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Sex Modification in *Asparagus officinalis* L.[1]

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Abstract. **Treatment of spears of pistillate asparagus (XX) with 5000 mg/liter gibberellic acid (GA_3) plus 1000 mg/liter 6-benzyl-amino-9-(tetrahydro-2-pyryl)-purine (PBA) or 2000 and 5000 mg/liter GA_3 alone induced development of stamens with sterile anthers. Spears of XY staminate genotype treated with 10 mg/liter PBA or PBA plus 50 g/liter glucose had more hermaphroditic flowers with ovules than untreated flowers; seedless fruits developed after pollination. YY staminate genotype developed pistils with styles following treatment of spears with 100 mg/liter PBA or PBA plus 50 g/liter glucose. Some ovules had well developed integuments and chalaza but no embryo sacs. PBA reduced stamen length and increased anther sterility.**

Asparagus is a dioecious species. Sexual dimorphism is controlled by a single genetic factor (or sex chromosome) conventionally termed X and Y (15). YY is staminate and homogametic, XY is staminate or occasionally andromonoecious and heterogametic; XX is pistillate. Staminate plants have flowers with mostly rudimentary pistils containing degenerated ovules; occasional flowers of andromonoecious plants are bisexual with rudimentary pistils and stamens present in unisexual flowers; pistillate flowers have stamens with collapsed anthers lacking pollen. Such flowers may be potentially hermaphroditic (11). In this paper we shall use interchangeably staminate–male referring to XY and YY genotypes and pistillate–female for XX genotypes.

Staminate plants are most desirable for commercial production because of greater yield, vigor, and longevity. These characteristics may be correlated with low fruit production (10). The few hermaphroditic flowers on andromonoecious males (XY) are selfed to produce YY males. Selected YY male and XX female clones are crossed to produce F_1 all male (XY) hybrid seed used in cultivation. Staminate and pistillate plants are propagated through tissue culture and crown division to give a limited number of genetically identical but heterozygous parents which when crossed produce a variable F_1 progeny.

The efficiency of asparagus breeding could be improved by developing inbred lines by self pollination of selected vigorous, disease-resistant XX females and YY males. Such lines might be crossed to obtain heterosis and uniformity in the F_1 progeny. Self-pollination of XX and YY genotypes is not presently possible, but might be if sex expression could be modified (11). Cytokinins such as PBA, 6 benzylamino purine (BA), and zeatin induce pistil development in *Vitis* (12), *Cannabis sativa* (4, 5), and *Cleome* (6), respectively. GA_3 induces staminate flower development in *Cannabis sativa* (4), *Carica papaya* (8), *Citrullus lanatus* (1), *Cucumis sativus, Cucumis melo* (14), and *Luffa acutangula* (3). However, GA_3 inhibits pistil development in *Cleome* (6); and induces femaleness in *Ricinus communis* (16).

Morphology, embryology and sex expression of some asparagus genotypes studied were determined previously (11). This study reports the effects of PBA, GA_3, and glucose on sex expression of asparagus.

Materials and Methods

PBA, GA_3, and glucose were applied at different concentrations to spears of XX, XY, and YY genotypes:

Sex genotypes	Clone
XX	Md10
XY	155II
YY	14-4

Length of spears at treatment varied from 5-25 cm, corresponding with a majority of flower buds having anthers and ovules with sporogenous tissue and at the megaspore mother cell stage. Materials were applied once as a 1 min dip or as a spray to runoff in water with 0.1% Tween 20.

The clones were grown in a greenhouse with temperatures of 27-29°C (day) and 17-19° (night). Cool White fluorescent tubes were used for 4 hr to extend the short photoperiod with high light intensity from November – March.

A completely randomized design was used. Mean treatment values were found on 30 flowers per treatment.

