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Production of potato monohaploids (2n = x = 12) through prickle pollination

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Summary. Data are presented on the potential of gynogenesis for the production of monohaploids and on factors affecting their frequency and relative vigour. Diploid *Solanum tuberosum* L. and *S. tuberosum* × *S. phureja* Juz et Buk hybrids were used as maternal parents and selected *S. phureja* clones as prickle pollinators with embryo-spot and nodal band as dominant seed and plant marker. About 2 million seeds were screened for absence of embryo-spot. After raising plants from phenotypically spotless seeds, further screening for absence of nodal bands and for ploidy level was carried out. Finally more than 500 monohaploid plants from three genetically different groups of maternal parents were obtained. Frequency and vigour of the monohaploids were clearly dependent on their maternal genotypes. The data also indicated an effect of the pollinator genotype, the physiological stage of the maternal plant and the environment on monohaploid frequency. On the basis of these results the possibility of breeding for a higher monohaploid production rate and for more stable and vigorous monohaploids is discussed. Furthermore, gynogenesis and androgenesis are compared. It is suggested that both should be used in order to obtain monohaploids from sufficiently various diploid breeding material.

Key words: Monohaploids – Prickle pollination – Gynogenesis – *Solanum* – Potato

Introduction

The first successful experiments for the specific purpose of producing monohaploid potato plants (2n = x = 12) took place in the early seventies. Van Breukelen et al. (1975, 1977) used prickle pollination (gynogenesis) and Irikura (1975) and Foroughi-Wehr et al. (1977) successfully tried anther culture (androgenesis).

Owing to their high level of heterozygosity, autotetraploid potato cultivars may comprise deleterious alleles at many loci without any harmful effects on growth and performance. On the other hand, such alleles are fully expressed in monohaploid plants. The same holds true for mutant alleles, whether dominant or recessive. If monohaploids survive, they not only are free from deleterious genes, but also have a harmonious combination of genes. Hence, selection for viable and relatively vigorous monohaploids implies selection for exceptional, excellent gene combinations.

It is only through identical doubling of monohaploid genomes that homozygous diploid or polyploid potatoes can be produced. Such homozygotes have special potentialities for potato breeding and research, as pointed out by Van Breukelen et al. (1975).

After the pioneering work in the early seventies, anther culture has been studied most extensively as to its potential for the production of monohaploids, mainly at the Max Planck Institute for Breeding Research at Cologne, W. Germany (Sopory 1977; Jacobsen 1978; Binding et al. 1978; Sopory and Tan 1979; Uhrig 1983, 1985; Wenzel and Uhrig 1981; Wenzel et al. 1979, 1981, 1982). This led Ross (1986) to the premature conclusion that "gynogenesis, as compared with androgenesis, is much less effective".

In the present paper it will be attempted to restore the balance by presenting data on the potential of gynogenesis for monohaploid production and to discuss some factors affecting the frequency of gynogenetic monohaploids and to examine the merits and limitations of both gynogenesis and androgenesis.

Materials and methods

The diploid plant material taken for the production of monohaploids derives from the species *Solanum tuberosum* L. and

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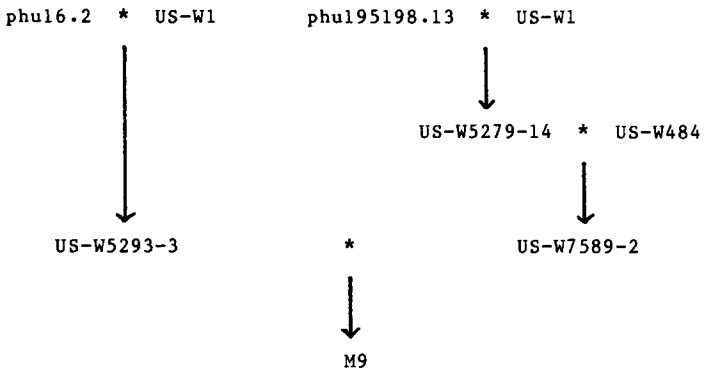

Fig. 1. Ancestry of the diploid hybrid clone M9. phu = *S. phureja;* US-W1 and 484 are dihaploids from cv. 'Katahdin' and 'Merrimack', respectively

