Therefore, it should not be necessary to have large pods to obtain the benefits of numerous heavy beans toward high yield—many thin-husked pods could achieve the same results.

The results clearly demonstrated the existence of large differences in combining ability for yield, pod, and bean characteristics. Although additive genetic effects are of major significance, there was also considerable genetic variability due to nonadditive effects. Based on GCA effects and the mean performance of the hybrids for their yield, pod, bean and disease-resistant characteristics, 20 specific crosses were considered promising (Table 4). A new cacao cultivar composed of these 20 high-yielding lines was reproduced in biparental seed gardens and was recommended to growers for commercial planting. The chance of recovering such recombinants with high yield and desirable pod and bean characteristics is high because both additive and nonadditive genetic variation were considered in the selection.

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Unreduced Pollen in a Wild Tetraploid Relative of Sweetpotato

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Abstract. Nonreduction of pollen mother cells was observed in a wild tetraploid morning-glory related to the sweetpotato (*Ipomoea batatas* L. Lam.). Techniques for identifying and determining the frequency of expression of the trait are straightforward. Previous strategies for transfer of germplasm from tetraploid ($2n = 4x = 60$) species to the hexaploid ($2n = 6x = 90$) sweetpotato involved crosses with diploids ($2n = 2x = 30$) to obtain triploids that then were doubled to $6x$. Nonreduction of pollen mother cells probably represents the natural mechanism for raising $4x$ to $6x$ since pollination of $2x$ ovules with unreduced pollen ($4x$) should give rise to $6x$ progeny without need for somatic doubling. Plants carrying this trait should be useful as bridging types for introgressing genes from wild $4x$ species into sweetpotato. A wide range in nonreduction (up to, 74%) was recovered in progeny of controlled crosses among selected plants, but data were not sufficient to estimate gene action.

During the past two decades there has been an increasing interest in the evolutionary origin of sweetpotato, a major food of the tropics that ranks seventh in carbohydrate production among the major world crops (FAO, 1981). Taxonomic relationships of *Ipomoea* section Batatas have recently been re-

viewed by Austin (1988), who concluded that *I. triloba* and *I. trifida* are the closest extant relatives of the sweetpotato. Shiotani (1988) has presented a review of the most recent phylogenetic studies in his discussion of genomic structure and gene flow in the section. Strategies for the use of exotic germplasm in sweetpotato breeding were recently summarized (Iwanaga, 1988). It is generally accepted that the sweetpotato originated in northwestern South America.

The sweetpotato is a hexaploid morning-glory with 90 somatic chromosomes and the related species are tetraploid ($2n = 60$) or diploid ($2n = 30$). Strategies in phylogenetic studies

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Fig. 1. (A) A large unreduced pollen grain (center) of Ac. 81.2 next to normal-size grains. (B) A normal PMC in tetrad stage and a dyad resulting from nonreduction. (C) A triad indicating nonreduction is due to second division failure. (D) Metaphase I demonstrates Ac. 81.2 is tetraploid with most chromosomes pairing as bivalents.

Table 1. Stained pollen (%) and frequency of nonreduction observed in pollen (%) and sporads at the normal tetrad stage for pollen mother cells of Ac. 81.2 plants.

Year	Plant	Pollen ^a			Sporads ^z			
		Stained ^y (%)	Unreduced stained ^y (%)	n	Dyads (%)	Triads (%)	Tetrads (%)	n
1986 ^x	1	90.0	34.4	200	---	---	---	0
1987 ^w	4	84.2	4.7	1100	5.2	0.0	94.8	846
	5	90.8	6.9	1100	11.2	0.0	88.8	475
1988 ^v	6	60.8	0.5	400	0.0	0.0	100.0	450
	7	38.1	3.0	700	0.2	0.0	98.8	945
	8	45.0	0.0	300	0.0	0.0	100.0	500
	12	69.4	3.0	800	0.0	1.6	98.4	737
	13	93.8	0.6	500	0.0	0.0	100.0	717
	14	51.8	1.2	500	1.1	0.0	98.9	546
	15	85.0	0.3	300	0.0	0.2	99.8	417
	17	61.7	0.0	300	0.0	0.0	100.0	500
	18	94.0	0.0	200	0.0	0.0	100.0	485
	19	89.7	0.0	300	0.0	0.0	100.0	100
	21	74.7	0.5	600	0.6	0.0	99.4	1000
	23	95.3	0.0	300	0.1	0.0	99.9	800
	24	91.2	20.6	1270	22.8	0.2	77.0	545
	25	99.0	1.0	200	0.2	0.0	99.8	600
	26	91.0	5.0	500	14.2	0.0	85.8	500
	28	62.8	1.0	500	13.4	0.2	86.4	494
	29	94.8	0.2	500	6.7	0.0	92.3	1000
	30	86.7	14.1	1100	24.1	0.0	75.9	744
	31	97.0	0.7	600	0.6	0.0	99.4	500
	32	38.3	1.0	400	2.2	0.0	97.8	500

