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# From tumour to tuber; tumour cell characteristics and chromosome numbers of crown gall-derived tetraploid potato plants (*Solanum tuberosum* cv. 'Maris Bard')

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**Summary.** *Agrobacterium tumefaciens* strains, known to induce tobacco crown galls that spontaneously develop shoots, were used to induce galls on cultured shoots of a tetraploid potato cultivar (*Solanum tuberosum* cv. 'Maris Bard'). Shoots also appeared spontaneously from the induced potato galls, although only after 2–4 months. The shoots were excised and cultured separately. Some of these frequently developed side-shoots from their axillary buds. They did not form roots and they produced opines, a strong indication that they were transformed and carried T-DNA. Grafts of the transformed plants were still able to develop tubers. Most of the tumour-derived shoots, however, formed roots, did not produce opines and were indistinguishable from the parental plants on the basis of morphology and chromosome numbers (48 chromosomes per cell). The results are discussed in relation to the origin of previously described variation among protoplast-derived potato plants and with respect to genetic engineering of tetraploid potato cultivars.

**Key words:** *Agrobacterium tumefaciens* – Crown gall – Chromosomes – Potato – Variation

## Introduction

Various ways have been described to transfer a limited amount of specified genetic information into plant cells from which it has been possible to recover stably transformed plants (Braun and Wood 1976; Wullems et al. 1981; Krens et al. 1982; De Greve et al. 1982; Ackermann 1977; Chilton et al. 1982).

A number of ways of obtaining transformed plants involve manipulation of protoplasts; others make use of a natural transfer system of genetic information as is employed by *Agrobacterium tumefaciens* (Smith and Townsend 1907) and *A. rhizogenes* (Riker et al. 1930). Cells transformed by these bacteria develop into either crown gall tissue or hairy root tissue, respectively. The transformation takes place after infection of wounded plant tissue with a bacterial suspension. Only dicotyledonous plants are susceptible and only *Agrobacterium* strains that carry a large plasmid, a Ti- or an Ri-plasmid are virulent (De Cleene and DeLey 1976; Van Larebeke 1975; White and Nester 1980). In the course of transformation a plasmid segment, the T-region, is transferred into the plant cell where it is stably integrated into the nuclear genome (Chilton et al. 1977, 1982; Thomashow et al. 1980; Willmitzer et al. 1980). Once inserted it is called T-DNA. Evidence exists that T-DNA coded products in crown gall tissue are involved in plant hormonal activities and opine synthesis (Ooms et al. 1981; Garfinkel et al. 1981; Leemans et al. 1982; Schroder et al. 1981). Usually, transformed plants derived from crown gall cells do not form a root system (Wullems et al. 1981), presumably due to sustained activities of some of the T-DNA coded products. Loss of T-DNA during meiosis results in the reappearance of normal plant morphology in the offspring (Yang and Simpson 1981). Furthermore, a specific *A. tumefaciens* mutant strain induced crown galls from which morphologically normal, but transformed tobacco plants have been recovered, at low frequency (De Greve et al. 1982). In these plants only the T-DNA segment coding for the opine synthesising enzyme LpDH was present. The reported frequency was low because many plants recovered from tobacco galls derive from normal untransformed cells, also present in the tumour (Sacristan and Melchers 1977).

It has been reported that potato plants recovered from protoplasts of tetraploid potatoes exhibit morphological variation – to a large extent this is probably due to aneuploidy (Shepard et al. 1980; Thomas et al. 1982; Karp et al. 1982). In the present study, transformed and untransformed plants were obtained from crown galls induced by infection with *A. tumefaciens*. These plants were examined for morphological and karyotypic variation and most of them were found to be normal. This is discussed in relation to the cause of variability among plants derived from protoplasts and its implications for genetic engineering of the potato.

