Boosting the Quality and Potency of St. John's-wort

Above: Plantlets of *Hypericum perforatum*. Photo by Peggy Greb. (K8943-20)

PEGGY GREB (K8942-1)

Plant physiologist Donna Gibson examines callus cultures of *Hypericum perforatum* that will be used to optimize production strategies.

St. John's-wort is one of the most widely used herbal crops in the world. Today, the herb is popularly used as a natural alternative for the treatment of depression in cases where antidepressants like Prozac or many other standard drugs are typically prescribed.

"The importance of St. John's-wort as a dietary supplement has significantly increased in the last few years. This is evidenced by the fact that the yearly market for the herb has topped over $210 million in the United States alone and over $570 million worldwide," says Agricultural Research Service plant physiologist Donna M. Gibson. She is in the Plant Protection Research Unit at the U.S. Plant, Soil, and Nutrition Research Laboratory in Ithaca, New York.

St. John's-wort is a plant that grows in the wild and is harvested for its active ingredients, which include at least 10 components that may contribute to its pharmacological effects. It is not yet known which specific component or mixture is the active ingredient, so the material is characterized on the basis of its leading components, especially the hypericins.

Now Gibson and plant physiologist Camilo Canel, who is in the ARS Natural Products Utilization Research Unit at Oxford, Mississippi, are studying the plant using the tools of biotechnology and biochemistry to zero in on the most potent forms of *Hypericum*. Both researchers' goals are to provide safer and accurately labeled forms of the herb.

Adulteration of St. John's-wort in commercial products usually occurs when species of *Hypericum* other than *H. perforatum* are used. Other factors that may affect the quality of the herb are environmental factors such as light, moisture, altitude, and latitude; the plant part; plant developmental stage; and harvesting and handling practices.

New Method for Analyzing Hypericin

Although the efficacy of the herb has not been clinically demonstrated, the potency of St. John's-wort is perceived to be tied to its hypericin content.

To measure the herb's potency, Gibson, along with Cornell University Department of Plant Pathology graduate student Tara Sirvent, have developed a new method of analyzing the hypericin compounds. This method is faster than current methods of analysis that are insufficient or laborious to perform.

Gibson says their method takes just 15 minutes to separate hypericin from pseudohypericin, a related compound, in crude extracts using high-performance liquid chromatography (HPLC). She says that other methods require much longer times and more complex mixtures for adequate separation.

"Our HPLC method permits the fast quantitative analysis of hypericins in crude acetone or methanol samples," says Gibson. She and Sirvent also studied the photoconversion of protohypericins—the precursor compounds—as well as the stability of hypericin and pseudohypericins as a function of light, time, and storage conditions.

According to Gibson, "Since hypericins are typically used as the measure of extract potency and since market pricing for this herbal product has switched from a yield basis to a price based on specific product ingredients, producers may receive $2,000 to $3,000 more per acre if their crops have higher hypericin levels."

Gibson's lab is currently completing a survey of wild-collected *H. perforatum* samples from the Pacific Northwest. The survey's purpose is to ascertain the range of variation that might be due to environmental influences.

Genetic Fingerprinting

Canel is using genetic fingerprinting to screen plants at the seed level. His goal is to identify genetic markers capable of differentiating between closely related *Hypericum* species.

"Unlike phytochemical profiles and morphological features, DNA sequence normally remains unaltered during the development of the plant and under various environmental conditions," he says. Genetic fingerprinting can be used at any stage of the herbal manufacturing process, but it is especially suited to the seedling level.

Canel believes this research will lead to a seed-certification program for cultivated *H. perforatum* that requires applying genetic techniques. His work on young leaves of *H. perforatum* is a model for locating genetic markers that can be used to find the best sources of compounds in other herbal products manufactured from cultivated plants.

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Cornell University graduate student Tara Sirvent prepares dried *Hypericum perforatum* for extraction and analysis.

Genetically certified plant material and products made from them can be expected to have a higher market value than uncertified material or material certified by other means. Certification of St. John's-wort at the seed level would not only establish the authenticity and purity of the starting material, but would also document its source and provide information about seed viability and germination rates.

Canel is developing a fingerprinting kit based on the genetic differences he has observed. He hopes to make it available for commercial use soon.

According to Gibson, since hypericin is a supplement, it doesn't have Food and Drug Administration approval. However, it is currently being evaluated in the first long-term, controlled clinical trials directed by the National Institutes of Health.

Gibson was recently notified that her research proposal on using plant cell biotechnology to produce hypericin and hyperforin—another hypericum compound also linked to antidepressive effects—has been awarded funding from USDA's Foreign Agricultural Service.—By **Hank Becker,** ARS.

This research is part of New Uses, Quality, and Marketability of Plant and Animal Products, an ARS National Program (#306) described on the World Wide Web at http://www.nps.ars.usda.gov/programs/cppvs.htm.

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