parts

| | Pericarp | | | | Partition | | | | Locule | |
| | AP | | SP | | AP | | SP | | | |
	6-cm	8-cm	6-cm	8-cm	6-cm	8-cm	6-cm	8-cm	6-cm	8-cm
IAA	23	49	21	43	16	31	38	45	13	12
GA_1	2.8	0.6	8.3	2.2	11	8.2	12	3.9	13	8.5
Z	1.3	1.1	1.4	0.77	1.7	0.5	4.6	0.72	2.5	0.28
ZR	14	18	14	17	20	9.2	20	12	55	10

*AP and SP solutions were collected by the centrifugation method. Phytohormone levels were determined by LC-MS using internal standards. Data from published report[21].

by sink activity produced by developing seeds[31].

(2) Changes of endogenous ABA levels within fruits[15]

The axis region of tomato is rich in vascular bundles with phloem, from which assimilate may be unloaded for the young developing fruit. ABA concentrations of the pericarp, axis and locule tissue were very high in the early stage (ca. 19 DAF) (Fig. 2). If the theory of Brenner[3] is applied, ABA may stimulate phloem unloading in the axis and promote sink activity in pericarp and locule tissue during the high growth rate phase.

2. Endogenous IAA, GA and CKs in AP of parthenocarpic fruits

The early growth of most fruits is divided into three phases[8]; ovary development, cell division, and cell expansion. In this study 6-cm-fruit corresponds to the middle of the cell expansion phase and 8-cm-fruit corresponds to the end of the cell expansion phase.

(1) Synthesis sites and roles for IAA

IAA may be mainly synthesized within cells of partitions during the expansion stage in parthenocarpic tomato fruits. In pollinated tomato fruits, IAA might be synthesized in the developing seeds, because those (including surrounding tissues) had the highest IAA concentration[16,31]. On the other hand, in parthenocarpic fruits the nucellar tissue of seed-like structures might produce the auxin necessary for growth[7]. However, in our study, in locules (including the seedlike structures), the IAA concentrations were lower than in pericarps and partitions (Table 1). In partitions, the SP solutions had higher IAA concentrations than the AP solutions. Moreover, in pericarps the AP solution in 8-cm-fruit had the highest concentration of IAA. Thus it is suggested that the SP of partitions might be the sites of IAA synthesis during the expansion stage and later the AP of pericarps might also participate.

(2) Roles and catabolization for GA_1

Gibberellic acid$_1$ (GA_1) was identified, but GA_4

could not be detected in either pollinated or parthenocarpic fruitlets of tomato, suggesting that the early-non-hydroxylation pathway might not function in tomato fruits[22]. Thus only GA_1 levels as active GA were analyzed.

The major role of GAs in fruit growth might be the promotion of cell expansion[6]. The results in which the GA_1 concentrations detected in all parts of 6-cm-fruit (cell expansion phase) were higher than in 8-cm-fruit may support a major role for GAs in cell expansion (Table 1).

In the AP of pericarps, there might be a mechanism for decreasing GA_1. GA_1 could be transported considerably from SP to AP through the cell membrane because of the lipophilic property of GA_1[4]. However, in pericarps, the GA_1 concentrations in the AP solution were one-third those in the SP solution in both 6- and 8-cm-fruit (Table 1).

(3) Synthesis sites and roles for CKs

The synthesis of trans-zeatin (Z) may occur mainly in the SP of partitions in parthenocarpic tomato fruits, because in 6-cm-fruit, its Z concentration was the highest of all the plant parts analyzed (Table 1). Additionally, the high permeability of benzyladenine from SP to AP through the cell membrane[23] also suggests a high rate of Z synthesis within cells.

Conclusion

The series of investigations using tomato plants have presented various knowledge of phytohormones. In shoots, it is shown that the IAA concentration in the AP solution is higher than that in the SP solution. In parthenocarpic fruits, it is suggested that synthesis sites may partition cells for IAA and trans-zeatin. This knowledge may lead to progress in cultivation methods and breeding of tomato plants.

References

1. Bohner, J. & Bangerth, F. (1988) Effects of fruit set sequence and defoliation on cell number, cell size and hormone levels of tomato fruits (*Lycopersicon esculentum* Mill.) within a truss. *Plant Growth Regul.*, **7**, 141–155.

2. Brenner, M. L. et al. (1987) Analysis of apical dominance in relation to IAA transport. *HortScience*, **22**, 833–835.

3. Brenner, M. L. (1989) Hormonal control of assimilate portioning: regulation in the sink. *Acta Hort.*, **239**, 141–148.

4. Durley, R. C. & Pharis, R. P. (1972) Partition coefficients of 27 gibberellins. *Phytochem.*, **11**, 317–326.

5. Fos, M. et al. (2001) Role of gibberellins in partheno-carpic fruit development induced by the genetic system pat-3/pat-4 in tomato. *Physiol. Plant.*, **111**, 545–550.

6. Garcia-Martinez, J. L. & Hedden, P. (1997) Gibberellins and fruit development. *In* Phytochemistry of fruit and vegetables, eds. Tomas-Barberan, F. A. & Robins, R. J., Oxford Sci. Publications, Heidelberg, 263–285.