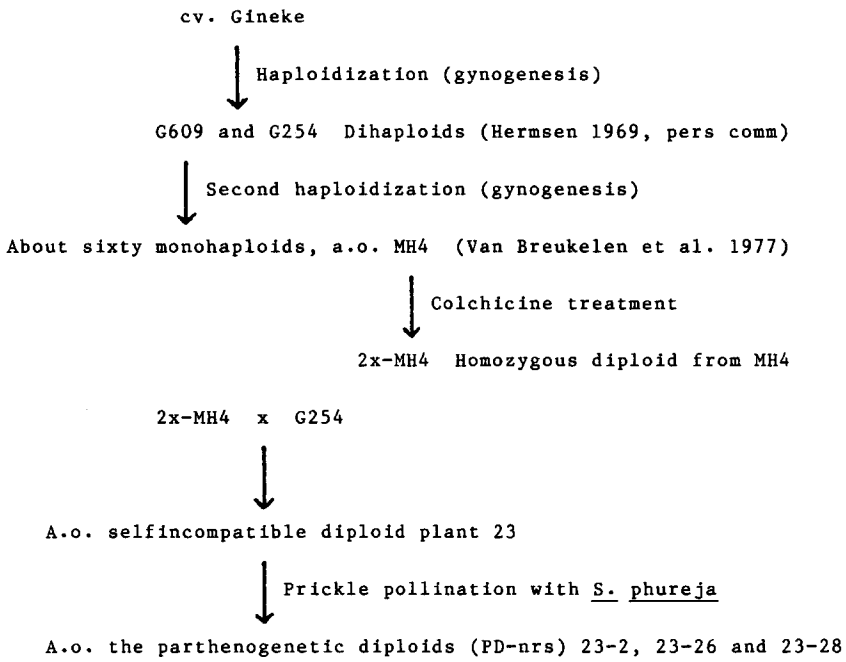

Fig. 2. Origin of three parthenogenetic diploid clones (PD-nrs)

S. phureja Juz. et Buk. Three independent (groups of) diploid clones are included in this study:

(i) M9

M9 is a diploid hybrid. Its ancestry is presented in Fig. 1. The US-W clones (5293-3 and 7589-2) were produced and selected by Dr. S. J. Peloquin and his coworkers in Wisconsin, USA and kindly provided via Dr. H. de Jong, Canada. These clones were intercrossed and M9 selected by the junior author in 1981.

(ii) PS10

PS10 is an F_1-hybrid from the cross *S. phureja* × diploid *S. tuberosum.* It was selected by the senior author for its monohaploid producing ability via gynogenesis. The *S. phureja* parent was bred by Dr. B. Maris for adaptation to long-day conditions; the diploid *S. tuberosum* parent was bred by Ir. N. van Suchtelen from pure *S. tuberosum* dihaploids. Both parental clones were kindly provided by the Foundation for Agricultural Plant Breeding (SVP), Wageningen.

(iii) PD23-2, PD23-26, PD23-28

These parthenogenetic diploid (PD) clones derive from cv. Gineke according to the scheme in Fig 2.

In addition to the three independent (groups of) clones, the following F_1 hybrid populations were included: PS10 × M9 (9 genotypes) and M9 × GPG (11 genotypes). GPG is the code for F_1-plants from spotless seeds of the backcross (G609 × IvP48) × G609. G609 is a highly fertile dihaploid from cv. 'Gineke' with a high monohaploid producing ability (Van Breukelen et al. 1975). IvP48 is a *S. phureja* clone selected by Hermsen and Verdenius (1973) for its superior dihaploid inducing ability and its homozygosity for embryo-spot.

The experiments were carried out during the period 1982–1985. All plants were grafted onto tomato rootstocks and grown in an air-conditioned greenhouse at high relative humidity.

The pollinators for monohaploid induction via gynogenesis were the *S. phureja* selections IvP35 and IvP48 (Hermsen and Verdenius 1973) and a recently selected improved pollinator IvP101 (Hermsen, in prep). All three pollinators are homozygous for the dominant seed marker embryo-spot, which is indispensable for selecting monohaploids via gynogenesis.