Results

Females (Table 1), were unaffected in stamen or flower length by PBA at 10 or 100 mg/liter. PBA at 1000 mg/liter increased flower length measured from base of receptacle to tip of petals at anthesis (Fig. 1D, 3C). GA_3 at 2000 and 5000 mg/liter and combinations of GA_3 and PBA significantly increased flower and filament length (Fig. 2B, C; 3B, D). GA_3 at 5000 mg/liter and GA_3 at 5000 mg/liter plus PBA at 1000 mg/liter treated flowers had intact anther walls, (Fig. 4). Untreated flowers showed collapsed anthers walls (Fig. 5).

Treatment of XY males with PBA at 10 mg/liter and PBA at 10 mg/liter plus glucose at 50 g/liter increased ovary width (Table 2). Stamen length was reduced by treatment with PBA at 10 mg/liter plus glucose at 50 g/liter (Fig. 6C). Both treatments induced a greater number of pistillate flowers with viable ovules (Figs. 7, 8). After pollination seedless fruits were produced.

Table 1. Effect of PBA and GA on flowers of female (XX) asparagus (Md 10).

Treatment (mg/liter)		Organ size (mm)[z]				
GA	PBA	Flower length	Stamen length	Filament length	Anther length	Anther width
0	0	3.63 a	1.63 ab	1.03 a	0.63 a	0.30 a
0	10	3.63 a	1.55 a	0.98 a	0.57 a	0.32 a
0	100	4.29 ab	1.95 abc	1.27 ab	0.68 a	0.32 a
0	1000	5.09 bc	2.54 abcd	1.58 abc	0.69 a	0.44 a
2000	0	5.76 c	2.80 abcd	1.96 bc	0.83 a	0.40 a
5000	0	6.11 c	3.30 d	2.37 c	0.93 a	0.38 a
5000[y]	1000[y]	6.38 c	2.87 bcd	2.20 c	0.66 a	0.35 a
5000[x]	1000[x]	5.65 c	3.08 cd	2.18 bc	0.90 a	0.40 a

[z]Mean separation in columns by Tukey's w-procedure, 5% level.
[y]In combination.
[x]24 hr between application; GA applied first.

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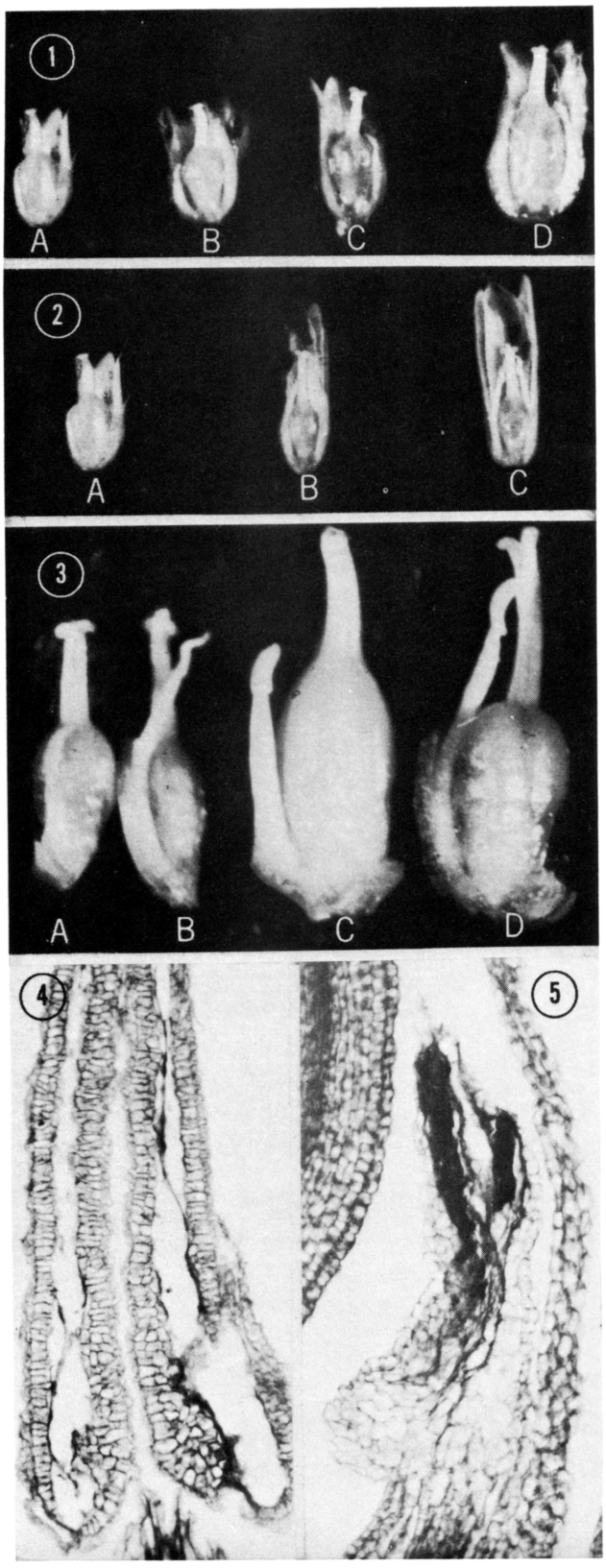

