

Aquatic Vegetable Production and Research in China

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ABSTRACT

Aquatic vegetables are all crop species growing in paddy fields, pools or lowlands, and have a very long cultivation history in China. There are mainly 12 fresh-water plant classes belonging to 12 families with about 1700 germplasm resources all over the country. They are : lotus (Nelumbo nucifera Gaertn), water bamboo (Zizania caduciflora Hand. Mazz.), taro (Colocasia esculenta Schott), water dropwort (Oenanthe stolonifera DC. C.), water spinach (Ipomoea aquatica Forsk.), arrowhead (Sagittaria sagittifolia L. var. sinensis Makino), water chestnut (Eleocharis tuberosa Roem. et Schult), water caltrop (Trapa spp.), gordon euryale (Euryale ferox Salisb.), water cress (Nasturtium officinale R.Br), common cattail (Typha caduciflora L.) and water shield (Brasenia schreberi Gmel). Aquatic vegetables are all enjoyed for their crisp, tasty qualities and special flavor, and most of them have exceptional health and appreciation value. Many of them originate in China, and they are an essential part of the Chinese plant germplasm resource treasury. China also produces more aquatic vegetables than any other country in the world. Nowadays, research focuses on germplasm resources and breeding, mechanisms of edible organ development and control, processing, and ecology. Facing the challenges of increasing demand for high quality vegetables, future prospects for production and research of aquatic vegetables are also discussed in this article.

Keywords: breeding, crop species, cultivation, edible organ development, germplasm resources, tissue culture Abbreviations: AAC, apparent amylose content; AP-PCR, arbitrarily primed polymerase chain reaction; 6-BA, 6-benzyl adenine; COD, chemical oxygen demand; 2,4-D, 2,4-dichlorophenoxyacetic acid; GA, gibberellin; MS, Murashige and Skoog medium; NAA, naphthalene acetic acid; POD, peroxidase; PPO, polyphenoloxidase; RAPD, random amplification polymorphism DNA; RVA, rapid visco analyzer; SSase, starch synthase; SSR, simple sequence repeat; UPGMA, unweighted pair-group method with arithmetic mean; ZT, zeatin

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INTRODUCTION

Aquatic vegetables, which cover all crop species growing in paddy fields, pools or lowland, have a very long cultivation

history in China. It has been proved that cattail, wild-rice, lotus and gordon fruit, among others were popular vegeta-bles during the early Qin and Han dynasties. The earliest record can be found in the book of Qi Min Yao Shu (important arts for people's welfare) in Chinese history, which gave a general introduction of the methods for aquatic vegetable cultivation (Kong 2005).

China is located in a Temperate Zone, subtropics and tropics, and there are many vegetable species existing in different places. Relatively, there are many more kinds of aquatic vegetable germplasm in the regions of East, South and Southwest China because of abundant water from many rivers and lakes. Only if there is water can aquatic vegetables be found in radical weather areas like Northeast and Northwest China, for the environment in water is much more stable. It has been investigated that there are mainly 12 fresh-water plant classes all over the country belonging to 12 families. They are: lotus (Nelumbo nucifera Gaertn), water bamboo (Zizania caduciflora Hand. Mazz.), taro (Colocasia esculenta Schott), water dropwort (Oenanthe stolonifera D. C.), water spinach (Ipomoea aquatica Forsk.), arrowhead (Sagittaria sagittifolia L. var. sinensis Makino), water chestnut (Eleocharis tuberosa roem. et Schult), water caltrop (Trapa spp.), gordon Euryale (Euryale ferox Salisb.), water cress (Nasturtium officinale R.Br), common cattail (Typha caduciflora L.), and water shield (Brasenia schreberi Gmel). Many of them originated in China, and are an essential part of Chinese plant germplasm resource treasury. Many of them have already been spread to many countries.

Aquatic vegetables are enjoyable for their crisp, tasty quality and special flavor. Most of them have acceptable health value, while some of have a high appreciation value. During the regulation of rural crop construction in China, ideal economic and ecological benefits have been achieved in some areas, when aquatic vegetables were grown instead of rice or inter-planting rotation was applied in some paddy fields. The exploitation and utilization of aquatic vegetables are efficient paths to enrich the countryside and relieve poverty in China.

SURVEY OF MAIN AQUATIC VEGETABLE SPECIES IN CHINA

Nelumbo nucifera Gaertn (lotus)

Belonging to lotus genus in the Nelumbonaceae family, *Nelumbo* Adas. Genus, lotus is a root-persistent perennial plant growing in water. Cultivar resources used in flower-seeing are named flower lotus, those cultivated for edible seed are named seed lotus, while lotus cultivated for their underground stems are termed rhizome lotus.

The existence of lotus in China has a very long history. In a classic poem it was written "There is Fusu on the hill, lotus flower in the marshy land". Furthermore, many old lotus seeds deep under ground were discovered in the Northwest of Pulandian of Liaoning province at the beginning of the 10th century A.D. following which germination and cultivation experiments were conducted (Xing 1983; Kong 2005); modern-day fresh lotus grew out, from which we deduce that China should be one of the origins of lotus since food lotus has a history of at least 2000 years. There are pieces of lotus in the tomb of Changsha "No. 1" of King Ma of the Han dynasty (about 160 B.C.), which was discovered in the 1970s (Xing 1983; Kong 2005).

