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Uijtewaal, B.A.; Jacobsen, E.; Hermsen, J.G.T.

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MORPHOLOGY AND VIGOUR OF MONOHAPLOID POTATO CLONES, THEIR CORRESPONDING HOMOZYGOUS DIPLOIDS AND TETRAPLOIDS AND THEIR HETEROZYGOUS DIPLOID PARENT

B. A. UIJTEWAAL[1], E. JACOBSEN[2] and J. G. TH. HERMSEN[1]

[1]Department of Plant Breeding (IvP), Agricultural University, P.O. Box 386, 6700 AJ Wageningen, the Netherlands
[2]Department of Genetics, University of Groningen, Haren, the Netherlands

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KEY WORDS

Solanum Tuberosum, Monohaploid potato, polyploidization, homozygotization, gene dosage effects.

SUMMARY

To study the joint effects of homozygotization and polyploidization in potato, the performance has been examined of five potato genotypes at three (x, 2x, 4x) and two genotypes at two (x, 2x) ploidy levels. Six out of the seven genotypes studied were compared with their heterozygous diploid parental clone. In this way comparisons could be made between i) the heterozygous diploid and its monohaploid derivatives, ii) three or two ploidy levels per genotye and iii) homozygous di- and tetraploids and their heterozygous diploid source.

Large variation could be detected between monohaploids obtained from one diploid source. A striking increase in vigour was observed with somatic chromosome doubling from x to 2x, but less clearly from 2x to 4x. The relatively vigorous diploids showed a weaker response to tetraploidization than the less vigorous ones. The heterozygous diploid exceeded all homozygous di- and tetraploid derivatives in performance. The results of this study suggest positive gene dosage effects for tuber production more than for leaf area and plant height. The observations on plant vigour in homo- and heterozygotes suggest that dominance effects are stronger than additive gene effects. Owing to sterility problems, homozygous potato clones will presumably be of little importance for practical breeding.

INTRODUCTION

In the autotetraploid potato homozygotization by repeated selfing is an extremely slow process: 27 generations of selfing are needed to reach 99% homozygosity. Furthermore severe inbreeding depression and decrease of fertility are common barriers to selfing for more than 3–5 generations. Therefore the pure effect of homozygotization can be studied to a limited degree only. Haploidization of a tetraploid has the same effect on homozygotization as three times selfing of that tetraploid and hence is accompanied by a considerable decrease in vigour and changes in performance regarding leaf shape, tuber production and yield (PELOQUIN & HOUGAS, 1958; VAN SUCHTELEN, 1966; GOREA, 1970; CAROLL & LOW, 1975, 1976). Besides the effect of 4x to 2x haploidization, that of mitotic 2x to 4x doubling on relative performance and tuber yield has been studied by FRANDSEN (1967a, 1967b), ROSS et al. (1967), ROWE (1967) and DE

Table 1. The diploid source parents M9 (UIJTEWAAL et al., 1987a) and H78.01 (DE VRIES et al., in prep.), seven monohaploids and their corresponding ploidy series.

Source	Monohaploids	Ploidy series
M9	839-19	(x,2x,4x)
	839-45	(x,2x,4x)
	839-61	(x,2x)
	839-79	(x,2x)
	849- 7	(x,2x,4x)
	849-30	(x,2x,4x)
H78.01	7322	(x,2x,4x)

MAINE (1985). In both directions the change in ploidy level is associated with a reduction in the degree of heterozygosity. This makes it hard to establish which proportion of the haploidization effects is due to the reduced ploidy level and which to the reduced level of heterozygosity.

The possibility of obtaining monohaploids from di(ha)ploids via gynogenesis or androgenesis opens up new perspectives. On the one hand this offers the opportunity to study the effect of haploidization of heterozygous diploids to monohaploids. On the other hand, pure polyploidization or gene dosage effects can be studied by comparing monohaploids with their corresponding homozygous diploids and tetraploids. In this paper attention is paid to both aspects by studying the relative performance of several monohaploids from one diploid clone, and by comparing the relative performance of the monohaploids, their heterozygous diploid parent and homozygous diploid and tetraploid derivatives of the monohaploids.

MATERIAL AND METHODS

Homozygous di- and tetraploid potato clones have been obtained via adventitious shoot regeneration on stem explant cultures (ROEST & BOKELMANN, 1980) of one androgenetic and six gynogenetic monohaploids (UIJTEWAAL et al., 1987a). Table 1 summarizes the material used in this study. The clone 7322, of which the origin is fully described by DE VRIES et al. (in prep.) was obtained at the Max Planck Institute, Cologne, West Germany.

