Theor. Appl. Genet. 63, 265–272 (1982)

Chromosome Variation in Protoplast-derived Potato Plants

A. Karp, R. S. Nelson, E. Thomas* and S. W. J. Bright
Biochemistry Department, Rothamsted Experimental Station, Harpenden, Herts. (UK)

Summary. Chromosomes have been studied in protoplast-derived potato plants of the tetraploid cultivars Maris Bard and Fortyfold. A high degree of aneuploidy was found amongst the regenerants of both cultivars but the nature of the chromosome variation differed. The Maris Bard regenerants were characterised by high chromosome numbers, a wide range of aneuploidy (46–92) and a low percentage of plants with the normal chromosome number (2n = 48), whereas a much higher proportion of the Fortyfold regenerants had 48 chromosomes and the variants were within a more limited aneuploid range. In both cultivars chromosome variation was found between calluses, within calluses and even within shoot cultures. The origin of the chromosome variation and the differences found between the two cultivars are discussed.

Key words: Potato protoplasts – Regeneration – Chromosome variation

Introduction

Potato plants regenerated from protoplasts of leaf mesophyll cells exhibit a wide range of phenotypic variation. The phenomenon was first reported in the tetraploid cultivar Russet Burbank for variation involving such agronomically important characters as tuber development, yield and resistance to certain pathogens (Matern et al. 1978; Shepard et al. 1980; Secor and Shepard 1981). These findings have aroused considerable interest with regard to the possible implications for plant breeding and in the interpretation of the nature of the variation (for a recent review see Larkin and Scowcroft 1981). As far as the latter aspect is concerned there is speculation that the basis of the permanent variation may involve gene mutation or changes in chromosome number or structure which were either present in the mesophyll cells or else induced during the regeneration process. Preliminary studies by Shepard and his coworkers (1980) on a small sample of 5 protoplast-derived plants showed that the chromosome number was normal (2n = 4x = 48) but they did not carry out an extensive cytological analysis of the regenerants.

In contrast, plants regenerated from protoplasts of dihaploids (2n = 2x = 24) were found to be phenotypically homogeneous and cytological analysis showed that virtually all the regenerants were tetraploid (2n = 4x = 48). Two aneuploids (2n = 47 and 2n = 49) were found amongst 48 plants studied although the frequency of aneuploids (checked in the callus) was found to increase when the callus phase was extended (Wenzel et al. 1979).

More recently, phenotypic variation has been reported for protoplast-derived plants of the tetraploid cultivar Maris Bard (Thomas et al. 1981). All regenerants differed from one another with respect to some of the ten morphological characters scored and only one of the plants resembled the parental Maris Bard type. In these experiments regenerants were obtained from several shoots developing on individual calluses, and since it is believed that each of the calluses originated from a single protoplast the source of the variation is probably within the callus phase itself rather than between the mesophyll cells within the leaf. In general, when shoot cultures were established (from each shoot forming on the callus) plants derived from the same shoot culture were phenotypically similar, suggesting that no variation was generated within the shoot culture. There was one exception, however, for which so far there is no explanation. These findings are in agreement with the general situation in plant micropropagation in which genetic stability is normal for

* We regret to report the death of Emrys Thomas since the initiation of this work

0040-5752/82/0063/0265/$ 01.60

axillary bud and shoot cultures whereas regenerants that have passed through callus phase show instability (D'Amato 1977).

The protoplast-derived regenerants of Maris Bard are also informative in showing, firstly, that the variation described for Russet Burbank is not an unique feature of this cultivar and, secondly, that the lack of variation found in the dihaploid regenerants of Wenzel et al. (1979) was not due simply to differences in technique because essentially the same culture method was used for Maris Bard.

The present work describes the results of a cytological survey of the Maris Bard regenerants. This was undertaken in order to evaluate the question of chromosome stability, or otherwise, and to relate any possible changes to particular stages in the regeneration process and, if possible, to the patterns of phenotypic variation found among regenerated plants.

In addition, chromosomes have been studied in regenerants of the cultivar Fortyfold. These have been derived from protoplasts using a different technique, essentially that of Shepard et al. (1980). Attempts to regenerate Maris Bard plants from protoplasts using this technique have as yet been unsuccessful. In this present paper only numerical variation will be described and structural chromosome changes will be dealt with in a separate study.

