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REVIEW

Review: genetically modified plants for the promotion of human health

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Abstract Plants are attractive biological resources because of their ability to produce a huge variety of chemical compounds, and the familiarity of production in even the most rural settings. Genetic engineering gives plants additional characteristics and value for cultivation and post-harvest. Genetically modified (GM) plants of the ''first generation'' were conferred with traits beneficial to producers, whereas GM plants in subsequent ''generations'' are intended to provide beneficial traits for consumers. Golden Rice is a promising example of a GM plant in the second generation, and has overcome a number of obstacles for practical use. Furthermore, consumer-acceptable plants with health-promoting properties that are genetically modified using native genes are being developed. The emerging technology of metabolomics will also support the commercial realization of GM plants by providing comprehensive analyzes of plant biochemical components.

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Introduction

Plants synthesize and accumulate in excess of 200,000 natural products (Fiehn 2002) that have been utilized as foods, pharmaceuticals, dyes, flavors and other materials for centuries. By comparison, bacteria and mammals together produce around 3500 natural products (Hall et al. 2002). Alkaloids, terpenoids, steroids, polyketides, phenylpropanoids and flavonoids are among the economically important compounds derived from plants. Although plants in their native form are familiar and attractive resources, transgenic techniques allow the movement of genes of interest between different plant species or even between kingdoms to target plants.

Agronomic traits such as herbicide tolerance, insecticides, virus resistance, disease-tolerance, and delayed ripening, were the initial focus of genetic engineering in plants, which are primarily beneficial to growers and the seed companies that pioneered the work (Fig. 1). These are the so-called ''first generation genetically modified (GM) plants'', and include the Flavr Savr^{TM} tomato that had been modified with a native gene inserted in reverse, giving it a delayed-ripening

trait, first commercialized in 1994 (http://www.who.int/foodsafety/publications/biotech/biotech_en.pdf). Large-scale commercial cultivation of GM plants began in 1996 with a total global area of 1.7 million hectares in six countries. Cropping acreage for GM plants rapidly expanded to 81 million hectares in 17 countries in 2004 and to 90 million hectares in 21 countries by 2005. When lab-based research is included, 63 countries have a stake in GM plants (Runge and Ryan 2004). In 2005, almost all GM acreage was occupied by first generation plants, dominated by herbicide-tolerance (71%), followed by insect-resistance (18%) and a combination of these traits (11%) (http://www.isaaa.org/).

The last decade has largely been dedicated to the development of ''second generation'' GM plants with traits beneficial to consumers such as high nutritional value, premium quality and low allergenicity (Table 1). The second generation includes soybeans with high oleic acid- or laurate-content and rice with high vitamin A or iron content, or low allergenicity. Development of a ''third generation'' as protein factories of vaccines and therapeutic medicines is in progress (Table 1). First generation GM plants mainly benefited producers by improving crop productivity, reducing agrichemical inputs and subsequently reducing labor costs. However, GM plants provided no obvious benefits for consumers over traditional plants. In contrast, the next generation of GM plants has been developed based on consumer benefits. Researchers and developers have been carefully choosing the traits to induce, including their origin, expression systems and selectable markers. In this review, we focus on second and third generation GM crops which can produce substances for promoting human health, including medicines and vaccines that have great potential for commercial application.

GM plants as a source of foods and as an expression system

Growing and harvesting plants for food, fiber or other materials is perhaps the oldest technology.

