OB stars offer new possibilities for probing the winds, at the same time raising new questions about the evolution of binary star systems containing compact objects. What news will the future bring?

Advances in space astronomy promise to make the next decade a most fruitful one for stellar wind studies. Even now, observations with the IUE satellite are greatly increasing the number of OB stars whose winds have been measured spectroscopically. Within the past few months the Einstein X-ray Observatory has revealed that most if not all OB stars are x-ray sources; more extensive observations of OB stars with this telescope, including searches for time variability, promise to reveal much about the nature of the winds and their embedded x-ray sources. Later in the 1980's, orbiting IR telescopes of greatly increased sensitivity will permit observations of the dynamical effects of stellar winds within the dense interstellar clouds where stars are born. Our ability to observe stellar winds in the UV and in x-rays will be greatly improved and extended to neighboring galaxies by the Space Telescope and by the proposed Advanced X-ray Astronomy Facility.

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Potato Protoplasts in Crop Improvement

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Potatoes, including sweet potatoes, yams, cocoa yams, and cassava, represent an important class of world food crops in which commercial propagation is asexual rather than through true seeds. Vegetative reproduction preserves essential varietal characteristics from one generation to the next, whereas, when seeds from these plants are used, progeny are only reasonable facsimiles of the parent. Despite their economic significance, these species have not been the beneficiary of the "green revolution" breeding technology, so successful for such crops as wheat, maize, and rice. In some examples, outright neglect is a contributing factor, but with the establishment of international crop development centers, improvement of these species is now receiving increased attention (*1*).

For potatoes, a concerted improvement program has been active for the past 75 years in both Europe and North America. Despite these efforts, no single potato cultivar has emerged with, for example, multiple resistances to most major diseases, quick adaptability to new geographic settings, and the embodiment of all horticultural traits, including yield potential, viewed as essential. In the United States, four cultivars constitute 72 percent of the total potato acreage (*2*); the most significant cultivar emerged in 1871 as a selection by the renowned botanist Luther Burbank (*3*). In Europe, "antique" varieties such as 'King Edward' and 'Bintje' continue

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in their popularity even though both are more than 50 years old.

The longevity of major cultivars implies that specific improvement of the potato by conventional or mutation breeding methods is problematical at best. Broertjes and van Harten (*4*) stated the case in a recent treatise:

> An essential condition for successful . . . breeding seems to be a considerable amount of realism with regard to the objectives as well as to the practical approach. As long as sufficient genetic variation can be obtained more easily or quickly in other ways, the mutation method should not be applied. However, the failure in The Netherlands to produce a suitable substitute for the famous cultivar Bintje in over 50 years, clearly demonstrates that even large amounts of conventional work do not always guarantee success.

Thus, when plant breeding efforts are limited in a crop such as potato by the nature or frequency with which specific modifications are realized, alternative technologies deserve increased attention. One of the simpler approaches has been the direct selection of spontaneous somatic variants (bud sports). The 'Russet Burbank' cultivar, for example, arose in the early 1900's as a sport in field plantings of 'Burbank' and today represents 39 percent of Canadian and U.S. potatoes (*5*). However, the frequency of spontaneous change is too low to be of consistent value, especially for disease-resistance characters. Attempts to boost both the incidence and range of variation in potatoes through treatment of tuber or shoot tip meristems with mutagens, although effective in generating higher frequencies of change, have nonetheless provided few improved cultivars (*6*).

In the 8 years since Takebe and coworkers (*7*) first established that morphologically normal plants could be raised from single tobacco leaf protoplasts, that is, cells without their walls, much attention has been directed toward the possible use of cell and protoplast techniques in crop improvement (*8*). Since progress in this area has been closely followed (*9*), we do not review it here. But, for vegetatively propagated plants like potato, enhancement through protoplast cultural methods appears especially promising. Side effects of genetic modification (or modifications) on sexual fertility are less significant in direct selection programs than in seeded crops, and variants can be readily reproduced through tubers. Moreover, there is precedent in another vegetatively propagated crop, sugarcane, where plant regeneration from single cells has led to improved cultivars (*10*).

Summary. Clonal populations regenerated from single-leaf cell protoplasts of the potato cultivar 'Russet Burbank' display a high frequency of variation for several horticultural and disease resistance characters. Observations over a period of three tuber generations suggest stable changes in tuber shape, yield, and maturity date, in photoperiod requirements for flowering, and in plant morphology. Enhanced resistance to early blight (*Alternaria solani*) and late blight (*Phytophthora infestans*) diseases also regularly occurs within regenerated populations. These findings are discussed in the context of possible application to varietal improvement, particularly as they pertain to asexually propagated plants.

