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Secondary metabolism in cultured red beet *(Beta vulgaris* L.) cells: Differential regulation of betaxanthin and betacyanin biosynthesis

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Abstract

Red beet cell lines exhibiting a range of cell colours were generated from secondary callus via specific induction methods. Phenotype colour ranged from white/green through yellow, orange and red to deep violet, representing all types of pigments found in red beet plant. Specific phenotypes could only be obtained through specific induction sequences and once established were stabilised by cultivation on a maintenance medium. The ratio of auxin (2, 4-D) to cytokinin (6-BAP) was an important factor in the control of these processes. All coloured phenotypes were linked, but could be classified into two main groups, one yellow-red and the other orange-violet, according to their different cellular morphologies. A certain amount of instability still existed within each group. Modification of the growth regulator composition could be used to interchange specific combinations of coloured phenotypes, depending upon the initial state of cellular differentiation. Use of the DNA-methylation inhibitor 5-azacytidine demonstrated that methylation plays a key role in the repression of genes encoding enzymes involved in betacyanin biosynthesis. Furthermore, the poly(ADP-ribose) polymerase inhibitor 3-methoxybenzamide blocked the induction of the same gene set in a concentration dependent manner without affecting cell growth.

Introduction

The mechanisms underlying cellular differentiation and the subsequent stabilisation of cell function are little understood. Observation of re-differentiation phenomena in cultured plant cells, maintained under strictly defined growth conditions, may provide an alternative approach to the study of mechanisms underlying the origin and maintenance of such differentiation events. It has frequently been observed that constituents (e.g. secondary metabolites) synthesised in intact plants are not always present in cultured tissues (callus and cell suspensions) of the same plant. In this context, numerous examples of specific phenotypic changes have been reported that concern variations in primary [1] and secondary metabolism [2-4]. This variability is largely heritable [5] and the frequency of these events is too great to be accounted for by simple base changes or deletions. In addition, the degree of variation can be influenced by components of the medium [6], but it occurs at a constant frequency under stable culture conditions [7]. A number of possible mechanisms exist by which variation occurs including, karyotypic changes [8,9], DNA rearrangements [10], gene amplification [11,12] and deletion/insertion events (transposable elements) [13,14], but no direct causal connection between the alteration and any phenotypic character has been demonstrated.

Betalains, a class of water soluble nitrogenous plant pigments characteristic of the *Centrospermae*, exhibit diverse patterns of colouration ranging from yellow to deep violet through vari-

ous shades of orange and red. The colour of red beet root *(Beta vulgaris* L.) is due to the presence of the deep red /violet betacyanins and the yellow betaxanthins contained within the cell vacuole. A betalain biosynthetic pathway (Fig. 1) has been proposed from tracer experiments [15,16].

The accumulation of betacyanins in cultured tissues was first reported in red beet calli by Constabel & Nassif-Makki [17] and has also been observed in cell cultures of *Portulaca grandiflora* [18], *Chenopodium rubrum* [19] and *Phytolacca americana* [20]. Only Weller & Lasure [21] have reported the occurrence of yellow cells in tissue culture.

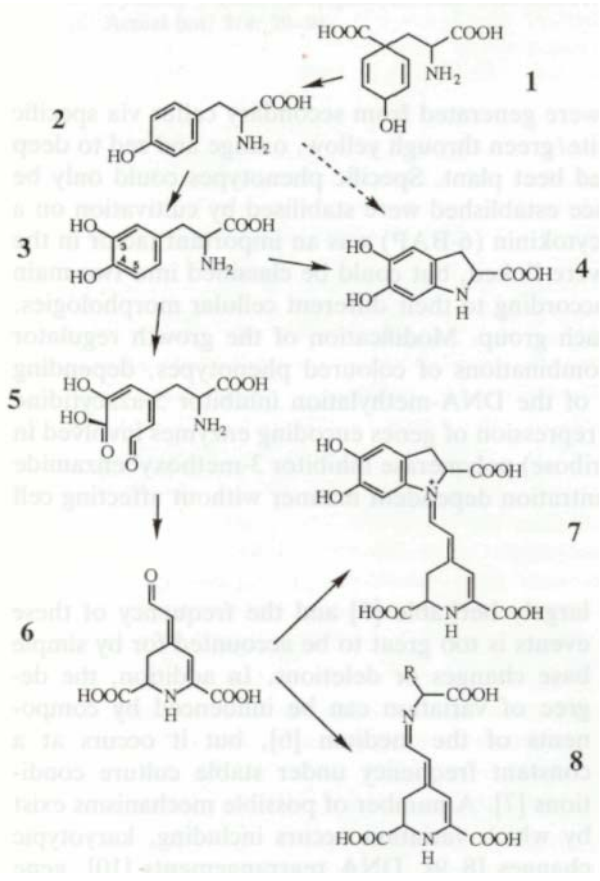

Fig. 1- The pathways of betalain synthesis. The pigment chromophore. betalamic acid [6], could be derived from dihydroxyphenylalanine (DOPA [3]) by extradiol-ring cleavage between C-4 and C-5 which would produce seco-DOPA [5| and subsequent bonding of nitrogen to C-3. The betalamic acid [6] may condense with the amino- or imino-group of aminoacids to give the yellow betaxanthins [8] or with the imino-group of cyclo-DOPA [4] to produce the violet betacyanins [7]. [1] arogenic acid. [2] tyrosine. [3] DOPA. [4| cyclo-DOPA [5] seco-DOPA. [6] betalamic acid. [7] betacyanins. |S| betaxanthins.

