J Plant Growth Regul (1991) 10:167–171

Journal of
Plant
Growth
Regulation



# Sucrose Metabolism and IAA and Ethylene Production in Muskmelon Ovaries

Sarah E. Lingle and James R. Dunlap*

USDA/ARS Subtropical Agricultural Research Laboratory, 2413 E. Highway 83, Weslaco, Texas 78596, USA

Received February 11, 1991; accepted May 13, 1991

**Abstract.** Changes induced by the pollination of ovaries may be mediated by phytohormones and involve sugar-metabolizing enzymes. In order to further explore these relationships, soluble sugars, sucrose-phosphate synthase (EC 2.4.1.14), sucrose synthase (EC 2.4.1.13), acid and neutral invertases (EC 3.2.1.26), indole-3-acetic acid (IAA), and ethylene were investigated in muskmelon (*Cucumis melo* L.) ovaries sampled before, during, and after anthesis. The fresh weight of ovaries increased 100% within 48 h after pollination, but did not change significantly in the absence of pollination. While sugar content per ovary increased after pollination, sugar content per mg protein was unaffected. Sucrose was not detected in nonpollinated ovaries 48 h after anthesis. Free IAA content was highest in ovaries sampled 48 h before anthesis. Pollination had no immediate effect on IAA content per mg protein in postanthesis ovaries. Although detected in all ovaries sampled, ethylene production increased significantly only in nonpollinated ovaries. Activity of sucrose-phosphate synthase was the same at all stages. The specific activities of sucrose synthase and the invertases were highest in nonpollinated ovaries. The increase in rate of sugar import into ovaries following pollination was not accompanied by an increase in the specific activity of any enzyme assayed, but was coincident with an increase in the total activity per ovary of sucrose synthase and acid invertase. There appears to be no direct relationship between sucrose-metabolizing enzymes, IAA or ethylene in developing pollinated ovaries, but the increase in sucrose cleavage activity in nonpollinated ovaries may be related to the increase in ethylene production.

In many fruits, such as muskmelon (*Cucumis melo* L.), fertilization of the ovary is necessary for proper fruit development. Without fertilization the ovary senesces and abscises from the plant. Phytohormones play an important role in ovary and fruit development before, during, and after pollination, and, in some cases, applications of exogenous hormones can replace fertilization and allow development of the fruit (Goodwin 1978).

The increase in growth rate that occurs following fertilization implies that the translocation of carbohydrate into the ovary is also enhanced. There has been much recent interest in the role that the enzymes of sucrose metabolism, especially the sucrose synthase pathway of sucrose breakdown, may play in the import of carbohydrate into a tissue (Hendrix 1990, Lowell et al. 1989, Sung et al. 1989).

A number of studies on sugar metabolism in muskmelon fruit have been reported (Hubbard et al. 1989, Lingle and Dunlap 1987, McCollum et al. 1988, Schaffer et al. 1987), but these were initiated after fruit were well established. Other work investigated changes in phytohormone levels during fruit or seed development (Brenner 1987, Dunlap 1989, Dunlap and Robacker 1990, Goodwin 1978 and references therein). However, the interaction between phytohormones and sucrose metabolism during pollination and fruit establishment has been largely ignored. Little is known of the period just prior to and immediately following anthesis. Muskmelon ovaries are very convenient for such studies, since several are produced on a plant, and they are large enough for easy handling. This study was conducted to explore the possible relationship of sugar content to activities of sucrose-metabolizing en-

* Present address: Texas A&M Agricultural Experiment Station, 2415 E. Highway 83, Weslaco, TX 78596, USA

Mention of a specific product does not imply an endorsement by the United States Department of Agriculture or Texas Aricultural Experiment Station over other suitable products.

zymes, and the phytohormones ethylene and indole-3-acetic acid (IAA) during the period just before and just after anthesis in muskmelon ovaries.