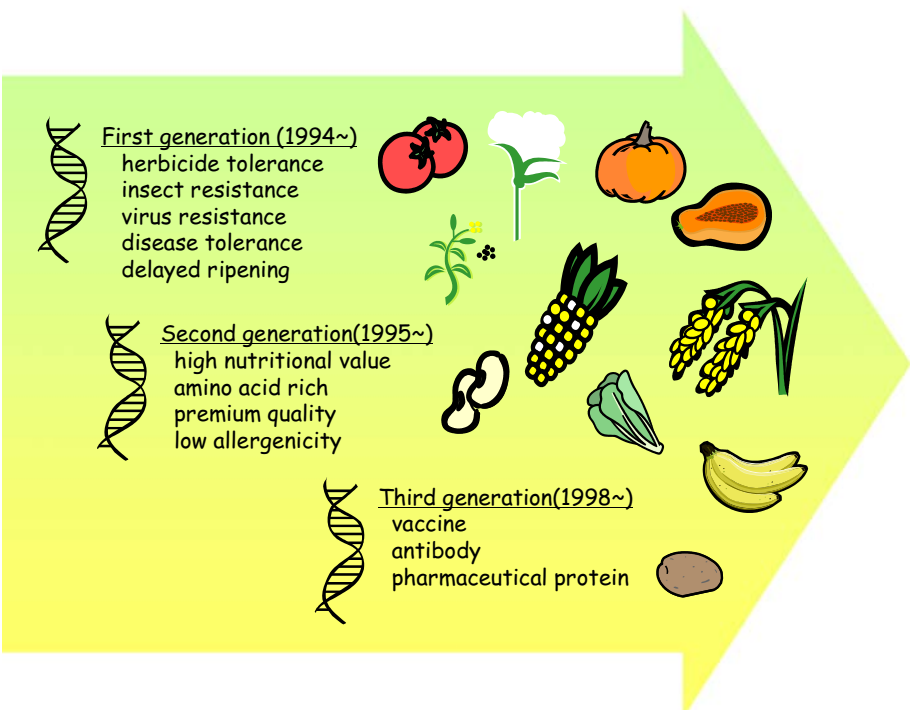

Fig. 1 Generations of genetically modified plants. The traits of each generation are shown

Table 1 GM plants reaching field or clinical trials

Generation	Purpose	Introduced trait (plant species)	Institution or company	Safety assessment completed year		
				US[a]	Canada[b]	EU[c]
second	high nutrient	high vitamin A content (rice)	SFIT*/UF*/Syngenta			
		ferritin-rich (lettuce)	CRIEPI*/NIAES*/STAFF*			
	amino acid rich	lysine rich (maize)	Monsanto	2005		2006
		tryptophan rich (rice)	NICS*			
	premium quality	high laurate content (canola)	Monsanto/Calgene	1995		
		high oleic acid content (canola)	Poineer Hi-Bred		1996	
		high oleic acid content (soybean)	Dupont	1997	2000	
		low linolenic acid (soybean)	Monsanto/AAC*		2000	
		pectin rich (tomato)	Zeneca/ Kagome, Japan			
	low protein	low gluterin (rice)	Orynova (Japan Tobacco), Japan			
	low allergen	low albumin (rice)	Mitsui Toatsu, Japan			
third	vaccine	against *E.coli* labile enterotoxin (potato)	Arizona State University, USA			
		against hepatitis B (potato)	Arizona State University, USA			
		against Norwalk virus (potato, tobacco)	Arizona State University, USA			
		against rabies (spinach)	DBI*			
	antibodies	non-Hodgkin's lymphoma antibodies (tobacco)	Large Scale Biology Corp. (http://www.lsbc.com)			
		dental caries antibodies (tobacco)	Planet Biotechnology (http://www.planetbiotechnology.com/)			
	others	lactoferrin production (maize)	Meristem therapeutics (http://www.meristem-therapeutics.com/sommaire_fr.php3)			
		lactoferrin production (rice)	Japan Agricultural Cooperatives, Japan			
		human intrinsic factor production (arabidopsis)	Cobento (http://www.cobento.com)			
		glucocerebrosidase production (unknown)	Protalix (http://www.protalix.com)			
		gastric lipase production (maize)	Meristem therapeutics (http://www.meristem-therapeutics.com/sommaire_en.php3)			

SFIT, Swiss Federal Institute of Technology, Switzerland; UF, University of Freiburg, Germany; CRIEPI, Central Research Institute of Electric Power Industry, Japan; NIAES, National Institute of Agro-Environmental Sciences, Japan; STAFF, Society for Techno-innovation of Agriculture, Forestry and Fisheries, Japan; NICS, National Institute of Crop Science, Japan; AAC, Agriculture and Agrifood Canada, Canada; DBI, Delaware Biotechnology Institute, University of Delaware, USA

