Nature of 'Pollinator' Effect in Potato (*Solanum tuberosum* L.) Haploid Production

STANLEY J. PELOQUIN, AUGUST C. GABERT and RODOMIRO ORTIZ*

Departments of Horticulture and Genetics, University Wisconsin Madison, 1575 Linden Drive, Madison, WI, USA

Haploids ($2n = 24$) of the common tetraploid ($2n = 48$) potato (*Solanum tuberosum* L.) provide promising material for attacking many problems concerned with the genetics, cytogenetics and breeding of this species. Interspecific $4x \times 2x$ crosses between *Solanum tuberosum* gp. *Andigena* or gp. *Tuberosum* cultivars as pistillate parents and *Solanum tuberosum* gp. *Phureja* as source of pollen (hereafter 'pollinator') have been used to produce maternally derived haploids through parthenogenesis. This paper discusses the nature of the 'pollinator' effect in haploid extraction. The 'pollinator' had a significant effect on haploid frequencies following $4x \times 2x$ crosses. The 'pollinator' effect seems to operate via the endosperm, in which haploid ($n = 2x$) embryos are associated with hexaploid endosperm. A superior 'pollinator' appears to have its effect by contributing two haploid (n) gametes to the central cell.

© 1996 Annals of Botany Company

INTRODUCTION

Haploids ($2n = 2x = 24$) of the common cultivated potato, *Solanum tuberosum* gp. *Tuberosum* ($2n = 4x = 48$), provide the opportunity for genetic research and breeding of this species (Peloquin, Werner and Yerk, 1990). A large number of haploids from a wide range of *Solanum tuberosum* gp. *Tuberosum* cultivars or advanced selections are essential to fully realize the potential of potato haploids for breeding and genetic research (Kotch and Peloquin, 1987).

The most common method to obtain potato haploids has been interspecific-interploidy $4x \times 2x$ crosses between a tetraploid seed parent and a diploid *Solanum tuberosum* gp. *Phureja* clone (hereafter 'pollinator') as pollen source (Peloquin, Hougas and Gabert, 1966; Hermsen and Verdenius, 1973). Different factors influence the frequency of haploid production in potato (Hougas, Peloquin and Gabert, 1964). Two of them are of great importance: (*a*) decapitation of pistillate parent (Peloquin and Hougas, 1959), and (*b*) the finding that the 'pollinator' effect was heritable (Gabert, 1963).

The 'pollinator' does not contribute chromosomes to the haploid embryo. Therefore, it is not immediately evident in what way the 'pollinator' influences haploid frequency. Nonetheless, the detection of superior 'pollinators' has provided the opportunity for cytological studies. The high haploid frequencies (20–40 haploids per 100 fruit) made it possible to determine developmental events following $4x$–$2x$ matings. Hence, the objective of this research was to investigate the nature of this 'pollinator effect' in potato haploid production.

* For correspondence at: IITA c/o Lambourn & Co., Carolyn House, 26 Dingwall Rd., Croydon, CR9 3EE, UK.

MATERIALS AND METHODS

Pollinations were made between tetraploid cultivars (pistillate parents) and pollen from *Solanum tuberosum* gp. *Phureja* clones following the method of Peloquin and Hougas (1959). Seeds were harvested from each fruit separately. The total number of pollinations, fruits and seeds were recorded. Haploid frequency was calculated as the ratio of number of haploids per 100 fruits and per 100 seeds. The identification of tetraploids and haploids was done by chromosome counting using root tips of each seedling.

A χ^2 test for homogeneity was carried out to test whether the two 'pollinators' (1·1 and 1·22) were equally efficient in the production of tetraploids and haploids after $4x \times 2x$ crosses.

RESULTS AND DISCUSSION

A summary of potato haploid production is shown in Table 1. The clone 1·1 was the superior 'pollinator' with a high seed set and high haploid frequency. Haploid extraction using the progeny derived from intermating superior 'pollinators' as 'pollinators' is indicated in Table 2. The clones 1·1, 1·13 and 1·22 were the superior 'pollinators' with more than ten haploids per 100 fruit.

These three clones were used for haploid extraction with tetraploid parents which were previously classified according to the ease of haploid extraction (Table 3). The clone 1·1 had the highest seed set among the 'pollinators' used. Its pollen was examined microscopically and it was found to have 5–10% $2n$ pollen (pollen with the sporophytic chromosome number). This early result suggested the functioning of $2n$ pollen as an important characteristic of a superior 'pollin-

TABLE 1. *Haploid extraction in* Solanum tuberosum *Gp.* Tuberosum* *using different '*pollinators*'*

