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Spontaneous electrophoretic and chromosomal variability in callus cultures and regenerated plants of celery*

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Summary. Reciprocal sexual crosses were performed to produce plants heterozygous at four nuclear loci, two of which coded for phosphoglucomutase (PGM) and shikimic acid dehydrogenase (SDH) isozymes and could be visualized in petiole callus tissue. Clones of one reciprocal always exhibited the heterozygous phenotype at both isozyme loci, but 25.8% of clones of the other exhibited loss of the fast migrating electromorph at the PGM locus while remaining 100% heterozygous at the SDH locus. No reversion to heterozygosity was observed and the variant phenotype was transmitted to regenerated plantlets. All regenerates were karyologically and developmentally abnormal, and studies of the inheritance of this variability were therefore not possible. Epigenetic change, point mutation, and somatic recombination were ruled out as possible causes due to gross incongruencies with the phenomenon. No consistent differences between normal and variant clones were detected with respect to chromosome number, structure, and anomalous disjunction.

Key words: Genetic variability – Chromosomal variability – Callus – Regeneration – Celery

Introduction

The use of in vitro somatic cell proliferation to clone single cells or tissues into populations of identical regenerated organisms presumes fidelity in DNA replication and equationality in mitosis. However, a large body of reports concerning DNA content and chromosome architecture at the cell level in cultured cells and tissues of a broad range of plant species has shown that spontaneous variability is more the rule than the exception (Partanen 1963; summarized in reviews by Sunderland 1973; D'Amato 1975; Bayliss 1980; Larkin and Scowcroft 1981). A smaller number of reports has demonstrated the transmission of spontaneous in vitro chromosomal variability into regenerated plants (Sacristan and Melchers 1969; Maliga et al. 1978, 1979; Orton 1980; Ogihara 1981; Browers and Orton 1982). The potential utility of such variability as novel raw materials for basic genetics and breeding has also been pointed out (Liu and Chen 1976; Skirvin 1978; Orton 1980, Larkin and Scowcroft 1981).

Although DNA content and chromosome numbers provide evidence for gross genetic changes, direct evidence of such changes at the informational level has not been reported in cultured plant cells, but has been implied from regenerated plants. Chromosome banding has been used to qualify chromosome loss and rearrangement (Ashmore and Gould 1981), but the technique has failed to emerge as an important diagnostic tool in plant cytogenetics as it has in mammals. Moreover, structural rearrangements have been observed which could obscure banding patterns (Kao et al. 1970; Orton 1980).

Advances in genetic technology in cultured mammalian cells have often suggested possible new approaches in plant systems. Examples are cell fusion and transformation. Electrophoretic isozyme markers have been used extensively in mammalian systems for over 10 years as proves for gene mapping and to

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elucidate possible mechanisms of neoplastic transformation (Ruddle and Creagan 1975). Plant callus and suspension tissues are void of morphological variability for possible use in genetic analyses. Variability in the expression of esterase and peroxidase isozymes in callus cultures of *Hordeum vulgare* × *H. jubatum* has been observed, but the genetic basis for isozyme expression in this hybrid and its relationship to spontaneous in vitro chromosomal variability could not be verified (Orton 1980).

Callus and cell cultures of celery have been reported to exhibit chromosomal variability (Browers and Orton 1982; Murata and Orton 1983). The present study was undertaken to examine whether changes in chromosomal number or structure could be associated with phenotypic changes at defined genetic loci.

Materials and methods

Plant material consisted of reciprocal F_1 hybrids between *Apium graveolens* L. var 'dulce' cv. 'Tall Utah 52-70 R' (R, Keystone Seed Co.,) and var. 'rapaceum' PI 169001' (Plant Introduction Station, USDA NE Region Geneva, NY) referred to as 112. The hybrids are hereafter designated as R×112 and 112×R (respectively R and 112 as female parents). A single plant each of 112 and R was used as both female and male in making the crosses and single plants of R×112 and 112×R were used as explant donors. R and 112 differed with respect to alleles at four known loci: *Pgm-1, Sdh-1, R-112,* and *A-112; Pgm-1* and *Sdh-1* are linked and separated by 39.2 map units (Arus and Orton 1983). Plants were derived from open-pollinated seed lots which give rise to uniform plant populations. However, a certain degree of segregation could be expected to have occurred since celery is not strictly self-pollinated. Therefore, the nuclear genotypes of R×112 and 112×R, while highly similar, were probably not genetically identical.