Materials and Methods

Details of the technique used to regenerate Maris Bard plants from protoplasts have already been described (Thomas 1981; Thomas et al. 1982) and only features relevant to the present analysis will be outlined here.

Protoplasts were isolated from plants grown as shoot cultures and were then cultured in agar until calluses were formed. Shoots were obtained from different points on some of the calluses and these were numbered for identification (P1–P41) and maintained as shoot cultures with those same numbers. Potted plants were obtained from the shoot cultures for cytological analysis and in some cases several plants were potted from the same shoot culture to provide replicates. In addition, a number of plants regenerated from cultured leaf pieces were included in the survey as a comparison.

Protoplasts of Fortyfold were isolated using the enzyme mixture and washing procedures described by Thomas (1981), from leaves of plants conditioned as recommended by Shepard (1980). Protoplasts were plated in the R medium (without agar), and subsequent protoplast-derived calluses induced to form shoots using the C and D media of Shepard (1980). Ten percent of the calluses gave rise to shoots, although more calluses possessed shoot primordia. After three months calluses were transferred onto Murashige and Skoog medium (Flow Labs. Ltd.) with 20 g/l sucrose, 0.25 mg/l 6-benzylaminopurine, 0.1 mg/l gibberellic acid (filter-sterilised) and 9 g/l agar ("Bacto-agar" Difco-Labs. Ltd.), at pH 5.6, on which the frequency of calluses with shoots increased to 30%.

Calluses obtained from the protoplasts were numbered for identification, as were (in order) the different plants arising from each callus, which were then maintained as shoot cultures, and the different plants obtained from the shoot cultures. The numbering system adopted for Fortyfold enabled each regenerant to be traced to the original shoot and callus from which it arose. The full description of each regenerant thus includes the callus number (given first), the shoot number (given second) and the number of the plant obtained from the shoot culture (given last). In addition some of the calluses fragmented prior to shoot formation and these were given a letter suffix e.g. 11 a, 11 b, 11 c, so that different subunits of the same callus could also be identified.

For cytological analysis, healthy root tips were pretreated with 0.002 M 8-hydroxyquinoline for 3–4 h at 18 °C and then fixed in 3 : 1 absolute alcohol : glacial acetic acid for at least 24 h. The roots were then hydrolysed in 1N HCl for 10 min at 60 °C and stained in Feulgen. Squash preparations were made in aceto-carmine. Counts were obtained from a minimum of 5 well spread cells and from at least two separate roots.

Results

(a) Maris Bard

The chromosome number of the cultivar Maris Bard was confirmed as $2n = 4x = 48$ by screening a sample of tuber derived plants. Ten plants taken from Maris Bard shoot cultures were also found to have 48 chromosomes (Fig. 1 a). In addition chromosomes were examined in eight Maris Bard plants regenerated from cultured leaf pieces. These all had 48 chromosomes and appeared karyotypically indistinguishable from normal Maris Bard (Fig. 1 b). Chromosome counts were then obtained for plants taken from 26 different shoot cultures initiated 12–18 months earlier from shoots which appeared on protoplast-derived calluses of Maris Bard (Thomas et al. 1982). Considerable variation in chromosome number was revealed (Table 1). Only one shoot culture (P40) gave plants which had the normal chromosome number and which appeared normal in karyotype, as far as preliminary investigations could determine (Fig. 1 c). Of the remaining shoot cultures, one (P35) gave plants with 46 chromosomes whilst the others gave plants with numbers that ranged from 51 (P1) to 93 (P31). Some chromosome numbers, for example $2n = 67$ (Fig. 1 d) were found in plants taken from only one shoot culture (P910) whereas half the shoot cultures gave plants with 88, 89 or 90 chromosomes, plants with 90 chromosomes (Fig. 1 e) being the most common. It was not possible to establish whether plants with the same chromosome number, for example from P8 and P23X ($2n = 88$, Fig. 1 f, g), had identical complements, due to the small size of the chromosomes and their lack of distinguishing features. Although identical chromosome numbers were observed in plants from more than one shoot culture the number of