## Materials and Methods

### *Plant Materials*

Netted muskmelon, cv 'Magnum 45' plants were grown in 12-L plastic pots containing a peat/vermiculite mixture in a glasshouse in Weslaco, TX. Plants were watered daily; nutrients were supplied by applying a commercial soluble fertilizer (N:P:K, 15:30:15). Nonpollinated ovaries were obtained by enclosing preanthesis flowers in a bag made from a nylon stocking. Other flowers were hand-pollinated at full anthesis to produce fertilized ovaries. Flowers were collected at 48 h before anthesis (petals emerging), at anthesis (petals fully opened), 48 h after pollination (petals closed, ovary swelling), and 48 h after anthesis without pollination (petals closed, ovary senescing). Petals, stamens, and pistils were removed and ovaries frozen at −80°C. For IAA analysis, tissue was lyophilized and stored at −80°C for no more than 30 days. Freshly harvested ovaries from each stage were used to determine ethylene production.

### *Sugar Content and Enzyme Activity*

Half of each ovary was diced, and homogenized at 4°C in a buffer of 50 mM K-phosphate (pH 7.5), 5 mM $MgCl_2$, 1 mM EDTA, 5 mM $Na_2SO_3$, 0.5% (wt/vol) casein, and 5 mM DTT. The homogenate was filtered through cheesecloth, centrifuged (15,000 *g*, 4°C), and 2 ml of the supernatant was desalted by centrifugation through a 5-ml column of superfine Sephadex G-25 preequilibrated with 25 mM Hepes-KOH (pH 7.5), 5 mM $MgCl_2$, 0.5 mM EDTA, and 5 mM DTT. Sucrose-phosphate synthase (SPS), sucrose synthase (SS), acid invertase, and neutral invertase were assayed immediately as described previously (Lingle and Dunlap 1987). Six replications (ovaries) were used per treatment.

The other half of each ovary was diced and extracted in the above extraction buffer without casein. An aliquot of the filtered homogenate was added to the same volume of 95% (vol/vol) ethanol. This was stored at −20°C until assayed for sugars using the method described by Hendrix and Peelen (1987). An additional aliquot of this extract was desalted as described above. Protein was determined on this portion using the method of Bradford (1976).

### *Phytohormones*

Free IAA in frozen ovaries was extracted and assayed by GC-SIM-MS as described in Dunlap and Robacker (1990). Ethylene production of freshly harvested ovaries was determined by sealing each ovary in a glass tube with a rubber serum cap. After 30–60 min, a 0.5-ml sample of the head space gas was analyzed by gas chromatography (Dunlap 1988). For both phytohormones, nine replications were used.

## Results

Following anthesis, fresh weight of the pollinated ovary doubled in 48 h (Table 1). No significant increase in fresh weight was observed in nonpollinated ovaries. Forty-eight hours after anthesis, nonpollinated ovaries were visibly yellow and had begun to senesce. Total protein content of ovaries increased significantly with pollination, but relative protein content per g of fresh weight decreased after pollination due to the fresh weight increase. The protein content and total protein of nonpollinated ovaries decreased by 50% within 48 h after anthesis.

**Table 1.** Fresh weight (FW) and protein content of pollinated or nonpollinated netted muskmelon ovaries before, at, and after anthesis.

| Stage | Fresh weight (g) | Protein | |
|---|---|---|---|
| | | mg/g FW | mg/ovary |
| Preanthesis | 0.434 | 8.46 | 3.57 |
| Anthesis | 0.467 | 8.26 | 3.89 |
| Pollinated | 0.969 | 6.49 | 6.28 |
| Nonpollinated | 0.592 | 3.56 | 2.13 |
| LSD $p < 0.05$ | 0.130 | 1.00 | 1.32 |

Total soluble sucrose and glucose content per ovary increased following anthesis and pollination (Fig. 1A), but the relative content per mg protein of those sugars (Fig. 1B) decreased slightly in pollinated ovaries 48 h after anthesis. In contrast, sugar content per ovary decreased after anthesis in nonpollinated ovaries (Fig. 1A), but the content of glucose and fructose per mg protein (Fig. 1B) remained approximately the same. Sucrose was not detectable in nonpollinated ovaries 48 h after anthesis.

Total SPS activity per ovary did not vary with development or pollination (Fig. 2A), while total SS and acid invertase activities per ovary increased with pollination, and neutral invertase activity declined in nonpollinated ovaries. However, the specific activity of all enzymes remained unchanged at 48 h after anthesis in pollinated ovaries (Fig. 2B), but increased significantly in nonpollinated ovaries.

