SELECTION FOR 2n GAMETES AND TUBERIZATION IN *SOLANUM CHACOENSE*[1]

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Abstract

A breeding scheme is outlined which broadens the genetic base of the potato by incorporation of germplasm from *S. chacoense* (Argentina) with Group Phureja and haploids of Group Tuberosum. Selection for adaptation to cultivation and production of 2n gametes is carried out at the diploid level, with the benefits of disomic ratios. *S. chacoense* provides increased genetic diversity, vegetative vigor and profuse flowering. About one-fifth of the 654 plants examined produced 2n eggs; clones with high 2n egg production may be obtained either by screening within particular introductions or by intercrossing known 2n egg producers. Only 16 of 1,212 plants screened produced 2n pollen — one by first division restitution. There is great variation among introductions and individuals for tuberization in the greenhouse. Selection for early tuberization in the field may be difficult due to extremely long stolons, but it would be facilitated by incorporation of the short stolon characteristic from the topiary mutant type *S. infundibuliforme*. Male sterile Tuberosum haploids hybridize with *S. chacoense* to give male fertile interspecific F_1 progeny, some of which produce 2n eggs or 2n pollen.

Resumen

Se delinea un esquema de mejoramiento dirigido a ampliar la base genética de la papa por incorporación de germoplasma de *S. chacoense* (Argentina) con el grupo Phureja y haploides del grupo Tuberosum. La selección para adaptación y la producción de gametos 2n es realizada a nivel diploide con los beneficios que supone la segregación disómica. *S. chacoense* aporta diversidad genética, vigor vegetativo y floración profusa. Alrededor de una quinta parte de las 654 plantas examinadas produjeron huevos 2n.

Clones con una alta producción de huevos 2n pueden ser obtenidos ya sea tamizando dentro de introducciones o inter-cruzando productores conocidos de huevos 2n. Solamente 16 de 1,212 plantas tamizadas produje-

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ron polen 2n, una por FDR. Existe una gran variación entre introducciones e individuos para tuberización en el invernadero. Selección para tuberización temprana en el campo puede ser difícil debido a los estolones extremadamente largos pero sería facilitado por incorporación de la característica estolones cortos del mutante topiary de *S. infundibuliforme*. Haploides de tuberosum con esterilidad masculina hibridizan con *S. chacoense* produciendo progenies interespecíficas F_1, que poseen fertilidad masculina. Algunos de estos progenies producen huevos 2n o polen 2n.

Introduction

The genetic base of the major North American potato cultivars is considered to be relatively narrow by many workers (3, 14). This is partly a result of the breeding and selection methods employed in the process of producing adapted varieties. Recent inputs of new variability from primitive cultivated forms and wild species have yielded many useful breeding lines (7, 13). Further major advances in potato breeding depend on thorough exploitation of exotic germplasm for specific traits and general genetic diversity.

Hougas and Peloquin (5) outlined an easy method of recovering haploids from most 4x cultivars, which facilitated the incorporation of the great store of variability present in the diploid, 2n=2x=24, tuber bearing Solanums (6). Hybrids between these haploids and related diploid species were remarkably vigorous and generally exhibited good fertility (11).

A new approach to potato breeding whereby intensive selection is carried out on haploids and haploid-species hybrids, following by polyploidization of the products, was proposed by Chase (1) in 1963. The work of Mok, Mendiburu & Peloquin (8, 10) involving crosses with *S. tuberosum* Group Phureja × haploid Group Tuberosum hybrids demonstrated that the most advantageous method of effecting polyploidization, in terms of the heterotic responses of the tetraploid progeny, is via 2n gametes formed by a first division restitution (FDR) mechanism. They attributed the outstanding vigor and yielding ability of the tetraploid families derived from 4x×2x and 2x×2x matings to two factors. First, the hybridization of Group Phureja individuals with male fertile haploids provides a large input of diversity, giving highly heterozygous offspring. Second, FDR 2n gametes preserve and transmit most of the heterozygosity and espistasis in these diploid hybrids by largely circumventing the disruptive effects of meiosis on the genotypic array.

An appropriate modification of Chase's analytic breeding scheme was formulated by Mendiburu, Peloquin and Mok (9) which would capitalize on genetic diversity, disomic inheritance, and bilateral sexual polyploidization. Germplasm from *S. chacoense* or other non-cultivated species,

Group Phureja, and haploids of Groups Tuberosum and Andigena or "Neo-Tuberosum" are initially selected for adaptation to cultivation, production of 2n gametes, and other desirable qualities. Breeding at the diploid level is greatly facilitated by the simple disomic ratios in 24 chromosome material. Intermediate diploid hybrids of (haploid × *S. chacoense*) and (Phureja × haploid) matings that produce 2n gametes by FDR are then intercrossed to produce highly diverse, heterotic tetraploids by bilateral sexual polyploidization.

S. chacoense is included in this scheme as a source of genetic diversity, vegetative vigor, profuse flowering, high fertility, and long tuber dormancy. *S. chacoense* hybridizes easily with both male fertile and male sterile haploids to give male fertile progeny, permitting the inclusion of a wide range of haploids as potential parents. Thus, *S. chacoense* broadens the genetic base in two ways.

