
VARIATION AT THE CELLULAR LEVEL

Stephen L. Sinden
Vegetable Laboratory, U.S. Department of Agriculture, ARS, Beltsville, MD 20705

James F. Shepard
Department of Plant Pathology, Kansas State University, Manhattan, KS 66506

Certain plant species have been propagated clonally over long periods of time using classical techniques. Variants occasionally appear, but usually in relatively low frequencies (31). Thus, “carbon copies” of the parent plant might have been expected when tissue explants and single cells were first isolated and regenerated into plants by cell-culture techniques. Most regenerated plants are, in fact, apparent exact copies of the parent plant (40, 41). However, some culture systems now available for commercial propagation and research purposes appear to produce cell cultures and regenerated plants with considerable phenotypic variability.

The relatively high incidence of variation in ploidy and chromosome number among cultured cells and in regenerated plants led D’Amato (7) to conclude that propagation by means of the true shoot apex is the only procedure that ensures varietal purity and genetic stability among in vitro-propagated plants. Some vegetatively propagated plants are known to exist as periclinal chimeras (33, 34), or genetic mosaics (4, 7, 15, 33). Variation among regenerated plants from cells or tissues of such plants would be anticipated. Conversely, instances of high-frequency variation among plants (protoclones) derived from populations of single cells (protoplasts), which are supposed to be genetically uniform, are indeed surprising (21, 29, 37).

Variation among plants derived from protoplasts isolated from a single leaf raises penetrating questions about the genetic homogeneity of somatic cells (4, 9, 31) and the nature of genetic modifications that may possibly be occurring during the cell-culture and regeneration processes (1, 2, 8, 33, 36). The wide range in frequency of variants recovered from various cell-culture and regeneration systems indicates that several, perhaps many, variants can affect the degree of variation at the cellular level and/or expression of cellular variation in regenerated plants, e.g., species, cultivar, culture medium, duration of culture, and cell source.

Variation among plants from cloned cell-cultures could pose problems for those seeking to use such technology for the rapid propagation of disease-free plants (3, 7, 8, 22). On the other hand, the

---

1 Visiting Scientist, Dept. of Plant Pathology, Kansas State Univ., Manhattan, KS 66506.
occurrence of new and novel sources of genetic variability could possibly be useful in certain varietal improvement programs. Several recent reports and reviews have focused on this novel variation and its potential for applications to plant improvement (12, 15, 16, 18, 29, 30, 31, 38).

Most variant plants derived from cell-culture systems probably will be inferior to the parental clone in regard to desirable horticultural traits because of the undirected nature of the genetic alterations (38). An occasional variant may exhibit one or more superior horticultural traits, perhaps derived from a novel genetic combination that cannot be generated by conventional breeding techniques (14, 15, 29). Such superior variants may be directly increased clonally as a new cultivar without further breeding and selection with vegetatively propagated plants. It is not too surprising that much of the effort to date to investigate the feasibility of improving plants by generating and/or isolating variability by means of cell-culture procedures has been directed towards 2 vegetatively propagated crops, i.e., sugarcane (15, 17, 18) and potato (29, 30, 31). Potential benefits that can accrue have been demonstrated already with the release of 'Velvet Rose' geranium, the first cultivar arising as a variant in a population of regenerated plants (34, 35).

Variation arising at the cellular level is particularly well-illustrated in the case of the potato protoplast system, where extensive phenotypic variability has been noted among populations of protoclones (plants regenerated from isolated leaf mesophyll protoplasts) (29). The potato appears to be one of the best-suited crop plants for investigations into the usefulness of variant selection for varietal improvement. It is vegetatively propagated, has worldwide importance as a food source and has been difficult to improve by classical breeding techniques; reliable methods are available for regenerating large populations of plants comprised of potentially distinct genotypes, i.e., the thousands of individual cells (protoplasts) that can be isolated from a single leaf of a donor clone; and there are several examples of the success of a similar varietal improvement technique, the selection of "sports" of commercial cultivars and their subsequent acceptance by growers as popular new varieties (29, 31).

