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Invited Review Article

Sweet potato Omics and Biotechnology in China

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Abstract

Sweet potato, *Ipomoea batatas* (L.) Lam., is an important food and industrial material crop throughout the world. It is also an alternative source of bio-energy as a raw material for fuel production. China is the biggest sweet potato producer in the world. Biotechnology offers great potential for improving disease, pest and stress resistance and nutritional quality of sweet potato. In the past decades, great progress in sweet potato omics and biotechnology has been made in China. An efficient system of embryogenic suspension cultures has been developed for a wide range of sweet potato genotypes. Somatic hybridization has been applied to overcome cross-incompatibility between sweet potato and its relatives, and has generated useful interspecific somatic hybrids. Novel mutants have been obtained by cell induced mutation and in vitro selection. Several genes related to stem nematode resistance, salt tolerance, carotenoid biosynthesis, and anthocyanin biosynthesis have been cloned. *Agrobacterium tumefaciens*-mediated transformation has been standardized for important cultivars, and has been used to produce transgenic plants resistant to diseases, stresses and herbicides. Molecular markers linked to a stem nematode resistance gene have been developed. This paper summarizes advances made so far in sweet potato omics and biotechnology in China and suggests future directions for research in omics and biotechnology of this crop in China.

Keywords: *Ipomoea batatas* (L.) Lam., plant regeneration, somatic hybridization, in vitro selection, gene cloning, genetic transformation, molecular marker.

Introduction

Sweet potato, *Ipomoea batatas* (L.) Lam., is an important food and industrial material crop throughout the world. It is also an alternative source of bio-energy as a raw material for fuel production (Zang *et al*., 2009). The improvement of this crop by conventional hybridization is limited because of its high male sterility, incompatibility and hexaploid nature (Dhir *et al*., 1998). Biotechnology offers great potential for improving disease, pest or stress resistance as well as the nutritional quality of sweet potato. Significant progress has been made in plant regeneration, somatic hybridization, gene cloning, genetic transformation and molecular markers in sweet potato. An efficient plant regeneration system is very important for the successful application of biotechnology to sweet potato improvement. The regeneration frequency in sweet potato is often genotype-dependent, ranging from 0 to 85% in tested cultivars (Jarret *et al*., 1984; Chee *et al*., 1992; Desamero *et al*., 1994; Gosukonda *et al*., 1995; Otani and Shimada, 1996; Al-Mazrooei *et al*., 1997; Wang *et al*., 1998; Santa-Maria *et al*., 2009). In most cases, however, plant regeneration at a high frequency has been restricted to one or a few genotypes. Much attention is being directed toward developing an efficient system of plant regeneration for a wide range of sweet potato genotypes. The successful application of somatic hybridization to crop improvement is highly dependent upon efficient plant regeneration from protoplasts. There were several reports on plant regeneration from protoplasts of sweet potato and its relatives (Murata *et al*., 1987; Sihachakr and Ducreux, 1987; Perera and Ozias-Akins, 1991; Belarmino *et al*., 1994; Murata *et al*., 1994; Dhir *et al*., 1998), but all these studies resulted in a low frequency of plant regeneration from protoplast-derived callus. Some intra- or inter-specific somatic hybrids have been formed (Murata *et al*., 1993; Belarmino *et al*., 1993; Wang *et al*., 1997). Kwak *et al*. (1995) and Kim *et al*. (1999, 2003) reported the isolation of a strong oxidative stress inducible peroxidase gene (*SWPA*2) from cultured cells of sweet potato and subsequently characterized its function in transgenic tobacco plants and cultured cells subjected to environmental stress. A MADS-box gene, *IbMADS*10, was cloned from sweet potato, which might be correlated with anthocyanin biosynthesis (Lalusin *et al*., 2006). Hamada *et al*. (2006) cloned the starch-branching enzyme Ⅰ gene (*IbSBE* Ⅰ) from sweet potato and this gene might work in concert with the AGPase large subunit during the primary phase of starch granule formation. Tanaka *et al*. (2009) cloned the *SRF*1 gene encoding Dof zinc finger transcription factor preferentially expressed in storage roots of sweet potato, and it is suggested that *SRF*1 modulates carbohydrate metabolism in storage roots through negative regulation of a vacuolar invertase gene. Transgenic plants expressing cowpea trypsin inhibitor (*CpTI*), snowdrop lectin, delta-endotoxin, soybean kunitz trypsin inhibitor (*SKTI*-4), sweet potato feathery mottle virus (SPFMV-S) coat protein, granule-bound starch synthase Ⅰ (*GBSS* Ⅰ), tobacco microsomal ω-3 fatty acid desaturase (*NtFAD*3), starch branching enzyme (*IbSBE*) or *bar* gene have been produced (Newell *et al*., 1995; Morán *et al*., 1998; Cipriani *et al*.,1999; Okada *et al*., 2001; Kimura *et al*., 2001; Wakita *et al*., 2001;