All parts of the lotus plant have value. Lotus root and seed as well as other parts, including flowers, pollen, leaves, nodes, and cotyledons in seeds can be processed into all kinds of products from which dishes, drinks, and health care food can be produced. The lotus rhizome is nourishing, with 100 g fresh of underground stem containing 1 g protein, 19.8 g carbohydrate, 19 mg Ca, 51 mg P, 0.5 mg Fe, and 25 mg Vitamin C (Wang 1991); furthermore it is considered to be a general medicine according to traditional Chinese medicine. The leaf can be used to feed fish or castor silkworms and is also being used in food package material. The fleshy underground rhizomes can be harvested all-year round, making them suitable for long-distance transfer (Zhao 1999).

Since lotus grows in mud underwater and since its

growth is steadier than crops in non-irrigated farmland, this plant has left its footprint from the northeast of China to Hainan island, and from the coast of the East Sea to the West plateau. However, vigorously-growing lotus occurs mainly south of the Yangtze River, especially in Jiangsu, Hunan, Hubei and Guangdong provinces, origin to a number of cultivars, plenty of cultivation experience and wide cultivation area.

Zizania caduciflora Hand. Mazz. (water bamboo)

As a cultivated species belonging to the wild rice genus in the grass family, water bamboo is a persistent-root perennial herb plant growing in shallows. It is a speciality of aquatic vegetables in China. The edible part is the abnormal, slender, and fleshy stem. When parasitized by sumt (Ustilago esculenta P. Hen), the stem is stimulated by the secretion of indoleacetic acid (IAA) from the germ, and abnormally enlarges into an abnormal stem (Lv 2001). Additional analysis shows that 18 amino acids, including glutamic acid and aspartic acid, several of which are necessary for the human body, could not be autosynthesized (Wang 1991; Kong 2005). Water bamboo has a good reputation as one of the "three famous dishes south of the Yangtze River", because it is very nourishing and delicious with special flavor. According to traditional Chinese medical science, water bamboo "can remove the heat of the breast and heart". It relieves cough and when boiled cures childrens' diarrhea. So, it is also a vegetable with medicinal value (Li 1982; Kong 2005).

Water bamboo is widely distributed throughout almost all provinces in China but is primarily commercially cultivated in regions south of the Yangtze River, especially in Zhejiang, Guangdong, Jiangsu and Taiwan provinces.

Colocasia esculenta Schott (taro)

Taro is a perennial herb belonging to the genus *Colocasia*, family Araceae. It originated in tropical swamps of South Asia.

The main edible parts of taro are the succulent, enlarged cormels. Some cultivars have an edible, fleshy leaf stalk or well-developed pedicels. The cormels can be used as the raw materials of starch and wine besides being consumed as a vegetable and food stuff. Furthermore, taro has some medicinal functions. It was said in the ancient books of *Herbal Medicine in the Tang Dynasty* that "Eating or drinking its cool juice can cure wet, hot diseases and hot, thirst diseases" (Li 1982; Kong 2005).

According to annals of some districts, taro grows in the areas from south China to north of the Yellow River valley in China. Among them, there are many varieties of taro which is most widely planted in Hainan province. So Hainan is probably the original center of domestication of cultivated taro.

Oenanthe stolonifera D.C. (water dropwort)

Water dropwort is a perennial, persistent rooted herb and belongs to the Umbelliferae family. Most parts of the young plant are edible such as stems, leaves and leaf stalks. Water dropwort was consumed as a vegetable in many regions throughout Chinese history. Every 100 g of edible parts contains 2.5 g of protein, 0.6 g of fat, 4 g of carbohydrate, 3.8 g of crude fiber as well as vitamins, carotenoids, nicotinic acid, minerals (Wang 1991). Water dropwort is also a kind of herbal medicine. Some ancient medical books said it can nurse breath and stimulate the appetite. Modern research shows that it can clean blood and prevent hypertension (Li 1982; Kong 2005).

Water dropwort originates from East Asia, and China is one of its original regions. Wild water dropwort grows in paddies, streams and moist lands along the Yangtze River and areas south of it. Many Chinese ancient books recorded the importance or presence of water dropwort, and from these reaccounts we can appreciate that people began to collect water dropwort 3000 years ago and to process it 2000 years ago. In the *Magic Peasants Herb Manual*, the skills for its cultivation were first mentioned. In fact, water dropwort was seldom cultured, but mainly grew wildly until the late Qing dynasty. Now water dropwort is spread throughout the wild in China, but only in the regions along Yangtze River and the south is water dropwort cultured for marketing (Zhao 1999). Sichuan and Yunnan provinces, in particular, are rich in water dropwort resources.

