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Plant cells selected for resistance to phosphate starvation show enhanced P use efficiency

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Summary. In many organisms, phosphate starvation induces multigene systems that act to increase the availability and uptake of exogenous phosphates. Tissue-cultured tomato cells were plated onto solid media containing starvation levels of phosphate. While most cells died, we identified isolated clumps of callus capable of near-normal rates of growth. Starvation-resistant cells were used to start suspension cultures that were kept under phosphate starvation conditions. A selected cell line showed constitutively enhanced secretion of acid phosphatase and greatly increased rates of phosphate uptake. These pleiotropic effects suggest modification of a regulatory apparatus that controls coordinated changes in the expression of a multigene system. The somaclonal variant cell line grew normally under phosphate-sufficient conditions, but did significantly better than unselected cells under phosphate-limited conditions. In vitro selection may be a useful system for developing phosphate ultraefficient crop plants.

Key words: Phosphate starvation – In vitro selection

Introduction

Phosphate starvation induces a set of complex metabolic and genetic responses in both eucaryotic and procaryotic cells (Torriani and Ludtke 1985). Using suspension-cultured tomato cells, my laboratory has shown that phosphate starvation induces the excretion of a specific acid phosphatase isozyme into the extracellular medium (Goldstein et al. 1988a, b). We have called this enzyme the excreted phosphate starvation inducible (epsi, pronounced eeψ) acid phosphatase (APase). Increased levels of the epsi-APase are observed shortly after transfer of the cells to an orthophosphate (Pi)-depleted medium and far in advance of starvation-induced inhibition of cell growth. More recently, we have shown that phosphate starvation induces the Golgi-mediated secretion of several media proteins (Goldstein et al. 1989b). In addition, Goldstein et al. (1988b) as well as others have observed that phosphate-starved cells show increased rates of Pi uptake from the media.

Bacteria and fungi have genetic systems that function to enhance phosphate availability and uptake. These coordinately regulated phosphate starvation inducible (psi) genes, collectively called a pho regulon, have been studied extensively (Torriani and Ludtke 1985). The molecular mechanisms for starvation rescue include enhanced solubilization and uptake of exogenous organic and mineral phosphates (Torriani and Ludtke 1985; Goldstein 1986; Goldstein and Liu 1987).

We wished to identify phosphate stress-tolerant plant cells and to determine whether the selected cells exhibited phenotypes characteristic of pho regulon mutants. Therefore, we used tissue culture selection methods to identify plant cells that were resistant to phosphate starvation.

Materials and methods

Plant material and in vitro selection

Suspension-cultured tomato cells (*Lycopersicon esculentum* cv VF-36) that had depleted the Pi in the medium (Goldstein et al. 1988a) were plated onto solid media containing 0.01 m*M* Pi. Approximately 1.5×10^6 cells were spread per plate. After 3 months, eight out of ten plates contained one or two normal-looking callus clumps, while the remaining tissue appeared necrotic.

A single callus clump, designated PSR-1, was selected for further study in liquid culture. Methods for liquid culture and

biomass measurement have been previously described (Goldstein et al. 1988a) except that, in order to maintain selection pressure, the concentration of Pi in the media was 0.01 m*M*.

Growth experiments

After 6 months of continuous selection, PSR-1 cells were inoculated into regular VF-36 medium (1.25 m*M* Pi) and grown to late log phase. PSR-1 and unselected late log-phase VF-36 cells were inoculated into VF-36 medium and grown to late log phase. Cell lines were transferred to medium containing 0.1 m*M* Pi and grown to stationary phase. Biomass accumulation as dry weight was measured daily in both growth experiments.

Pi uptake and excretion of epsi-APase

Rates of Pi uptake from the media and excretion of acid phosphatase into the media were measured for both cell lines. Pi uptake was measured using 3-day-old cells sampled from the growth experiments described above. The apoplast space was preequlibrated with cold Pi and Pi uptake measured using ^{32}P-orthophosphate following the methods previously described (Goldstein et al. 1989b).

The level of epsi-APase in the media was determined by immunoblotting. Media protein per lane represented an equal amount of biomass. Both media samples were from day 3 in the growth curve shown in Fig. 2a (see Results). Preparation of media proteins, immunoblotting, and visualization via binding to ^{125}I-protein A were conducted as previously described (Goldstein et al. 1989b), except that the specificity of polyclonal antisera AP3 was enhanced by repeated passage over a pineapple stem bromelain column (Sigma Chemical Co.) to remove antibodies directed against N-linked oligosaccharides with a terminal xylose.

