YIELD STUDIES ON ARIZONA HYBRID #1, BUFFALO GOURD

by

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A Thesis Submitted to the Faculty of the

DEPARTMENT OF PLANT SCIENCES

In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE
WITH A MAJOR IN HORTICULTURE

In the Graduate College

THE UNIVERSITY OF ARIZONA

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ACKNOWLEDGMENTS

The author's gratitude is extended to Dr. W. P. Bemis, Joe Scheerens, and Martha Rosemeyer for their continued professional, and sometimes more importantly, moral support in every aspect of this endeavor. Its completion was indeed a joint adventure.

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ABSTRACT

An experimental plot was established to study buffalo gourd yield as it related to sex expression. Indications were that gynoecious plants had higher yield potential than monoecious plants. Plants were observed over two growing seasons. Results of the first year's harvest showed that the gynoecious plants outyielded their monoecious counterparts both on a per plot and a per plant basis. Second year data revealed similar results on a per plot basis, but when examined on a per plant basis the difference was not significant. Monoecious plants appeared to match gynoecious plants in yield potential after the first season's growth. A possible explanation for the observed difference in the first year's data could be juvenility. A high degree of genetic variability exists in the currently available hybrid seed. When isogenic inbred lines are developed, a more accurate test of the hypothesis can be attempted.

CHAPTER 1

INTRODUCTION

The feral perennial buffalo gourd, *Cucurbita foetidissima* HBK, has been the object of an intensive domestication program due to its potential as an oil seed, protein and starch producer. Due to its origin in the semi-arid regions of southwestern North America, it is being considered as a possible crop adapted to desert environments.

Dr. Lawrence Curtis (1946) first described the potential of the native gourd as a crop species and suggested further research on the basis of four horticultural attributes: (1) the plants are perennial, (2) they grow on wastelands in regions of low rainfall, (3) they can produce an abundant crop of fruit containing seed rich in oil and protein, and (4) the fruit lends itself to mechanical harvesting. Further work on the domestication of the buffalo gourd has been the focus of the Buffalo Gourd Research Team at The University of Arizona. Under the guidance of an interdisciplinary team of researchers, germplasm was collected from diverse sources and a total of 145 accessions was planted in germplasm nurseries at the Agricultural Experiment Station in Tucson. Studies were conducted to identify plants with superior horticultural properties.

During these studies two sex types, monoecious and gynoecious, were observed in a wide range of the germplasm sources. As the normal sex expression in Cucurbita is monoecious, this genetic mutant which caused gynoecy was of major interest to future breeding developments. The obvious question was whether gynoecious plants could outyield monoecious plants.

Further research necessitated the creation of a relatively homogeneous seed supply and an attempt to produce hybrid seed was initiated. Open-pollinated seed from previously selected superior plants and seed from selected crosses were used to establish hybrid seed production plots. At each hybrid seed production plot only one line was chosen as the pollinator line. Gynoecious plants in this line were sacrificed so that only monoecious plants remained. All other lines grown at a location were designated as seed parent lines. Only gynoecious plants were saved in these lines. Since gynoecious plants are male sterile, and the plants are isolated from each other, the only pollen available within a plot should come from the pollinator line. Thus, all seed collected off the seed parent lines should be hybrid seed, a cross between two lines (Scheerens 1978).

The seed designated Arizona Hybrid #1 was the result of a cross between accession 158-2, germplasm collected from Patagonia, Arizona, used as the seed parent, and accession 142-1, germplasm collected from Jerome, Arizona, and used as the pollinator line. In the present study, an experimental plot was established using Arizona Hybrid #1 seed to determine the relationship of yield versus sex expression. The results were intended to verify the earlier indications of superior yielding ability of gynoecious plants. In order to eliminate the confounding effects of background genotype, the experiment was conducted on a per plot basis. In this way a clear measure of fruit yield based on sex type could be determined without having to consider the effect of superior or inferior plants.