Shepard and Totten (28) began the development of techniques for regenerating potato plants from mesophyll protoplasts with the view that "it might be simpler to selectively improve a popular variety by variant selection than to create a new one through conventional breeding" (29) if it were possible to create or identify genetic variability in a population of protoclones from a popular cultivar. More than 10,000 protoclones have been regenerated from 'Russet Burbank', the most popular American potato cultivar. Genetic variation resulting from any basic investigation of culture-associated variability, or to the exploitation of the variability for cultivar improvement, is efficient and repeatable procedures for isolating protoplasts or cells and regenerating large numbers of plants. Consequently, the original methods developed for potato protoplasts have been tested continually and refined over several years of study (28–32).

Two general categories of variations have been observed in populations of protoclones (30, 31). A low and variable frequency of clearly abnormal plants, termed "wild aberrants," are always detected. These variants display severe anomalies, such as gross morphological changes and greatly reduced vigor, generally associated with deviations from the normal chromosome number in other regenerated plants, e.g., tobacco (24). A 2nd category of variants, termed "phenotypic variants," includes plants that appear very similar or identical to parental 'Russet Burbank' on cursory examination in the greenhouse. The very interesting and potentially useful variation for disease resistance (29, 31, 31) and horticultural characters (27, 39, 30) has been uncovered among these phenotypic variants.

Extensive variation in a wide array of horticultural characters was observed initially and documented in field trials with an original population of 1700 protoclones. The stability and potential usefulness of several of the variant traits has been demonstrated clearly in continuing field tests at several locations over 5 growing seasons with protoclones selected for desirable field-performance characteristics, including a replicated experiment with 65 of the original protoclones (27).

There are some protoclones in field plantings that have more compact and efficient canopies, with shorter internodal lengths—a trait that could give greater production efficiency per hectare of plantings (29). The protoclones also exhibited a wide range of tuber initiation dates, from a few days earlier to as much as 4 weeks later than the parental 'Russet Burbank'. Some protoclones produce nearly all U.S. No. 1 tubers, while others produce mostly No. 2 tubers (27) and 2 protoclones produce tubers with a smooth white skin instead of the brown russet skin that is so characteristic of 'Russet Burbank' (27, 29).

Variation for traits such as a photoperiod requirement for flowering and number of flowers and seedballs produced in field plantings has been noted also (27, 29). These traits are of no consequence to the grower, but they could be important in transferring 'Russet Burbank' characters to other cultivars through sexual matings or in cytological investigations and inheritance studies to ascertain the basis of observed phenotypic variability.

Significant variation was found in 22 of 35 complex, quantitative characters measured in a replicated field experiment (27). Each of the 65 protoclones diverged from the parent in at least a single character and not one protoclone was exactly like any other. However, most of the 65 protoclones closely resembled 'Russet Burbank' in vine morphology and general tuber characteristics. The original objective of demonstrating that it is possible to improve one character selectively while simultaneously preserving all or most of the desirable features of the parental cultivar seems to have been achieved in the potato protoclone system.

Breeding for disease resistance is a major objective in most breeding programs. Differences in resistance-susceptibility were detected and were consistent over 2 or more tuber generations when protoclone populations were screened for resistance to early blight [Alternaria solani (Ell. & G. Martin) Sor.] and late blight [Phytophthora infestans (Mont.) de Bary]. A few protoclones were affected less severely than the parent by A. solani (19), while about 2.5% of the 800 protoclones were less susceptible to late blight (29). A protoclone should have considerable worth as a parental stock or potential commercial cultivar if essential field-performance characters are found in combination with an enhanced level of disease resistance in one or more of the variants.

The contribution of protoclone selection to potato improvement will not be known until the results of continuing field tests have been completed. But, the existence of interesting and novel phenotypic variability among protoclones is definitely established and the prospects for exploiting the variation for varietal improvement appear promising.