Seeds were sown in December, 1979 and young petioles were removed from 8-week-old plants grown in a greenhouse at 22±2 °C under ambient light, prewashed for 1 h in distilled water with one drop of "tween-20" per 100 ml, surface sterilized in 0.6% sodium hypochorite with one drop of "Tween-20" per 100 ml for 20 min under agitation, and washed twice in sterile distilled water. Callus tissues were initiated approximately 6 weeks after placing 1 cm petiole segments on 0.9% agar-solidified (Difco Bacto) MS medium (Murashige and Skoog 1962) supplemented with 0.5 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D, Sigma), 0.6 mg/l furfurylaminopurine (kinetin, Sigma), and 3% w:t sucrose. Callus was thereafter maintained by transfer at 3 to 4 week intervals onto the same medium with 0.1 mg/l 6-benzylaminopurine (BA, Sigma) substituted in the place of kinetin. Cultures were maintained in sealed 100×15 mm petri dishes at 22±0.5 °C in the dark. Regeneration was accomplished by transferring 18-month-old callus tissue to solid MS medium (2% sucrose) lacking hormones, under constant light at 25 °C. About 2 to 3 weeks later, when distinct globular structures were present, tissues were transferred to the same medium with 0.1% w:v activated charcoal, which enhanced further development.

Clones referred to in this study were generated as follows: On December 4, 1980, 150 spatially-separated groups of spatially adjacent cells (100 to 200 per group) each from R×112 and 112×R callus cultures were plated separately on maintenance medium. On January 21, 1981, small samples (100–200 mg) of callus tissue of clones were transferred to fresh medium, and samples from the remaining tissue were used for phenotype determination. Clones were thereafter maintained by dividing in half and transferring to fresh medium every 2 weeks until March 27, 1981 when eight representative clones of each distinct phenotype were chosen and 10 spatially separated groups of adjacent cells, 100–200 cells per group, were plated onto maintenance medium and maintained thereafter by mass transfered every two weeks as above.

On June 10, 1981, callus tissue of the 112×R source culture was introduced into liquid culture and maintained by 1:1 transfer with fresh medium at 10–14 day intervals. On March 15, 1982, the supernatant of the resulting suspension culture was plated onto solid medium. Approximately one hundred colonies which presumably arose from single cells and small clumps of cells were selected and maintained separately on solid medium as described above.

To visualize isozymes, approximately 100 mg of fresh callus tissue was homogenized in 50 µl 0.1 m tris buffer at pH 8.0, with 1.0% w:v reduced glutathione (Sigma), and taken up to saturation into 3×10 mm Beckman electrophoresis paper wicks. Wicks were then inserted sequentially into a 12% w:v horizontal starch gel. The gel buffer was 0.05 M DL histidine at pH 7.0 and the tray buffer was 0.15 M tris and 0.05 M citrate (pH 7.0). Crude extract was eluted from the wicks by applying a potential of 150 V, which produced a current of 25 mA, for 20 min at 4.0±0.5 °C. The wicks were removed and gels were run for 3.5 h at a potential of 200 V. The cathodal section of the gel was discarded, the anodal section was sliced three times horizontally, and the top and bottom slices were discarded. Of the remainder, the top slice was stained for phosphoglucomutase (PGM) activity in an 0.1 m tris buffer, at pH 7.5, containing 0.5 g/100 ml $MgCl_2$, 40 units/100 ml glucose-6-phosphate dehydrogenase (Sigma), 50 mg/100 ml glucose-1-phosphate (Sigma), 50 mg/100 ml 3-4, (5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT, Eastman), 10 mg/100 ml NADP (Sigma), and 10 mg/100 ml phenazine methosulfate (PMS, Sigma). The remaining slice was stained for shikimic acid dehydrogenase (SDH) activity in 0.1 M tris buffer, at pH 7.5, containing 50 mg/100 ml shikimic acid (Sigma), 50 mg/100 ml MTT, 10 mg/100 ml NADP, and 10 mg/100 ml PMS. Staining was complete after incubating for 1–2 h at room temperature in the dark and gels were then fixed in 50% ethanol. It was observed prior to this study that both *PGM-2* and *SDH-1* are expressed in an identical manner in both differentiated tissues of whole plants and callus tissues. Details of the inheritance and linkage of these loci will be addressed elsewhere (Arus and Orton 1983; Orton and Durgan, in preparation).

