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Selection and Genetics of Nectary Development in Cytoplasmic Male Sterile *Brassica campestris*

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Abstract. **Nectary development in cytoplasmic male sterile (cms) *Brassica campestris* L. was partially restored through 3 cycles of selection for nectary size and number. No major anatomical differences between nectaries of normal and cms plants were apparent under light and scanning electron microscopes (SEM). Half-sib family analysis of nectary development showed negligible additive genetic variance but a prominent maternal effect. Differential response to selection observed in 3 pedigrees suggests the possibility of capitalizing on nuclear-cytoplasmic diversity for the improvement of nectary function.**

The discovery of cytoplasmic male sterility in *Raphanus sativus* L., radish, by Ogura (10) has provided an alternative approach to the self-incompatibility system in hybrid brassica production (9). Cytoplasmic male sterile (cms) *Brassica oleracea* L. (cabbage) and *B. napus* L. (oilseed rape) were first produced by Bannerot et al. (1) through substituting the *Brassica* nuclei into the radish (R1) cytoplasm. Cytosterile *B. campestris* was subsequently generated by repeated backcrossing of *B. campestris* to cms *B. napus* (13). Though R1-cytoplasm-induced male sterility in *Brassica* species is environmentally stable, several R1-cms-associated physiological and morphological abnormalities can limit the potential usefulness of the R1-cms system in commercial seed production (8, 14). One of the major abnormalities is a partial to complete suppression of nectary development in the cms parents.

Experience with cms in onion and carrot hybrid seed production indicates that honey bee (*Apis mellifera* L.) foraging preferences among male fertile and male sterile lines often result in low seed yields on the cms seed parents (7). In cms carrot, asynchrony of floral events and inferior nectar quality, quantity and aroma are major causes of low bee visitation (4). Though the importance of adequate pollination in hybrid seed production is apparent to plant breeders, there have been few studies on the effectiveness of selecting for higher nectary function in insect-pollinated crops. Teuber et al. (11, 12) reported that the nectar volume produced in male fertile alfalfa was under additive genetic control and presumably amenable to selection; however, it is not known whether nectary development suppressed by a cms system can be restored through selection. We have approached the problem of insect pollination in R1-cms *B. campestris* by initially selecting for the number and size of nectaries to provide a morphological basis for further improvement of other nectary functions such as nectar, flavor, and aroma that are crucial for bee attraction.

This report describes a selection system in restoring normal nectary development in R1-cms *B. campestris* and the progress made after 3 cycles of selection. Data are presented on the relative contribution of genetic and environmental components in nectary development, and may provide insight in developing more effective selection schemes.

Materials and Methods

Cytosterile *B. campestris* ssp. *pekinensis* Rupr. (Chinese cabbage) and *B. campestris* ssp. *chinensis* L. (pak choy) were obtained from the advanced populations of our R1-cms oriental vegetable breeding program. Plants were induced to flower by vernalization of moistened seeds held at 4°C for 24 days under low fluorescent light and then grown under continuous light of 200 $\mu E\ s^{-1}m^{-2}$ and irrigated with Hoagland's solution twice weekly in a 25°C greenhouse. Nectary development was scored using a 0–9 scale based on the number and size of nectary glands (Fig. 1). The scale normally was used as a 6-point scale (0, 1, 3, 5, 7, 9); however, intermediate scales (2, 4, 6, 8) could be added as necessary. Nectaries were observed using a 10× handlens or a dissecting microscope. Nectary function of an individual plant was rated by averaging the scores of the first 5 flowers.

