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Growth and Water Relations of Cultured Tomato Cells after Adjustment to Low External Water Potentials[1]

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ABSTRACT

Cultured cells of tomato, *Lycopersicon esculentum* Mill. cv VFNT-cherry, have been selected for resistance to water stress (low water potential) imposed by the addition of polyethylene glycol to the culture medium. The ability of nonselected cells to grow in media with low water potentials changes dramatically with the age of the cells (with respect to days following inoculation) whereas there is little effect of the age of selected cells on growth over the same media water potentials. The increased resistance of selected cells has limited stability in the absence of stress, indicating that resistance is established by a slow reversible adaptive process.

Increased resistance (growth) in the presence of water stress appears to result from considerable osmotic adjustment by the cells. Growth cycle-dependent changes in resistance of nonselected cells are correlated with osmotic potential changes which are associated with the normal cell growth pattern in culture. Lowered osmotic potential is maintained by selected cells throughout the entire growth cycle and may explain the growth cycle independence of growth of selected cells on polyethylene glycol-containing media. Osmotic adjustment of resistant cells at stationary phase can be as much as 40 bar. Turgor is maintained by resistant cells (as high as 21 bar) in media with low water potentials at least partly at the expense of cell expansion.

We recently have reported the isolation of cultured cell lines of tomato (*Lycopersicon esculentum* Mill. cv VFNT-cherry) which are resistant to water stress imposed by PEG (2, 12). During the isolation of these lines, the initial growth of the cells in medium containing PEG was somewhat variable. In an effort to understand why the ability of these cells to grow in medium with low water potential caused by the addition of PEG is not always the same, we examined the relative resistance to PEG of cells at different stages in their growth cycle. We found that dramatic changes in the resistance of the cells occur as they progress through their growth cycle. Furthermore, once the cells have become more resistant (selected) to the PEG, changes in resistance through the growth cycle are greatly diminished.

Changes in cellular activities during growth cycles of cultured microorganisms have been known to occur for some time (for example, see Ref. 17). A number of reports have shown growth cycle-related changes in various characteristics of higher plant cells including plating efficiency (20), nutrient uptake (11), accumulation of nitrogen (25), ethylene production (15), and secondary product formation (14). The activities of a number of enzymes vary also during the growth cycle (7–9, 11, 21, 23, 24, 28). Although the activities of certain enzymes, *e.g.* nitrate reductase (9), may be related to nutrient availability, for the most part, such growth stage-dependent variation in phenotype is not well understood except to say that these changes are 'developmental' and that they may reflect differential expression of genetic information. A greater understanding of the factors which influence differential expression of tolerance to water stress will be important toward determining genetic mechanisms of adaptation to stress of higher plant cells. In addition, the potential usefulness of somatic cell selection techniques for the improvement of crop tolerance to stress remains uncertain until such phenotypic variation is understood better.

We report here several characteristics of PEG-induced water stress resistance of cultured tomato cells. The resistance character exhibits wide phenotypic variation over the course of the cell growth cycle, and appears to have an epigenetic basis. The data which are presented further support the hypothesis that resistance of cell populations selected in culture is based on adaptation rather than true selection of pre-existing resistant genotypes. Our findings indicate that resistance appears to be based on substantial osmotic adjustment which is maintained throughout the cell growth cycle, at least partially, by a failure of the resistant cells to continue cell enlargement in the presence of considerable turgor.

MATERIALS AND METHODS

Cell Cultures. Cell suspensions of tomato, *Lycopersicon esculentum* Mill. cv VFNT-Cherry, were used in all experiments. Stock culture maintenance and routine transfers were performed as previously described (2).

Analysis of PEG Resistance throughout the Cell Growth Cycle. Cells in stationary phase were harvested by sterile collection on sintered glass filters. Four-L flasks containing 2 L of medium each were inoculated at a density of 8 g/L with the cells collected by filtration. These cells were then incubated on gyratory shakers as described previously (2). Cells were collected by sterile filtration from these flasks after various days following inoculation. These cells were used then to inoculate 125-ml culture flasks containing 25 ml medium with various concentrations of PEG to determine their ability to grow in medium with low water potentials caused by the addition of PEG. Nonselected cells growing in 4-L flasks containing 2 L medium each with no PEG were collected by filtration and resuspended in fresh medium with no PEG before inoculation into medium with different concentrations of PEG to measure tolerance. Selected cells growing in 15, 20, or 25% PEG were resuspended in fresh medium containing PEG at the concentration which was used for selection (15, 20, or 25% PEG) before measuring tolerance. Since the volume of the cell inoculum in all cases was less than 5% of the total volume, the PEG concentration changes due to the inoculum were ignored. Cells used in these PEG dose-response determinations were allowed to grow for 17 d

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unless otherwise noted before measuring fresh and dry weight gained as described earlier (2).

Cell Volume Measurements. Average cell volumes were determined by measuring the volume displacement and number of cells in a given weight of cells. Typically, 1 g cells was suspended in a known volume of appropriate medium in a 5-ml pipette with the tip removed and the other end sealed. The volume of medium displaced by the cells was determined to the nearest 0.05 ml. The total number of cells in an equal weight of cells was determined using a hemocytometer after separation by incubation in 15% chromic acid for 10 min at 70°C. The total volume divided by the total cell number was used as the average cell volume. The technique overestimates the volume of cells since the intercellular volume is ignored. However, satisfactory relative volume values are obtained.