CHAPTER 2

LITERATURE REVIEW

The Plant

The buffalo gourd, *Cucurbita foetidissima* HBK (1817), is native to semi-arid and arid lands of southwestern North America and can be found wild from Nebraska to California, and south to Guanajuato in Mexico (Bailey 1943). Plants are initially established from seed but the primary mode of reproduction is asexual. Large homogeneous colonies are established by adventitious rooting at the nodes of vines. These trailing ground-cover vines may reach lengths of six meters and as many as 20 vine initials may be produced from a single crown. Vines are frost sensitive but the plant survives as a perennial due to its exceptionally large storage root. Roots have been recorded reaching depths of 2.5 meters with fresh weights of 45kg (Bemis et al. 1978a). Average root weights are typically 3 to 6kg after two seasons of growth. Leaves are grey-green in color and harsh to the touch due to a covering of short stiff hairs. They are typically entire, ovate to sagittate in shape, with a base width of 10-13cm and a mid-rib length of 20-25cm (Bemis et al. 1978a). Crushed green foliage emits a distinctive odor, hence the origin of the specific name, *foetidissima*.

Fruits in the form of pepos, ranging from 7-10cm in diameter, can be produced in abundance in one season's growth. It is not unusual for a single plant to produce anywhere from 100 to 200 fruit in one growing season. The number of seed contained per fruit ranges from 200 to 300, with a mean weight per 100 seed of four grams. Flowers are produced individually at the nodes of vines and are either pistillate or staminate (Bemis et al. 1978a). Large, conspicuous yellow-orange flowers appear in early spring, the first usually being male, followed later by female flowers. Cross-pollination is necessary for fruit and seed development and is accomplished in native populations by the solitary squash and gourd bees, Peponapis and Xenoglossa (Hurd, Linsley, and Whitaker 1971).

Cultural Aspects

A study of cultural practices related to buffalo gourd is currently in progress in the areas of consumptive water use and disease problems. The minimum annual water requirement for crop production has been tentatively estimated at 250mm. The plant survives with as little as 150cm but under summer conditions with temperatures of 40 degrees centigrade and higher, it would rarely flower and fruit (Vasconcellos et al. in press). Field observations suggest that the gourd is highly salt tolerant. Disease problems have appeared in the form of: (1) virus, one of which has been identified as squash mosaic virus and the other appears to be cucumber mosaic virus, and (2) a fungal-bacterial c mplex of *Fusarium/Erwinia*, causing storage root decay (Rosemeyer 1980).

Potential Uses

The economic value of buffalo gourd was originally considered in light of its ability to produce oil and protein-rich seeds in minimal rainfall regimes. The crude protein component of whole seed analyzed by Curtis and Rebeiz (1974) on a 50-plant sample was reported as ranging from 22.2 to 35.1%. Shahni et al. (1951) found a protein value of 42.1% for undecorticated seed meal from which the oil has been extracted. Berry et al. (1976) analyzed a decorticated and oil free seed meal sample at 75% crude protein. Protein quality was examined in a feeding study with mice in which threonine and methionine were found to be the limiting amino acids. With amino acid supplementation the protein quality of the seed was greatly enhanced (Thompson et al. 1978). Oil content analyses showed variation of Curtis' 50-plant sample. He reported values ranging from 25.6 to 42.8% for crude oil from whole seed. Shahani et al. (1951) investigated the fatty acid composition of a 66g seed sample collected from fruit of wild plants in Texas. Linoleic acid comprised 65.3% of this oil sample. The presence of this desirable polyunsaturated fatty acid at high levels is evidence of the potential value of buffalo gourd seed as a source of edible oil.

Vasconcellos et al. (in press) reported a mean crude fat content of 39.0% for selected hybrids as compared to a mean of

32.9% for the wild counterparts. Principle fatty acid components were: linoleic acid, 58%; oleic acid, 27%; palmitic acid, 9%; stearic acid, 4%. They suggested an edible oil with acceptable properties could be produced by processing crude buffalo gourd oil using triple refining. In a feeding study with mice, crude oil was evaluated as a dietary component. Normal growth was observed for all levels ranging up to 11% oil in the total isocaloric, isonitrogenous diets (Bemis, Berry, and Weber 1977).

Subsequent study of the large tuberous root has yielded a second economic possibility in the presence of root starch in potentially commercial amounts. Starch can be readily isolated from the roots and recovered in amounts varying from 50 to 56% of the dry weight (Berry et al. 1975). Buffalo gourd starch suitability for food use has been established by a feeding study with mice (Dreher in press). Gelatinized gourd starch was equal to corn and tapioca starch in digestibility. Recent interest in ethanol producation for fuel led to a study on the use of buffalo gourd starch as a fermentation substrate with promising results (Gathman et al. 1979).