[a] http://www.cfsan.fda.gov/~lrd/biocon.html

[b] http://www.efsa.eu.int/science/gmo/gm_ff_applications/catindex_en.html

[c] http://www.hc-sc.gc.ca/fn-an/gmf-agm/appro/index_e.html

Later generation GM plants can be either consumed directly as foods, can be used to produce desirable extractable oils, or can serve as an efficient protein expression system. However, to move from research to practical application, there are several hurdles to clear. In case of direct consumption, it is critical to know the impact and potential market for a given target trait and to have a comprehensive understanding of the biosynthetic pathway and genetic regulation of the introduced product. It is also important to know about the expression system in the desired organelle, organ, or tissue at each developmental stage and to achieve a high accumulation level. In the case of nutriceuticals, a relatively constant and predictable product accumulation level must be established to assure that the introduced trait does not adversely affect the host plant under agronomic conditions. Plants in an applied role must also meet all of the applicable regulatory conditions, and must meet acceptable local agronomic and social conditions for cultivation. As a protein expression system, there are additional infrastructure requirements, such as an extraction and purification system as well as storage and transport. Golden Rice, which is one of most promising second generation GM foods, provides an excellent case study for each of these issues. Further, we explore two other novel consumer-oriented GM crops that show promise in meeting social and economic needs.

GM crops with high vitamin A content: Golden Rice

Golden Rice was named for its bright yellow β-carotene-producing endosperm. Vitamin A (retinol) is a fat-soluble micronutrient and is mainly contained in eggs, liver and butter. Vitamin A precursors such as β-carotene, and other carotenoids, are produced in green and yellow vegetables. After uptake, β-carotene and the other carotenoids are oxidatively cleaved in the intestinal mucosal brush border or liver to form the isoprenoid retinol. Carotenoids from a plant origin have an advantage over retinol from animal sources, because excessive retinol may cause a toxic excess in vitamin A, whereas carotenoids can be converted as needed to meet metabolic requirements.

Vitamin A deficiency (VAD) causes night-blindness, xerophthalmia, keratomalacia, bone growth deficiencies, and weakens the immune system. The clinical effect of VAD is inversely related to the age of patient, and the mortality of children with severe VAD can reach 50%. The World Health Organization (WHO) reported that VAD is a global public health problem especially for children and affects 140–250 million preschool children in more than 118 countries (http://www.who.int/vaccines-diseases/en/vitamina/advocacy/adv01.shtml). In developing countries vitamin A, preferably in the form of carotenoid-enriched foods, is needed to solve the VAD problem.

The carotenoid biosynthetic pathway has been well-studied in bacteria and plants. In plants, carotenoids are synthesized from geranylgeranyl-diphosphate (GGPP) in plastids. Phytoene synthases (PSY) catalyze the formation of phytoene (15-*cis*-phytoene) from two molecules of GGPP. Phytoene is converted to ζ-carotene (9,15,9′-*dicis*-ζ-carotene) by phytoene desaturase and further to prolycopene (7,9,9′,7′-*tetracis*-lycopene) by ζ-carotene desaturase. Carotene *cis*-*trans*-isomerase catalyzes the *trans*-isomerization of prolycopene to *all-trans*-lycopene and lycopene cyclase (LCY) converts *trans*-lycopene to α-carotene or β-carotene. α- and β-carotene are converted to zeaxanthin and lutein, respectively by hydroxylases (Al-Babili and Beyer 2005, Breitenbach and Sandmann 2005).

Golden Rice was first engineered with the insertion of the PSY gene from daffodil (*Narcissus pseudonarcissus*) and the bacterial phytoene desaturase (CrtI) gene from *Erwinia uredovora* (Ye et al. 2000). Bacterial CrtI can catalyze three enzymatic steps from phytoene to *all-trans*-lycopene. The PSY gene is under the control of an endosperm-specific glutelin promoter. To localize the product in plastids, CrtI was designed as a fusion with the transit peptide of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) small subunit and is under the control of the cauliflower mosaic virus 35S promoter. An alternative construct was made by co-transformation with constructs carrying the PSY/CrtI gene as

described above and the LCY gene under the control of a glutelin promoter.