Osmotic Potential Measurements. Osmotic potentials were determined essentially as described earlier (12). Solutions of NaCl were prepared at concentrations equivalent to 5-bar increments according to CRC physical handbook data. The cells were allowed to equilibrate with the NaCl solutions for 30 min before the percentage of cells showing plasmolysis was determined by examination with a microscope.

RESULTS

Resistance to PEG-Induced Water Stress throughout the Cell Growth Cycle. When nonselected cells are taken from different stages of the growth cycle and their resistance to PEG is examined, it can be seen from Figure 1A that resistance is highest near the exponential phase of growth and declines during all other stages. During a short period, 7 to 10 d after inoculation, the cells are capable of growing fairly well in medium containing PEG as high as 15 and 20% and even exhibit some growth in 25% PEG (Fig. 1A). Cells taken after about 15 d following inoculation, however, will not grow in medium with 15% PEG or higher, although their growth in 0% PEG medium is not diminished. The overall tolerance of these cells to PEG is indicated by the LD_{50} which is defined as the PEG concentration required to inhibit weight gain by 50% of the maximum weight gain observed. The LD_{50} of nonselected cells is shown at various stages of the growth cycle (Fig. 1B).

If cells which have been selected to grow on 15, 20, or 25% PEG are examined in this way, it becomes apparent that certain changes in resistance during the growth cycle which are seen with nonselected cells (Fig. 1A) are not exhibited by the selected cells (Fig. 1, D, G, and J). The growth of these cells is dependent on their growth cycle stage only at PEG concentrations above the level of selection (20% PEG in Fig. 1D, 25% PEG in Fig. 1G, and 30% PEG in Fig. 1J). The LD_{50} of the cells increases as the PEG concentration used for selection is increased and the LD_{50} is not influenced greatly in the selected cells by the growth cycle stage as it is in nonselected cells (Fig. 1, B, E, H, and K).

In addition, cells which have been selected at 20% PEG lose their ability to grow in medium with 5 or 0% PEG while those selected at 25% PEG will not grow in medium with 15% PEG or less. This characteristic of not growing in medium with substantially higher water potentials than the medium to which the cells are accustomed is apparently the result of osmotic downshock (12). Similar osmotic downshock occurs when recently (within 10 generations) selected (in 20% PEG) cells are taken from the exponential growth phase (where cells have the most negative osmotic potential and are the most resistant) and placed in 0 or 5% PEG medium (data not shown). This indicates that resistance to PEG and susceptibility to downshock are both related to osmotic adjustment.

Dry weight gain of nonselected cells under stress is also very dependent on the growth cycle stage and generally is maximal when fresh weight gain is maximal. However, in contrast to fresh weight gain, the dry weight gain of the cells actually can be greater with stress than without stress when cells in the middle of their growth cycle are used as inoculum. Fresh weight gain generally is more reduced than dry weight gain by water stress (10). This is indicated by the higher LD_{50} for dry weight gain of all the cells examined (Fig. 1, B, E, H, and K) and the failure of selected cells to attain the final fresh weight of which nonselected cells are capable while they do attain similar or greater dry weights (Figs. 1 and 5).

Characteristics of the Growth Cycle Effect on Resistance. If cells are taken from various stages in their growth cycle and used to start new growth cycles in both 0 and 15% PEG medium, the following results are observed (Fig. 2). The growth curves in 0% PEG (Fig. 2, A and B) are very similar and there is very little change in either the growth rate in linear phase or the length of the lag period (Fig. 3A). However, the growth curves in 15% PEG (Fig. 2, C and D) indicate that cells taken from exponential phase have a substantially shortened lag period (Fig. 3B). In addition, the growth rate in 15% PEG is reduced 2-fold if cells from stationary phase are used as inoculum. This effect was seen in the original attempts to select cells in 15% PEG (2). These results indicate that a greater proportion of the inoculum survives the initial stress if the inoculum cells are in exponential phase of growth. In addition, if cells used for inoculum are taken from stationary phase, the cells surviving the initial stress exposure can grow only at a reduced rate in the 15% PEG. Although there is a slight increase in the proportion of nonviable cells at the onset of stationary phase (Fig. 4), this is unlikely to account for both the increased survival rate during exponential phase and the reduced growth rate of surviving cells from stationary phase. However, both of these phenomena would be explained by the fact that the average osmotic potential of the cells changes during the growth cycle. Or, in other words, the cell population is composed of cells with different osmotic potentials which can change as the cell proceeds through a culture cycle. At any time in the growth cycle, three types of cells may exist. Cells whose osmotic potentials were not low enough to prevent cytorrhysis would die (in effect this would bring about selection). Other cells would not have osmotic potentials low enough to grow rapidly but would survive and grow at various reduced rates. Other cells would not be affected because their osmotic potentials would be low enough not to be growth limiting. By changing the ratios of these kinds of cells, both the lag and growth rate in the PEG medium could be affected.