All clones were propagated in vitro via shoot tip culture and after rooting transferred to the greenhouse at the end of January. The experiment includes 12 plants per genotype at each ploidy level. The plants were cultured in 24 cm pots in the greenhouse under normal daylight conditions and temperatures varying from 20–30 °C.

For comparison of plant vigour, the parameters plant height, leaf area per leaf, leaf area per plant and tuber production were examined. Flower bud development and male and female fertility were examined to study the potential use of homozygous clones in crosses. For effects on plant morphology, length/width-ratio of the terminal and lateral leaflet and internode length were examined. For the determination of leaf area per leaf the mean value of the fifth and the sixth full-grown leaves as measured with a photo-electric leaf area meter were taken. Pollen fertility was estimated by lactophenol acid fuchsine staining according to SASS (1964). Female fertility was tested

by pollination of open flowers with mixtures of pollen from the *S. phureja* pollinator lines IvP48 and IvP101 (HERMSEN & VERDENIUS, 1973; HERMSEN, in prep.).

RESULTS

The performance of six monohaploid clones relative to their common diploid parental clone M9 has been determined (Table 2). For comparison the absolute values of M9 were used. It is apparent that the ploidy change from diploid to monohaploid is accompanied by a striking loss of vigour. As expected, the monohaploid showed a significantly lower relative value for plant height (0.54), internode length (0.39), leaf area per leaf (0.10) and leaf area per plant (0.12) than the heterozygous diploid (1.00). Tuber production in terms of tuber weight per plant was almost completely lost. A small difference was found between M9 and the monohaploids for the number of leaves per plant. Within the group of monohaploids considerable variation could be detected for the characters investigated. It is apparent that for plant height the clones 849-7 and 839-61 were superior, a phenomenon which was only parly due to a larger internode length. For leaf area per leaf as well as total leaf area per plant, 839-61 was superior. This was mainly due to a relatively large number of relatively big leaves. In general the length/width ratio of the terminal leaflets was somewhat smaller than that of the lateral leaflet, again with the exception of clone 839-61. The relative values for nine characters of mitotically doubled homozygous di- and tetraploids in relation to the original monohaploid (Table 3, part A) and the performance of these homozygous di- and tetraploids in relation to the heterozygous parental clone M9 (Table 3, part B) have been determined. It is apparent that the mean values of the homozygous clones showed hardly any increase by raising the ploidy level from 2x to 4x. In fact the homozygous 4x appeared, only in leaf area per leaf and tuber weight per plant, to be superior over the homozygous 2x plants. In general it can be said that, except for tuber weight, the increase in vigour from x to homozygous 2x is much larger than the increase from 2x to 4x. The relative increase in tuber weight per plant with raising ploidy levels from 2x to 4x was larger than the increase from x to 2x (Table 3 and Fig. 1). Except for the number of leaves per plant the heterozygous diploid parental clone had a better overall performance than the homozygous diploids and tetraploids. The biggest effect of homozygotization was detected for leaf area per leaf and per plant and for tuber production (Fig. 1).

The terminal leaflet showed hardly any differences in L/W-ratio at different ploidy levels. However, for the lateral leaflet a negative relation between L/W-ratio and ploidy level was observed.

Observations on plants of the ploidy series of genotype 7322 showed the same tendencies (Table 4) as found for the polyploidized monohaploids of M9 (Table 3). Because of the absence of the diploid parental clone of 7322, its homozygous di- and tetraploids could not be compared with their own heterozygous source.

All clones produced flower buds, and three of them produced open flowers. Pollen stainability of the homozygous clones was very low: 25% for 849-30(2x), 10% for 7322(2x) and 15% for 7322(4x), whereas the heterozygous diploid M9, cultured under the same conditions as the homozygous clones showed 85% stainability. Female fertility of these open flowers was tested by pollination. Pollination of about 100 flowers

Table 2. Performance of the heterozygous diploid M9 (part A) and relative performance (in relation to M9) of six monohaploid derivatives (part B) for nine quantitative characters. The standard deviation is given in parentheses.

	Genotype	Plant height	Leaf area per leaf	Number of leaves	Leaf area per plant	L/W-ratio of the terminal leaflet	L/W-ratio of the lateral leaflet	Internode length	Frequency of tuber producing plants	Tuber weight per plant
A)	M9	92.6 (cm)	7963 (mm^2)	65	5241 (mm^2)	1.80	1.90	3.8 (cm)	1.00	33.5 (g)
B)	839-19 (x)	0.48	0.09	1.00	0.09	0.91	1.05	0.39	0.27	0.01
	839-45 (x)	0.50	0.07	1.02	0.07	1.02	1.13	0.29	0.29	0.01
	839-61 (x)	0.67	0.20	1.35	0.27	1.26	1.14	0.47	0.08	0.02
	839-79 (x)	0.51	0.08	1.17	0.09	0.95	1.07	0.42	0.18	0.01
	849- 7 (x)	0.65	0.07	1.05	0.08	0.87	0.98	0.42	1.00	0.10
	849-30 (x)	0.44	0.07	1.40	0.10	1.04	1.07	0.32	0.00	0.00
	Mean (x)	(0.54)(0.09)	0.10(0.05)	1.16(0.18)	0.12(0.08)	1.01(0.14)	1.07(0.06)	0.39(0.07)	0.30(0.36)	0.02(0.04)