Preliminary Work (Historical Development of Gynoecy)

Initial field research on the domestication of the feral gourd took place in Lebanon under the direction of Curtis (*in* Curtis and Rebeiz 1974). Results of six years of field work indicated a tremendous amount of genetic variation in this species, ranging from vine habit to sex expression (Bemis et al. 1967b).

Other known species of Cucurbita are monoecious. However, Curtis (in Curtis and Rebeiz 1974) reported the existence of a genetic mutant in his experimental breeding population. This variant caused the male flowers to abort in the early stages of growth, leavin the plant entirely female, or gynoecious. He classified these plants based on the development of antherless buds as follows:

Type I. Male blossoms 2-3mm in length, never enlarging and never opening.

Type II. Male blossoms 2cm in length, with short stems, rarely opening, drying and remaining on plant.

Type III. Male blossoms normal size, 10-12cm, never opening.

Type IV. Male blossoms containing anthers but no pollen, never opening, reaching half normal size, anthers turning brown, flowers drying and persisting on plant.

Yousef (1976) observed 90 F_1 populations in their second growth season and reported that the majority of plants needed more than one season to express their sex type, because a large proportion of first season plants failed to develop any flower buds. As an indicator of sex expression, he used the type of developing male flower bud. Plants with only normal male flower buds eventually develop female buds and become monoecious. Those developing only abortive male buds will become gynoecious (Yousef 1976).

Flowering pattern was reported to have four phases: (1) non-flowering, (2) male, (3) mixed, and (4) vegetative for both monoecious and gynoecious plants, with male buds being antherless on gynoecious plants (Yousef 1976). The ratio of male to female buds was lower in gynoecious plants and decreased progressively with plant age. Onset of female flower buds on gynoecious plants was earlier than on monoecious plants. The average number of female flower buds on gynoecious plants was much higher than on monoecious plants. The number of nodes to the first female flower was lower in gynoecious plants (Yousef 1976).

Observations of female plants over three consecutive seasons gave Curtis (in Curtis and Rebeiz 1974) evidence to conclude that the antherless trait (gynoecy) was due to genetic inheritance and not environmental factors. Based on data for 560 plants segregating 431 monecious to 129 gynoecious (a close fit for a 3:1 monogenic ratio), he reported the antherless trait to be controlled by a single gene. He further assumed that the monoecious plants in his population could be heterozygous for the trait and hypothesized that gynoecy was due to the homozygous recessive condition.

Yousef (1976) observed the segregation of F_1 populations which resulted from crossing 10 gynoecious lines by nine monoecious lines into 1:1 ratios and concluded that all the monoecious plants were heterozygous. His results supported Curtis' hypothesis of gynoecy being recessive to monoecy, and the trait being controlled by one gene.

A recent study by Bemis et al. (1978b) suggested an alternative theory to the inheritance of the gynoecious character. Forty-seven accessions were collected from diverse locations and their progeny scored for sex type. Progeny populations were of two types: those which consisted only of monoecious plants, and those which contained monoecious and gynoecious plants in 1:1 ratios. They described the latter condition as monogynodioecious and proposed the tentative hypothesis that gynoecy is controlled by the dominant heterozygous condition and monoecy is expressed by the homozygous recessive state.

Sex Expression Related to Yield

The variation in sex expression exhibited by the buffalo gourd is of primary importance in terms of yield as only pistillate flowers can produce fruit. Early research indicated that higher yield potentials could be expected from gynoecious plants. In a preliminary search for horticulturally superior plants, Curtis and Rebeiz (1974) identified five individuals from a population of 730 seedlings which were superior in their production of fruit and seed. Four of these five were gynoecious plants.

After breeding advances the seed production from the highest seed-producing gynoecious plants was shown to be statistically higher than the highest seed-producing monoecious plants. Curtis and Rebeiz (1974) chose 16 gynoecious plants and 16 monoecious plants based on their seed-producing ability and examined them for number of fruit per plant and grams of seed per plant over two growing seasons. Gynoecious plants the first year outyielded monoecious plants in mean number of fruit per plant by 116.1 to 76.8. The second year yield showed means of 202.3 for gynoecious to 159.7 for monoecious plants. Mean grams of seed per plant were calculated for the second year data at 1719.3 to 1092.0g, gynoecious to monoecious respectively.