7. George, W. L., Scott, J. W. & Splittstoesser, W. E. (1984) Parthenocarpy in tomato. *Hort. Rev.*, **6**, 65–84.

8. Gillaspy, G., Ben-David, H. & Gruissem, W. (1993) Fruits: a developmental perspective. *Plant Cell*, **5**, 1439–1451.

9. Guerrero, J. R. et al. (1999) Influence of 2,3,5-triiodobenzoic acid and 1-N-naphthylphthalamic acid on indoleacetic acid transport in carnation cuttings: relationship with rooting. *J. Plant Growth Regul.*, **18**, 183–190.

10. Iino, M. & Carr, D. J. (1982) Estimation of free, conjugated, and diffusible indole-3-acetic acid in etiolated maize shoots by the indole-α-pyron florescence method. *Plant Physiol.*, **69**, 950–956.

11. Iino, M. (1991) Mediation of tropisms by lateral translocation of endogenous indole-3-acetic acid in maize coleoptiles. *Plant Cell Environ.*, **14**, 279–286.

12. Iwahori, S. (1965) High temperature injuries in tomato. IV. Development of normal flower buds and morphological abnormalities of flower buds treated with high temperature. *J. Jpn. Soc. Hort. Sci.*, **34**, 33–41.

13. Iwahori, S. (1967) Auxin of tomato fruit at different stages of its development with a special reference to high temperature injuries. *Plant Cell Physiol.*, **8**, 15–22.

14. Kaldeway, H. (1984) Transport and other modes of movement of hormones (mainly auxins). *In* Encyclopedia of plant physiology, new series, Vol. 10, ed. Scott, T. K, Springer-Verlag, Berlin, 80–148.

15. Kojima, K. et al. (1993) Distribution of abscisic acid in different parts of the reproductive organs of tomato. *Sci. Hort.*, **56**, 23–30.

16. Kojima, K., Sakurai, N. & Tsurusaki, K. (1994) IAA distribution within tomato flower and fruit. *HortScience*, **29**, 1200.

17. Kojima, K. (1995) Simultaneous measurement of ABA, IAA and GAs in citrus–role of ABA in relation to sink ability. *JARQ*, **29**, 179–185.

18. Kojima, K. et al. (1995) Relationships between growth properties and endogenous ABA, IAA and GA of citrus varieties for rootstock. *Engei gakkai zasshi (J. Jpn. Soc. Hort. Sci.)* **63**, 753–760 [In Japanese with English summary].

19. Kojima, K. (2001) Property of HPLC column and mass spectrum of LC-MS for phytohormone analysis. *JARQ*, **35**, 149–154.

20. Kojima, K., Ohtake, E. & Yu, Z. (2002) Distribution and transport of IAA in tomato plants. *Plant Growth Regul.*, **37**, 249–254.

21. Kojima, K. et al. (2003) Distribution of indole-acetic acid, gibberellin and cytokinins in apoplast and symplast of parthenocarpic tomato fruits. *Plant Growth Regul.*, **41**, 99–104.

22. Koshioka, M. et al. (1994) Analysis of gibberellins in growing fruits of *Lycopersicon esculentum* after pollination or treatment with 4-chlorophenoxyacetic acid. *J. Hort. Sci.*, **69**, 171–179.

23. Laloue, C., Pethe-Terrine, C. & Gueern, J. (1981) Uptake and metabolism of cytokinin in tobacco cells. *In* Metabolism and molecular activities of cytokinins, eds. Guern, J. & Peaud-Lenoel, C., Springer-Verlag, Berlin, Heidelberg, 80–96.

24. Mapelli, S. et al. (1978) Relationship between set, development and activities of growth regulators in tomato fruits. *Plant Cell Physiol.*, **19**, 1281–1288.

25. Monselise, S. P., Varga, A. & Bruinsma, J. (1978) Growth analysis of the tomato fruit, *Lycopersicon esculentum* Mill. *Ann. Bot.*, **42**, 1245–1247.

26. Naylor, A. W. (1984) Functions of hormones at the organ level of organization. *In* Encyclopedia of plant physiol. New series Vol. 10, ed. Scott, T. K., Springer-Verlag, Berlin, Heidelberg, 195–200.

27. Okada, K. et al. (1991) Requirement of the auxin polar transport system in early stages of Arabidopsis floral bud formation. *Plant Cell*, **29**, 1337–1343.

28. Sakurai, N. & Kuraishi, S. (1988) Water potential and mechanical properties of the cell wall of hypocotyls of dark-grown squash (*Cucubita maxima* Duch.) under water stress condition. *Plant Cell Physiol.*, **29**, 1337–1343.

29. Tsurusaki, K., Masuda, Y. & Sakurai, N. (1997) Distribution of indole-3-acetic acid in the apoplast and symplast of squash (*Cucurbita maxima*) hypocotyls. *Plant Cell Physiol.*, **38**, 352–356.

30. Tucker, D. J. (1981) Axillary bud formation in two isogenic lines of tomato showing different degrees of apical dominance in relation to IAA transport. *Ann. Bot.*, **48**, 837–843.

31. Varga, A. & Bruinsma, J. (1976) Roles of seeds and auxins in tomato fruit growth. *Z. Pflanzenphysiol. Bd.*, **80**, 95–104.

32. Venis, M. A. et al. (1990) Impermeant auxin analogues have auxin activity. *Planta*, **182**, 232–235.