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. 1988 a, b). We have called this enzyme the excreted phosphate starvation inducible (epsi, pronounced eeO) 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 co-ordinately 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. 1988 a) were plated onto solid media containing 0.01 mM Pi. Approximately $1.5 \times 10^6$ 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 mM.

Growth experiments

After 6 months of continuous selection, PSR-1 cells were inoculated into regular VF-36 medium (1.25 mM 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 mM 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 preequilibrated 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 antiserum 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