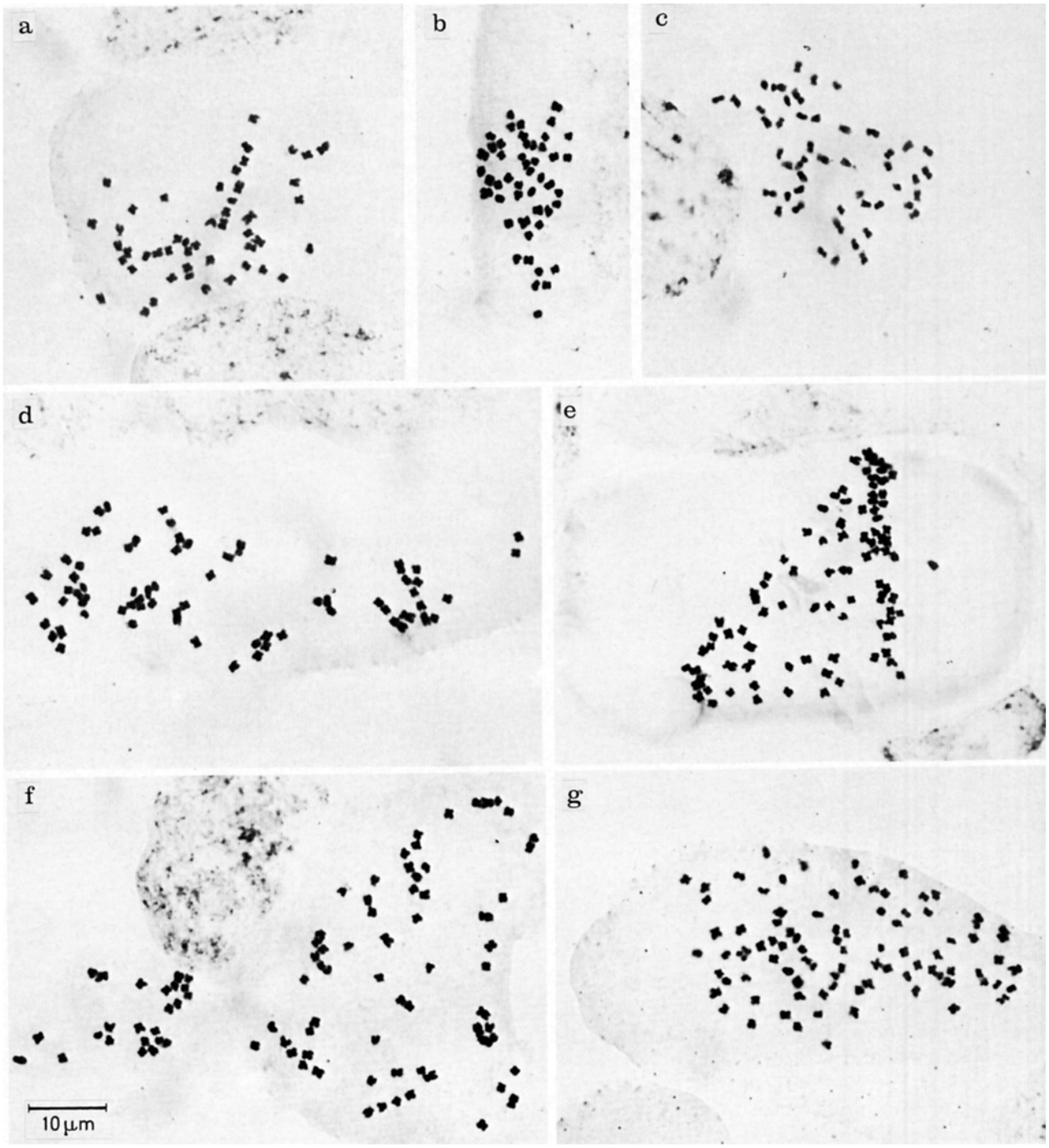

Fig. 1 a – g. Chromosomes in somatic cells from root-tips of Maris Bard plants. **a – c** normal tetraploid cells (2n = 48) as found in plants: **a** from axillary bud shoot cultures; **b** regenerated from cultured leaf pieces and **c** from the P40 shoot culture obtained from a protoplast-derived callus. **d – g** examples of aneuploidy in regenerant plants taken from other shoot cultures produced from protoplast-derived calluses: **d** 2n = 67, found in plants taken from P910 shoot culture; **e** a plant with 2n = 90 chromosomes from P12 shoot culture and **f – g** two plants with 88 chromosomes but which are derived from different shoot cultures (P8 and P23X)

