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## **Cassava, a potential biofuel crop in China**

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## Abstract

**Cassava is ranking as fifth among crops in global starch production. It is used as staple food in many tropical counties of Africa, Asia and Latin America. In China, although not yet being a staple food, cassava is of major economic importance for starch for a large area of southern China, especially in the provinces of Guangdong, Guanxi, Yunnan and Hainan. Recently, cassava-derived bioethanol production has been increasing due to its economic benefits compared to other bioethanol-producing crops in the country. We discuss here the possible potentials of cassava for bioethanol production.**

## 1. Introduction

### 1.1. Cassava

Cassava (*Manihot esculenta* spp. *esculenta*) is a starch-containing root crop of worldwide importance as food, feed and nonfood products [Figure 1]. More than 70% of this production is in the subtropical and tropical regions between 30° N and 30° S of Africa, Latin America and Asia by small-scale farmers. The crop is widely grown as staple food and animal feed in these regions with a total cultivated area over 18 million hectares [1,2]. Cassava is a high starch producer with levels of up to 90% of its total storage root dry weight and is one of the most important sources of calories in the tropics. Starch is synthesized and deposited in the underground tuberous storage roots, which can measure up to 1 m in length and over 10 cm in diameter. The high starch production attributes

together with the unique properties of its starch make cassava suitable for particular food and nonfood applications [1,2]. It is consumed daily by more than 600 million people. It provides a cheap source of dietary carbohydrate energy ( $720 \times 10^{12} \text{ kJ day}^{-1}$ ) ranking fourth after rice, sugarcane and maize, and fifth among starch crops in global production [<http://faostat.fao.org>]. It gives reasonable yields in dry and poor soils and does not require high management level and cost compared to other major food crops.



**Figure 1.** Field-grown cassava plants (*M. esculenta* spp. *esculenta*).

Photo by Anna Westerbergh

Cassava originates from South America and has been domesticated for more than 5000 years from its wild ancestor *Manihot esculenta* ssp. *flabellifolia* [3]. Selection during domestication has resulted in many morphological, physiological and biochemical differences between cassava and its wild ancestor. Some traits, such as increased size of tuberous roots, higher starch content in tuberous roots [Figure 2], and vegetative propagation through stem cuttings are results of human selection. Interestingly, some progenies in the second (F<sub>2</sub>) generation from a cross between cassava and *Manihot esculenta* ssp. *flabellifolia* showed considerably higher tuberous root weight than the cassava parent [4]. These interspecific hybrids may constitute important genotypes for improved root yield from which selection can be made in future cassava-breeding programs. In addition, high genetic diversity and morphology have been found within cassava [5,6], reflecting the genetic potential for plant modification. Here, we discuss the potentials of cassava in the biofuel sector and point to some research areas that need to be addressed to fully exploit cassava starch production for both food and non-food applications.

## **1.2. Cassava as a biofuel crop**

Many characters of cassava such as high drought and heat tolerance, little requirement to agricultural fertilizers, and high starch content make it one of the most attractive plants for starch production in the future. With increasing population pressure and climate change it is predicted that the production of cassava will increase over the next few decades, and, as a result, cassava is now an international priority for crop improvement. Even though cassava is mainly grown by small-scale farmers, its use for agro-industrial

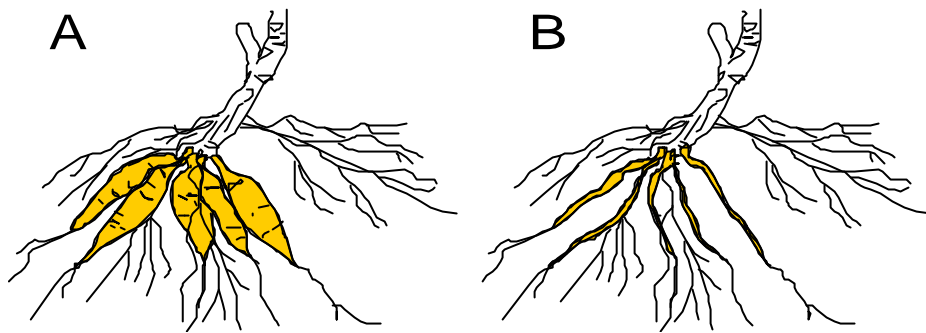
processing in Asia is increasing [7]. Apart from its traditional role as a food crop, cassava is likely to increase its value by becoming an important biofuel crop. The high yields of starch and total dry matter in spite of drought conditions and poor soil, together with low agro-chemical requirements, result in an energy input that represents only 5–6% of the final energy content of the total cassava biomass. This translates to an energy profit of 95%, assuming complete utilization of the energy content in the total biomass. The energetic and economic aspects of using cassava as a biofuel crop are well documented. For instance, a direct comparison of bioethanol production from different energy crops was reviewed by Wang, 2002 [8, Table 1]. A detailed study on the biofuel conversion performance and its related energy input in this crop compared to other energy crops has also been presented [9]. The conclusion was that cassava compared favorably to other crops such as maize [9], sugarcane and sweet sorghum [8, Table 1]. Indeed, the annual yield of bioethanol was found to be higher for cassava than for any other crops, including sugarcane.