Fig. 1-5. Flowers of female (XX) asparagus (Md10); Fig. 1, PBA treatments: A, control; B, 10 mg/liter; C, 100 mg/liter; D, 1000 mg/liter, 5×; Fig. 2, GA_3 treatments: A, control; B, 2000 mg/liter; C, 5000 mg/liter, 5×; Fig. 3, Pistil and one (of six) stamen: A, control; B, GA_3 5000 mg/liter; C, PBA 1000 mg/liter; D, GA_3 5000 mg/liter plus PBA 1000 mg/liter, 13×; Fig. 4-5, Longitudinal sections of anthers from asparagus flowers; Fig. 4, GA_3 5000 mg/liter, 120×; Fig. 5, control, 120×.

Table 2. Effect of PBA and glucose on flowers of male (XY) asparagus 155 II.

Treatment (mg/liter)		Organ size (mm)[z]		
PBA	Glucose	Ovary width	Stamen length	Anther length
0	0	1.18 a	3.03 b	0.96 b
10	0	1.54 b	2.80 ab	0.55 a
10[y]	50000[y]	1.51 b	2.16 a	0.60 a

[z]Mean separation in columns by Tukey's w-procedure, 5% level.
[y]In combination.

PBA at 100 mg/liter or PBA at 100 mg/liter plus glucose at 50g/liter promoted development of pistils with viable ovules in YY males (Table 3). This genotype is characterized by rudimentary pistils without styles (Fig. 9A). PBA at 100 mg/liter reduced flower length and width and increased ovary length and width and style length. PBA at 100 mg/liter plus glucose at 50 g/liter stimulated style and pistil development and induced smaller stamens, filaments and anthers; different degrees of anther sterility were observed (Fig. 9). There was great variation in sex expression of treated flowers probably because of the different developmental stage of flower buds in an asparagus spear at treatment. Few flowers treated with PBA plus glucose were morphologically pistillate (Fig. 9G); their pistils contained