Free IAA content was highest in ovaries harvested 2 days before anthesis (Fig. 3A). With the onset of anthesis, IAA content decreased nearly 70%. An increase in total free IAA content between anthesis and 2 days after anthesis in pollinated ovaries was the result of the increase in fresh weight (Table 1); relative IAA content remained the same in pollinated and nonpollinated ovaries (Fig. 3B). Ovaries were capable of producing ethylene before and after anthesis (Fig. 3A and B), but ethylene production by nonpollinated, senescing ovaries was much greater than in ovaries at any other stage of development.

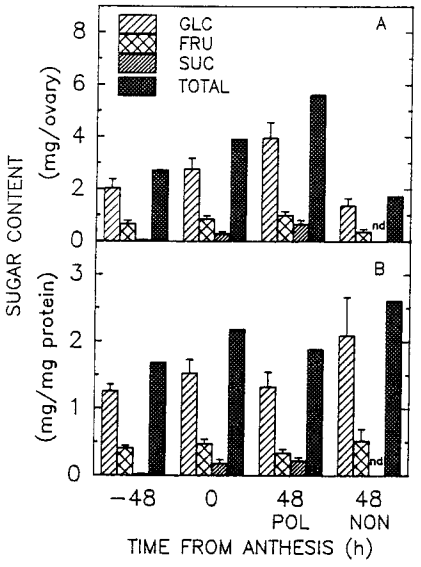


**Fig. 1.** Content soluble glucose (GLC), fructose (FRU), and sucrose (SUC) per ovary (**A**) and per mg protein (**B**) in pollinated (POL) or nonpollinated (NON) netted muskmelon ovaries before, at, and after anthesis. Bars represent ± SE.

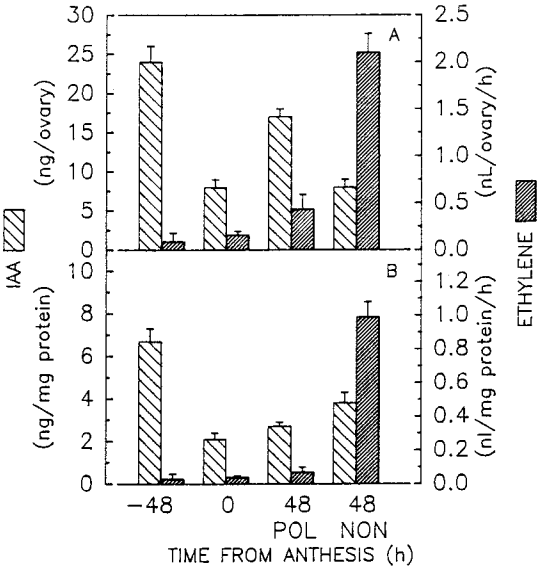


**Fig. 3.** Total IAA content (**A**) and content per mg protein (**B**) and ethylene production in pollinated (POL) or nonpollinated (NON) netted muskmelon ovaries before, at, and after anthesis. Bars represent ± SE.

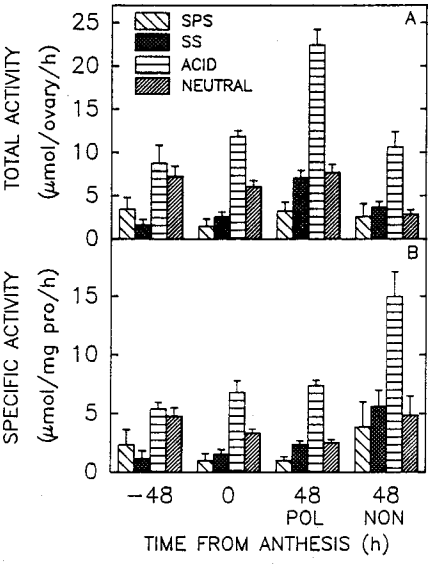


**Fig. 2.** Total (**A**) and specific (**B**) activities of SPS, SS, acid invertase (ACID), and neutral invertase (NEUTRAL) in pollinated (POL) or nonpollinated (NON) netted muskmelon ovaries before, at, and after anthesis. Bars represent ± SE.

