arises to have more funds made available to Agricultural Departments to extend their virus testing facilities.

Buds are tied in with rubber bands 120 mm long and 3.5 mm wide leaving the bud itself uncovered. These are a lot faster than tapes to tie and deteriorate in about three weeks saving the labour of cutting them off. Wherever possible we endeavour to bud high enough to leave some leaf growth below the bud.

Immediately after budding, 50% of the tree is cut off and 15 days later the stock is cut to just above the bud, a good proportion of which should then be pushing; any sucker growth is left until the bud is approximately 150 mm (6 inches) long. It is then completely trimmed off, and this is when the tree really starts to grow.

Pest Control. Temik, a granulate, is incorporated into the soil immediately after suckering. This translocates into the tree and gives very good control of thrips and other insects that would check or otherwise damage the young growth. Gusathion is used late in the season for tip moth control.

Digging. This commences early in June (early winter) using a U-shaped blade behind a high-rise tractor. We endeavour to dig the whole crop prior to delivery starting.

Being in an area isolated from all orchards, and able to rotate, disease control is relatively easy, but because of the fact that a lot of the trees go into old orchard situations all trees are dipped in a No-Gall solution immediately after digging.

In conclusion, if you are prepared to accept the extra worries of producing a one-year tree, (because everything has to be done when the tree is ready , not when you are ready), you can produce a tree that is very acceptable to the trade. It is dug with a minimum of root damage, and no loss of bottom buds shooting and dying because of shading; it is a tree that can be adapted to any of the training methods used in orchards today.

PLANT BIOTECHNOLOGY, SOMACLONAL VARIATION, AND VARIETAL IMPROVEMENT

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INTRODUCTION

Plant improvement and varietal selection originated with the dawn of human society. Under the pressure of deliberate

selection many of our domesticated species are distinctly different from their wild relatives. For example, the cabbage, the cauliflower, and brussel sprouts are all derived from the same species, *Brassica oleracea*, as a consequence of deliberate selection for the specialised development of the leaf, flower, and axillary buds, respectively.

Any varietal improvement program has a set of defined objectives. From an agricultural viewpoint, improvement focuses on those variables which maximise yield and economic return, such as disease resistance, earliness and maturity characteristics, drought tolerance, and features which enhance mechanisation and reduced energy dependent inputs. At the other end of the spectrum, the floriculture and nursery industry may see the maintenance of uniformity on the one hand, and spectacle and uniqueness of new cultivars on the other, as the desiderata.

Whether the concern is large scale, efficient production of food and fibre crops, or the use of plants to enhance our aesthetic environment, plant breeding has and will figure prominently. In essence, plant improvement is a numbers game. Unique or rare gene combinations have to be identified and recovered from a large population of plants. Such specific gene combiantions may result from recombination in a hybrid gene pool. Unique mutants may occur spontaneously or be induced by mutagenesis. The skill or art of the plant breeder lies in his ability to rapidly identify those variants he considers useful. His job is made easier if techniques can be devloped to enhance the frequency of potentially useful variants.

Plant biotechnology, which includes plant cell culture and the exciting area of genetic engineering, has the potential of greatly amplifying both the amount of genetic variability available to plant breeders and the power of selecting useful genotypes.

PLANT CELL CULTURE

For an increasing number of species, cell lines can be induced to proliferate under defined culture conditions. Plant tissue culture media consists of defined amounts of inorganic salts, trace elements, vitamins, a carbon source for energy, and phytohormones. Virtually any part of a plant can be induced to form a cell line, including the root and stem section, hypocotyl, cotyledons, leaves, and even immature haploid pollen grains.

Cultured plant cells have the enormous advantage of totipotency, i.e., rapidly growing undifferentiated cell lines can be induced to form shoot and root primordia which develop into

fully fertile plants. The principal determinant of this differentiation process is the relative levels of auxins and cytokinins in the culture medium. Generally, a high ratio of cytokinin to auxin induces shoot formation while the converse tends to favour root initiation. For any given species, the culture conditions which favour rapid undifferentiated cell proliferation as against plant regeneration is arrived at empirically.