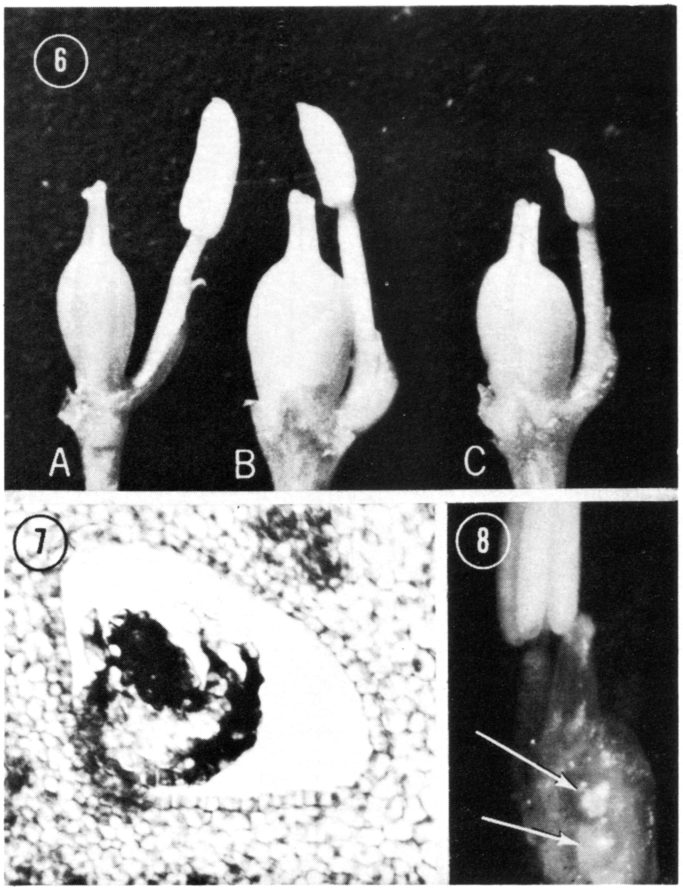

Fig. 6-8. Flowers of XY male (155II); Fig. 6, pistil and 1 (of 6) stamen: A, control; B, C, PBA at 10 mg/liter plus glucose at 50 g/liter, 16×; Fig. 7, longitudinal section of degenerated ovule, 400×; Fig. 8, viable ovules of PBA plus glucose 50 g/liter treated flower, 30×.

Table 3. Effect of PBA, glucose and sucrose on flowers of male (YY) asparagus (14-4).

Treatment (mg/liter)			Organ size (mm)[z]							
PBA	Glucose	Sucrose	Flower length	Flower width	Ovary length	Ovary width	Style length	Stamen length	Filament length	Anther length
0	0	0	5.32 c	2.53 c	1.31 b	0.79 a	0.00 a	3.52 b	2.10 bc	1.42 d
100	0	0	4.71 b	2.40 b	1.34 b	0.94 b	0.22 b	2.44 a	1.71 ab	0.73 b
0	50000	0	5.43 c	2.50 bc	1.37 b	0.91 ab	0.00 a	3.23 b	2.18 c	1.05 c
100[y]	50000	0	4.05 a	2.21 a	1.08 a	0.86 ab	0.20 b	1.95 a	1.42 a	0.53 a
100[y]	0	50000	4.30 ab	2.18 a	1.13 a	0.85 ab	0.05 ab	1.90 a	1.39 a	0.52 a

[z]Mean separation in column's by Tukey's w-procedure, 5% level.
[y]In combination.