The selection procedure of monohaploids was as follows. From the bulk of seeds obtained from crosses between diploids and pollinators spotless seeds were selected and sown in flats. After emergence, the plants with "nodal bands"

(controlled by the same genes as embryo-spot) were removed. In non-hybrid seedlings the ploidy level was first estimated by counting the number of chloroplasts in the guard cells of the stomata according to Frandsen (1968); later the number of chromosomes was counted in root tip cells according to Henderson and Lu (1968).

All monohaploid lines were maintained in vitro in shoot tip cultures on MS-medium (Murashige and Skoog 1962) with 20 g sucrose/l and 20 g mannitol/l at 10 °C and low light intensity. In this way monohaploid lines could be maintained from 1983 till now without the occurrence of spontaneous doubling. Maintenance via tubers was often not possible since most lines failed to produce them.

Monohaploid plants were screened for vigour in the greenhouse and under in vitro conditions on MS-medium with 20 g sucrose/l. As vigour strongly depends on environmental conditions, the data for vigour can only serve as a measure for the relative vigour of the monohaploids in a given environment.

Results

About 2 million seeds have been screened for absence of embryo-spot. Seeds with no clear embryo-spot were also classified as "spotless". About 33,000 spotless seeds were selected. Of these only 75% germinated, probably because the seeds containing a partly developed embryo or no embryo at all were unavoidably classified as spotless. About 19,000 seedlings could easily be recognized as hybrids owing to the presence of nodal bands. In addition to 1,500 lethals, 4,000 diploids and a few tetraploids, more than 500 monohaploid plants were obtained from the 6,000 parthenogenetically developed seedlings. From the five parental lines that have been pollinated in more than two years the results are summarized in Table 1. In 1982–1984 a mixture of pollen from both IvP35 and IvP48 was used and in 1985 IvP101 was also included as a pollinator.

It is apparent that the parental genotypes differ in their ability for producing viable monohaploids. One can also note that both the frequency of monohaploids per 100 berries and monohaploids per 1,000 seeds differ in the successive years. This may be due to (i) the physiological stage of the parental plants, (ii) the environmental conditions and/or (iii) the types of pollinator used. Tables 2 and 3 demonstrate that these

Table 1. Numbers and frequencies of monohaploids from five different diploid clones obtained over the years 1982 through 1985

Year	Source	No. of berries	Average no. of seeds per berry	No. of monohaploids		
				Total	per 100 berries	per 1,000 seeds
1982	M9	415	277	76	18.3	0.66
1983	M9	483	260	84	17.4	0.67
	23-2	8	268	1	12.5	0.47
	23-26	159	187	12	7.5	0.40
	23-28	218	115	7	3.2	0.28
	PS10	104	263	3	2.9	0.11
Total and means		972	216	107	11.0	0.51
1984	23-2	781	214	65	8.3	0.39
	23-26	130	218	11	8.5	0.39
	23-28	214	198	11	5.1	0.26
	PS10	411	252	27	6.6	0.26
Total and means		1,536	223	114	7.4	0.33
1985	M9	361	314	42	11.6	0.37
	23-2	97	278	14	14.4	0.52
	23-26	10	237	1	10.0	0.42
	PS10	150	384	22	14.7	0.38
Total and means		618	324	79	12.8	0.39
'82–85'	M9	1,283	227	202	15.7	0.57
	23-2	886	222	80	9.0	0.41
	23-26	299	202	24	8.0	0.40
	23-28	432	156	18	4.2	0.27
	PS10	665	284	52	7.8	0.27
Total and means		3,565	244	376	10.5	0.43

possibilities are relevant. In Table 2 large differences in monohaploid frequencies can be observed when pollinations are carried out during different stages of plant development or at different times during the season.

In Table 3 a comparison is made between the monohaploid inducing ability of IvP(35+48) and IvP101 in the year 1985. From these data it may be concluded that for the parthenogenetic induction of monohaploids in diploid *S. tuberosum* and *S. tuberosum* × *S. phureja* hybrids, IvP101 is the better pollinator, i.e. it has a higher monohaploid induction rate (MIR) than the combination of IvP35 and IvP48. IvP101 exceeds the IvP(35+48) mixture in (i) number of berries per 100 pollinated flowers, (ii) number of monohaploids per 100 berries and (iii) number of monohaploids per 1,000 seeds. These results correspond with those obtained from the induction of dihaploids in tetraploid potato cultivars using the same pollinators (Hermsen, in prep.).

