

# Application of sweet and taste modifying genes for development in plants: current status and prospects

Shahina Akter · Md. Amdadul Huq · Yu-Jin Jung · Yong-Gu Cho · Kwon-Kyoo Kang

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**Abstract** Sweet and taste modifying proteins are natural alternatives to synthetic sweeteners and flavor enhancers, and have been used for centuries in different countries. Use of these proteins is limited due to less stability and availability. However, recent advances in biotechnology have enhanced their availability. These include production of sweet and taste modifying proteins in transgenic organisms, and protein engineering to improve their stability. Their increased availability in the food, beverage or medicinal industries as sweeteners and flavor enhancers will reduce the dependence on artificial alternatives. Production of transgenic plants using sweet and taste modifying genes, is an interesting alternative to the extraction of these products from natural source. In this review paper, we briefly describe various sweet and taste modifying proteins (such as thaumatin, monellin, brazzein, curculin and miraculin), their properties, and their application for plant development using biotechnological approaches.

**Keywords** Sweet gene, Taste modifying proteins, Monellin, Thaumatin, Brazzein

## Introduction

Demand of agricultural crops is increasing worldwide with the increase of population. Hence, scientists are always trying to develop crop plants to enhance yield, resistance to various

diseases and to add nutritional value. Therefore, the use of sweet and taste modifying genes for development of different crop varieties like maize, tomato, rice, potato, wheat, barley, or different fruits would be a good way. The necessity of healthy, natural and low calorie sweeteners production is increasing (Faus 2000) because every day many of world population are attacked by many diseases like caries, hyperlipemia, obesity and type II diabetes as a result of consumption of high caloric food many of which are comprised of sugars as well as carbohydrates. Sometimes these substances, cause other side effects such as, brain tumors, bladder cancer, heart failure, even mental disorders (Kant 2005; Sun et al. 2006). For this reason, there is an exquisite, ongoing exploration for alternative sweeteners which can overcome such limitations. It was thought that macromolecular substances do not have any taste but it was changed after discovering sweet and taste modifying proteins (Witty 1998). Lifestyle related diseases like, obesity, diabetes, hypertension, hyperlipidemia etc. have risen as a major problem in developed countries. These diseases might be involved with intake of high calorie foods. Therefore, low calorie sugar alternatives can be used in foods, beverages, and medicines. Sweet tasting proteins could be a potential alternative as low-calorie sugar.

There are many reports about the identification and characterization of sweet and taste modifying proteins from different plants. Among these, the main sweet and taste modifying proteins have been narrated as: thaumatin (Wel and Loeve 1972), monellin (Wel 1972; Moris and Cagan 1972), brazzein (Ming and Hellekant 1994), miraculin (Kurihara and Beidler 1968), curculin (Yamashita et al. 1990). Sweet and taste modifying proteins are natural and have been in cultural use for centuries and earned more popularity to the public than artificial ingredients. These proteins have been the subject of interest to the food industry for many years. However, they have not been widely used because of their limited availability. Many scientists tried to overexpress these sweet and taste modifying proteins using bacteria, yeast or fungi (Moralejo et

<sup>†</sup>These authors contribute equally to this work as co-first author.

A. Shahina · H. Md. Amdadul · Jung Y.-J. · Kang K.-K. (✉)  
Department of Horticultural Life Science, Hankyong National University, Anseong 17579, Korea  
Institute of Genetic Engineering, Hankyong National University, Anseong, 17579, Korea  
e-mail: kykang@hknu.ac.kr

Cho Y.-G.  
Department of Crop Science, Chungbuk National University, Cheongju 28644, Korea

al. 1999; Daniell et al. 2000; Masuda et al. 2004), but there are very few reports about the overexpression of these proteins in different agricultural crop plants. Present biotechnological advances would be helpful to produce transgenic plants containing a protein capable of inducing a sweet taste phenotype. Herein, we tried to discuss briefly about various sweet and taste modifying proteins with their characteristics and their application and production in plants.