In a report on 50 selected buffalo gourd plants, Curtis and Rebeiz (1974) compared gynoecious to monoecious plants for fruit number and grams of seed per plant. Only plants which produced 100 fruit or more were included. Range for gynoecious plants was 100 to 260 fruit per plant with a mean of 173. Seed weight per plant ranged from 1000 to 2500g with a mean of 1448g. For monoecious plants fruit number and grams of seed per plant were from 110 to 227 (mean 159) and 1000 to 1700 (mean 1158) respectively. Yousef (1976, p. xi) compared the development of female flower buds on monoecious versus gynoecious plants and reported the values for gynoecious plants to be "far higher than for monoecious plants, indicating that the gynoecious plants produce higher yield."

Scheerens (1979) studied first year yields on an individual plant basis spanning eight different strains of buffalo gourd, including the seed parents of Arizona Hybrid #1. His results revealed a great deal of variation among individual plants within plots for both number of fruit per plant and seed weight per plant. The range in variability of the seed parents which make up the hybrid shows the extent of variation that must exist in Arizona Hybrid #1. The high level of genetic variability masked any differences in yield due to sex type which might have existed (Appendix A).

CHAPTER 3

MATERIALS AND METHODS

The experimental plot, consisting of approximately $1600m^2$, was located at the University of Arizona Agricultural Experiment Station in Tucson. On 20 June 1978, the plot was mechanically seeded to Arizona Hybrid #1 and later thinned to .5m in-row spacing with 1m rows. The experimental design utilized four blocks with two sub-lots per block. Each sub-plot encompassed an area of approximately $200m^2$. As flowers appeared, plants were marked for sex expression and rogued as follows:

1. In gynoecious sub-plots every plant producing a male flower was staked and removed. The population after roguing consisted of all female plants plus unknown plants which failed to flower in their first season.
2. In monoecious sub-plots every plant producing a male flower was staked and the non-staked plants were removed. The population after roguing was 100% monoecious plants.

After roguing, a final count of plants per plant was taken on 7 October. Gourds were harvested and counted by hand on 18 November 1978.

The following year plants emerged from perennial roots in mid-February. They were observed for sex expression upon budding, and in May and June gynoecious plots were rogued to remove any monoecious plants which had developed from unknown plants of the previous year. Counts were made on new plants arising from adventitious rooting from the previous year's growth on 28 February. A final harvest and count was made on 15 October 1979.

Further study was necessary to determine the influence of sex type on the parameters of fruit size, seed weight per fruit, and seed weight per 100 seed. The first year 15 fruit from each of the eight sub-plots were randomly selected and cut in half. The diameter of each was measured and the fruit halves submerged in water to ferment, which facilitated the removal of seed following the method of Scheerens et al. (1978). Upon removal from the fruit the seed lots were washed and air dried on paper plates. The procedure was repeated the following year with one alteration. Fruit halves were soaked in .2M HCl as a substitute for the natural fermentation to speed the decay of placental tissue.

Analyses of variance were performed on all data utilizing methods described for randomized complete block experiments according to Steele and Torrie (1960).

CHAPTER 4

RESULTS

First Year Yields

Results of the first year yield study are presented in Table 1. Total fruit yield on a per plot basis is given for the final plant stand. The discrepancy in the number of gynoecious plants compared to the number of monoecious plants in the final plant stand is due to the late seeding date and the method of roguing. Many plants failed to flower in their first season due to the late planting date. Plants which did not flower were rogued out of monoecious plots, while in gynoecious plots the non-flowering plants were not removed.

Total number of fruit harvested in the first year from gynoecious plots was 1807. Individual plot yields ranged from 273 to 623 fruit. Their monoecious counterparts yielded 438 total fruit with a range from individual plots of 68 to 155 fruit.

Mean number of fruit per plant was calculated for each plot. Range for gynoecious plots was from 2.5 to 6.2, while the monoecious plots ranged from 1.2 to 2.8 mean fruit per plant.