Relationship between Osmotic Adjustment, Cell Size, and Resistance. Inasmuch as the average osmotic potential of nonselected cells changes during the growth cycle and becomes most negative during the stage of highest resistance (Fig. 5), it seems likely that changes in resistance during the growth cycle largely are due to osmotic changes in the cells. The lowest osmotic potential attained by the nonselected cells does not quite equal the most positive value observed for cells selected at 25% PEG (Fig. 5) and the data shown in Figure 5 represent the most negative osmotic potentials observed for nonselected cells in several experiments. Both cell populations, however, exhibit a similar pattern in osmotic potential changes. The shift to more negative values of the cell osmotic potentials at all stages of the growth cycle (Fig. 5) also would explain why the resistance to PEG becomes dependent on the growth cycle stage only at higher PEG levels once the cells become adapted. The osmotic potential of adapted cells would not become more positive than the critical value needed to sustain growth at early and late stages of the growth cycle until the level of PEG in the medium was high enough to reduce turgor below the critical value. At this PEG level, the turgor of stationary phase cells would be growth limiting. If the water potential values of medium which are required to cause limited growth of stationary phase cells at various levels of adaptation are estimated from the data in Figure 1 and plotted against the mean osmotic potential of cells at the

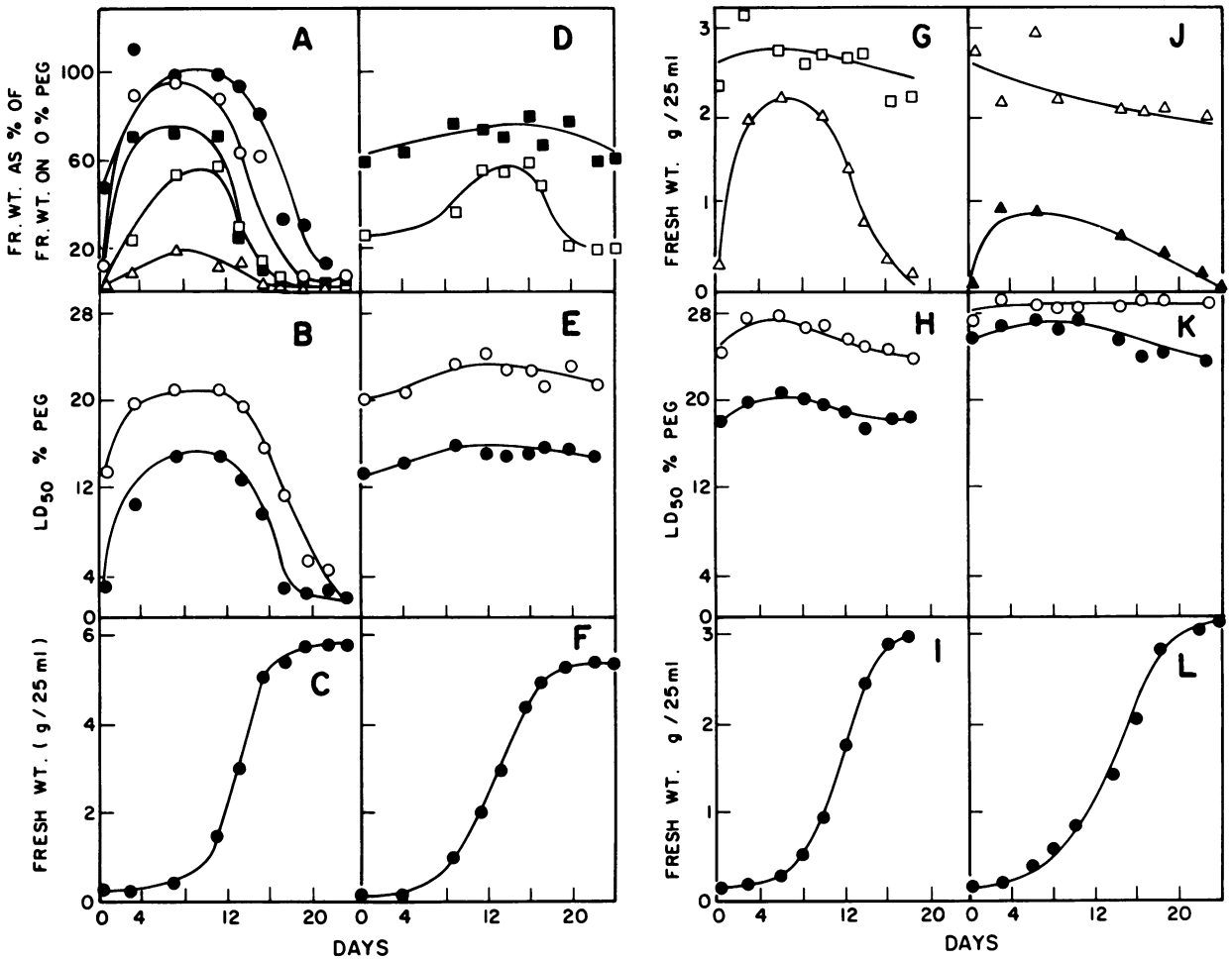

FIG. 1. Growth characteristics of nonselected tomato cells (A, B, and C) and tomato cells selected at 15% PEG (D, E, and F), 20% PEG (G, H, and I), and 25% PEG (J, K, and L), respectively, as a function of growth cycle stage. Cells selected at 15, 20, and 25% PEG were grown for approximately 100 generations on media containing 15, 20, and 25% PEG, respectively, before studying their growth characteristics. Cells taken after various days following inoculation were subcultured into media containing increasing concentrations of PEG. The cells were allowed to grow for 17 d, at which time fresh and dry weights were determined. All inoculum densities were 0.2 g fresh weight (25 ml)$^{-1}$ of culture medium. All fresh weights are shown as g (25 ml)$^{-1}$ of culture medium. Shown in A and D are the fresh weight gain in medium with various PEG concentrations as percentage of the fresh weight gain on medium without PEG. Shown in G and J are the actual fresh weight gains in medium with various PEG concentrations as cells selected at 20 and 25% PEG did not grow on 0% PEG medium. In D, G, and J, only the PEG concentrations between which growth became dependent on the growth cycle stage are shown. (●), 5% PEG; (○), 10% PEG; (■), 15% PEG; (□), 20% PEG; (△), 25% PEG; and (▲), 30% PEG. The actual fresh weight