## Discussion

The increase in fresh weight of fertilized ovaries 48 h after pollination (Table 1) signaled the beginning of the rapid growth phase. In a previous study, melon fruit grown under similar conditions weighed 300–500 g by 10 days after anthesis (Lingle and Dunlap 1987). This increase in fresh weight of rapidly growing organs, such as ovaries and young fruits, can mask changes in metabolite concentration on a cellular level. For this reason, data for this study have been expressed both on a per ovary and a per mg protein basis.

Ovary glucose content was more than twice the fructose content (Fig. 1B). This contrasts with other reports that showed approximately equal glucose and fructose in developing muskmelon fruit 10 days after anthesis (Lingle and Dunlap 1987, McCollum et al. 1988). This imbalance may be the result of a conversion of galactose to glucose in the ovary. *Cucumis* is a stachyose-transporting species (Zimmerman and Ziegler 1975). Gross and Pharr (1982) showed that the galactose produced in the breakdown of stachyose is readily converted to glucose in the peduncle of cucumber (*C. sativus*) fruit. Galactose was not assayed in the present study, and free galactose was detectable by HPLC only in full-slip melon fruit (S. E. Lingle unpublished observations).

After anthesis, the increase in fresh weight of pollinated ovaries (Table 1) was paralleled by an increase in glucose and sucrose content per ovary (Fig. 1A). However, the relative sugar content per mg protein decreased only slightly in the 48 h following anthesis in pollinated ovaries (Fig. 1B).

Since an increase in growth rate and volume without an increase in sugar import would dilute the sugar within the tissue, the rate of sugar import must have increased following pollination. A case has been made that sucrose-metabolizing enzymes play a key role in determining the rate of sugar import in sink tissues, such as ovaries or fruits (Hendrix 1990, Lowell et al. 1989, Sung et al. 1989). However, the increase in the rate of sugar import into ovaries following pollination suggested by these data was not accompanied by any changes in the specific activity of any enzyme assayed (Fig. 2B). Perhaps the increase in the total activity of SS and acid invertase in pollinated ovaries 48 h after anthesis (Fig. 2A) helped trigger the increase in sugar import. Another possibility is that some of the enzymes involved in biosynthesis of membranes and cell walls also contribute to sink strength.

In nonpollinated ovaries sugar content per ovary declined (Fig. 1A) within 48 h after anthesis, but relative content per mg protein of glucose and fructose was essentially stable (Fig. 1B) due to the protein loss during senescence (Table 1). However, there was a decrease in glucose and fructose content per ovary (Fig. 1A), and a loss of sucrose, 48 h after anthesis in nonpollinated ovaries. These decreases were accompanied by increases in the specific activities of SS and the invertases in the senescing, nonpollinated ovaries (Fig. 2B). Both the decrease in soluble sugar and increase in specific sucrose cleavage activity may be related to an increase in respiration rates that frequently accompany senescence (Solomos 1988), and were coincident to the increase in ethylene production by nonpollinated ovaries (Fig. 3).

Changes in free IAA did not appear to be related to changes in enzyme activities or sugars, since relative IAA content declined before pollination (Fig. 3B), while relative sugar contents (Fig. 1B) and specific activities of enzymes related to sucrose degradation (Fig. 2B) remained the same, and enzyme-specific activities increased in nonpollinated ovaries (Fig. 2B), while IAA content remained unchanged (Fig. 3B).

In contrast to results reported for mung bean (Yoshii and Imaseki 1981) and other tissues (Mattoo and Aharoni 1988), changes in ethylene production and IAA in ovary tissue appear to be unrelated. The burst of ethylene production in nonpollinated ovaries (Fig. 3) more likely represents part of the signal associated with senescence of the plant tissue (Noodén 1988).

Pollinated ovaries 48 h after anthesis are greater sinks for carbohydrate than ovaries before and at anthesis, since relative sugar contents per mg protein did not change significantly despite dilution by increased volume. This increase was accompanied by an increase in total but not specific activity of sucrose degradative enzymes SS, acid invertase, and neutral invertase. A significant increase in the specific activities of sucrose-metabolizing enzymes in senescing ovaries suggests an increase in respiratory activity in those ovaries. There appears to be no direct relationship between sucrose-metabolizing enzymes, IAA, or ethylene in developing pollinated ovaries, but the increase in sucrose cleavage activity in nonpollinated ovaries may be related to the increase in ethylene production.

