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Use of *Ipomoea trifida* germ plasm for sweet potato improvement. 3. Development of 4x interspecific hybrids between *Ipomoea batatas* (L.) Lam. (2n = 6x = 90) and *I. trifida* (H.B.K) G. Don. (2n = 2x = 30) as storage-root initiators for wild species

G. Orjeda, R. Freyre * and M. Iwanaga **

International Potato Center (CIP) P.O. Box 5969, Lima, Peru

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Summary. More than 28,000 pollinations were carried out between 5 *Ipomoea batatas* and 41 diploid *I. trifida* accessions of diverse origins to obtain 4x interspecific hybrids. From the resultant 730 seeds, 248 plants were finally obtained. Ploidy level determination of the progeny showed unexpected results: 52 individuals were hexaploid, 5 were pentaploid, 190 were tetraploid, as expected, and one was not determined. The existence of 5x and 6x progenies from 6x × 2x crosses not only confirmed the presence of 2n gametes but also their successful function in gene flow between ploidy levels and polyploidization within this genus. The progeny and their cultivated parents were planted in an observation field. The cultivated parents produced 0.49 kg/plant or less. Most 4x progenies did not produce storage roots or had very poor yields; nonetheless, and despite their cultivated parents' poor yields, 8 genotypes yielded between 0.81 and 1.50 kg/plant.

A new scheme, using the 4x interspecific hybrids, is proposed for evaluating 2x and 4x wild accessions of the section Batatas to which the sweet potato belongs. Other possible uses of the 4x hybrids in breeding and genetics of the sweet potato are also discussed.

Key words: *Ipomoea batatas* – *Ipomoea trifida* – Interspecific hybrids – Polyploidy – 2n gametes

Introduction

Sweet potato, *I. batatas* (L.) Lam. (2n = 6x = 90) belongs to the section Batatas genus *Ipomoea,* as do 11 wild species, none of which produces edible storage roots. *I. trifida*, a wild species of the section, has been designated to be the most probable ancestor of the cultivated sweet potato (Kobayashi 1984; Nishiyama and Teramura 1962; Austin 1988). It is also the only species that is able to produce fertile progeny when crossed with sweet potato. For these reasons it is considered to be a key wild species for sweet potato evolution and breeding.

All of the species of the section Batatas have chromosome numbers that are multiples of x = 15. Sweet potato is a hexaploid, with 90 chromosomes, whereas the other species of the section are either diploid or tetraploid; that is, they have 30 or 60 chromosomes, respectively. There also exists a hexaploid wild cytotype whose taxonomic identification is under discussion (Nishiyama 1959, 1963; Kobayashi 1984; Sakamoto 1976; Austin 1978, 1988; Jones 1967a; Martin et al. 1974).

Most of the important objectives in sweet potato breeding are related to its storage roots: earliness, yield, eating quality, nutritive value, storage ability, and insect resistance (Iwanaga 1988). There are two main constraints in using wild species for sweet potato breeding: first, none of the wild species of the section produces sizable storage roots. For this reason they cannot be directly evaluated for desirable characteristics related to storage roots. Second, there is a difference in ploidy level between the wild (2x, 4x) and cultivated species (6x). This difference acts as a reproductive barrier to obtaining sufficient progeny with which the wild parent can be evaluated through progeny testing.

We therefore propose an alternative scheme for evaluating sweet potato wild species in general, and 2x or 4x *I. trifida* in particular, for characteristics related to storage roots (Fig. 1). This alternative scheme has three primary objectives. Firstly, the development of a 4x population that has storage-root production and is able to in-

* *Present address:* Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824, USA
** To whom all correspondence should be addressed. *Present address:* Genetic Resources Unit, Centro International de Agricultura Tropical (CIAT) A.A.6713, Cali, Colombia

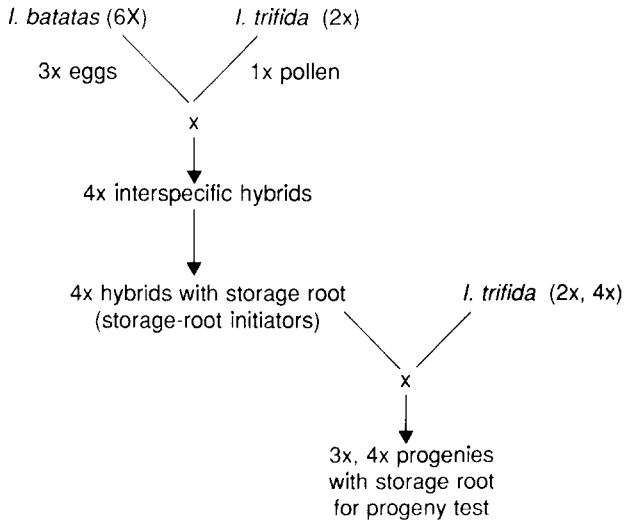

Fig. 1. Alternative scheme for evaluating 2x and 4x sweet potato to wild species via storage-root initiators and progeny testing

