A transgenic plant expressing miraculin: a case study of the production of a functional recombinant protein in plants

H. Ezura and H.-J. Sun

Gene Research Center, University of Tsukuba, Tennodai 1-1-1, Tsukuba, Ibaraki 305-8572, Japan

Introduction

Plants constitute the main food resource for animals and humans, which is why the foremost mission of agriculture is to produce plants in sufficient quantities to feed the world. This problem has become acute in the face of the demographic explosion, the erosion of arable land, and the development of intensive farming, which causes environmental damage. Genetic engineering of plants can offer good solutions to these problems. The development of genetic transformation technology for plants has enabled the expression of foreign genes in different plant species, making it feasible to use plants as bioreactors to produce recombinant proteins. Plants are among the most economical producers of biomass. They require only sunlight, water, carbon dioxide, and some minerals, but provide an optimal system for the expression of recombinant proteins that are free from contamination by bacterial toxins or animal pathogens. Moreover, they possess the eukaryotic protein modification machinery, allowing subcellular targeting, proper folding, and post-translational modifications. Recently, several academic and industrial laboratories have begun experimenting with transgenic plants as novel manufacturing systems. There are several advantages to using plants as "bioreactors", namely, the production of functional recombinant proteins from plants is less expensive than conventional fermentation, it is easy to scale up plant production, and it eliminates possible animal source contamination when used to produce proteinaceous drugs. Transgenic plants are being used to produce desired functional recombinant proteins, such as vaccines, antibodies, mammalian hormones, biopharmaceuticals, and food additives. Our laboratory is trying to produce functional recombinant proteinsintransgenic plants. Here, we show the preliminary results of "miraculin" production as an example of the use of transgenic plants to produce functional recombinant proteins.

What is miraculin?

A shrub native to tropical West Africa called *Richadella dulcifica* yields red berries that can convert a sour taste into a sweet taste. For example, lemons taste sweet, like oranges, after chewing the pulp of the red berries. Due to this unusual property, the berry has been called the "miracle fruit". The active ingredient in the berry, miraculin, causes citric acid, ascorbic acid, and acetic acid, which are normally sour, to be perceived as sweet after being held in the mouth. It was first isolated by Kurihara and Beidler (1968). The complete amino acid sequence of miraculin has been determined (Theerasilp *et al.*, 1989). Edman degradation showed that miraculin has 191 amino acid residues, with a calculated relative molecular mass of 24,600. Native miraculin appears to be a tetramer or a dimer that is held together by an interchain and three intrachain disulfide bridges. Both forms have taste-modifying activity. Up to 13.9% of the glycoprotein is sugar. The 42^{nd} and 186^{th} asparagine residues are connected to sugar chains including glucosamine, mannose, galactose, xylose, and fucose. Miraculin by itself does not elicit a sweet response. Like curculin, however, it can modify a sour taste into a sweet taste. Kurihara and Beidler (1968) named the protein the "taste-modifying protein".

Transgenic plants expressing miraculin

In recent years, there has been an increasing demand for "low-calorie" sweeteners. These are important for persons affected by diseases linked to the consumption of sugar, *e.g.*, diabetes, hyperlipidemia, and caries. Most sweet substances, including all popular sweeteners, are low molecular mass compounds with widely different chemical structures. Therefore, it was surprising when higher-molecular-mass taste-modifying proteins were discovered that interact with the taste receptors in a potent and specific manner.

Currently, six alternative, high-intensity sweeteners have been approved by European Union regulatory bodies (Grijspaardt-Vink, 1996): aspartame, saccharin, cyclamate, neohesperidine DC, acesulfame-K, and thaumatin. The first five compounds in this list are low-molecular-weight entities that are obtained by traditional organic synthesis technology. The last one, thaumatin, is a protein.

West Africans have traditionally used sweetness- and taste-modifying proteins to improve the flavor and suppress the bitterness of food and drink. They are used to improve the flavor of maize dishes, such as *agidi*, and in beverages, such as palm wine or tea. In modern times, they have found uses in the food processing industry as sweetening agents, flavor enhancers, and animal fodder supplements (Michael, 1998). These proteins can act at extremely low concentrations, and because of this low effective dose they are effectively non-cariogenic and are acceptable for diabetics in flavor and sweetening formulations. With the commercialization of thaumatin, there has been an increase in these compounds. There are seven known sweetness- or taste-modifying proteins: thaumatin, monellin, mabinlin, pentadin, brazzein, curculin, and miraculin. In recent years, their genes have been cloned and sequenced, and in many cases, they have been expressed in foreign hosts (Faus, 2000).

In this study, we attempted to express the taste-modifying protein miraculin in plants as a potential alternative sweetener. A synthetic gene encoding miraculin was assembled and inserted into a plant expression vector. The miraculin gene has been introduced into vegetable crops, such as lettuce, tomato, and strawberry, using *Agrobacterium*-mediated genetic transformation. In a preliminary characterization, a recombinant miraculin protein was detected in some lines of transgenic lettuce (Figure 1). The complete characterization of the recombinant miraculin is under way.

Perspective

Sweetness- and taste-modifying proteins are obviously attractive alternatives to some of the more traditional artificial sweeteners. Thaumatin is currently used commercially as a sweetener, flavor enhancer, and pharmaceutical additive, and in chewing gum and animal feed. The taste-modifying protein miraculin can be used to control the palatability of foods or to develop new food products. Taste-modifying proteins are a natural alternative to artificial sweeteners and have been used in some cultures for centuries. The stability and availability of these proteins has limited their use, but recent advances in biotechnology should increase their availability. These advances include production in transgenic organisms and protein engineering to increase stability. Taste-modifying proteins will be available for much wider use in the food industry, and will reduce the dependence on synthetic alternatives.

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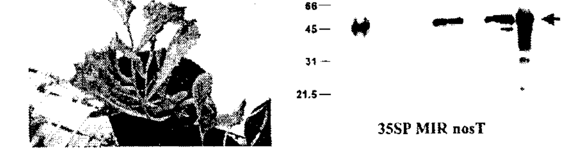

Figure 1. Production of transgenic lettuce plants expressing miraculin protein.
A: Transgenic lettuce plant, B: Detection of recombinant miraculin in transgenic lettuce. C: non-transgenic plant, 1-9: transgenic plants, P: protein from miracle fruits.