A large variation was found among the monohaploid plants obtained. The most vigorous plants reached a length of 170 cm and branched profusely, whereas the least vigorous ones stopped growing already at a height of 10 cm. Between these extremes there was a large number of plants that either grew well but did not branch, or branched excessively but did not grow taller than about 40 cm. Most of the morphological characters were variable: length of the fifth leaf varied from 1 to 18 cm; the length/width ratio of the top leaflet of the fifth leaf ranged from 1.4 to 2.8 and the length of the internodes varied between 1 and 4.5 cm. Tuberization was also variable. Some plants did not tuberize at all, whereas others did, even under long-day conditions. Whenever tubers were formed, they generally varied in size from 3 to 13 mm but tubers of 25 mm in diameter were also observed.

About 60% of the plants produced flower buds, which generally dropped before anthesis. Three monohaploids flowered. They did not produce viable pollen and did not form berries after pollination of eight flowers with diploid pollinator lines.

The parental lines differed greatly in monohaploid production rate (MPR) as well as in quality of the monohaploids they produced (MQL = monohaploid quality level) (Table 4).

To qualify the MQL of the diploid lines, estimates of maximum as well as minimum relative vigour are given for the monohaploids from each parental line. The relative vigour is expressed in a scale from one to ten. It has been calculated as the height of the plants in cm after 3–4 months in relation to their age in days ([height/age] × 10). A correction of at most two scale

Table 2. Numbers and frequencies of monohaploids obtained from clone M9 during 1983

Date	No. of berries	Average no. of seeds per berry	No. of monohaploids		
			Total	per 100 berries	per 1,000 seeds
3/6	73	247	29	39.7	1.61
29/6	57	254	1	1.7	0.07
5/7	43	128	3	7.0	0.55
12/7[a]	24	56	0	–	–
19/7	156	247	25	16.0	0.65
2/8	128	322	21	16.4	0.51
29/8	26	307	5	19.2	0.63

[a] During the day of pollination the maximum temperature in the greenhouse was 43 °C

Table 3. Numbers and frequencies of monohaploids obtained from six different lines using different pollinators

Source	Pollinator	No. of berries	No. of berries per 100 pollinated flowers	Average no. of seeds per berry	No. of monohaploids		
					Total	per 100 berries	per 1,000 seeds
M9	IvP35/48	230	28	304	27	11.8	0.38
	IvP101	131	33	331	15	11.4	0.35
PD23-2	IvP35/48	61	68	242	2	3.3	0.13
	IvP101	36	77	340	12	33.3	0.98
PD23-26	IvP35/48	6	75	297	0	–	–
	IvP101	4	80	147	1	25.0	1.70
PS10	IvP35/48	98	84	388	9	9.2	0.24
	IvP101	52	76	377	13	25.0	0.66
PS10 × M9-1	IvP35/48	20	59	83	1	5.0	0.60
	IvP101	21	68	68	4	19.0	2.80
PS10 × M9-2	IvP35/48	12	32	382	3	25.0	0.65
	IvP101	13	41	294	2	15.4	0.52
Total	IvP35/48	427	38	306	42	9.8	0.32
	IvP101	257	43	315	47	18.3	0.58

Table 4. Data showing the quality of monohaploids from five diploid lines (pooled data of the years 1982–1985)

Source	*n*	Vigour			% (sub)lethal
		Max.	Min.	Mean	
M9	168	9	1	4.3	17
PD23-2	70	9	1	4.4	12
PD23-26	23	9	1	4.4	18
PD23-28	17	8	1	4.1	6
PS10	34	4	1	1.4	35

n = number of viable plants after three months; (sub)lethal = dying within three months.
For calculation of vigour, see text

units was carried out depending on their degree of branching. A non-destructive way of estimating vigour was thus obtained.