duce the formation of storage roots in their progeny with 2x and 4x wild species. Such a 4x population could be obtained by crossing *I. batatas* with 2x *I. trifida*. Secondly, the use of this population as storage-root initiators with many different 2x and 4x wild species accessions. Finally, the evaluation, by progeny testing, of hybrids between initiators and wild accessions that need to be evaluated.

In this paper we report how the first part of our alternative scheme was achieved; that is, how we produced interspecific hybrids. We also describe their performance in the field.

Materials and methods

Five sweet potato cultivars (2n = 6x = 90), selected for their flowering capability and self-incompatibility, were used as female parents in crosses with 41 diploid accessions of *I. trifida* of diverse origins. Between January and September of 1987, 28,214 hand pollinations were carried out under greenhouse conditions to avoid insect pollination. Capsules were harvested 30–40 days after pollination.

The male and female plants were induced to flower by using a short-day treatment (9 h light), as explained in the first of this series of articles (Iwanaga et al. 1991). Female plants were also periodically checked for their self-incompatibility by Martin's method (1959) to circumvent the need for emasculation.

The seeds thus obtained were disinfected successively in different solutions and scarified by hand before sowing. The resultant seedlings were planted in a nursery plot at San Ramón, Peru (lat. 11°08'S, 800 m.a.s.l., and temperature range of 14° and 31 °C). An observation field was established adjacent to the nursery plot by taking five cuttings from each seedling. The plot size was 1 m in length and the distance between rows was also 1 m. Five cuttings were planted in each plot with 0.2 m distance between cuttings of same genotype, and harvest was made at 150 days after planting the cuttings in the observation field.

The ploidy level of the 6x × 2x progeny was determined by chromosome counts in root-tip cells at metaphase. Cuttings were made from each genotype and placed in vials with water. Five days later the root tips were collected and pretreated with 8-hydroxyquinoline (0.002*M*) for 4 h at 12 °C and then fixed with Farmer's solution for 24 h. Hydrolysis was made by submerging the root tips in 1*N* HCl at 60 °C for 20 min. The samples were washed with tap water and then stained. Observations were made by using the acetic-orcein squash method. Counting exact chromosome numbers was often found to be difficult, but approximate ploidy level determination was carried out without much trouble.

Results

Seed set and female fertility

From the 28,214 flowers pollinated, 730 seeds were harvested: 312 germinated, and 248 seedlings were finally obtained; that is, 1 plant from every 114 flowers pollinated. Table 1 shows the number and percentage of pollinations, seeds obtained, germinated seeds, and thriving plants from each female used. There was a large difference among female parents in the number of flowers pollinated. This difference was a result of the different flowering ability of each accession and, consequently in the number of flowers available for pollination. The number of seeds obtained per 100 pollinations was, on average, 2.59. When the seeds germinated, this value decreased to 1.11, and 0.88 plant/100 pollinations. All of these values showed a wide range of variation, depending on the sweet potato accession used, and are indicative of differing female fertility in interspecific crosses.

The average germination rate of the obtained seeds was 42.7%, with 312 seedlings produced from 730 seeds. Ultimately, only 248 plants grew well and were planted in the field. These plants represent 34.0% of the total seed produced and only 0.88 plant/100 pollinations. The most profusely flowering accession, RCB2117H, was the least successful in producing progeny, with only 88 plants from 13,771 pollinations (0.64 plant/100 pollinations). In contrast, the least profusely flowering accession, RCB213IN, was the most successful, producing 1 plant from 17 pollinations (5.88 plant/100 pollinations).

Ploidy level of the progenies

Ploidy level determination showed unexpected results (Table 2): of the 247 individuals examined, 52 (21.1%) were hexaploids (2n = 6x = 90), 5 individuals were pentaploids (2n = 5x = 75), and 190 were as expected, tetraploids. Thus, 23% of the progenies turned out to have unexpected ploidy levels (6x and 5x).

A failure in the self-incompatibility system of DLP 627 was suspected because 40 of its 70 progenies were hexaploids. To clarify this question, an additional 500 self-pollinations were carried out on this clone. Using Martin's method (1959) we did not observe any pollen tubes growing in the style, and no capsule developed.