Plant propagators and the nursery industry were quick to see the advantages of tissue culture in terms of rapid propagation of desirable genotypes. There is now a large number of plant species which are amenable to rapid propagation by tissue culture technology (10). In recent times geneticists and plant breeders have successfully applied these techniques to many important agriculture crops. The objective was to augment the armoury of conventional plant improvement. Exciting consequences have followed this development.

SOMACLONAL VARIATION

It often happens that valuable scientific applications emerge from unexpected quarters. Because a tissue culture cycle was seen essentially as cloning a particular genotype it became the accepted dictum that all plants arising from tissue culture should be exact copies of the parental plant. Phenotypic variants which occurred among regenerated plants were dismissed as "artifacts of tissue culture". The variants were viewed as transitory consequences from exposure to phytohormones and were labelled as epigenetic events which somehow made them unworthy of further scientific interest. Now it seems this was a premature and erroneous judgement. Tissue culture apears to be an unexpectedly rich and novel source of genetic variability. This variation is called somaclonal ("protoclonal") variation and has been reviewed in detail by Larkin and Scowcroft (3).

(A) **Agricultural species**

(i) *Sugarcane.* The first real developments to utilize tissue culture generated variation as a plant breeding tool began in the experiment station of the Hawaiian Sugar Planters' Association. This initial work, followed by research in Fiji and Taiwan, has resulted in sugarcane genotypes with increased resistance to several diseases including downey mildew, Fiji virus disease, and eyespot disease.

Our own research to explore the potential of somaclonal variation to improve Australian sugarcane cultivars began in 1979. We were concerned with disease resistance, in which eyespot disease *(Helminthosporium sacchari)* figured promi-

nently. All lines were derived from the cultivar, Q101, which is agronomically valuable, except that is susceptible to eyespot disease. Following prolonged culture (up to 15 months) a large number of plants were regenerated and subsequently grown under glasshouse conditions.

To facilitate screening a large number of somaclones, a leaf bioassay was developed to quantify the sensitivity of the leaves of a given plant to a standardised concentration of the fungal toxin responsible for leaf damage. The sensitivity is measured as the initial rate of leakage of electrolytes from leaf discs briefly exposed to the toxin. This metric proved to be highly repeatable and consistent for a given cultivar (4).

The distribution of toxin sensitivity among these somaclones is depicted in Figure 1. Many of the somaclones proved to be resistant or essentially immune to the effects of the toxin and the mode of the distribution is significantly shifted to the resistant side of Q101. All 52 somaclones of Q47, a highly resistant cultivar, retain their resistance. Most importantly these resistant somaclones of Q101 retain their resistance through subsequent cane generations. Second and subsequent tissue culture cycle somaclones derived from resistant somaclones also tend to retain toxin insensitivity.