Beyond the origin of variability in protoclone populations nor the genetic mechanisms responsible for phenotypic differences are fully understood; however, certain factors affecting the frequency of variation within the potato systems and within cultural and regeneration systems for other species have been identified. Alterations in chromosome number and other karyotypic abnormalities are a common occurrence in cultured cells (8, 33, 36). The frequency of variant cells often is related directly to the duration of in vitro culture. Calli or suspension cultures maintained over prolonged periods often regenerate plants with higher or lower ploidy levels, aneuploidy, or other karyotypic abnormalities (8, 9, 23, 25, 33).

For example, all of the tobacco plants regenerated from 5-year-old callus cultures were both abnormal in morphologic characters and either aneuploid or polyploid (24). Maize plants regenerated from young callus were mostly cytologically identical to the original explants and showed little phenotypic variation (12), whereas all plants from a 3-year-old callus were abnormal (13). Cytological analyses of these abnormal maize plants revealed that a segment of one of the chromosomes was deleted, a karyotypic alteration that presumably arose during the extended culture period (13, 14). The mean chromosome number in Hordeum spp. cells more than doubled between 4 and 10 months of culture and various other karyotypic abnormalities developed during the culture cycle (23). Cytological studies offer direct evidence that at least some of the variability in regenerated plants is due to genetic changes that occurred in cultured cells (1, 21, 24).

There also are reports of "epigenetic" changes in cultured cells which are transmitted stably to daughter cells in culture, but are not manifested in regenerated plants (6, 20). Karyological aberrations

838

HORTSCIENCE, VOL. 18(6), DECEMBER 1983
encountered in cultured cells do not necessarily persist in regenerated plants. Polyploidy of cultured cells in *Hordeum* was eliminated completely in regenerated plants and aneuploidy and chromosomal aberrations were reduced greatly (23). Selection for normal karyotypes within a population of callus cells during regeneration apparently is a common occurrence (1, 8, 25, 26).

Phenotypic variations among regenerated plants do not always appear to be related to cytological modifications of the karyotype. All variant sorghum regenerates had a normal chromosome complement (11), normal chromosome numbers, as well as normal meiotic pairing of chromosomes, also were found in a sample of variant tobacco regenerates (5). No definite relationship between changes in numbers of chromosomes and morphological variation among sugarcane variants was apparent by cytological analyses even though the variants had widely differing chromosome numbers (17).

No deviations from the normal chromosome number were detected in a sample of 5 potato protoplasts exhibiting variation for horticultural characters, “phenotypic variants” (29). More detailed cytological studies have, however, indicated that subtle karyotypic irregularities, such as abnormal pairing of chromosomes during meiosis, may occur in some of these protoplasts (B.S. Gill, L.N.W. Karn, and J.F. Shepard, unpublished data). In contrast, most of the clones in a sample of “wild aberrant” protoplasts examined in preliminary cytological studies were identified as aneuploids (30).

Protoplasts obviously are subject to the same karyotypic alterations during their culture as are cells of callus or suspension cultures derived from somatic or germinal explants and quite possibly the aneuploidy and polyploidy detected in “wild aberrant” protoplasts developed during the cultural phase of protoplast regeneration. Other explanations may be required for the more subtle variation for quantitative characters (horticultural and field-performance traits) encountered among protoplasts (27, 29, 31). Alternative hypotheses regarding possible mechanisms to account for the variability in the potato system and in other systems include: 1) isolation of preexisting genetic differences (genetic mosaics) among populations of somatic cells from a single tissue by the culture and regeneration processes (4, 15, 33); 2) mutagenic activity of the culture medium or of cellular constituents released by dying cells (29, 31); 3) chromosomal rearrangements; 4) transposable genetic elements; 5) somatic gene rearrangements, such as somatic crossing-over and sister chromatid exchange; 6) gene amplifications and deletions; and 7) cryptic virus elimination (16, 32).

