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Review

Somaclonal Variation – a Novel Source of Variability from Cell Cultures for Plant Improvement

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Contents

Summary. It is concluded from a review of the literature that plant cell culture itself generates genetic variability (somaclonal variation). Extensive examples are discussed of such variation in culture subclones and in regenerated plants (somaclones). A number of possible mechanisms for the origin of this phenomenon are considered. It is argued that this variation already is proving to be of significance for plant improvement. In particular the phenomenon may be employed to enhance the exchange required in sexual hybrids for the introgression of desirable alien genes into a crop species. It may also be used to generate variants of a commercial cultivar in high frequency without hybridizing to other genotypes.

Key words: Plant cell culture – Somaclones – Interspecific hybrids – Gene amplification – Virus elimination

1 Introduction

The assembly of genetic variability is vital to any plant breeding enterprise. Without a continual input of 'new' genes, progress, as measured by improved agronomic suitability, cannot be made.

Plant breeders have been quick to recognize potentially useful sources of genetic variability. To augment the incorporation of such useful genes into commercial species, sophisticated genetic techniques, such as alien gene transfer in wheat, have been developed.

Plant cell culture has often been hailed as one of the more significant potential adjuncts to plant improvement. This is usually seen in terms of the ability to apply cellular selection for recovering useful genetic variants, anther culture to speed the attainment of homozygosity, somatic hybridization for recombining genomes of sexually incompatible species and more recently the possibility of specific gene addition or modification by recombinant DNA techniques (Scowcroft 1977; Nitzsche and Wenzel 1977; Thomas et al. 1979; Kado and Kleinhofs 1980). These approaches have made only limited direct contribution to genotype improvement of commercial species to date.

Often potentially valuable contributions of science emerge from unexpected quarters. Plant cell culture has provided a new and exciting option for obtaining increased genetic variability relatively rapidly and without sophisticated technology. We refer to variability generated by the use of a tissue culture cycle.

A tissue culture cycle involves the establishment of a more or less dedifferentiated cell or tissue culture under defined culture conditions, proliferation for a number of cell generations and the subsequent regeneration of plants. In other words one imposes a period of essentially dedif-

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ferentiated cell proliferation between an explant and the next plant generation. The ease and efficiency with which such manipulations can be made varies enormously from species to species. The rapidly expanding list of species which are amenable to passage through a tissue culture cycle has been reviewed (see Scowcroft 1977; Murashige 1978; Thomas et al. 1979). The initiating explants for a tissue culture cycle may come from virtually any plant organ or cell type including embryos, microspores, roots, leaves and protoplasts.

Historically a culture cycle was seen essentially as a method of cloning a particular genotype. It was often considered a sophisticated method of asexual propagation enabling a more rapid rate of propagation. Thus it became the accepted dictum that all plants arising from a tissue culture should be exact copies of the parental plant (the palingenetic expectation). However, phenotypic variants were frequently observed amongst regenerated plants. These were usually dismissed, somewhat embarrassingly, as 'artefacts of tissue culture'. Sometimes the variants were viewed as consequences of the recent exposure to exogenous phytohormones and sometimes they were labelled 'epigenetic' events which somehow made them unworthy of further scientific scrutiny. Recent evidence has shown this judgement to be premature and possibly erroneous.

Tissue culture per se appears to be an unexpectedly rich and novel source of genetic variability.

Plants regenerated from stem callus have been referred to as 'calliclones' (Skirvin and Janick 1976a) and those from leaf protoplasts as 'protoclones' (Shepard et al. 1980). We propose the more general term 'somaclones' for plants derived from any form of cell culture. 'Somaclonal variation' is then the variation displayed amongst such plants. Reference to this in vitro variability was made in the reviews by Morel (1971), Murashige (1974), Green (1977, 1978), Skirvin (1978), and Thomas et al. (1979). Exciting recent results however justify a fuller assessment of its significance.

Somaclonal variation seems not to be species or organ specific and variation among somaclones has been observed for a wide array of characters. Doubtless the genetic mechanism(s) which give rise to such variation will prove a productive field of investigation. We shall also argue that somaclonal variation can provide a valuable adjunct to plant improvement.

Some authors have made passing reference to the apparent homogeneity of regenerated plants (Williams and Collins 1976; Reuther 1977; Vazquez et al. 1977; Wenzel et al. 1979). We contend that the failure to observe gross changes or abnormalities in morphology does not negate the possibility of genetic variations which careful and specific analysis might have revealed. We have chosen a sample of the cases from the literature which demonstrate somaclonal variation in a substantial way, plus a sample indicating the possible breadth of the phenomenon.

2 Variation in Cultures

A phenomenon frequently encountered is the spontaneous appearance of variation amongst subclones of the one parental cell line. Chaturvedi and Mitra (1975) described two subclones of *Citrus grandis* callus which differed in their morphogenetic pattern. Under identical culture conditions one would consistently form numerous embryoids while the other would form shoots. Selby and Collin (1976) studied 20 subclones of the cultures of each of three onion varieties. They were found to vary greatly in the following parameters: growth rate (a 3-fold difference), friability, sliminess, pigmentation (from none to deep red), and alliinase activity. Clonal variation in culture morphology and growth rate has often been described (Blakely and Steward 1964; Sievert and Hildebrandt 1965; Davey et al. 1971; Snijman et al. 1977) as has subclonal differences in pigmentation (Davey et al. 1971; Stickland and Sunderland 1972; Mitzukami et al. 1978).

Changes to auxin habituation (Gautheret 1955; Spiegel-Roy and Kochba 1975) and cytokinin habituation (De Marsac and Jouanneau 1972; Meins and Lutz 1979; Meins et al. 1980) have also been frequently observed in cell cultures. Umiel et al. (1978) observed differences in expression of streptomycin resistance among subclones of a resistant tobacco line.

Tabata et al. (1978) and Zenk (1978) have reviewed the phenomenon of callus subclones varying in their ability to produce alkaloids and other secondary metabolites. For example, when 143 colonies of a *Solanum laciniatum* culture were examined individually for the steroidal alkaloid, solasonine, they varied from 0% to > 3% on a dry weight basis (Zenk 1978). Similarly Tabata and Hiraoka (1976) and Tabata et al. (1978) observed dramatic differences in nicotine production in subclones of *Nicotiana rustica* and *N. tabacum* cultures. When plants were regenerated from high nicotine variants the plant tissues did not have more nicotine than control plants (indeed somewhat less). However, fresh cultures initiated from these plants did again show the high nicotine content (about 6-fold the control cultures). No attempt was reported to see if this characteristic was transmitted to the sexual progeny.

Zenk et al. (1977) also found *Catharanthus roseus* colonies plated from the one culture to differ in their contents of ajmalicine and serpentine. The content ranged from none to concentrations exceeding the parent plant.

In addition there have been many reports on the isolation of variant cell lines resistant to many different antimetabolites. The occurrence of these variants has recently been reviewed by Widholm (1977) and Maliga (1978). The antimetabolites most frequently used are toxic amino acid analogues, toxic levels and combinations of amino acids, nucleic acid base analogues, protein synthesis inhibiting antibiotics and herbicides. In most of this work mutagenic

treatments have not been used. The variant lines have occurred spontaneously in culture and been isolated by selection. In some cases the variant phenotype has persisted into regenerated plants where the mode of inheritance could be analysed (Carlson 1973; Marton and Maliga 1975; Gengenbach et al. 1977; Bourgin 1978; Chaleff and Parsons 1978a, b; Müller and Grafe 1978; Hibberd et al. 1980; Lawyer et al. 1980; Sung and Jacques 1980).

No adequate explanation has ever been advanced for the occurrence of culture variation. To say that the culture environment itself is mutagenic may in the broad sense be true since stable variants arise from it. However, as we now hope to show, the frequency of somaclonal variation is much too great to be accounted for by simple base changes or deletions caused by some chemical mutagen component of the medium. We propose that this ubiquitous culture heterogeneity is the result of more drastic genetic rearrangements which are also responsible for a high frequency of genetic variation amongst regenerated plants (somaclones).