Callus tissue was prepared for cytological observation by placing a small sample of tissue, 10–14 days after transfer, onto a microscope slide and dispersing cells in a drop of modified carbol fuschin staining solution (Kao 1975). After 10 min, cells were fixed and nuclei well stained. Preparations were squashed with a glass coverslip and sealed with dental wax and could be stored for up to 2 weeks under refrigeration.

Cells in the cytological analyses were sampled from a wide spatial range of each culture entity to insure independence and to estimate the total range of variability as accurately as possible. Chromosome counts were taken from 20 to 25 cells of each culture entity and were unambiguous. A sample size of 100 was used to score cytological aberrations at late anaphase/early telophase.

In histological observations of regenerating embryoids and plantlets, structures at various stages were fixed in 5:2:1

ethanol : glacial acetic acid : chloroform for 24 h at 25 °C. They were passed through three changes of 95% ethanol at 1 °C, 24 h per change, and embedded in JB-4 plastic using recommended procedures (Polysciences, Inc., Warrington, PA, USA). Embedded specimens were glued to wood stubs and 5 μM sections were cut with a glass knife on a Sorvall JB-4 microtome. Sections were rehydrated on a glass slide with distilled water, heat evaporated, stained for 2 s in 1% toluidine blue, and rinsed for 15 min in distilled water. Slides were made permanent with euporal.

Results

Although extreme care was taken to isolate tissue to generate the clones equally and without damage, a significant proportion showed little or growth: 30.7% in 112×R and 36.7% in R×112. On January 21, 1981, all growing clones of R×112 exhibited the heterozygous phenotype at both loci (Table 1). Phenotype determinations conducted on these clones approximately 6 and 12 months later revealed no changes. However, while all 112×R clones retained the heterozygous phenotype at the *Sdh-1* locus on January 21, 1981, tissue samples from 25.8% of the clones had an altered phenotype, PGM-2^S (i.e. the fast-migrating PGM-2 band was not present; Fig. 1).

On March 27, 1981, eight each of representative PGM-^{FS} and PGM-2^S 112×R clones were chosen to characterize further, one of which was later discarded due to contamination. Ten subclones of each selected clone were isolated using the procedures described in the Materials and methods section. On May 6, 1981, the PGM-2 phenotype of subclones was determined. In 6 of 15 clones, a complete change of phenotype was observed from the January 21 determination (clones 8, 41, 42, 45, 48, and 57). The 8, 42, and 45 clones (PGM-2^{FS}) had been previously determined to be PGM-2^S. This phenotypic change could have been a consequence of inadequate sampling of chimeras or bona fide reversion events, but they constituted the only possible instances of PGM-2^S–PGM-2^{FS} reversion observed among the extensive studies of PGM-2^S clones. Apparent mixtures of PGM-2^{FS} and PGM-2^S tissues were detected in clones 38, 40, 83, 86, and 89. The PGM-2 phenotype of the remaining 4 clones was consistent with the January 21 determination. The PGM-2 phenotype of these subclones was stable as determined 1, 2, and 5 months later, with the exception of two PGM-2^{FS} subclones (one each of clones 42 and 48) which changed to PGM-2^S.

On August 27, 1982, the PGM and SDH phenotypes of the clones derived from 120 colonies of the 112×R suspension culture (Materials and methods) were determined (two replications). All of the clones were PGM-2^S, SDH-^{FS} (Table 1).