gain in g nonselected cells on 0% PEG of cells which were taken from 0 d following inoculation to 22 d after inoculation were, in order of increasing days: 6.2, 6.5, 7.1, 6.9, 6.6, 6.2, 6.4, 6.1, 6.0, 6.1. The actual fresh weight gain in g of cells selected at 15% PEG on 0% PEG of cells which were taken from 0 d following inoculation to 24 d after inoculation were, in order of increasing days: 6.2, 5.6, 6.2, 6.2, 6.1, 5.9, 6.8, 6.1, 6.1, 6.3. Shown in B, E, H, and K are the LD_{50} (defined as the PEG concentration required in the medium to inhibit fresh weight (●) or dry weight (○) gain by 50% of the maximum weight gained) of nonselected cells and of cells selected at 15, 20, and 25% PEG, respectively. Cells were taken from various stages of the growth cycles shown in C (nonselected cells), F (cells in 15% PEG), I (cells in 20% PEG), and L (cells in 25% PEG) to measure relative growth in various concentrations of PEG. All data points represent the mean of at least two measurements of separate cultures.

different levels of adaptation (Fig. 6), it seems that the cells must maintain about 4 to 6 bar of turgor pressure or growth in fresh medium is limited. This then results in the observed lag and reduced growth over the 17-d growth period used to determine resistance of cells to PEG.

It appears that the relationship between turgor and growth in these cells is not simple, however, and the growth rate is inversely correlated with turgor if cells at different stages of growth or different levels of adaptation are compared. For instance, there is a linear relationship between water potential of the medium and osmotic potential of the cells at stationary phase (Fig. 7). Very interestingly, as the cells become adapted to higher stress levels, they maintain not a constant amount, but an increasing level of turgor which is proportional to the water potential of the medium. Mean cell size of the cell population also decreases as the cells are selected at higher PEG levels (Fig. 7). Inasmuch as the highly adapted cells have higher turgor and grow to smaller mean cell volumes, turgor is actually inversely correlated with growth if these very different cells are compared.

Cell volume is not strictly related to cell osmotic potential but does decrease rapidly above a specific level of osmotic adjustment (about 12 bar). After placing into fresh medium, cells at this same level of adjustment (shown as initial in Fig. 7) begin to increase turgor as adjustment increases, reflecting an inability of the cells to complete expansion following division. It appears that reduction in mean cell volume is a result of adjusting to low external water potential and may not be necessarily a means of resistance to low external water potential *per se* as implied by past investigations (6) since cells which have undergone osmotic adjustment and exhibit reduced volume would be able to maintain turgor even if