By the latter approach, the carotenoid content of edible rice endosperm was 1.6 μg/g dry weight (Ye et al. 2000). According to United Nations Administrative Committee on Coordination/subcommittee on nutrition (UN ACC/SCN, The United Nations System Chief Executive's Board for Coordination at present), average availability in the world is 777 μg retinol equivalents (RE) per person per day (Beaton et al. 1993) and minimum average requirements are around 250 μg RE per person per day (http://www.unsystem.org/scn/archives/rwns02vol1/index.htm). RE is a concept introduced by the UN Food and Agriculture Organization (FAO)/WHO Expert Group in 1967 and is defined as 6 μg β-carotene or 11.9 μg of the other provitamin A carotenoids in food sources (World Health Organization 1967). In 2004, WHO/FAO recommended conversion factors to retinol from usual mixed vegetable diets of 1:14 for β-carotene and 1:28 for other provitamin A carotenoids. (http://whqlibdoc.who.int/publications/2004/9241546123_chap2.pdf). At 1.6 μg/g, the carotenoid content of Golden Rice would not satisfy minimum nutritional requirements even if consumed as a staple food. However, in 2005, Golden Rice2 was developed in collaboration with the biotechnology company, Syngenta, and the carotenoid content was increased up to 23-fold (37 μg/g of dry weight) compared to the original Golden Rice (Paine et al. 2005). This content is close to a realistic level for palliating VAD in children (Paine et al. 2005). The higher β-carotene content was achieved by choosing the maize PSY gene rather than the PSY genes from *Arabidopsis*, daffodil or the carotenoid-accumulating vegetables such as tomato, bell pepper and carrot. To make Golden Rice more responsive to environmental concerns, an antibiotic resistance marker was used for initial selection and was later replaced with a phosphomannose-isomerase sugar-based selection system, and co-transformation techniques have been adopted for production of marker-free transgenic plants.

To adapt Golden Rice2 to local cultivation conditions in Southeast Asia, which is one of the regions where widespread clinical VAD has been reported, β-carotene synthesis genes were introgressed into two *Indica* varieties, IR64 and MTL250 from *Japonica* cultivar Taipei309 (Hoa et al. 2003). IR64 is a commonly planted cultivar throughout the rice-growing regions and MTL250 is widely cultivated in Vietnam. The Golden Rice project is in progress with the participation of the Philippines, Vietnam, India, Bangladesh, China and Indonesia (http://www.goldenrice.org/index.html).

Thus, Golden Rice has the potential to positively impact human health because of its high carotenoid accumulation level for practical application, adaptation for local cultivation in cultivars with which rural farmers are familiar, and because much is known about the expression of carotenoid biosynthetic genes in the desired organelle and organ. Further investigation will answer the remaining scientific questions about Golden Rice (Al-Babili and Beyer 2005).

GM plants with one gene to get two traits: carotenoids and flavonoids

Golden Rice is transformed with genes from maize and bacteria to acquire one trait, high β-carotene content. Increased levels of the antioxidant chlorogenic acid were achieved by one gene modification (Niggeweg et al. 2004). Recently, Davuluri et al. (2005) reported GM tomatoes with high levels of carotenoids and flavonoids produced by alteration of a single transcription factor. PAP1 and MYB12, members of the R2R3-MYB family, are transcriptional regulators of the phenylpropanoid biosynthetic pathway (Borevitz et al. 2000) and of the flavonol-specific pathway (Mehrtens et al. 2005), respectively. However, the transcription factor, DE-ETIOLATED1 (DET1) used in the high antioxidant GM tomato, can influence the two distinct secondary metabolic biosynthetic pathways that produce carotenoids and flavonoids (Davuluri et al. 2005). Flavonoids are a group of well-studied plant secondary metabolites that, among other functions, serve as flower and fruit pigments and as precursors of plant defense compounds. Flavonoids also play an important

role for human health by their antioxidant activity (Kong et al. 2003, Nakajima et al. 2004, Tohge et al. 2005a) and may be involved in protection against cancers, cardiovascular disease and age-related diseases (Schijlen et al. 2004).

DET1 was first isolated from *Arabidopsis* as a negative regulator of light-mediated development (Pepper et al. 1994). DET1 from *Arabidopsis* is an ortholog of HIGH PIGMENT-2 (HP-2) from tomato (Mustilli et al. 1999). The mutant phenotype of *high pigment-2* (*hp-2*) is characterized by high levels of anthocyanin and chlorophyll accumulation in fruits and roots, but not in leaves, compared to wild type (Mustilli et al. 1999), and dwarfing and bushiness (when tomato DET1 was constitutively overexpressed) (Davuluri et al. 2004).