Pollinator	Fruit (F)	Seeds (S)	S/F	Haploids (H)	H/100 F (%)	H/100 S (%)
1 Gp. *Phureja* PI 225682	1160	1614	1·4	5	0·3	0·3
1·1 PI 225682	1285	12047	9·4	147	11.4	1·2
1·2 PI 225682	2077	2136	1·0	3	0·1	0·1
2·1 PI 225682	408	774	1·9	5	1·2	0·6
2·3 PI 225682	1603	1965	1·2	6	0·4	0·3
2·4 PI 225682	1766	3257	1·8	20	1·1	0·6
3·2 PI 225682	487	558	1·1	6	1·2	1·1
3·4 PI 225682	844	1413	1·7	2	0·2	0·1
4·8 Gp. *Phureja* PI 195198	297	396	1·2	1	0·3	0·3
4·11 PI 195198	520	691	1·3	7	1·3	1·0
4·14 PI 195198	604	814	1·3	1	0·2	0·1
7·4 Gp. *Phureja* PI 225672	911	1141	1·3	2	0·2	0·2

* *Solanum tuberosum* Gp. *Tuberosum* cultivars: Cherokee, Chippewa, Early Gem, Irish Cobbler, Katahdin, Kennebec, Merrimack, Osseo, Red Beauty, Red Pontiac, Red Warba, Russet Burbank, Sebago, Tawa and Triumph.

TABLE 2. *Haploid extraction in* Solanum tuberosum *Gp.* Tuberosum *using progeny from intermating superior '*pollinators*'*

Pollinator	Fruit (F)	Seeds (S)	S/F	Haploids (H)	H/100 F (%)	H/100 S (%)
1·1	848	4010	4·7	93	11·0	2·3
1·3	156	114	0·7	2	1·3	1·8
1·13	639	1087	1·7	80	12·5	7·4
1·15	822	308	0·4	2	0·2	0·6
1·16	711	207	0·3	5	0·7	2·4
1·17	582	319	0·6	8	1·4	2·5
1·18	956	927	1·0	10	1·0	1·0
1·19	720	1026	2·7	6	0·8	0·6
1·22	1283	2787	2·2	151	11·8	5·4
1·24	925	1085	1·2	2	0·2	0·2
1·25	1247	1428	1·2	16	1·3	1·1

TABLE 3. *Haploid extraction from three different* Solanum tuberosum *Gp.* Tuberosum *tetraploid cultivars* *using superior* '*pollinators*'

Gp. *Tuberosum* Parent	Pollinator	Fruit (F)	Seeds (S)	S/F	Haploids (H)	H/100 F (%)	H/100 S (%)
Chippewa	1·1	740	8452	11·4	58	7·8	0·7
	1·13	292	383	1·3	27	9·2	7·0
	1·22	778	1323	1·7	51	6·6	3·9
Merrimack	1·1	295	3974	13·5	79	26·8	2·0
	1·13	185	327	1·8	44	23·8	13·5
	2-2-22·1	129	228	1·8	33	25·6	14·5
W-231	1·1	363	8006	22·1	146	40·2	1·8
	1·13	250	910	3·6	100	40·0	11·0
	4·4	202	786	3·9	78	38·6	9·9

* *Solanum tuberosum* gp. *Tuberosum* cultivars characterized according to their frequency to produce haploids following $4x \times 2x$ crosses: Chippewa (low), Merrimack (medium) and W-231 (high).

ator', since the best 'pollinator' had $2n$ pollen. However, superior 'pollinators', such as 1·22, with high haploid frequencies did not have $2n$ pollen. This result questioned the association between a superior 'pollinator' with the functioning of $2n$ pollen.

Haploid frequency was influenced by the maternal (or pistillate) parent as was shown in the early work of Hougas *et al*. (1964). Merrimack had more haploids than the other cultivars of *S. tuberosum* gp. *Tuberosum* (Table 3). Hougas *et al*. (1964) suggested that haploid frequency could be

TABLE 4. *Frequency of haploids and tetraploids following* 4x × 2x *crosses.*

Parent	Pollinator	Fruit (F)	Seed (S)	S/F	Haploids (H)	H/100 F (%)	H/100 S (%)	Ratio 4x:haploid
Gp. *Andigena* (PI 230457)	1·1	25	311	12·4	21	84·0	6·8	14:1
	1·22	31	37	1·2	24	77·4	64·8	1:2
Gp. *Andigena* (PI 253705)	1·22	19	18	1·0	14	73·7	77·8	1:4

χ^2 for tetraploid:haploid ratios on PI 230457 (1·1 *vs.* 1·22) = 94·085; $P < 0.001$.

related to the genetic load, i.e. the frequency of lethal recessive alleles, in the pistillate parent.

A comparison of superior 'pollinators' for haploid extraction (Table 4) indicates that pollinators with $2n$ pollen *vs.* without $2n$ pollen (1·1 *vs.* 1·22) gave equal haploid frequencies. The seed set was higher in 1·1 due to the functioning of $2n$ pollen; however, the progeny was mainly tetraploid.