## Materials and methods

Shoot cultures of 'Maris Bard' were grown in glass jars on agar-solidified (Fisons; 0.8% agar) Murashige and Skoog medium (Flow Laboratories) supplemented with 20 g/l sucrose, but without hormones (MS-H; Murashige and Skoog 1962) at 25 °C, 12 h day length. They were propagated by subculturing stem pieces with a single leaf at about four week intervals. *A. tumefaciens* strains were grown on agar-solidified (1.8% Difco Bacto agar) TY medium (5 g trypton; 3 g yeast extract per litre) at 29 °C. The *Agrobacterium* strains used, i.e. T37, LBA4060, LBA1501, were obtained from the *Agrobacterium* collection of the Molbas research group in Leiden, The Netherlands. Small holes were made in the stems of the potato shoots of similar cross-sectional diameter using a drawn out and subsequently broken pasteur pipette. Tumours were induced by *Agrobacterium* infection through these holes. Three to four months after infection, galls were excised and grown on agar solidified MS-H medium supplemented with 200 μg/ml carbenicillin to suppress bacterial growth. Two months later, shoots were excised from the tissue and subcultured on fresh, agar-solidified medium of the same composition. Once plants appeared free of bacteria, as judged by the absence of bacterial growth in the absence of antibiotics, they were potted up and grown in a growth room (18 °C; 85% humidity; 12 h daylength). Tumour cell-derived shoots that had been grafted onto stems of normal potato shoots were grown in the same growth room.

The presence of lysopine dehydrogenase (LpDH) activity in tumour-derived shoots was determined when the shoots were transferred the first or second time after excision from the tumour tissue. A microassay was used (Oten and Schilperoort 1978) in which an extract of about 10 mg tissue was mixed with arginine and pyruvate in the presence of NADH to allow the synthesis of octopine if LpDH were present in the extracts. Sometimes incubations were carried out overnight. All gall-derived plants were assayed at least twice.

Stem explants of 'Maris Bard' shoot cultures (2–5 mm long) were grown on MS medium with 0.12 mg/l 2.4 D and 0.5 mg/l zeatin. After three months shoots had developed from the explants and these were excised and grown on MS-H medium.

Chromosome numbers in root tips were determined, both of tumour-derived plants and of explant-derived plants, as described elsewhere (Karp et al. 1982). For each plant at least five well spread cells derived from a number of roots were used to establish the chromosome number. Moreover, in each preparation, many more cells were quickly scanned to search for obvious deviations and/or abnormalities.

## Results and discussion

The stems of shoot cultures of a number of tetraploid potato cultivars ('Maris Bard', 'Maris Piper', 'Desiree', 'King Edward', 'Pentland Crown' and 'Record') were wounded and infected with one of three *A. tumefaciens* strains (T37, LBA4060, LBA1501). All three strains were known to induce galls on stems of tobacco plants from which shoots developed spontaneously (Braun and Wood 1976; Ooms et al. 1981). Shoots also developed spontaneously from most of the galls induced on stems of all the potato varieties tested although only two to four months after infection (Fig. 1 a). Once established, the galls were excised and grown in vitro (Fig. 1 b). This allowed the tumour-derived shoots to develop further and considerably enhanced the subsequent survival of subcultured shoot tips.

We examined in greater detail the shoots of three 'Maris Bard' galls. All gall-derived shoot tips were subcultured and numbered individually, such that the plant (e.g. 'Maris Bard'), the inducing strain (e.g. LBA4060), the specific gall (e.g. A) and the shoot (e.g. 5) could be recognized (Mb-4060A-5).

Of the total 35 subcultured shoot tips, four failed to form roots, four died, whilst the remainder developed a normal root system. The axillary buds of shoots without roots frequently developed into side-shoots but they