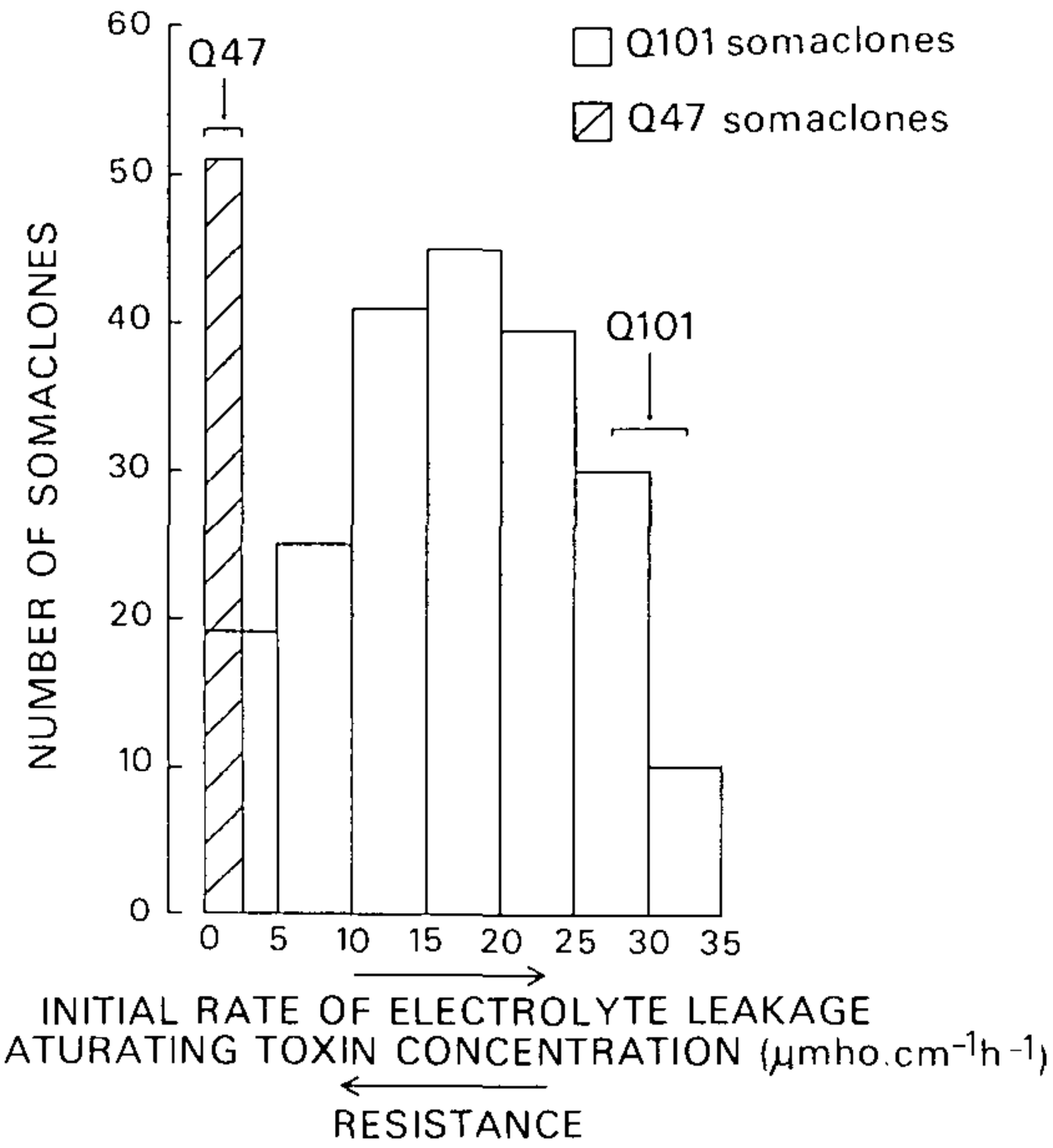

Figure 1. The distribution of reaction to eyespot toxin of Q101 somaclones, and Q47 somaclones. The parental reactions are as indicated by arrows.

Recent research in Taiwan has found variation among sugar cane somaclones from 8 cultivars in characters such as yield, sugar content, stalk number and weight, fibre percentage, and several leaf characters. Under stringent field experiments some of these somaclones showed significantly increased sugar yield relative to their parental cultivar. This research has also recovered resistant somaclones from parents susceptible to either downey mildew or culmicolous smut disease.

(ii) *Tobacco.* Because tobacco has been amenable to cell culture and plant regeneration for some time it might be expected that somaclonal variation has been observed in this species. And indeed this is so.

Burk and Matzinger (1) derived by anther culture a series of dihaploid lines from a tobacco cultivar which had previously been inbred for 15 generations. It would be expected that the 41 dihaploid lines were identical both with each other and the parent from which they were derived. However, significant variability was observed for all the economically important characters examined, namely: yield, grade index, days to flowering, leaf characters, alkaloid content, and reducing sugars. Surprisingly, the variability observed was as great as that normally associated with a segregating F_2 population from a cross between two different cultivars.

Recent work has also shown that even when specific loci controlling chlorophyll synthesis are considered, the "mutation rate" among regenerating plants is a staggering 3 to 4%.

(iii) *Potato.* Perhaps the most significant demonstration of the potential value of somaclonal variation was provided by Shepard *et al.* (8) in potato. In North America the 70 year old cultivar, Russet Burbank, represents 39% of the potato crop. This cultivar, along with most "old" potato cultivars, though not particularly remarkable in any specific character, has been retained because it is a good "all-rounder". Nevertheless, each year about 22% of the world potato crop is lost through disease. The sterility or very low fertility of many cultivars discourages their use in breeding programs.