Table 1. Chromosome numbers of plants from protoplast-derived shoot cultures of Maris Bard

Plant	Chromosome number	Plant	Chromosome number
P1	51	*P13*	90
P2	54, 86	*P15*	89
P23X	88	*P16*	65
P3	88	*P22*	89
P4	90	*P30*	88
P45X	72	*P31*	93
P5	90	*P32*	90
P6	70	*P33*	92
P7	89	*P35*	46
P8	88	*P36*	89
P910	67	*P37*	92
P11	82	*P40*	48
P12	90	*P41*	92

Table 2. Chromosome counts for replicate plants from the same shoot culture

Shoot culture	Chromosome number of replicate			
	(i)	(ii)	(iii)	(iv)
P1	51	51	51	51
P2	86	54	54	–
P8	88	88	88	88
P40	48	48	48	48
P41	92	92	92	92

different counts obtained (Table 1) exceeded the number of original calluses.

The chromosome numbers were obtained for plants taken after the shoot cultures had been maintained for more than one year. It was therefore necessary to establish whether plants taken from the earliest passages of the shoot cultures contained the same chromosome numbers, or whether the counts described were actually a consequence of changes occurring during time in shoot culture. Tubers produced by plants taken from the earliest passages of five shoot cultures (P40, P41, P35, P910 and P2) were therefore sprouted and chromosome counts obtained from the roots. In the cases of P40 and P41 such roots had the same chromosome number ($2n=48$ and $2n=92$, respectively) as found in plants derived from later passages (Table 1) indicating chromosome stability during the 12–18 month period. In the case of P35, however, the roots had 47 chromosomes (instead of 46, Table 1) indicating that a chromosome had been lost during the time in culture. In the case of P910 the roots were found to have 86 chromosomes (instead of 67, Table 1) and it is evident that extensive chromosome loss occurred in this shoot culture. Roots produced by tubers of plants derived from early passages of P2 had 99 chromosomes (instead of 54 and 86, Table 1) again indicating chromosome loss during the 12–18 months. These results show that changes did occur in some of the shoot cultures but also that these changes consisted only of chromosome loss and that high chromosome numbers were present in the early passages.

To establish whether the chromosome numbers were stable after a year in shoot culture a number of plants were potted from each of 5 different shoot cultures. Table 2 shows that, with the exception of P2, plants derived from the same shoot culture had the same chromosome number whether chromosome numbers were high (P8 and P41), low (P1) or "normal" (P40). P2 was unusual in that plants derived from the shoot culture could be identical (e.g., $2n=54$, two plants) or could be entirely different ($2n=86$).

(b) Fortyfold

The chromosome number of the cultivar Fortyfold was confirmed as $2n=4x=48$ by examining a sample of plants grown from tubers.

Table 3 shows the counts for plants from 26 different Fortyfold calluses. In most cases the count quoted corresponds to the first plant from the first shoot obtained from each callus but occasionally this died and a count from the next available shoot is given. The table clearly shows chromosome numbers varied between calluses; 30% of the plants had the correct chromosome number (Fig. 2a) but the remainder were aneuploids ranging between 46 and 49. The majority of the aneuploids had lost rather than gained chromosomes, and monosomics were most frequent ($2n=47$, Fig. 2b). An exception to this aneuploid range was callus 20 which gave a count of 93 chromosomes (Fig. 2c). Due to the nature of potato chromosomes

Table 3. Chromosome numbers of plants regenerated from 26 separate protoplast-derived calluses of Fortyfold