Ipomoea aquatica Forsk (water spinach)

Water spinach is an annual or perennial herb belonging to the *Ipomoea* genus in the Convolvulaceae family. It was indicated in *The Annals of Guangzhou* that for more than 1600 years ancient Chinese people picking water spinach as food (Kong 2005). The edible part of this vegetable is the young stem and leaf. It can grow upland, in wetlands, in both shallow and deep water, and it grows better in an environment with sufficient water. These findings are based on practical cultivation for which no experimental data exists. Water spinach is sent to the market earlier if planted under protection in early spring. Because water spinach is heat-tolerant and it tends to grow in wet environments, it is the main vegetable in off season when water culture is used in spring and summer.

Water spinach is also distributed in the tropical zones of Africa, South-east Asia and America (Liu *et al.* 1996; Kong 2005). China plays an important role in the popularization of water spinach all over the world.

Sagittaria sagittifolia L. var. *sinensis* Makino (arrowhead)

Arrowhead is a perennial herb growing in paddies. It belongs to *Sagittaria* genus, Alismataceae family. It originated in China. Among the *Sagittaria* genus, only Chinese arrowhead (*S. trifolia* var. *sinensis*) is taken as vegetable usually (Kong 2005). Arrowhead is broadly spread-out in China, from the north in Heilongjiang province to the south in Hainan province, and in almost all regions except those with a water shortage. But only in Jiangsu, Zhejiang, Fujian, Guangdong, Guangxi and Sichuan provinces it is cultivated as a vegetable.

The edible part of arrowhead is the cormel, enjoyed by many people. It is seen as one of the five excellent local specialties in Guangzhou city and as a tribute in Shanghai city and Jiangsu province. Arrowhead is highly nutritious. It contains 5.6 g protein, 25.7 g carbohydrate, 0.2 g fat and 0.9 g crude fiber per 100 g of fresh cormels, and many mineral elements, vitamins, and choline (Wang 1991). Modern research found that arrowhead is rich in vitamin B and a repressor of pancreatic protein enzyme and that eating it often can accelerate the calcification of pulmonary tuberculosis (Wang 1991). In addition, it aids in the treatment of nyctalopia, pancreatitis, diabetes, tracheitis and urethra infection (Li 1982; Kong 2005).

Eleocharis tuberosa Roem. *et* Schult (water chestnut)

Water chestnut, which grows for its sweetish underground cormels, is a perennial herbal plant belonging to the sedge family (Cyperacea). China is one of the origins of water chestnut. It is widely grown in Korea, Japan, India and America. The cormels are crisp and abundant in juice. Water chestnut can be used as a vegetable and a fruit, and its raw materials can be processed, its starch can be extracted and its fruit canned. It also has a high medicinal value, widely used as medicine in China (Li 1982; Zhao 1999).

The earliest record of water chestnut was in the book of Er Ya (about 2 centuries B.C.) (Ye 1992; Kong 2005). Nowadays, it is spread widely over the whole of China except in the cold regions. The leading production areas are the Yangtze Valley and the South-east coastal regions. The wellknown cultivars of water chestnut are 'Su Water Chestnut' in Jiangsu, 'Hangzhou Big Red Coat' in Zhejiang, 'Xiaogan Water Chestnut' of Hubei, 'Guilin Mati' in Guangxi province, and so on.

Euryale ferox Salisb. (gordon euryale)

A native of east Asia including China, gordon euryale is a perennial herbal plant belonging to the *Euryale* genus of the Nymphaeaceae family. Gordon euryale seed (the kernel) is edible and rich in nutrients: 9.8-11.8 g proteins, 75 g carbohydrates, 0.2 g crude fiber, 0.3 g fat, 39 mg Ca, 86 mg P and 1.2 mg Fe in each 100 g dry kernel (Wang 1991), and abounds with glutamic, arginic, leucinic and aspartic acids (Wang 1991). It is a delicious dish for its creamy sensation and rich fragrance. It functions as a Chinese medicine by nourishing the spleen, dispelling diseases caused by dampness and benefiting the kidneys (Li 1982). Its tender footstalks are used as a vegetable.

Gordon euryale seeds were dug out from the ruins of the New Stone Age located in Qindun of Haian county in Jiangsu province. The earliest cultural account of gordon euryale was in "Qi Ming Yao Shu" of the Qi dynasty (Kong 2005). Gordon euryale is widely distributed in the south and north of China while it is more popular in Jiangsu and Zhejiang provinces for economic reasons. So, Jiangsu and Zhejiang province are probably the original center of cultivated gordon euryale of domestication.

Nasturtium officinale R. Br (water cress)

Water cress is a semi-aquatic annual or biennial herbal plant belonging to the *Nasturtium* genus of the Cruciferae family. It is easy to grow and can be harvested in several seasons throughout the year with a high yield and marketed for a long period, especially in spring and autumn when the varieties of fresh vegetable are relatively rare, especially in the southern provinces of China.

Water cress is highly nutritious. According to the *Chinese Herb Medicine Handbook*, water cress eliminates internal heat, moistens the lungs and stops coughing, making excreta and urine move freely and overcomes fatigue (Li 1982; Kong 2005).