Davuluri et al. (2004) used three fruit-specific promoters, P119, 2A11 and TFM7, for tomato DET1 expression to avoid severe developmental disturbances. Fruit-specific suppression of tomato DET1 by RNA interference (RNAi) resulted in high accumulation levels of both carotenoid and flavonoid compounds in tomatoes (Davuluri et al. 2005). These promoters allow expression during the early fruit developmental stages, but each of the stages is distinct. P119 expresses from the earliest green fruit stage to red ripe stage, 2A11 expression is highest during ripening, but is also transient in 2–3 cm green fruits, and TFM7 expression extends from immature green fruit development to full size green fruit. Transgenic tomatoes with three kinds of promoters have a high content of β-carotene (up to 2-fold), lycopene (up to 10-fold) and flavonoids (chlorogenic acid, flavonol derivatives and naringenin-chalcone). Among three promoters, P119 was dominant in β-carotene and chlorogenic acid contents. TFM7 plants had relatively high flavonol accumulation levels. These phenomena may reflect the slight differences that the expression stage has on the level of each compound, suggesting the great importance of promoter choice. Long et al. (2006) reported that mutants defective in carotenoid biosynthesis structural genes do not change flavonoid/phenylpropanoid content and vice versa. The only exceptions are the high pigment mutant (*hp-1*) and its progeny LA3771. *hp-1* has a mutation in the UV-DAMAGED DNA-BINDING PROTEIN 1 gene, which is also a negative regulator of light signaling from tomato (Liu et al. 2004). The reason that these genes alter both carotenoid and flavonoid contents is unclear and requires further investigation. However, these transformants are good hosts for breeding. Since the RNAi technique makes use of a plant's native genes, GM crop plants using this technology may fall under less stringent regulatory requirements and may be more easily accepted by consumers.

Vaccine-producing plants: transgenic rice for pollen allergy

Plants are a reservoir of valuable pharmacological compounds and people have utilized plants as therapeutic products for thousands of years. In this decade, plants may fill a more direct role as producers of vaccines, antibodies or protein-based signal molecule therapeutics. Takagi et al. (2005) reported GM rice expressing two T-cell epitope peptides of Cry j I and Cry j II allergens of Japanese cedar (*Cryptomeria japonica*) pollen (Takagi et al. 2005).

Allergen proteins are degraded to small peptides after uptake and the epitopes are exposed on the cell surface of antigen presenting cells for recognition by specific T-cell receptors. After recognition, T cells transfer signals through T-helper cells (Th1 and/or Th2) to B cells that produce IgE antibody. Th1 and Th2 produce interferon (IFN-γ) and interleukins (IL-4, IL-5 and IL-13), respectively, as cytokines, regulatory proteins for the immune response cascade. IL-4 stimulates the production of IgE antibodies in B cells and IFN-γ represses it. The process of interaction of IgE antibody with mast cells is called sensitization. Once sensitization is completed, the binding of allergen to IgE antibody on mast cells causes a severe hypersensitivity called Type I allergy (Broide 2001).

GM rice designed to counter cedar-pollen allergy expresses two major T-cell epitopes derived from Cry j I and Cry j II as a fusion protein with the seed protein glycinin, A1aB1b, (A1aB1b-Crp1 and -2) under the control of the rice seed major protein glutelin promoter. Accumulation of the A1aB1b-Crp1 and -2 protein was

7 μg per grain. Oral feeding of GM A1aB1b-Crp1 and -2 rice to mice prevents the production of allergen-specific IgE and IgG antibodies and inhibits the production of allergen-induced Th2 cytokine, IL-4, IL-5 and IL-13. Histamine release levels in sera of mice fed with GM A1aB1b-Crp1 and -2 rice was low when compared those in mice with non-transformed rice.