The Potato Problem

The so-called potato comprises a diverse group of more than 160 *Solanum* species that bear tubers (modified stems), but the commercial version belongs to a single subspecies, *S. tuberosum* ssp. *tuberosum* (*11*). Virtually all potato cultivars are tetraploid ($2n = 4X = 48$), and it is not altogether certain whether this form arose from an amphiploid hybrid or a simple autotetraploid (*12*). Recently, it has become possible to create, parthenogenetically, dihaploid ($2n = 2X = 24$) and monohaploid ($n = X = 12$) lines from crosses between cultivars and selected genotypes of *S. phureja*, an effective pollinator (*13*). Thus, under the general heading of "potato," there has been described an extensive array of *Solanum* species, or within *S. tuberosum* ssp. *tuberosum*, a range of dihaploid and monohaploid lines. In this article, we limit our emphasis to tetraploid cultivars and certain of the practical and genetic considerations that influence the development of improved forms.

Our knowledge of potato genetics is fragmentary in comparison with the genetics of corn and tomato. Nevertheless, as has been summarized by Howard (*14*), there are a number of consistent genetic features. For maximum plant vigor, potato cultivars must be extremely heterozygous, and they are apparently so for the majority of characters identified thus far. Appreciable reductions in interallelic heterozygosity, as, for example, through selfings, regularly depress vegetative vigor in the progeny. The genome of subspecies *tuberosum* seems to be largely simplex (*Aaaa*), which is significant in that mutation of a dominant allele, if not directly expressed, will favor the expression of recessive alleles at that locus.

The number of horticultural traits, many with complex inheritance, that must be amalgamated during the synthesis of an acceptable cultivar is substantial. The yield, shape, size, quality, and storage characteristics of tubers are critical features along with general growth characteristics of the foliage, environmental requirements for the onset of tuberization, and susceptibility to disease. In a typical breeding program in the United States, 60,000 to 80,000 seedlings are grown each year for the purpose of identifying one with promise. Many cultivars, such as 'Russet Burbank,' are effectively sterile, and even when partially restored, low fertility discourages their use as breeding lines in improvement programs. In recent years, the greatest emphasis in breeding has been on increasing host plant resistance to diseases; and for good reason, about 22 percent of the world's potato crop is annually sacrificed to disease (*15*). In the United States alone, this amounts to a monetary figure exceeding $200 million. Progress in obtaining resistance has been slow. Attempting to alter a specific type of genetic vulnerability in a cultivar is regularly accompanied by a compromise in some erstwhile attribute and, in any case, resistance to a single disease still leaves many other pathogens that must be coped with by growers.

By conventional technology, performing one-step improvements in potatoes while otherwise preserving all remaining characters is simply not possible. Yet, because of the vast number of specific traits present in a commercially established cultivar, it might be simpler to selectively enhance a popular variety than to create a new one. If such a capability were available, it could be useful not only for modifying existing cultivars, but also for ameliorating selections from breeding programs that are limited by one or two critical flaws.

Protoplast Culture and Regeneration

By their single-cell nature and the relative ease with which they may be cultured, plant mesophyll cell protoplasts have simplistically been likened to microbes as objects for genetic manipulation (*16*). In theory, it is necessary only to induce a desired mutation within a

protoplast population, allow ample time for expression, and then apply appropriate selection pressure to recover first a cell and, ultimately, a plant having a specified modification. Such a course of events would be possible, however, only if the cell is a true haploid, which is rare in plants, and the trait is simply inherited.

Although protoplast-level selection has led in a few instances to the regeneration of a modified plant (*17*), none has involved a major crop. A primary reason for this, apart from genetic limitations, is that it has generally not been possible to obtain the sustained division of cultured mesophyll protoplasts; even when this hurdle has been overcome, it has been difficult to define conditions that promote plant regeneration, particularly in crops such as wheat, rice, corn, soybeans, and, until very recently, potatoes.

Protoplast culture is a specialty within the much broader field of plant tissue culture. As for many other plants, potato tissues have been subjected to some form of in vitro culture, such as meristem regeneration, for many years. Only since 1976, however, have there been reports of efficient plant regeneration from single cells of callus origin (*18*), and these procedures have been successful with but a few cultivars and dihaploid lines. Moreover, potentially useful variation has only recently been suggested (*19*) in plants regenerated from potato callus cultures, and none has been confirmed under field conditions. There have been more observations of phenotypic diversity among potato plants grown from meristem tips than from callus cells although a genetic explanation for this is lacking (*20*).

An alternative to more traditional methods of tissue culture for potato involves direct enzymatic dissociation of leaves into mass populations of viable protoplasts. Leaf cells of at least some plant species, such as tobacco, are thought to be genetically stable (*21*) and, therefore, are particularly desirable objects for genetic modification. First, however, it was necessary to define cultural conditions whereby potato plants can be reliably obtained from individual protoplasts.

In 1977, we reported methods (*22*) for the regeneration of plants from mesophyll protoplasts of the potato cultivar 'Russet Burbank.' Later (*23*), the procedures and culture media were refined and found to be suitable for several other commercial cultivars as well (*24*). Unlike tobacco, conditions necessary for regenerating potato cultivars are multifaceted and relatively complex. Plants to be used as sources of protoplasts must be grown under precisely controlled conditions of nutrition, temperature, light intensity, and photoperiod; otherwise protoplasts fail to undergo division regardless of the culture medium used. After isolation from properly grown plants, protoplasts are placed in a semisolid culture medium to induce growth and proliferation. However, the most effective medium for initiating protoplast division, cell layer medium, is unsuitable for sustaining proliferation beyond the four- to six-cell stage. To overcome this limitation, we devised a culture plate system whereby a second medium of different composition, reservoir medium, would slowly diffuse into dish segments that contained protoplasts (Fig. 1). This combination of media along with a mechanism for a slow exchange permits both the onset and continuation of cell proliferation with maximal efficiency.