Water cress originated in the east part of the Mediterranean, and there are also wild types in south subtropical regions. The Romans and Persians were the first to use water cress as food. It then spread into England and France in the early 14th century, later to the USA, South Africa, Australia, and New Zealand. It was taken to Japan in about 1780. It was recoded in the book of *Guangdong Annals* in 1935 that water cress was planted in China in the early part of the last century (Kong 2005). Nowadays, water cress is widely planted in the whole of China excepting Northeast China. Guangdong province is now the largest growing area.

Trapa spp. (water caltrop)

Water caltrop is an annual leaf floating aquatic herbal plant, belonging to the *Trapa* genus of Trapaceae family. The edible part is the fruit. Fresh water caltrop fruit contains abundant carbohydrates, proteins, various vitamins and minerals. It can be used as a fruit, vegetable, and also in the processing of raw materials such as noodle and wine. It can be used as the raw material of drugs. Ergosterol and β -sitosterol are found in water caltrop, which seem to be effective against cancer of the stomach and womb to some extent (Li 1982; Kong 2005).

Using different cultivating methods, water caltrop can be planted in deep waters such as lakes and pools, and also in shallow waters such as channels and paddy fields. The charred two-horn water caltrop was discovered in "Hemudu" ruins in the New Stone Period in Yuyao of Zhejiang province (Sun 1981), emphasizing the existence of water caltrop in China a long way back in history. Water caltrop is widely spread throughout China. Both wild type and domestic type of water caltrop have existed for a long time. Wild water caltrop was recorded in many of county annals such as those of Zhejiang, Jiangxi, Jiangsu and Shangdong provinces until the Qin dynasty. Although there is still wild water caltrop in Heilongjiang province, it is more popular in the south of the Yangtze River for commercial culture.

Typha caduciflora L. (common cattail)

Common cattail belongs to the Typha genus, Typhaceae family. It originated in China and has a long history of cultivation and application. Only Chinese use it as a vegetable although it is widespread throughout the world. It can be divided into three types according to its different edible parts: Pseudo-stem wrapped by a leaf sheath, rhizome or shortened stem. It can be fried, braised, steamed or made into a soup.

An observation of the biological characteristics reveals that the rhizome of edible common cattail is very different to the pseudo-stem of the edible type. "Jianshui grasssprout", which is a rhizome edible plant, belongs to the broad-leaf common cattail genus (*Typha caduciflora* L.), while "Huai'an common cattail" and "Minghu common cattail", which are pseudo-stem edible types, belong to the water-candle species (*Typha angustifolia*), which is also called narrow-leaf common cattail (Kong 2005). Common cattail was widely spread throughout China, and it has been recorded in almost all counties' annals.

Brasenia schreberi Gmel (water shield)

Water shield is a cultivated species in the *Brasenia* genus, *Cabombaceae* family and is a persistent-root perennial aquatic herbal plant. The edible part of water shield is the tender bud with transparent glia and the developing young leaf. Water shield is abundant in carbohydrates, celluloses, vitamins, proteins, carotenoids, amino acids and many mineral elements (Wang 1991). So, it is a delicious table dish in China for its dark green color, creamy-in-mouth feeling, fragrant and distinctive flavor. It has a high medical value. It is used to improve the secretion of gastric juice, prevent the liver from lesions, curing dysentery caused by heat, jaundice, swelling and pain, malignant boils, and also prevents cancer (Li 1982; Kong 2005).

Water shield is widely distributed in China from the Tangwang River Valley 47° N to the southwest provinces such as Yunnan, Guizhou and Sichuan, but it is more important in Jiangsu and Zhejiang provinces for its commercial culture.

AQUATIC VEGETABLE RESAERCH IN CHINA

Germplasm resources

By random amplified polymorphic DNA (RAPD) analysis, Nelumbo accessions were clustered into three clusters: flower lotus, seed lotus and rhizome lotus. This is similar to the traditional horticultural classification. No difference was observed between Nelumbo lutea and flower lotus accessions (Nelumbo nucifera). Flower lotus, seed lotus, and rhizome lotus might have derived from different wild lotus (Guo et al. 2004). Each lotus accession had its unique fingerprinting pattern (Han et al. 2004). Random primers and SSR primers had been selected in the ancient "Taizi lotus" (about 580±70 years ago) and the modern Chinese red flower lotus from Hebei, Harbin (wild population), Jiangxi and Hunan provinces (cultivar). According to the UPGMA analysis of MEGA program, "Taizi", Harbin and Hebei lotus were gathered to a single branch in the dendrogram. They genetic distance of "Taizi" and Hebei lotus was very close (0.05). Compared with the modern Chinese lotus, the ancient "Taizi" lotus only lacked one locus, so it was still classified as belonging to Nelumbo nucifera (Zou et al.

1998; Peng *et al.* 2004). The genetic differences between single and double cropping varieties of *Zizania caduciflora* are larger than these between varieties in the same cropping type (Yu *et al.* 2005).