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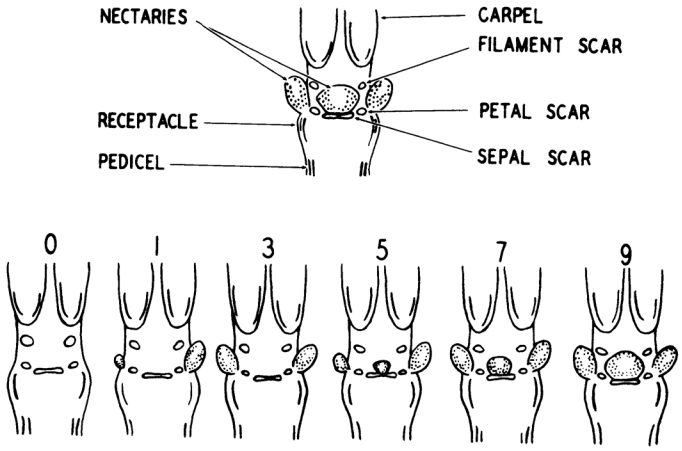

Fig. 1. Morphological scale for the assessment of nectary development and functions in cms *Brassica campestris*. Top: Diagram of flower base of *B. campestris* indicating the position of nectaries. Bottom: 0 = no nectaries; 1 = 1 or 2 partially developed nectaries, usually small; 3 = 2 partially developed nectaries close to normal size; 5 = 3 partially developed nectaries, 1 usually smaller; 7 = 4 partially developed nectaries from small to nearly normal size; 9 = 4 fully developed nectaries.

In advancing the cms breeding lines, only plants with highest nectary ratings in a backcross family were selected for further backcrossing. Three cycles of selection were completed.

In order to assess the effectiveness of the selection scheme, nectary development of families from 3 pedigrees were monitored over successive backcross generations. The pedigrees, A, B, and C represented 3 series of families backcrossed to *B. campestris* recurrent parents PHW64033, PHW64040, and PI419106, respectively. The average nectary function of a family was calculated by scoring 5 flowers from 10 individual plants per family.

The choice of nectary morphology as a selection criterion was based on the assumption that nectaries of cms plants, though reduced in size and number, were structurally and functionally similar to normal nectaries. In order to substantiate our hypothesis, nectaries of normal and cms plants were examined under light and scanning electron microscopes (SEM). Nectaries of about 1000 plants were surveyed for nectary secretion under 25× stereomicroscope.

For scanning electron microscopy, specimens were prepared following a modified procedure of Erickson and Garment (3). Normal flowers and cms flowers with a nectary rating of "3" were fixed for 3 hr in fresh 2.5% glutaraldehyde in 0.1 M phosphate buffer, followed by postfixation for 2 hr in 1% osmium tetroxide in 0.1 M phosphate buffer, and then dehydrated through a graded alcohol series. Flowers with sepals and petals removed were mounted on aluminum stubs, coated with gold-palladium, and viewed in a JOEL-JEM-US scanning electron microscope.

The relative significance of the genetic and environmental effects in nectary development were examined using a half-sib family analysis to partition the phenotypic variance of nectary development (6). Twenty-two cms plants representing 6 different *B. campestris* recurrent parents—PI419130, PI419106, PHW64040, PHW64166, PHW64237, and PHW64241—were randomly chosen from the 7th cms *B. campestris* backcross generation. These plants were crossed to 2 *B. campestris* pollen donors, PHW64614 and PHW64630, with normal nectary function (scale 9). Nectary morphology of 20 progenies from each of the 44 crosses were scored and the variance components were analyzed.

Results and Discussion

Based on the survey of nectaries of over 1000 cms and normal flowers, we found that gland size and number were more definitive features than the amount of nectar secreted. Though variation in nectary size and number among flowers on a single plant occurred, the variation seldom exceeded more than one unit on the 0 to 9 scale. In the early generations, prior to conscious selection, nectaries of breeding lines were either absent or rudimentary. After 3 generations of selection, we have developed families having at least 2 nectaries of size comparable to those on a normal parent (scale 3) and occasionally there were plants with 4 well-developed nectaries (scale 7). Among the 64 lines in our advanced breeding population, 21 (33%), 6 (9%), and 4 (6%) were rated as 3, 5, and 7 on the nectary scale, respectively. Nectaries of scales 3 to 5 or higher in cms plants were usually associated with nectar secretion (Fig. 2).