Within 2 weeks, cultured potato protoplasts resynthesize their cell walls, undergo numerous cell divisions, and give rise to small calli, which are masses of relatively undifferentiated cells. To induce plant regeneration, calli are exposed to a sequence of three media, each of which serves a distinct developmental function. The first, medium C, condi-

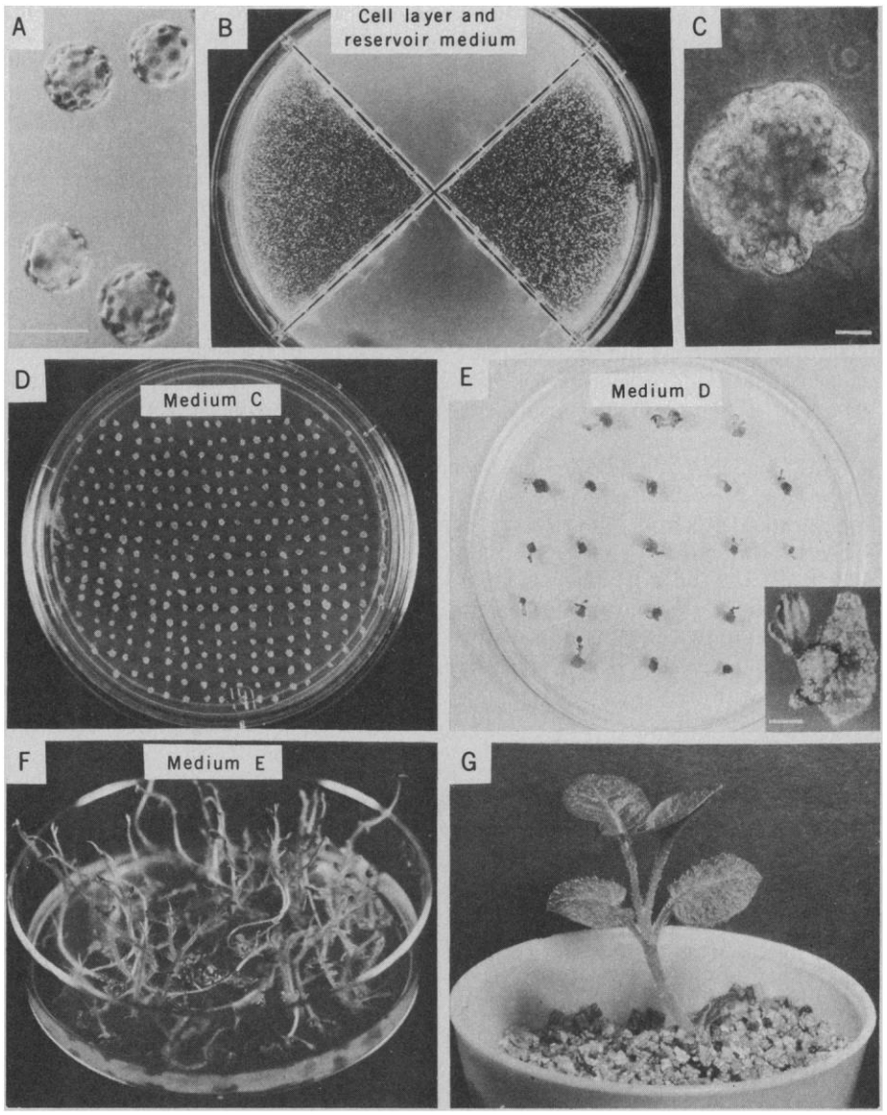


Fig. 1. Plant regeneration sequence from mesophyll protoplasts of potato. (A) Freshly isolated protoplasts. Scale bar, 50 μm. (B) Culture plate (100 mm) 14 days after protoplast plating. Top and bottom compartments contain reservoir medium and are connected to quadrants with cell layer medium by multiple holes. Developing protoplast-derived calli are present in the left and right segments of the petri dish. (C) A single protoplast-derived callus from the plate in (B) at higher magnification. Scale bar, 100 μm. (D) "Conditioned" calli 14 days after transfer to medium C. (E) Emergence of shoots from protoplast-derived calli after 8 weeks on medium D. A shoot primordium at higher magnification is shown in the inset. Scale bar, 1 mm. (F) Shoot elongation and rooting in petri dish (100 by 25 mm) containing medium E. (G) Transplanted regenerate in 40-mm pot containing vermiculite.

tions calli during a 14-day growth period, stimulates chlorophyll synthesis, and influences their responsiveness to succeeding steps. Calli from medium C are induced to form shoot meristems after transfer to medium D. The final stages of development, shoot elongation, and adventitious root formation, occur when tissues are placed on medium E (Fig. 1).