Although the data were not subjected to rigorous statistical tests, notable differences were apparent 112×R clones with respect to chromosome number (Table 2). Some clones were completely hypodiploid (counts of 9 to 21; sublines 42, 89) while most others consisted primarily of mixtures of diploid (2n=22) and hypodiploid cells. Some clones exhibited 25% or more of cells in the polyploid range (≧ 37; clones 40, 100) while others exhibited no detectable polyploidy (clones 42, 83, 86, 89). Modal clusters of chromosome numbers were generally observed in the 17–21 (hypodiploid) and 37–43 (hypotetraploid) ranges.

No patterns of concomitant PGM-2 phenotype and karyological makeup could be discerned. For example, clones 3, 48, and 78 always exhibited the PGM-2^S phenotype, but chromosome constitution was quite different. Clone 3 consisted entirely of hypodiploid or hypotetraploid cells while both 48 and 78 contained presumptive diploid and tetraploid cells. Clones 88 and 100 were PGM-2^{FS}, although 88 was primarily hypodiploid and 100 was about half hypodiploid and half hypotetraploid. Likewise, the incidence of cell division anomalies was not associated with PGM-2 phenotype (Table 2).

Table 1. PGM-2 and SDH-1 phenotypes among clones of reciprocal F_1 crosses

Callus derived from (reciprocal heterozygote)	No. with phenotype					
	F	FS	S	F	FS	S
R×112 (January 21, 1981)	0	96	0	0	96	0
(February 4, 1982)	0	96	0	0	96	0
112×R (January 21, 1981)	0	69	24	0	93	0
(August 27, 1982)	0	0	120	0	120	0

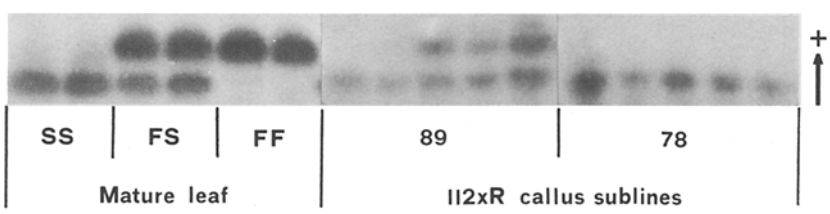

Fig. 1. PGM-2 phenotypes of mature leaf and calus tissue. Left six lanes depict those of mature leaf tissue; *lanes 1 and 2 PGM-2^{SS}*; *lanes 3 and 4 PGM-2^{FS}*; *lanes 5 and 6 PGM-2^{FF}*. Right lanes depict representative PGM-1 phenotypes of 5 subsamples each of 112×R *clones 89 and 78*. Clone *89* consisted of a mixture of PGM-2^S and PGM-2^{FS} tissues while clone *78* was uniformly PGM-2^S

Table 2. PGM-2 phenotype, chromosome numbers, and frequency of cytological anomalies at anaphase/early telophase among 15 112×R clones from which subclones were isolated March 27, 1981. Tissues for cytology were fixed at the time subclones were isolated. PGM-2 phenotypic determinations were conducted on May 6, 1981

Clone	PGM-2 Phenotypes of 10 subclones	No. of cells out of 20 with chromosome number											No. of anaphase/telophase cells/100 exhibiting			
		15	16–18	19	20	21	22	23–29	30–35	36–40	41–43	44	45	Chromatin bridges	Lagging chromosomes	Multipolar divisions	Fragments
3	10 (S)	0	3	4	5	6	0	0	0	1	1	0	0	13	8	2	0
41	10 (S)	0	1	5	8	6	0	0	0	0	0	0	0	13	0	0	0
48	10 (S)	0	1	0	7	0	8	1	0	1	1	0	1	48	11	2	0
57	10 (S)	0	1	1	2	6	5	1	0	2	2	0	0	26	9	3	3
78	10 (S)	0	0	1	6	7	5	1	0	0	0	1	0	40	2	3	3
8	10 (FS)	2	0	1	3	9	2	0	1	0	1	0	1	48	3	0	0
42	10 (FS)	0	0	0	1	2	9	8	0	0	0	0	0	59	12	2	0
45	10 (FS)	0	0	0	1	5	8	3	0	0	0	2	1	65	1	0	3
88	10 (FS)	0	2	2	6	7	2	0	0	1	0	0	0	15	6	0	2
100	10 (FS)	0	0	3	4	4	0	0	0	4	3	1	0	55	0	1	2
38	8 (S) + 2 (FS)	0	0	3	4	3	1	2	0	1	5	0	1	18	0	0	0
40	4 (S) + 6 (FS)	0	2	2	9	4	0	0	0	2	1	0	0	25	2	2	0
83	3 (S) + 7 (FS)	2	2	6	2	4	3	1	0	0	0	0	0	52	13	0	4
86	9 (S) + 1 (FS)	0	1	0	2	6	10	1	0	0	0	0	0	23	2	3	5
89	6 (S) + 4 (FS)	0	0	0	3	17	0	0	0	0	0	0	0	8	1	0	6