Since a large part of the planned research on the obtained monohaploids had to be carried out in vitro, all lines were screened for vigour in shoot tip cultures and for the potential of protoplast isolation and fusion. Material in both the greenhouse and in vitro was screened for stability of ploidy level. Stem and leaf pieces were incubated in vitro for callus induction and chromosome doubling to obtain, from each monohaploid, a homozygous series of ploidy levels (x, 2x, 4x). The results of both the in vitro work and the production and selection of homozygous lines will be published in future papers.

Conclusions and discussion

Only one diploid clone (M9) was studied for monohaploid production rate (MPR) during one entire season (1983). The results suggest a clear intra-seasonal variation of this character. An adequate explanation for this phenomenon cannot be given at this stage of research. No correlations could be found between MPR and such environmental conditions as greenhouse temperature and light intensity in the period from five days before to two days after pollination. The physiological condition of the plants is likely to be important. In the 1983 season, with a relatively high average outdoor temperature (17.8 °C) in June, July and August, the very early pollinations seemed to be most successful in inducing parthenogenetic development of embryos. Intra-seasonal as well as inter-seasonal variation could be detected.

Little is known about the mode or modes of origin of monohaploids in potato. In theory two processes are possible: (i) one sperm nucleus fertilizes the secondary embryo-sac nucleus, whereas the other one, which normally fertilizes the egg cell, degenerates leaving an unfertilized egg cell in a vital triploid endosperm. This is the process known in maize (Chase 1969); (ii) both sperm nuclei fertilize the secondary embryo-sac nucleus leading to tetraploid endosperm or, when dealing with 2n pollen, to hexaploid endosperm. This is the process suggested for the induction of dihaploid potato lines (Von Wangenheim et al. 1960). The involvement of both processes may be most likely.

Since monohaploids obtained from one parental line are a gametic sample of that line, the quality of monohaploids may provide an estimate of its breeding value. The high frequency of vigorous monohaploids from the clones M9, PD23-2, PD23-26 and PD23-28 may suggest a large number of genes for vigorous growth in these clones. On the other hand, PS10, which itself is highly vigorous, did hardly produce any well-growing monohaploids. This line probably owes its large vigour to its highly heterozygous condition (intra-locus interaction). In monohaploids there is no heterozygosity and in the PS10 monohaploids this lack is not compensated for by positive gene action nor by inter-locus interaction. On the contrary, recessive genes for (sub)lethality reveal themselves in these monohaploids (Fig. 3).

To investigate which will be the result of crossing a line with average MPR and low MQL with a line of high MPR and high MQL, we crossed PS10 with M9. Twelve random plants from the F_1 were taken for testing MPR. Since three plants died, only nine could be investigated. The results are shown in Table 5.

Whereas over the years the monohaploids from PS10 and M9 showed an average vigour of 1.4 and 4.3 respectively, the mean MQL of the F_1 PS10×M9 (MQL = 2.0) is lower than the midparent vigour (MQL = 2.9). In fact only one monohaploid was as vigorous as the better monohaploids of M9. So the diploid F_1 plants, although vigorous, were hardly able to produce well growing monohaploids. Apparently the positive genes from M9 could not compensate for the negative genes from PS10. Some F_1-plants were able to produce monohaploids in high frequencies, but this was associated with low average vigour and high mortality in the first three months.

The results of a cross between a line with high MPR and high MQL (M9) and a line with low MPR and high MQL (line GPG) are shown in Table 6.

Although in this experiment the total number of monohaploids obtained is low, it may be concluded that the quality of the monohaploids is rather good, while the production rate is lower than the mean rate of both parents.

In summary, the tendency is that: (i) high MPR× low MPR gives low MPR; (ii) high MQL×high MQL gives high MQL; (iii) low MQL×high MQL gives low MQL.