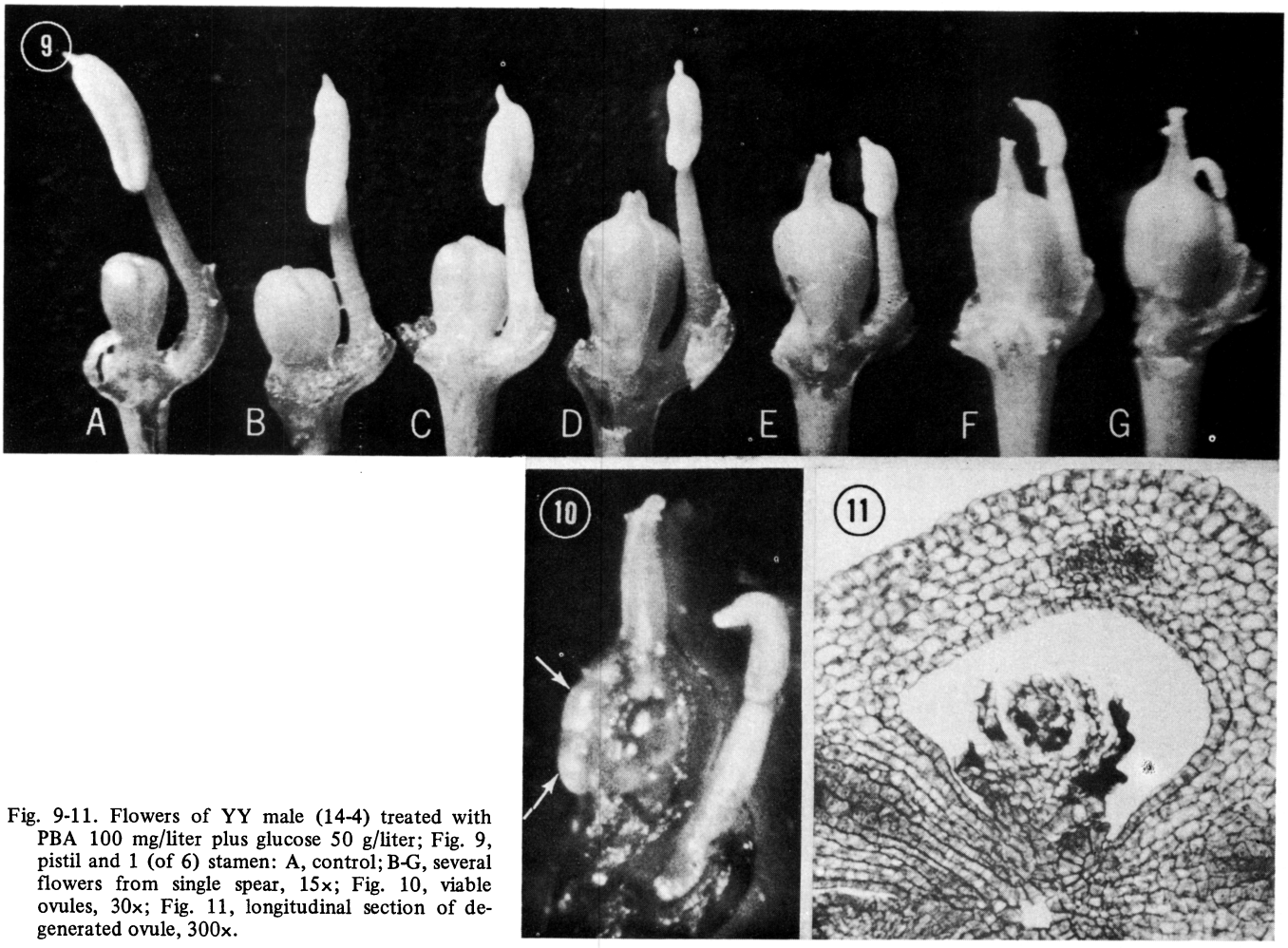

Fig. 9-11. Flowers of YY male (14-4) treated with PBA 100 mg/liter plus glucose 50 g/liter; Fig. 9, pistil and 1 (of 6) stamen: A, control; B-G, several flowers from single spear, 15×; Fig. 10, viable ovules, 30×; Fig. 11, longitudinal section of degenerated ovule, 300×.

well developed ovules (Fig. 10). However, microscopic studies indicated that no embryo sac was present. In contrast, the controls had flowers with degenerated ovules (Fig. 11). Frequency distribution of pistil measurements per number of flowers treated with PBA plus glucose indicate that style length increased in most pistils but few showed well developed styles and larger ovaries. Treated flowers had higher number of pistils with wider ovaries (Fig. 12).

Summary and Discussion

Several growth regulators have been tested in attempts to induce changes in sex expression in various plants but only one ineffective result has been reported for asparagus (7).