Results

Preliminary identification of phosphate starvation-resistant callus on solid media is shown in Fig. 1. Figure 2a shows that, under unstressed conditions, both cell lines exhibited equivalent rates of growth. However, the PSR-1 cells accumulated significantly more biomass in a Pi-limited environment (Fig. 2b).

PSR-1 cells grown + or –Pi showed an approximate sevenfold increase in the rate of Pi uptake versus VF-36 cells grown under Pi-sufficient conditions. Unselected

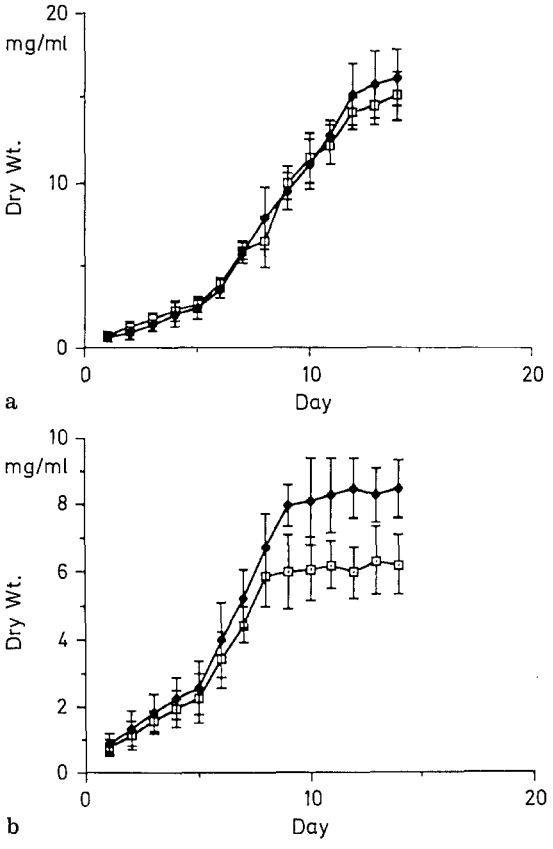

Fig. 2. **a** Growth of PSR-1 (◆) versus unselected VF-36 (□) on media containing 1.25 m*M* Pi. **b** Growth of PSR-1 (◆) versus unselected VF-36 (□) on media containing 0.1 m*M* Pi

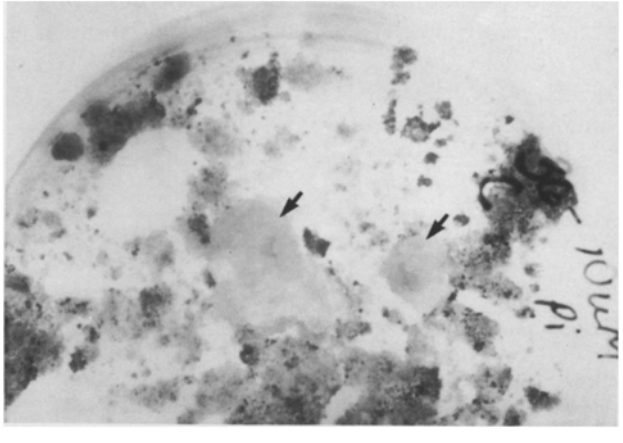

Fig. 1. Suspension-cultured tomato cells were plated onto media containing 10 μ*M* Pi. With the exception of two normal-looking callus clumps, all cells on this plate are dead. The larger clump was used to start the suspension-cultured cell line PSR-1

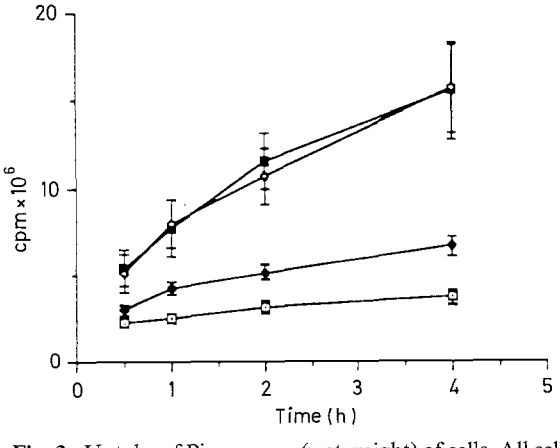

Fig. 3. Uptake of Pi per gram (wet weight) of cells. All cells were sampled at day 3 of the growth curves shown in Fig. 2. Therefore, initial media Pi concentration was either 0.1 m*M* (■, *PSR-1;* ◆ VF-36) or 1.25 m*M* (◇, PSR-1; ⊡ VF-36)