When potato monohaploids have to be produced on a large scale, whether through anther culture or prickle

Fig. 3. Representative monohaploid seedlings from two diploid potato genotypes photographed 4 months after sowing (bar = 10 cm). Upper and middle row: two random samples each of 7 different monohaploids from diploid M9, each sample taken from a different angle; lower row: random sample of 8 different monohaploids from diploid PS10. For M9 and PS10, see text

Table 5. Frequencies and quality of monohaploids of nine F_1-plants from the cross PS10 × M9 in the year 1985

Source	*n*	No. of monohaploids		Vigour			% (sub)lethal
		per 100 berries	per 1,000 seeds	Max.	Min.	Mean	
PS10 × M9- 1	2	6.9	0.41	1	1	1.0	50
- 2	3	6.0	0.16	2	1	1.3	25
- 3	4	5.6	0.53	5	2	2.5	33
- 4	14	11.9	0.74	4	1	1.4	26
- 6	1	1.3	0.08	1	–	1	50
- 8	1	11.1	1.05	5	–	5	0
- 9	0	–	–	–	–	–	–
-10	16	18.6	1.98	9	1	2.5	47
-11	1	1.8	0.10	2	–	2	0
Total	42	7.7	0.43			2.0	37

n = number of viable plants after three months; (sub)lethal = dying within three months
For calculation of vigour, see text

Table 6. Frequencies and quality of monohaploids of 11 F_1-plants from the cross M9 × GPG in the year 1985

Source	*n*	No. of monohaploids		Vigour			% (sub)lethal
		per 100 berries	per 1,000 seeds	Max.	Min.	Mean	
M9 × GPG- 1	2	3.3	0.15	6	4	5.0	0
- 2	4	7.4	0.24	8	1	4.0	0
- 3	4	20.0	0.45	5	3	3.8	0
- 4	5	11.6	0.59	7	2	5.0	0
- 5	0	–	–	–	–	–	–
- 6	1	16.7	0.40	2	–	2	0
- 7	0	–	–	–	–	–	–
- 9	1	1.1	0.42	9	–	9	0
-10	1	3.8	0.07	4	–	4	0
-11	1	2.0	0.05	1	–	1	0
-12	6	4.5	0.24	4	1	2.7	33.3
Total	25	4.2	0.15			4.6	10.7

n = number of viable plants after three months; (sub)lethal = dying within three months
For calculation of vigour, see text

pollination, these conclusions are very important. Even when the induction mechanisms for androgenesis and gynogenesis are totally different and coded by different genes (resulting in different MPR-values), the MQL of the parental line is a critical trait for the frequency and potential use of monohaploids. This means for the scheme of Wenzel et al. (1979) that, in trying to obtain monohaploids or homozygous diploids from lines that have some desired characters (e.g. genes for resistance) but low MQL, a lot of problems have to be dealt with. When crossing such lines with high MQL lines, only slow progress will be made, if conclusion (iii) would turn out to be generally valid.

Using the anther culture technique, Uhrig (1985) got 2 monohaploids out of 313 regenerants that were obtained from about 700 anthers. This means 6.4 monohaploids per 1,000 regenerants and 0.29 monohaploids per 100 anthers. All plants had to be regenerated and screened for ploidy level as there was no marker available for the selection of monohaploids. The supposed advantage of producing homozygous doubled lines directly via anther culture (Wenzel et al. 1979) ceases to exist if one takes into account that a series of markers is necessary to detect completely homozygous diploids. In addition to homozygous diploids, heterozygous diploids (originating from 2n gametes) and parent-like diploids (originating from somatic anther tissue) may also be obtained. It is obviously more efficient to insert one marker in a pollinator than to insert several in the parental material since one

pollinator probably is sufficient to obtain monohaploids from a number of parental lines. Thanks to the available marker embryo-spot in the pollinator lines used, it was relatively easy to obtain 0.6 monohaploids per 1,000 seeds and 18 monohaploids per 100 berries (Table 3) from several diploid lines using the prickle pollination technique. The statement that androgenesis in itself has more potential for monohaploid production than gynogenesis because an anther contains more gametes than an ovary is depreciated by these results.

Furthermore, using the anther culture technique, a lot of valuable genotypes may be lost because they lack the in vitro regeneration capacity, which as such is a useless selection criterion in a breeding scheme starting with homozygous lines.

The genetic aspects of monohaploid production deserve careful consideration. Both gynogenetic and androgenetic monohaploids can be obtained from a limited number of specific genotypes. The genetic basis of gynogenesis and androgenesis may be different. If so, both prickle pollination and anther culture should be applied in order to obtain monohaploids from sufficiently various diploid breeding material.

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