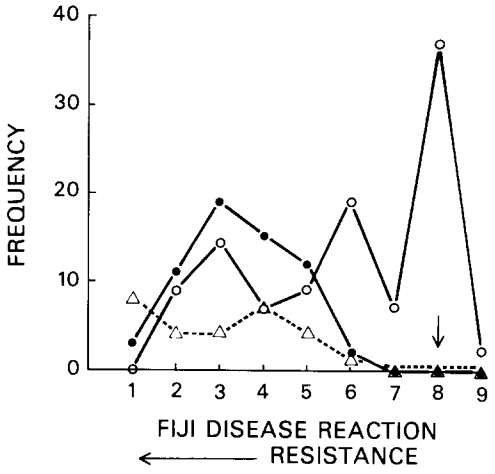

Fig. 1. Data from Krishnamurthi (1974). The distribution of Fiji disease reaction of the somaclones generated from three parental lines LF66-9601 (●—●), Pindar (○—○), and LF60-3879(△—△). All three parental lines are highly susceptible (8) as indicated by the arrow

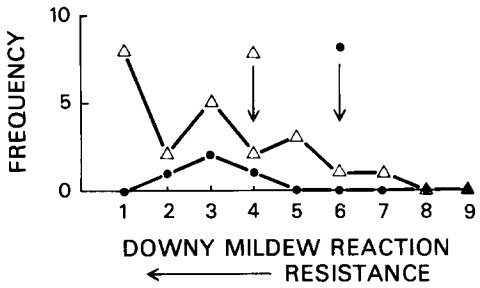

Fig. 2. Data from Krishnamurthi (1974). The distribution of downy mildew reaction of somaclones from cultivars LF60-3879 (△—△) and LF51-124 (●—●). The parental reactions are indicated by arrows

3 Substantive Examples of Somaclonal Variation

3.1 Sugar Cane

The potential usefulness of somaclonal variation for plant improvement first became apparent in sugar cane. In the Hawaiian Sugar Planters' Association Experimental Station's annual reports of 1967, 1968, 1969 and 1970 mention is made of variability among plants derived from sugar cane tissue cultures. Further details of this work were reported by Heinz and Mee (1969, 1971) and Heinz (1973). Variation was observed in morphological, cytogenetic and isozyme traits. In Taiwan there were also early reports of morphological variation in stooling and erectness amongst sugar cane somaclones (Liu et al. 1972).

Following the exciting indications of variability in Hawaii, work was begun in Fiji in particular to seek resistance to Fiji disease virus (Krishnamurthi 1974; Krishnamurthi and Tlaskal 1974). Beginning in 1970 they screened the somaclones of a number of varieties for their reaction to Fiji disease (a leafhopper transmitted virus) and Downy Mildew (*Sclerospora sacchari*). In every case some somaclones were identified with increased resistance to both Fiji disease and Downy Mildew. Some examples of the variation generated by a tissue culture cycle are illustrated in Figs. 1 and 2. There appears to be a predominant shift towards increased resistance for both diseases. This is apparent even when the donor variety is already reasonably resistant (LF60-3879, Fig. 1). Some of the Pindar somaclones resistant to both Fiji disease and Downy Mildew have been tested for yield in Fiji (Krishnamurthi 1974) and independently in Australia (John Waldron, pers. com,.). They did not show a reduced sucrose yield compared to Pindar, indeed some showed a slightly increased yield though this was not statistically significant.

An independent series of somaclones (more than 4000) have been produced in Hawaii to screen for Fiji disease resistance. The first 735 of these which were derived from varieties with high susceptibilities (> 7 on the 1-9 disease rating scale) contained 18% with susceptibilities of < 5 (Heinz 1976).

Nickell and Heinz (1973) refer to tentative results of R.E. Coleman. Beginning with 15 sugar cane varieties susceptible to mosaic virus disease Coleman initiated cultures and regenerated somaclones. Resistance to the disease was found in 116 of these somaclones. They were virtually immune after more than 8 inoculations and extensive growth under field conditions favourable to the establishment of mosaic virus disease.

In Hawaii somaclones were also found which vary greatly in their reaction to eyespot disease (*Helminthosporium*

sacchari) (Heinz 1973; Heinz et al. 1977). It had been intended to induce mutations with methyl-methanesulphonate (MMS) or ionizing radiation during culture but they found the background variation was just as high as in the mutagenized cultures (Fig. 3).

In our own work we are confirming the very extensive variation among sugar cane somaclones to eyespot disease susceptibility. We have developed a leaf essay for quantifying the sensitivity of the leaves of a given plant to a standardized concentration of the fungal toxin responsible for leaf damage. The sensitivity is measured as the initial rate of leakage of electrolytes from leaf discs pre-exposed briefly to the toxin. This metric proves to be highly repeatable and consistent for a given cultivar (Larkin and Scowcroft

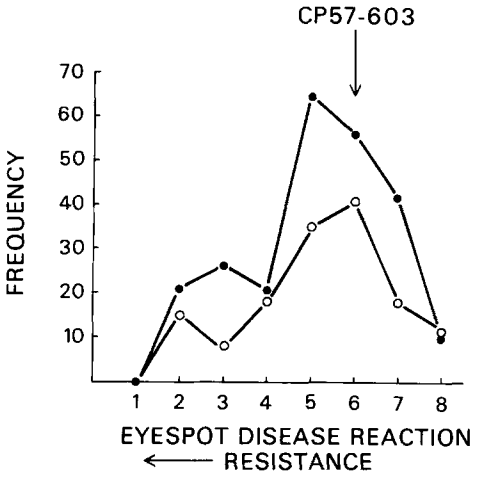

Fig. 3. Data from Heinz et al. (1977). The distribution of the eyespot disease rating of somaclones from CP57-603 cultures with (●—●) or without (○—○) mutagenesis. The reaction of the parental line is indicated by the arrow

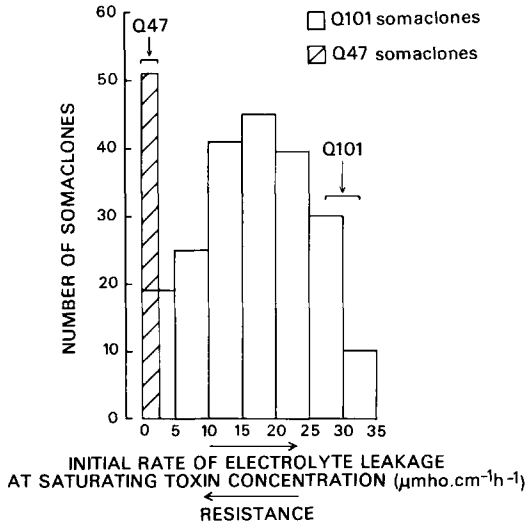

Fig. 4. The distribution of reaction to eyespot toxin of Q101 somaclones and Q47 somaclones. The parental reactions are as indicated by arrows

1981). Cultures were initiated from Q101 sugar cane which is an agronomically valuable Australian cultivar whose major fault is high susceptibility to eyespot. After more than 8 months in culture, plants were regenerated and grown in soil under glasshouse conditions. To date 260 such Q101 somaclones have been assayed for their eyespot toxin sensitivity. The variability is illustrated in Fig. 4.

The mode of this distribution is significantly shifted to the resistant side of the Q101 parental reaction (30 ± 2 $\mu mho \cdot cm^{-1} \cdot h^{-1}$). A high percentage (8.9%) of the somaclones are highly resistant or nearly immune ($0\text{-}5$ $\mu mho \cdot cm^{-1} \cdot h^{-1}$). These resistant somaclones retain the same level of resistance into later cane generations. They are indistinguishable from the parental Q101 in gross morphology and are now being evaluated under field and glasshouse disease testing conditions. It is of interest that all 52 somaclones of Q47, a highly resistant cultivar, are also resistant.