Callus number	Chromosome number	Callus number	Chromosome number
1	47	*36*	48
2	47	*37*	46
3	48	*38*	48
6	47	*39*	48
8	48	*40*	46
10	49	*67*	47
11	46	*68*	46
19	49	*72*	47
20	93	*73*	47
22	49	*74*	46
23	48	*75*	48
24	48	*76*	47
29	46	*77*	47

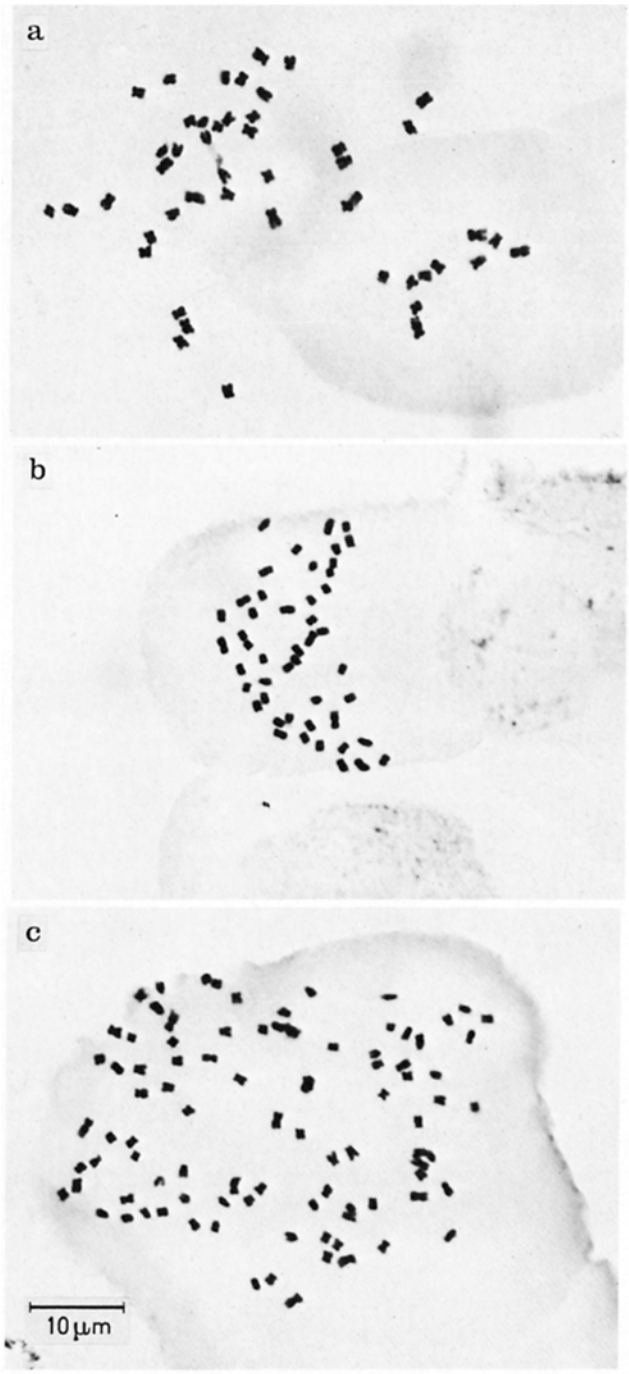

Fig. 2a – c. Chromosomes in somatic cells from root-tips of Fortyfold regenerants. **a** a cell from a plant with the correct chromosome number; 2n = 4x = 48; **b** a monosomic with 2n = 47 and **c** an aneuploid with the high chromosome number of 2n = 93

further detailed work is necessary before the aneuploids can be classified but it was clear in some cases that it was not the same chromosome(s) causing the aneuploidy.

When different shoots from the same callus were analysed for eleven different calluses (Table 4) considerable variation was also revealed. The table shows that different shoots from the same callus can have different chromosome numbers and this is true both for shoots from the same part of the callus (19a, where 6 shoots were counted) and for shoots from different subparts of the same callus (19c, e, f). It is also apparent, however, that in some instances the same product can be obtained from the same callus. This is shown by callus 20 where both shoots had 93 chromosomes. This could be due either to both shoots having originated from a very small region of the callus or to the change to 93 chromosomes having occurred very early on in the history of the callus.