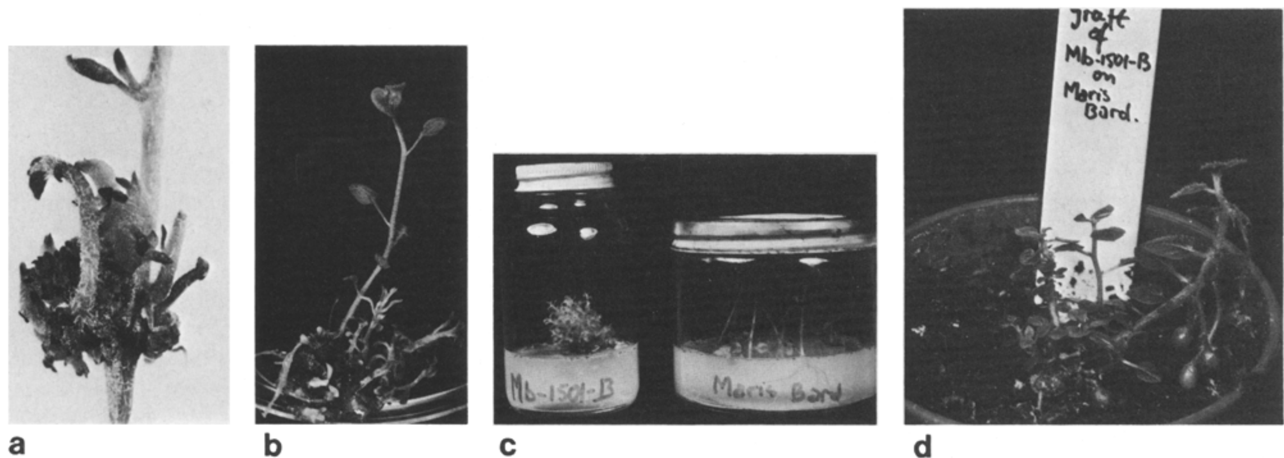


**Fig. 1 a–d.** Different stages in the development of shoots derived from crown-galls on stems of potato shoots of cv. 'Maris Bard'. **a** a potato stem tumour induced by *Agrobacterium* strain T37 and photographed four months after induction; **b** shoots spontaneously developed from crown gall tissue excised from the stem of a cultured shoot. The tissue was induced by *Agrobacterium* mutant strain LBA1501; **c** shoot cultures of normal 'Maris Bard' shoots (*right*) and of "tumour shoots" (*left*). Note the absence of root formation, frequent development of side shoots and short appearance of the potato 'tumour shoots'; **d** graft of a tumour cell-derived potato shoot on the stem of a normal 'Maris Bard' shoot. Note the spontaneous development of aerial tubers

remained relatively short (Fig. 1c). T-DNA coded LpDH activity was found in all four of these shoots (e.g. Mb-1501B in Fig. 1 lane 2a, b), strongly suggesting that the plants were derived from tumour cells. Two of the shoots were grafted onto stems of normal 'Maris Bard' shoots, and within three months stolons and subsequently small tubers developed from some of the axillary buds of the grafts (Fig. 1d).

Of the crown gall-derived shoots that did form a normal root system none showed LpDH activity (e.g. Mb-4060-15 in Fig. 2 lane 3a, b), suggesting that they were derived from untransformed cells. From the 21 root-forming shoots that were recovered from gall Mb-4060A, two shoots were clearly morphologically abnormal. One, Mb-4060A-5, formed curly leaves and the second one, Mb-4060A-25, spontaneously developed each of its axillary buds into side shoots. The chromosome numbers in root-tips of these two shoots were determined. Mb-4060A-25 had the normal complement of 48 chromosomes, but Mb-4060A-5 had 47 chromosomes only (Fig. 3), which could explain its abnormal morphology. However, when we determined the chromosome number in the 11 side shoots of Mb-4060A-25, i.e. in the roots of subcultures of these side shoots, the two side shoots that had appeared first (Mb-4060A-25 a/b) had 47 chromosomes only. The remaining side shoots (Mb-4060A-25 c/k) had 48 chromosomes. Moreover, in a number of preparations of root tips from Mb-4060A-25 and of Mb-4060A-25 f, we found a dicentric chromosome (Fig. 3c). It is noted that dicentric chromosomes have been reported to occur frequently in habituated tobacco tissue (Mouras and Lutz 1980), but to what extent these observations are related is unknown. Of the remainder of the apparently normal 21 shoots, we examined eight shoots all of which had 48 chromosomes. The normal character of these shoots was confirmed further when the shoots were grown to maturity in a growth chamber. All tumour-derived plants with normal chromosome numbers were morphologically indistinguishable from Maris Bard plants grown under the same conditions.

Essentially the same results as described for shoots Mb-4060A-1/25 were obtained for shoots from two other galls. All five shoots derived from gall Mb-4060B had 48 chromosomes and gave morphologically normal mature plants. Furthermore, three out of a total of five shoots recovered from gall Mb-1501B did not form roots whereas the remaining two grew into morphologically normal mature plants.