In the present study, we describe the establishment of red beet cell lines displaying white, green, yellow, orange, red and violet pigmentations, a pattern of colouration similar to that exhibited by the flowers of betalain production species, and representing the overall pathways of betalain metabolism. These data show that genetic pathways which may be repressed at the whole plant level may, under given conditions. be activated in tissue culture.

Materials and methods

Media composition

Two basal media were used, H1 and J1, both of which were supplemented with sugars and growth regulators (Table 1). The pH was adjusted to 5.8 with KOH, and 6 g l^{-1} Agar (Merck) was added prior to autoclaving. Iron was prepared as a complex of EDTA as follow: 4.1 g of $FeCl_2$ $4H_2O$ and 8.1 g K_2EDTA $2H_2O$ were dissolved separately in 500 ml H_2O. Both solutions were boiled, allowed to cool and mixed. The volume was made up to 1 l. This solution was incorporated to the medium at the time of preparation.

Plant material and establishment of in vitro cultures

Cell lines used in this study were derived from the red beet cultivar *Bikores monogerm.* Seeds were surface sterilised by immersion in ethanol for 30s followed by immersion in a 25% calcium hypochlorite solution containing 0.25% Tween 80 and finally washed, thoroughly, in sterile water. Seeds were soaked in liquid MS [22] medium in the dark at 30°C. After 3 days the seeds were again sterilised by successive immersion in

- ethanol for 30 s,
- 0.05% $HgCl_2$ containing 0.25% Tween 80 for 10 min,
- 5% K_2EDTA containing 1.5% H_2O_2 for 10 min, and finally rinsed 3 times in sterile water.

Sterile seeds were sown on the surface of solidified MS [22] medium and incubated at 27°C under a 16 h photoperiod using daylight Grolux Sylvania WS (wide spectrum) fluorescent tubes (fluence 6mW/m^2).

Seedling tissues were vegetatively propagated by subculturing the apical meristem every four weeks onto growth regulator-free H130 medium (Table 1).

Callus initiation and maintenance

Calli were initiated from seedling hypocotyl and cotyledonary explants and from the leaves and petioles of plantlets by transfer to solidified MS [22] and 5H110 media (Table 1) containing either 300 or 500 μg l^{-1} 6-benzyladenine (BA) and supplemented with 2,4-dichlorophenoxyacetic acid (2, 4-D) at a range of concentrations: 50, 100, 300 μg l^{-1}. Tissues were maintained at 27°C under low intensity illumination (6 mW/m^2). Callus initiation occurred after 3-4 weeks. Friable strongly proliferating sectors of primary callus were transferred to induction media containing reduced growth regulators concentrations. (Table 1) The coloured sectors were then subcultured every 7 days on to fresh media.

The inhibitors 3-methoxybenzamide (3-MBA; Aldrich) and 5-azacytidine (5-azaC; Sigma) were dissolved in liquid culture medium at 60°C and filter sterilised prior to incorporation into autoclaved medium.

Table 1. Composition and labelling of the media used for callus induction and culture.

	H1	J1
Macronutrients (mg l^{-1}):		
NH_4NO_3	825	825
KNO_3	950	1900
$CaCl_2 2H_20$	200	150
$MgSO_4 7H_2O$	185	185
KH_2PO_4	85	85
$FeCl_2 4H_2O$	10.25	10.25
$K_2EDTA_2H_2O$	20.25	20.25
Xylose	-	250
Micronutrients: Half strength MS [22] microelements		
Vitamins (mg l^{-1}) :		
Pyridoxine HCI	0.68	0.68
Thiamine HCI	0.68	0.68
Nicotinic acid	0.68	0.68
Ca2+ Panthothenat	0.68	0.68
Biotine	0.068	0.068
Folic acid	6.8	6.8
Meso-Inositol	110	110

Sugars (g l^{-1}):

	Medium type	Mannitol	Sucrose
1	5H110	5	10
2	5J110	5	10
3	H130	-	30
4	J130	-	30

Growth regulator combinations (μg l^{-1}):

	2,4 D	6-BAP
1	100	500
2	50	500
3	20	100
4	20	50
5	100	-
6	50	-
7	20	-

Isolation of betalains

Cells were weighed, frozen in liquid nitrogen, lyophilized and the dry weight determined. Prior to extraction, cells were ground to a fine powder using a mortar and pestle. Pieces of storage root, from the same beet variety, were prepared following the same procedure.