It was reasoned that the 15 clones may have contained karyological differences unrelated to the PGM-2 phenotype, due to relatively distant clonal relationships. To minimize the problem of background variability, subclones of one of the clones isolated on March 27, 1981 exhibiting mixtures of PGM-2^{FS} and PGM-2^{S} cells were contrasted. It was reasoned that background variability should be minimized because the tissues were related by a very small group of cells. Clone 40 was used for this analysis, consisting of 6 PGM-2^{FS} subclones and the remainder PGM-2^{S} (Table 3). These phenotypes remained stable until August 27, 1982 when subclone 4 changed from PGM-2^{FS} to PGM-2^{S}.

Among 25 sampled metaphase cells of each of the PGM-2^{FS} subclones, a considerable degree of variability was again observed with respect to chromosome number. All of these subclones had cells with hypodiploid chromosome numbers (16 to 21) and nearly all had one or more polyploid cells. In contrast PGM-2^{S} subclones were highly heterogeneous with respect to chromosome number. Subclones 3 and 8 were similar, and different from PGM-2^{FS} subclones in that they were completely void of cells with 21 or 22 chromosomes. Subclone 7 was mainly hypotetraploid, but subsequent counts not reported have revealed a significant representation of hypodiploid cells, particularly $2n=20$ and 21. Subclone 10 exhibited chromosome numbers which were not strikingly different from the PGM-2^{FS} subclones. In some, the chromosome number and structural rearrangement data among subclones of clone 40 failed to establish a clear distinction between PGM-1^{FS} and PGM-1^{S} subclones. Moreover, subsequent, more detailed karyological analyses have also failed to establish consistent differences in chromosome number and structure between PGM-2^{FS} and PGM-2^{S} subclones. However, the analyses lacked sufficient resolution to identify reciprocal translocations or small deletion events involving small chromosome segments.

Attempts were made to regenerate plants from all of the clones listed in Table 2. Most showed no organized response under conditions which have normally yielded somatic embryogenesis in celery cultures (Williams and Collin 1976; Orton 1983). Green bipolar embryoid structures were observed regenerating from callus cultures of clones 57, 89, 40-subclone 4, and 40-subclone 5. In all of these cases, somatic embryoids occurred in a discrete sector (or sectors) of the culture, and not from all callus tissue, suggesting that variability with respect to competence to regenerate was present within clones. Those of 40-subclone 4 and 40-subclone 5 (Table 3) were grossly abnormal, rarely having recognizable shoots or roots. Embryoids of clones 57 and 89 were morphologically normal until axis elongation was initiated. When

Table 3. PGM-2 phenotypes and chromosome numbers among 10 subclones of 112×R-40. Tissues for cytological studies were fixed on May 6, 1981

Subclone	PGM-2 phenotype	No. of cells with chromosome number (25 total)											
		15	16–18	19	20	21	22	23–29	30–35	36–40	41–43	44	45
1	FS	0	3	2	4[a]	13	1	0	0	1	0	0	1
2	FS	0	1	1[a]	7	13	1	0	1	0	1	0	0
4*	FS	0	0	3[a]	4	17[a]	0	0	0	0	0	0	1
5	FS	0	2	2	8	11	0	0	0	0	2	0	0
6	FS	0	0	0	5[e]	9[a]	10	1	0	0	0	0	0
9	FS	0	2[f]	2[a]	8[a]	8[b]	3	0	0	2	0	0	0
3	S	0	1	3	19	0	0	0	0	2	0	0	0
7	S	0	1	0	0	0	0	0	1	15[c]	6	0	2
8	S	0	4[b]	7[d]	10	0	0	0	1	3[a]	0	0	0
10	S	1	1[a]	2	5	11	4[a]	1	0	0	1	0	0