Table 3. Performance of homozygous di- and tetraploids relative to their original monohaploid (part A) and to their heterozygous diploid source M 9 (part B) for nine quantitative characters. The standard deviation is given in parentheses.

	Genotype	Plant height	Leaf area per leaf	Number of leaves	Leaf area per plant	L/W-ratio the terminal leaflet	L/W-ratio the lateral leaflet	Internode length	Frequency tuber pro-ucing plants	Tuber weight per plant
A)	839-19 (2x)	0.90	1.24	1.18	1.45	1.02	0.81	1.27	0.74	1.00
	(4x)	1.11	2.30	0.94	2.13	0.89	0.68	1.87	1.85	12.67
	839-45 (2x)	1.40	4.67	1.14	5.29	0.99	0.83	1.64	3.45	3.67
	(4x)	1.81	4.79	0.68	3.27	0.96	0.67	1.91	1.00	17.33
	839-61 (2x)	1.46	1.38	1.05	1.44	0.91	0.78	1.11	1.14	0.33
	839-79 (2x)	2.06	2.46	1.13	2.80	1.02	0.79	1.56	0.55	5.33
	849- 7 (2x)	1.67	2.46	1.43	3.49	1.01	0.91	1.25	0.45	2.33
	(4x)	1.06	4.79	0.99	4.69	0.98	0.72	1.13	0.82	4.79
	849-30 (2x)	1.53	2.43	0.70	1.71	0.91	0.90	1.25	0	0
	(4x)	1.72	1.99	0.89	1.79	1.06	0.87	1.92	0	0
	Means (2x)	1.51(0.38)	2.44(1.23)	1.11(0.24)	2.70(1.51)	0.98(0.05	0.84(0.06)	1.35(0.21)	1.22(1.22)	2.11(2.08)
	(4x)	1.43(0.40)	3.47(1.53)	0.88(0.14)	2.97(1.31)	0.97(0.07)	0.72(0.11)	1.71(0.39)	0.92(0.76)	6.80(7.77)
B)	Means (2x)	0.82 (0.26)	0.21 (0.08)	1.26(0.18)	0.26(0.12)	0.98(0.09)	0.89(0.05)	0.55(0.13)	0.31(0.37)	0.05(0.09)
	(4x)	0.73(0.15)	0.26(0.10)	0.98(0.23)	0.24(0.08)	0.93(0.14)	0.76(0.13)	0.61(0.11)	0.39(0.35)	0.19(0.20)

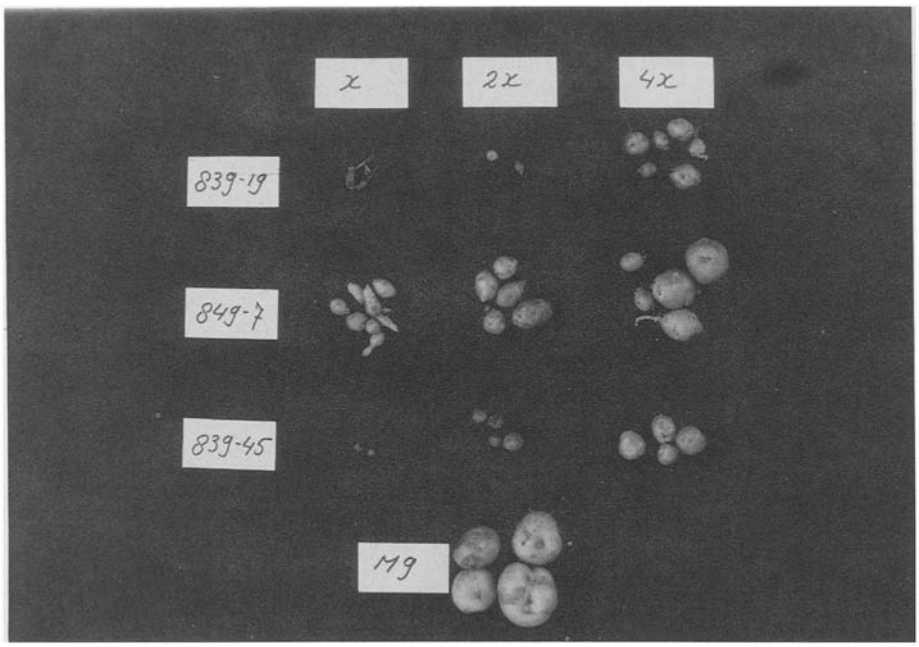

Fig. 1. Mean tuber production per plant of three different genotypes at three ploidy levels and of their heterozygous diploid parent M9.

of 7322(2x) gave two berries containing 15 seeds in total. These seeds gave rise to diploid hybrid plants. Pollination of tetraploid 7322 (50 flowers), and diploid 849-30 (ten flowers) did not give any berry set.