The powder was extracted, on ice, with 200 ml of methanol: HCl 0.2% (80:20) per g dry weight. After 30 min incubation, the mixture was centrifuged at 20,000 *g* for 30 min at 2°C. The pellet was re-extracted under the same conditions and the pooled supernatants evaporated to dryness in a Rotavapor (Büchi) under reduced pressure at 35°C. The residue was dissolved in 50 volumes deionised water (NANOpure, Barnstead) and centrifuged at 10,000 *g* for 20 min at 2°C. The aqueous betalain extract was stored at -20°C prior to analysis.

Betalains separation

Apparatus. The HPLC system (Spectra Physics) consisted of an SP8750 organiser equipped with a ternary valve; an SP8700 XR ternary controller, a dynamic SP8500 mixer; a Rheodyne automated injector fitted with a 20 μl injection loop; a guard column (20 x 4.6 ID mm) containing LiChroprep RP18 gel; a 3 μm spherical

packing Hyperchrome column (250 x 4.6 ID mm) (Bischoff) containing Hypersil ODS RP18 (Shandon). An HP ChemStation (Hewlett Packard) was used for column eluent analysis. Detection was routinely undertaken at 280, 310, 424, 480 and 536 nm.

Procedure. HPLC grade water (NANOpure, Barnstead) was used for the preparation of the mobile phase buffer systems. Buffer A contained 50 mM NaH_2PO_4 and 2.5 mM triethylamine and was adjusted to pH 4.20 with phosphoric acid. Buffer B consisted of a 40% acetonitrile solution. The system was operated at a flow rate of l.0 ml min-1 using the analytical gradient program shown:

Time(min)	% Buffer A	% Buffer B
0	100	0
15	70	30
20	40	60
25	20	80
30	20	80

Pigment identification. Peaks were identified by co-migration with standards. Betaxanthin standards were prepared following the procedure described in [23] from crystallised betanin extracted from red beet. The concentration of individual compounds was determined from their respective molar extinction coefficients:

Compound	λ max(nm)	ε
DOPA	280	3,000
Tyr	280	1.400
Dopamine	280	2,700
Betalamic acid	424	25,000
Betaxanthins	480	48,000
Betacyanins	536	56,000

Results and discussion

Establishment of coloured cell lines

Once plated on solid media, all explants, except those from petioles, exhibited marked increases in size and became red in colour. Observation of the tissue surfaces revealed a mosaic of red and non-coloured epidermal cells. Primary callus developed at the cut edges of the explants within 4 weeks; these were mainly composed of white and green cell masses with a few red sectors and were very compact. No qualitative differences in callus phenotypes were observed with regard to the origin of the explant tissue or the composition of the media used for callus initiation.

The actively growing calli were transferred to fresh media supplemented with growth regulators at a range of concentrations (Table 1). During subcultivation of the secondary calli, groups of cells displaying green, yellow, orange, red and violet pigmentation appeared over the callus surface, depending on the composition of the medium used. Gradually, with repeated sub-culturing the individual callus phenotypes were stabilised (Fig. 2). The continuous accumulation of alkaloid-type pigments throughout the growth cycle was indicative of constitutive betalain biosynthesis.

The different callus phenotypes were associated with cells of markedly different morphologies (Fig. 3), which could be classified into two groups:

—white, orange and violet phenotypes, which consisted of a mixture of small cell aggregates and single, elongated cells which arose from the periphery of the cell clumps (Fig. 3, O and V) and

—green, yellow and red phenotypes, which were composed of clusters of small spherical cells (Fig. 3, Y and R).

In addition, small roots exhibiting orange and violet pigmentations were occasionally observed on old orange and violet calli, respectively (results not shown) suggesting that the program of development leading to either the orange or violet phenotype is part of a morphogenetic program. Consequently, orange and violet calli appeared to be mosaics of different stages of differentiation, possibly reminiscent of root organisation.

A strict relationship was observed between cell phenotype and the composition of the induction medium as described in Fig. 4. The tree lineage gave the subculture history of all cell lines ob-