^zSee Fig. 1.

^yStained includes normal and large grains; unreduced stained is relative to all grains.

^xFirst plant observed with nonreduced pollen.

^wFrom seed of original collection of Accession 81.2.

^vFrom open-pollinated seed of plant 1 (6-17) and plant 4 (18-32).

Table 2. Stained pollen (%) and frequency of nonreduction observed in pollen (%) and sporads (%) of plants from intercrosses among selected Ac. 81.2 plants (Dec. 1988 to Apr. 1989).

(Cross)-Plant	Pollen ^a			Sporads ^a			
	Stained ^b (%)	Unreduced stained ^b (%)	n	Dyads (%)	Triads (%)	Tetrads (%)	n
(23 x 19)-1	24.6	0.0	1000	---	---	---	0
-2	76.2	0.0	1000	---	---	---	0
(23 x 25)-1	81.6	2.0	1000	2.1	0.7	97.2	431
-2	66.1	1.4	1000	1.5	0.0	98.5	271
-3	52.3	0.3	1076	0.4	0.0	99.6	500
(23 x 29)-1	41.5	0.5	1000	---	---	---	0
(25 x 23)-1	63.4	0.5	1000	2.1	0.4	97.4	468
-2	79.8	0.0	1861	0.0	0.0	100.0	404
(24 x 5)-1	52.0	1.6	500	---	---	---	0
-2	72.5	9.3	1100	---	---	---	0
-3	90.8	3.1	1000	---	---	---	0
-4	90.4	9.4	1000	11.5	0.2	88.3	452
-5	62.9	6.3	1000	25.4	0.2	74.4	503
-6	64.6	7.1	2000	17.4	0.0	82.6	500
(4 x 5)-1	64.0	19.0	600	---	---	---	0
-2	93.3	2.8	1000	---	---	---	0
-3	93.6	7.2	1000	---	---	---	0
(26 x 30)-1	91.9	1.3	1000	---	---	---	0
-2	77.9	6.6	1513	18.7	0.0	81.3	300
-3	91.2	74.2	2106	86.6	2.4	11.0	500
-4	41.4	2.2	2200	20.5	0.2	79.3	616
-5	78.9	8.8	1000	29.8	0.2	70.0	500
(30 x 26)-1	66.3	7.0	1681	13.8	0.0	86.2	891
-2	74.0	39.9	2000	70.9	0.0	29.1	700
-3	75.4	3.2	3000	13.0	0.3	86.7	1100
-4	47.9	1.4	1000	5.9	0.0	94.1	455
-5	69.5	2.3	1000	8.6	0.2	91.2	406
(26 x 5)-1	87.3	48.9	1000	67.0	0.0	33.0	558
-2	92.3	19.5	1000	44.0	0.0	56.0	500
-4	84.0	7.8	1000	10.5	0.0	89.5	209
-5	87.6	22.5	1000	41.8	0.0	58.2	500
(30 x 5)-1	68.3	1.6	2000	15.4	0.0	84.6	500
-3	71.0	3.8	500	---	---	---	0

^aSee Fig. 1.

^bStained includes normal and large grains; unreduced stained are relative to all grains.

generally have been to cross diploid with tetraploid types to obtain triploids that were then doubled somatically to hexaploid (Shiotani, 1988). The derived hexaploids could be crossed with sweetpotato with varying degrees of success, which gave clues to genomic relationships. In some cases hexaploids were crossed to tetraploids to obtain pentaploids, and, in some cases, spontaneous gametic doubling occurred.