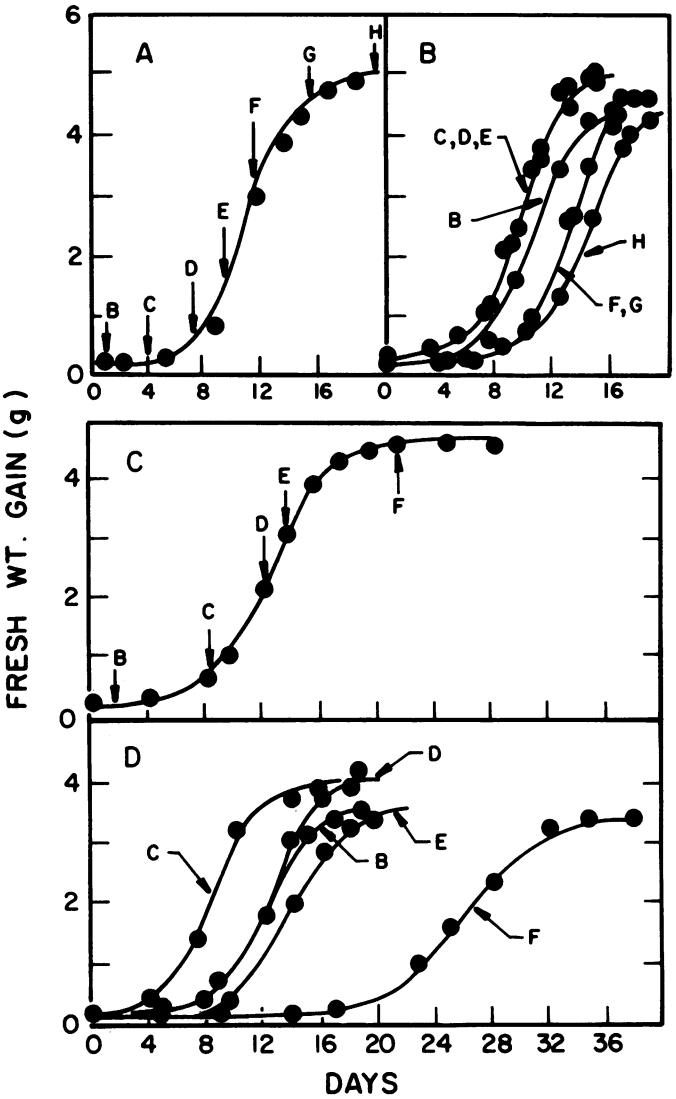

FIG. 2. Growth kinetics on medium with and without PEG of nonselected tomato cells taken from different stages of their growth cycle on medium without PEG. A, Indicates from which point in the growth cycle cells were used for inoculum for the growth cycles shown in B on medium without PEG. Position in the growth cycle (in d following inoculation) of inoculum cells for each cycle shown is indicated by an arrow designated by the letter corresponding to the resultant growth curve. C, Indicates the growth cycle of cells grown in medium without PEG used for inoculum into medium with 15% PEG (D). Positions of the inoculum cells in the growth cycle (in d following inoculation) and their resulting growth curves in 15% PEG are indicated by corresponding letters. All growth cycles were started by inoculation with 0.2 g fresh weight cells (25 ml)$^{-1}$ medium. Points on each growth curve represent measurements taken from the same culture at the times indicated.

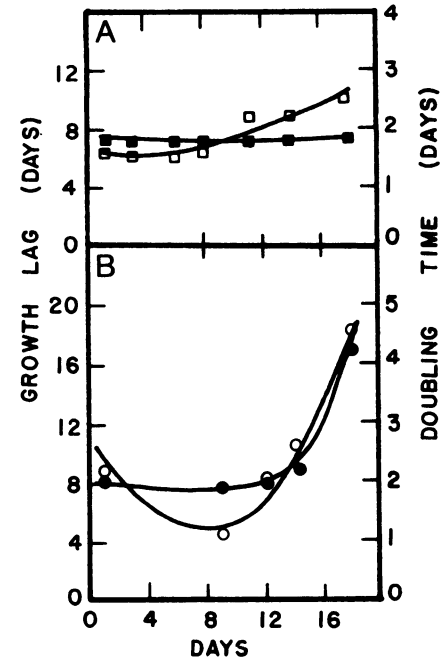

FIG. 3. Changes in lag period and doubling time resulting from the use of cells from different growth cycle stages (in d following inoculation) for inoculum (data from Fig. 2). Age of cells (in d following inoculation) used for new inoculation is indicated on the abscissa. The lag period and the doubling time for fresh weight gain are shown on the ordinates. A, Nonselected cells in 0% PEG medium reinoculated into 0% PEG medium, (□) lag; (■), doubling time. B, Nonselected cells in 0% PEG medium reinoculated into 15% PEG medium; (○), lag; (●), doubling time.

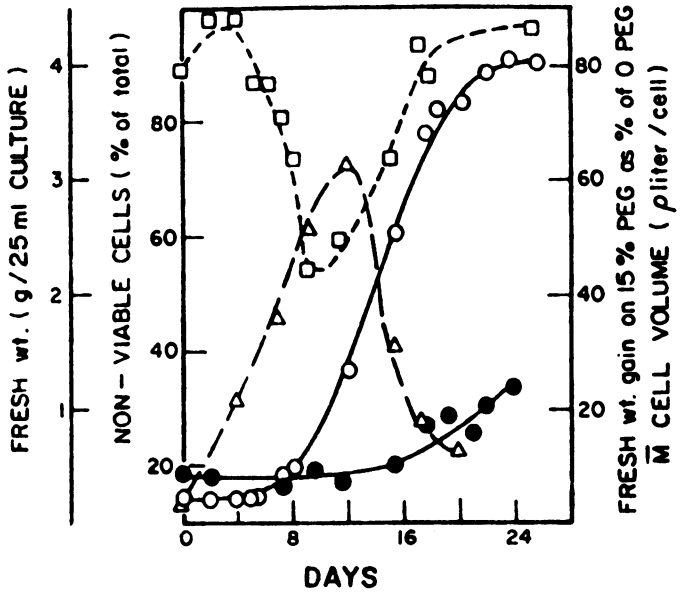

FIG. 4. Changes in fresh weight gain on 15% PEG as percentage of fresh weight gain on 0% PEG (□- - -□), per cent nonviable cells (●—●), and mean cell volume (△— —△) as a function of growth (○—○) cycle stage in d following inoculation. Cells taken at various times following inoculation were reinoculated into 0 and 15%, 0.2 g fresh weight (25 ml)$^{-1}$ PEG medium and allowed to grow for 17 d before determining fresh weight gain. All data shown are from the same culture, for which the growth (○——○) following inoculation is shown.

their volume were as great as nonadjusted cells. This is not to imply, however, that a change in cell size or geometry cannot be an adaptive response to water stress as elegantly discussed by Nobel (22). Failure of the selected cells to expand as much as nonselected cells also indicates that maintenance of high turgor in itself is insufficient under these circumstances to promote growth and that a lack of wall loosening must limit cell expansion instead. Thus, the high turgor is perhaps related to changes in cell wall structure which occurs during adaptation. It is apparent, however, that changes in resistance which occur during the growth cycle and those which are a result of 'selection' reflect considerable osmotic adjustment by the cells as well.