Several features of medium C and medium D are of significance in the promotion of shoot morphogenesis. Most of these requirements relate to specific concentrations of phytohormone and nutritional components. In comparison with other systems are two additional aspects of these media that also are determinative of shoot morphogenesis. First, the osmotic pressure of both must be maintained at between 200 and 400 milliosmoles per kilogram with 0.2 to 0.3*M* D-mannitol. Without this, calli do not assume the intense green color that necessarily precedes shoot meristem development, and instead maintain a proliferative growth habit. Second, the concentration of the exogenous carbon source, such as sucrose, glucose, or maltose, must not exceed the minimum capable of sustaining growth. Amounts greater than 0.008*M* progressively inhibit shoot morphogenesis and 0.05*M* sucrose will stimulate root in lieu of shoot morphogenesis.

It is probably significant that our scheme for protoplast regeneration differs substantially from those described for other forms of potato including dihaploid lines and miscellaneous *Solanum* species (*25*). We have tested several of these methods with 'Russet Burbank' but have not been successful in regenerating plants with them.

Horticultural Variation

We have examined more than 10,000 protoplast-derived clones (protoclones) of 'Russet Burbank' potato. Since we had used no overt treatments with chemical or physical mutagens, and because expression of some forms of genetic change, such as aneuploidy, are difficult to visualize in potato (*26*), we originally anticipated that regenerated plant populations would essentially consist of carbon-copy replicas of the parent. From results with tobacco and several other plants (*27*), that assumption appeared entirely reasonable. Furthermore, a recent report by Wenzel *et al.* (*28*) describing identical protoplast regenerates from two dihaploid potato lines provided still further support. Even so, several genetic features of the 'Russet Burbank' cultivar favor the possibility that improved forms might still emerge in a regenerate population. First, like all other cultivars, 'Russet Burbank' presents an exceptional array of characters for analysis, and simplex inheritance (*Aaaa*) could, in part, obviate concerns that only in true haploids would there be expression of most point mutations. Second, that 'Russet Burbank' itself arose as a somatic mutant from 'Burbank' (*29*) confirms that in potato spontaneous changes do occur and are readily expressed.

When the first 1000 'Russet Burbank' protoclones were grown under greenhouse conditions, it was apparent that all were not identical. Several plants displayed anatomical anomalies generally associated with pronounced chromosomal disturbances, including malformed leaflets and stems, altered color patterns, and reduced vigor. When these individuals were discarded, the remainder closely resembled the parent in gross morphology during their first vegetative cycle although some subtle phenotypic differences could be detected. However, when plants were raised under field conditions, from tubers of seemingly identical regenerates, clonal variation became much more apparent. One population, which initially consisted of 1700 clones, has been reduced to 60 via selections during three seasons in the field. From these evaluations, stable variation is suggested for many horticultural characters (*23*). Some of the more prominent examples include the following.

1) *Compact growth habit*. In comparison with several other cultivars, 'Russet Burbank' is relatively indeterminate in growth habit; it produces elongated stems that may continue to grow throughout the season. Several protoclones displayed more compact vines than the parent with shorter internodal distances. The number and surface area of leaves, however, were not reduced, and this resulted in a fuller and potentially more efficient canopy.

2) *Maturity date*. At what point during the growing season potato plants will begin to set tubers is subject to several genetic and environmental factors. The 'Russet Burbank' cultivar is considered relatively late and, therefore, requires a longer production period than certain other cultivars. Protoclone populations, in contrast, have exhibited a full range of variation in their earliness of tuber set. Some required fewer days than the 'Russet Burbank' while others initiated tuberization up to 4 weeks after the parent. This variation has been consistent over three growing seasons at five locations; but significantly, and again in comparison with the mother clone, the trait is more environmentally influenced, hence, site-dependent, in some protoclones than in others.

3) *Tuber characteristics*. A recurring problem in the commercial production of the 'Russet Burbank' potato is its tendency to develop misshapen tubers under suboptimal growth conditions (*30*). The frequency of undesirable tubers can be quite high; in one field plot, during 1978, it exceeded 50 percent, whereas several protoclones displayed uniform tuber shape with no secondary malformations (Fig. 2). In two plots cultivated the same year we also found that the numbers and total weight of tubers differed from clone to clone and that a few showed the potential for substantially outperforming the normal 'Russet Burbank.' Two protoclones, M-218 and M-261, produced tubers with a smooth white skin rather than the characteristic brown, russet skin. Another, M-153, consistently formed tubers with a more elongate profile than the parent.

4) *Photoperiod requirements*. Under controlled conditions of light intensity and temperature, young 'Russet Burbank' plants initiate flowering only when given relatively long photoperiods of about 16 hours, and even then, most flower buds abscise before development is complete. Berries resulting from any remaining flowers are subject to a similar fate. Few reach maturity, except during unusual years in the field, before they abscise. In breeding programs where 'Russet Burbank' is to be used as a female parent, lack of berry development is a serious problem. Several protoclones required only a 13-hour photoperiod to initiate flowering and were able to retain flower buds through blooming (Fig. 3). Moreover, some variant protoclones produced up to 100-fold more berries than the parent in comparable field trials. In no case, however, and consistent with mother clone, were berries found to contain viable seeds.

