Use of *Ipomoea trifida* (HBK.) G. Don germ plasm for sweet potato improvement. 1. Development of synthetic hexaploids of *I. trifida* by ploidy-level manipulations

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Corresponding Editor: J. P. Gustafson
Received July 10, 1990
Accepted October 10, 1990

IWANAGA, M., FREYRE, R., and ORJEDA, G. 1991. Use of *Ipomoea trifida* (HBK.) G. Don germ plasm for sweet potato improvement. 1. Development of synthetic hexaploids of *I. trifida* by ploidy-level manipulations. Genome, **34**: 201–208.

Crosses were made between 21 tetraploid accessions and 41 diploid accessions of *Ipomoea trifida*, obtaining a total of 9185 triploid seeds from 215 different cross combinations. Doubling the somatic chromosome number of the triploids was attempted by colchicine treatment on young seedlings to obtain synthetic hexaploids. A total of 787 axillary buds of 316 triploid plants were treated with a 0.5% colchicine solution for 24 h, applied to cotton plugs surrounding the buds. The survival rate of the treated buds was, on average, 41%. The ploidy level in germ-layer L_2 was determined in 258 clones by evaluating a pollen sample from each clone. Fifty-five clones were selected for high stainability and pollen size. The selected genotypes were meiotically analyzed. Twenty-two of them were identified as hexaploid and 33 as triploid with $2n$ pollen production. The present study is the first report on $2n$ pollen production in triploid plants of *Ipomoea* species. The use of these triploid clones with $2n$ pollen production and synthetic hexaploid clones for sweet-potato breeding is discussed.

Key words: *Ipomoea batatas*, *Ipomoea trifida*, colchicine, triploids, $2n$ pollen.

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Des croisements ont été faits entre 21 accessions tétraploïdes et 41 accessions diploïdes de l'*Ipomoea trifida*, lesquels se sont traduits par la production d'un total de 9185 graines triploïdes dérivées de 215 combinaisons différentes de croisements. Le doublement du nombre chromosomique somatique des triploïdes par traitement à la colchicine a fait l'objet d'une tentative avec de jeunes plantules, en vue de produire des hexaploïdes synthétiques. Un total de 787 bourgeons axillaires chez 316 plantes triploïdes ont reçu une application de tampons de coton imbibés d'une solution de colchicine à 0,5% durant 24 h; ces tampons entouraient complètement les bourgeons. Le taux de survivance des bourgeons traités a été, en moyenne, de 41%. La détermination du niveau de ploïdie a été faite dans l'assise germinale L_2 chez 258 clones, en évaluant un échantillon de pollen pour chaque clone. Cinquante-cinq clones ont été sélectionnés pour leur colorabilité élevée et la dimension des graines de pollen. Les génotypes sélectionnés ont été soumis à une analyse méiotique. Trente-deux d'entre eux ont été identifiés comme hexaploïdes et 33 comme triploïdes avec une production de pollen $2n$. La présente étude constitue un premier rapport de production de pollen $2n$ chez des plantes d'une espèce d'*Ipomoea*. L'emploi de ces clones triploïdes avec production de pollen $2n$ et des clones hexaploïdes synthétiques dans les programmes d'amélioration de la patate sucrée sont discutés.

Mots clés : *Ipomoea batatas*, *Ipomoea trifida*, colchicine, triploïdes, pollen $2n$.

[Traduit par la rédaction]

Introduction

Sweet potato, *Ipomoea batatas* (L). Lam. ($2n = 6x = 90$), is an important crop in Asia, Africa, and America, ranking seventh in food production among all food crops of the world, with the production of approximately 135 million metric tons (Food and Agriculture Organization of the United Nations (FAO) 1987). This crop is important for poor farmers since it can yield well under suboptimum conditions. Sweet potato is a versatile plant: it can be used for direct human consumption as a staple food or leafy vegetable, as a source of starch and alcohol, for processed products (i.e., canned and dried products), and for animal feed.

The principal objectives in sweet-potato breeding are to overcome production and utilization constraints. Many such constraints have been reported (Lin *et al.* 1983; Martin and Jones 1986). To achieve these breeding goals, proper germ plasm is needed. Excellent cultivated germ-plasm collections are available around the world (International Board For Plant Genetic Resources. (IBPGR) 1986). On the other hand, collection and use of wild germ plasm has been rather limited (Kobayashi and Miyazaki 1977). Because of the potential value of wild germ plasm for sweet-potato improvement, recent efforts have been made to collect wild germ plasm from centers of genetic diversity (Huaman and De la Puente 1988).