Lat and Lantin (1976) in the Philippines examined only 9 somaclones from the sugar cane cultivar CAC57-13. In replicated field trials some of these showed significant differences from the parental variety in cane diameter, stalk length and weight. Liu and Chen (1976, 1978a, b) in Taiwan have found significant variations amongst sugar cane somaclones from 8 varieties in characters such as cane yield, sugar yield, stalk number, length, diameter, volume, density and weight, percent fibre, auricle length, dewlap shape, hair group, and attitude of top leaf. Some of these somaclones in replicated randomized complete block field experiments showed significant improvements over the parental performance and over the performance of Taiwan's major variety. For example somaclone 70-6132 was higher than its donor parent for cane yield, sugar yield and stalk number by 32%, 34% and 6% respectively. This somaclone showed an improvement for these three characters over Taiwan's best variety (F160) by 20%, 16% and 8% respectively. Liu and Chen (1978b) have also recovered resistant somaclones from susceptible donors for both Downy Mildew and also culmicolous smut diseases.

It is quite clear that somaclonal variation is very easily obtained in sugar cane. It is extensive, affects many important characters and shows promise for the improvement of varieties particularly those with single defects.

3.2 Potato

The commercial potato belongs to the *tuberosum* subspecies of *Solanum tuberosum* and is essentially autotetraploid though displaying some segmental allotetraploidy. It seems that heterozygosity is essential to maximize plant vigour. Despite considerable breeding programmes in North America and Europe the most widely grown potato varieties continue to be 'old' varieties (> 50 years old). The reasons for the failure of new releases to supplant the old ones

are complex. Simmonds (1969) argued that the varieties which have lasted 'are good all-rounders; they are generally unremarkable in individual characters but are markedly defective in none; in short, they are not-very-imperfect.' Each year about 22% of the world potato crop is lost to disease. The sterility or very low fertility of many cultivars also discourages their further use in breeding programmes.

In North America the 70 year old variety 'Russet Burbank' represents 39% of the potato crop. Shepard et al. (1980) argued that it might be simpler, if possible, to selectively improve a popular variety than to create a new one. They seem to have demonstrated this by the use of somaclonal variation. Screening over 10000 somaclones ('protoclones') produced from leaf protoplasts of 'Russet Burbank', they found significant and stable variation in: compactness of growth habit (some with a fuller and more efficient canopy); maturity date (some which set tubers earlier than 'Russet Burbank'); tuber uniformity (some more uniform in size and shape); tuber skin colour (some were white as compared to the brown of the parent); photoperiod requirements (some required only a 13 h photoperiod to initiate flowering whereas 'Russet Burbank' required a 16 h photoperiod); fruit production (some somaclones produced 100 fold more berries than 'Russet Burbank' in comparable field trials). It is of particular importance that somaclones were recovered which were resistant to disease pathogens. Five of 500 somaclones were more resistant to *Alternaria solani* toxin than the parent and of these, four showed field resistance to early blight. About 2.5% (20 of 800) somaclones screened were resistant to late blight (*Phytophthora infestans*) some of which were resistant to multiple races of this pathogen (Matern et al. 1978; Shepard et al. 1980).

These variant somaclones have retained their phenotype through a number of vegetative generations. Some 60 somaclones have been identified having one or more significant agronomic improvements on the parent cultivar. Some of these are being evaluated as potential new cultivars.

By contrast Wenzel et al. (1979) observed phenotypic variability from protoplast-derived somaclones of potato dihaploids only after extended culture periods. They refer to increased aneuploidy in such material and ascribe the variation to the culture-induced aneuploidy. It is not possible to rationalize the difference between these results and those of Shepard et al. (1980). It may well be important that many loci in the donor were in the simplex configuration (Aaaa) in Shepard et al. (1980) but in Wenzel et al. (1979), who used dihaploids, such loci would be genetically duplex, (AAaa). Also only 192 regenerated plants were examined in the latter as compared with more than 10000 in the former investigation.

Interestingly, Behnke (1979, 1980) using cultures of the same dihaploids as used by Wenzel et al. (1979) has been able to select variant lines (and plants) resistant to the toxic culture filtrates of *Phytophthora infestans*. Mutagenesis did not greatly enhance the rate of appearance of the variants. Some of them could be regenerated and the leaves were also resistant. Presumably by selecting for variants in cultures she was effectively able to screen much larger numbers of 'individuals' (cells) than the 192 individual protoplast-derived plants examined by Wenzel et al. (1979) for variants.

3.3 Tobacco

In tobacco there have been a number of reports indicating genetic variation among regenerated plants. Mousseau (1970) observed a much greater variation in CO_2 adsorption and chlorophyll content amongst somaclones than amongst seed progeny. Popchristov and Zaganska (1977) also reported somaclonal yield variability. Ten out of 100 somaclones were considered valuable improvements. Oinuma and Yoshida (1974) conducted triplicated randomized-block field experiments to compare seed progeny of three inbred tobacco cultivars to three doubled haploid lines derived by anther culture from each of these cultivars. The somaclonal lines differed significantly between themselves and from the parental line in almost all characters examined including: days to flowering, plant height, stem diameter, total leaf number, leaf length, leaf width, yield and total alkaloids. There was no significant variation in any of these characters within the somaclonal lines.

Devreux and Laneri (1974) provide data on anther culture derived somaclones which again indicates extensive variation. Their donor material was from 6 inbred cultivars of tobacco. The isogenic lines produced by anther culture (the haploids were subsequently doubled) were uniform within themselves but there was extensive variation between these lines (somaclones). Characters examined included leaf shape, leaf number, plant height, type of inflorescence and yield. Some lines showed dry weight yields up to 10% greater than the original cultivar. These same authors attempted to enhance the level of variation by irradiating (5-2000 R) the flower buds prior to anther culture. The frequency of variants did not increase with dose. Indeed the highest frequency (11.7% were variant in one of the few characters examined) occurred with the lowest dose. The no-mutagen control data are not given. This may be interpreted as resulting from the fact that the variation is being generated by the culture cycle itself. The frequency is already so high that mutagenesis is not seen to have an effect.

Burk et al. (1979) obtained anther-culture derived dihaploids from a line heterozygous for each of three single gene-controlled characters (tobacco mosaic virus, potato virus Y and root knot nematode resistance). The segregation amongst more than 1100 lines for TMV resistance

was significantly disparate (in favour of resistance) from the expected 1:1. Similarly the segregation for root knot resistance amongst 619 dihaploids was significantly disparate in favour of susceptibility.

A more substantive demonstration of tobacco somaclonal variation has come from Burk and Matzinger (1976). Their parental plant was the highly inbred variety, 'Coker 139', which had gone through 15 generations of self-fertilisation since its release as a pure line. Dihaploids were derived by anther-culture from this highly inbred line. Five spontaneously doubled haploids were included with 41 colchicine doubled haploids as a control on the effect of colchicine. The selfed progeny of these lines and Coker 139 were evaluated in a randomized block field design with six replications. Each block contained 20 plants. Significant variability between the somaclonal lines was observed in all the characters examined: yield, grade index, days to flowering, plant height, leaf number, leaf length, leaf width, total alkaloids and reducing sugar content. The dihaploid lines which had not been exposed to colchicine showed as much variability as those that had been. There was no significant variation within lines. It seems very unlikely that there could be any residual heterozygosity in their parental plant and yet the dihaploid somaclones dis-

