

The effects of inbreeding on the parental values of potato dihaploids

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Summary

Dihaploids obtained from a somatically chromosome-doubled dihaploid potato were crossed with *Solanum phureja* clones. To test the effect of inbreeding, measurements were made of their seed production and the tuber yield, tuber number and mean tuber weight of their offspring. On average, seed production of the second generation dihaploids was higher than that of the original dihaploid progenitor. Progeny tuber yield and its components were little different from those of the original dihaploid's progeny. Tuber flesh quality, as measured by lack of blemishes, was better in the offspring of second generation dihaploids.

It is suggested that the negative effects of producing second generation dihaploids are minor compared with producing the first generation dihaploid from a tetraploid, because most deleterious recessives have already been unmasked. The results indicated residual variation within the original dihaploid which could be exploited for plant breeding purposes.

An indicator of inbred status, alternative to the inbreeding coefficient, is suggested which could be applied to both diploids and tetraploids.

Key words: Yield, somatic chromosome doubling, tuber shape, tuber flesh, monogenicity coefficient

Introduction

A scheme for increasing the homozygosity of potato genotypes proposed by De,Maine & Jervis (1989) used dihaploidisation and somatic chromosome doubling to avoid problems with selfing due to male sterility and self-incompatibility. Inbred lines, such as those produced by this method or by selfing or sib-mating, are valuable for plant breeding and genetic research. They can be used, for example, as true breeding parents to pass agronomic characters efficiently into potential cultivars. Inbreeding depression could seriously limit the value of an inbreeding scheme if it affected key agronomic characters such as tuber yield and its components. The offspring of inbred lines would indicate whether any of the deleterious effects of inbreeding were passed on to subsequent generations as predicted by Mendoza & Haynes (1973). Genetic study of a second generation of dihaploids resulting from dihaploidisation of a chromosome doubled dihaploid of a tetraploid breeder's line [G4657(17)] were used to examine the effects of inbreeding resulting from this method. Dihaploid production does not result in alleles identical by descent occurring in the genome; it is not strictly inbreeding as defined by Falconer (1960). The exposure of homozygous deleterious recessives in dihaploids however results in much weaker plants compared with their tetraploid parents. Somatic chromosome doubling results in allele duplication so that

the next generation of dihaploids will be even more highly inbred. A measure of inbreeding is required for use with material which can be either diploid or tetraploid.

A dihaploid and dihaploids derived from its chromosome doubled derivative were crossed with *Solanum phureja* clones to determine the effects of inbreeding on their progeny. The results are discussed in relation to the potential value of inbred material produced by this route.

Materials and Methods

Dihaploids were obtained from PDH40X2, a somatically chromosome-doubled derivative of the dihaploid PDH40. Seven of them were used in crosses with *Solanum phureja* clones. They were PDHs 777, 903, 904, 911, 913, 917, 921 and the original dihaploid PDH40. Test-crosses were carried out with IVP48 and, where enough flowers were available, with three other *S. phureja* clones [DB228(70), 71P10, 41P25] selected for their good yield and tuber size. The number of berries and seeds produced were counted. It was intended to raise 72 seedlings per progeny but the maximum number actually produced was 65, which were planted in 10 cm square pots filled with soil-less compost. Each progeny was divided into four blocks of a maximum of 17 seedlings each, and these were randomised throughout the glasshouse. The following year one tuber of each seedling was planted in the field at 30 cm spacing within rows and 75 cm between rows, in the progeny blocks.

At harvest, tuber yield was measured and the number of tubers counted. A score on a one (poor) to nine (excellent) scale for overall appearance was also assigned. One large tuber from each plant, except for the IVP48 progenies, was cut open, the flesh examined for hollow heart and other blemishes and a score assigned on a scale of one (poor internal condition) to nine (excellent). IVP48 produced offspring with very small tubers which were not considered comparable with the larger tubers chosen to indicate flesh quality in other material so that scores on these seedlings were not assigned. A quantitative tuber shape score on a scale of one (round) to six (long) was assigned to each plant in the progenies of IVP48. In addition the numbers of 'round' and 'long' tubered seedlings in six shape categories were counted to obtain information on the segregation of this character. The shape scores of the dihaploids were 3 (oval) for all except PDH777 which scored 4 (long oval) and that for IVP48 was 1 (round).