The genus *Ipomoea* section *Batatas* contains 12 species, two named hybrids, and one unnamed hybrid (Austin 1988). In this section, *I. trifida* (HBK.) G. Don is considered as the wild species most closely related to sweet potato. It constitutes a polyploid series, with recognized diploid ($2n = 2x = 30$) and tetraploid ($2n = 4x = 60$) cytotypes, and possibly also hexaploid ($2n = 6x = 90$) cytotypes. The hexaploid accession K123 has been considered as *I. trifida* by some investigators (Nishiyama 1959; Nishiyama and Teramura 1962; Shiotani and Kawase 1987, 1989) and as

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Printed in Canada / Imprimé au Canada

I. batatas by others (Jones 1967; Martin *et al.* 1974; Austin 1977, 1988).

K123 has been used in sweet-potato breeding in Japan as a source of resistance to nematodes, leading to the release of successful cultivars (Sakamoto 1970, 1976). The variety Minamiyutaka has one-eighth of its genetic background from K123 and is resistant to root-lesion nematodes (*Pratylenchus* spp.). Moreover, this cultivar has a high starch content and high yield, possibly as a result of heterosis caused by the introgression of alien germ plasm. The same accession has been used in a breeding program in China and one cultivar with high yield and starch content was recently released (Q.H. Xue, personal communication). Regardless of the exact taxonomic classification of K123, the production of successful cultivars using K123 in the two major sweet potato producing countries is a significant achievement. It demonstrates the importance of exotic germ plasm for the introduction of specific traits such as nematode resistance and to widen the genetic background of cultivated germ plasm.

Hexaploid *I. trifida* germ plasm is clearly valuable, but K123 is the only such germ plasm with the same ploidy level as the cultivated sweet potato. Further success cannot be expected by using just one particular accession. On the other hand, the numerous diploid and tetraploid accessions of *I. trifida* (Sakamoto 1970; Huaman and De la Puente 1988) represent a valuable source of germ plasm for sweet-potato improvement but cannot be used directly owing to their difference in ploidy levels. When tetraploid or diploid accessions are crossed with sweet potato (hexaploid), progeny are expected to be pentaploid or tetraploid. The odd-ploidy pentaploid progeny should have low fertility and produce aneuploid progeny when backcrossed with sweet-potato cultivars. Tetraploid progeny would present similar problems, thus complicating further introgression work. Kabayashi (1978) proposed four approaches for ploidy-level manipulations, but no serious attempt was made to produce hexaploid *I. trifida* germ plasm through ploidy-level manipulations.

The present paper reports the production of triploid hybrids ($2n = 3x = 45$) that combine the genetic content of tetraploid and diploid accessions of *I. trifida* from diverse geographic origins. Somatic chromosome doubling of these triploids was attempted to obtain synthetic hexaploids that could be subsequently crossed with sweet potato. The traditional method of colchicine treatment (Ross *et al.* 1967) already used in *Ipomoea* (Jones and Kobayashi 1968) was applied with some modification.

Materials and methods

Tetraploid and diploid accessions of *I. trifida* collected by Japanese expeditions in Central America and northern South America were kindly provided by the Kyushu National Agricultural Experiment Station Collection in Ibusuki, Japan. They were maintained in a greenhouse at International Potato Center (CIP) in Lima, Peru. In the greenhouse, the average maximum and minimum daily temperature was 29 and 15°C, respectively. As these plants have a climbing growth habit, metal spirals were placed in the pots to provide support for the vines.

The plants were placed under a short-day photoperiod (9 h light) for 1 month to induce flowering. Crosses were made with controlled pollinations between 21 tetraploid accessions as females and 41 diploid accessions as males to obtain triploid hybrids of *I. trifida*. All female parents were previously checked for their self-incompatibility (Martin 1959), so no emasculation was made before crossing. Using the hybrid seeds thus produced, somatic chromosome doubling was attempted by three methods of colchicine treatment of seedlings to produce synthetic hexaploid hybrids.

Experiment 1

A total of 149 triploid *I. trifida* seedlings from eight different families were grown in the greenhouse. All shoots but the apical ones were pruned from the seedlings. When the plants were 40 days old, the tip of the apical shoot was also cut out, and the upper two axillary buds were surrounded with cotton plugs and identified with a label. A 0.5% colchicine solution was applied to the cotton plugs, which were kept saturated for 24 h. After this, the cotton plugs were carefully removed and the treated buds were thoroughly rinsed with water. This procedure was repeated with 10 other plants used as controls, but only water was applied to the cotton plugs.