Using arbitrarily primed polymerase chain reaction (AP-PCR), Chinese Sagittaria can be divided into three groups composed of seven species and three varieties or forms, except for Sagittaria natans. The three groups are: group 1, with only one species, S. guayanensis; group 2, composed of S. tengtsungensis, S. wuyiensis, S. lichuanensis and Sagittaria sp.; in group 3 S. potamogetifolia, S. pygmaea, S. trifolia var. trifolia, S. trifolia var. sinesis, S. trifolia longiloba. These results are congruent with those based on morphological and karyotypical ones (Du et al. 1998).

According to the cell shape of the exocarp and the sculpture of the fruit surface, Chinese water chestnut are distinguished into two types: (1) smooth type, of which the fruit coat is smooth and without a differentiated surface cell (e.g., *Eleocharis soloniensis*); and (2) reticulate type, which consists of two subtypes according to the configuration of anticlinal cell walls and the contour of the cell lumen. In the first subtype the anticlinal cell wall is straight or slightly undulate, and the cell shape is hexagonal or transversely long hexagonal (e.g., *Eleocharis yokoscensis* and *E. retro-flexa*), while in the second subtype the anticlinal cell wall is conspicuously crenate to serrate, and the cell shape is long hexagonal or polygonal. The genus *Eleocharis* is a natural group which is characterized by a general resemblance of an achene microstructure (Liu *et al.* 1999).

Breeding

Aquatic vegetables can be propagated asexually by buds or sexually by seed (or fruits, e.g. *Trapa* spp.). Systematic selection is the main breeding method used by many aquatic vegetables (Kong *et al.* 1994; Ke *et al.* 1997). *Nelumbo nucifera* has also been used in artificial crossing or onboard space satellites to obtain new varieties (Kong *et al.* 1994; Xie *et al.* 2004).

Ecology

Modern studies show that aquatic plants can remove pollutants and improve the water environment. The average removal of chemical oxygen demand (COD), ammonia and total phosphorus by Zizania caduciflora and Typha angustifolia L. were 39.9%, 72.7%, 58.5% and 37.9%, 59.4%, 48.6% respectively (Li et al. 2006). The dissolved oxygen in water in which Z. caduciflora and T. angustifolia are cultured are more stable, and the water temperature is lower than that in which there are no aquatic plants (Li et al. 2006). The forté of Oenanthe stolonifera D. C. (water dropwort) is to reduce the pH value of water. Different aquatic plants have different merits in removing pollutants in water and in improving the water environment (Dai et al. 2006).

Edible organ development and control

The changes in endogenous hormones, salicylic acid (SA) and polyamines were studied during lotus (Nelumbo nucifera Gaertn) rhizome and water bamboo gall swelling. 2.2-3.6 ng/g.FW zeatin (ZT) may start rhizome enlargement and promote cell division; SA (0.08-0.34 µg/g.FW), IAA (5.0-28.0 ng/g.FW) and $GA_3(0.15-0.35 \text{ ng/g.FW})$ all took part in the enlargement of the louts rhizome, furthermore, GA3, IAA and SA content increased quickly from the 1 and 2 internode stage, respectively; and all reached the highest at the 3 internode stage; IAA content was significantly correlated with SA at P=0.01 during rhizome development, so, they may possibly co-operate in the process. Spermine and putrescine content remained high throughout lotus rhizome enlargement while cadavarine and spermidine content remained low, and thus possibly had little effect on rhizome development (Li et al. 2006a). The differences of ZT, GA₃, IAA and ABA contents and their changes were obviously between cultivars in the swelling period of the gall; ZT maybe a key hormone in gall development of Zizania caduciflora (water bamboo) (Jiang et al. 2005). Before the gall of Z. caduciflora expands, total sugar accumulates while reducing sugar and starch increase continuously and the greatest amount of carbon assimilates were observed to be accumulated in the shortened stem compared with that in other organs. When the Zizania gall began to expand, the carbohydrate in other organs stopped being accumulated. Most carbohydrates stored in the shortened stem were remobilized. The shortened stem serves as a storage organ for remobilized assimilates for the growth of the gall. Starch is the main form of stored carbohydrate, and most $^{14}\mathrm{C}$ assimilates were prefixed in the shortened stem before gall development. During the period of Zizania gall expansion, most prefixed ¹⁴C was remobilized into it (Jiang *et al.* 2003; Cheng et al. 2004).

