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Sources and Frequency of Somaclonal Variation

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Plant tissue culture was introduced to the public as an asexual method to propagate plants rapidly and reliably. Many ornamental, fruit, and vegetable crops are now routinely propagated commercially in vitro. The ease of tissue culture propagation has revitalized certain ornamental industries and facilitated the rapid introduction of new cultivars. In spite of new cultivars and in spite of these successes, some commercial propagators are now advertising that their plants are "not from tissue culture," while others continue to advertise that their plants are tissue culture-derived (e.g., *American Nurseryman*, Apr. 1990, Classified Section, "Daylilies").

What is going on? Commercial nurseries are now encountering the ubiquitous phenomenon of somaclonal (tissue culture-derived) variation (Anonymous, 1989; Knuttel, 1989). Since all cells of an organism are derived from a single cell, researchers assumed that plants derived from cells of a mother plant would yield plants identical to the mother plant. However, according to Scowcroft (1985), who has researched somaclonal variation since it was formally acknowledged, "clonal uniformity is now recognized as the exception rather than the rule."

WHAT IS SOMACLONAL VARIATION?

Somaclonal variation is variation among regenerated plants that occurs as a result of tissue culture of any type. It may arise from pre-existing or induced variation. Some scientists add another aspect to the definition and require that somaclonal variation be heritable through a sexual cycle. Unfortunately, it is not always possible or feasible to demonstrate heritability because of complex sexual incompatibilities, seedlessness, polyploidy, or long generation times. Hence, it may be difficult or impossible to verify the heritable nature of somaclonal variation for these crops.

Such variation is a serious problem confronting researchers or propagators who require uniformity. However, the natural variability associated with tissue culture represents a pool upon which selection pressure can be imposed to isolate unique forms of a clone. The amount

of variation that can be expected will vary with the clone, age of the culture, use of mutagenic agents, and selection pressure applied to cell suspensions or callus cultures of clones for stress conditions such as salt level, herbicides, microorganisms or their byproducts, and specific metabolites (Skirvin, 1978). The exploitation of such natural and induced variation seems especially applicable to old woody plant cultivars, such as 'Bartlett' pear (*Pyrus communis* L.) and 'Delicious' apple (*Malus domestica* Borkh.) (introduced in 1770 and 1893, respectively) (Skirvin, 1977), which could be expected to have accumulated many mutant cells that may have stabilized into chimeras of various complexity.

Skirvin and Janick (1976a) were among the first to emphasize the importance of clonal variation in genotype improvement of horticultural species. Shepard et al. (1980) demonstrated extensive variability among plants regenerated from cultured leaf protoplasts (protoclones) of 'Russet Burbank' potato (*Solanum tuberosum* L.). In investigating the extent and possible significance of this source of genetic variability, Larkin and Scowcroft (1981), who have reviewed the topic extensively, first suggested that the term "somaclonal variation" be used to describe the phenotypic variation observed among plants regenerated after a passage through tissue or cell culture. Tissue culture-related variants have also been referred to as calliclones (Skirvin and Janick, 1976a), phenovariants (Sibi, 1976), and protoclones (Shepard et al., 1980). However, the term somaclonal variation describes the system best and has justifiably become the standard term to describe the phenomenon.

The amount of variation that can be encountered in vitro has been described for banana (*Musa ×paradisi* L.) by Smith and Drew (1990) in a review of Australian tissue culture programs and prospects. They reported that up to 90% of the in vitro-derived banana plants in Australia were off-types under field conditions. The off-types primarily consisted of dwarf "choke-throat" plants. This choke-throat condition prevents the bunch from emerging from the banana shoot and produces closely packed, undersized fruit. Although the choke-throat off-types were devastating to the banana tissue culture industry (Walduck et al., 1988), as reported by Smith and Drew (1990), a few dwarf plants with normal fruiting characteristics have been observed (Smith and Hamill, 1991). According to Smith and Drew (1990),

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banana breeders in Australia have used conventional breeding protocols with this triploid parthenocarpic fruit crop for more than 60 years and isolated no commercially acceptable cultivars. They hope that new phenotypes will be selected among the banana somaclones and eventually introduced as cultivars.

Somaclonal variation is observed in all crops; some commercial tissue culture companies maintain greenhouse space for conserving and growing out off-types in an effort to obtain marketable plants (Michael Kane, Univ. of Florida, Gainesville, personal communication).