No anatomical difference between nectaries of normal and cms plants were apparent under the SEM (Fig. 3). Though not rigorously measured, stomatal structure and distribution appeared to be similar in cms and normal plants. The shrunken appearance of the nectary surface under the SEM probably was due to the collapse of the delicate nectary walls during specimen preparation.

A survey of the change in the average nectary development of backcross families in 3 pedigrees suggested that the recovery of nectary development was favored by certain genotypic combinations. Over 3 generations of selection, pedigree C (recurrent parent PI 419106) and pedigree B (recurrent parent PHW 64040) showed more rapid gain toward normal nectary development than pedigree A (recurrent parent PHW 64033) (Fig. 4).

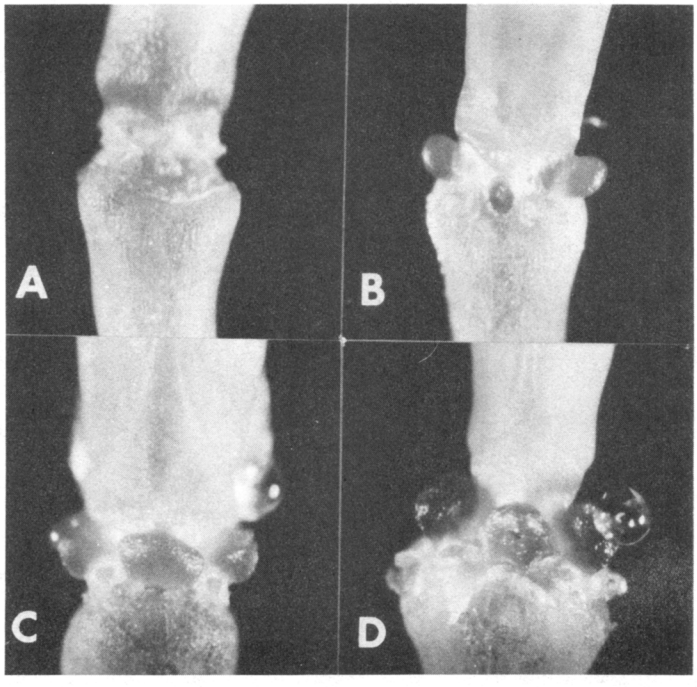

Fig. 2. Nectaries in cms *Brassica campestris* (23× magnification). **A)** no nectaries, scale 0; **B)** 2 partially developed nectaries, scale 3; **C)** 4 partially developed nectaries, scale 7; **D)** fully developed nectaries in male fertile *B. campestris*.