Statistical analyses revealed a significant difference (P=0.05) in yields of gynoecious over monoecious plots for both

Table 1. Fruit Yield of Arizona Hybrid #1 (1978--First Year)

Block	Final Plant Stand		Total Yield per Plot		Mean Yield per Plant	
	Gyn	Mono	Gyn	Mono	Gyn	Mono
A	111	52	273	68	2.5	1.3
B	101	52	623	106	6.2	2.0
C	115	91	489	109	4.3	1.2
D	102	55	422	155	4.1	2.8
Total			1807	438		
Mean			451.7	109.5	4.3	1.8

final yield totals per plot and mean number of fruit per plant (Table 2).

Second Year Yields

Total fruit yields on a per plot basis for the 1979 harvest is given in Table 3. Total fruit harvested from the gynoecious plots was 8406. Individual plot yields ranged from 1662 to 2467 fruit. Yield from monoecious plots totalled 4821 fruit with a range from 881 to 1241.

Mean number of fruit per plant was again calculated for each plot. The range for gynoecious plots was from 21.3 to 26.2. For monoecious plots the range was from 9.8 to 34.6 mean fruit per plant.

Table 2. Analysis of Variance: Fruit per Plot; Mean Fruit per Plant (1978--First Year)

	Fruit per Plot				Mean Fruit per Plant			
Source	df	SS	MS	F	df	SS	MS	F
Sex type	1	234,270	234,270	24.9*	1	11.6	11.6	10.94*
Blocks	3	39,125	13,041.6	1.4	3	5.5	1.8	1.6
Error	3	28,231	9,410.3		3	3.2		
Total	7	201,626			7	20.3		

*Significant at 0.05 level.

Table 3. Fruit Yield of Arizona Hybrid #1 (1979--Second Year)

Block	Final Plant Stand		Total Yield per Plot		Mean Yield per Plant	
	Gyn	Mono	Gyn	Mono	Gyn	Mono
A	97	52	2467	1241	25.4	23.9
B	94	52	1998	1799	21.3	34.6
C	87	91	2279	881	26.2	9.8
D	77	55	1662	900	21.6	16.4
Total			8406	4821		
Mean			2101.5	1205.2	23.6	21.2

Statistical analyses performed on final yield totals on a per plot basis revealed a significant difference in yield of gynoecious over monoecious plots. However, when the final plant stand is considered and mean number of fruit per plant is examined, the F-test revealed no significant difference in gynoecious over monoecious yields (T ble 4).

Second year data included plants which had developed from adventitious roots the previous season. Statistical analyses failed to reveal a difference in gynoecious versus monoecious yield for the 1979 yields on a per plant basis when adventitious plants were counted along with the final stand. Also, no significant difference in the ability to root asexually was found between sex types.

Table 4. Analysis of Variance: Fruit per Plot; Mean Fruit per Plant (1979--Second Year)

	Fruit per Plot				Mean Fruit per Plant			
Source	df	SS	MS	F	df	SS	MS	F
Sex type	1	1,606,528	1,606,528	11.1*	1	12.0	12.0	.16
Blocks	3	488,767	162,922	1.1	3	133.6	44.5	.59
Error	3	432,335	144,112		3	225.5	75.2	
Total	7	2,527,530			7	371.1		

*Significant at 0.05 level.

Sex Type Influence on Fruit Parameters

In order to assure that sex type did not influence seed weight or fruit size, comparative data were taken on mean seed weight per fruit, seed weight per 100 seed, and mean fruit size for both years. Values are presented in Table 5. F-tests showed no significant difference between gynoecious and monoecious plants for these characters. From these data it can be concluded that an increase in fruit number plant indeed represents an increase in seed yield. Estimates of seed yield per hectare for a first year crop are approximately 158kg/ha and for a second year crop approximately 1150kg/ha.