Experiment 2

In this case, 167 triploid *I. trifida* plants from 65 different families, and 10 control plants were used. The same procedure used in the first experiment was repeated, but the colchicine treatment was applied on 20-day-old seedlings, which had not developed secondary branches.

Experiment 3

For plants in experiment 1, after 1 month, it was observed that many treated buds did not show any growth and thus appeared to be dead. Forty-five plants that presented such buds were reused for ploidy-level duplication. Another shoot coming from a lower axillary bud was permitted to grow on each plant, and the colchicine treatment was applied to the upper two buds, using the same procedure as in experiment 1.

In all three experiments, shoots coming from the treated buds were propagated, each one constituting a clone. Ploidy levels in each clone were determined for the three different germ layers, namely L_1, L_2, and L_3 (Frandsen 1967; Hermsen and De Boer 1971):

Evaluation in germ-layer L_1

A piece of epidermis from the bottom side of a young leaf of each clone was placed on a microscope slide and stained with 1% KI. Then, the number of chloroplasts in stomatal guard cells was counted. Previous evaluation had determined that there are highly significant differences in the number of chloroplasts between ploidy levels. They averaged 10 and 16 chloroplasts per pair of stomatal guard cells for triploid and hexaploid ploidy levels, respectively.

Determination in germ-layer L_2

After the evaluation of germ-layer L_1, the plants were placed under a short-day treatment to induce flowering. After approximately 1 month, they were returned to the greenhouse, and a pollen sample from one flower from each clone was prepared by staining with 1% aceto-carmine glycerol (Marks 1954).

From previous studies in nontreated triploid, tetraploid, and hexaploid genotypes of *I. trifida*, the expected pollen sample characteristics, such as pollen stainability and average diameter, had been determined for each ploidy level, as follows.

Triploid—This odd-ploidy level is generally sterile. When pollen is found in the sample, it is often in a small amount and with stainability rarely above 5%. Most pollen grains have poor staining and have deformed shapes. Pollen diameter is variable, ranging from 13 to 45 units, with a mode of approximately 35 units (0.083 mm).

Tetraploid—Abundant pollen grains are found in a sample, generally with more than 70% stainability. Pollen diameter is fairly uniform, measuring approximately 40 units (0.095 mm).

Hexaploid—Pollen sample characteristics are similar to those of the tetraploid, but pollen diameter is greater, ranging between 45 and 50 units (0.107–0.119 mm).