Disease Resistance

Early blight. The fungus *Alternaria solani* causes an often troublesome disease of potato known as early blight. The malady has been classed as a senescence-induced disorder (*31*) because older or stressed leaves are considerably more damaged by the pathogen than are juvenile tissues. Because of this irregular level of susceptibility, resistance screening by fungal inoculation is difficult, especially when young plants are tested. In

other plants, toxins liberated by a pathogen in culture have been used to help identify resistant individuals (*32*). This approach has the additional advantage that, if the toxin is sufficiently pure, it may also be applicable in protoplast, cell, or callus level selection, a potentially more efficient process (*33*). Far greater numbers of individuals may be tested at the single-cell stage than at the plant stage with less effort and expense.

Matern and Strobel prepared crude toxin from culture filtrates of *A. solani* and applied it to leaves of 500 of our protoclones from 'Russet Burbank' (*34*). Five clones proved significantly less susceptible to damage from the toxin preparation than the parent. One protoclone was more sensitive than the parent, but the remainder were equivalent in reaction. Later, all except one of the toxin-resistant clones also showed enhanced resistance to infection by the fungus (Fig. 4A), and this trait was passed through two tuber generations.

We have attempted to purify the *A. solani* toxin complex to select for resistant mesophyll protoplasts. We were unable to do so by previously described methods. Since our *A. solani* cultures were nonetheless virulent, we examined the possibility that other toxins might be present in short-term cultures of the fungus. A fraction was identified (*35*), which, when supplied to petioles and drawn into leaflets by transpiration pull, elicited symptoms closely resembling those of the early blight disease. Copious lesion formation occurred on exposed leaves followed by overall yellowing and death (Fig. 4B). Only modest progress has been made in further purifying the toxic principle (or principles), which apparently has a low molecular weight, about 1000 daltons, and is water soluble. It is now being tested in culture systems for possible application as a selective agent.

Late blight. The fungus *Phytophthora infestans* causes the potentially devastating "late blight" disease of potato. In the 1840's, it combined with an unfortunate political circumstance to create the calamity known as the Irish potato famine. Today, the disease remains a threat in those geographic areas where extended periods of cool moist weather persist during the growing season, and frequent applications of fungicides are necessary to prevent serious loss.

Genetic resistance to *P. infestans* is recognized in either of two basic forms (*36*). Major (or R) gene resistance is a dominant, simply inherited trait that confers near total protection to the plant. Here, the organism is only able to elicit a hypersensitive reaction, characterized by small necrotic lesions on inoculated foliage, and cannot develop further or reproduce by sporulation. Unfortunately, *P. infestans* exists as a multitude of pathogenic races, and a major resistance gene for race 0, for example, of the organism is totally ineffective against all other races of the fungus. For some time, R genes derived from *S. demissum* have been incorporated into new commercial cultivars by plant breeders. However, protection from these sources has proved transitory. Soon after R genes have been deployed in field plantings, different races of the pathogen appeared and these quickly overcame the host plant resistance.

A second resistance mechanism is thought to result from the cumulative action of a series of minor genes. Each has a small effect, but in concert they offer additive and nondiscriminatory protection against at least several and perhaps all races. Minor gene resistance is not expressed as the hypersensitive reaction characteristic for R gene resistance. Rather, the infecting organism is partially compatible with the host, may become established, and even sporulate. But, depending upon the nature and relative degree of resistance, pathogen development is slowed to the degree where it is less able to destroy the plant. If of sufficient magnitude, this field type of resistance offers several advantages over the R gene form. It is effective against a broader range of pathogenic races, does not as readily encourage the emergence of new ones, and is longer lasting. But, its polygenic and perhaps epistatic nature renders plant breeding efforts ex-

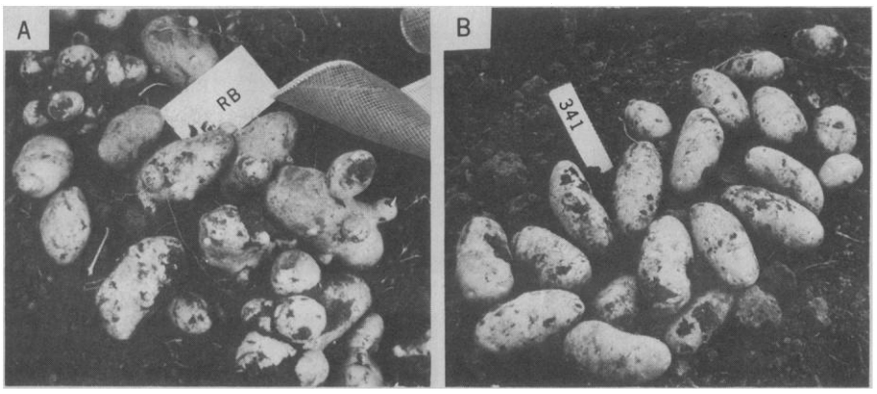
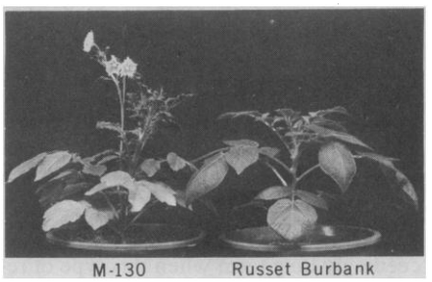