Oral tolerance is an accepted mechanism leading to immune tolerance (Faria and Weiner 2005). Other attempts to create an edible vaccine were examined using potato, and banana fruits with expression of the hepatitis B surface antigen (Richter et al. 2000, Kumar et al. 2005). Ma et al. (2005) reviewed pharmaceutical proteins for potential medical use derived from plants. Vaccines for diarrhea, hepatitis B and rabies, and antibodies for non-Hodgkin's lymphoma, colorectal cancer and dental caries, have been submitted for phase I or phase II clinical trials in humans (Ma et al. 2005, http://www.molecularfarming.com/PMPs-and-PMIPs.html) (Table 1).

The future of GM plants

Public acceptance of genetically altered crops is one of the biggest obstacles to commercial application. First generation GM plants may only slowly gain public favor because of concern about unintended environmental and dietary effects (Frewer et al. 2004). A feasible solution may be to include comprehensive metabolite analysis by metabolomics (Rischer and Oksman-Caldentey 2006). Metabolomics is defined as ''the technology geared towards providing an essentially unbiased, comprehensive qualitative and quantitative overview of the metabolites present in an organism'' (Hall 2006). Metabolomics is now rapidly developing by a combination of a variety of techniques such as gas chromatography mass spectrometry (GC–MS), liquid chromatography mass spectrometry (LC–MS), Fourier transform mass spectrometry (FT–MS), quadrupole time-of flight mass spectrometry (QTOF–MS) and nuclear magnetic resonance (NMR) (Bino et al. 2004, Kikuchi et al. 2004, Oksman-Caldentey and Saito 2005, Hall 2006). Metabolomics is being applied not only to functional identification of novel genes (Hirai et al. 2004, 2005, Tohge et al. 2005b) but also to the investigation of compositional similarities between GM and conventional crops (Catchpole et al. 2005, Baker et al. 2006). Consumers may yet be skeptical about GM plants, despite the accumulation of scientific data due to the inherent sociological or psychological factors associated with the risks of new technology. However, the redistribution of benefits to consumers by the next generation of GM plants and the reassurance of a sound evaluation by metabolomics could be encouraging for their practical implementation.

As common recombinant protein expression systems, bacteria, yeast, mammalian cell culture and transgenic animals are well known. In terms of commercial application, the cost of cultivation and operation, biomass, safety and scalability are important factors (Raskin et al. 2002). In addition to these, protein folding and post-translational modifications such as glycosylation are also significant problems when producing recombinant proteins (vaccines and antibodies). It turns out that plants are relatively good systems for the production of active and effective protein products (Raskin et al. 2002).

Among commercialized GM plant species, soybean accounts for 60% of the GM cultivation area, followed by maize, cotton and canola (http://www.isaaa.org/). In addition to these plant species, rice, squash, papaya, sugar beets, potato and tomato are available in the market as GM crops (http://www.who.int/foodsafety/publications/biotech/biotech_en.pdf). Apple, mango, banana, pineapple, barley, sweet potato, lettuce and coconut are also under investigation (http://www.isaaa.org/kc/). Transformation systems will be improved and most higher plants may be able to serve as hosts for genetic engineering.

World crop production increased steadily and significantly in the post-war years through the development of agricultural techniques such as conventional breeding, pesticides, chemical fertilizers, and increased cultivated acreage through arable reclamation and irrigation. However, explosive improvements in traditional agricultural techniques like the ''Green Revolution'' and cultivated acreage increases can no longer be expected. In 2005, world population has reached

6.5 billion (http://www.prb.org/pdf05/05WorldDataSheet_Eng.pdf) and is predicted to increase to 8.9 billion by 2050. For food production sufficient to feed a growing world population, breakthroughs of agricultural techniques are expected and needed. GM agricultural crops are one of the avenues to overcome the world's food crisis and environmental problems.

Along with food production, healthcare issues are of crucial significance to people in underdeveloped regions. In the case of Golden Rice, the ability to produce large amounts of β-carotene in familiar crop species and cultivars will provide tangible benefits to meet social and economic needs by alleviating the human and social costs associated with blindness and other vitamin A-associated disabilities. GM plants in subsequent generations can serve as nutriceuticals and edible vaccines without requiring industrial facilities on a broad scale. The research and development of new GM plants that originate from the needs of impoverished, undernourished or developing societies can be an integral part of a sustainable, rational means to address the needs of the world's burgeoning population. Implementation of these technologies must take place in close partnership with all stakeholders for the establishment of safety assessment systems and in collaboration between the public and private sectors.

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