Reliance on spontaneous gametic or somatic doubling in a natural system is not a very satisfactory explanation for the 2n = 30, 60, and 90 series of the genus. A reproductive system under genetic control and repetitive in time and space, such as gametic nonreduction, would provide a more likely mechanism for natural polyploidization. Therefore, a study of meiotic activity of pollen mother cells (PMC) in a collection of tetraploid *Ipomoea* spp. was conducted in hopes of finding an explanation for polyploidization within the genus. One plant was found that appeared to express nonreduction. This paper reports this observation and confirmatory studies.

Materials and Methods

In the initial 1986 screening process three or four plants from each of 20 species collections were studied, but plants were not preserved at the end of the growing season. Nonreduction was

first observed in 1986 in a collection from Calegonoma (Loja), Ecuador, made by C.M. Rick on 7 Aug. 1980 and sent to me with the designation SAM 5316. That collection was assigned the U.S. Vegetable Laboratory sweetpotato accession number 81.2.

In 1987, the remaining seed from the original collection of Ac. 81.2 provided two additional plants. In 1988, open-pollinated seed from two of the original field-grown plants were started in the greenhouse and later moved to trellises in the field. Controlled intercrosses of selected plants were made for use in 1989 studies.

After examination of dried specimens of Ac. 81.2, D.F. Austin, Florida Atlantic Univ., Boca Raton, considered it to be similar to collections identified as *I. trifida* by Japanese scientists (Shiotani, 1988). However, he did not consider this collection, or the previous ones, as true *I. trifida*, but rather within the variation of *I. batatas* and thus tetraploid *I. batatas*.

Flower buds collected between 8:00 and 10:00 AM were prepared for microscopic examination by fixation in Carnoy's solution (3 ethyl alcohol : 1 glacial acetic acid) for 24 hr. Observations of sporads at the tetrad stage of PMCs were made with special attention paid to frequencies of dyads (Fig. 1). Standard aceto-carmin squash techniques were used for meiotic

Table 3. Chromosome pairing at metaphase I of pollen mother cells of Ac. 81.2.

Plant ^a	Pairing (mean per cell)				No. cells	2n chromosome number
	I	II	IV	VI		
1	0.0	23.0	3.5	0.0	18	60
19	0.0	28.8	0.6	0.0	13	60
24	0.0	27.2	1.4	0.0	10	60
28	0.0	27.4	1.3	0.0	10	60
30	0.0	26.4	1.8	0.0	10	60
31	0.0	28.4	0.8	0.0	10	60
(23 x 25)-2	0.2	25.9	2.0	0.0	10	60
(23 x 25)-3	0.0	28.0	1.0	0.0	15	60
(23 x 29)-1	0.8	25.9	1.7	0.1	10	60
(25 x 23)-1	0.0	29.4	0.3	0.0	10	60
(24 x 5)-3	0.1	25.8	2.1	0.0	20	60
(24 x 5)-6	0.0	29.8	0.1	0.0	8	60
(26 x 30)-2	0.0	26.4	1.8	0.0	11	60
(26 x 30)-3	0.4	27.2	1.3	0.0	10	60
(26 x 30)-4	0.0	27.4	1.3	0.0	10	60
(26 x 30)-5	0.0	28.4	0.8	0.0	10	60
(30 x 26)-1	0.0	28.4	0.8	0.0	10	60
(30 x 26)-3	0.0	26.6	1.6	0.0	10	60
(26 x 5)-1	0.0	27.8	1.1	0.0	10	60
(26 x 5)-2	0.0	27.3	1.3	0.1	20	60
(26 x 5)-5	0.2	27.5	1.2	0.0	10	60
Mean	0.1	27.3	1.3	0.0	11.7	60

^aSee Tables 1 and 2.

Table 4. Pollen measurements of various ploidy levels of *Ipomoea* section Batatas.