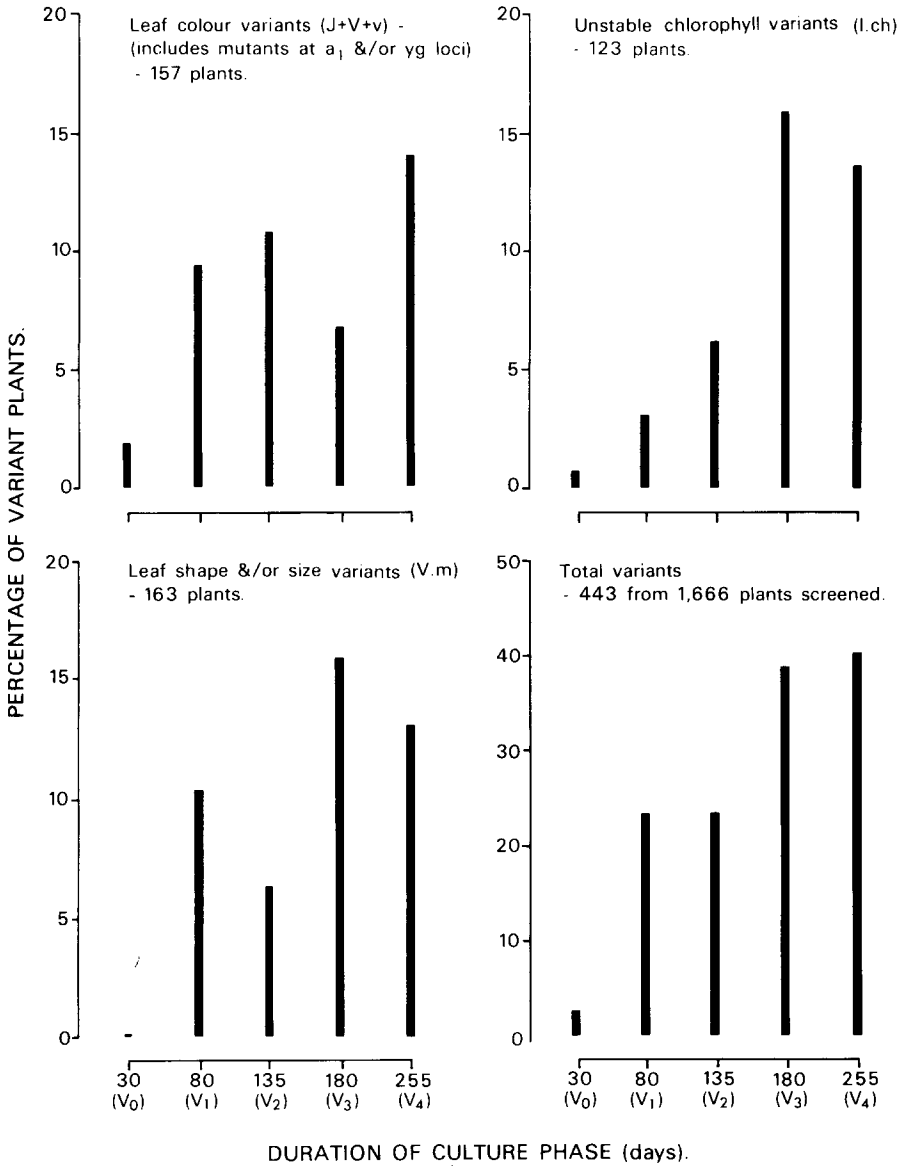

Fig. 5. Percentage of variant tobacco plants among those regenerated from cotyledon explants (30 days culture, V_0), protoplast colonies (80 days, V_1) and for plants regenerated from successive callus subcultures after 135 (V_2), 180 (V_3) and 255 (V_4) days. Distributions are derived from Table 1 of Barbier and Dulieu (1980) with variant classes (J, V, v, I.ch., V.m) and regeneration pathways (V_0-V_4) as defined by the authors. The leaf colour variants (J, V, v) have been pooled since they represent mutations and/or deletions at the a_1 and/or yg loci of which 113 (V and v types) were genetically analysed

played as much variability as normally associated with a segregating F_2 population from a cross between 2 different cultivars.

Arcia et al. (1978) also argue against residual heterozygosity in the Burk and Matzinger (1976) parental material. For some heterozygosity to have been maintained through so many selfing generations (probably in excess of 20) would require heterozygotes to exhibit significant advantage in the seedling stage prior to picking and transplantation. Such an advantage is not observed. Collins and Legg (1980) remain unconvinced that there is no residual heterozygosity whose elimination in the dihaploids results in the variability. However, they offer no evidence for such heterozygosity.

Studies by De Paepe (1980) with *Nicotiana sylvestris* seem to discount entirely the explanation based on residual heterozygosity. He found extensive variability amongst diploid plants obtained from binucleate microspores. A second cycle of androgenesis from the dihaploids generated even further variation. A comparative study with DNA reassociation kinetics between variant dihaploids and the parental suggested some form of genome reorganization in the dihaploids.

Burk and Chaplin (1980) have endeavoured to combine somaclonal variability with hybrid segregation variability. They observed exceptional variation among dihaploids derived from anther-culture of the F_1 of VY32 × Coker 86. They claim that the levels of variation were more than expected simply by segregation at heterozygous loci. Lines were obtained with yields well in excess of both parents and with combination of the disease resistances of the parents (tobacco mosaic virus, potato virus Y and root knot nematode).

Most recently Barbier and Dulieu (1980) have made a painstaking and detailed analysis of a quantitatively staggering amount of variation among plants regenerated by cultured cells of tobacco (Fig. 5). From a hybrid, heterozygous for 2 loci (a_1, yg) which affect chlorophyll synthesis (yellowish-green phenotype) they found that the frequency of mutations (reversions to a wild type allele or deletions) among 1,666 plants regenerated was 3.5% and 3.6% at each locus respectively. This estimate was obtained by genetically analysing 113 more or less green variant plants. The authors acknowledge that this is an underestimate resulting from misclassification of variants as parental types at the plantlet stage.

In their experiment they observed the frequency of variants regenerated directly from cotyledon explants (30 days in culture), from cotyledon derived callus cultured in an undifferentiated state for 135, 180 or 255 days from callus initiation to plantlet formation and among plants regenerated from cotyledon protoplasts (80 days from protoplast culture to plants). They found that the frequency of mutations at the a_1 and yg loci was at a maximum in plants derived from protoplasts and early callus cultures, and remained stable among plant regenerated from older callus. For these they conclude that the mutational event pre-existed in the cotyledon cells either as a functional mutation or as a lesion in the DNA of the latent cotyledon cell which on dedifferentiation is repaired and expressed following mitotic segregation. It would seem difficult to distinguish between variation preexisting in the cotyledon cells and that generated during early culture phases.

On the other hand the unstable chlorophyll variants and leaf shape and size morphological variants did increase in frequency with prolonged culture. A cytogenetic analysis also revealed some polyploidisation, but this was independent of the class of variant observed.

In very definite concordance with our theme of somaclonal variation, Barbier and Dulieu (1980) conclude: –

'Compared to the genetic stability observed in plant populations grown from seeds, the number of genetic changes was very high among regenerated plants. A proportion of these changes could either preexist in the differentiated cells or occur during dedifferentiation. It is likely that regeneration allows expression of potential variability accumulated in cotyledon resting cells after the last cell cycle; variability also accumulates in cultured cells.'

3.4 Rice

Nishi et al. (1968) and Henke et al. (1978) both reported phenotypic variants amongst somaclonal plants regenerated from rice callus. The variations were in traits such as number of tillers per plant, number of fertile tillers per plant, average panicle length, frequency of fertile seed, plant stature and flag leaf length.