## Various sweet and taste modifying proteins

### Thaumatococcus

Thaumatococcus is a sweet protein and was first isolated from fruit of West African plant, *Thaumatococcus daniellii* in 1972 (Van der Wel and Loeve 1972; Witty and Higginbotham 1994). It is not only sweet tasting protein but also works as a flavoring. Thaumatococcus is a mixture of some forms, thaumatococcus I, thaumatococcus II, thaumatococcus a, thaumatococcus b and thaumatococcus c with a total molecular mass of 22 kDA. Here the thaumatococcus I and thaumatococcus II are major components and the remaining are minor components. The major forms are consisting of 207 amino acids (AAs) (Fig. 1). Only these two major thaumatococci have been studied

in detail. The sweetness of thaumatococcus does not degrade even above 100°C at pH 5.5. On the molar basis, thaumatococcus is 100,000 times sweeter than sucrose and 3,000 times on weight basis (Van der Wel and Arvidson 1978; Bernard et al. 1996; Masuda and Kitabatake 2006). Previously, thaumatococcus was used by native of West African as sweeteners and flavor enhancers in food products (Etheridge 1994). It does not create tooth decay and even safe for diabetic patients. From 1975 to 1985, a minimum of eighteen studies were done and results indicated no safety concerns (Higginbottom 1985). Although it has many advantages, the production of thaumatococcus is difficult and limited. Alternative production through genetic engineering has been done for commercial use to demonstrate the identical properties of the sweetness of thaumatococcus protein (Szwacka et al. 2002; Daniell et al. 2000; Masuda et al. 2004).

### Monellin

The sweet protein monellin was isolated from fruit of *Dioscoreophyllum cumminsii* Diels. plant, native to West Africa (Moris and Cagan 1972). Natural monellin protein is consisting of 94 AAs. It has two polypeptide chains, A and B, where A chain consisting of 44 amino acid (AA) and B chain consisting of 50 AA (Fig. 1). Both chains are held together by

#### Amino acid sequence of Thaumatococcus I (207 AA)

ATFEIVNRCSYTVWAAASKGDAALDAGGRQLNSGESWTINVEPGTNGGKIWART  
DCYFDDSGSGICKTGDCGGLLRCKRFRPPTTLAEFSLNQYKDYDISNIKGFNVF  
MNFSPITTRGCRGVRCAADIVGQCPAKLKPAGGGCNDACVTFQSEYCCCTTGKCGP  
TEYSRFFKRLCPDAFSYVLDKPTTIVTCPGSSNYRVTFPCPTA

#### Amino acid sequence of Monellin

##### A-Chain (44 AA)

REIKGYEYQLYVYASDKLFRADISEDYKTRGRKLLRFNGPVPPP

##### B-Chain (50 AA)

GEWEIDIGPFTQNLGKFAVDEENKIGQYGRITFNKVIKRCMKKTIYEEN

#### Amino acid sequence of Brazzein (54 AA)

QDKCKKVYENYPVSKCQLANQCNYDCKLDKHARSGECFYDEKRNLCICDYCEY

#### Amino acid sequence of Miraculin (191 AA)

DSAPNPVLDIDGELKRTGTNYIYVPLRDHGGGLTVSATTPNGTFVCPPRVVQTRK  
EVDHDRPLAFFPENPKEDVVRVSTDLNINFSAFMPNPGPETISSWCRWTSSTVWRL  
DKYDESTGQYFVTIGGVKFKIEEFCGSGFYKLVFCPTVCGSCKVKCGDVGIVIDQK  
GRGRLLALSDKPFAFEFNKTVYF

#### Amino acid sequence of Curculin (114)

DNVLLSGQTLHADHSLQAGAYTLTIQNKCNLVKYQNGRQIWAANTDRRGSGCRLT  
LLSDGNLVIYDHNNDVWGSACWGDNGKYALVLQKDGRFVIYGPVLSLGPNG  
CRRVNG

**Fig. 1** Amino acid sequence of sweet and taste modifying proteins. Sequence was collected from the Swiss-Prot biological database of protein

non-covalent interaction (Tancredi et al. 1992; Kohmura et al. 1990). Although monellin loses its sweetness at high temperature, linkage of these two chains can overcome this problem without changing the sweet taste (Kim et al. 1989). Monellin protein is approximately 2800 times sweeter than sucrose on the weight basis and also 100,000 times sweeter than sucrose on the molar basis (Moris and Cagan 1972; Moris and Cagan 1973). Single chain monellin are of two types, SCM (Single chain monellin) and MNEI (Structural mutant of single chain monellin) namely. SCM is made up of 94 AAs, which is acquired by joining the C-terminus of B chain to N-terminus of A chain. On the other hand, MNEI is made up of 96 AAs which is acquired by the linkage of B and A chains via glycine residues (Masuda and Kitabatake 2006). Monellin could be used for its sweet taste for those people who are suffering from high blood sugar levels. For available production of monellin sweet protein there are many attempt already done (Reddy et al. 2015; Liu et al. 2016; Kondo et al. 1997).