This report is concerned with the evaluation of many introductions of *S. chacoense* for 2n gamete production and tuberization.

Materials and Methods

Individuals from 48 introductions of *S. chacoense* were screened for production of 2n eggs and pollen. 2n pollen was identified by microscopic examination of pollen grains stained with acetocarmine in glycerol (12). Plants that produce 5% or more large pollen grains were considered to be 2n pollen producers. 2n eggs were detected by means of 2x×4x crosses. The number of seeds/fruit (s/f) resulting from these crosses is a direct indication of the level of 2n egg production. The pollen source employed was either tetraploid *S. chacoense* or W231, an advanced selection from the University of Wisconsin potato breeding program. All pollinations on field grown plants were made on cut-stems in an air conditioned greenhouse. Average seeds per fruit data presented are calculated on the basis of the number of plump seed with parthenocarpic berries excluded. Most families from the 2x×4x crosses consisted of only presumptive tetraploids as identified by general morphology, pollen grain size and morphology, and the number of chloroplasts per guard cell.

Samples of 18-20 individuals from each of the 48 accessions were grown in the greenhouse and the tubers were harvested after approximately six months of growth. The number of tubers and size of the largest tuber were recorded for each plant, and a weighted index was computed. Three parameters were considered to have some bearing on the average ability of individuals in an accession to produce tubers: the proportion of plants tuberizing to any degree, the average size of the largest tuber, and the average number of tubers produced. The parameters were weighted according to their relative importance. Accessions were grouped into

categories of low, medium, and high for the three parameters, and assigned index values as shown below:

	low	medium	high
percent tuberizing	1	5	9
largest tuber size	1	3	5
number of tubers	1	2	3

The composite tuberization index for an introduction is simply the sum of the values for the three parameters.

Crosses were made among selections of *S. chacoense* that produce 2n gametes, and also between male-sterile Tuberosum haploids and 2n gamete producing clones of *S. chacoense*. The resulting progenies were evaluated for 2n gamete production, and the percent pollen stainability was determined for the haploid × *S. chacoense* hybrids.

Results and Discussion

2n gamete Search — Sixteen plants of the 1,212 screened produced 5% or more 2n pollen. They represent 9 of the 48 introductions. An additional 17 plants were found that produced less than 5% 2n pollen. The incidence of 2n pollen in 14 of the 48 introductions is presented in Table 1. Parallel spindles at Anaphase II were observed in pollen mother cells of one plant

TABLE 1. — *Frequency of 2n gametes in several* S. chacoense *introductions.*

	2n Pollen		2n Eggs			
Introduction	Number of individuals screened	Number with 5% or more 2n Pollen	Number of individuals screened	Number with 2n Eggs	Average Seeds/Fruit	Range Seeds/Fruit
P.I. 230580	20	0	34	5	2.6	1.0 - 9.0
P.I. 230582	54	6	17	5	1.5	1.0 - 4.0
P.I. 265576	64	2	31	6	4.0	1.0 - 8.0
P.I. 275139	24	1	24	7	3.1	1.0 - 8.0
P.I. 320282	71	0	18	1	1.0	1.0
P.I. 320283	28	0	24	8	3.0	1.0 - 17.0
P.I. 320291	46	0	30	1	2.0	2.0
P.I. 320293	27	1	22	6	1.2	1.0 - 3.0
P.I. 320294	50	0	23	12	6.3	1.0 - 35.0
WRF 267	49	0	38	5	1.8	1.0 - 5.0
WRF 1885	13	1	24	0	0	0
Wei s.n.	11	1	19	6	6.1	1.0 - 17.7
Haw 3187	11	1	24	2	1.5	1.0 - 2.0
Hof 1533	15	0	33	4	4.4	1.0 - 12.0

from the accession Wei s.n. Approximately 40% of the stainable pollen from this plant was 2n. Parallel spindles have previously been found in *S. chacoense* in two plants of P.I. 230580, and in three diploids and a triploid from P.I. 320294 (12). The presence of the FDR mode of diplandrogenesis

in several introductions adds considerably to the desirability of *S. chacoense* for use in the analytical breeding scheme.

One hundred twenty-eight plants of the 654 examined produced 2n eggs; they were found in 38 of the 48 introductions screened. The details of 2n egg production in 14 introductions are presented in Table 1.

Tuberization in the Greenhouse — The data on tuberization and the derived index values are presented for eight introductions in Table 2. These were selected to indicate the range in values for the tuberization parameters *values*. The parameters were found to be uncorrelated for this population.

TABLE 2. — *Tuberization values of several* S. chacoense *introductions.*

Introduction	Percent Tuberized	Mean # Tubers/Plant	Mean size (cm) Largest Tuber Per Plant	Index Value
WRF 311	80*	23.1	2.0	17
P.I. 209412	22	7.5	1.2	3
WRF 317	95	24.4	2.0	17
WRF 1855	6	33.0	2.2	9
WRF 309	65	34.9	1.6	11
WRF 368	11	4.0	1.6	5
P.I. 217451	20	17.8	2.8	9
P.I. 320292	15	11.0	1.2	4

*There were 18-20 individuals in each introduction.