**Table 1.** Comparison of bioethanol production from different energy crops. Source: [8].

Crops	Yield (tonne ha <sup>-1</sup> year <sup>-1</sup> )	Conversion rate to bioethanol (L tonne <sup>-1</sup> )	Bioethanol yield (L ha <sup>-1</sup> year <sup>-1</sup> )
Sugarcane	70	70	4,900
Cassava	40	150	6,000
Sweet sorghum	35	80	2,800
Maize	5	410	2,050
Wheat	4	390	1,560
Rice	5	450	2,250

Hence, the interest in production of cassava starch-derived bioethanol is progressively increasing in China and the rest of world. In this review, we mainly address on biological

issues of cassava as a biomass for biofuel production and some of its economic aspects in China.



**Figure 2.** Roots of cassava (A) and its wild ancestor *M. esculenta* ssp. *flabellifolia* (B).

## 2. Cassava in China

In China, around 440, 000 hectares of cassava is cultivated, with annual yields of ca. 9,110, 000 tonnes of fresh weight of storage roots [10-12]. The harvested storage roots are mainly used for production of starch, including modified starch. Production of bioethanol from cassava is just at a beginning [13,14]. As a feedstock, cassava holds a top potential for bioethanol production in Asia. For maize-derived bioethanol in China [15], production of 1 tonne of ethanol requires 3.2 tonnes of maize kernels. That corresponds to a cost of around 3,456 CNY, based on a price of 1,080 CNY per tonne of maize. The same calculation for sugarcane-derived bioethanol shows that 13.5 tonnes of sugarcane stems are required for 1 tonne of bioethanol production at a cost of around 2295 CNY, assuming a price of 170 CNY per tonne of sugarcane stems. As a comparison, cassava-derived bioethanol requires 6.6 tonnes of cassava storage roots for 1 tonne of ethanol. This corresponds to a cost of around 1716 CNY using a price of 260 CNY per tonne

harvested cassava roots. Apart from the economic benefits, the environmental and geographic concerns should also be taken into account. The main arable land in China is used for staple food production, such as rice, wheat, potato and maize. Thus high-quality land is in short supply and should not be allocated for production of biofuels. Presently, cultivation and production of cassava is mainly located in four provinces of south China, Guangdong, Guanxi, Yunnan, and Hainan. The capacity for cassava cultivation in these provinces is estimated to be over 1.5 million hectares [12]. This is a far cry from today's 0.4 million hectares that is devoted to cassava cultivation. Thus the cultivated area for cassava in China is small, only 1.4 % of that of the world, with a relatively low yield around 16 tonnes per hectare of storage roots (fresh weight), as compared to 31 tonnes per hectare in India, 25 tonnes in Cook Islands and 22 tonnes in Thailand [<http://faostat.fao.org>]. Because of this, China is still the number one country for import of cassava for processing to starch products [11]. However, the potential for cassava cultivation in China is substantial. In addition to increasing production in the Guangdong, Guanxi, Yunnan, and Hainan provinces, large potential acreage for cassava cultivation can be found in other regions. A cursory inspection suggests that such a region can be found up to 30° N in the country. This territory is south of the Yangzi River and includes at least 5 additional provinces, Guizhou, Hunan, Jiangxi, Zhejiang and Fujian. These five provinces are characterized by vast areas of mountainous land or low-quality land that are not arable for other subtropical crops such as rice but can be used for cassava cultivations. Moreover, cassava is a particularly sustainable crop for small-scale farmers. Thus, cassava will bring a tremendous benefit to the small-scale farmers, the majority of farmers in this area, without significantly negative effects on their present agriculture.