### Brazzein

To date, brazzein is the smallest sweet protein which was isolated from the *Pentadiplandra brazzeana* Baillon plant fruit. It is 9500 times sweeter than sucrose on the molar basis and 500 to 2000 times sweeter on weight basis (Ming and Hellekant 1994; Assadi-Porter et al. 2000). Two forms of brazzein were detected in the fruit of *Pentadiplandra brazzeana* Baillon plant. The major form (~80%) consists of 54 AAs (Fig. 1) which has pyroglutamic acid at the N-terminus. On the other hand, the minor form (~20%) is identical to the major form except for the N-terminal residue, which is not pyroglutamic acid. The molecular mass of brazzein is 6.4 kDa. Brazzein has heat stability and its sweetness does not degrade even heating at 80°C for 4 h. It is thought that it is

because of lack of free sulfhydryl groups as well as for four intramolecular disulfide bonds (Caldwell et al. 1998a; 1998b). For commercial use, the extraction of brazzein from its natural source is not easy as it is costly. Consequently, brazzein has been cloned and sequenced and attempt has been taken to express it in different host cells (Faus et al. 2000). To date, sweet protein brazzein has been produced in different organisms (Assadi-Porter et al. 2000; Lee et al. 2010; Assadi-Porter et al. 2010; Berlec et al. 2006; Berlec and Strukelj 2009; Lamphear 2005; Jo et al. 2013).

### Miraculin

The taste modifying protein miraculin was isolated from red berries of *Richadella dulcifica* which is native to West Africa. The indigenous peoples use these berries to improve the taste of acidic maize food or for sweetening sour quencher. The active component of the berry is miraculin protein which is tasteless and has the property to make sweet taste of sour tasting product but salty, bitter and sweet tastes are not modified by it. Compared to sucrose, miraculin can induce 3000 times sweetness on the weight basis. Miraculin is very stable protein which does not change its sweetness property at 5°C (pH 4) for more than 6 months. The miraculin is consisting of 191 AAs (Fig. 1) including seven cysteines and 3 intrachain and one interchain disulfide bonds were determined (Kurihara and Beidler 1969; Theerasilp and Kurihara 1988; Gibbs et al. 1996; Kurihara and Nirasawa 1997).

### Curculin

According to Yamashita et al. (1990), the protein curculin has unique properties, both sweet taste and taste modifying. This protein was extracted from fruit of *Curculigo latifolia* which

**Table 1** The properties of various sweet and taste modifying proteins

Features	Thaumatococin	Monellin	Brazzein	Curculin	Miraculin
Plant	<i>Thaumatococin daniell</i>	<i>Dioscoreophyllum cumminsii</i> Diels.	<i>Pentadiplandra brazzeana</i> Baillon.	<i>Curculigo latifolia</i>	<i>Richadella dulcifica</i>
Source	Fruit	Fruit	Fruit	Fruit	Fruit
Year first isolated	1972	1972	1994	1990	1989
Country of origin	West Africa	West Africa	Tropical Africa	Western Malaysia	West Africa
Protein type (taste)	Sweet	Sweet	Sweet	Taste modifier	Taste modifier
Sweetness relative to sucrose (molar basis)	100,000x	100,000x	9500x	20,000x	400,000x
Sweetness relative to sucrose (weight basis)	3000x	2800x	500–2000	550x	Tasteless
Molecular weight	22 kDa	11 kDa	6.4 kDa	12 kDa	28 kDa
Number of amino acids	207	94	54	114	191

is native of Western Malaysia. No other sweet protein can exhibit both sweet and taste modifying properties. Curculin is insoluble in deionized water but soluble in salt solution. Amino acid sequence analysis demonstrated that it has 114 AAs (Fig. 1) and having 4 disulfide bridges and 4 cysteine residues were also found. The molecular weight 12 kDa was determined. On the weight basis, curculin is 20,000 times sweeter than sucrose and 550 times sweeter on weight basis (Gibbs et al. 1996; Suzukia et al. 2004). The properties of above mentioned sweet or taste modifying proteins have been summarized in Table 1.