Fig. 2 (above). Tuber harvested from 1978 field tests. (A) Tubers from parental 'Russet Burbank' (*RB*) plants. (B) Tubers from protoplast-derived clone M-341 (*341*). Greater uniformity in size and shape occurred for tubers of M-341 than in the parent. Fig. 3 (right). Differing photoperiod requirements for flowering between young plants of protoclone M-130 and parental 'Russet Burbank.' Both plants were grown under 13-hour photoperiods of 10,000 lux illumination for 4 weeks at 20°C.



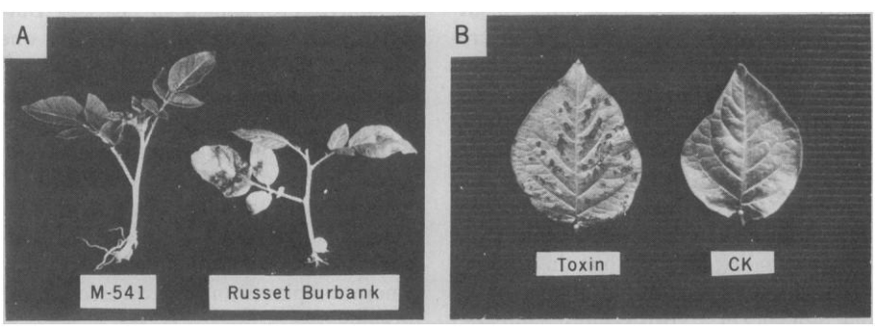


Fig. 4. Reactions to the early blight fungus *Alternaria solani*. (A) Resistance in protoclone M-541 and susceptibility in 'Russet Burbank' cuttings. Plants were inoculated with 100,000 conidia per milliliter and incubated for 8 days at 24°C. The 'Russet Burbank' plant displays characteristic symptoms including necrotic lesions and overall chlorosis while M-541 does not. (B) Reaction of 'Russet Burbank' potato leaves to culture filtrates (*Toxin*) and control preparations (*CK*) from *A. solani*. Leaflet on the left illustrates typical necrotic lesion development.

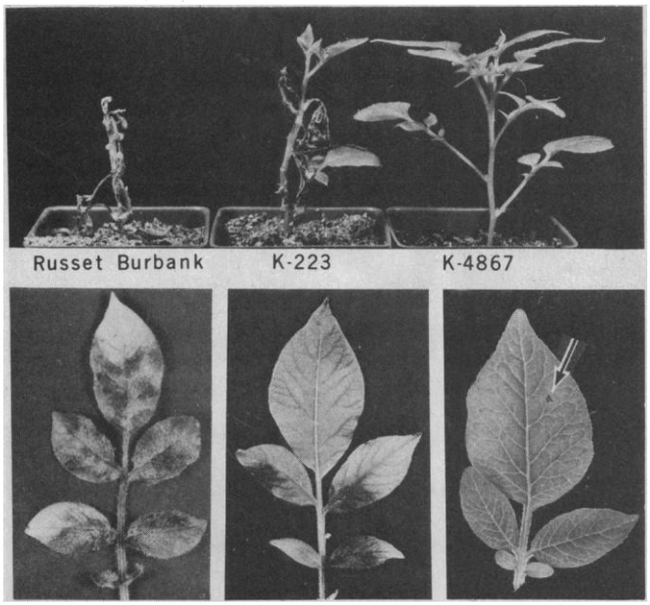


Fig. 5. Reactions of 'Russet Burbank' and two protoclonal plants to *Phytophthora infestans* 14 days after inoculation. Protoclone K-223 was classed as moderately resistant, while K-4867 was classed as extremely resistant to race 0 of the fungus. Below each plant a respective leaf is shown, which illustrates differential responses to the organism 5 days after inoculation. For the leaf from K-4867, the arrow denotes a necrotic lesion that contrasts with the profuse fungal sporulation evident on the 'Russet Burbank' leaf.

ceedingly long term when this type of resistance is to be incorporated into a cultivar. As a result, there has been a heavy reliance on resistance R gene in potato, often at the expense of any preexisting minor resistance genes (*37*).