Statistical analyses of the quantitative data were carried out using the computer package Genstat (Lawes Agricultural Trust). Taken altogether the variance of the yield data of the eight dihaploids crossed with IVP48 was found to increase with the mean and so logarithms were used for the analysis of variance.

Results and Discussion

Tables 1 (a) and (b) present data from 2× progenies grown in the field in 1991 derived from crossing PDHs 40, 911 and 913 with four *S. phureja* clones. There were no significant differences ($P > 0.05$) between the dihaploids for progeny mean yield ($P = 0.76$) or its components, tuber number ($P = 0.42$) and tuber weight ($P = 0.24$), and for overall appearance score ($P = 0.35$). There were significant differences ($P < 0.05$) for the number of seeds per berry ($P < 0.001$) between dihaploids and between the *S. phureja* parents for tuber number ($P = 0.003$), tuber weight ($P = 0.004$) and appearance score ($P < 0.001$) and significant parent interaction differences for tuber number ($P < 0.025$), tuber weight ($P < 0.01$) and seeds per berry ($P < 0.001$). There were significant differences between dihaploids ($P = 0.025$) for progeny mean internal condition score with PDH40 producing offspring with poorer quality tuber flesh than dihaploids derived from PDH40X2 ($P < 0.05$).

Table 1. Means and variances of variates measured on diploid progenies and two dihaploids from PDH40X2, pollinated by four *S. phureja* clones, 1991

a) Tuber yield per plant and its components in 2× progenies

	Clone	Yield (g)		Tuber number		Tuber wt (g)		N
		Mean	Variance	Mean	Variance	Mean	Variance	
Dihaploids	PDH40	705	153052	15.0	58.3	56.4	1018.8	264
	PDH911	711	124185	16.8	71.2	47.8	422.6	291
	PDH913	670	160760	17.2	100.1	45.2	469.6	280
<i>S. phureja</i>	DB228(70)	774	139173	14.4	44.7	58.8	679.9	212
	71P10	707	158481	12.2	5.2	64.0	1079.3	215
	41P25	705	126720	15.1	49.3	50.3	629.4	220
	IVP48	581	159621	24.9	160.7	22.1	159.5	188

N = number of seedlings

b) Appearance score and internal flesh condition in diploid progenies. Seed production of crosses

	Clone	Score		Internal condition		Seed/berry		No. berries
		Mean	Variance	Mean	Variance	Mean	Variance	
Dihaploids	PDH40	1.9	0.449	8.3	5.01	31.4	1270	26
	PDH911	2.0	0.481	8.8	1.33	103.9	3244	43
	PDH913	2.0	0.631	8.8	1.76	104.0	1336	10
<i>S. phureja</i>	DB228(70)	2.4	0.689	8.9	0.62	101.3	2941	15
	71P10	2.3	0.791	8.5	3.39	78.9	3489	9
	41P25	2.0	0.597	8.5	3.81	50.4	1107	7
	IVP48	1.0	0.005	—	—	78.0	3891	48

Table 2 presents data from 1991 field-grown 2× progenies derived from crossing PDH40 and seven dihaploids derived from PDH40X2 with a *S. phureja* clone, IVP48. There were significant differences between dihaploids in progeny yield ($P = 0.02$), progeny mean tuber number ($P = 0.04$) and tuber shape scores ($P = 0.002$). There were no significant differences in progeny mean tuber weights ($P = 0.17$). Table 3 summarises the numbers of seeds per berry produced from crossing the eight dihaploids with IVP48. There were significant differences between the dihaploids ($P < 0.001$).