Hanneman (4) was able to make little or no progress in recurrent selection for field tuberization in a bulk population of *S. chacoense*. This may be partly due to the difficulty of recovering tubers from the mat of stolons the plants produced. Considerably more progress was made in bulk populations of Tuberosum haploids × *S. chacoense*. In three generations the proportion of plants tuberizing increased from 15% in the unselected group to 56% in the groups undergoing recurrent selection for tuberization.

Mendiburu, Peloquin and Mok (9) recognized the possibility that Tuberosum haploid — *S. chacoense* hybrids might require further intercrossing and selection for short stolons and early maturity. The additional manipulations following the synthesis of the F_1 hybrid will be at the expense of a portion of the initial heterozygosity of the hybrid. This will be reflected in the proportionally lower heterozygosity of the 2n gametes produced by the secondary haploid-species hybrids. They concluded that adaptation of *S. chacoense* or other wild species previous to hybridization with haploids is therefore preferable from this standpoint.

The discovery of mutant plants of *S. infundibuliforme* with very short stolons (2) opens up the possibility of making field harvests much more simple and accurate. The monogenic nature of the mutant makes transfer of

the short stolons characteristic into *S. chacoense* a straightforward exercise in Mendelian genetics. The vigor and smooth tubers of *S. chacoense* may be masked by the effects of the mutant gene, but these traits should be expressed in the haploid-species hybrid. Unfortunately, the long stolons of native *S. chacoense* will also reappear in these progenies. This trait has shown variation in intensity in *S. chacoense* × cultivar offspring. There should therefore be little difficulty in selecting against it in both the intermediate diploid hybrids and in the final tetraploids.

Hybrid Families — Hybridization between 2n gamete producers proved to be a valuable method of obtaining new 2n gamete producing individuals. A total of twelve progenies from four crosses was evaluated for 2n egg production, and five of these set seed in a 2x×4x cross. Of these five, three individuals had a considerably higher level of 2n egg production (23-32s/f) than any of the six parents used in the study (2-14s/f). It is evident that individuals with high 2n egg production can be obtained from small progenies.

Hybrids between male sterile Tuberosum haploids and *S. chacoense* were generally very vigorous, flowered profusely, and tuberized under long-day conditions. More important was the high level of male fertility as determined by pollen stainability. The mean pollen stainability varied from 48% to 91% among the nine families, a great improvement over the extremely low pollen stainability of the haploid parents. Equally important was the identification of seven individuals with 2n pollen and five with 2n eggs.

Literature Cited

1. Chase, S.S. 1963. Analytic breeding in *Solanum tuberosum* L. — a scheme utilizing parthenotes and other diploid stocks. Can J Genet Cytol 5:359-363.
2. den Nijs, T.P.M., E.F. Leue, and S.J. Peloquin. 1977. A heritable compact plant type in *Solanum infundibuliforme*. Am Potato J 54:493.
3. Glendinning, D.R. 1974. Neo-Tuberosum: new potato breeding material. 1. The origin, composition, and development of the Tuberosum and Neo-Tuberosum gene pools. Potato Res 18:256-261.
4. Hanneman, R.E., Jr. Personal communication.
5. Hougas, R.W. and S.J. Peloquin. 1958. The potential of potato haploids in breeding and genetic research. Am Potato J 35:701-707.
6. Hougas, R.W. and S.J. Peloquin. 1960. Crossability of *Solanum tuberosum* haploids with diploid *Solanum* species. Eur Potato J 3:325-330.
7. Hougas, R.W. and R.W. Ross. 1956. The use of foreign introductions in breeding American potato varieties. Am Potato J 33:328-339.
8. Mendiburu, A.O. and S.J. Peloquin. 1977. The significance of 2n gametes in potato breeding. Theor Appl Genet 49:53-61.
9. Mendiburu, A.O., S.J. Peloquin and D.W.S. Mok. 1974. Potato breeding with haploids and 2n gametes. In: Haploids in higher plants, K. Kasha (ed.), Univ. Guelph p249-258.

10. Mok, D.W.S. and S.J. Peloquin. 1975. Breeding value of 2n pollen (diplandroids) in tetraploid×diploid crosses in potatoes. Theor Appl Genet 46:307-314.

11. Peloquin, S.J., R.W. Hougas, and A.C. Gabert. 1966. Haploidy as a new approach to the cytogenetics and breeding of *Solanum tuberosum*. In: Chromosome Manipulations and Plant Genetics, Ralph Riley, K.R. Lewis (eds.), Oliver and Boyd, Edinburgh. p. 21-28.

12. Quinn, A.A., D.W.S. Mok and S.J. Peloquin. 1974. Distribution and significance of diplandroids among the diploid Solanums. Am Potato J 51:15-21.

13. Ross, H. 1965. The use of wild Solanum species in German potato breeding of the past and today. Am Potato J 43:63-80.

14. Simmonds, N.W. 1976. Potatoes. In: Evolution of Crop Plants, N.W. Simmonds (ed.), Longman Group Ltd. p. 279-283.