As might be expected, the mean cell size of the population reaches its lowest level just before the linear phase of growth and subsequently increases to its maximum level (Fig. 4) at stationary

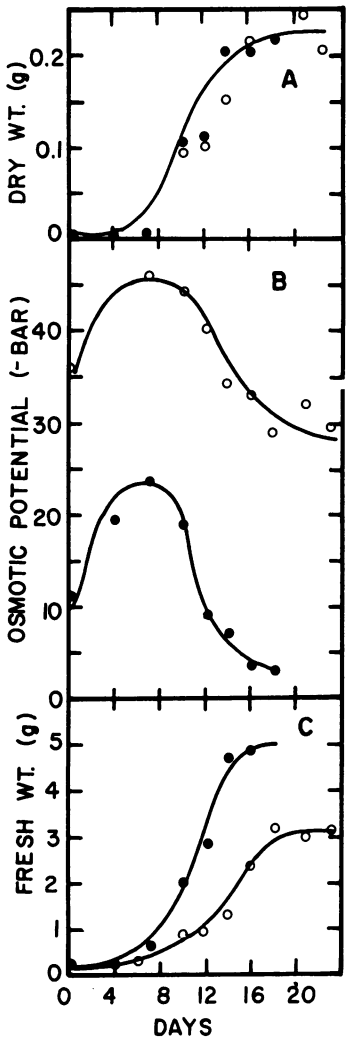

FIG. 5. The osmotic potentials of cells taken from various stages of the growth cycle in d following inoculation. The osmotic potentials indicated represent the −bar of solutions resulting in plasmolysis of 50% of cells examined. Dry and fresh weight gain is shown also. (●), Nonselected cells; (○), cells selected at 25% PEG. Nonselected cells are growing in 0% PEG medium; selected cells (25% PEG) are growing in 25% PEG medium.

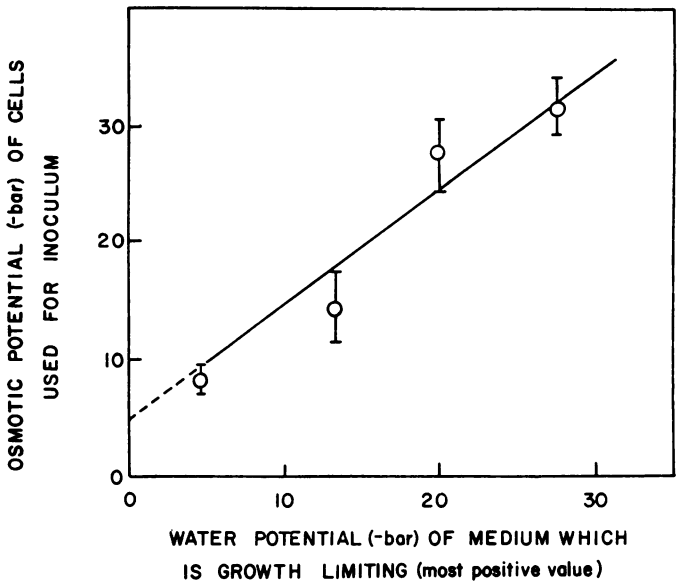

FIG. 6. Shown is the relationship between the osmotic potential of stationary phase cells adapted to four PEG levels (0, 15, 20, and 25%) and the water potential of the medium which is required to cause each cell type to exhibit growth on PEG which is dependent on the growth cycle stage. Cells with any given osmotic potential (ordinate) on the line shown will not exhibit growth which is dependent on the growth cycle stage from which inoculum cells are taken as long as the medium into which the cells are inoculated has a water potential more positive than the corresponding value taken from the abscissa. The bars indicate the highest and lowest osmotic potentials of cells in the examined population for each cell type.

phase. A similar pattern of cell volume change during the growth cycle occurs with nonselected and selected cells (data not shown). The reduction in mean cell size during the growth cycle must result from the cell division process. Following the rapid division phase, during which the osmotic potential falls to a minimum, presumably as a result of nutrient uptake (Fig. 5), the cells begin to expand rapidly (Fig. 5). As the cells expand, the osmotic potential becomes more positive (Fig. 5), presumably because solute accumulation does not keep pace with dilution, and as a result, the resistance of the expanding cells decreases (Fig. 4; also see Fig. 1). The inability of the selected cells to expand to the full volume of nonselected cells may be a consequence of the adaptation mechanism as discussed earlier. However, again the reduced cell volume itself is not a prerequisite for survival at the higher stress levels since cell populations selected at 25% PEG have been observed to contain some cells as large as those found in the nonselected populations. It would be interesting to determine whether the minority of cells which continue to full expansion after division represent a subpopulation genetically distinct from the other cells.