Table 2. Means of variates for diploid progenies of PDH40 and dihaploids of PDH40X2 crossed with the *S. phureja* clone IVP48, 1991

Clone	Log Yield (g)		Tuber number		Tuber wt (g)		Shape score		N
	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance	
PDH40	5.966	1.028	21.8	316.3	25.0	192.6	4.1	8.33	65
PDH777	6.513	0.263	32.0	184.1	24.3	56.8	6.1	5.50	47
PDH903	6.035	1.490	33.0	461.4	19.6	141.0	5.3	8.20	23
PDH904	5.666	1.947	18.3	99.0	26.3	350.3	4.3	6.95	53
PDH911	5.932	1.194	23.5	118.0	21.8	110.6	5.4	9.08	58
PDH913	5.914	1.725	29.1	270.5	20.3	171.9	5.1	8.21	64
PDH917	5.440	2.548	18.4	132.0	22.3	202.7	4.8	8.82	42
PDH921	5.289	2.158	21.2	1000.6	21.2	255.0	4.1	7.91	62

N = number of seedlings

Table 3. *Seed production of PDH40 and dihaploids of PDH40X2 in crosses with IVP48, 1991*

Clone	Number of seeds per berry		Number of berries
	Mean	Variance	
PDH40	12.1	120.0	14
PDH777	21.7	271.0	6
PDH903	7.3	2.0	3
PDH904	23.0	111.0	6
PDH911	101.9	3042.0	31
PDH913	138.0	337.0	3

There is some evidence that genes present in PDH40 which affect seed production segregate on dihaploidisation. Such genes may affect sporophyte or gametophyte developmental processes which affect fertility (e.g. pollen tube growth). Similarly, there are second generation dihaploids producing offspring with a higher or lower level of character expression for yield and its components compared with PDH40. The evidence suggests that dihaploidisation resulted in the unmasking of recessive alleles which increase or decrease the level of expression of a character. It indicates the complexity of the genetic architecture which governs quantitatively inherited characters in this material.

Dihaploids derived from a doubled dihaploid are assumed to be more homozygous compared with the original dihaploid as allele duplication took place on chromosome doubling. Doubled dihaploids are also inbred to a degree depending upon the inbred status of the dihaploids they were derived from. The inbreeding coefficient F is inadequate as an indicator of inbreeding status in material of varying ploidy. As defined by Falconer (1960), F is the probability of an allele at a locus being identical with any allele at the same locus. It does not show any increase in the proportion of alleles which are identical by descent in successive generations of doubled dihaploids produced by inbreeding. For instance, if $F = 0$ for G4657(17) and for its dihaploid PDH40, $F = 1$ for PDH40X2 because each of the original PDH40 alleles has a duplicate. Dihaploids derived from PDH40X2 have $F = \frac{1}{2}$ but the next generation of doubled dihaploids have the same inbreeding coefficient of 1 as PDH40X2. It is suggested that a coefficient which is the probability of all alleles at a locus being identical by descent would be a better index of the level of inbreeding in polyploids and material with variable ploidies. This may be called the coefficient of monogenecity (M). Thus, for PDH40, $F = 0$ and $M = 0$ as no alleles are identical by descent. In PDH40X2, $F = 1$ because each allele has an identical partner, but $M = \frac{1}{2}$ (0.33) because this is the probability that all four alleles are identical by descent at a locus. The dihaploids produced from PDH40X2 have $F = \frac{1}{2}$ and $M = \frac{1}{2}$. The next generation of doubled dihaploids, which result from these inbred dihaploids would have $F = 1$, $M = \frac{5}{8}$ (0.56). M , therefore, is equivalent to F for the dihaploid ($2\times$) generations but the M of the doubled dihaploids is equal to the F of the subsequent dihaploid generation. This follows because the doubled dihaploids are subjected to allele duplication (chromosome doubling) before giving rise to the next generation of dihaploids. Table 4 presents F and M for alternating diploid and tetraploid generations produced by five cycles of dihaploidisation and chromosome doubling according to the scheme described by De,Maine & Jervis (1989). It shows that M also indicates an increasing level of inbreeding in the tetraploids in succeeding generations. F , on the other hand, only shows progressive inbreeding for the diploids and remains at 1 for all generations of tetraploids.