Table 1. Numbers and percentages of pollinations, seed set, and seeds germinated and percentage of plants obtained from interspecific crosses between 5 *Ipomoea batatas* and 22 2x *I. trifida* accessions

Female[a]	Pollinations (no.)	Seeds (no.)		Plants obtained (no.)
		Obtained	Germinated	
ARB 389	566	25 (4.42)[b]	23 (4.06)[b]	23 (4.06)[b]
DLP 627	7,048	206 (2.92)	88 (1.25)	70 (0.99)
EEC 251	6,812	120 (1.76)	75 (1.10)	66 (0.97)
RCB2117H	13,771	378 (2.74)	125 (0.91)	88 (0.64)
RCB213IN	17	1 (5.88)	1 (5.88)	1 (5.88)
Total	28,214	730 (2.59)	312 (1.11)	248 (0.88)

[a] Each female was crossed with many different 2x males, so results are summarized for females
[b] Percentages are expressed in parentheses

Table 2. Number of individuals with different ploidy level in the progeny of each female after interspecific crosses between *Ipomoea batatas* (6x) and *I. trifida* (2x)

Female	Ploidy level				Total
	4x	5x	6x	?	
ARB 389	23	0	0	0	23
DLP 627	27	3	40	0	70
EEC 251	54	2	9	1	66
RCB2117H	85	0	3	0	88
RCB213IN	1	0	0	0	1
Total	190	5	52	1	248

Table 3. Yield distribution of 248 clones derived from interspecific crosses between *Ipomoea batatas* (6x) and *I. trifida* (2x). [a] San Ramón, Peru, 1988

Yield (kg/plant)	Ploidy level				Total
	4x	5x	6x	?	
+[b]	4	0	0	0	4
0	35	1	3	0	39
0.01–0.20	106	0	13	0	119
0.21–0.40	20	1	7	0	28
0.41–0.60	10	1	7	0	18
0.61–0.80	7	0	4	1	12
0.81–1.00	4	2	7	0	13
1.01–1.50	4	0	9	0	13
>1.50	0	0	2	0	2
Total	190	5	52	1	248

[a] Yield of progenitors (kg/plant): RCB2117H, 0.28; DLP 627, 0.49; EEC 251, 0.10
[b] + = Cuttings died in the field

Yield in observation field

Table 3 shows the yield distribution of the progenies according to their ploidy levels and the yields of the three most profuse progenitors, RCB2117H, DLP 627, and EEC 251. The cultivated progenitors had very low yields: DLP 627 had the highest with 0.49 kg/plant; RCB2117H, 0.28 kg/plant; and EEC 251, only 0.10 kg/plant. Yield distribution was very wide among the 52 6x progenies. There were 3 genotypes that did not produce storage roots, 13 produced less than 0.21 kg/plant, while the remaining genotypes were distributed over all of the yield classes: there were even 2 genotypes that yielded more than 1.50 kg/plant. The majority of the tetraploid genotypes did not produce storage roots at all (35 genotypes, or 18.4%.) or produced only few (106 genotypes or 55.8% produced less than 0.21 kg/plant). In spite of the low yields of the cultivated parents and the inability of the wild parents to produce storage roots, ten 4x genotypes produced between 0.41 and 0.60 kg/plant; another seven between 0.61 and 0.80; and a further eight, between 0.81 and 1.50 kg/plant. Comparing the yields of the progeny with those of their respective parents, 70% of the 4x progenies yielded more than their cultivated parents (data not shown).

Discussion

The ovary in the sweet potato is superior, bicarpellate, and bilocular. Each locule possesses two ovules separated by a pseudoseptum (Kokubu et al. 1982). Under normal fertilization and embryogenesis, four seeds per pollinated flower should be expected to set. Nonetheless, this rarely occurs, even in intraspecific compatible crosses in which many pollen grains germinate on the stigma (Kokubu et al. 1982). In intracultivar crosses in which the same sweet potato cultivars were used (e.g. 'ARB 389', 'DLP 627', and 'RCB2117H'), seed set varied between 240.36 and 23.89 seeds per 100 pollinations for the most and least successful combinations, respectively (Orjeda 1990). Seed sets in interspecific crosses were dramatically reduced relative to those of intraspecific crosses, which was expected. The lowest set obtained in interspecific crosses was 1.76 seeds per 100 pollinations, and the highest was only 5.88 seeds (Table 1).

The low seed set (2.59 seeds per 100 pollinations) obtained from crosses between *I. batatas* and 2x *I. trifida* is to be expected, mainly because of the ploidy level difference. Kobayashi (1978) reported a comparable low seed set in crosses between sweet potato and 2x *I. trifida*. However, although the ploidy level difference acted as a reproductive barrier, it was not strong enough to completely restrict the gene flow between these related species. Thus, a low frequency of introgression from *I. trifida* into sweet potato occurred.