Table 4. Chromosome numbers for plants taken from shoot cultures in eleven cases where several arose from the same callus or sub-callus

Callus	Sub-callus	Shoot	Chromosome number
2	a	*1*	47
	a	*2*	47
	a	*3*	47
3	a	*1*	48
	a	*2*	44
6	a	*1*	47
	a	*4*	47
	b	*5*	47
	c	*2*	46
10	a	*6*	47
	b	*1*	49
11	a	*1*	46
	d	*4*	47
	e	*3*	44
	f	*6*	46
19	a	*1*	49
	a	*2*	48
	a	*7*	47
	a	*8*	48
	a	*9*	48
	a	*10*	47
	c	*15*	45
	e	*4*	49
	f	*6*	49
20	a	*2*	93
	a	*4*	93
23	a	*1*	48
	a	*2*	47
24	a	*1*	48
	a	*3*	47
29	a	*1*	46
	a	*2*	46
	a	*3*	46
74	a	*1*	46
	b	*2*	46

Table 5 gives the chromosome numbers for different plants derived from the same shoot culture (11a/1). Surprisingly, although the majority of plants scored had

Table 5. Chromosome counts for different plants from the 11a/1 shoot culture

Plant number	Chromosome number
11a/1/3	46
11a/1/4	70/94
11a/1/8	46
11a/1/9	46
11a/1/10	46
11a/1/12	46
11a/1/13	46
11a/1/14	46
11a/1/15	46
11a/1/16	93
11a/1/20	94
11a/1/23	46

the same chromosome number (2n=46), which also corresponds to the count originally obtained from this callus (Table 3), three of the plants had more than twice as many chromosomes. One of these (11a/1/4) consistently gave two counts (2n=70 and 2n=94) but more work is required before comment can be made on the nature of this plant.

Discussion

Cytological analysis of protoplast-derived regenerants of the cultivars Maris Bard and Fortyfold has revealed extensive variation in chromosome number. In Maris Bard the number of different aneuploid shoot cultures exceeded the number of original calluses indicating that chromosome variation was present between calluses and also probably within calluses, although the latter remains an inference as no record was made of which shoots came from which calluses. In the case of Fortyfold, where a more precise record was made of the origin of each regenerant, the results clearly showed that chromosome variation was present both between (Table 3) and within calluses (Table 4). Variation was also found within shoot cultures for both Maris Bard (P2) and Fortyfold (Table 5).

The nature of the variation differed for the two cultivars. Maris Bard regenerants were characterised by high chromosome numbers, a wide range of aneuploidy (46–93) and a low percentage of normals, whereas a much higher percentage of the Fortyfold regenerants had 48 chromosomes and the variants were within a more limited aneuploid range (2n=48±2). For Maris Bard it would appear that chromosome doubling occurred followed by loss to give a large range in chromosome number. High chromosome numbers were present in early passages of the shoot cultures which indicates that chromosome doubling occurred prior to initiation of the shoot cultures. Chromosome loss, however, appears to have occurred both prior to initiation of the shoot cultures and also (in some of the shoot cultures) during time in culture. In contrast, aneuploidy has mostly occurred independent of chromosome doubling in the Fortyfold regenerants.

These differences could either be cultivar-dependent or else the consequence of using two different methods of regeneration. The two methods differed essentially in three respects – the source of the protoplasts, the regeneration protocol and the hormones used, and these can be briefly described.

For Maris Bard small shoots were maintained as shoot cultures on medium containing 0.5 mg/l BAP. Whole shoots were then used for protoplast isolation. Essentially the same medium was used until callus formation and this contained 1 mg/l 2,4-D and 0.5 mg/l zeatin (Thomas 1981).

In contrast, for Fortyfold, protoplasts were isolated from fully expanded leaves taken from plants grown in defined growth room conditions without added hormones (Shepard 1980). The protocol used involved more changes of media and 0.5 mg/l zeatin and IAA were only present in the last regeneration medium.