Based on these results, two alternatives can be considered for formation of the hexaploid endosperm associated with the diploid embryo in the haploid from a tetraploid potato, (*a*) functioning of $2n$ pollen with one gamete going to the polar nuclei and the other gamete not functioning, or (*b*) functioning of normal (n) pollen with both gametes going to the polar nuclei and leaving none to fertilize the egg, which develops through parthenogenesis. It was impossible to get direct evidence to support either of these two alternatives because the potato is not an easy plant for cytological studies of double fertilization. However there was indirect evidence to elucidate the origin of hexaploid endosperm associated with haploids.

The first evidence was the finding that 30% of the developing seed with hexaploid endosperm had unfertilized eggs after $4x \times 2x$ crosses with $2n$ pollen producers. It is hard to accept fertilization of the egg failing so often if $2n$ pollen was present at the time of fertilization. Conversely, it is easy to account for this failure on the basis of both haploid gametes uniting with the polar nuclei and leaving none available to fertilize the egg.

The second evidence involved the occurrence of haploids in fruits with varying number of seeds. The haploids were equally frequent in fruits having many or few seeds. This also supports the haploid pollen alternative. In effect, one would expect more seeds per fruit if $2n$ pollen functions frequently because the rate of survival of tetraploid embryos should be higher than that of triploid embryos.

Finally, both hypotheses ($2n$ *vs.* haploid pollen in superior 'pollinators') were tested using the following rationale. If $2n$ pollen in the superior 'pollinator' was necessary, the haploid frequency will be dependent on two events, (*a*) partheno-genetic development of the egg, and (*b*) $2n$ pollen always uniting with polar nuclei; however, the frequency of tetraploids, after $4x \times 2x$ matings, would only depend on the functioning of $2n$ pollen. Therefore, the frequency of tetraploids should always be greater than the frequency of haploids. Conversely, if there is no relationship between functioning of $2n$ pollen and haploid development, one

should expect to find more haploids than tetraploids after $4x \times 2x$ crosses with superior 'pollinators'.

Haploid frequencies, after $4x \times 2x$ crosses, using superior 'pollinators' with significant and with very little $2n$ pollen were useful to test both hypotheses (Table 4). The clone 1·1 (with $2n$ pollen) had fruits with both tetraploids and haploids. The clone 1·22 (with very little $2n$ pollen) had many fruits with only haploids. Further, both 'pollinators' had the same haploid frequencies. The extra seeds are mainly tetraploid if clone 1·1 was used as pollinator. Conversely, more haploids than tetraploids were found if 1·22 was used as 'pollinator'. Therefore, the evidence supports the hypothesis of two haploid gametes uniting with polar nuclei as the basis for the 'pollinator' effect.

The endosperm associated with a haploid embryo was always hexaploid. This clearly indicates: (*a*) the union of the two chromosome sets from the 'pollinator' with the polar nuclei, and (*b*) a lack of fertilization of the egg. Both are necessary for the development of a haploid sporophyte. Moreover, this finding supports the endosperm balance number hypothesis (Johnston *et al.*, 1980), which states that a balance between 2 maternal : 1 paternal EBNs are necessary in the endosperm for normal seed development. Therefore, the 'pollinator' appears to influence haploid frequency via its effect on the endosperm.

LITERATURE CITED

Gabert AC. 1963. *Factors influencing the frequency of haploids in the common potato* (Solanum tuberosum *L.*). Ph.D Thesis. University of Wisconsin-Madison.

Hermsen JGTh, Verdenius J. 1973. Selection from *Solanum tuberosum* group *Phureja* of genotypes combining high frequency haploid induction with homozygosity for embryo spot. *Euphytica* **22**: 244–259.

Hougas RW, Peloquin SJ, Gabert AC. 1964. Effect of seed-parent and pollinator on the frequency of haploids in *Solanum tuberosum*. *Crop Science* **4**: 593–595.

Johnston SH, den Nijs TPM, Peloquin SJ, Hanneman RE Jr. 1980. The significance of genic balance to endosperm development in interspecific crosses. *Theoretical and Applied Genetics* **57**: 5–9.

Kotch GP, Peloquin SJ. 1987. A new source of haploid germplasm for genetic and breeding research. *American Potato Journal* **64**: 137–141.

Peloquin SJ, Hougas RW. 1959. Decapitation and genetic markers as related haploid in *Solanum tuberosum. European Potato Journal* **2**: 176–183.

Peloquin SJ, Hougas RW, Gabert AC. 1966. Haploidy as a new approach to the cytogenetics and breeding of *Solanum tuberosum.* In: Riley R, Lewis KR, eds. *Chromosome manipulations and plant genetics*. Edinburgh: Oliver and Boyd, 21–28.

Peloquin SJ, Werner JE, Yerk GL. 1990. The use of potato haploids in genetics and breeding. In: Gupta PK, Tsuchiya T, eds. *Chromosome engineering in plants*. Part B. Essex, UK: Elsevier, 79–92.