Somaclonal variation is not limited to the plant kingdom. There have been hundreds of reports of cell line variants among animal tissue cultures. In fact, the phenomenon of somatic variation was so well known in animal systems that an entire book was devoted to the topic in 1964 (Harris)—long before the topic was discussed in plant systems.

TYPES OF SOMACLONAL VARIATION

Somaclonal variation appears to result from both pre-existing genetic variation within the explants and variation induced during the tissue culture phase (Evans et al., 1984). There appear to be two types of somaclonal variation: heritable and epigenetic. Heritable variation is stable through the sexual cycle or repeated asexual propagation; epigenetic variation may be unstable even when asexually propagated. Somaclonal variation can involve either single or multiple genes and can be due to alterations in DNA bases, genes, chromosomes, or entire sets of chromosomes (Orton, 1984).

Epigenetic variation is also known as developmental variation. It includes persistent changes in phenotype that involve the expression of particular genes (e.g., the gene for thorniness of juvenile *Citrus*) (Hartmann and Kester, 1983). Explants from mature parents adapt to the in vitro environment by becoming more juvenile in a step-wise manner. Tissue cultures may be at any stage of development, from mature to fully juvenile. Plants regenerated from such tissues will vary, depending on the developmental stage that the tissue had attained when the stimulus to regenerate was received. Shoot regeneration from dedifferentiated cultures can yield an immature form of the parental clone that may or may not be stable over time. For instance, *Eucalyptus* cultures could yield regenerants with sessile leaves, a juvenile trait. However, with time, such juvenile traits cease to be expressed and the parental type reappears.

The best known example of epigenetic variation is the loss of auxin, cytokinin, or vitamin requirements by callus (Jackson and Lyndon, 1990; Meins, 1982; Skirvin, 1978). These changes in requirements are collectively known as tissue or cellular habituation. Other epigenetic changes include extreme vigor ex vitro (Swartz et al., 1981) that is probably associated either with the reversion to juvenility discussed earlier or with virus elimination (Abo El-Nil and Hildebrandt, 1971). Tissue culture-derived plants behave like seedlings and grow vigorously until flowering has been induced. At that time, the plant often reverts to the phenotype of the parental plant. This temporary sort of vigor has been exploited by the tissue culture industry to produce vigorous young plants that transplant easily, grow rapidly, and become established more quickly than conventionally propagated plants (Swartz et al., 1981). Transient dwarfism is probably epigenetic also and may be due to a carryover of growth regulators from the tissue culture medium. Many dwarfed plants revert to a normal growth habit after a season or two of growth under field or greenhouse conditions (McPheeters and Skirvin, 1989; Moore et al., 1991).

An example of heritable somaclonal variation is the development of pure thornless blackberries from chimeral parents. 'Thornless Evergreen' (TE) is a thornless mutant of the European thorny apomictic 'Evergreen' blackberry (*Rubus laciniatus* Willd.) (Darrow, 1931; Waldo, 1977). TE is a periclinal ("hand-in-glove") chimera in which a layer of mutant (thornless) cells surrounds a core of wild-type (thorny) tissue. TE blackberry is grown widely in the Pacific northwestern United States and is one of the most economically important blackberry cultivars in the United States (Moore and Skirvin, 1990).

Although the TE plant appears completely thornless, its histological thornlessness is not stable since the plant produces thorny adven-

titious root suckers. This instability is due to the fact that TE is a periclinal chimera with its histogenic layers arranged such that a layer of L_1 -derived mutant thornless tissue overlies wild-type thorny tissue. Root cuttings of TE arise from L_{III} -derived internal tissue that has the thorny genotype and therefore "reverts" back to the thorny condition. In addition, gametes of TE that arise from the L_{II} -derived tissue express the thorny, not the thornless, character in crosses. The chimeral nature of TE is maintained commercially by layerage and cuttage (Darrow, 1931; McPheeters and Skirvin, 1983; Waldo, 1977). These asexual systems produce new plants from pre-existing axillary buds and thus maintain the chimeral status.

Rapidly proliferating TE shoot-tip cultures formed many shoots in vitro (McPheeters and Skirvin, 1983). Nearly half of the TE regenerants were dwarfed and had a unique bent petiole phenotype. Root segments from these dwarfed plants produced thornless sucker plants; roots of normal-sized vigorous plants produced thorny suckers. The pure thornless blackberries had probably developed as adventitious shoots from TE epidermal cells; the full-sized plants were still chimeras and of axillary bud origin. Because the dwarfed plants were pure thornless, their gametes were expected to carry the thornless character. When a sample of these pure thornless plants were hybridized and selfed, the bent petiole character was found to be linked to a dominant thornless gene (S_{te}) that was transmitted sexually (Hall et al., 1986a).