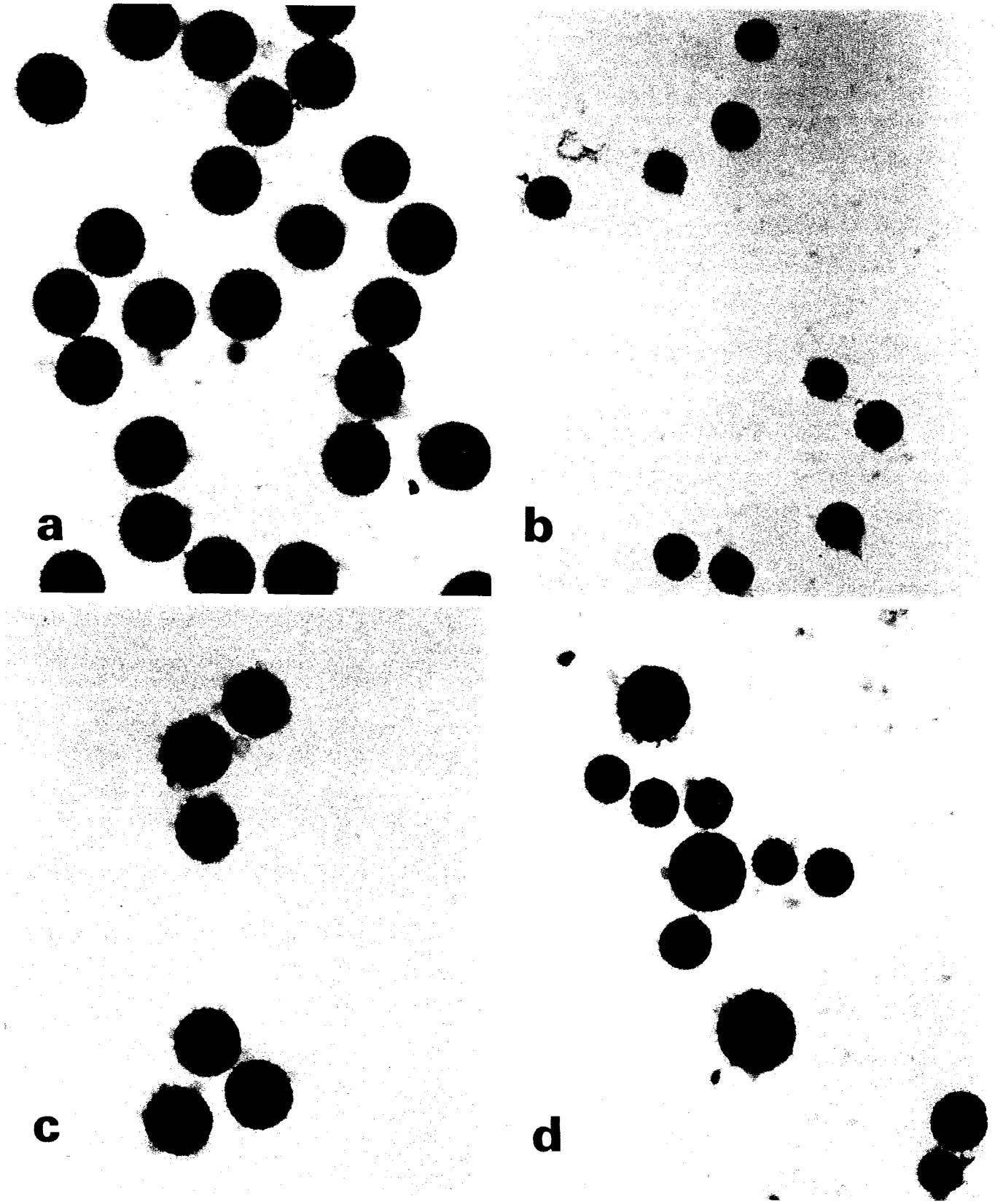

FIG. 1. Four types of pollen sample observed in colchicine-treated triploid plants of *I. trifida*. (*a*) Pollen of hexaploid plants with high pollen stainability. (*b*) Pollen of typical triploid with small size and poor stainability. (*c*) Pollen of tetraploid plants with high stainability plants. (*d*) Pollen of triploid plants with 2*n* pollen production.

Clones selected on the basis of their pollen sample were used to study meiosis. Flower buds of different sizes from each clone were fixed in Farmer's solution (95% ethanol – glacial acetic acid (3:1)) for at least 24 h. Anthers were extracted using dissecting needles and a stereoscopic microscope. They were then squashed in a drop of aceto-orcein (2% orcein in 45% acetic acid) and

TABLE 1. Survival rates of colchicine-treated buds of triploid *I. trifida* plants for the three experiments where colchicine was applied to plants of three different ages

	Expt. 1	Expt. 2	Expt. 3	Total
No. of treated buds	290	317	180	787
No. of surviving buds	104	125	95	324
%survival	36	39	53	41

observed under a microscope, to search for pollen mother cells (PMCs) in the tetrad stage at the end of the second division. A minimum of 100 meiocytes were checked for each clone to estimate the percentages of dyads, triads, tetrads, or abnormal types (with more than four microspores per PMC or with abnormal shapes).

Determination in germ-layer L_3

Cuttings were made from each clone and were placed in jars with water, where they started to form roots. Several days later, root tips were collected from the cuttings and pretreated with 8-hydroxyquinoline (0.002 M) for 4 h at 12°C and then fixed in Farmer's solution for at least 24 h. Hydrolysis was performed with 1 M HCl at 60°C for 20 min, and then root tips were stained and observed using the aceto-orcein squash method.

Results

Production of triploid hybrids

A total of 12 586 pollinations were made between tetraploid and diploid genotypes of *I. trifida* in 299 different cross combinations. The number of pollinations per family varied from a minimum of 1 to a maximum of 422. A total of 9185 seeds were obtained in 215 families, coming from 15 different tetraploid females and 31 diploid males.

Colchicine treatment

Results for the three experiments are summarized in Table 1. Of 787 buds treated, 324 buds developed into shoots and, thus, the average survival rate of the treated buds was 41%. Buds in experiment 3 had the highest survival rate (53%). There was a lack of uniformity in the growth of buds treated with 0.5% colchicine and, in general, the growth was much slower than that of control buds treated with water.