DISCUSSION

Although this study has been carried out in only one season, tendencies in the relative performance of potato plants can be indicated.

It appeared that reducing the ploidy level from diploid to monohaploid has a dramatic effect on plant growth performance and tuber formation. Similar effects were found for the change from the tetraploid to the diploid level by PELOQUIN & HOUGAS (1960). In the present experiment the extent of this effect was clearly dependent on the parameter studied. Compared to the diploid parental clone, plant height and internode length decreased with 46 and 61%, respectively. The decrease was much stronger for leaf area per leaf and per plant and for tuber production, 90%, 88% and 98% respectively, whereas the number of leaves per plant and their length/width-ratio remained more or less the same. The six monohaploids derived from M9 represented a gametic sample visualized on the plant level. The variation observed within the group of monohaploids is a direct consequence of the heterozygosity of M9 and hence of the variation of its gametal genotypes. The monohaploid clone 839-61 showed the smallest decline in plant growth performance, yet tuber production was low.

Comparison of the monohaploids with their mitotically doubled and twice doubled

Table 4. Relative performance of the genotype 7322 on the homozygous diploid and tetraploid level in relation to its monohaploid origin for nine quantitative characters.

Genotype	Plant height	Leaf area per leaf	Number of leaves	Leaf area per plant	L/W-ratio of the terminal leaflet	L/W-ratio of the lateral leaflet	Internode length	Frequency of tuber producing plants	Tuber weight per plant
7322 (2x)	1.43	2.12	1.42	3.04	1.08	0.91	1.11	0.91	0.06
(4x)	1.43	2.73	0.98	2.69	1.12	0.75	1.22	1.81	0.41

homozygous counterparts showed that this pure polyploidization effect strongly varies per genotype. For some genotypes the optimal ploidy level, in terms of plant height or leaf area, seemed to be the diploid, and for others the tetraploid level. No or only minor differences in growth performance, including tuber production, were found by ROWE (1967) and DE MAINE (1985) when comparing heterozygous diploids with their mitotically doubled counterparts.

There is an overall increase in mean performance with raising the ploidy level from x to 2x. Yet, hardly any increase and sometimes even a decrease in performance was detected for the polyploidization step from 2x to 4x, except for tuber production. This suggests positive gene dosage effects for tuber production. All genotypes showed a reduction of L/W-ratio of the lateral leaflet with raising ploidy level. This is in agreement with the data presented by VAN BREUKELEN et al. (1975). However, large variation could be detected for L/W-ratio of the lateral leaflets of the monohaploid genotypes, i.e. 2.17 to 1.86. L/W-ratio of the terminal leaflet did not show any relation with ploidy level. The fact that the heterozygous diploid M9, except for the number of leaves per plant, exceeds all homozygous di- and tetraploids in performance, suggests that for these combinations of alleles heterozygotization is more important than polyploidization. In other words dominance effects may have a larger influence on performance than additive or gene dosage effects.

Fertility was very low in the doubled and twice doubled homozygous clones. Only in the diploid 7322 some female fertility was detected. This strongly limits the use of homozygous clones in crossing schemes. It may also imply that protoplast fusion will be necessary to obtain homozygous triploids and the production of a homozygous trisomic series may not be possible by crossing a homozygous triploid with a corresponding homozygous diploid.

In further analytic breeding research, apart from the polyploidization effect as described in this paper, numerous heterozygotization effects can be studied by the combinations of genomes of 2, 3 or 4 different monohaploids. If a sufficient level of fertility is available, a number of these genome combinations may be obtained by crossing. For other combinations protoplast fusion will be necessary. This means that for this material an efficient protoplast fusion system and a suitable detection method for hybrid fusion products are needed. Isozymic markers and preferential plant regeneration of hybrid fusion products may offer some opportunities (UIJTEWAAL et al., 1987b). However, ploidy instability as determined in leaf material of protoplast source plants (UIJTEWAAL, 1987) and after plant regeneration from protoplast derived calli (UIJTEWAAL, in prep.) may still be a critical factor.

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