Pollen source	Ploidy	Pollen diam. ($\times 10^{-2}$ mm)	No. grains
Diploid	x	9.0 $\pm$ 0.4	100
Tetraploid	2x	10.2 $\pm$ 0.5	382
Hexaploid	3x	11.6 $\pm$ 0.6	200
Unreduced tetraploid	4x	13.9 $\pm$ 0.9	191

studies and notes were taken on chromosome pairing at metaphase I (MI). Flower buds for pollen studies were collected on the day before anthesis and held overnight at room temperature with pedicels in water. The number of stained and nonstained pollen grains following treatment with aceto-carmine were recorded along with frequencies of normal size and large (unreduced) grains (Fig. 1). Pollen size measurements (in millimeters) were obtained from diploid *I. trifida* (x), tetraploid Ac. 81.2 (2x), hexaploid sweetpotato (3x), and nonreduced pollen of Ac. 81.2 (4x).

Controlled crosses were made under greenhouse conditions with flowers emasculated between 3:00 and 5:00 PM and pollinated between 8:00 and 10:00 AM the following morning (Jones, 1980). Seed were harvested as they matured.

Results and Discussion

The unreduced pollen count for plant 1 may be high because concepts of what constituted unreduced pollen were not well-developed at that point (Table 1). A PMC disease that causes distortions of pollen grains and sporads was evident in some plants (Jones, 1964). This confounding effect tended to obscure the presence of two sizes of pollen in some plants. Some large pollen grains were more oblong than round in plant 1, but this shape did not appear to be related to distortion by PMC disease.

Information from plant 1 was not considered sufficient to conclude that nonreduction was the cause of the large grains.

Plants grown in 1987 showed no symptom of PMC disease and raised the confidence level that nonreduction was occurring (Table 1). Observations of PMCs confirmed the presence of dyads following second division when normally there would be only tetrads (Fig. 1). When open-pollinated seedlings of plants 1 and 4 were grown in 1988, some were so severely affected by the PMC disease that they could not be used. In subsequent evaluations, only disease-free plants were included. The frequency of nonreduced pollen grains and dyads was highly correlated (0.86 ± 0.17 , $n = 22$). Progeny from controlled intercrosses of several plants demonstrated that the nonreduction observed earlier was heritable, but data were insufficient to define the number of genes involved (Table 2). Some plants either failed to flower or flowered so infrequently that data from them were incomplete. The frequency of large pollen grains was again highly correlated with frequencies of dyads (0.95 ± 0.09 , $n = 22$).

Chromosome numbers were obtained for 21 plants and all were tetraploid with $2n = 4x = 60$ (Table 3). Meiosis was regular with predominantly bivalent pairing, and most cells had some quadrivalents with infrequent univalents and hexavalents (Fig. 1). Thus, the two genomes appear to be partially nonhomologous, suggesting Ac. 81.2 may be an allotetraploid.

In progeny from controlled crosses (Table 2), nonreduction was noted in 30 of 33 plants that flowered and ranged as high as 74% unreduced pollen. Triads noted in 13 of 44 plants for which sporad data were available (Tables 1 and 2, Fig. 1) indicated that unreduced pollen was due to PMC second-division failure.

Size differences of pollen from different ploidy levels (Table 4) may be useful in preliminary evaluations of new collections. Unreduced pollen from tetraploid plants (4x) appeared equally viable to normal (2x) pollen when stained with aceto-carmine. After a little experience, recognition of unreduced pollen became rather routine, making screening for the trait relatively easy compared to other cytological procedures (Fig. 1). Sporad evaluation was not as easy due to the difficulty of recognizing buds in the proper stage of PMC development. However, dyads were easily differentiated in preparations of the proper stage.

The observation of nonreduction in this collection and the high frequency of recovery in progeny from crosses of plants exhibiting various levels of expression suggest this trait may be a mechanism for polyploidization within the genus (Harlan and deWet, 1975). If so, other wild collections should be found to express the same trait. When unreduced pollen (4x) fertilizes normal ovules (2x), the resulting offspring would be 6x, thus providing a mechanism for the increase of ploidy level from 4x to 6x directly, without need for somatic doubling. Therefore, this trait should prove useful for raising the ploidy level of any compatible tetraploid collection to the hexaploid level, thus facilitating hybridization with domesticated sweetpotato. The potential for obtaining hexaploids from tetraploid parents should be enhanced by use of plants such as Ac. 81.2-(26 x 30)-3 (Table 2) that have high frequencies of nonreduction as the pollen parent.

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