### Application of sweet and taste modifying genes for plant development

In ancient times the indigenous people used the plant parts which contain different sweet and taste modifying proteins to sweeten or modify their food's taste. Sweet and taste modifying proteins, such as thaumatin, monellin, brazzein, miraculin and curculin earned great appeal instead of sucrose to change food taste or to enhance flavor. Moreover, they can be used safely for people who are suffering from diabetes or have problem of sugar intake. But to meet the public demand of these sweet proteins from its original natural source is difficult and expensive. For easy availability, these proteins have been cloned into bacteria, fungi or in plants to express recombinant sweet protein. Generally tomato itself does not produce thaumatin protein. For the first time in 2003, thaumatin gene was cloned into tomato to develop transgenic tomato to check whether it can change the tomato fruit taste or not. After *Agrobacterium*-mediated transformation, two transgenic tomato lines were developed to express biologically active thaumatin in tomato fruits. After sensory evaluation assay, T2 generation was found sweeter than the control fruits and this result indicated that thaumatin can modify the taste of tomato fruit (Bartoszewski et al. 2003). The transgenic plants of *Solanum tuberosum* produced biological active thaumatin and induced sweet taste. It happened after the transformation of preprothaumatin II gene into *S. tuberosum* (Witty 1990). Not only in tomato or potato, thaumatin gene was also transformed into cucumber (*Cucumis sativus* L.). Thaumatin II protein was accumulated in transgenic cucumber fruits and finally sweet taste was determined (Szwacka et al. 2002). In 2002, another study demonstrated the recombinant sweet protein thaumatin production in pear. After the transformation of thaumatin II gene, the regeneration leaves of pear plants gone through organoleptical test and result indicated induced sweet taste of thaumatin (Lebedev et al. 2002). There are many report proved that gene

transferred transgenic crop plants showed constitutive overexpression of proteins that involved in plant defense system (Van Loon 1997; Zhu et al. 1996; Datta et al. 1999). Depending on the plant development strategy, in 2005 thaumatin II gene was transferred via *Agrobacterium* and transgenic strawberry plant was developed to check the resistance to *Botrytis cinerea* infection. The transgenic strawberry lines were selected for bioassay and results demonstrated that there was an increased resistance against *Botrytis* infection (Schestibratov et al. 2005). The expression of thaumatin sweet protein was not only checked in plants systems but also in other organisms, such as *Escherichia coli* (Faus et al. 1996; Edens et al. 1982; Makrides 1996; Hannig et al. 1998; Baneyx 1999; Daniell et al. 2000), *Bacillus subtilis* (Illingworth et al. 1988), *Streptomyces lividans* (Illingworth et al. 1989), yeast (Edens et al. 1984), yeast *Pichia pastoris* (Masuda et al. 2004), yeast *Saccharomyces cerevisiae* (Weickmann et al. 1994), fungus *Aspergillus oryzae* (Hahm and Batt 1990) *Aspergillus niger* var. *awamori* (Faus et al. 1998), *Aspergillus awamori* (Moralejo et al. 1999), fungus *Penicillium roquefortii* (Faus et al. 1997).

The expression of another sweet protein monellin in different plant systems has been reported. In 1992, the monellin gene was transferred into tomato and lettuce plants to produce transgenic lines. The transgenic line demonstrated that monellin sweet protein was expressed in the fruit and leaf of tomato and lettuce and expressed the recombinant monellin protein also enhanced the flavor of fruit or leaf (Peñarrubia et al. 1992). Reddy et al. (2015) transformed synthesized monellin gene into tomato. The transgenic tomato demonstrated high level of monellin sweet protein expressing higher level of sweetness (Reddy et al. 2015). The recombinant monellin sweet protein expression was reported in other organisms like bacteria such as *E. coli* (Chen et al. 2005; Liu et al. 2016; Reddy et al. 2015), yeast (Kim and Lim 1996), *Candida utilis* (Kondo et al. 1997), *S. cerevisiae* (Liu et al. 2015). The smallest sweet protein brazzein has been transformed into foreign hosts like plant, bacteria or yeast to overexpress it. Lamphear et al. (2005) transferred brazzein sweet gene into maize. The high level of brazzein was expressed in transgenic maize seed and it demonstrated that the maize germ flour sweetness was the same with original brazzein sweetness. This result suggested that maize could be a cost-effective expression system for commercial production of brazzein sweet protein. Brazzein expression also checked in different expression systems, such as *S. cerevisiae* (Guan et al. 1995), *Kluyveromyces lactis* (Jo et al. 2013), *E. coli* (Fariba et al. 2008; Fariba et al. 2000), mice (Yan et al. 2013).