The seed set was 2.59 per 100 pollinations, and the germination rate was 42.7%: these results mean that the reproductive barriers in the interploidy crosses lead more to the abortion of seeds than to germination failure. Thus, indications are that the seeds, if formed, tend to be normal or complete. The barrieres involved may have been the double fertilization process or the development of the endosperm at an early stage. Contrasting results were obtained for crosses from *I. batatas* and synthetic 6x *I. trifida* clones (Freyre et al. 1991). From such interspecific, intraploidy crosses, 36.2 seeds per 100 pollinations were obtained, indicating that no major difficulties exist in these interspecific crosses. However, when these seeds were scarified, only 5.3% were viable, even though the shape of the seeds was normal: seed germination rather than seed set was a problem.

I. batatas is a hexaploid so its gametes have three sets of chromosomes; *I. trifida* is a diploid and its gametes have only one set. When these species are crossed, tetraploid progeny is to be expected. Surprisingly high frequencies of progenies with unexpected different ploidy levels (6x and 5x) were found among the progenies of this cross combination (Table 2).

Because self-fertilization was prevented, the only explanation for the existence of hexaploid progeny is the presence of 2n eggs and parthenogenesis in *I. batatas* female parents. There is evidence that 2n eggs occur in this genus (Ling 1984; Eckenwalder and Brown 1986; Iwanaga et al. 1991; Freyre et al. 1991).

In the work described in this paper, the 5x progeny must have arisen from the fertilization of normal eggs (3x) of *I. batatas* by 2n pollen grains (2x) coming from the 2x male parent. These individuals not only confirm the presence of 2n pollen in the diploid male parent *I. trifida* (Orjeda et al. 1990), but also its success in polyploidization and in gene flow between two related species with different ploidy levels.

The 4x interspecific hybrids have a normal appearance, and their growth habit is intermediate between the wild and cultivated plants. It is expected that some of these plants may produce storage roots because of the genetic input of their cultivated parent.

The 4x interspecific hybrids generated in this research have very interesting, useful features. They have a lower ploidy level than the cultivated sweet potato, but produce storage roots. They also combine the wild with the cultivated gene pools, opening up a new possibility for exploiting wild sweet potato germ plasm.

Three of the four genomes of the hybrids must come from their cultivated parent and the other genome from 2x *I. trifida*. Because of this fact, some hybrids would have genes for storage-root production that come from the cultivated parent, and therefore would be able to transmit these genes to their progeny with other wild 2x or 4x species. The 3x or 4x progeny obtained could produce storage roots, as was evident in our preliminary study (Orjeda 1990). In this way, useful genes for traits related to storage roots coming from the wild parent could be expressed, and progeny testing could be performed.

Once specific 2x and 4x wild accessions are selected through progeny testing, they can be crossed to obtain 3x *I. trifida*. Desirable traits captured in the 3x individuals, which combine genes of selected 2x and 4x individuals, can be transferred into the 6x gene pool for cultivar improvement by two approaches: somatic polyploidization, using colchicine, or sexual polyploidization, using 2n gametes (Iwanaga et al. 1991). Prescreening of 2x and 4x wild accessions, which are becoming abundant (Huaman and De la Puento 1988), would increase efficiency in the use of that germ plasm for seet potato improvement. In essence, the present approach is similar to the concept of the analytic breeding scheme (Chase 1963) which has been successfully applied for a couple of polysomic polyploid crops, such as potatoes (Peloquin et al. 1989) and alfalfa (Bingham 1980).

The good yields observed in some 4x hybrids indicate the possibility of developing 4x sweet potato cultivars for commercial use. There are two major advantages of such 4x cultivars over the present 6x cultivars. First, the lower ploidy level could facilitate breeding efforts because tetrasomic inheritance is simpler than hexasomic. Second, the use of related species becomes easier. Further improvement of the present 4x population for agronomic traits as well as male and female fertilities are being carried out to assess the feasibility of developing 4x cultivars (Orjeda 1990).

The 4x genotypes can also be used to obtain diploid ($2n = 2x = 30$) genetic stocks with storage-root production through haploid induction. Those 2x genotypes could be used for basic inheritance studies of important traits, which is extremely complicated at the 6x level (Jones 1967b; Kumagai et al. 1990). They also could be used to make a genetic linkage map, through restriction fragment length polymorphisms, of genes related to agronomic traits, such as yields, that are not present in 2x wild germ plasm.

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