More extensive results attended by careful analysis have come from Oono (1978a, b). Beginning with homozygous material (75 seeds from a selfed doubled haploid) he examined about 800 somaclones derived from callus initiated from those seeds. All of these lines were examined immediately and over the subsequent two selfing generations. In particular, chloroplast content, flowering date, plant height, fertility and morphology were considered. Only 28.1% of the plants were considered normal (parental) in all these characters! There was wide variation in seed fertility, plant height and heading date. Chlorophyll deficiencies were seen in the second generation of 8.4% of the lines which is a comparable frequency to that expected from X-ray and γ-irradiation. Sectorial analysis of plants derived from a single seed callus showed that at least most of the variations were induced during culture and were unlikely to pre-exist amongst the 75 homozygous seeds used to initiate the experiment. In the second selfed generation after somaclone regeneration some of the mutant characters were segregating and some were fixed. It was estimated that mutations affecting these five traits were induced in culture at a rate of 0.03-0.07/cell/division. Oono (1975)

had also previously observed similar variations in haploid lines derived from the one pollen callus.

3.5 *Oats*

Somaclonal variation was also apparent from oat (*Avena sativa*) tissue cultures. Cummings et al. (1976) reported altered phenotypes in plant height, heading date, twin culms, yellow leaf stripes, awn morphology and fertility. Many of these variants were transmissable to later generations (both true breeding and segregating lines occurred). An extension of this work (McCoy et al. (1978) has also indicated a high frequency of cytogenetic abnormality. The most frequently encountered alterations involved heteromorphic bivalents and ring chromosomes at meiosis indicating that deletions and non-homologous exchanges had occurred. Tripolar divisions, lagging chromosomes and micronuclei were occasionally observed. The frequency of plants showing these variations increased with the duration in culture. For example with the cultivar Tippicanoe the frequency increased from 11% to 50% as the culture phase extended from 4 to 20 months. Similarly with the cultivar Lodi the frequency of variant plants increased from 50% to 87% (McCoy 1979).

3.6 *Maize*

Green (1977) described the regeneration of 85 maize plants from a callus initiated from an immature F_1 hybrid embryo. Examining gross morphology and karyotype only, they observed 6 plants with the abphyl syndrome (decussate leaf and ear arrangement giving twice the number of leaves on a normal number of nodes), 2 plants with twin stalks from a single node, 2 plants with reduced pollen fertility, 1 plant was mosaic with monosomy for chromosome 5 and 1 plant with tetraploid sectors in the tassel. These two mosaic plants had normal pollen fertility (Green et al. 1977).

Additionally Green et al. (1977) examined 11 regenerated plants from a 3 year old maize callus. All of these plants had the abphyl syndrome and were about three feet tall at maturity. They possessed a heteromorphic chromosome 6 bivalent at pachynema in which one chromosome 6 in each plant had lost the distal one-third of the long arm.

Maize somaclonal variation has also been observed to affect the mitochrondial genome. Selection for resistance in cultures of T-cytoplasm maize (sensitive to the southern corn leaf blight T-toxin of *Drechslera maydis* Race T) by recurrent sublethal exposure to T toxin resulted in the recovery of toxin-resistant plants. These same plants were also fertile in contrast to the male-sterility of the original parent (Gengenbach et al. 1977). Brettell and Ingram (1979) and Brettell et al. (1980) have confirmed these results and indicated that the frequency of occurrence of these resistant variants was very high even when toxin was not added to the cultures prior to regeneration (35 out of 60). The restored male-fertility and toxin resistance were shown to be cytoplasmically inherited.

3.7 *Barley*

There is some preliminary evidence from Foroughi-Wehr et al. (1979) indicating that barley plants derived from microspore culture of homozygous material show unexpected phenotypic variation. As many as 10-15% of the plants were variant. Although they were classified as tetraploid-like in appearance their progeny were completely sterile which is not expected of a tetraploid. They concluded from observations of chromosome breaks and reciprocal translocations that the variation is not due simply to chromosome number alterations.

Deambrogio and Dale (1980) have reported a greatly enhanced variance for four characters amongst barley plants derived from callus cultures as compared to control plants. Interestingly the increased variation was only apparent after 4 weeks of culture on 4mg/l 2,4-D but not at lower concentrations. This does not necessarily indicate a mutagenic effect of 2,4-D but may reflect a faster growth rate allowing the normal culture mechanisms for genetic change to operate within this time.

3.8 *Brassica sp.*

Grout and Crisp (1980) reported that the propagation of cauliflower by adventitious meristems on the roots results in some phenotypic variants. Such meristems arise by proliferation of somatic cells on the root surface. The variants include: altered leaf wax; multiple branching of the stem; precocious flowering from the apex, stem or leaf; abnormal cupped leaves; reduced laminae in leaves; spontaneously aborting vegetative buds; exceptionally slow growth; failure to flower; unusually large pollen grains. Most of the plants including the variants were karyotypically normal ($2n = 18$) although a few plants showed mixaploidy in the roots. One plant (a variant) had $n = 8$ or 9 in meiosis with bridges and fragments at anaphase I and micronuclei in telophase II. Ploidy changes in stem pith derived plants have been reported previously by Horák et al. (1975).

Although Grout and Crisp (1980) do not present any evidence of their own they do refer to unpublished results of L.E. Watts showing that the variations derived by their method are heritable. Interestingly from over 4000 plants derived from culture of the cauliflower curd they claim to

have observed no variants. This could be a consequence of the curd being simply a mass of proliferated apical meristems (Crisp and Walkey 1974). It may indicate the importance of distinguishing adventitious meristems (or callus-derived shoots) from apical meristems, with respect to their significance for somaclonal variation.

Unexpected variation has also been observed amongst doubled haploids of rape (*Brassica napus*) obtained by microspore culture (Hoffman 1978; Wenzel 1980). Variants occurred for lateness of flowering. For example 8 out of 63 derived from a spring rape required cold to induce flowering. Greatly altered glucosinolate content was also found. Hoffman (1978) also observed extensive variability in growth habit and gross morphology amongst about 50 plants derived by stem embryogenesis from a single microspore derived plant. In this phenomenon epidermal cells undergo rapid embryogenic proliferation.

3.9 Pelargonium

Skirvin and Janick (1976a, 1976b) have observed a remarkable degree of variability in plants regenerated from the tissue cultures of 5 *Pelargonium* cultivars. These somaclones were compared to plants propagated from stem cuttings which were all indistinguishable from the parental plants. Variability in the callus derived somaclones occurred in all of the examined phenotypes: leaf shape, size, and form, flower morphology, plant height, fasciation, pubescence, anthocyanin pigmentation and essential oil composition. A lower frequency of less extensive variation was also observed with root cuttings as a result of the chimeral nature of some of the parents. However the cultivar 'Attar of Roses' is not chimeral and all of its root cuttings (and stem cuttings) were uniform. In dramatic contrast, 28 out of 55 somaclones from 'Attar of Roses' were variants. This would indicate that the variability from the tissue culture cycle is independent of any chimeral assortment.

Chromosome counts did uncover ploidy changes but only in a small proportion of the variants. One of the variants has been released as a new cultivar called 'Velvet Rose' (Skirvin and Janick 1976b). In some of their material they observed a tendency for increased variability with increased duration of the culture cycle. They also presented some indications of increased stability when variants were taken through a second tissue culture cycle.

Abo El-Nil and Hildebrandt (1972) also observed variation in geranium (*P. hortorum* var. 'Lady Ester') plants derived from anther culture. They presumed all changes to be due to elimination of a virus. More recently Tokumasu and Kato (1979) regenerated homogeneous plants from somatic tissue of diploid anthers. Amongst these plants were two variants for essential oil composition.

3.10 Other species

A study of the tissue culture literature shows many passing comments on the occurrence of abnormalities amongst regenerated plants. Rarely were these abnormalities examined further. Some examples include carrot (Ibrahim 1969), *Chrysanthemum* (Ben-Jaacov and Langhans 1972), lily (Stimart et al. 1980), carnation (Hackett and Anderson 1967), and clover (Beach and Smith 1979). Gamborg et al. (1977) found variants amongst sorghum culture regenerants. Some had reduced fertility, some had altered leaf morphology and growth habit. All these variant sorghum somaclones were normal in karyotype (2n = 20).