Although it is difficult to dismiss cultivar-dependence two points favour different culture techniques as the cause of the difference in chromosome variation. Firstly, regenerants from cultured leaf pieces of Maris Bard had 48 chromosomes and secondly, there is a large amount of evidence in the literature indicating the influence of culture conditions on the nature of chromosome stability in callus and cell cultures (Bayliss 1980).

Plants regenerated from dihaploid protoplasts were also found to have doubled up in chromosome number (Wenzel et al. 1979) but, although the Maris Bard technique was essentially that of Wenzel the two results cannot be directly compared because of the difference in ploidy level in the starting material. The effect of ploidy level on the nature of chromosome stability in calluses has been shown, for example, by Sacristan (1971) in *Crepis capillaris*, who found a higher frequency of change in ploidy in callus derived from a haploid plant compared with callus derived from a diploid plant over a year in culture.

The precise cause of the difference in chromosome variation between the Maris Bard and Fortyfold regenerants is difficult to identify because of the number of possible variables. It does seem, however, that the culture method adopted can have a strong effect on the chromosome stability of the regenerants, and a better understanding of the influence of the different variables may lead to appropriate improvements in the technique so that a higher degree of chromosome stability can be achieved.

The finding of chromosome variation raises the question of its origin. Three possibilities will be explored.

It could be that changes in chromosome number occur as part of the differentiation of the leaf and that in forcing the cells into regeneration of a whole plant these changes are revealed. This would imply that the Maris Bard shoot cultures contained cells with more than the tetraploid chromosome set, whereas the fully expanded leaves used as a source of Fortyfold protoplasts must have contained euploid/aneuploid cells. Although this explanation for the differences found between the two cultivars may not seem convincing we have, as yet, no grounds for excluding the possibility that it contributed to the observed chromosome variation. Endoduplication of chromosomes, concomitant with differentiation, has been found in 80% of angiosperm species studied so far (D'Amato 1978) but remains to be demonstrated in potato. The presence of aneuploid cells in differentiated tissue has yet to be described. Plants regenerated from cultured leaf pieces of Maris Bard, plants taken from Maris Bard shoot cultures and also most plants regenerated from crown galls on Maris Bard, following infection by *Agrobacterium tumefaciens* (Ooms et al. in preparation) were all found to have 48 chromosomes. This indicates that some of the cells, at least, must have had the correct chromosome number. The resolution of this problem can only come from measurement of DNA content and/or chromosome counting of cells in the tissues used for protoplast isolation, or better still, of the protoplasts themselves.

Chromosome variation was found both between and within calluses for both Maris Bard and Fortyfold and a second possibility is that the variation originates in the callus. There is considerable evidence that chromosome changes do occur in the callus phase (Orton 1980; Constantin 1981). The possibility that the calluses are not derived from single protoplasts cannot be completely excluded, however, and it could be that protoplasts differing in chromosome constitution clump to form a chimaeric callus from which shoots with differing chromosome numbers develop. This problem would also be resolved if the protoplasts can be shown to be identical in chromosome constitution, in which case even if the calluses were produced from aggregates this would not explain the chromosome variation found within the callus.

The third possibility is that the variation originates in shoot cultures. Maris Bard shoot cultures (whether controls or protoplast-derived) which gave plants with 48 chromosomes continued to do so even after a year. Most of the shoot cultures which gave aneuploid plants were found to be stable when looked at after 12 to 18 months but instability was observed in P2. Furthermore, cases of chromosome loss over 12–18 months were revealed when tubers from plants derived from early passages of the shoot cultures were examined. For Fortyfold, chromosome variation was clearly demonstrated in the 11a/1 shoot culture. We believe that the variation observed in shoot cultures is a consequence of the aneuploid nature of the original shoot, and that aneuploid shoot cultures, although often stable at any one time, cannot be relied on to consistently produce shoots with the same chromosome number. This would also imply, however, that although variation can be generated in shoot cultures it does not originate there.

Whatever its origin the chromosome variation is clearly of consequence. The chromosome variation in the Maris Bard regenerants correlates well with the variation in morphology described for growth cabinet grown plants (Thomas et al. 1981). As examples, P40 (found to give plants of $2n=48$) was the only shoot culture which gave plants resembling the parental Maris Bard and P2 (found to be chromosomally "unstable") was described as giving plants of varied morphology. The glass house grown Fortyfold regenerants also showed morphological variation, although more of the plants appeared normal, or "near-normal", than for Maris Bard.