Table 4. *The Inbreeding (F) and Monogenicity (M) coefficients for five cycles of dihaploidisation and chromosome doubling involving diploid and tetraploid generations*

Generation	F	M
1a	0.00	0.0
1b	1.00	0.33
2a	0.33	0.33
2b	1.00	0.56
3a	0.56	0.56
3b	1.00	0.70
4a	0.70	0.70
4b	1.00	0.80
5a	0.80	0.80
5b	1.00	0.87

Notes:

1. Generations with 'a' suffix are dihaploids (2×), those with 'b' suffix are the chromosome-doubled dihaploids (4×) derived from them.
2. F = the frequency of genotypes carrying two alleles at a locus which are identical by descent.
3. M = the frequency of genotypes which are monogenic at a locus, i.e. genotypes where all alleles are identical by descent.
4. Assumes no chromosome crossing over.

If, as suggested by Mendoza & Haynes (1973), the deleterious effects of inbreeding can be passed from parents to their offspring, the progenies of dihaploids derived from PDH40X2 may be expected to be lower yielding than that of PDH40. Single degree of freedom contrasts between PDH40 and the dihaploids of PDH40X2 showed that, overall, there were no significant differences between them for any character apart from seed yield, where PDH40 yielded less than the rest ($P < 0.05$). Progenies of some second generation dihaploids were higher (tuber) yielding and some had higher parental values than the original dihaploid for tuber internal condition scores. Although the within progeny variances of the second generation dihaploids may have been expected to be less than that of PDH40, because the former were more homozygous, they were not much different. This may be because of a high occurrence of recessive alleles in the inbred material so that the heterozygosity of the other parent was exposed in the offspring (De,Maine & Fleming, 1991), counteracting the effects of homozygosity in the dihaploids. On the other hand, it is possible that there is natural selection against gametes that are homozygous because recessive lethal alleles are unmasked in such gametes.

Qualitative tuber shape data were tested for goodness of fit against two genetic models. The first model was a major gene for shape where the allele for roundness is dominant to that for long, and the second was where there was co-dominance between the two alleles. It appears from the literature on potato tuber shape genetics that authors differ on whether they class intermediate shaped tubers as rounds or longs (for literature review see De Jong & Burns, 1993 and see also van Eck *et al.*, 1994). Hence, all six shape categories were manipulated in all possible ways to group the data to provide two shape classes (round and long) in the case of the dominance model, or three classes (round, intermediate and long) in the case of the co-dominance model. No data groupings gave a good fit for all progenies. Five out of eight progenies had data not significantly different ($P > 0.05$) from that expected from both the dominance and co-dominance models. For the dominance model, data groupings giving the best fit were categories one to four (round, round oval, oval and long oval) regarded as rounds, and categories five and six (long and very long) longs in the best

case. For the co-dominance model, the best fit groupings were category one – rounds, categories two to four – intermediate, and categories five and six – longs. The results of analysing tuber shape as a qualitative trait, therefore, were inconclusive.

The data presented and discussed in this report shows that there were no significant deleterious effects on the progenies obtained from dihaploids subjected to inbreeding by chromosome doubling and haploidisation. Yield, its components and internal tuber condition were not reduced and, overall, the seed yield was higher from the inbred dihaploids than from the non-inbred. Also, some inbred dihaploids had progenies which had higher mean yields and tuber numbers than that of the original dihaploid.

These findings resemble those of De Jong & Rowe (1971) who, working with progenies derived from selfed interspecific (and presumably therefore highly heterozygous) $2\times$ potatoes, found very little inbreeding depression after the first generation of selfing. The low vigour of first generation dihaploids, compared to the tetraploid clones from which they were derived, is well known. The results of De Jong & Rowe (1971) and those presented here indicate that inbreeding depression is largely spent on the initial unmasking of deleterious recessives, whether it be by selfing or by dihaploidisation. Subsequent generations, on average, do not show such a large reduction in vigour. Chromosome doubling and dihaploidisation, therefore, can be used to produce inbred diploid and tetraploid lines which can transfer useful agronomic traits to their offspring. Within progeny variances were not reduced however, and this may indicate that further inbreeding is required in order to produce parental material which gives more uniform progenies. It remains to be seen whether the level of inbreeding required to give such parents results in much more serious deleterious effects than those seen in the materials reported here.

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