*Acknowledgments.* We wish to thank Ms. Karen Robacker for the GC-SIM-MS analysis of hormones and Mr. Paul Thompson, Jr. for the enzyme and sugar analyses.

## References

Bradford MM (1976) A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. Anal Biochem 72:248–254

Brenner ML (1987) Photosynthate partitioning and seed filling. In: Davies PJ (ed) Plant hormones and their role in plant growth and development. Martinus Nijhoff, Boston, pp 474–493

Dunlap JR (1988) Regulation of ACC-dependent ethylene production by excised leaves from normal and albino *Zea mays* L. seedlings. J Exp Bot 39:1079–1089

Dunlap JR (1989) Hormonal relationships to fruit set in muskmelon. Proc. Cucurbitaceae 89: Evaluation and Enhancement of Cucurbit Germplasm, pp 183–185

Dunlap JR, Robacker KR (1990) Abscisic acid alters the metabolism of indole-3-acetic acid in senescing flowers of *Cucumis melo* L. Plant Physiol 94:870–874

Goodwin PB (1978) Phytohormones and fruit growth. In: Letham DS, Goodwin PB, Higgins TJV (eds) Phytohormones and related compounds: A comprehensive treatise, vol. II. Elsevier/North Holland, New York, pp 175–214

Gross KC, Pharr DM (1982) A potential pathway for galactose metabolism in *Cucumis sativus* L., a stachyose transporting species. Plant Physiol 69:117–121

Hendrix DL (1990) Carbohydrates and carbohydrate enzymes in developing cotton ovules. Physiol Plant 78:85–92

Hendrix DL, Peelen KK (1987) Artifacts in the analysis of plant tissues for soluble carbohydrates. Crop Sci 27:710–715

Hubbard NL, Huber SC, Pharr DM (1989) Sucrose phosphate synthase and acid invertase as determinants of sucrose content in developing muskmelon (*Cucumis melo* L.) fruits. Plant Physiol 91:1527–1534

Lingle SE, Dunlap JR (1987) Sucrose metabolism in netted muskmelon fruit during development. Plant Physiol 84:386–389

Lowell CA, Tomlinson PT, Koch KE (1989) Sucrose-metabolizing enzymes in transport tissues and adjacent sink structures in developing citrus fruit. Plant Physiol 90: 1394–1402

Mattoo AK, Aharoni N (1988) Ethylene and plant senescence. In: Noodén LD, Leopold AC (eds) Senescence and aging in plants. Academic Press, New York, pp 241–280

McCollum TG, Huber DJ, Cantliffe DJ (1988) Soluble sugar accumulation and activity of related enzymes during muskmelon fruit development. J Am Soc Hort Sci 113:399–403

Noodén LD (1988) Whole plant senescence. In: Noodén LD, Leopold AC (eds) Senescence and aging in plants. Academic Press, New York, pp 391–439

Schaffer AA, Aloni B, Fogelman E (1987) Sucrose metabolism and accumulation in developing fruit of *Cucumis*. Phytochemistry 26:1883–1887

Solomos T (1988) Respiration in senescing plant organs: Its nature, regulation and physiological significance. In: Noodén LD, Leopold AC (eds) Senescence and aging in plants. Academic Press, New York, pp 111–145

Sung S-JS, Xu D-P, Black CC (1989) Identification of actively filling sucrose sinks. Plant Physiol 89:1117–1121

Yoshii H, Imaseki H (1981) Biosynthesis of auxin-induced ethylene. Effects of indole-3-acetic acid, benzyladenine and abscisic acid on endogenous levels of 1-aminocyclopropane-1-carboxylic acid (ACC) and ACC synthase. Plant Cell Physiol 22:369–379

Zimmerman MH, Ziegler H (1975) List of sugars and sugar alcohols in sieve-tube exudates. Appendix III. Encycl Plant Physiol 1:480–503