Shepard *et al.* (8) argued that it might be simpler to selectively improve a popular cultivar than to create a new one. Screening over 1000 somaclones produced from leaf protoplasts of 'Russet Burbank', they found significant and stable variation in compactness of growth habit, maturity date, tuber uniformity, tuber skin colour, photoperiod requirements, and fruit production. Some characteristics, such as greater tuber uniformity and early onset of tuberisation, were agronomic improvements over the parent, Russet Burbank.

It is most significant that some 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.

These variant somaclones have retained their phenotype through a number of vegetative generations. Sixty-five selected somaclones have now been analysed in detail for variability under field conditions (7). Among 35 characters analysed statistically significant variation was found for 22 characters. All clones differed from Russet Burbank for at least 1 character and one somaclone differed for 17 characters. The modal class of 15 clones differed from Russet Burbank in 4 characters.

(iv) *Rice.* Among rice somaclones, variants have been reported in characters 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.

Oono (5) has made a detailed analysis of some 800 somaclones derived from a homozygous line of a selfed doubled haploid. Chloroplast content, flowering date, plant height, fertility, and morphology were examined in each of these somaclonal derivatives and in each of two subsequent selfed generations. For these characters only 28% of the plants were normal with respect to the parental phenotype. 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 form 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.

(v) *Other species.* Table 1 provides a list of other agriculturally important species in which variation has been observed among plants regenerated from cell culture. Increasingly, this cell culture approach is being explored as an adjunct to the improvement of agriculture species.

(B) **Horticultural and Floricultural Species.**

(i) *Pineapple.* Among some 450 somaclones of pineapple (11), variation was observed in spine and leaf colour, wax

Table 1. Additional species displaying somaclonal variation

Species	Variant characters
Oats	plant heights, heading date, leaf striping, twin culms, awn morphology
	heteromorphic bivalents, ring chromosomes
Maize	abphyl syndrome, pollen fertility
Barley	plant height, tillering, fertility
Sorghum	fertility, leaf morphology, growth habit
Onion	bulb size and shape, clove number, aerial bulbil germination
Rape	flowering time, glucosinolate content, growth habit

secretion, foliage density, leaf width and leaf spine formation. The origin of the cell culture explant had a dramatic effect on the occurrence of variation. If the explant for the initiation was syncarp or slip, nearly 100% of the somaclones were variant, whereas if the crown was used only 70% of the somaclones were variant.

(ii) *Pelargonium*. A remarkable degree of variability has been observed among plants regenerated from 5 different cultivars of *Pelargonium* (8). In contrast, plants propagated from stem cuttings were all indistinguishable from the parent plants. Somaclonal variation was observed in leaf shape, size and form, flower morphology, plant height, degree of fasciation, anthocyanin pigmentation and essential oil composition. From one cultivar, Attar of Roses, 28 out of 55 somaclones examined were variant.

Cytological examination of the somaclonal variants did reveal some ploidy changes but only in a small proportion of them. In some of the cell culture lines there was a tendency for increased variability with increased duration of the culture cycle. From this program a new cultivar, Velvet Rose, has been released.

(iii) *Begonia*. In several floricultural species, such as *Begonia*, adventitious bud technique is used for rapid clonal propagation. This technique is based on the phenomenon that adventitious shoots induced on petioles of detached leaves originated from single cells. This technique has been extended to the induction of adventitious shoots on leaf explants.

In a mutation breeding program with *Begonia* × *hiemalis*, leaf explants of 2 different cultivars were either not irradiated or irradiated with X-rays (6). Adventitious shoots were then induced on leaf disc explants from both X-ray treated and unirradiated leaves. From these shoots a second cycle of adventitious shoot formation was initiated and adventitious shoots so derived were subcultured to produce plantlets and finally flowering plants. These somaclonally derived plants

were examined for variation in the colour, size and form of flowers and leaves.

Among a total of 894 plants examined from the 2 cultivars, 266 were considered mutant. While X-rays caused a higher frequency of mutant plants, a high frequency was also found among plants derived from unirradiated leaf disc explants. The frequency from non-irradiated leaves of one cultivar was a staggering 43% whilst in the other, 7% mutant plants were found. The majority of these variants were solid mutants.