### 3. Research priorities

One of the main factors that determine the economic viability of biofuel production is the efficiency of photosynthetic CO<sub>2</sub> assimilation into storage and structural compounds by the plant. Cassava has a high photosynthetic capacity under favorable field conditions. For example, it has a photosynthetic rate of 40 to 50 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> under high solar radiation, which is double that of rice (around 20 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>). During the major part of its life cycle cassava has accessory photoassimilates for the growth of stems and roots and so has a large potential for production of more storage roots [3]. Thus an important research direction is to increase sink strength for allocation of carbon to starch synthesis in the storage roots. The work by Ihemere *et al.* (2006) [16] where a bacterial ADP-glucose pyrophosphorylase (AGPase), insensitive to allosteric inhibition, was introduced into cassava, demonstrates the applicability of this strategy. They showed that cassava plants expressing the bacterial AGPase had an overall increase in starch synthesis and enlarged storage roots. However, to capitalize on the full potential for high-yield starch production in cassava requires a comprehensive understanding of the regulatory mechanisms behind source-sink interactions, sink strength, carbon partitioning, and storage root formation.

Another important research area deals with the input costs for the conversion of plant biomass to biofuel. The energy conversion efficiency from cassava starch to bioethanol is still low. The average starch content in cassava fresh storage root is around 24%-32%. With current technology, about 6.6 tonnes of fresh storage root may be converted into 1

tonne of ethanol [15]. The energy conversion efficiency is only about 50% and one bottleneck that needs to be addressed is the relative insolubility of cassava starch.

It is worth pointing out that since the storage root is a non-reproductive organ, cassava lends itself to extensive metabolic engineering without jeopardizing reproduction.

### **3.1. Modifying carbon partitioning and sink strength**

Improvements in storage compound production require a thorough understanding of the metabolic fluxes in plant sinks and of the mechanisms that regulate source-sink interactions.

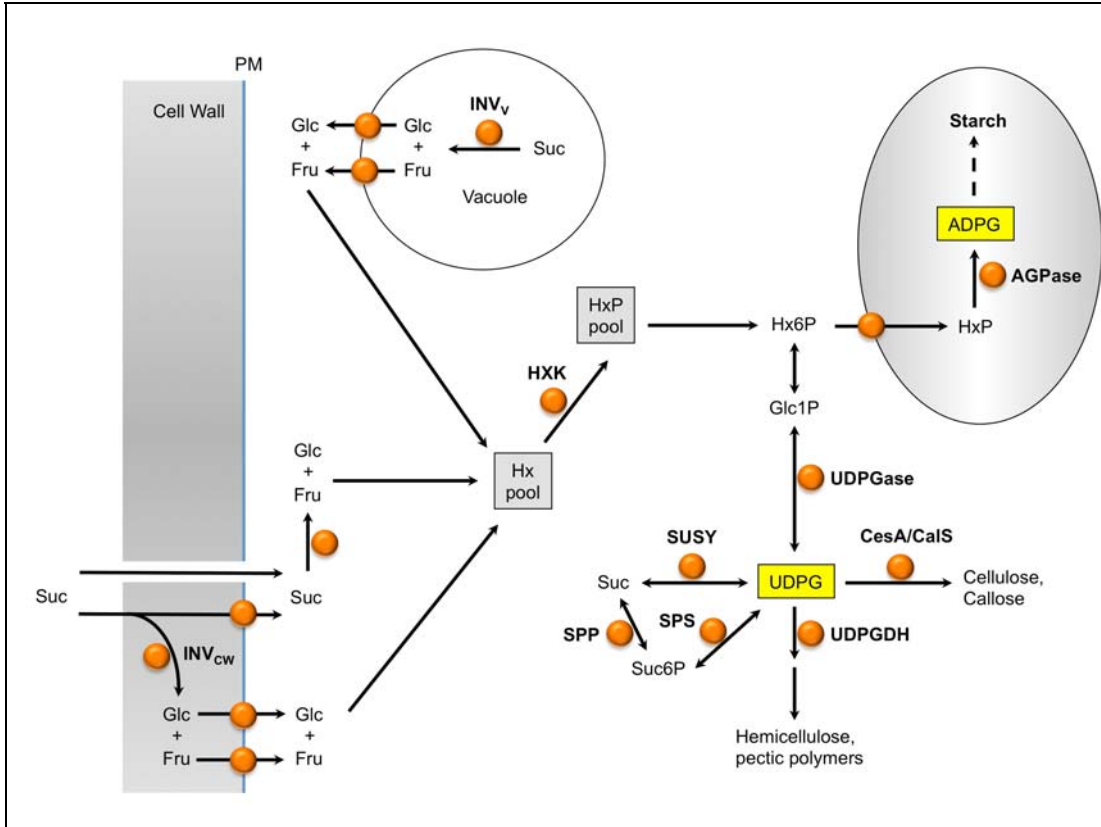
When we understand these regulatory networks it should be possible to engineer current crops to allocate more carbon to storage at the expense of cellulose, and vice versa, to produce cell walls with less lignin, and to redirect carbon flux from starch to oil in tubers as a means to produce novel biodiesel crops. We also don't understand the mechanisms that control feedback inhibition of photosynthesis by sink demand. Insight into this phenomenon should allow us to engineer crops to produce more biomass.