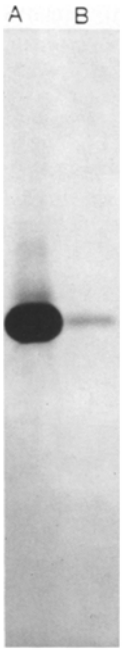

Fig. 4. The level of epsi-APase in the media was determined by immunoblotting. Excretion of this psi enzyme by 3-day-old cells of PSR-1 (a) was greatly enhanced over unselected VF36 (*B*). Both samples were from the growth curve shown in Fig. 2a. The high level of Pi present in this media would normally limit epsi-APase production to the level observed in *lane B*

cells grown –Pi showed a more moderate 2.6-fold (approximate) psi enhancement in Pi uptake rates relative to unstressed controls (Fig. 3).

Figure 4 shows that PSR-1 cells were excreting large quantities of acid phosphatase 3 days after inoculation into Pi-sufficient medium, while secretion of this enzyme was strongly inhibited in the unstressed controls.

Discussion

The goal of this experiment was to develop an in vitro system to study the relationship between resistance to Pi starvation and the physiological genetics of Pi uptake and use efficiency. Selection for Pi stress-resistant cells on solid media was successful. A suspension culture started from a single clump of stress-resistant callus accumulated 14 mg/ml of dry weight at the end of 6 months of growth under starvation levels of Pi. This was equivalent to a 12- to 14-day-old late log-phase culture of unstressed cells.

To compare relative growth rates of selected and unselected cells lines at unstressed levels, PSR-1 cells were transferred from 0.01 m*M* Pi media to 1.25 m*M* Pi medium and put through one cycle of growth to late log phase. It took PSR-1 23 days to reach late log phase. In the second cycle of Pi-sufficient growth, PSR-1 cells grew as well as controls. Further experiments verified that the PSR-1 cells were capable of accumulating significantly more biomass than controls under Pi-deficient conditions (Fig. 2).

In addition to an enhanced ability to grow under limited Pi, the starvation-resistant cell line showed simultaneous changes in two traits characteristic of pho regulon induction: enhanced secretion of the epsi-APase and an increased rate of Pi uptake. We have previously shown that Pi-sufficient conditions repress the epsi-APase in VF-36 cells (Goldstein et al. 1988a, b, 1989b). The VF-36 cells assayed in this study showed only low levels of the epsi-APase at day 3. The PSR-1 cells excreted large amounts of APase in spite of the presence of what should have been inhibitory levels of Pi in the external medium (Fig. 4). Likewise, the PSR-1 cells showed a greatly enhanced rate of Pi uptake from the medium under both Pi-sufficient and -deficient conditions (Fig. 3). Control cells showed a more moderate phosphate starvation inducible enhancement of Pi uptake, as previously reported (Goldstein et al. 1989b). In both cases, the Pi use-efficiency phenotype appeared to result from the failure of high levels of exogenous Pi to repress the expression of inducible (or derepressable) starvation rescue systems.

The data presented here may have implications for the development of biorational P fertilizers for agriculture. Many attempts have been made to use both organic and mineral P-solubilizing organisms as P biofertilizer agents (Goldstein 1986). In a related area of research, plant breeders have identified genetic variability in mineral nutrient utilization, but have been unable to develop varieties that differ dramatically in their fertilizer use efficiencies (Englestad and Terman 1980; Ozanne 1980). While a great deal of work remains to be done, the data reported here strongly suggest the existence of a higher plant pho regulon. Elucidation of the genetic and biochemical components of this regulon may provide us with one of the first mechanistic examples of a multigene biophysical stress-tolerance/resistance system in higher plants. The genetic components of this system may, in turn, provide the molecular bases for the development of a biorational P fertilizer system for crop production. At the very least, it is reasonable to suggest that crop plants may be selected in vitro for enhanced P use efficiency.

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