The 'Russet Burbank' cultivar has low field resistance to *P. infestans*; but, under extreme environmental and inoculum pressures, it is insufficient to prevent destruction by any virulent race of the pathogen. No major or R genes are present. To ascertain whether useful variation might occur in regenerated plants as pertains to the late blight disease, vegetative cuttings were prepared from 800 protoclones. After they had developed roots and attained a height of 6 to 8 cm, they were assayed along with 'Russet Burbank' control cuttings by foliar inoculation with race 0 of *P. infestans* (*38*). Under these conditions, all parental plantlets collapsed and died by day 12 as did most protoclone cuttings. However, 20 protoclones, approximately 2 percent, were not killed. Some of the infected survivors appeared to be near death but recovered due to regrowth of a basal bud, while others showed only scattered necrotic lesions (Fig. 5). Between these extremes, the remaining survivors displayed increased levels of petiole and stem resistance. Pathogen development was slower than in 'Russet Burbank,' and, depending on the clone, invasion of the main stem was either infrequent or did not occur. Furthermore, infected leaves showed a greater tendency to abscise before petiole infection was complete, and this also reduced the frequency of stem infections in resistant protoclones. After these initial effects of inoculation had passed, surviving plantlets continued to grow without further damage. The inoculation process was twice repeated with additional rooted cuttings from first generation plants and the results were consistent. Some of these 20 clones were then tested against a second pathogenic race (1,2,3,4) of *P. infestans* with the results summarized in Table 1. Most race 0 resistant protoclones examined thus far were also less susceptible to the effects of race 1,2,3,4 than the parental 'Russet Burbank.' Tubers were collected from uninoculated plants of each resistant clone, and their progency proved as resistant as in the first generation.

Whether mature plants from these protoclones will express equivalent degrees of *Phytophthora* resistance either under controlled or field conditions remains to be determined. Nevertheless the evidence to date does suggest that:

1) Protoclones with an apparent normal morphology can be raised from mesophyll protoplasts of 'Russet Burbank' potato, and during at least one phase of their development, some are strikingly more resistant to multiple races of *P. infestans* than is the parent. Similar variation in susceptibility has not been observed within populations of comparable plantlets of the mother clone. The resistance character (or characters) is passed through tubers of first generation plants and is expressed in the progeny.

2) The nature of the observed resistance to *P. infestans* remains to be described, but it more closely resembles minor than major gene reactions. In even the most resistant protoclone, lesions incited by the fungus would slowly increase in size and support some sporulation.

3) Resistance to a specific host-pathogen interaction (late blight) and to a senescence-related (early blight) disorder seem unrelated. None of the protoclones resistant to *A. solani* displayed enhanced resistance to late blight although reciprocal tests have not been performed.

Significance of Clonal Variation

Despite current optimism for the potential of protoplast or cell culture systems in crop improvement (*39*) current results with regenerated potato plants must be cautiously interpreted. Establishing the horticultural worth of a new cultivar, a bud sport, or, in this case, a protoclone, requires performance evaluations over several years at many sites, and complex tuber quality factors (such as specific gravity and glycoalkaloid content) must be satisfied. Furthermore, just as in breeding programs, protoclones with an acknowledged improvement in one character must be exposed to adequate environmental pressures to eliminate any that are simultaneously deficient in some other critical feature. None of even our most advanced selections has fulfilled all these necessary standards, and clones resistant to early blight or late blight have yet to experience an in-field onslaught of the respective pathogen under diverse environmental conditions. In terms of varietal improvement, therefore, our results must be viewed as preliminary.

Apart from practical considerations the mere appearance of high-frequency phenotypic variation for the several traits examined in 'Russet Burbank' protoclones raises interesting genetic questions, such as the source of variation particularly in the absence of intentional mutagenesis. At present, the matter is strictly speculative, but it is pertinent that many vegetatively propagated plants express spontaneous mutations directly and often. Among potato plants in the field, the frequency has been placed at between 1.5×10^{-3} and 1.2×10^{-5} (*40*), depending on the character and the variety. Sweet potatoes (*Ipomoea batatas*) are capable of even higher frequencies. In the 'Centennial' cultivar, for example, two plants per 100 differed in some morphological feature (*41*), implying that some of the variation observed in regenerated potatoes may have preexisted within individual leaf cells before their transformation into proto-

plasts. In leaves of some plant species, there are considerable alterations in the ploidy level of cells as the leaf ages, expands, or is exposed to different environmental circumstances (*42*). Since readily detectable genetic changes occur regularly, other alterations, such as point mutations, inversions, translocations, deletions, additions, and substitutions may also exist in leaf cells. Many of these forms of change have been recognized in cultured plant tissues for some time (*43*), and culturing mesophyll protoplasts could, theoretically, contribute more of these modifications. Another important consideration in protoplast culture is the minimum cell density requirement. Only in exceptional cases (*44*) have protoplasts developed when cultured singly. Usually, and with potato as well, 5×10^3 to 10×10^3 protoplasts per milliliter of culture medium are needed before proliferation will occur. During the initial period of culture, some protoplasts die and they, as well as survivors, release into the medium a range of substances, such as flavonoids, that are potentially mutagenic to embryonic cells (*45*).