Wakasa (1979) produced 448 somaclones of pineapple (*Ananas comosus*). Many variants were observed in spine and leaf colour, wax secretion, foliage density, leaf width, and leaf spine formation. The explant source had a dramatic effect on the occurrence of variation. If the explant for the initiation of cultures was syncarp or slip nearly 100% of the somaclones were variants. If the explant was the pineapple crown only 7% of the somaclones were morphologically variant. This explant effect on the frequency of variations might imply that the variations preexit in these tissues. Alternatively one might argue that the explant has an effect on the rate of cell proliferation or the degree of dedifferentiation in subsequent cultures.

Novák (1980) indicated that garlic (*Allium sativum* L.) somaclones from shoot tip callus and leaf base callus showed great phenotypic variability. From an examination of 808 plants 12 diploid somaclone lines were selected with improved bulb shape and size compared to the parental line.

Somaclonal variation has also been encountered in lettuce (*Lactuca sativa* L.) (Sibi 1976) for plant morphological traits. It appeared from selfing analysis and diallel crosses that the variations were maternally inherited.

4 Enhanced Variation in Interspecific Hybrids

There is some evidence to suggest that the variability observed in somaclones may be caused at least in part by chromosome breakage and reunion events (see later). If tissue culture generates an environment for enhancing such events then it may provide the means for obtaining the genetic exchange needed between two genomes in an interspecific hybrid. It is often desirable but frequently difficult (e.g. cotton) to introgress particular genes from an alien genome into that of a commercial crop cultivar. Even if a hybrid can be produced it often proves difficult to obtain any pairing (and subsequent exchange) between the crop and alien chromosomes. A tissue culture cycle of the hybrid material may enhance the frequency of the requisite exchange.

Ahloowalia (1976, 1978) produced a triploid hybrid embryo by a diploid *Lolium perenne* × tetraploid *L. multiflorum* cross. This embryo was cultured to produce callus and more than 2000 plants were regenerated from the callus over a period of five years. The plants showed a wide variation in leaf shape, size, floral development, growth vigour, survival and perenniality. Some of the variants represented combinations of parental characteristics which were agronomically valuable and, according to the author, had not been observed in hybrids which had not been through a tissue culture cycle. The seed progeny also showed the variation.

The first series of hybrid rye-grass plants regenerated were triploid (2n = 21) but many subsequent plants were 2n = 20 with one being 15 and one 18. Meiotic chromosome behaviour suggested the occurrence of reciprocal translocations, deletions and inversions.

Similarly Kasperbauer et al. (1979) examined over 1200 somaclones of the F_1 between *Lolium multiflorum* × *Festuca arundinaceae*. No obvious morphological variation was apparent in somaclones regenerated after 6-16 weeks. However after about 40 weeks phenotypic variants were apparent. Some karyotype variation was observable but the phenotypic variants were also found amongst somaclones with a normal 28 chromosome karyotype.

Orton (1980a) and Orton and Steidl (198) have found that *Hordeum* tissue culture causes polyploidy, aneuploidy and chromosomal rearrangements. Most significantly the somaclonal plants derived from cultures of the sterile hybrid *H. vulgare* × *H. jubatum* had enhanced multivalent formation, whereas the original hybrid showed nearly complete asynapsis of the chromosomes from the different genomes. Exchange between these two genomes will be made possible if breakage and reunion events occur during culture and also by the relaxation of pairing suppression between homoeologues in the meiotic behaviour of plants derived from those cultures. There was much morphological and isozyme variability amongst the somaclones derived from cultures of this hybrid. A number of the regenerates were haploid and generally of *H. vulgare* appearance. Hence *H. jubatum* chromosomes had been eliminated. The *H. vulgare*-type haploids had *H. vulgare* bands when analysed for esterase and glutamateoxaloacetate transaminase isozymes. Yet 2 of the 5 examined also showed a few *H. jubatum* bands indicating that some interspecific exchange had occurred prior to chromosome elimination (Orton 1980b).

5 The Origin of Somaclonal Variation

Any discussion of possible mechanisms for somaclonal variation at this time will need to be speculative. Nevertheless there are a number of hints available. It may of course be that different processes are at work in different species or that a number of processes are operating simultaneously in the one culture. The major benefit of speculations about mechanism is that it breeds further experimentation.

The most desirable endpoint would be to both understand the processes involved and be able to control them such that variation can be enhanced when required or suppressed when our goal is simply to propagate useful genotypes.

5.1 Karyotype Changes

In the past it was usually assumed that variants amongst somaclones were the consequence of gross karyotype changes such as aneuploidy or polyploidy (Murashige 1974; Thomas et al. 1979). Indeed gross karyotype alterations have very frequently been observed in tissue cultured plant cells (Murashige and Nakano 1967; Heinz et al. 1969; Sacristan and Melchers 1969; Kao et al. 1970; Malnassy and Ellison 1970; Horak et al. 1971; Bayliss 1973, 1980; Bennici 1974; Sree Ramulu et al. 1976; D'Amato 1978; Skirvin 1978; Roy 1980).

However in potato, Shepard et al. (1980) reported that the five protoclones examined (with promising high tuber yields) all appeared to have normal karyotype. It will require a more extensive and detailed cytological study before they can be definitive about the variants not being the result of complementary nulli-tetrasomic change.

It is well documented in sugar cane that chromosome number shifts can occur in cultures and culture-derived plants (Heinz et al. 1969, 1977; Krishnamurthi and Tlaskal 1974; Liu and Chen 1976; Liu et al. 1977). However a painstaking analysis of a somaclonal series from Pindar sugar cane could find no correlation between karyotype alterations and Fiji-disease reaction (Krishnamurthi and Tlaskal 1974). Similarly Liu and Chen (1976) were able to conclude from a cytological study of their sugar cane somaclonal variants that there was 'no apparent correlation between morphological modifications and changes in chromosome number.'

All of the sorghum somaclones of Gamborg et al. (1977) had a normal karyotype, 2n = 20, even those with leaf and growth habit abnormalities. Skirvin and Janick (1976a) found ploidy changes only in a small proportion of their *Pelargonium* variants. Oinuma and Yoshida (1974) used only confirmed doubled haploids in their study of genetic variation in tobacco from anther culture. Burk and Chaplin (1980) also found normal chromosome number and meiotic pairing behaviour in a random sample of their variant tobacco somaclones. No irregularities such as multivalents were observed at meiosis in F_1 hybrids between variant tobacco dihaploids and their donor parent (Gerstel et al. 1974). This would discount gross chromosome rearrangements or karyotype changes as the explanation for the tobacco somaclonal variation Most of the cauliflower

somaclones of Grout and Crisp (1980), including the variants, had normal karyotype (2n = 18). Similarly all of the lettuce and most of the garlic and *Lolium* × *Festuca* somaclonal variants were of apparently normal karyotype (Sibi 1976; Novák 1980; Kasperbauer et al. 1979). Barbier and Dulieu (1980) observed some polyploidization of tobacco somaclones but this occurred independently of the changes at loci affecting chorophyll synthesis.

It would seem that gross karyotype changes, while possible from cultures, are not necessary for the appearance of somaclonal variation. It is also likely that many gross karyotype changes will be selected against during plant regeneration, especially in diploids.

5.2 *Cryptic Changes Associated with Chromosome Rearrangement*

In contrast to gross changes in chromosome numbers, more cryptic chromosome rearrangements may be responsible for genetic variation in cultured cells. Jelaska et al. (1978) reported that the chromosomes of cultured *Vicia faba* cells can undergo changes which alter their Giemsa C-banding pattern. Chromosome breakage and reunion, multicentrics and translocations have been observed in the plants derived from cultures of barley (Foroughi-Wehr et al. 1979; Orton 1980a). In plants from ryegrass cultures the meiotic chromosome behaviour suggested the presence of reciprocal translocations, deletions and inversions (Ahloowalia 1976, 1978). Cummings et al. (1976) and McCoy (1979) observed heteromorphic pairs (deletions or translocations) and ring chromosomes (nonhomologous translocations) in oat somaclones. Heteromorphic pairs were also found in maize somaclones (Green et al. 1977). Chromosome irregularities such as breaks, acentric and centric fragments, ring chromosomes and micronuclei were observed in the mixaploid garlic somaclones (Novák 1980). Although these phenomena were not observed in the diploid and potentially useful garlic somaclonal variants nevertheless similar phenomena early in culture may have played a role in generating the variability.