Until the Maris Bard and Fortyfold aneuploid regenerants are classified and until some more precise assessment of the morphological variation (from the results of field trials) is at hand the only conclusion that can be drawn is that some of the observed morphological variation (at least) can be accounted for by variation in chromosome number. The Fortyfold aneuploids may prove to be useful in investigating the genetic constitution of the chromosomes in the complement. Although their potential value is still to be assessed, it may be that not all of the chromosome variation is undesirable.

Acknowledgements

The authors thank the Scottish Crop Research Institute for supplying tubers of Fortyfold. We also acknowledge the support of an SERC grant to Robin Nelson and are grateful to Julian Franklin and Jan Roberts for growing the plants.

Literature

Bayliss, M.W. (1980): Chromosomal variation in plant tissues in culture. Intern. Rev. Cytol. Suppl. **11A**, 113–144

Constantin, M.J. (1981): Chromosome instability in cell and tissue cultures and regenerated plants. Environ Exp. Bot. **21**, 359–368

D'Amato, F. (1977): Cytogenetics of differentiation in tissue and cell cultures. In: Applied and fundamental aspects of plant cell, tissue, and organ culture (eds. Reinert, J.; Bajaj, Y.P.S.), pp. 343–357. Berlin, Heidelberg, New York: Springer

D'Amato, F. (1978): Chromosome number variation in cultured cells and regenerated plants. In: Frontiers of plant tissue culture (ed. Thorpe, J.A.), pp. 287–295. Intern. Ass. Plant Tissue Culture, University of Calgary Offset Printing Services, Calgary, Canada

Larkin, P.J.; Scowcroft, W.R. (1981): Somaclonal variation – a novel source of variability from cell cultures. Theor. Appl. Genet. **60,** 197–214

Matern, U.; Strobel, G.; Shepard, J.F. (1978): Reaction to phytotoxins in a potato population derived from mesophyll protoplasts. Proc. Natl. Acad. Sci. USA **75,** 4935–4939

Orton, T.J. (1980): Chromosomal variability in tissue cultures and regenerated plants of *Hordeum.* Theor. Appl. Genet. **56,** 101–112

Sacristan, M.D. (1971): Karyotypic changes in callus cultures from haploid and diploid plants of *Crepis capillaris* (L.) Wallr. Chromosoma **33,** 273–283

Secor, G.A.; Shepard, J.F. (1981): Variability of protoplast-derived potato clones. Crop Sci. **21,** 102–105

Shepard, J.F.; Bidney, D.; Shahin, E. (1980): Potato protoplasts in crop improvement. Science **208,** 17–24

Shepard, J.F. (1980): Mutant selection and plant regeneration from potato mesophyll protoplasts. In: Genetic improvement of crops/emergent techniques (eds. Rubenstein, I.; Gengenbach, R.L.; Philips, R.L.; Green, C.E.), pp. 185–219. Univ. of Minnesota Press, Minneapolis, MN

Thomas, E. (1981): Plant regeneration from shoot-culture derived protoplasts of tetraploid potato (*Solanum tuberosum* cv. Maris Bard). Plant Sci. Lett. **23,** 84–88

Thomas, E.; Bright, S.W.J.; Franklin, J.; Lancaster, V.; Miflin, B.J.; Gibson, R. (1982): Variation amongst protoplast-derived potato plants (*Solanum tuberosum* cv. Maris Bard). Theor. Appl. Genet. **62,** 65–68

Wenzel, G.; Schieder, O.; Przewozny, T.; Sopory, S.K.; Melchers, G. (1979): Comparison of single cell culture derived *Solanum tuberosum* L. plants and a model for their application in breeding programs. Theor. Appl. Genet. **55,** 49–55

Accepted July 1, 1982
Communicated by R. Riley

Dr. A. Karp
Mr. R. S. Nelson
Dr. S. W. J. Bright
Biochemistry Department
Rothamsted Experimental Station
Harpenden, Herts AL5 2JQ (UK)