A sample of 150 pure thornless TE plants was moved to a field where they exhibited continuous somaclonal variation for plant vigor, height, erectness, leaf morphology, fertility, and flower and prickly number (McPheeters and Skirvin, 1989). Among the somaclones, a pure thornless and fully fertile form of TE has been selected. This unique plant is slightly smaller than the parent plant and produces thornless, not thorny, adventitious root suckers. The somaclone has been tested for its commercial potential in Oregon for several years (L. Martin, Dept. of Horticulture, Arkansas State Univ., personal communication). It produces fruit that appears identical to that of the parent type. The clone is now being patented and will soon be formally introduced as a new cultivar. A similar dominant thornless gene has been identified from 'Thornless Loganberry' (Hall et al., 1986b) and named S_p (Rosati et al., 1986). The pure thornless type was released to the public as 'Lincoln Logan' (Hall et al., 1986c).

VALUE OF SOMACLONAL VARIATION FOR PLANT IMPROVEMENT

To have an impact on a plant breeding program, an in vitro scheme must either produce a plant useful for breeding or be useful directly. A few somaclones have been released as named cultivars (Table 1), but many somaclones are still being tested. Ostry and Skilling (1987), for instance, screened poplar regenerants with *Septoria musiva* and isolated stable putative somaclones with resistance to this fungus. They pointed out that these plants should not be considered heritable somaclones until genetic analyses had demonstrated it. However, in an economically valuable asexually propagated crop such as poplar (*Populus* L.), even a stable epigenetic change of an elite poplar could be of value. Furthermore, Ostry and Skilling state that "somatic variation may be a better means of tree improvement than either direct

Table 1. A sample of named horticultural cultivars derived from tissue culture via somaclonal variation.

Crop	Source
Ornamental	
<i>Eustoma grandiflorum</i>	Griesbach and Semeniuk, 1987; Griesbach et al., 1988
<i>Hemerocallis</i> Yellow Tinkerbell	Griesbach, 1989
<i>Paulownia tomentosa</i>	
Somaclonal Snowstorm	Marcotrigiano and Jagannathan, 1988
<i>Pelargonium</i> Velvet Rose	Skirvin and Janick, 1976b
<i>Torenia</i> UConn White	Brand and Bridgen, 1989
Fruit	
<i>Rubus</i> Lincoln Logan	Hall et al., 1986b, 1986c
Vegetable	
<i>Apium</i> UC-T3 Somaclone	Heath-Pagliuso et al., 1989
<i>Ipomoea batatas</i> Scarlet	Moyer and Collins, 1983

gene insertion or *Agrobacterium*-mediated transformation because of the many [regulatory and] technical difficulties that must be overcome with those methods.”

The power of somaclonal variation for plant improvement is that new traits are developed in the best cultivars available. Most scientists perform somaclonal variation research with elite individuals or clones with known potential as cultivars or breeding lines. The variation observed in such material can result from various sources (discussed below), including the induction of expression by genes that are present in the genome but that had been suppressed at some stage of development. However, one should be wary of assuming that characters propagated through repeated asexual generations are stable. For instance, Maretzki (1987) isolated a somaclone of sugarcane (*Saccharum officinarum* L.) with resistance to eye-spot. The character proved to be stable through 10 years of asexual propagation, but the trait was recently lost (Smith and Drew, 1990), which suggests either the epigenetic nature of resistance or a resistant strain of the fungus.

ORIGIN OF SOMACLONAL VARIATION

The causes of somaclonal variation are not well understood and have not been elucidated. However, among the heritable types of variation, single base-pair changes, chromosome deletions, translocations, and changes in ploidy have been encountered (D'Amato, 1991; Evans and Sharp, 1986). Variation is not limited to nuclear genes; Gengenbach and Umbeck (1982) have used restriction enzyme analysis of isolated mitochondrial DNA to demonstrate variation in mitochondrially controlled male sterility. The broad spectrum of variation available through both nuclear and cytoplasmic characters suggests that all classes of variation could be recovered and used for crop improvement.