Such chromosome rearrangement can result in loss of genetic material which may result in phenotypic variants. As well as affecting the gene in which the chromosomal break occurs, neighbouring genes, particularly those for which transcription may be coordinately regulated, will also be affected. If reunion or transposition to a different site occurs then distant gene functions may also be altered. This phenomenon, generally known as the position effect, is well documented in *Drosophila* (Lewis 1950; Spofford 1976) and is thought to occur in plants such as *Oenothera* (Catcheside 1947). Cryptic changes can not only result in the loss of genes and their functions but also the expression of genes which have hitherto been silent. For example, a rearrangement may delete or otherwise switch off a dominant allele allowing the recessive allele to affect the phenotype. Siminovitch (1976) refers to this phenomenon as culture-induced hemizygosity. If portions of the genome become effectively haploid many recessives will be expressed.

The detailed genetic analysis of tobacco somaclonal variants by Barbier and Dulieu (1980) seemed to indicate the occurrence of deletions at specific loci.

5.3 *Transposable Elements*

In procaryotes transposable genetic elements are stretches of DNA which can move from one locus in the genome to another independently of extensive sequence homology (see reviews by Kleckner 1977; Bukhari et al. 1977; Calos and Miller 1980). The excision and reinsertion of the genetic element can directly affect the expression of neighbouring structural genes. Moreover imprecise excision of bacterial transposable elements may generate rearrangements (deletions, inversions) of adjacent chromosomal sequences.

In eucaryotes genetic evidence suggests that certain unstable mutants may be explained by transposable elements. In maize, controlling elements, a concept developed by McClintock (1956), have many genetic properties which are analogous to those generated by transposable elements in bacteria (Peterson 1970; Peterson and Weber 1969; Fincham and Sastry 1974; Starlinger 1980). Genetic instability of the white locus in *Drosophila* also suggests the presence of a transposable element (Green 1977). Other mutable loci have been described in plants such as *Antirrhinum*, tobacco hybrids and soybean (see Fincham and Sastry 1974; Sand 1976), tomato (Hagemann 1958) and *Petunia* (Potrykus 1970). Transpositional events in mitochondrial DNA have also been implicated in the spontaneous reversion to fertility of S male sterile cytoplasm in maize (Levings et al. 1980). In no case has a causal relationship been established between genetic instability and the physical excision and/or insertion of DNA sequences.

Physical evidence for the mobility of certain types of repetitive DNA has been obtained in yeast (Cameron et al. 1979) and *Drosophila* (Finnegan et al. 1978; Strobel et al. 1979; Potter et al. 1980). Some of these mobile sequences are known to contain terminal repeats. No specific genetic effect of these transpositional events was reported.

Recently, mutational events at the his 4 locus in yeast (Chaleff and Fink 1980) have been correlated with the physical insertion of a transposable element (Roeder and Fink 1980). This element is homologous to a previously reported (Cameron et al. 1979) family of repetitive yeast DNA sequences. In addition His^+ revertants, which presumably result from the excision of the element, can con-

tain chromosomal aberrations such as deletions, translocations, transpositions or inversions.

The transposition-like events observed in eucaryotes such as *Drosophila*, do occur in somatic cells, and in maize the abolition of gene function and its occassional reactivation (possibly by insertion/excision events) is clonally inherited and is expressed as altered kernel variegation patterns.

It is thus conceivable that transpositional events could play a role in somaclonal variation. The tissue culture environment may be highly conducive for DNA sequence transposition. Weill and Reynaud (1980) referred to the high mutability and consequent adaptiveness in somatic tissues as 'somatic Darwinism'. More speculatively, Doolittle and Sapienza (1980) and Orgel and Crick (1980) have argued that transposable elements and certain classes of middle repetitive DNA may be ubiquitous and that their prime function is to ensure their own survival in the genome (hence the term 'selfish DNA').

Variability generated by sequence transposition is an effective means of enhancing adaptability to new environments such as occurs in cell culture. In plants the opportunity exists for the integration of such putatively generated variants into germ line cells.

5.4 Somatic Gene Rearrangements

The most well characterized example of somatic gene rearrangement is in the mouse immunoglobulin genes (Brack et al. 1978; Molgaard 1980). During differentiation of mouse embryonic cells to plasma cells the chromosomal genes undergo extensive rearrangement. Various regions of the embryonic DNA are eliminated during ontogeny and the functional regions are 'translocated' together. Similarly during sea urchin ontogeny there are extensive DNA sequence translocations occurring (Dickinson and Baker 1978). It is assumed that the germ line cells are unaffected. It would seem likely that such somatic gene rearrangements will also be found in higher plants. If so then the development of plants from somatic cells by tissue culture allows such rearrangements to exist in the new germ line.

5.5 Gene Amplification and Depletion

It has become clear in the last few years that specific genes in higher organisms can amplify themselves during differentiation or in response to environmental pressures, i.e. the number of copies of that gene per haploid genome increases. Depending on how gene expression is regulated this could mean that the production of mRNA and protein from that gene is increased. In mouse cell cultures the dihydrofolate reductase (DHFR) gene was found to have amplified as much as 200 fold in lines selected for resistance to methotrexate which inhibits DHFR activity (Schimke et al. 1977). Such amplification was a consequence of sequential selection at progressively higher concentrations of methotrexate. Similarly Strom et al. (1978) have observed amplification of specific genes in chicken embryos during differentiation of cartilage and neural retina tissues.

Wahl et al. (1979) selected hamster cells resistant to N-(phosphonacetyl)-L-aspartate which is a transition state analogue inhibitor of aspartate transcarbamylase. Analysis showed these lines to have amplified the appropriate CAD gene by up to 191-fold. CAD is the multifunctional protein which catalyzes the first three reactions of de novo UMP synthesis (carbamyl synthetase, aspartate transcarbamylase, and dihydroorotase).

In plants there is also evidence that such processes can occur. Cullis (1973, 1975) and Cullis and Goldsborough (1980) observed changes in the DNA of the flax variety, Stormont Cirrus, in response to different environmental conditions. Reassociation kinetics showed that the large plant form (L) had a class of moderately repeated DNA not present in either the small (S) or normal (P1) forms. S had 70% fewer ribosomal cistrons than L or P1. In other species also, such as wheat, rye, hyacinth, maize, *Vicia*, melon, and tobacco, there is evidence of ribosomal RNA gene amplification and depletion (Flavell 1975; Siegel 1975). Nagl (1979) has reviewed the evidence for differential DNA replication in plants.

If such amplification or depletion of DNA sequence copies also can occur in plant cell cultures it may account at least in part for somaclonal variation. Though not proven, several cases of selection in plant cell cultures are consistent with gene amplification. Selection for resistance to high levels of *Drechslera maydis* Race T toxin in toxin sensitive (T cytoplasm) maize cultures resulted from serial selection at progressively higher toxin levels (Gengenbach and Green 1975). Similarly Nabors et al. (1980) required 11 successive selection cycles to obtain high level resistance to salinity in tobacco cultures.

Some recent observations in soybean suspension cultures suggest that depletion of DNA sequences can occur in plant tissue cultures. After prolonged growth on medium with maltose as carbon source the cells had lost one third of their ribosomal genes (Jackson 1980). Of the two types of ribosomal repeats present in soybean, one (with the more complex, non-transcribed spacer) is lost within two generations of slow culture growth (on maltose). This sequence can be slowly reamplified again by returning to sucrose medium but only if the period on maltose has not been too long (Jackson and Lark, pers. comm.).