If genetic mechanisms are responsible for the observed phenotypic variation, the form of the alterations that have occurred must be taken into account. Aneuploidy would first be suspected because in many plants, particularly diploids, chromosome loss or addition (or both) influences phenotype (*46*). However, certain polyploid plants, including potato, are less sensitive than others to aneuploidy. Trisomy, for example, is not necessarily expressed as obvious morphological change in tetraploid potatoes (*47*). Still, it is anticipated that more extreme types of aneuploidy would be expressed as altered phenotypes. Cytological examination of five protoclones that showed promise for producing higher tuber yields than the parent indicated that in root tip cells there were 48 chromosomes. Thus, even though we cannot be certain that all chromosomes are the same as in the parent, pronounced aneuploidy does not seem to be a prerequisite for phenotypic variation. If 'Russet Burbank' is truly simplex at key genetic loci, especially those of a regulatory nature, point mutations should thus remain a potential mechanism for change in regenerated plants of this cultivar despite its tetraploid nature.

Contributions to some of the phenotypic diversity might also be anticipated from other sources such as extranuclear inheritance. For example, Gengenbach *et al.* (*48*) regenerated corn plants resistant to the toxin of race T of *Helminthosporium maydis* from callus of a sensitive corn line. Available evidence suggests the change to be mitochondrial in origin. Finally, the possibility that epigenetic phenomena provide at least some of the alternative phenotypes deserves consideration. Meins (*49*) presented evidence of transitory variation in regenerated tobacco plants, which was more easily accounted for by differential gene expression than mutation. By propagating variants of this type asexually, epigenetic phenomena might continue through several somatic generations. Examples of this are few in plants, however, and difficult to verify through sexual crosses unless the trait is simply inherited and not subject to epistatic effects.

Table 1. Relative reactions of tuber progeny from protoclonal plants resistant to race 0 or race 1,2,3,4 of *Phytophthora infestans*. Abbreviations: ++++, highest degree of resistance with few necrotic lesions; +, the lowest compared with 'Russet Burbank'; S, a susceptible reaction equivalent to the parent; NT, not tested.

Clone	Reaction	
	Race 0	Race 1,2,3,4
K-4576	+++	+++
K-2571	+++	S
K-3017	+++	NT
K-3289c	+++	+++
K-1467	+++	+++
K-4005	+	+++
K-4867*	++++	NT
K-223*	++	NT
'Russet Burbank'*	S	S

*See Fig. 5.

Incorporation of several alterations into a single protoclone is a major goal, and experiments designed to test the possibility involve the regeneration of protoplasts from protoclones selected for an improved characteristic. For example, subclones of two high yielding protoclones (M-341 and M-92) are now being transplanted to soil. All will be screened for possible *Phytophthora* and *Alternaria* resistance. Protoplasts from late blight-resistant protoclones K-3688 and K-3289c have been cultured with the aim of selecting for a second enhancement among regenerates. If any of these experiments proves successful, it should be possible to repeat the process for yet a third trait and perhaps a fourth. Were the methods to be combined with specific protoplast selection techniques, such as the use of *Alternaria* toxin, prospects for rapid and efficient improvement of the cultivar would be further increased. At present, the possible outcome of such approaches is uncertain. There could, for example, be a negative correlation between *Phytophthora* resistance and maturity date (*37*) or some other character.

Whether any of these findings apply to other cultivars or plant species remains to be seen. It is probable that considerable phenotypic variation, although perhaps with different frequency, will occur in regenerated plants of additional potato cultivars, especially if they have been vegetatively maintained for extended periods. A long history of vegetative propagation might have permitted the slow accumulation of a greater repository of genetic variation, although unexpressed in the plant, than would be present in a very recent seedling. Pertinent in this context is a report by Wenzel *et al.* (*28*) with protoplast regenerates from two dihaploid potato clones. They obtained plants of uniform morphology and inferred that exposure of mesophyll protoplasts to cell suspension culture was a necessary additional step to achieve variability. Thereafter, they suggested that the high frequency of variation reported for *Alternaria* resistance in our protoclones of 'Russet Burbank' might have resulted from chimerical starting material. We propose that neither of those postulates explains the apparent contradiction in results. Each of the regenerates they obtained from mesophyll protoplasts of dihaploid plants was tetraploid. The process of parthenogenetic dihaploidization with subsequent chromosome doubling reduces the level of heterozygosity in the original genome and erases all simplex inheritance. Should chromosome duplication, whether in the leaf or in culture, precede a mutational event, the expression of minor genetic changes, such as point mutations, would be far less likely. Thus, results with older cultivars and those with doubled-dihaploid potatoes are not directly comparable; rather, results from other cultivars and other heterozygous crop species will first be needed.

We are now attempting to regenerate sweet potatoes and cassava from leaf protoplasts to ascertain whether high frequencies of variation will be realized in these plants as well. To date, we have recovered protoplast-derived calli in high efficiency and are now defining conditions suitable for shoot morphogenesis. If protoplast-derived clones from these species are as variable as those from potato, it should lend support to the notion that a somatic cell population directly isolated from the plant represents a vast source of genetic variation of interesting practical and genetic consequence.

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