* Changed to PGM-2^S as per August 27, 1982 phenotype determination
[a] One cell with one dicentric; [b] Two cells with one dicentric; [c] Three cells with one dicentric; [d] Four cells with one dicentric; [e] Two cells with 2 dicentrics; [f] One cell with 3 dicentrics

embryoids were 5 to 15 mm long and had recognizable root and presumptive shoot structures, growth ceased and chlorosis was observed. Histological observations of regenerating embryoids of clones 57 and 89 revealed a progressive decline in meristematic activity at the apex and primary root, and an increase in meristematic activity of cortical cells proximal to the apex. These regions appeared to develop further into discrete independent globular embryoids, which presumably began a new cycle of development and decline.

Callus of clone 57 was PGM-2^S while that of 89 consisted of a mixture of PGM-2^{FS} and PGM-2^S tissues (Table 2). The phenotypes of plantlets regenerating from clone 57 were all PGM-2^S, while those of 89 were all PGM-2^{FS}. Chromosome numbers in root tips of subline 57 regenerates were almost all $2n=21$, and comparisons of ten karyotypes suggested that they were monosomic for one long acrocentric chromosome (Fig. 2). Chromosome counts were more variable among subline 89 regenerates, but most (77%) were $2n=21$. Karyotype analyses of six $2n=21$ cells of clone 89 showed the presence of a long submetacentric chromosome not in the original karyotype and possible rearrangements involving the metacentric chromosomes. By comparison, plantlets regenerated from 5-month-old callus of a *Pgm-2*^{FS}, *Sdh-1*^{FS} 112×R F_2 plant were morphologically, electrophoretically, and karyologically normal (Table 4, Fig. 2).

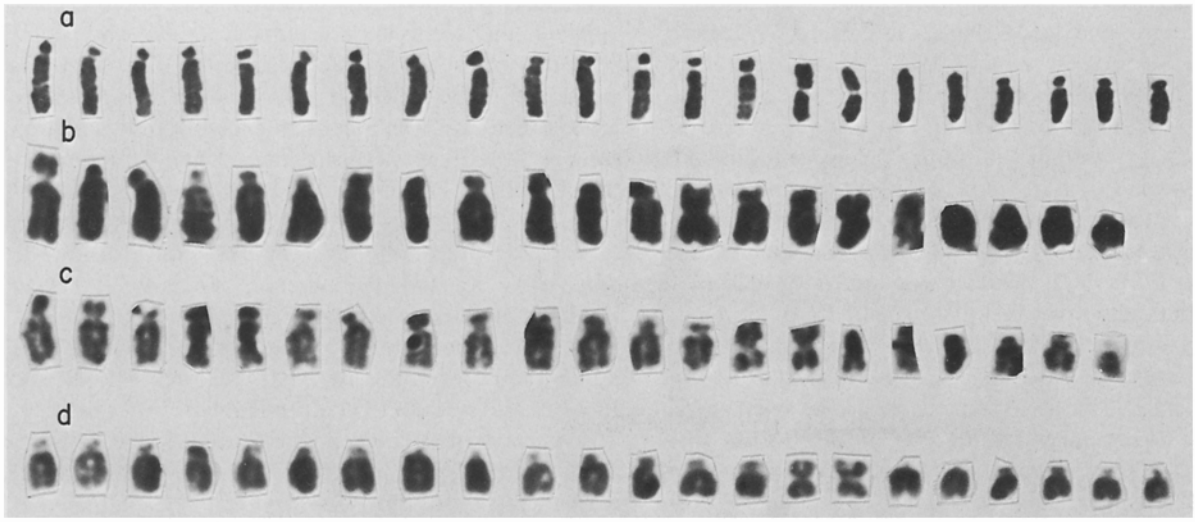

Fig. 2a–d. Root tip karyotypes of plantlets regenerated from callus cultures: **a** native karyotype of seed-propagated celery; **b** abnormal plantlet regenerating from callus of clone 89 (PGM-2^{FS}); **c** abnormal plantlet regenerating from callus of clone 57 (PGM-2^S); **d** normal plantlet regenerating from callus of 112×R F_2-5