Pre-existing variation

Multicellular explants. Explants derived from a single clone, seed, or seedling are assumed to be genetically uniform, permitting the conclusion that spontaneous genetic instability occurs in vitro (Orton, 1983). However, this assumption is not always valid, because explants may include several types of cells, such as phloem, parenchyma, cortex, and xylem parenchyma. These cells may also vary in their ploidy level. To account for the lack of uniformity in multicellular explants, Bright et al. (1983) suggested that explants derived from sources other than protoplasts be called “complex cultures” in recognition of their multicellular origin.

Chimeras. Many plants are known to be chimeras. They consist of cell sectors or tissues that differ in genetic constitution and that have developed from a meristem containing layers or sectors of mutated tissue (Hartmann and Kester, 1983). Dermen (1960) has described many chimeras, and Whitham et al. (1984) wrote a fascinating discussion of the evolutionary value of chimera formation and maintenance in woody plants.

The arrangement of the genetically different tissues within the plant meristem affects chimera stability. The most stable chimera is the periclinal chimera. However, even these chimeras can exhibit instability by cell displacement and replacement (Stewart and Dermen, 1970), resulting in natural variation known as sports. Chimera plants grown in tissue culture can yield variants in high percentages. For instance, as discussed earlier, McPheeters and Skirvin (1983) found that nearly half of the shoots obtained from tissue culture of a chimera thornless blackberry were dwarfed and pure thornless. Much of the variation was attributed to chimera segregation. Although some types of somaclonal variation can be explained in terms of chimera segregation, regenerants may include somaclonal variants resulting from variation other than simple chimera segregation. To test for pre-existent somaclonal variation, regenerants may be subjected to another round of in vitro regeneration. Clones with pre-existent variation should yield more variability in the first generation than in the second, etc. After the second or third generation, one would expect that pre-existing variation either would be eliminated or stabilized. Subsequent variation is more likely to be tissue culture derived.

The most common cause of chimera dissociation is adventitious

shoot production (Marcotrigiano, 1990). Adventitious shoots can be produced either in vivo or in vitro. Adventitious shoots usually arise from a single cell or a few cells that could be derived from a specific tissue (Broertjes, 1969; Broertjes and Keen, 1980). If adventitious shoots can be traced to a single cell, then any system that yields adventitious shoots can result in chimera segregation. Adventitious shoot formation, the same process that results in chimera segregation, also is related to the induction of somaclonal variation (Karp, 1989). It is well known that shoots of preformed origin (e.g., axillary buds) show much less variation than those that arise from adventitious budding systems, such as organogenesis or embryogenesis (Karp, 1989). Most of this variation is probably due to pre-existing variation or variation induced during callus formation, not the shoot formation process itself.

Conversely, clonal stability is best achieved by avoiding adventitious shoot formation (Karp, 1989; Skirvin, 1978). Therefore, in moderately proliferating, well-cared-for, shoot-tip cultures, one would expect little or no variation because propagules develop from axillary buds and thereby maintain the integrity of their histogenic layers. Such has been the case for nonchimera blackberries. In fact, the use of commercial tissue culture proliferation techniques to propagate genetically thornless nonchimera blackberry plants is a rapid, relatively risk-free method of producing uniformly vigorous and productive true-to-name plants (Swartz et al., 1983).

Tissue culture-induced variation

Callus is so often associated with somaclonal variation that some callus-derived variants have been called “calliclones” to denote their callus origin (Skirvin and Janick, 1976a). [The first tissue culture-derived cultivar of any species was a calliclone of *Pelargonium* known as ‘Velvet Rose’ (Skirvin and Janick, 1976b)]. One reviewer even ventured that variation is “... only associated with regeneration through a callus or cell suspension phase and not with true micropropagation or meristem-tip culture” (Karp, 1989). The relationship between callus and somaclonal variation is so strong that many commercial laboratories try to avoid callus development at any stage of propagation.

Callus initiation in vitro occurs most readily on cut or exposed surfaces in contact with medium. Callus initiation is probably analogous to wound responses observed in vivo, which activates transposable elements and stimulates the appearance of stress-induced enzymes and specific by-products (McClintock, 1984).

However, variation is not limited to callus regenerants. Evans (1988) reports considerable variation for morphological traits (flower shape, leaf shape, plant height), pollen viability, and chromosome number among adventitious *Nicotiana glauca* Link & Otto plants regenerated directly from leaf explants without a callus intermediate. Variation also occurs among plants regenerated via embryogenesis.