Determination in germ-layer L_1

Chloroplast counts in stomatal guard cells were performed in one leaf sample from each of the 324 shoots derived from treated buds. More than one-half of them were determined to be triploids. Only 16 plants were hexaploid, according to their chloroplast number. In all other cases, no conclusive determination of ploidy levels could be made. Often chloroplast numbers showed deviation from the typical expected numbers. Some plants seemed to be chimeras, with the number of chloroplasts corresponding to both ploidy levels. Moreover, chloroplast numbers that corresponded to a lesser ploidy level than triploid or greater than hexaploid were also found. It seems that the existence of unexpected tetraploid plants in the treated material (as was later demonstrated) further complicated the analysis.

Determination in germ-layer L_2

A total of 258 clones of the 324 that survived the colchicine treatment (80%) were evaluated to determine the ploidy level in germ-layer L_2. The rest did not flower or died before reaching the flowering stage. As expected, pollen samples corresponding to triploid and hexaploid ploidy levels were found as well as unexpected tetraploid pollen samples (Figs. 1*a*, 1*b*, and 1*c*). In addition, many samples were found in which there was a high proportion of sterile pollen grains as well as others that were stained and had a greater diameter than hexaploid pollen, measuring approximately 55 units (0.131 mm) (Fig. 1*d*). These genotypes were tentatively defined as triploid with 2*n* pollen production. In other words, they were not doubled and were otherwise sterile triploids, but they produced stainable pollen as a result of meiotic restitution. 2*n* pollen is a meiotic product that bears the sporophytic rather than the gametic chromosome numbers (Mendiburu and Peloquin 1976; Veilleux 1985). Thus, 2*n* pollen of a triploid plant have the 3*x* chromosome number.

TABLE 2. Results of the evaluation of 258 clones derived from colchicine-treated axillary buds of triploid plants of *I. trifida* for their ploidy level in germ-layer L_2 by pollen observation

Ploidy level	No. of clones
Triploid	88
Male sterile (no pollen)	28
Triploid with low frequency of 2*n* pollen	58
Tetraploid	29
Hexaploid or triploid with high frequency of 2*n* pollen	55
Total	258

A total of 174 genotypes (68%) were determined to be triploid. These included genotypes in which no pollen was found in the sample, genotypes with sterile pollen grains, and also triploid plants with a low frequency of 2*n* pollen (Table 2). Twenty-nine genotypes (11%) were determined to be tetraploid on the basis of their pollen size and high pollen stainability. When there was a high percentage of stainability of pollen grains in the sample, it was difficult to differentiate according to pollen size between hexaploid and triploid with a high frequency of 2*n* pollen. Because of this, 55 genotypes (21%) that had high stainability and pollen diameter bigger than 45 units (0.107 mm) were grouped together in the last category.

The 55 clones were selected to study their meiosis to determine their ploidy level in germ-layer L_2. It was observed that the tetrad stage of PMCs was frequently found in flower buds 5–8 mm long. In this stage, it was possible to classify microspores into dyads, triads, tetrads, or abnormal types and find the percentage for each class (Fig. 2). Since direct counting of the chromosome number at diakinesis or metaphase I was not possible owing to technical difficulties, the following criteria were used to differentiate the 55 clones into two groups (hexaploid versus triploid with 2*n* pollen production). The presence of dyads and triads gives strong indication of 2*n* pollen production in the material in question. Since there was the possibility of 2*n* pollen production in the hexaploid plants, data of pollen stainability were also used to differentiate hexaploid from the triploid with 2*n* pollen production group. Genotypes that presented more than 75% tetrads, or more than 90% pollen stainability, were considered as hexaploid. Those that had neither characteristic, and presented dyads, were considered as triploid with 2*n* pollen production.