Cultivar and medium composition are 2 variables that influence the frequency of variant recovery within certain cell-culture and regeneration systems. For example, the cultivar on resulting variability in regenerated plant populations have been observed with sugarcane (15, 17), geranium (34), and potato (29, 40). Observed differences in variability have been related in some cases to different degrees of preexisting genetic heterogeneity and chromosomal instability among the donor cultivars (7, 9, 15, 35). Plants regenerated from callus of a sugarcane variety known to exist in the field as a chromosomal mosaic exhibited greater variability than plants derived from another cultivar that was considered more homogenous in chromosome number (15). The degree of variation in plants from geranium callus cultures was partially dependent upon the chimeral nature of the donor cultivars (34). The degree of variation among regenerated plants was even greater in both of these systems, regardless of source cultivar, when older callus cultures were regenerated, indicating that karyotypic alteration occurring during cell culture was another factor also contributing to the observed variability.

A difference in variation frequency resulting from protoplast regeneration between 2 genotypes of potato has been reported (27, 29, 40). Variability among protoplasts derived from an experimental dihaploid line was low and was observed only when cells were cultured for an extended period (40). Interestingly, all plants regenerated from the dihaploid protoplasts were tetraploid. All protoplasts from the other genotype, a commercial cultivar and putative autotetraploid, exhibited, in contrast, some form of variation (27, 31). In addition to the differences in ploidy levels of source plants, there were also differences between the 2 studies in the methods of isolation, culture, regeneration, and testing for the occurrence of subtle phenotypic variations. Thus, the difference in variation frequency between these 2 potato cultivars may not have been entirely a function of genotype.

Hormone composition of the culture medium can influence frequency of karyotypic alterations in cells (2, 7, 33) and subsequent variant recovery in some systems. A high concentration of 2,4-D greatly increased variability among regenerated *Hordeum* plants, compared to culture in the same medium with lower levels of the auxin (10). Substitution of 2,4-D for NAA as the auxin source in the culture medium for tobacco also influenced the frequency of abnormal plants (32). It was not entirely clear in these latter 2 studies whether the effects of the hormones were direct, inducing greater variation among the cultured cells, or indirect, affecting the length of time to regeneration or the selection pressure for normal karyotypes during shoot regeneration.

There are indications that the source of the cells or explants for culture systems also may influence resulting variation (7). Pineapple plants from crown-tissue explants exhibited almost no variation, whereas nearly 100% of the plants from syncarp or slip tissues were identified as variants (39). Preliminary results indicate that potato plants regenerated from ‘Russet Burbank’ leaf callus exhibit considerably less phenotypic variability than do the protoplasts, although the frequency of wild aberrants does not appear to be affected (31).

Additional studies obviously are needed to define the effects of the many factors that could affect variability within particular cell-culture systems. Knowledge of treatments that would reduce variability within culture systems intended for clonal propagation would be helpful to plant propagators, while means of manipulating other culture systems to increase genetic variability or to alter the types of genetic modification produced may be needed in order to exploit some of the systems to their maximum potential for varietal improvement purposes. It is the same cell-culture-associated phenotypic and genetic variability, which may present potential problems for plant propagators in some cases, that offers a promising new approach to plant improvement in others.

**Literature Cited**


---

**PHASE CHANGE AND INTRA-CLONAL VARIABILITY**

Wesley P. Hackett

Department of Environmental Horticulture, University of California, Davis, CA 95616

There is considerable opportunity for intra-clonal variability due to nongenetic or epigenetic causes because of the well-known and striking age-related changes in developmental patterns in plants propagated from seed. It is well-known that a juvenile phase exists to nongenetic or epigenetic causes because of the well-known and physiological and biochemical basis of phase change and the factors that affect it to be able to maintain or manipulate phase-change-related variation. The ontogenetic basis of phase change will be considered first.

**Ontogenetic basis of phase change**

It can be demonstrated where it has been possible to analyze