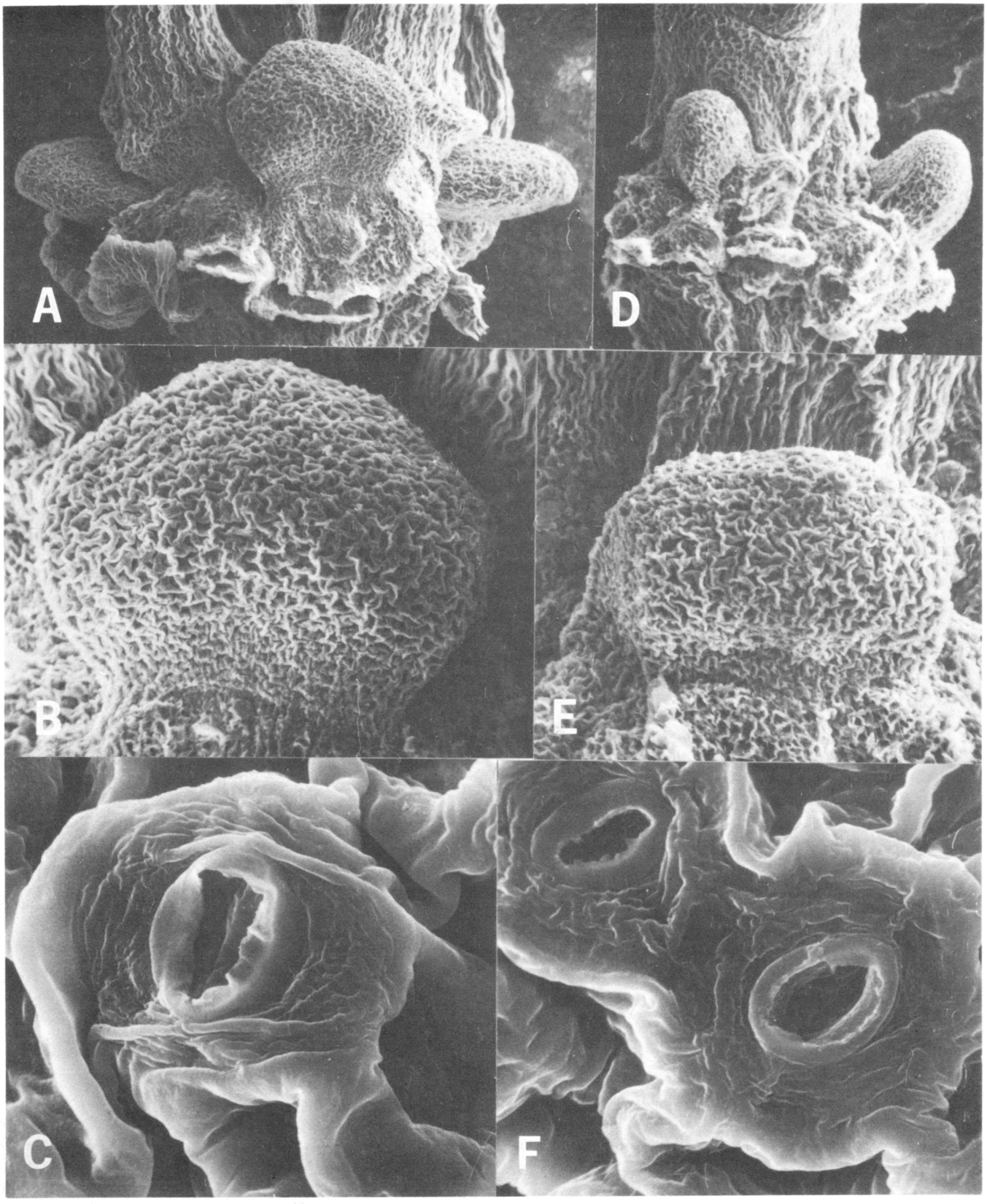

Fig. 3. Scanning electron micrographs of nectaries of male fertile and cms *Brassica campestris*. A, B, and C = nectaries of normal flowers at 88×, 234×, and 3075×, respectively. D, E, and F = nectaries of cms flowers at the same magnifications as A, B, and C. C and F = close-ups of the stomates. Note the similar anatomical features of nectaries from normal and cms flowers.

The analysis of variance and composition of mean squares of the half-sib family analysis is given in Table 1. Male effect was found to be not signficiant, whereas effect of female within male was highly significant. To partition the total phenotypic variance, the male and female observational components were estimated as follows: $\sigma^2 m$ = (MS m − MS f/m)/fn and $\sigma^2 f/m$ = (MS m − MS w)/n where MS m, MS f/m, and MS w were the corresponding mean squares of male, female within male,