(iv) *Other species*. Somaclonal variation has also been observed in *Chrysanthemum* for such characteristics as flower colour and the temperature required for flower induction (2) and also in daylily and carnation.

Among vegetable crops variation has also been observed in somaclonally-derived plants of onion, lettuce, and tomato.

ORIGIN OF SOMACLONAL VARIATION

An understanding of the causes of somaclonal variation is important, first to be able to enhance the level of variation where increased variability is the objective. Second, where the goal is to clonally propagate a cultivar to produce uniform progeny the ability to suppress the phenomenon could be very desirable.

Doubtless, many different genetic processes operate to generate somaclonal variants. A discussion of some of the possible processes is to be found in Larkin and Scowcroft (3). Gross karyotypic changes and chromosomal rearrangements have been documented in both cell cultures and regenerated plants. However, these gross changes seem to reflect the capacity of cell culture to generate varation rather than as an underlying cause of phenotypic variability. While abnormal karyotypes have been observed in somaclones of potato and pelargonium, for example, these represent only a small proportion of the total phenotypic variants observed.

Cryptic chromosomal rearrangements leading to translocations, deletions and inversions, for example, could have a genetic consequence affecting an individual phenotype. For example, a small rearrangement may delete or otherwise switch off a dominant allele thereby allowing the recessive allele to affect the phenotype.

There is evidence in higher organisms, including plants, of a phenomenon where particular stretches of DNA (transposable elements) can move from one locus to another at relatively high frequency. The transposition of such elements from one chromosomal location to another generates mutations. A tissue culture environment may be conducive to sequence

transposition. This appears to be the case in animal cell cultures. Such a high frequency transposition would generate extensive phenotypic variability.

It has been shown for higher organisms, including plants, that the quantity of a specific gene product can be increased or decreased simply by amplification or diminution of the gene copy number. The artificial nature of a tissue culture environment may impose sufficient selection pressure to cause both amplification of some genes and diminution of others. This would obviously affect the phenotype of plants regenerated from the cell lines so affected.

CONCLUSIONS

Somaclonal variation among plants regenerated from cell culture is extensive both in the range of species and diversity of characters affected. It has already been recognised as being a significant source of genetic variability for varietal improvement. Several research programs have already been initiated to exploit its potential usefulness.

On the negative side, somaclonal variation has also been recognised as a hindrance to product uniformity by those who utilise cell culture for the rapid propagation of floricultural and ornamental species. In this context the frequency of somaclonal variation appears to be enhanced in older cell lines. Thus, an expedient approach to reduce the incidence of somaclonal variation is to continually reinitiate explants from the parental genotype and to regenerate plants only from relatively recently explanted material.

Somaclonal variation may find its greatest usefulness in concert with selection for desirable mutations at the cellular level. It is no longer surprising that in the recovery of cell culture mutants the frequencies have been relatively high and mutagenic treatments often enhance the frequency only marginally if at all. Many agronomic traits are known or suspected of having a cellular basis. These include disease resistance, particularly where a host-specific toxin is involved, tolerance of adverse soils (salinity, metal toxicity), herbicide and temperature stress. For each of these attributes genotypes have already been recovered following cell culture selection. Cellular selection and somaclonal variation collectively provide promising new technology from plant improvement.

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LABOUR REDUCTION TECHNIQUES FOR PROPAGATING AZALEAS AND MAGNOLIAS BY CUTTINGS

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Preparation and Pasteurization of Medium. One part German peat and three parts coarse river sand are mixed in a 6 cu.yd. concrete mixer. This is driven by a 3 h.p. electric motor. The drum revolves at 3/4 rpm.

Heat pasteurisation of the medium is performed in the revolving drum by a diesel oil burner. (Fig 1.) This is best described as a "conduction heater" or an "indirect flame heater."

A vented mild steel pipe, approximately 6 ft. long, is attached to the burner and carries the heat inside the drum. When mixing, the drum revolves in a counter-clockwise direction, pulling the soil to the right hand side of the drum. The heat is directed into the air space on the left hand side of the drum. The hot air then warms the steel drum. The medium is warmed by contact with the hot air and heated metal surface inside the steel drum. Hence the reason for the above names.