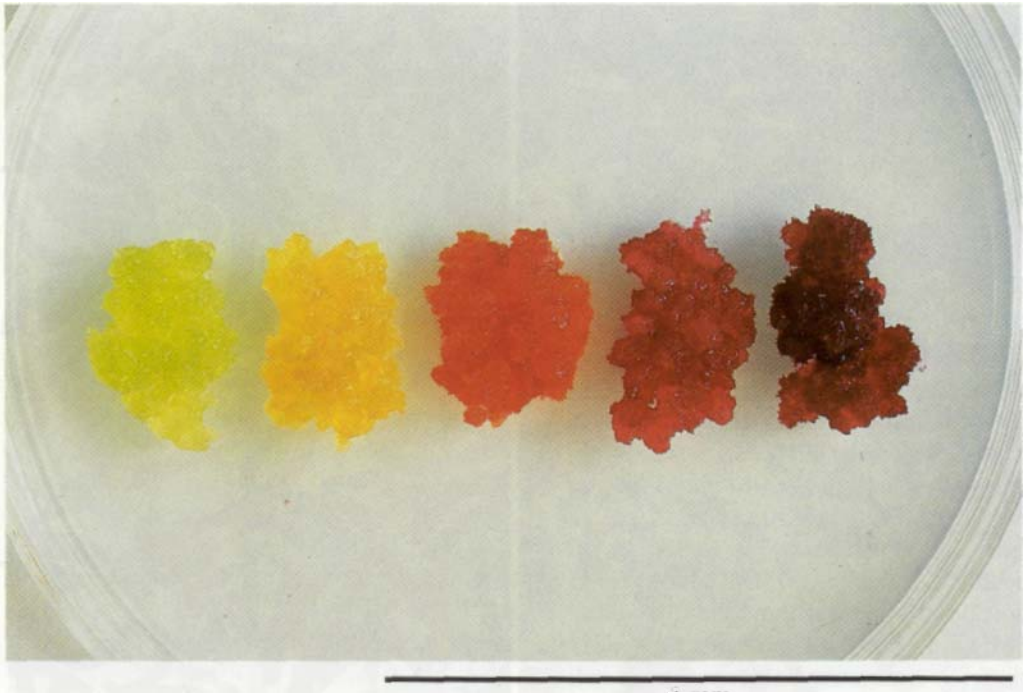

Fig. 2- The five basic inherited stable phenotypes of red beet cell cultures. Left to right: green [G], yellow [Y], orange [O|, red [R] and violet |**V**|**.**

tained from cotyledonary (C) or leaf (L) explant of three distinct plantlets (namely 3, 6. 7). The yellow phenotype from explant C7 was obtained by maintaining cells on 5H110 medium supplemented with 0.l mg l^{-1} 2,4-D whereas the same phenotype was obtained at a concentration of 0.02 mg l^{-1} 2,4-D from explant L3. However, this line was very aggregated and grew slowly in this culture condition. The orange and violet phenotypes could only be generated on 5J110 supplemented with 0.1 mg l^{-1} BA and 0.02 mg 1^{-1} 2,4-D. Once established, some phenotypes were supported by media different to that used for their initiation. For example, the yellow phenotype was maintained on 5H110 medium containing 0.05 mg 1^{-1} of 2,4-D, while the orange phenotype was maintained on H130 medium containing 0.02 mg l^{-1} 2,4-D. In contrast, the violet cell line from the C6 explant (Fig. 4) became destabilised when transferred to H130 medium supplemented with 0.02 mg l^{-1} 2,4-D and an increased sucrose content, with cellular pigmentation becoming variable. The violet cell line was. therefore, maintained on 5J110 medium under the original regime of growth regulators.

Individual cellular phenotypes appeared not only to be a function of their immediate environment, but also of their culture history. When given the appropriate environmental stimuli, such as growth regulators and medium composition, red beet cells appear to respond by switching on or off a series of reactions which commit the cells to a specific developmental pathway. In so doing, these cells become committed to the expression of gene sequences characterised by specific patterns of betaxanthin and betacyanin production and accumulation. Once engaged on a specific pathway of differentiation cells become committed to the specific coloured state associated with that pathway.

The selective expression of betaxanthin and betacyanin appeared to occur through a limited number of discrete, stable, differentiated states, since only four coloured phenotypes could be isolated and not a range of phenotypes exhibiting a continuous range of colouration. Orange and red phenotypes, which were not directly related (Fig. 5), could be maintained on media of the same composition (H130 + 0.02 mgl^{-1} 2,4-D) indicating that once a cell line has been directed