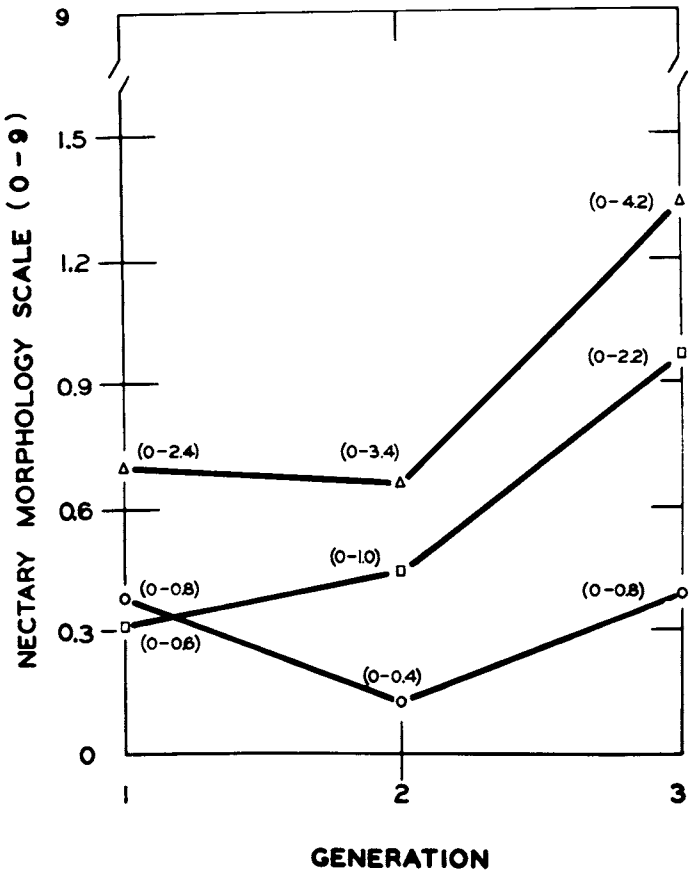

Fig. 4. Developmental response of cms *Brassica campestris* nectaries to 3 generations of selection in 3 pedigrees A (○—○), B (□—□), and C (△—△). Morphological scale is an assessment of nectary development in cms *B. campestris* with 0 being the absence of nectaries and 9 being normal. Each point represents the average scale of 10 plants examined in a family. Numbers in brackets indicate the range of scales found among plants in each family.

and progenies within female; f = number of female per male, and n = number of progenies per female. Assuming no epistasis, the relationships between the observational (σ^2) and causal components (V) were deduced and summarized in Table 2. Additive genetic variance (V_A) as estimated by the male component was slightly negative (-0.07) and was treated as zero for the purpose of analysis. Though variance by definition cannot be negative, it is statistically probable to obtain a negative variance component in the partitioning of the total variance. Variance due to dominance (V_D) and environment ($V_{EC} + V_{EW}$) constituted all the phenotypic variance estimated.

Table 1. Analysis of variance and composition of mean squares for half-sib family analysis of nectary development in cytoplasmic male sterile *Brassica campestris*.

Source	df	Mean square[z]	Composition of mean square[y]
Male (m)	1	5.76^{NS}	$\sigma^2w + n\sigma^2 f/m + fn\sigma^2m$
Female within male (f/m)	42	37.53**	$\sigma^2w + n\sigma^2f/w$
Within progenies (w)	836	1.89	σ^2w

[z]NS = nonsignificant, ** = significant at 1% level.
[y]f = number of females per male; n = number of progenies per female.

Table 2. Estimates of variance components of half-sib family analysis of nectary development in cytoplasmic male sterile *Brassica campestris*.

Source	Component: Observational	Component: Causal[z]	Estimate	Percentage
Male	σ^2n	$1/4\ V_A$	0	0
Female	σ^2f/m	$1/4\ V_A + 1/4\ V_D + V_{EC}$	1.78	48.5
Progeny	σ^2w	$1/2\ V_A + 3/4\ V_D + V_{EW}$	1.89	51.5
Total	σ^2T	$V_A + V_D + V_{EC} + V_{EW}$	3.67	100

[z]Abbreviations according to Falconer (6): V_A = additive genetic variance; V_D = dominance genetic variance; V_{EC} = environmental variance due to common environment; and V_{EW} = environmental variance within progeny.