Growth regulators. Growth regulators such as 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzylaminopurine (BA) have been implicated in the induction of variability (Evans, 1988; Griesbach et al., 1988; Shoemaker et al., 1991), but their direct relationship to this phenomenon is still debated.

Although the effect of specific growth regulators on variation remains unclear, most tissue culturists agree that variation rates are increased as the overall concentrations of growth regulators rise. High growth regulator concentrations also can alter the frequency of ploidy changes vs. point mutations. The excising process itself is probably enough to stimulate wound responses that growth regulators enhance (McClintock, 1984).

Cultivar. The amount of variation encountered in vitro is not the same for all cultivars of a species (Kurtz and Lineberger, 1983). Some cultivars show excessive variation, others are relatively stable. For instance, Hwang and Ko (1986) reported an overall variation rate of 3% among banana cultures, but the rate of variation for ‘Cavendish’ bananas was up to 20%.

Cultivar age. Although older asexually propagated clones would be expected to show more preexisting variation than newer clones, there seems to be little correlation between clonal age and the amount of variation observed in a clone. Shepard et al. (1980) were among the first to report somaclonal variation. They found thousands of variants

Ploidy level. Since the ability to regenerate in vitro is a heritable trait in tomato (*Lycopersicon esculentum* Mill.) (Evans and Sharp, 1983; Kurtz and Lineberger, 1983), differences in cultivar response also could have a genetic basis. Variability seems to be highest among polyploid and high-chromosome species. One of the first important reports of somaclonal variation involved the isolation of callus lines from sugarcane, a plant with high chromosome numbers (Heinz and Mee, 1969). Heinz and Mee obtained callus lines that varied in their growth habits and color. Each of these lines was later found to have a different number of chromosomes. Similar differences were noted among regenerated plants (Heinz and Mee, 1971).

Explant source. When exploring a new species or cultivar for somaclonal variation, it is a good idea to use several different explants and compare progeny from each. Not all explants should be assumed to exhibit variation equally. In general, variation is less likely to be observed from preformed shoots (axillary buds, shoot tips, and meristems) than from explants that have no preformed shoot meristems, such as leaves, roots, or protoplasts (discussed earlier). For instance, protoplasts of 'Russet Burbank' potato (Shepard et al., 1980) yielded thousands of variable progeny from which two or three advanced lines have been selected (Gary Secor, Dept. of Plant Pathology, North Dakota State Univ., Fargo, personal communication) for possible introduction. R.M.S. recently visited S. Ke (Wuhan Institute of Botany, People's Republic of China), who showed him protoclonal kiwifruit [*Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson var. *deliciosa*] endosperm that varied in sex expression, growth habit, vigor, and leaf morphology. These results were published recently (Gui et al., 1993; Tsai et al., 1992).

When maintenance of clonal stability is mandatory (such as in a government germplasm repository), cultures can be stored in sealed containers under refrigeration or in liquid nitrogen (Reed, 1990; Reed and Lagerstedt, 1987). A 100-year project has been established in Oregon (Sherman, 1987) to study the effects of long-term storage of strawberry (*Fragaria ×ananassa* Duch.) meristems.

Proliferation rate. Cultures proliferated at excessive rates show more variation than those grown at moderate rates. For instance, Smith and Drew (1990) report that pineapple [*Ananas comosus* (L.) Merrill] can be propagated by conventional cuttings and separation systems at four to five plants per year. In vitro, the rate can be 30 to 50

Selection pressure. Selection pressure in vitro has been used to select cell lines with resistance to disease, herbicides, particular chemical compounds, etc. Bingham and McCoy (1986) reported the selection of alfalfa cell lines with resistance or tolerance to ethionine, salt, and a fungal culture filtrate. However, among plants regenerated from these cultures, only the disease-resistant character was expressed at the whole-plant level. Widholm (1988) has reviewed the use of tissue culture to select cell lines of value to researchers and industry. He states that direct selection for single gene traits is more likely to be successful than for those with multigenic control.

Hammerschlag (1990) and Hammerschlag et al. (1991) have isolated peach [*Prunus persica* (L.) Batsch.] clones with putative resistance to *Xanthomonas campestris* pv. *pruni*. Some of these clones have continued to exhibit disease resistance for 2 years, while in others, the trait has proved transient. Seeds are now being collected from these plants to study the heritability of the trait among somaclones (Hammerschlag et al., 1991).