Morphological studies of treated XX spears indicate that flower, filament and anther length were increased by GA_3; similar effects and conversion to staminate flowers have been previously reported only in the Cucurbitales *(Carica)*, especially in the Cucurbitaceae *(Luffa, Citrullus, Cucumis)*. PBA reduced flower, anther and filament length while style and ovary length increased in XY genotypes. Such contrasting effects were not noted by Negi and Olmo (13), who used PBA to convert staminate flowers to hermaphrodites in one genotype of *Vitis vinifera*. Treatment of asparagus with PBA, especially if combined with glucose, showed the most promise of modifying sex in the YY genotype, which had degenerating ovules at megaspore mother cell stage. Converted flowers had well developed ovules which lacked

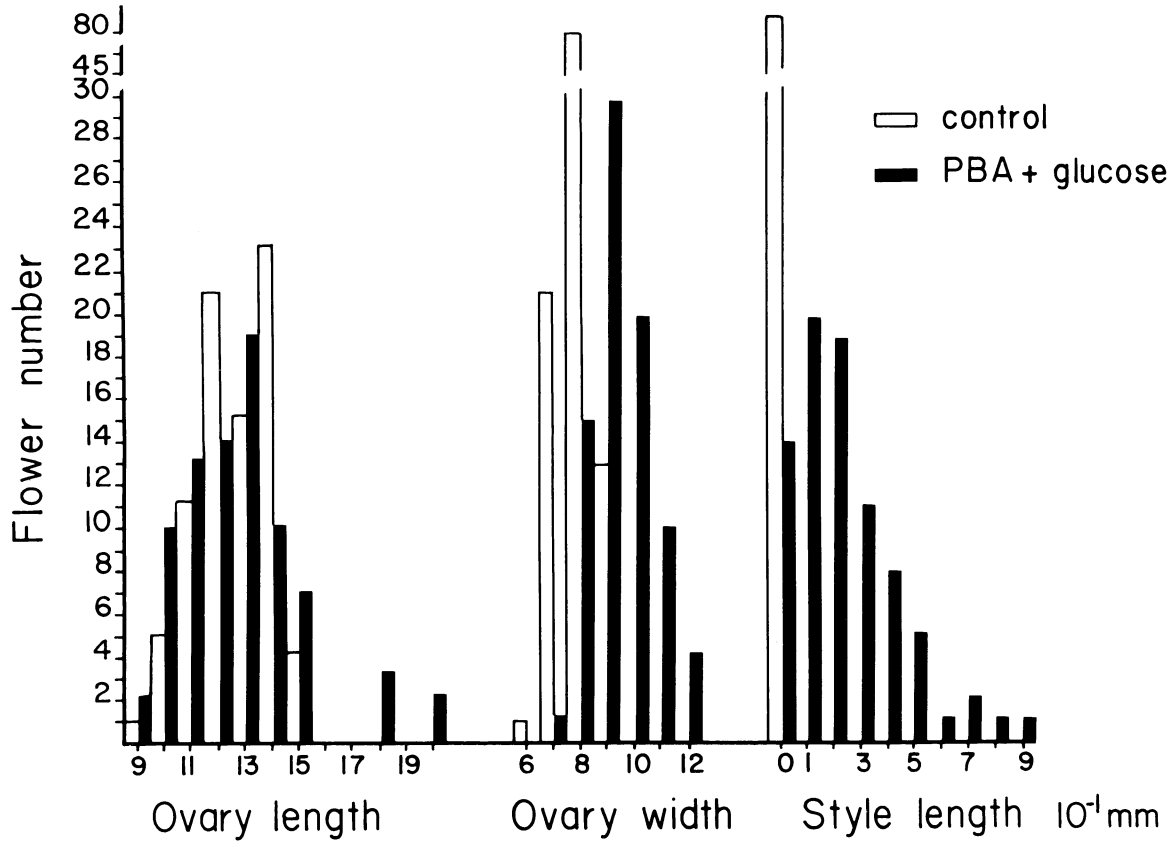

Fig. 12. Frequency distribution of ovary and style measurements per number of flowers in YY male (14-4), treated with PBA at 100 mg/liter plus glucose at 50 g/liter.

megagametophytes. The fact that morphological changes toward femaleness resulted from this treatment suggests a direction for future attempts to induce functional pistillate flowers in XY or YY genotypes.