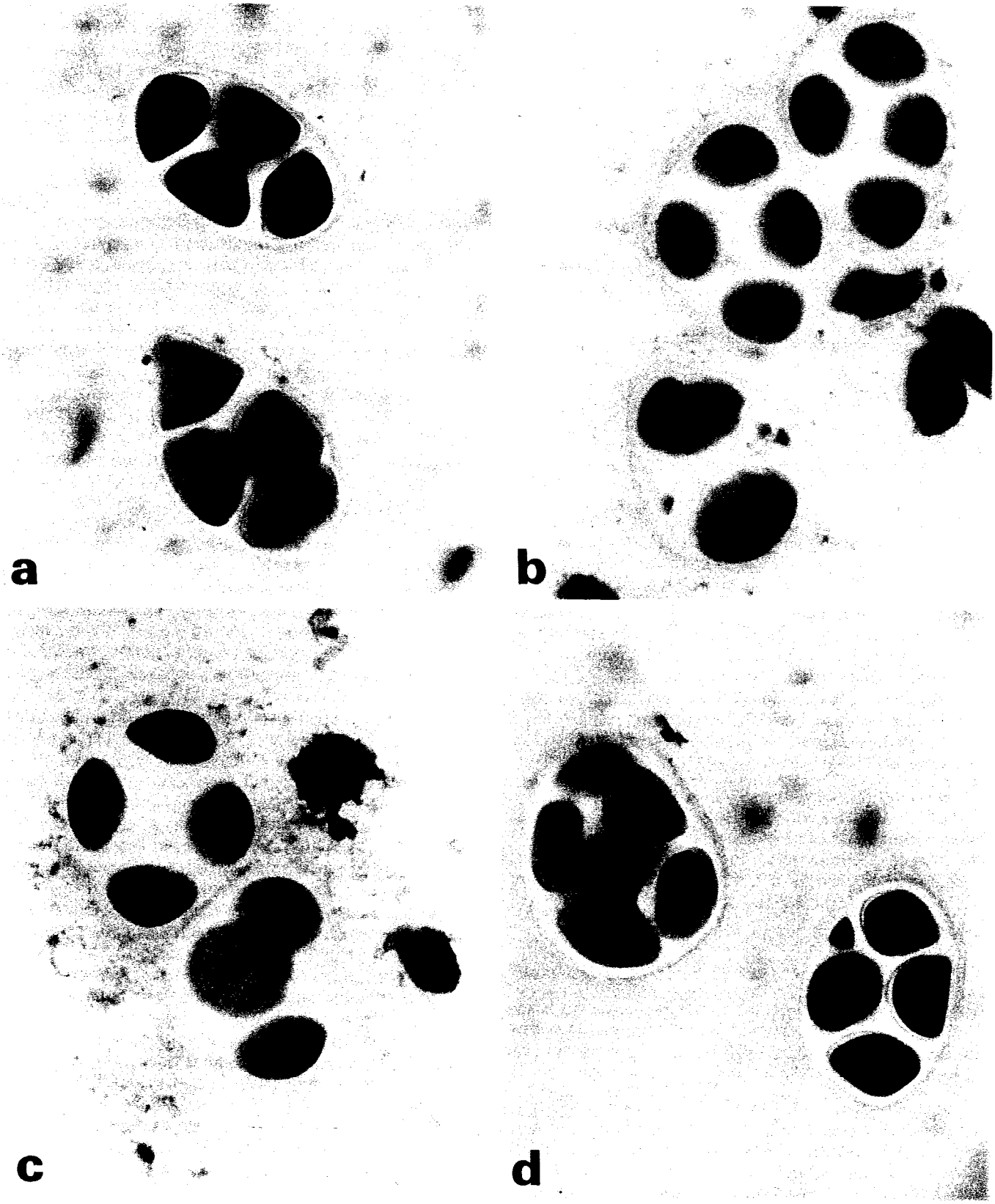

FIG. 2. Different types of microspores at the tetrad stage in colchicine-treated triploid plants of *I. trifida*. (*a*) Normal tetrad. (*b*) Two tetrads and one dyad. (*c*) One tetrad and one triad. (*d*) Abnormal types with more than four microspores per meiocyte.

According to the above-mentioned criteria, 22 clones were classified as hexaploid and 33 as triploid with 2*n* pollen production. Of 22 hexaploid clones 9 produced some frequency of dyads, and giant pollen corresponding to 2*n* pollen from hexaploid plants was found in some of them. Dyad frequency was variable among the 33 triploid clones with 2*n* pollen production, ranging from 1 to 92%. Nevertheless, the fact that all 2*n* pollen producers exhibited dyad forma-

TABLE 3. Comparison of results of germ layer L_2 evaluation in the three experiments of colchicine treatment for triploid plants of *I. trifida*

	Expt. 1	Expt. 2	Expt. 3	Total
No. of buds evaluated	67	114	77	258
No. of hexaploid plants	2	18	2	22
No. of triploid plants with 2*n* pollen production	7	13	13	33