The peaks of fructose, glucose and total soluble sugar, and sucrose content were reached at the early, middle, and end stages of lotus rhizome development, respectively. Total starch, amylose and amylopectin content in lotus rhizome increased quickly after the middle stage, while total starch content accounted for 11.1% to 12.7% of the fresh rhizome at the end stage, and 70% of total starch was amylopectin. Total starch content in the matured lotus rhizome was determined by ADPGPase and SSase activities at the middle and early stages; amylopectin content was determined by branching enzyme activity at the middle stage during lotus rhizome development (Li et al. 2006b). The characteristics of starch in the rhizomes of four matured lotus varieties indicated that: the sort order of apparent amylose content or AAC from the highest to the lowest were: "Mei ren hong", "E lian No. 1", "E lian No. 4", "Wu zhi No. 2". Pellet and ellipse starch granules were found in the lotus rhizome, and the number of pellet starch granules in "Wu zhi No. 2" and "E lian No. 4" (about 80%) was more than in "E lian No. 1" and "Mei ren hong" (about 50%). The rapid visco analyzer or RVA profile indicated that the starch gelatinization characteristics of "Wu zhi No. 2" was similar to "E lian No. 4", that their peak viscosity of both high and cool viscosity was far below the peak viscosity, that the consistency was very low and that the setback was negative. But in "E lian No. 1" and "Mei ren hong", the peak viscosity of both low and cool viscosity was close to or even higher than the peak viscosity, while consistency and setback were significantly higher than the other two varieties. AAC in the lotus rhizome was negatively correlated with peak viscosity and breakdown, with significant coefficients of -0.9748** and -0.9793**, respectively, but positively correlated with consistency, setback, cool viscosity, peak time and pasting temperature with significant coefficients of 0.9860**, 0.9804**, 0.9339*, 0.9392* and 0.9130*, respectively. The difference of starch granule form and configuration led to the difference in the starch gelatinization characteristics (Li et al. 2006c).

A large quantity of storage proteins are accumulated in mesophyllous cells of lotus cotyledons at different rates, and various morphological and structural protein bodies are composed by different patterns from 10 to 28 days. There are eight main protein subunits, and their molecular weights range from 98×10^3 to 16×10^3 (Tang *et al.* 1998a, 1998c). Further studies show that storage protein gene expression in lotus seed is controlled by development. The appearance of an allosteric phenomenon of mesophyllous cells in the cotyledon is earlier than the beginning of storage protein gene expression, mainly displayed as follows: The volume of the nucleus increases, the external form is irregular, the distribution of chromatin is heterogeneous, the nucleolus disintegrates, the local nuclear membrane disappears, and it intensifies as development progresses (Tang *et al.* 1998b).

The activities of peroxidase (POD) and polyphenoloxidase (PPO) in lotus rhizome tissues increased with swelling and delaying in harvesting date of the lotus rhizome, and they were significantly different in different cultivars (Shou *et al.* 2005). Phenolics such as dopa, catechol, gallic acid, D(+) catechin and L(-) epicatechin were isolated and identified in the lotus root, and gallic acid was the optimum substrate of PPO (Wang *et al.* 2004). In *Zizania caduciflora* the activity of catalase and superoxide dismutase declined continuously during stem swelling, but POD activity increased slowly at an earlier swelling stage, and rapid rose occurred afterwards (Cheng *et al.* 2004).

Tissue culture

Nelumbo nucifera Gaertn, Sagittaria sagittifolia L. var. sinensis Makino, and Eleocharis tuberosa Roem. et Schult are usually propagated asexually with rhizomes and corms, but it is not the most efficient way. So, an effective micropropagation protocol was established. The favorable medium for induction and regeneration of Chinese arrowhead shoot tips are Murashige and Skoog medium (MS) + 1.0 mg.L⁻¹ 6-benzyl adenine (6-BA) + 0.3 mg.L⁻¹ α -naphthalene acetic acid (NAA) and $MS + 3.0 \text{ mg.L}^{-1}6\text{-BA} + 0.1 \text{ mg.L}^{-1}$ NAA, respectively (Zhu et al. 2006). Shoot tips of Chinese water chestnut can be cultured and propagated on MS me-dium $+ 0.1 \text{ mg.L}^{-1}$ kinetin (KT) and MS $+ 2.0 \text{ mg.L}^{-1}$ 6-BA + 1.0 mg.L⁻¹ NAA, and bulblets could be induced from plantlets by culture in MS medium + 1.0 mg.L⁻¹ 6-BA + 90 g.L⁻¹ sucrose for 60 days; all bulblets were over 0.2 g and could be preserved in 0°C-15°C for 6 months with a germinating rate of over 90% (Cao *et al.* 1999). The best differentiation medium for lotus shoot tips was MS medium supplemented with 1.6 mg.L⁻¹ 6-BA, or for explants having one unfolded leaf and one immature leaf with one terminal or axillary bud for sub-culture on MS medium with 0.3 mg.L⁻¹ 6-BA, 50 g.L⁻¹ sucrose for 30 days resulted in the most efficient propagation (Li et al. 1995, 1998).

Post-harvest physiology and processing

The amount of total phenols, free phenols and catechol in fresh cut lotus root slices during the storage period increased initially and decreased afterwards; free phenols, which are the mainly catechol, accounted for more than 70% of total phenols. The best combination of browning inhibitors selected for fresh cut lotus roots was 4.0 mg.mL⁻¹ L-cystene + 1.5 mg.mL⁻¹ EDTA-Na + 2.0 mg.mL⁻¹ Zn(AC)₂ (Yu *et al.* 2003). Chlorophyllous decomposition of *Z. caduciflora* treated by gibberellic acid and 2,4-D declined, and respiration rate and POD activity were also suppressed, while the fibrotic rate declined following treatment with 1000 mg.kg⁻¹ malic hydrazide for 15 minutes, then storage in 0°C ± 0.5°C for 60 days (Chen *et al.* 2003).