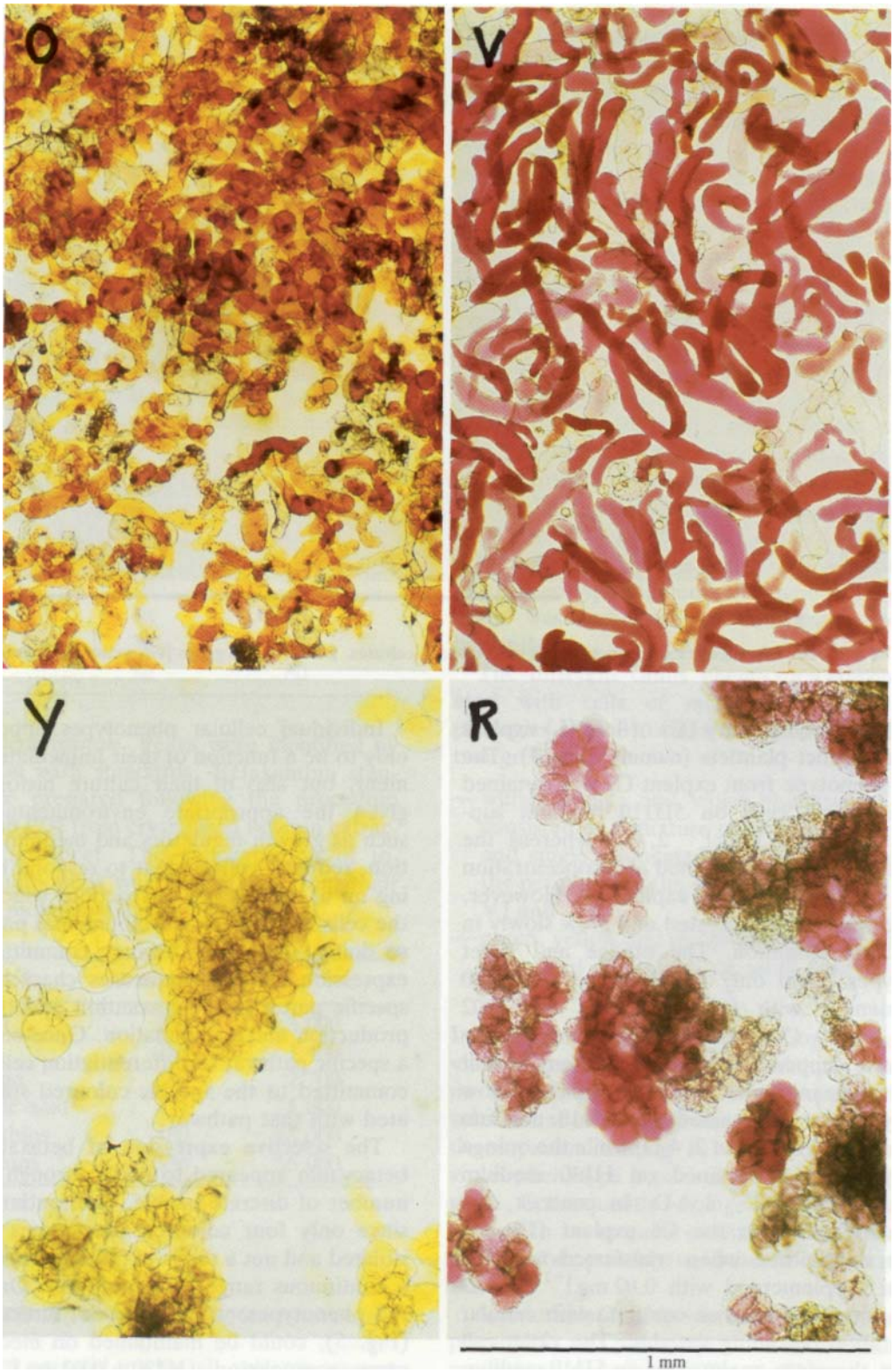

Fig. 3. Micrographs of red beet cells: from top left to bottom left (clockwise): orange [O]. violet [V], red [K] and yellow [Y] cells showing the morphology corresponding to each coloured phenotype. Due to the high friability of the calli, cells scattered spontaneously when dropped in a 7% mannitol solution.

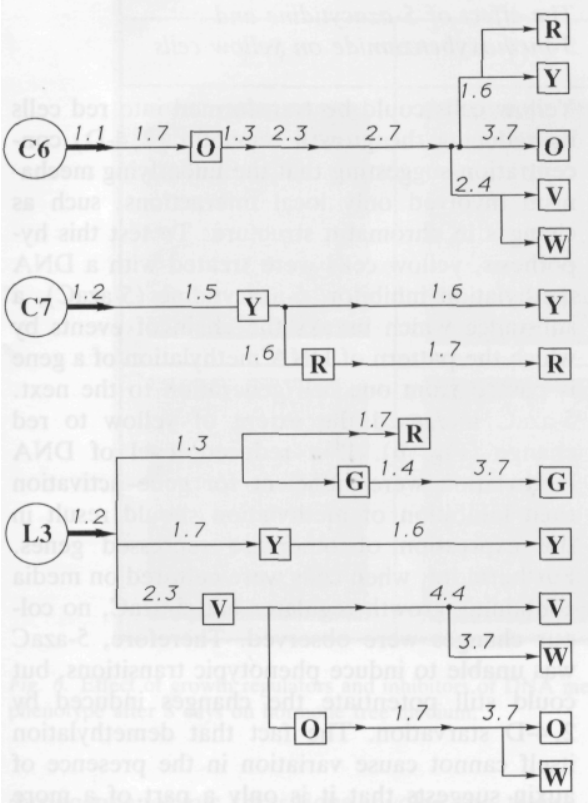

Fig. 4. Highly schematic diagram describing the establishment of all phenotypes ([O] orange, [Y] yellow, [R] red, [V] violet, [G] green, [W] white) from three distinct explants (C: cotyledon; L: leaf). See Table 1 for media composition: the first digit represents the medium type and the second the growth regulator composition. The length of the thick arrow represents 4 transfers.

along a particular developmental pathway that any subsequent developmental changes were restricted. The orange and red cell lines appeared to exhibit a differential perception of the same environmental stimuli, indicating some degree of cell autonomy inherent to the two differentiated states.