TABLE 4. Distribution of the 55 clones, which were selected for their either hexaploid or triploid with 2*n* pollen production in germ-layer L_2, for their ploidy levels in L_2 and L_3 germ layers

	L_2		
	Triploid with 2*n* pollen	Hexaploid	Total
L_3			
Triploid	32	10	42
Hexaploid	1	12	13
Total	33	22	55

NOTE: For this 2 × 2 contingency table $\chi^2 = 19.41$ and is highly significant at the 0.01 level.

tion as well clearly supported our interpretation that they were triploid with 2*n* pollen production. Preliminary observations indicated the occurrence of parallel spindles at anaphase II and this could be the possible mechanism of 2*n* pollen production in the triploid plants.

The results of this study in the 55 selected genotypes, for each of the experiments of colchicine treatment, are given in Table 3. The highest doubling rate was obtained in experiment 2.

Determination in germ-layer L_3

By chromosome counts in root tips of the selected 55 clones, it was determined that 42 were triploid and 13 were hexaploid. Ploidy levels in germ-layers L_2 and L_3 of the selected clones are given in Table 4: 32 clones (58%) are triploid in germ-layer L_3 and triploid in germ-layer L_2; 12 clones (22%) are hexaploid in both germ layers; 10 clones (18%) are triploid in germ-layer L_3 and hexaploid in germ-layer L_2; only one clone (2%) is hexaploid in germ-layer L_3 and triploid in germ-layer L_2. Of 13 genotypes that were hexaploid in germ-layer L_3, 12 genotypes were also hexaploid in germ-layer L_2: a statistically significant level of coincidence in the doubling of L_2 and L_3 germ layers was observed.

Discussion

In crosses between tetraploid and diploid genotypes of *I. trifida*, the number of seeds obtained represented 61 seeds per 100 pollinations, which would mean that there is no major problem in these interploidy crosses, and in the production of hybrid triploid seed. This is in contrast to the "triploid block" encountered in 4*x*–2*x* crosses in *Solanum* species (Marks 1966; Hanneman and Peloquin 1968). Thus, our results indicate a strong possibility of naturally occurring triploid hybrids since both tetraploid and diploid cytotypes are often sympatric in nature. Some proportion of the progeny from 4*x* × 2*x* crosses in *I. trifida* can be expected to be tetraploid owing to 2*n* pollen production in the diploid *I. trifida* male progenitor (Orjeda *et al.* 1990). Of 258 genotypes evaluated for their germ-layer L_2, 29 were tetraploid, apparently indicating a high frequency of 2*n* pollen production in some of the diploid males.

Spontaneous duplication of chromosome numbers during adventitious shoot regeneration *in vitro* was successfully used for *Solanum* species (Jacobsen 1977). We applied the *in vitro* method for doubling the ploidy level of our triploid seedlings, but no success was obtained (Freyre 1989). On the other hand, the use of colchicine was simple and successful, because large numbers of genotypes can be treated with this method and because it resulted in the production of genotypes with doubled chromosome numbers. This method was especially effective when applied to axillary buds of 20-day-old plants, as in experiment 2. In this experiment, 317 buds were treated, and the survival rate was 39%. Of 114 shoots that were evaluated in germ-layer L_2, 18 were hexaploid, which represents a doubling rate of 16%, and accounted for 82% of the total of 22 hexaploids obtained from the three experiments using colchicine. In a previous study in *Ipomoea* by Jones and Kobayashi (1968) a similar rate of success (67% survival and 16% doubling) was found.

The disadvantage of producing periclinal ploidy chimeras with the use of colchicine is of no great importance for the production of hexaploid *I. trifida*. The fundamental objective is to obtain clones doubled in germ-layer L_2, so that the 3*x* gametes produced can be used for crosses with sweetpotato cultivars. In addition, mortality after colchicine treatment was not very high and, moreover, the ease of production of triploid hybrid seeds provides sufficient numbers of seeds and plant material for colchicine treatment.

Colchicine acts on the peripheral germinal layer towards the interior, which means that it acts sequentially in L_1, L_2, and lastly L_3 germ layers. Data obtained for ploidy level in germ-layer L_1 are not conclusive and are of no practical use for selecting clones doubled in germ-layer L_2. On the other hand, a study of independence of events using χ^2 in a table of contingency has proved that chromosome duplication in L_2 and L_3 germ layers is not independent. If duplication is confirmed in germ-layer L_3, then it is almost definite that duplication of germ-layer L_2 has also occurred. In the present case, 12 of 13 clones that were doubled in germ-layer L_3 were doubled in germ-layer L_2. Therefore, with a ploidy level evaluation in germ-layer L_3, an identification of hexaploid clones should result in clones that are also hexaploid in germ-layer L_2. Coincidence in success of doubling in the three layers has been reported in other plant species (Langton 1974; Frandsen 1967).