Focal points in this research should be to map the regulatory steps in starch synthesis in the cassava storage root and to identify the transcription factors and other regulators involved in source-sink communication during storage root maturation. For example, it will be of interest to learn if the SUSIBA transcription factor family, identified as important components of the sugar-signaling pathway in cereals [17,18] have orthologs in cassava. Other candidates are Snf-related kinase 1 (SnRK1), hexokinase (HXK) and trehalose 6-phosphate (T6P), although affecting these targets is expected to result in a wider array of

pleiotropic effects [19,20]. As shown by Baguma *et al.* (2008) [2] the exogenous application of inhibitors, hormones and sugar analogs offer excellent help in the investigation of sugar-signaling pathways in the cassava storage root. Another powerful approach that should be tested here is the employment of antisense oligodeoxynucleotide (ODN) inhibition, which has proven to be a potent diagnostic tool and rapid antecedent to transgenic studies in cereals [18,21-23].

It is known that photosynthesis is subject to inhibition by metabolic restrictions in sink sites, and higher sink activities, e.g. starch or cellulose synthesis, provide a pulling mechanism that relieves source tissues of this feedback inhibition [24]. In addition to AGPase, a number of other enzymes are potential targets for enhancing sink strength and directing carbon flux toward starch synthesis. Figure 3 gives a simplified overview of central carbon metabolism in a sink organ such as the cassava storage root. Of particular interest are sucrose synthase (SUSY), UDP-glucose pyrophosphorylase (UDPGase) and UDP-glucose dehydrogenase (UDPGDH). They are all pivotal enzymes in central carbon metabolism. In the plants studied so far, SUSY is present in two distinct forms, one soluble and one that is transiently associated with the plasma membrane (PM), internal membranes, or actin filaments [25]. The soluble form of SUSY is generally involved in the respiration process, whereas the PM-bound form takes part in the synthesis of cell wall components, providing UDPG for the cellulose and callose synthase machinery. It is likely that SUSY associated with Golgi similarly directs UDPG to MLG synthesis [25]. Another SUSY form, associated with the amyloplast, is involved in starch synthesis, probably by affecting carbon partitioning and increasing sink strength. It becomes important to find out if recruitment of SUSY to the

amyloplast occurs also in cassava and to what extent this association can be increased at the expense of soluble and plasma membrane-bound fractions.



**Figure 3.** A model of central carbon metabolism in cells of the cassava storage root. AGPase, ADP-glucose pyrophosphorylase; CesA/CalS, cellulose and callose synthases; Hx, hexose; HXK, hexokinase; HxP, hexose phosphate; Hx6P, hexose 6-phosphate; INV, sucrose invertase (cell wall, cytosolic and vacuole isoforms); Fru, fructose; Glc, glucose; PM, plasma membrane; Suc, sucrose; Suc6P, sucrose 6-phosphate; SUSY, sucrose synthase; SPP, sucrose phosphate phosphatase; SPS, sucrose phosphate synthase; UDPGase, UDP-glucose pyrophosphorylase; UDPGDH, UDP-glucose dehydrogenase.