Since in most instances gain in selection is usually accounted for by the presence of additive genetic variance, the observed gain in nectary development in the absence of additive genetic variance suggested by our mating experiment poses some interesting questions relating to the genetic basis of nectary suppression in a cms system. The inability to detect additive genetic variance could be due to an inadequate representation of male lines in the mating experiment. As only 2 males were used, it was possible that the males chosen had the same breeding values that rendered the male effect nonsiginificant in our analysis. If indeed additive genetic variance in nectary development is negligible, then about one half of the total phenotypic variance needs to be accounted for by the dominance and common environment components. Though the effects due to dominance and common environment are confounded in the present design, the significant female effect is likely to be due largely to maternal effect, which represents the common environment, rather than to dominance. This interpretation seems logical since the suppression of nectary development is a R1-cms-associated trait in *B. campestris*, and may be subject to a substantial cytoplasmic influence. It is unlikely, however, that nectary development is entirely controlled by the R1 cytoplasm since gain through selection would be impossible in the absence of cytoplasmic diversity. The improvement of nectary development in the breeding population and the differential response to selection observed in 3 pedigrees suggest that there is considerable cytoplasmic and nuclear interaction. By crossing the lines with partially restored nectary function to a wide range of male genotypes, one should be able to generate a diversity of cytoplasmic nuclear interaction which would permit further improvement of nectary function.

If dominance variance is of minor significance, then the other half of phenotypic variance (51.5%) would be due to random environmental effects (V_{EW}). Since plants were grown under a relatively constant greenhouse environment, the large environmental variance could result from the inherent variation in scoring nectary development with an arbitrary scale.

Though nectary improvement has been shown to be responsive to our selection scheme, it should not be assumed to be a substitute for field evaluation as a predictor of cms parents, since many floral characters are known to be involved in insect attraction and pollination under the natural environment (2). Since the cms lines are devoid of pollen, nectar quality and aroma become even more critical factors for bee attraction. Though we observed a close association between nectar secretion and well-developed nectaries of cms plants, it is not known whether the nectar quality is comparable to the normal. A knowledge of the

chemical constituents in nectar from normal and cms plants will provide a basis for the selection of nectary quality. Nectar characterization by high-performance liquid chromatography (5) will be valuable in detecting subtle differences in nectar quality among the cms lines.

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Variation in *Sechium edule* in Central America

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Abstract. **Genetic variation for 11 fruit characteristics of Central American chayotes (*Sechium edule* Sw.) was similar in Costa Rica, Honduras, Guatemala, and Mexico. Costa Rica possessed many types belonging to the extremes of the total range. Fruit size descriptors were correlated strongly.**

The chayote (xu-xu, christophine, or vegetable pear) is a popular vegetable throughout the tropics, mainly concentrated in the more elevated areas (500–1500 m) with an annual well-distributed rainfall of more than 2000 mm. The young fruits are eaten as a boiled vegetable and when ripe as a source of carbohydrates, notably as a component of a hospital diet. The young stems and leaves, called "quelites" in Costa Rica, are used in soups; the tuberous roots are eaten boiled or fried.

The genus *Sechium* is constituted by only one species, *Sechium edule*, and is native to southern Mexico and Central America (4). However, more recent studies suggest the presence of more species in the genus (J. León, personal communication).

Bukasov (2) confirmed a single species and adds the argument that the natural distribution of *Frantzia tacaco*, a species closely related to chayote, is restricted to Costa Rica. Brücher (1) preferred to include *Frantzia* in the genus *Sechium*. He also described a wild form of *Sechium edule*, found in a cloud forest in the state of Merida in Venezuela.

In the past, some of the fruit characteristics (descriptors) of chayote were used to divide the phenotypic diversity into cultivars. Cook (3) described 5 cultivars in Puerto Rico. Lagos (5) divided all the types into 5 groups, and Lionti (7) used the fruit color and shape to characterize the various types. León (6) mentioned that at least 25 cultivars exist in Central America, using size, color and shape of the fruit, pulp texture, and number of spines as descriptors.

In this study it will be shown that the variation is continuous for some of the characteristics of the fruit, mainly caused by the reproduction system and by the way the chayote is multiplied. Furthermore, the variation for fruit characteristics will be shown to be similar in the 4 countries in which collections were made and that the "center of diversity" includes Costa Rica.

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