5.5 *Somatic Crossing Over and Sister Chromatid Exchange*

Somatic crossing over in heterozygotes results (50% of the time) in twin-spotting. This was first characterized in *Drosophila* but has also been demonstrated in leaves in tobacco, tomato, *Antirrhinum majus*, *Tradescantia hirsuticaulis*, soybean and *Gossypium barbadense* (Evans and Paddock 1976; 1980). Estimates of frequency based on soybean and tobacco range from 5.74×10^{-5} to 7.70×10^{-6} somatic cross-overs per 'spot-capable mitosis'. A 'spot-capable mitosis' is one occurring developmentally at a time such that the twin spot will be visually discernible (Evans and Paddock 1976).

Environmental factors and certain agents are known to increase the frequency. For example 1600 R of X-irradiation increased the frequency in soybean 282-fold. Mitomycin C also specifically induces somatic crossing over (Evans and Paddock 1980). Some of the somaclonal variation may be explained in terms of the tissue culture environment enhancing the frequency of somatic crossing over. This would be particularly so if the exchanges were asymmetric or even between non-homologous chromosomes.

Somatic cell sister chromatid exchange, if it is asymmetric, can also lead to deletion and duplication of genetic material. The estimates of frequency of sister chromatid exchange are very high (e.g. 20.6 per barley cell per division) (Schubert et al. 1980). Asymmetric exchanges have been observed in *Vicia faba* and were thought to occur in AT-rich segments (Schubert and Rieger 1979). If such asymmetric exchanges were somehow induced in tissue culture even in unique sequences the result would be somatic segregation for duplications and deletions. Unequal sister chromatid exchange has been implicated in explaining rDNA inheritance patterns in *Drosophila* (Tartof 1973). Petes (1980) demonstrated unequal sister chromatid exchange in rDNA sequences of yeast resulting in the elimination of an inserted gene in one segregant and its duplication in the other segregant.

5.6 *Cryptic Virus Elimination*

In order to explain the variation in disease reaction in somaclones of sugar cane and potato a radically different mechanism to those already discussed may be proposed (Adrian Gibbs, pers. comm.). There are many examples where prior infection of a plant with a virus results in an enhanced susceptibility to a fungal disease (Latch and Potter 1977; Potter 1980). For example, infection with maize dwarf mosaic virus renders wheat and maize more susceptible to root and stalk rotting fungi, maize and sorghum more susceptible to *Helminthosporium maydis*, and wheat more susceptible to *Puccinia recondita*. By contrast barley yellow dwarf virus renders rye-grass more resistant to *Puccinia coronata*.

Viruses may be present in plants and cause no symptoms. Kassanis et al. (1977) described a virus they called beet cryptic virus which was present in sugar beet in high numbers. The plants showed no symptoms. Such cryptic viruses may be widespread in other plant species as well.

Tissue culture and especially meristem culture is a recognized technique for freeing plants of viruses. We may propose, for example, that the susceptibility of Q101 sugar cane to the eyespot fungus is a consequence of the presence of a cryptic virus which gives no obvious symptoms. Many of the Q101 somaclones may have been freed of the cryptic virus by the tissue culture cycle and subsequently prove to be resistant to eyespot.

Such a possibility deserves further investigation. However one might expect that this mechanism would result in somaclones which are either resistant or susceptible. The observation of a distribution showing the whole spectrum of reactions would seem to be inconsistent with cryptic virus elimination.

6 Conclusion

Many authors (Heinz et al. 1977; Liu and Chen 1976, 1978a; Skirvin 1978; Hoffman 1978; Oono 1978b; Brettell and Ingram 1979; Thomas et al. 1979; Shepard et al. 1980; Barbier and Dulieu 1980) have given substantial acknowledgement to the existence of variability among plants regenerated from cell cultures. Generally their direct or allusive judgements imply that somaclonal variation is artefactual noise, confined to vegetatively-propagated plants, produced by karyotype changes or occurs only in chimeric tissues. Oono (1978b), Skirvin (1978), Thomas et al. (1979), Shepard et al. (1980) and Barbier and Dulieu (1980) have suggested that the phenomenon may prove to be of some value as a tool in plant improvement. The reluctance of most to give greater credence or significance to the variation probably reflects how deep-rooted is the expectation of palingenesis.

We have argued that somaclonal variation is widespread, being found not only in asexually propagated species but also in seed propagated, self-fertilizing species. Although the most extensive examples to date have been in non-diploids (sugar cane, potato and tobacco) it has been observed in diploid species also. The spectrum of characters affected can be diverse and the frequency of variant occurrence comparatively high.

There is a wealth of possible mechanisms that could account for the origin of somaclonal variants. Some of the possible mechanisms and some of the examples of variability appear to be most readily fitted to a model where the variations preexist in the somatic cells of the explant.

Other mechanisms, and examples, most readily fit a model where the variability is only generated during the tissue culture phase. The development and application of molecular genetic techniques together with the more sophisticated manipulation of cultured plant cells will doubtless identify and characterize specific genetic mechanisms.

Somaclonal variants are already having some impact on the improvement of sugar cane and potato cultivars, and in the breeding of new floricultural varieties. We envisage an increasing role of somaclonal variation as an adjunct to conventional plant improvement. This demands an efficient and sustained capacity to regenerate plants from cell cultures. Recent research has demonstrated that such a requirement is realisable in previously recalcitrant crop plants such as wheat (Shimada and Yamada 1979), rice (Oono 1978b) and important legumes (Bingham et al. 1975; Beach and Smith 1979; Phillips and Collins 1979; Kao and Michayluk 1980; dos Santos et al. 1980) possibly including soybean (Saka et al. 1980).

Somaclonal variation may find its greatest application for plant improvement in concert with selection for desirable mutations at the cellular level. It should no longer be surprising that in the recovery of cell culture mutants the frequencies have been so high and mutagenic treatments have often failed noticeably to enhance the frequency. Cellular selection is conceivable for the recovery of variants resistant to antimetabolites such as amino acid analogues, antibiotic drugs, pathotoxins, herbicides and physiological stress (see Maliga 1978; Thomas et al. 1979; Brettell and Ingram 1979). Many agronomically important attributes are known or suspected of having a cellular basis. These include resistance to host-specific toxins such as those found in some of the *Drechslera*, *Pseudomonas* and *Alternaria* pathogens, tolerance of adverse soils (salinity, metal toxicity), herbicide tolerance, tolerance to temperature stresses and waterlogging. Somaclonal variation, cellular selection and early rapid screening of regenerants collectively provide a powerful option for plant improvement.

In section IV we discussed the enhanced variability amongst interspecific hybrids following a tissue culture cycle of the hybrid tissues. The examples (in *Lolium* and *Hordeum*) are most easily interpreted as the consequence of the tissue culture cycle allowing far more genetic exchange between the two genomes than normally occurs during a meiotic cycle. We suggest this enhanced exchange results from some of the same processes operating to produce other forms of somaclonal variation. The genetic factors which can limit or completely suppress the association and exchange between different genomes in germ line cells appear to break down in somatic cell cultures. Very frequently, breeder's attempts to introgress alien genes into crop plants are frustrated by a lack of exchange between the crop and alien genomes in the hybrids. A brief callus phase of the hybrid embryo may be sufficient to overcome these barriers.

Finally, though enthusiastic about its possibilities, we advocate temperance about somaclonal variation lest a Pandora's box be opened. Genetic variability is valuable only if it is handled skilfully and the useful variants selected and evaluated judiciously. If somaclonal variation is as extensive as we believe, simply taking the first somaclone with the desired attribute would be myopic – many other genetic changes may also have occurred which are detrimental. A series of independently derived, presumptively beneficial somaclones must be evaluated under relatively stringent field conditions.

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