Stability of the phenotypes

Under culture maintenance conditions, the differently coloured cell lines exhibited a high degree of phenotypic stability. Periodically, however, some cells were spontaneously changed from one coloured state into another. The nature and extent of these phenotypic interconversions was limited. For example, red cell clusters appeared more frequently over the surface of yellow calli than did yellow cells over red calli. Such transitions, however, were not observed between orange and violet calli. Yellow as well as red cells clusters developed on the green phenotype, while violet cells only appeared on orange phenotypes. Only orange cells and white cells appeared on callus of the violet phenotype (Fig. 5).

These data were indicative of the presence of specific phenotypic interconversion sequences, with certain phenotypes not being directly interconvertible (Fig. 5). In terms of phenotypic interconvertability, the red cell line was more closely related to the yellow and green lines than to the orange and violet cell lines. The changes of phenotypes appeared to be restricted to those differentiated states which had been initiated under similar environmental conditions (Fig. 4) and, which it was assumed, had followed similar developmental processes. Certain inherited states, i.e. the yellow and orange or the red and violet, were so divergent in character that they could be considered as separate clones. Stabilisation and maintenance of the individual phenotypes could only be achieved by subculturing the variant cells on specific media; under these conditions the phenotypic trait was inherited by the progeny of the cells. The requirement for a specific medium to maintain a particular phenotype indicated that the individual differentiated states were not completely autonomous. A fundamental property of these transitions is that they were asymmetric with some of the trans-

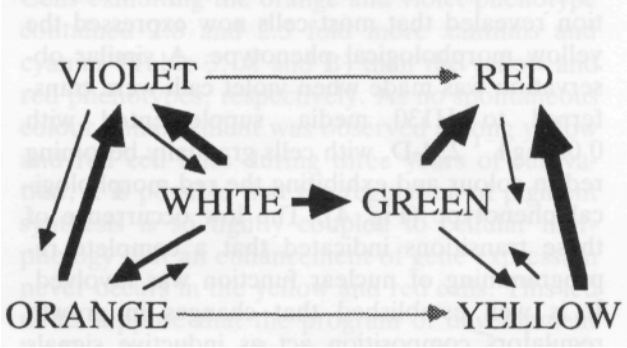

Fig. 5. Observed phenotypic interconversions. Size of the arrows symbolizes the frequency of transition. Thick arrows represent transitions that occur with high frequency; thin arrows, those that occur with low frequency; dotted arrows, those that are extremely rare or doubtful.

formed states being reversible, but with considerable variations in frequency.

Modifications in culture medium composition induced specific phenotypic interconversions, giving rise to chimera. Yellow calli, from explants C6 and L3, became progressively red when maintained on media containing a reduced 2, 4-D content; red sectors appeared on these yellow calli within one to two cell generations, possibly indicating that the hormone induced phenotypic conversion was dependent upon the replication of DNA and that the process was, therefore, associated with cellular proliferation [7]. Similar changes in cell phenotype were observed when calli from other cell lines were maintained on modified media. Orange cell clusters appeared when the violet L3 cell line was maintained on cytokinin deficient medium, while violet cells appeared when calli from the orange cell line were transferred to 5J110 medium containing BA and 2, 4-D. The transitions, which always occurred between morphologically related, phenotypes, represented modulations of specific differentiated states. The change from yellow to red may have resulted when a factor (e.g. medium growth regulators composition) modulated the activity of gene(s) involved in the control of betacyanin biosynthesis.

Although spontaneous interconversions were mainly restricted to closely related phenotypes (Fig. 5), transdifferentiation [24] of the two morphological types also occurred. Orange calli transferred to 5H110 medium supplemented with 0.05 mg l^{-1} 2, 4-D, gradually became less pigmented. After ten weeks, microscopic examination revealed that most cells now expressed the yellow morphological phenotype. A similar observation was made when violet calli were transferred to H130 media, supplemented with 0.02 mg l^{-1} 2, 4-D, with cells gradually becoming red in colour and exhibiting the red morphological phenotype (Fig. 4). The low occurrence of these transitions indicated that a complete re-programming of nuclear function was involved. It is well established that changes in growth regulators composition act as inductive signals for morphogenesis during plant development [25]. The data presented here suggest that this is also true for the secondary metabolic pathways of in vitro cultured plant cells.