Table 4. Root tip chromosome counts and PGM-2 phenotypes among regenerated plants

Clone	PGM-2 Phenotype	No. of plantlets with chromosome number						
		19	20	21	22	24	42	Total
57	PGM-2^{S}	2	0	48	0	0	1	51
89	PGM-2^{FS}	1	3	27	3	1	0	35
112×R F_2-5	PGM-2^{FS}	0	0	0	33	0	0	33

Discussion

When 112×R tissues were introduced into culture, an instability was observed at the *Pgm-2* locus resulting in a gradual shift over two and a half years from PGM-2^{FS} in the original explant tissue to 100% PGM-2^{S}. The *Sdh-1* locus was completely stable in 112×R cultures and phenotypes at the *R-112* and *A-112* loci could not be determined since normal plants could not be regenerated. In contrast, phenotypes at both *Pgm-2* and *Sdh-1* were stable in R×112, which differed from 112×R with respect to cytoplasm and segregation at an undertermined number of nuclear loci.

The bulk of evidence suggests that stable events occur in single PGM-2^{FS} cells which result in the loss of the activity of the fast-migrating electromorph. Since no convincing instance of reversion was observed, it would tentatively appear that the conversion is unidirectional. As the frequency of independent conversion events increases over time, the corresponding frequency of PGM-2^{S} cells increases to complete takeover. Alternatively, the frequency of such events may be quite rare, and PGM-2^{S} cells in culture have some selective advantage over PGM-2^{FS} cells. Among six PGM-2^{FS} subclones of 112×R clone 40, only one PGM-2^{FS}–PGM-2^{S} conversion was observed over 18 months of continuous culture. However, putative mixtures of PGM-2^{FS} and PGM-2^{S} tissues would, except in cases where PGM-2^{S} cells predominated, have been identified as PGM-2^{FS}. Some quantitative variation in PGM-2^{F} activity was noted in PGM-2^{FS} clones, but densitometric measurements were not made. Hence, the five clone 40 subclones which appeared to be stable over this period could, in fact, have undergone PGM-2^{FS}–PGM-2^{S} conversion events resulting in mixtures. Further separation of these tissues into constituent clones would serve to test this possibility.

Table 5 lists the possible causes of the altered PGM-2 phenotype observed in this study. Epigenetic mechanisms, such as differential gene transcription, transcript processing, translation, and activation or degradation of protein could all conceivably result in a hemizygous PGM-2 phenotype in a normal diploid cell. Differences in isozyme activity have been observed among different tissues of the same plant (Woodman and Freeling 1981). Such differences have been shown to persist in corresponding callus and suspension cultures of *Phaseolus vulgaris* (Arnison and Boll 1974, 1975). Moreover, different glutamate dehydrogenase phenotypes were observed in carrot cultures under different 2,4-D regimes, concluded to be due to induced developmental changes (Lee and Dougall 1973). The genetic contribution to the total variation in the studies with bean and carrot was unknown, although Arnison and Boll (1975) speculated that some of the variation could have been a consequence of karyological instability. Woodman and Freeling (1981) showed that preferrential tissue-specific allele expression in heterozygotes at the *Adh-1* locus in maize was due to cis-acting control sequences. While it is conceivable that a similar phenomenon was responsible for differential PGM-2 expression in the present study, some evidence is clearly contradictory: 1) no activity at the PGM-2^{F} band position was detected, while differential ADH-1 expression in maize was shown to be quantitative; 2) the ADH-1 phenomenon was observed to have reciprocal properties in maize, while no such reciprocity was observed in this study; 3) no differential allele expression has been seen in any of the extracts of tissues from seedlings or mature plants, and 4) the hemizygous PGM-2^{S} phenotype was expressed in regenerated plants (of clone 57) at developmental stage corresponding to that in which the normal PGM-2 phenotype is expressed by heterozygotes. Hence, differential allele expression as a function of differentiated state seems, in this case, highly unlikely.