The taste modifying protein miraculin expression has also been checked in plant expression system. For example, in

**Table 2** Overexpression of sweet and taste modifying proteins in various plants. CaMV35S, Cauliflower mosaic virus 35S promoter

Gene	Plants	Promoter	Reference
Thaumatococin	Tomato	CaMV35S	Bartoszewski et al. 2003
	Solanum tuberosum	CaMV35S	Witty 1990
	Cucumis sativus	CaMV35S	Szwacka et al. 2002
	Pear	CaMV35S	Lebedev et al. 2002
	Strawberry	CaMV35S	Schestibratov et al. 2005
	Tobacco	CaMV35S	Pham et al. 2012
Monellin	Tomato	E8	Peñarrubia et al. 1992
	Lettuce	CaMV35S	Peñarrubia et al. 1992
	Tomato	E8	Reddy et al. 2015
Brazzein	Maize	Embryo-preferred	Lamphear et al. 2005
Miraculin	Lettuce	CaMV35S	Sun et al. 2006
	Tomato	CaMV35S	Sun et al. 2007
	Tomato	E8	Hirai et al. 2011
	Strawberry	CaMV35S	Sugaya et al. 2008

2007 miraculin gene was transformed into tomato. It was demonstrated that miraculin protein was expressed in both fruit and leaves of transgenic tomato lines and its biological activity was similar with the natural miraculin. It was also observed that the sweetness of transgenic line fruit or leaves was stable (Sun et al. 2007). In another study, Hira et al. (2011) transformed miraculin gene into tomato and the results demonstrated that miraculin protein overexpressed in the transgenic tomato. Sun et al. (2006) expressed taste modifying protein as a potential alternative sweetener and they found that the transgenic lettuce showed strong sweetness-inducing activity. In 2008, transgenic strawberry plants were developed to overexpress miraculin protein and the transgenic strawberry lines showed potential expression of miraculin in strawberry fruits (Sugaya et al. 2008). Miraculin expression was also demonstrated in other foreign hosts like, *E. coli* (Matsuyama et al. 2009). Very little study was done on curculin protein. The sweet and taste modifying protein curculin expressed in bacterial systems (Suzuki et al. 2004). In this system, it was observed that the sweet and taste modifying activities were exhibited by the recombinant curculin protein. Overexpression of sweet and taste modifying proteins in various plants is shown in Table 2.

### Future prospects

Successful transformation of sweet and taste modifying proteins indicate that these proteins could be used to develop various crop varieties which can fulfill the demand of natural sweet and taste modifying compounds. Bulk sweeteners and flavorsome

ingredients such as sugars are essential in food and food processing and contribute many benefits. However, intake of high calorie food causes obesity and related problems mainly due to consumption of refined sugars, which ultimately leads to higher probability of heart diseases, type II diabetes, sleep apnoea, certain types of cancer and osteoarthritis. Moreover, artificial substances such as chemical intense sweeteners and flavor enhancers have attracted public dissatisfaction. For example cyclamate was suspected of causing health problems (Price et al. 1970) and was the subject of a ban in the UK and USA (Anonymous 1969). Synthetic sweeteners may be replaced by natural sweet and taste modifying proteins that confer similar properties to food and are perceived by the public as a more natural alternative. Since they are natural, they could be safer than aspartame and other sweeteners. The ease of using genetic engineering techniques with currently used microorganisms and plants in the food, medicine and beverage industries can lead to mass production of the proteins and the generation of large numbers of variants with more desirable taste and physical properties by deleting, substituting or inserting various amino acids. Biotechnological advantages make it easy to transfer and modify these sweet and taste modifying genes into any type of plants, to analyze their expression and to purify the target sweet and taste modifying compounds. It is not too much far that these natural compounds will produce at a large scale in food, beverage and pharmaceutical industries which could be used to improve the flavor of various foods, crops, fruits, beverages such as palm wine or tea and medicines. They also could be used in the food processing industry as sweetening agents, flavor enhancers, and animal fodder supplements. The expression of different sweet and taste modifying

proteins in plants would improve the flavor, taste, and nutritional value of various crops and fruits like tomato, potato, apple, orange, banana, rice, wheat, maize, barley, and other vegetable crops.

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