Stability of Resistance in the Absence of Stress. We reported earlier that cells selected at 15% PEG did not retain their enhanced resistance when returned to medium without PEG (2). If cells selected at higher levels of PEG are returned to medium without PEG, they fail to grow (12). However, they may grow in 0% PEG if they have not been exposed to the high PEG concentration for too long (less than approximately 20 generations) and if they are taken from stationary phase of growth. If the resistance of these cells to PEG is examined after growing for 15 generations in 20% PEG followed by 10 generations in 0% PEG, the following results are seen (Fig. 8). They appear to be less resistant than cells which were grown in 20% PEG for many generations (Fig. 1, G and H) but slightly more resistant than cells never exposed to PEG (Fig. 1, A and B). After 10 more generations in 0% PEG, these cells exhibit resistance to PEG very similar to nonselected cells (data not shown). Thus, it seems that the enhanced resistance to low water potentials exhibited by the selected cells is an epigenetic characteristic which is slowly acquired over extended exposure to water stress and is gradually lost after removal of the stress.

DISCUSSION

The driving force for plant cell expansion or growth is the water potential gradient across the cell membrane (16). Factors which influence this gradient can be expected to affect the growth process. Inclusion of PEG into the medium of cultured plant cells will diminish a gradient favoring water movement into the cell and, if the PEG concentration is high enough, the gradient will be reversed and cause water to leave the cell. Likewise, cellular processes which influence the gradient will affect water movement and growth. Specifically, changes in cell osmotic potential through solute accumulation can produce gradients which result in turgor pressure and drive growth (16). Diurnal osmotic changes (1) and those associated with developmental processes such as seed germination (4) are known to cause specific growth patterns.

The observed changes in growth in PEG medium as the cells

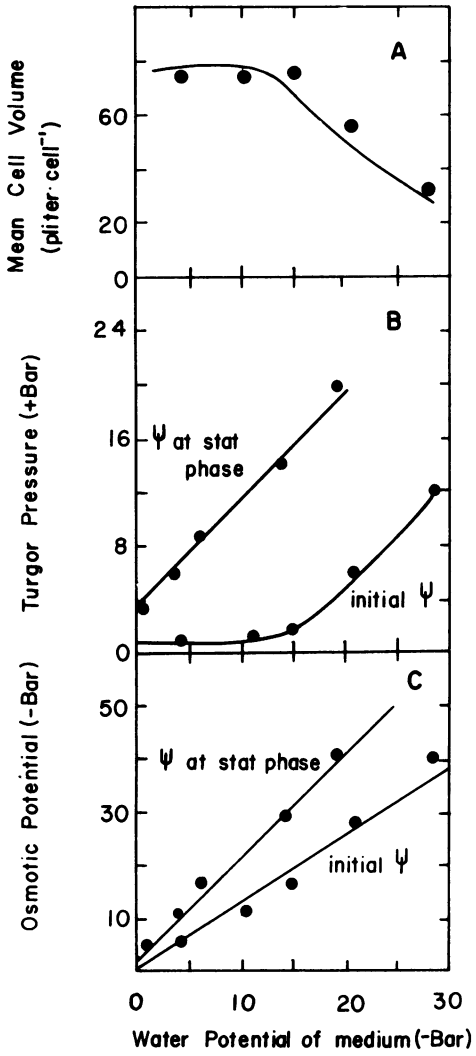

FIG. 7. Relationship between water potential of medium of cells selected at increasing levels of PEG and mean cell volume (A), cell turgor potential (medium water potential − cell osmotic potential) (B), and cell osmotic potential (C). Water potential values of medium were taken before inoculation (initial) and at the time of cell osmotic potential determination (at stationary (stat) phase).

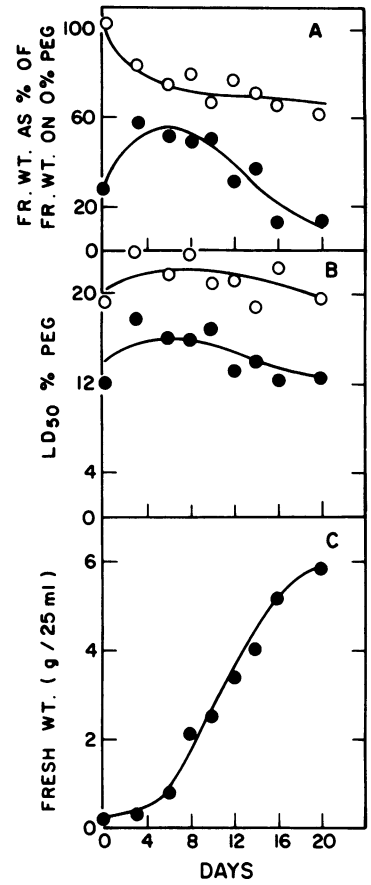

FIG. 8. Growth characteristics of cells selected at 20% PEG and subsequently returned to 0% PEG medium. Conditions are as described in Figure 1. Cells were grown in 20% PEG medium for 15 generations followed by 10 generations in 0% PEG before starting the growth cycle in 0% PEG from which cells were taken to test PEG tolerance. Shown in A is the fresh weight gain in medium with 10% (○) and 15% (●) PEG, respectively, as percentage of the fresh weight gain on medium without PEG. Shown in B are the LDS_{50} values (defined as the PEG concentration required to inhibit fresh (●) and dry weight (○) by 50% of the maximum gained). Shown in C is the growth of cells which were taken at various stages of the growth cycle to measure relative growth in various concentrations of PEG. All points are the means of at least two measurements of separate cultures.

progress through the growth cycle (Fig. 1) could be explained by the observed changes in cell osmotic potential (Fig. 5) which are perhaps caused by rapid solute accumulation following cell division since the cells reach their smallest mean volume near this stage of high osmotic adjustment (Figs. 4 and 8). Changes in cell viability do not explain the growth stage dependence of PEG tolerance since there is little change in viability during the stages where PEG tolerance increases greatly (Fig. 4). Although there is a loss of viability at the latter stages, which might result in the observed increase in growth lag, viability changes alone should not cause the growth rate change on PEG medium observed with cells from stationary phase since no such change in growth rate was observed when stationary phase cells were inoculated into 0% PEG medium (Figs. 2 and 3).