The mechanism of cytokinin action suggested by Negi and Olmo (13) did not consider nutrient deficiency as a factor in normal development of rudimentary pistils. Based on observations by Goldschmidt and Huberman (9), who studied carbohydrate distribution within the flower, and by Borkowska and Borkowski (2), who found high cytokinin activity in pistillate flowers of *Cucumis sativus* in contrast to low activity in staminate flowers, we suggest that exogenous cytokinins may supplement a low cytokinin activity in staminate flowers, inducing development of rudimentary pistils. The actively developing pistils then could constitute a sink, diverting some nutrients and photosynthate from other floral parts to the pistils. This possibility is supported by the observation that following treatment with PBA the pistil of a staminate flower enlarges, but perianth parts and stamens of the same flower are smaller.

Literature Cited

1. Bhandari, M. C. and D. N. Sen. 1973. Effect of certain growth regulators on sex expression of *Citrullus lanatus* (Thumb.) Mansf. *Biochem. Physiol. Pflanz.* 164:450-453.
2. Borkowska, B. and J. Borkowski. 1975. Endogenous cytokinin-like compound and growth inhibitors in pistillate and staminate cucumber flowers. *Bul. Acad. Polon. Sci. Ser. Sci. Biol.* 23:291-294.
3. Bose, T. K. and J. P. Nitsch. 1970. Chemical alteration of sex expression in *Luffa acutangula*. *Physiol Plant.* 23:1206-1211.
4. Chailakhyan, M. K. and V. N. Khryanim. 1978. The influence of growth regulators absorbed by the root on sex expression in hemp plants. *Planta* 138:181-184.
5. _____ and _____. 1978. The role of roots in sex expression in hemp plants. *Planta* 138:185-187.
6. deJong, A. W. and J. Bruinsma. 1974. Pistil development in *Cleome* flowers III. Effect of growth regulating substances on flower buds of *Cleome iberidella* Welw. ex oliv. grown *in vitro*. *Z. Pflanzenphysiol.* 73:142-151.
7. Flory, W. S., Jr. 1932. Genetic and cytogenetic investigation in *Asparagus officinalis* L. *Genetics* 17:432-467.
8. Ghosh, S. P. and S. P. Sen. 1974. The modification of sex expression in papaya *Carica papaya* L. *J. Hort. Sci.* 50:91-96.
9. Goldschmidt, E. E. and M. Huberman. 1974. The coordination of organ growth in developing citrus flowers: a possibility for sink type of regulation. *J. Expt. Bot.* 25:534-541.
10. Green, W. J. 1980. Asparagus. *Ohio Agr. Exp. Sta. Bul.* 3(9):241-244.
11. Lazarte, J. E. and B. F. Palser. 1979. Morphology, vascular anatomy and embryology of female, heterogametic and homogametic male flowers of *Asparagus officinalis*. *Amer. J. Bot.* 66:753-764.
12. Negi, S. S. and H. P. Olmo. 1971. Induction of sex conversion in male *Vitis*. *Vitis* 10:1-19.
13. _____ and _____. 1972. Certain embryological aspects of cytokinin SD 8339 in converting sex of a male *Vitis vinifera* (Sylvestris). *Amer. J. Bot.* 59:851-857.
14. Peterson, C. E. and L. D. Andher. 1960. Induction of staminate flowers on gynoecious cucumber with gibberellin GA_3. *Science* 131:1673-1674.
15. Reimann-Philipp, R., B. Zilm, and H. Eresen. 1959. Cytologische Untersuchungen zur Identifizierung der Geschlechtschromosomen bei *Asparagus officinalis* L. in Verbindung mit Züchtung rein männlicher Sorten. *Z. Pflanzenzucht.* 42:295-304.
16. Shifriss, O. 1961. Gibberellin as sex regulator in *Ricinus communis*. *Science* 133:2061-2062.