The effect of 5-azacytidine and 3-methoxybenzamide on yellow cells

Yellow cells could be transformed into red cells by reducing the growth regulator (2, 4-D) concentration suggesting that the underlying mechanism involved only local interactions, such as changes in chromatin structure. To test this hypothesis, yellow cells were treated with a DNA methylation inhibitor, 5-azacytidine (5-azaC), a substance which breaks the chain of events by which the pattern of DNA methylation of a gene is passed from one cell generation to the next. 5-azaC increased the extent of yellow to red change (Fig. 6). If a reduced level of DNA methylation were sufficient for gene activation then inhibition of methylation should result in the expression of otherwise repressed genes. Furthermore, when cells were cultured on media containing growth regulator and 5-azaC, no colour changes were observed. Therefore, 5-azaC was unable to induce phenotypic transitions, but could still potentiate the changes induced by 2,4-D starvation. The fact that demethylation itself cannot cause variation in the presence of auxin suggests that it is only a part of a more general regulatory process. This raises the question of whether these genes (and possibly those involved in the metabolism of all betalains) are under negative regulation [26].

3-Methoxybenzamide (3-MBA), a poly(ADP-ribosyl) polymerase inhibitor [27], inhibited the 5-azaC induced transition of callus phenotypes in a concentration dependent manner (Fig. 6). These data suggest that poly(ADP-ribosyl) polymerase was involved in the changes of callus phenotypes. 3-MBA at a concentration of 1 mM completely inhibited the change of phenotype with no apparent effect upon culture growth.

The enzyme poly(ADP-ribosyl) polymerase exists in the nuclei of most multicellular organisms and catalyses the transfer of ADP-ribose moities from NAD^+ to proteins. Poly(ADP-ribosyl) polymerase has been implicated in numerous cellular functions including DNA repair, cellular differentiation, formation and transformation of active chromatin structures [27-30]. In the absence of specific information, it is only possible to speculate that the mechanisms which effect the alteration of gene expression

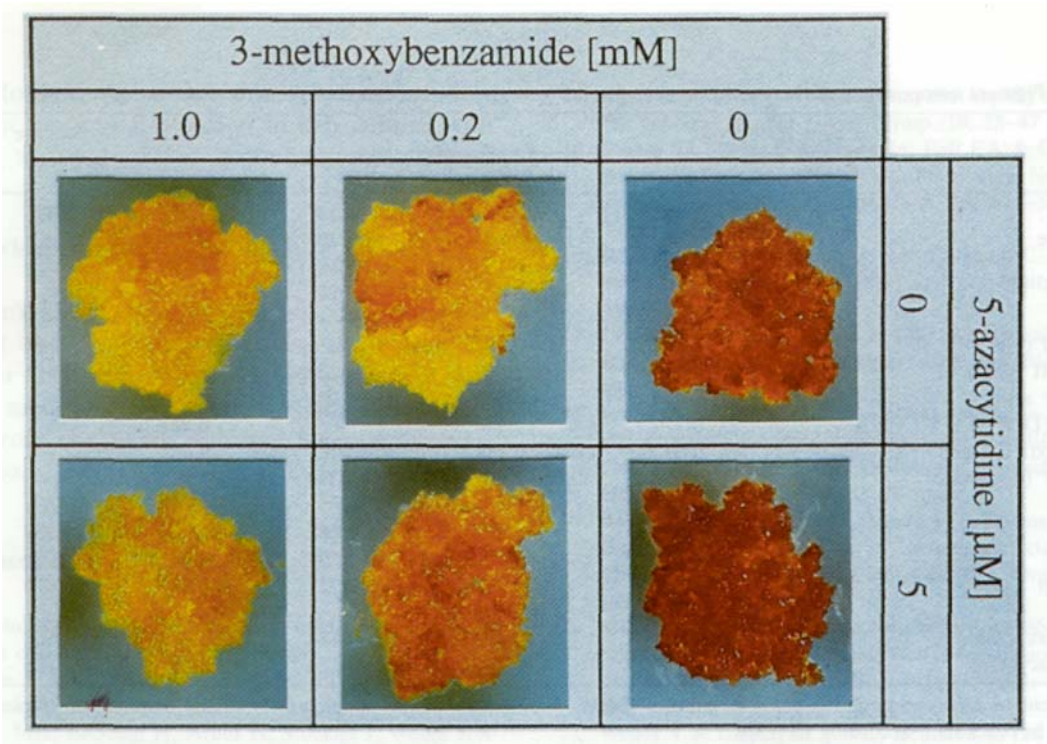

Fig. 6. Effect of growth regulators and inhibitors of DNA methylation and polyADP-ribosylation on the fate of the yellow [Y] phenotype after 8 days on hormone free medium.

during phenotypic transitions involve the transposition of DNA or the specific rearrangement of genes. Chromosome rearrangements such as translocations, inversions, breakage and fusion have been observed in cultured plant cells from a number of species [2, 14, 31-33]. Such rearrangements could result in the relocation of particular genes, either within the same chromosome or to another chromosome. The formation of new heterochromatin-euchromatin junctions could propagate new functional properties along the chromosome [34, 35]. The cis-inactivation of DNA sequences by neighbouring heterochromatin (the so called position effect | has been particularly studied at the white locus in the fruitfly *Drosophila melanogaster* [36]

Pigment composition

The pigment composition presented in Table *2* shows that the specific cell colours associated with each phenotype resulted from both quantitative and qualitative differences in betalains contents.