Hundreds of kinds of edible products such as salted lotus roots, fresh-kept lotus roots in whole, water-boiled lotus roots, canned water-chestnut, compound dish and lotus root powder, among others have found a market niche in Japan, South Korea, USA and other countries.

FUTURE PRODUCTION AND RESEARCH

Germplasm collection and diagnostics

There are abundant aquatic vegetable germplasm in the vast expanse of territory and multifarious physiognomy of China. Inevitably, many germplasm resources from some provinces have been ignored. So, aquatic vegetable resource collection and diagnostics are still important, basic work that needs to be continued.

Molecular biology studies on important phenotypic traits and molecular-assisted breeding

Most aquatic vegetables are usually propagated asexually, so systematic selection is so far the main breeding method. The development of molecular markers and molecular linkage maps have facilitated marker-assisted selection for both qualitative and quantitative traits of aquatic vegetables. New aquatic vegetable accessions obtained by molecular breeding will become possible.

Isolating and application of health-beneficial compounds from aquatic vegetables

According to traditional Chinese medical science, most aquatic vegetables have been used historically to treat and prevent disease. As people's health requirements increase, the utilization and development of aquatic vegetables for human health may have a very tremendous prospect. Studies on isolating and application of health-beneficial compound in aquatic vegetable have become increasingly important.

ACKNOWLEDGEMENTS

We thank Professor Qingdong Kong for his helpful references. This work was supported by the Jiangsu Science and Technology Department (BG2005313).

REFERENCES

- Cao B-S, Cai H, Li L-J, Ding C-M (1999) Induction of corms *in vitro* in Chinese water chestnut. Acta Horticulturae Sinica 26, 335-336
- Chen W-X, Gao H-Y, Zhou Y-J, Zhu M-Y, Zhu D-L (2003) Effect of plant growth regulators on physiological quality of preserves Zizania caduciflora. Acta Agriculturae Zhejiangensis 15, 185-188
- Cheng L-J, Guo D-P, Zhu Z-J, Sun Y-Z (2004) Study on the biochemical changes during stem gall formation in *Zizania caduciflora*. *Acta Agriculturae Nucleatae Sinica* 18, 457-461
- Dai B, Hu X-B (2006) Study on water purification with economical aquatic plants. *Journal of Anhui University of Technology* 23, 47-49
- Du G-W, Yi Q-M, Chen J-K (1998) Phylogenetic relationship of Chinese Sagittaria species based on AP-PCR analysis. Acta Phytotaxonomica Sinica 36, 216-221
- Guo H-B, Ke W-D, Li S-M, Peng J (2004) Cluster analysis of Nelumbo accessions based on RAPD markers. Journal of Plant Genetic Resources 5, 328-332
- Han Y-C, Diao Y, Zhou L, Liu J-Y, Zhou M-Q, Hu Z-L, Song Y-C (2004) Construction of the DNA fingerprinting in *Nelumbo. Journal of Wuhan Bota*nical Research 22, 193-196
- Jiang J-Z, Qiu J-J, Han X-Q, Cao B-S, Zhu Q-S (2004) Changes of endogenous hormone contents of different parts during development of Zizania caduciflora. Journal of Wuhan Botanical Research 22, 245-250
- Jiang J-Z, Cao B-S, Huang K-F, Zhang Q, Han X-Q, Zhu Q-S (2005) Changes of non-structural carbohydrate and endogenous hormones during *Zizania* galls swelling. *Acta Horticulturae Sinica* **32**, 134-137
- Jiang J-Z, Cao B-S, Qiu J-J, Han X-Q, Zhang Q, Zhu Q-S (2003) Studies on accumulation and allocation of carbohydrate in *Zizania caduciflora*. Acta Horticulturae Sinica 30, 535-539
- Ke W-D, Fu X-F, Huang X-F, Peng J, Zhou G-L, Ye Y-Y (2000) Systematic cluster analysis of some *Nelumbo nucifera* Gaertn. germplasm resources and its application on breeding. *Acta Horticulturae Sinica* 27, 374-376
- Ke W-D, Kong Q-D, Peng J (1997) A study on classification and breeding of double harvesting water bamboo (Zizania caduciflora). Journal of Wuhan Botanical Research 15, 262-268
- Kong Q-D (2005) *The Aquatic Vegetable Cultivars and Resources of China*, Hubei Science and Technology Press, Wuhan, China, pp 3, 61, 101, 149, 175, 219, 248, 269, 293, 303, 313, 339
- Kong Q-D, Fu X-F, Yang B-G, Ye Y-Y, Ke W-D (1994) A preliminary study on lotus breeding method. *Seed* **73**, 31-33
- Li L-J, He X-D, Zhao Y-W, Ding C-M (1995) A preliminary study on shoot tip culture of lotus. *Journal of Jiangsu Agricultural College* 16, 31-34
- Li L-J, Pan E-C, Xu C, Ye Z-R, Cao B-S (2006a) Changes of endogenous hor-

mones, polyamines and salicylic acid content during rhizome development of *Nelumbo nucifera gaertn. Acta Horticulturae Sinica* **33**, 1106-1108