### 3.2. Regulation of storage root formation

Cassava is a perennial starch crop. In commercial or farming production, the cassava plants are reproduced by planting hardwood stem cuttings for the regeneration and development of both roots and leaves. In a normal growth condition, the adventitious

roots are formed first from the nodes at the base of axillary buds 5-7 days after planting, which is then followed by the formation of rootlets from a recently formed callus at the base of the cutting. The buds also begin to sprout and enlarge 5-8 days after planting with the first leaves appearing by day 13. During the first two months, the cassava plant mainly develops stems, leaves and a fine root system. It is known that the development of storage roots begins with secondary growth in several fibrous roots (ranging from 3 to over 15 storage roots per plant) and starch deposition has initiated around 25-40 days after planting in many cultivars, but visible root bulking is noticeable only when root thickness reaches about 5 mm, usually 2-4 months after planting [3].

The number of storage roots and storage root formation govern the cassava yield. This factor has been heavily addressed in the cassava breeding history. In fact, it is exactly the character where cassava differs from its ancestor *flabellifolia* [Figure 2]. It is a mystery as to what factors determine storage root formation, but it is generally believed that the process of storage formation is regulated either genetically (cultivar-dependent) or environmentally (growth conditions). While the mechanisms underlying tuberization in stem tuber crops such as potato are well studied [26], the knowledge about the mechanisms underlying tuberization in root tuber crops such as cassava is lacking. It can be assumed that storage root formation is controlled by unique genetic and biochemical mechanisms, and a main goal should be to develop a model for the factors involved in storage root formation in cassava, taking into account both internal genetic factors and external environmental influences. For example, it is critical to find out if phloem unloading of sucrose to the cassava storage root cells is symplastic or apoplastic. In

potato, tuberization involves a switch from apoplastic to symplastic unloading [27]. This rearrangement coincides with major changes in gene expression and metabolic processes; SUSY transcript levels increase, invertase activities go down, and the hydrolysis of sucrose by invertases is replaced by SUSY-sucrolytic reactions (see Figure 3). It will be of interest to investigate if similar changes result from, or trigger, the switch from fibrous root to storage root formation in cassava. The findings from such research projects should help to identify candidate genes controlling storage root formation in cassava. The results will not only provide insight into the genetic and biochemical mechanisms underlying storage root formation in “root tuber crops”, but will provide knowledge for the improvement of high yielding cassava varieties that will increase the potential of cassava as a future biofuel crop.

### **3.3. Improved saccharification**

One way to improve saccharification is by increasing the content of amylopectin-bound phosphate groups in the cassava starch. This will render the starch more water soluble and hence better suitable for enzymatic hydrolysis. The degree of phosphorylation in cassava starch is very low (less than 10% of that in potato [28]). To enhance the phosphate levels, one can increase the activity of the gene for the endogenous glucan-water dikinase (GWD), or, alternatively, introduce a heterologous GWD gene [29].

### **3.4. Redirection of carbon flux from polyglucans to oil**

Ethanol and biodiesel are the two major bio-based alternatives to fossil fuels in transportation. Compared to biodiesel, the net energy balance (energy output minus energy

input) for bioethanol is considerably lower, mainly due to the energy losses in the conversion of starch to ethanol. The lipids that comprise most plant oils are highly reduced forms of carbon and therefore represent the most energy-dense plant-storage compounds. Production of novel high-yielding oil-accumulating plants, such as cereals and tuber crops, could result in a new biofuel source that would reduce loss of carbon associated with fermenting sugars to ethanol [30]. By identifying the genetic switch(es) of sucrose utilization between starch and glycolysis and subsequent acetyl-CoA and fatty acid production in the storage root cells, it should be possible to redirect the carbon flow to oil instead of starch.

#### **4. Conclusions**

Analyses of bioethanol production in China from different crops such as maize, sugarcane and cassava show cassava in top in terms of economic benefits. Cassava has high efficiency of photosynthetic CO<sub>2</sub> assimilation, high tolerance to heat and drought, and little requirements for fertilizers. Also, it is considered a non-staple food in China. These properties make cassava a highly attractive biofuel crop in the country. However, the cassava plant can be further improved by metabolic engineering, e.g. to increase the efficiency of photosynthetic CO<sub>2</sub> assimilation into storage and structural products, and to reduce input costs for the conversion of cassava biomass to biofuel. In this article we address these targets and discuss prioritized research strategies.

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