The characteristic colouration of the yellow and orange cell lines was due to the presence of betaxanthins (predominantly vulgaxanthin II) in the cell vacuole, while that of the red and violet strains was due to betacyanins (mainly betanin). Nevertheless, there existed no significant difference among betaxanthins and betacyanins types. Cells exhibiting the orange and violet phenotype contained 2.8 and 2.5 fold more xanthins and cyanins (Table 3, A and B) than the yellow and red phenotypes. respectively. As no spontaneous colour enhancement was observed among yellow and red cell lines during three years of cultivation, it is possible that the regulation of pigment synthesis is so tightly coupled to cellular morphology that an enhancement of gene expression never occurs in the yellow and red cells. This led us to suppose that the program of development leading to the coloured phenotypes include mechanisms able to potentiate cell-type-specific activity of the genes encoding the biosynthesis of

Table 2. Pigment composition of the basic inherited phenotypes and the storage root (cultivar *Bikores monogerm).*

	Callus phenotype					Storage Root
	Green	Yellow	Orange	Red	Violet	
DOPA	1.98	2.08	17.45	6.53	14.65	8.45
Dopamine	9.61	96.07	136.88	217.28	170.77	-
Tyrosine	6.18	8.98	6.19	4.18	7.14	-
Tyramine	10.38	9.81	6.47	24.25	5.71	-
Vulgaxt. I (glu*)	-	0.096	0.197	-	-	-
Vulgaxt. II (gln*)	-	1.813	5.519	0.122	1.276	2.517
Betalamic acid	-	0.326	0.743	0.088	0.493	-
Dopaxt. (DOPA)	-	0.119	1.146	-	0.231	-
Portulacaxt. II (tyr)	-	0.072	0.154	-	0.087	-
Miraxt. V (dopamine)	-	1.177	3.061	0.344	1.833	-
Betanin		-	0.732	8.688	22.629	20.355
Betanidin		-	-	0.209	0.287	-
Isobetanin		-	0.022	0.721	2.903	0.819
Total betaxanthins	0	4.278	12.210	0.848	3.201	2.626
Total betacyanins	0	0	0.754	11.222	28.016	21.187

The content of each compound is given in μmoles/g DW. Aminoacids of the upper moiety of the betaxanthins molecules are indicated in brackets (*according to [23]).

betaxanthin and betacyanin or that the genome is compartmentalised in functional units (gene-activity patterns) [37] as opposed to genes per se. Whether the same sets of genes were expressed in the yellow and orange phenotypes or the red and violet, respectively, or whether betalains biosynthesis was encoded by a multigene family of which different members were expressed in different phenotypes [38] is still an open question.

Beside this control of colour enhancement, the phenotypic changes within the yellow-red and the orange-violet groups resulted from a qualitative modulation with regard to the class of pigment synthesised. The yellow to red and the orange to violet changes corresponded to an activation of the betacyanin and an attenuation of the betaxanthin biosynthetic pathways. As betacyanins and betaxanthins share a common chromogen, betalamic acid (Fig. 1), so there exists a strong association between the biosynthetic pathways of these two classes of pigment. An increase in the level of cyclo-DOPA would result in an increased synthesis of betacyanins relative to betaxanthins. However, Table 3, C and D shows that the total pigment content of the red and violet phenotypes was approximately 2.5 fold higher than that of the related yellow and orange cell lines. Thus, not only was cyclo-DOPA available for biosynthesis of betacyanins but also the synthesis of betalamic acid was enhanced in the red and violet phenotypes. It is possible, then, that the synthesis of betalamic acid is coordinated with that of cyclo-DOPA in the red and violet phenotypes. In analogy with known models [39], clustered genes in one locus or cis-acting regulatory elements could account for such coordinated biosynthetic regulation [13, 40].

The position-effect which we postulate to be the cause of the variegations observed in the present study can be considered as a form of epigenetic switch. A clearer understanding of the mechanisms underlying the control of phenotypic transitions will be possible when molecular probes, specific to betalain metabolism, are available. The system presented here provides a promising

Table 3. Ratio between the pigments concentration of the different coloured phenotypes.

A Betaxanthins ratio [orange/yellow] = 2.85
B Betacyanins ratio [violet/red] = 2.50
C Total pigment ratio [red/yellow] = 2.82
D Total pigment ratio [violet/orange] = 2.41

model for the elucidation of mechanisms underlying the observed variation in cell culture with respect to alkaloid-type secondary metabolites.

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