Table 5. Comparison of Mean Seed Weight/Fruit, Seed Weight/100 Seed, Fruit Size, and Total Seed Yield/Plot in Gynoecious and Monoecious Buffalo Gourd

	Mean Seed Weight(g)/Fruit*		Seed Weight(g)/ 100 Seed		Mean Fruit Size(mm)*		Total Seed Yield(kg)/Plot	
Block	Gyn	Mono	Gyn	Mono	Gyn	Mono	Gyn	Mono
1978								
A	11.6	11.6	4.4	4.4	65.9	68.9	3.2	0.8
B	9.5	9.5	4.2	4.0	66.6	63.8	5.9	1.0
C	13.1	9.6	4.9	4.3	69.1	65.4	6.4	1.0
D	11.6	13.8	4.3	4.7	67.3	66.6	4.9	2.1
Mean	11.5	11.1 ns**	4.2	4.3 ns**	67.2	66.2 ns**	5.1	1.2
1979								
A	13.6	13.2	5.0	4.8	72.7	70.2	33.6	16.4
B	12.5	14.2	4.6	5.4	71.6	73.7	25.0	25.6
C	15.8	11.5	5.2	4.5	74.2	70.1	36.0	10.1
D	14.9	14.1	4.7	4.8	71.3	72.1	24.8	12.7
Mean	14.2	13.2 ns**	4.9	4.9 ns**	72.5	71.5 ns**	29.8	16.2

*Calculated on a 15-fruit sample.

**Non-significant gynoecious versus monoecious.

CHAPTER 5

DISCUSSION AND SUMMARY

Discussion

First year yields followed the expected outcome. Gynoecious plants outyielded monoecious plants both on a per plot and a per plant basis. Second year yields showed similar results on a per plot basis, but when the yield per plant was considered results did not support the hypothesis. Testing with similar plant densities would be advantageous.

A dramatic increase in fruit production was observed in the second year. Gynoecious plots produced a near five-fold increase, while monoecious plots exhibited a ten-fold increase in number of fruit produced.

Differences observed in first year results between sex types might be due to juvenility in that plants require more than one season to express their sex type. In the first year, monoecious plants produce more male flowers and some plants never reach the female flowering stage. By the second year, these monoecious plants produce female flowers and seem to equal the fruit-producing potential of the gynoecious plants. A second possible explanation could be that there is a crowding effect whereby the high plant populations of gynoecious plots suppressed the per plant yields. The data suggest the possibility that actual yields per plant of gynoecious versus monoecious sex types are virtually the same after the first season's growth.

Although the experiment was designed to minimize the effect of genetic variability, data were collected from plants in which a high degree of variation still exists. More accurate tests may be available when inbred lines are developed. By using two isogenic inbred lines differing only in sex type, the ultimate test of this hypothesis would be possible.

Summary

Yield studies were conducted on the feral perennial buffalo gourd to determine whether the mutant gynoecious sex type had a higher fruit producing potential than the monoecious type. An experimental plot was designed to test the effect of sex expression on yield. Four blocks of two sub-plots each, one rogued to all monoecious and the other to all gynoecious plants, were observed over two growing seasons. Results were examined on a per plot and a per plant basis. Additional data were collected on fruit size, mean seed weight per fruit, and seed weight per 100 seed, to assure that sex type did not influence these parameters.

First year results showed that gynoecious plants outyielded monoecious plants on both per plot and per plant bases. Sex type did not influence the size of fruit nor the weight of seed.

Second year data showed gynoecious plants outyielding monoecious plants on a per plant basis but not on a per plant basis. Sex type did not influence fruit size nor seed weight.

New plants arising from adventitious rooting on second year studies did not influence results. Bemis (1979) suggested that adventitious plants rarely fruit during the first season. Sex type did not influence the ability of plants to root asexually.

In conclusion, it appears that on a per plant basis gynoecious plants outyield monoecious plants during their first year of growth. However, in subsequent seasons of growth, sex type by itself may not assure higher yields.

APPENDIX A

SUMMARY OF SEED PRODUCTION PLOT YIELDS PER PLANT (NUMBER OF FRUIT/PLANT; SEED WEIGHT/PLANT) FOR SEED PARENTS OF ARIZONA HYBRID #1, BUFFALO GOURD, 1979*

Plot No.	Sex	No. Plants	$\bar{x}$ No. Fruit/Plant	Range	$\bar{x}$ Seed Weight(g)/Plant**	Range(g)
142-1	Gyn	10	27.1	1-67	201.6	3.2-533.4
158-2	Gyn	10	37.5	0-90	334.9	0-900.0
142-1	Mono	10	21.9	0-79	333.7	0-1374.6
158-2	Mono	10	27.0	0-97	217.3	0-617.7

*Scheerens (1979).

**Seed weight for individual plants estimated from a 15-fruit sample.

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