# Plant protein secretion on tap

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Transgenic plants have shown tremendous potential as photosynthetic bioreactors for the production of foreign proteins such as antibodies[1] and streptavidin[2]. But harvesting these recombinant molecules can be problematic because they typically are sequestered inside plant cells and require tissue disruption and extraction treatments for purification, often with mixed results. In this issue, Ilya Raskin and colleagues[3] get to "the root" of the problem, both by making clever use of endoplasmic reticulum (ER) targeting signals to shuttle recombinant molecules into the plant's default secretory pathway and by exploiting the ability of plants to secrete copious amounts of proteins from their root systems. Combining these tricks with hydroponic propagation, they have come up with "rhizosecretion," a system by which large amounts of active recombinant proteins are secreted into hydroponic media. This system not only allows recombinant proteins to be continually collected over the lifetime of the plant, but also substantially reduces the time and labor involved in their harvest and purification.

Using ER targeting signals to shuttle proteins to the cell's exterior is now a well-established trick. For example, recombinant proteins can be directed to the extracellular spaces (apoplast) of leaves and subsequently leached from the extracellular spaces by infiltration of a buffer, which can then be collected by gentle centrifugation. Alternatively, cultured plant cells can be engineered to secrete recombinant proteins directly into the culture medium. Neither approach is ideal, however, the former being rather time-consuming and labor intensive, and the latter requiring a carbon source and sterile conditions, making the process prohibitively expensive. By comparison, rhizosecretion seems simple. Why has it not been reported before? Apparently, no one has thought to look.

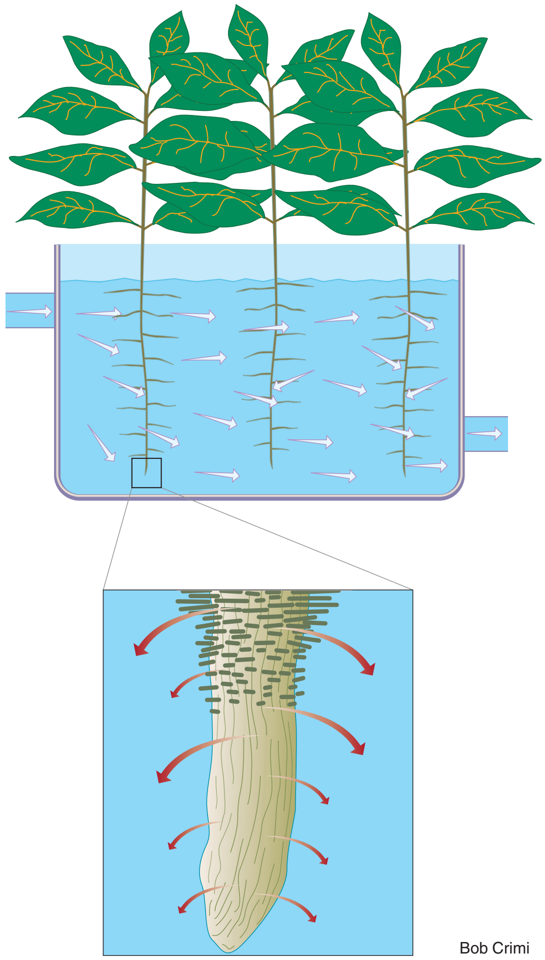


**Figure 1. Schematic of rhizosecretion in plant hydroponic environment. As the hydroponic solution is circulated around the rhizosphere, proteins secreted by the roots are either collected or used to convert other molecules into more useful compounds.**

Although this study is limited to single-plant cultivation, the potential for scaleup is obvious. The advantages of large-scale rhizosecretion would be twofold: the continual production of recombinant proteins and the apparent ease of protein purification. One can envision a hydroponic system (see Fig. 1) in which roots are bathed or sprayed with a hydroponic solution, which is then collected for both flow-through protein purification and nutrient addition, as needed. In fact, a continuous and rapid means of protein purification would be essential, as long-term protein survival in a hydroponic environment could be problematic. The hydroponic chamber could even become a bioreactor of sorts, with precursor proteins placed in the hydroponic solution converted to new molecules by rhizosecreted enzymes.

In the present study, Raskin and colleagues fuse green fluorescent protein (GFP), human placental alkaline phosphatase, or bacterial xylanase to an ER targeting signal, and express the respective recombinant proteins in hydroponically grown transgenic tobacco. In all cases, they show that the proteins were secreted into the hydroponic media to high levels and were present in a biologically active form. Experiments with GFP confirmed that secretion to the medium was specifically associated with presence of the ER targeting signal.

Based on the above observations and related information in the literature, rhizosecretion makes sense and clearly works. But what are the limitations? Is rhizosecretion an economically viable system for large-scale production of recombinant proteins?

A crucial test of this new technology is how it stacks up to existing methods—notably, seed expression of recombinant proteins[2]. Transgenic crops such as wheat or corn can be propagated on a massive scale, literally producing fields of recombinant protein—all conveniently stored in dry seed form until purification. Can hundreds of thousands of plants be similarly propagated in the hydroponic rhizosecretion system?

On the other hand, rhizosecretion may have some advantages over conventional technologies, particularly in terms of purification. As the authors note, rhizosecretion can be optimized to yield much higher levels of protein than the reported levels. Roots secrete relatively few proteins, so rhizosecreted proteins are already somewhat purified. Ease of purification is often a critical step, requiring extensive manipulations at a high cost. This is particularly important in that trace amounts of impurities can in some cases make a product unacceptable.

As more information becomes available on ER targeting, secretion, enhanced gene expression, and flow-through or continual protein purification, rhizosecretion could become a very valuable approach, perhaps one day allowing large-scale recombinant protein production in plants on tap.

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1. Firek, S. et al. *Plant Mol. Biol.*, **23**, 861–870 (1993).
2. Hood, E.E. et al. *Mol. Breed* **3**, 291–306 (1997).
3. Borisjuk, N.V. et al. *Nat. Biotechnol.* **17**, 466–469 (1999).