Cultural conditions. Genomic irregularities in plant and animal cell cultures occur most often when cells are removed from the stabilizing and controlling influences exerted by an intact organism and placed into an unnatural environment within a culture vessel. When a plant is excised and wounded in preparation for transfer to tissue culture, the control systems that regulate a whole plant are interrupted. For instance, in a whole plant, callus is a wound response that rarely results in sufficient organization to produce shoots or roots. However, in tissue culture, callus is a common phase through which excised plant parts commonly pass before organizing into a meristem that can yield roots, shoots, or both. Growth regulators and other *in vitro* stimuli mediate these events. It is not surprising, therefore, that adventitious shoot formation results in abnormal phenotypes.

In the earliest tissue cultures, off-types were noted but generally rogued as undesirable. Later, visual analyses of regenerants were inadequate to appreciate the extent of variation. With biochemical and chromosomal analyses, the magnitude of change sometimes was as high as 100% (Orton, 1983), but rates of 15% to 20% were common (Evans and Sharp, 1983). These findings are in contrast to naturally occurring mutation rates of 1 in 100,000 to 1,000,000 for a given locus. Larkin et al. (1984) reported 3% to 26% variation for fertility in wheat (*Triticum aestivum* L.); Evans (1988) reported the frequency of single-gene mutations was about one mutant in every 20 to 25 regenerated plants. Orton (1987) reported that 100% of celery (*Apium graveolens*

L.) lines isolated in vitro varied from the parental chromosomal condition. Although rates of somaclonal variation seem high, one should remember that somaclonal variation is a random phenomenon that can occur at any location in the genome. These random changes are pooled to arrive at the total somaclonal variation rates presented in most literature. The true rate of somaclonal variation is difficult to ascertain because there are so many individual genes to examine.

Many somaclones are identical, suggesting a common origin. Davies et al. (1986) showed that certain types of variation occurred repeatedly within groups of *Paspalum dilatatum* Poir. plantlets derived from the same explant source. They postulated that the abnormalities occurred at high rates because variation arose early in callus cultures and was proliferated and unintentionally selected as callus "sectors" through subsequent subcultures. Lee and Phillips (1987) made a similar observation with *Zea mays* L. They found that the same culture often yielded several plants that segregated for phenotypically identical variants. Such variation probably occurred early in culture initiation, leading to an overestimation of the frequency of somaclonal variation.

Based on literature and our professional experience, a more realistic level of somaclonal variation expected in vitro is probably about 1% to 3%. This does not mean that a researcher should expect 1% to 3% of their regenerants to mutate at a particular locus, but it does mean that 1% to 3% of the regenerants will vary from their parent in some physical or biochemical manner. Although this rate of variation sounds less useful than the 25% to 100% levels discussed earlier, the researcher should remember that all of this variation occurs within a clone. Selection pressure can result in isolating unique forms of the parental clone. Conventional breeding procedures involving backcrossing can also yield improved forms of a clone, but the process can be time consuming, unsuccessful, and only work for plants that are self-compatible. Many important food crops, such as apple and pear (*Pyrus* spp. L.), are either self-incompatible or exhibit severe inbreeding depression.

Although variation rates of 1% to 3% may be realistic, even those rates are difficult to explain at the molecular level. For instance, there is a set of genes in wheat for the gliadins, which are involved in storage protein quality. Larkin et al. (1984) used the gliadins to quantify large amounts of variation and demonstrate the extent of variation available through in vitro procedures. However, Novoselskaya et al. (1987) traced variation in 50 gliadin-coding genes that could be distinguished by electrophoretic bands on gels. They found little variation, as did Maddock et al. (1985). Novoselskaya et al. (1987) and Metakovsky et al. (1987) suggested that the rate of variation observed among the gliadins was too high to be sensible. For instance, if we assume that, in terms of the genome, the gliadins are no different than any other site on chromosomes, then a change in one or two of the 50 gliadin genes represents a 2% to 4% rate of mutation throughout the genome. When this level is extrapolated to the whole genome, the rate of variation is so high that no viable offspring should be expected. However, it is also possible that the gliadin genes are inherently unstable under particular conditions, biasing conclusions from these studies.

In summary, somaclonal variation can be used for plant improvement. To date, somaclonal variation has been a random process that will become more useful as researchers learn to control and direct it to achieve specific goals. The development of regeneration protocols for recalcitrant species will facilitate access to natural and induced variations.

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