- Li L-J, Zhang X-D, Pan E-C, Sun L, Xie K, Cao B-S (2006b) Relationship of starch synthesis with its related enzyme activities during development of lotus (*Nelumbo nucifera* Gaertn). *Scientia Agricultura Sinica* 39, 2307-2312
- Li L-J, Zhang X-D, Shen X-P, Sun L, Xie K, Gu L, Cao B-S (2006c) Studies on starch RVA profile and starch granule shape in rhizome of *Nelumbo nucifera* Gaertn. Acta Horticulturae Sinica 33, 534-538
- Li L-J, Zhao Y-W (1998) Rapid propagation of plantlets cultured from lotus shoot tips. *Journal of Nanjing Agricultural University* 21, 113-115
- Li S-Z (1982) Ming dynasty. Liu H-R (Ed) *The Compendium of Materia Medica*, People's Medical Publishing House, Beijing, China, pp 269, 350-354
- Li R-H, Guan Y-T, He M, Hu H-Y, Jiang Z-P (2006) Pilot scale study on riparian *Phragmites communis, Zizania caduciflora* and *typha angustifolia* zones treating polluted river water. *Environmental Science* 27, 493-496
- Liu J-Q (1999) Achene micromorphological characters of *Eleocharis* from China and its taxonomic significance. *Chinese Journal of Applied and Envi*ronmental Biology 5, 578-584
- Liu Y-M, Ye Y-Y, Kong Q-D (1996) Studies on the water spinach germplasm in China. *Journal of Changjiang Vegetables* 3, 1-4
- Lv J-L (2001) Vegetable Culture, Agricultural Press, Beijing, China, 270 pp
- Peng Y-L, Han Y-C, Wang L, Teng C-Z, Zhou M-Q, Hu Z-L, Song Y-C (2004) Genetic diversity in lotus (*Nelumbo*) accessions revealed by AFLP technique. *Molecular Plant Breeding* 2, 823-827
- Shou S-Y, Zai W-S, Huang X-Z (2005) Activities changes of browning enzymes during rhizome swelling of lotus. Acta Agriculturae Nucleatae Sinica 19, 279-281
- Sun X-J (1981) The paleovegetation and paleoclimate during time of Homudu people. Acta Botanica Sinica 23, 146-151
- Tang P-H, Jiang H, Li Q-Y, Jiang Z-J, Zhou G (1999) Major subunits and accumulation pattern of lotus storage proteins. *Acta Botanica Sinica* 41, 176-180
- Tang P-H, Jiang Z-J, Jiang H, Zhao R-Q (1998a) The type, source and development of protein bodies in lotus cotyledon. *Journal of Beijing Normal University (Natural Science)* 34, 252-257
- Tang P-H, Jiang Z-J, Jiang H, Zhao R-Q (1998b) Storage protein genes expression and allostric phenomenon of mesophyllous cells in cotyledon of lotus. *Journal of Beijing Normal University (Natural Science)* 34, 380-384
- Tang P-H, Jiang Z-J, Mei C-H, Jiang H (1998c) The composition, solubility and quality of lotus seed. *Journal of Beijing Normal University (Natural Sci*ence) 34, 532-537
- Wang G-Y (1991) Book of Component Parts in Food, People's Medical Publishing House, Beijing, China, pp 10-16
- Wang Q-Z, Pen G-H, Jin Y, Li J, Yan S-L (2004) Extraction of polyphenol from lotus roots and its enzymatic browning substrate. *Journal of Analytical Science* 20, 38-40
- Xie K-Q, Yang L-B, Zhang X-L, Xu J-X, Jiang D (2004) Selection of lotus from seeds re-onboard in space satellite. Acta Agriculturae Nucleatae Sinica 18, 300-302
- Xing X-C (1983) On the place of origin of *Nelumbo nucifera*. Agricultural Archaeology 2, 248-250
- Ye J-Y (1992) A brief history of aquatic vegetable culture in China. Ancient and Modern Agriculture 1, 13-21
- Yu X-P, Zhang J-F, Lu Z-X, Chen J-M, Zheng X-S, Xu H-X, Chen L-Z (2005) DNA polymorphism of 12 selected water oat varieties (*Zizania caduciflora*) collected from Zhejiang. *Bulletin of Science and Technology* 21, 137-141
- Yu Z-F, Li N, Zhao Y-X, Kang R-Y (2003) Change of phenol substances and browning inhibition by inhibitor combination in fresh-cut lotus roots. *Journal* of Nanjing Agricultural University 26, 78-81
- Zhao Y-W (1999) Chinese Aquatic Vegetables, Agricultural Press, Beijing, China, pp 18, 81, 128
- Zhu H-L, Ke W-D, Wang L-P (2006) Tissue culture and mass propagation of Chinese arrowhead. *China Vegetable* 143, 15-17
- Zou Y-P, Cal M-L, Wang X-D, Xu B-M (1998) RAPD analysis of germplasm in ancient "taizi lotus" and modern Chinese lotus. Acta Botanica Sinica 40, 163-168