Differential gene amplification could conceivably generate a phenotype resembling loss of allele expression but this seems highly unlikely in the absence of selection pressure for the product of one allele as compared to the other. It is also unlikely that loss of allele expression was a consequence of point mutation since it would be necessary to postulate impausibly high selective advantage of PGM-2^{S} over PGM-2^{FS} cells.

Somatic recombination could conceivably be responsible for the phenomenon, since the expected products of somatic recombination between a centromere and a heterozygous marker are two clones of cells homozygous for different alleles. However, only the PGM-2^{S} and not the PGM-2^{F} phenotype was observed. This phenomenon could possible occur if the PGM-2^{F} allele were lethal or linked to a recessive lethal allele masked in the original heterozygote. The finding that PGM-2^{FF} F_2 progeny of the original 112×R F_1 plant were both viable and give rise to normal callus tissue (Orton, unpublished) refuses this possibility.

Table 5. Possible causes of loss of allele expression in heterozygous callus tissue and expected properties

Cause	Manifestation or consequence
1. Developmental (epigenetic) change	– May or may not revert at high rate – Involves homologous loci, not one allele or the other of the same locus
2. Unstable genetic change (gene amplification)	– Unstable in the absence of selection pressure
3. Stable genetic changes	
(a) Point mutation	– Rare in the absence of differential allele fitness
(b) Somatic recombination	– Reciprocal products
(c) Chromosome loss	– Hypoploidy, subvitality; consistent karyological differences between PGM-1^S sublines
(d) Deletion, inversion, translocation	– Chromatin bridges, chromosome, structural alterations, subvitality; consistent karyological and/or cytogentic differences between PGM-1^S and PGM-1^{FS} sublines.
4. Integration of insertion sequences	– May or may not be stable – May be allele-specific

The altered PGM-2 phenotype could have also been a consequence of chromosome loss or deletion. This would require the assumption that karyotype evolution was such that PGM-2^F cells were not generated or that they were selectively unfit as compared to PGM-2^S cells. Evidence for directional karyotype evolution has been reported previously in cultures of *Haplopappus* (Singh et al. 1975) and *Crepis* (Ashmore and Gould 1981). In contrast to these findings, a wide range of karyological variation was observed to persist in cultured celery cells.

Cultures of R×112, which exhibit no instability at the *Pgm-2* locus (Table 1), were consistently more variable with respect to chromosome numbers and structural alterations than equivalent 112×R cultures (M. Murata, personal communication). Moreover, no consistent gross karyological differences could be found between PGM-2^{FS} and PGM-2^S clones of 112×R although smaller changes would have been progressively more difficult to detect. Finally, the *Pgm-2* and *Sdh-1* loci are linked (Arus and Orton 1983), and chromosome loss would have resulted in concomitant phenotypic change in both, which was never observed. This would make a further assumption necessary that only subchromosomal fragment loss was responsible. While none of the evidence directly rules out karyological instability as the underlying cause of PGM-2 phenotypic instability, the sum of circumstantial evidence points to the conclusion that this is unlikely.

Attempts to investigate the genetics of the variant PGM-2^S phenotype have been stymied by the failure of these cultures to regenerate normally. It is highly unlikely that PGM-2^S cells arose as a consequence of transient or nongenetic causes since the phenotype is stable in culture and transmitted to regenerated plantlets. However, the characteristics of the phenomenon were incongruent with common sources of genetic variability (Table 5). It is still possible that selective inactivation by controlling element-like sequences could be responsible, but not enough is known about the activity of such elements to make conclusive judgements. The reciprocal effects suggest a possible relationship to the phenomenon of hybrid dysgenesis, described by Kidwell et al., (1977), which implicates the activity of insertion sequence in selective mutation. Attempts are in progress to isolate and clone the PGM-2 coding genes of celery, and to make comparisons among PGM-2^S and PGM-2^{FS} cell subclones of clone 40 to test this hypothesis.

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