The selection of genotypes on the basis of high pollen stainability and a diameter greater than 45 units (0.107 mm) was adequate for obtaining hexaploid genotypes or triploids with 2*n* pollen. This criterion for pollen size successfully

excluded 29 tetraploid genotypes that had high pollen stainability. In addition, this allowed us to eliminate triploid plants with a low frequency of 2*n* pollen. As reported in other plant species (see review by Veilleux, 1985), 2*n* pollen frequency in the present material was strongly affected by environmental factors (Freyre 1989). Therefore, it seems likely that other triploid plants with 2*n* pollen could have been selected if more than one pollen sample had been used. Nevertheless, it was decided that 55 genotypes was a sufficiently large sample to be used for further breeding studies.

The 22 synthetic hexaploids combine germ plasm coming from 11 tetraploid females and 17 diploid males of *I. trifida*. Considering the fact that only one natural hexaploid *I. trifida* accession has been available so far, they represent a new source of potentially valuable genetic diversity for sweet-potato improvement. Many of them were found to have resistance to root-knot nematodes, *Meloidogyne incognita* (Freyre 1989). Their female and male fertilities as well as their crossability with sweet potato are published in an accompanying article (Freyre *et al.* 1991).

Plants in which meiotic abnormalities can generally be expected, odd-ploids (monoploid, triploid, pentaploid, etc.) among them, are often found to produce 2*n* gametes, and in fact, polyploid gametes are often the only functional gametes produced by such plants (Veilleux 1985). In triploids, 2*n* gametes have been reported in several species, such as potato (Müntzing 1933; Lange and Wagenvoort 1973; Mok *et al.* 1975), *Rubus idaeus* (Pratt *et al.* 1958), *Dactylis glomerata* (Zohary and Nur 1959), wheat (Kihara 1944; Vardi and Zohary 1967; Waines *et al.* 1982), *Medicago sativa* (Bingham and Binek 1969; Smith *et al.* 1984), *Beta vulgaris* (Romagosa *et al.* 1988), and *Vaccinium* spp. (Dweikat and Lyrene 1988).

The present study is the first report on 2*n* pollen production in triploid plants of the genus *Ipomoea*. Since some of the control plants produced high frequencies of 2*n* pollen (Freyre 1989), it is clear that colchicine treatment *per se* did not induce 2*n* pollen production in the triploid hybrids. It is likely that parallel spindles (fused spindles) is the cytological mechanism responsible for 2*n* pollen production in the triploid genotypes, as reported in triploid potato hybrids (Mok *et al.* 1975). This reasoning is based on the existence of triads and the fact that other possible mechanisms cannot explain production of fertile pollen in triploid plants. Production of 2*n* pollen was observed in many diploid accessions of *I. trifida* (Orjeda *et al.* 1990) and, thus, production of 2*n* pollen in the triploid hybrid with diploid male progenitors is not surprising. However, 2*n* pollen frequencies in the diploid accessions were much lower than those of some triploid hybrids. Odd ploidy *per se* may enhance 2*n* pollen frequency in the triploid hybrids with gene(s) for 2*n* pollen production.

The presence of 2*n* pollen in triploid *I. trifida* would mean that it is possible to make crosses between these genotypes and sweet potato to produce interspecific hexaploid hybrids directly. In other words, we do not need to somatically double triploid hybrids to produce synthetic hexaploids of *I. trifida* which inherit the disadvantage of inbreeding depression due to the chromosome duplication. Sexual polyploidization is definitely an attractive alternative for sweet-potato breeding, as demonstrated in other polysomic polyploid crops such as potatoes (Mok and Peloquin 1975; Mendiburu and Peloquin 1977) and alfalfa (Bingham 1968, 1980). Fertility of 2*n* pollen from the triploid hybrids is being studied and will be reported in the accompanying article (Freyre *et al.* 1991).

Acknowledgements

The authors thank Dr. T. Carey, Dr. K. Watanabe, and Dr. J. Dodds of CIP for valuable suggestions that led to improvements in the manuscript.

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