These results show that a large proportion of the shoots derived from crown-gall, and which are capable of forming roots, have normal chromosome numbers and, when grown to maturity, probably are morphologically identical to the parental plant. This is in contrast with protoplast-derived plants among which much variation has been observed both in morphology

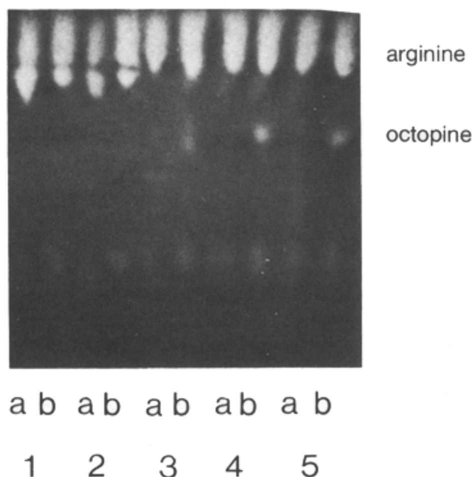


**Fig. 2.** Electropherogram of the products in reaction mixtures used to assay for LpDH activity in various potato tissues. *Lanes a* and *b* indicate reaction mixtures at t=0 and t=18 h of incubation, respectively. *Lane 1* 'Maris Bard'; *2* Mb-4060-15 *3* crown gall tissue induced by LBA1501 (Fig. 1a); *4* shoot of Mb-1501B (Fig. 1c); *5* grafted shoot of Mb-1501B (Fig. 1d)

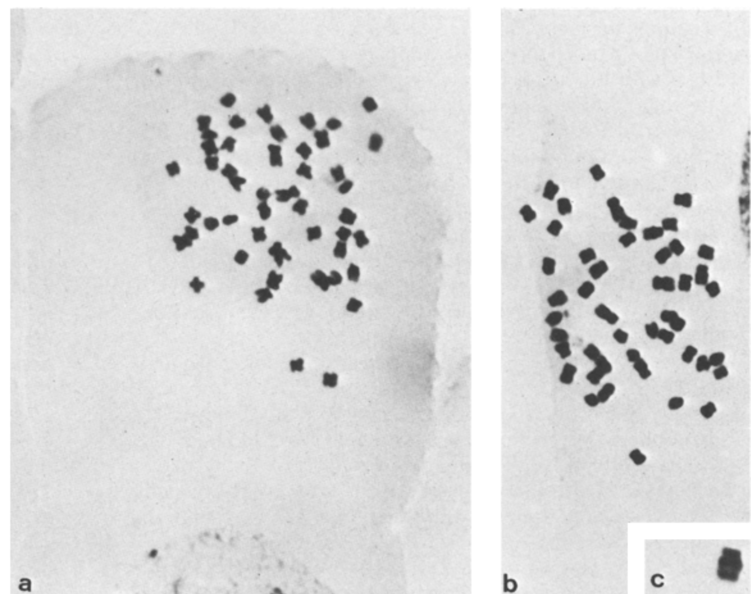


**Fig. 3a–c.** Chromosomes in somatic cells of root tips of 'Maris Bard' plants. **a** normal chromosome complement (2n=48) in tumour-derived plant Mb-4060-1 (magnification: 2016x); **b** aneuploid chromosome complement (2n=47) in Mb-4060-25a (2016x); **c** a dicentric chromosome as found in cells of Mb-4060-25 (4032x)

and chromosome numbers (Shepard et al. 1980; Thomas et al. 1982; Karp et al. 1982). Therefore, it is most likely that transformed potato plants, almost identical to the parental plant, can be obtained by screening for root-forming transformed shoots among tumour-derived shoots rather than among shoots derived from protoplasts. The results also suggest that chromosome variation among protoplast-derived potato plants is more likely to be caused by instability induced by the protoplast isolation procedures and/or subsequent culture methods currently employed, rather than by pre-existing variation in the chromosome constitution of the somatic cells in the plant. The same conclusion was reached when ten 'Maris Bard' potato plants obtained from stem segments cultured under tissue culture conditions on a shoot-inducing medium, were all found to be morphologically normal and to carry 48 chromosomes. Both results confirm and extend previous observations on the recovery of normal plants from (1) leaves cultured in vitro (Karp et al. 1982), (2) wounded roots (Miedema 1973) and (3) shoots recovered from the cut surface of potato plants treated with plant hormones (Thomas et al. 1982).

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