Expansion (or volume increase) of the cells in the cultured cell population can be compared with typical plant cell populations such as in an expanding leaf where similar growth dynamics exist (27). Following cell division, there is an expansion phase which is driven by turgor resulting from a water potential gradient across the cell membrane. This expansion apparently is not balanced by solute accumulation in order to maintain constant osmotic potential, and even leaf osmotic potential becomes more positive as leaf expansion proceeds (see Ref. 13). However, the cultured cells are able to re-initiate cell division and start the process over again upon subculture, whereas the intact leaf represents a terminal process.

An integration between cell osmotic dynamics and cell wall chemistry changes exists in higher plants which coordinates the growth process (5, 16). Of course, the role of phytohormones, *e.g.* auxin, in regulating this coordination mainly through effects on wall properties (3, 5) is well established. However, it seems that the selected or adapted cells have altered the coordination between osmotic and wall properties resulting in considerably slower growth (volume or fresh weight gain) in the presence of given water potential gradients or turgor. This is illustrated by the fact that cells selected at increasing PEG levels have higher turgor (Fig. 7) but gain fresh weight more slowly and reach a smaller maximum volume (fresh weight) as seen in Figure 5. Others have reported recently (18, 26) such slower growth rates after osmoregulation in soybean and rice. Meyer and Boyer (18) indicate that this may reflect an altered coordination between expansion and osmotic adjustment. In addition, they suggest that the reduced

growth rate resulting from such alterations protects the adaptive value of the osmotic adjustment which might otherwise be lost if rapid cell expansion occurred. This is an interesting hypothesis and the results reported here support their results. However, the selected or adapted cells fail to expand to what appears to be their normal volume (that of nonselected cells) even though normal turgor (that of nonselected cells) could be maintained after such expansion. This can be explained partly by the fact that, upon subculture into fresh medium, the turgor will decrease because the fresh medium has a lower water potential. Yet the selected or adapted cells maintain higher turgor than is needed to prevent turgor loss below that of normal cells, even after subculture into fresh medium. For instance, cells selected at 30% PEG maintain about 21 bar of turgor at stationary phase. Upon subculture into fresh 30% PEG, this would drop to about 12 bar which is substantially more than the 1 to 5 bar of turgor retained by normal cells upon subculture in fresh 0% PEG medium. Thus, the mechanism preventing cell expansion seems to overprotect the adaptive value of the osmotic adjustment. The interesting questions remain as to the universality of such growth property changes in response to stress, the biochemical changes on which they are based, and the genetic mechanisms which control their expression in the cell.

It is quite apparent from the results shown here that higher plant cells are capable inherently of substantial osmotic adjustment. However, the stability of such adjustment is not only difficult to determine because of osmotic downshock behavior, but for the same reason could be considered undesirable. On the surface, it seems that an increased ability of the plant cells to adjust and readjust to the changing external water status and not any static cellular osmotic potential is what provides the necessary adaptive response under most cultural conditions of plants. However, if the stress is ever present as is the case of the selected cells, then stability of resistance in the absence of stress is not important but only adaptation to the level of stress present during culture is necessary for survival and growth.

In medium at −27 bar, the adapted cells have a fresh weight doubling time of about 8 d. This level of stress, even if applied gradually, is sufficient to kill 3-week-old tomato plants (data not shown). Consequently, it can be expected that the failure of the cells of tomato plants to adjust osmotically to such stresses is not an intrinsic characteristic of the cells themselves, but likely reflects certain limitations imposed on the cells by the developmental changes required to organize, form, and function as a whole plant, *e.g.* obtaining the reduced carbon necessary for osmotic adjustment via mechanisms like stomatal movements and photosynthetic reactions which are themselves quite sensitive to water stress. Thus, the substantial osmotic adjustment demonstrated by the cells adapted to PEG is considerably more than often noted in greenhouse- or field-grown plants by other investigators (1, 18). Nevertheless, Morgan (19) has reported that certain wheat genotypes show nearly 20 bar of osmotic adjustment under greenhouse conditions. The high levels of osmotic adjustment which cultured cells can achieve should facilitate investigations concerning the biochemical or metabolic changes which result in such osmotic changes. Furthermore, the nature of the control mechanisms which might limit osmotic adjustment in the whole plant could be further understood by determining the control processes for osmoregulation in the cultured cells.

Once more information is gained concerning the physiological and biochemical nature of osmotic adjustment in plant cells, the limitations of osmotic adjustment for improvement of crop performance under water stress will be better understood.

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LITERATURE CITED

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