Shimada *et al*., 2006; Otani *et al*., 2003, Yi *et al*., 2007; Choi *et al*., 2007). Low transformation efficiency has limited the successful application of genetic engineering in sweet potato improvement. Ukoskit *et al*. (1997) obtained a marker linked to a sweet potato root knot nematode resistance gene by using a bulked segregant analysis (BSA) – random amplified polymorphic DNA (RAPD) method. Using amplified fragment length polymorphism (AFLP) – analysis of molecular variance (AMOVA), Mcharo *et al*. (2005) later developed the markers linked to this gene. To date, three independent genetic maps of sweet potato have been reported (Ukoskit and Thompson, 1997; Kriegner *et al*., 2003; Cervantes-Flores *et al*., 2008). This paper summarizes advances made so far in embryogenic suspension cultures and plant regeneration, somatic hybridization and interspecific somatic hybrid production, cell induced mutation and mutant selection, gene cloning, genetic transformation and transgenic plant production, and development of molecular markers in sweet potato in China. Some future directions of research in omics and biotechnology of this crop in China are also suggested.

Embryogenic suspension cultures and plant regeneration

In China, tissue cultures of sweet potato began in the 1980s. Using approximately 100 sweet potato cultivars, several researchers reported plant regeneration in different tissues via organogenesis or somatic embryogenesis (Xin and Zhang, 1987; Liu *et al*., 1993; Tan *et al*., 1993; Liu *et al*., 1996; Liu *et al*., 1997). These results showed that sweet potato was recalcitrant to plant regeneration and in most cases the frequency of regeneration was very low. It was found that shoot apices of almost all genotypes could form bright-yellow and compact embryogenic callus on the medium supplemented with dichlorophenoxyacetic acid (2,4-D); however, the frequency of embryogenic callus formation remained very low in most genotypes (Liu *et al*., 1993; Tan *et al*., 1993; Liu *et al*., 1996; Liu *et al*., 1997). Chee and Cantliffe (1988, 1989), Chee *et al*. (1990), and Bieniek *et al*. (1995) succeeded in establishing embryogenic suspension cultures of sweet potato cv. White Star. Liu *et al*. (1996, 1997, 2001) improved on these results and developed an efficient system of embryogenic suspension cultures and plant regeneration for a wide range of genotypes, especially for commercial cultivars (Fig. 1). So far this embryogenic suspension culture system has been extended to more than 40 commercial cultivars of sweet potato and gives very high frequencies of plant regeneration ranging from 96.8% to 100% (Liu *et al*., 2001; unpublished data). The protocol includes:

(1) Induction of embryogenic callus: embryogenic calluses are induced from shoot apices about 0.5 mm in length on solid MS (Murashige and Skoog,1962) medium supplemented with 2.0 mg/l 2,4-D at 28℃ in the dark.

(2) Establishment of embryogenic suspension cultures: embryogenic callus is crushed into cell aggregates and free cells for initiating embryogenic suspension cultures in liquid MS medium containing 2.0 mg/l 2,4-D at 28℃ under 13 h of cool-white fluorescent light at 10 μmol $m^{-2}s^{-1}$. These cultures are maintained by subculturing at a 10-d interval.

(3) Proliferation of cell aggregates: sixteen to 20 weeks after initiation, cell aggregates 0.7-1.1 mm in size are transferred to solid MS medium with 2.0 mg/l 2,4-D for the proliferation of cell aggregates into embryogenic callus with somatic embryos at 28℃ in the dark.

(4) Regeneration of plants: embryogenic callus with somatic embryos is further transferred to solid MS medium with 1.0 mg/l abscisic acid (ABA) to induce the germination of somatic embryos and the regeneration of plants at 28℃ under 13 h of cool-white fluorescent light at 54 μmol $m^{-2}s^{-1}$.

Using the above embryogenic suspension cultures, it is possible to produce 100,000 to 120,000 plants in 35 to 38 weeks per embryogenic callus from a shoot apex. This regeneration system has great potential in somatic hybridization, in vitro selection of mutants, and genetic transformation of sweet potato.

Somatic hybridization and interspecific somatic hybrid production

The pioneering attempt to culture sweet potato protoplasts was made in the 1970s. Wu and Ma (1979) reported successful isolation of protoplasts and formation of callus. Liu *et al*. (1995) and Wang *et al*. (1997) observed low frequency plant regeneration from sweet potato protoplasts. High frequency plant regeneration was achieved in protoplast cultures of its relatives such as *I. triloba*, *I. lacunosa*, and *I. cairica* (Liu *et al*., 1991; Liu *et al*., 1995; Guo *et al*., 2006). The first interspecific somatic hybrid was produced between sweet potato cv. Kokei No.14 and *I. triloba* by fusing petiole protoplasts of two species using the polyethylene glycol (PEG) method (Liu *et al*., 1994). Using a similar protocol, Liu *et al*. (1998) and Wang *et al*. (2003) obtained a few interspecific somatic hybrid plants from the fused petiole protoplasts of sweet potato + *I. lacunosa* and sweet potato + *I. triloba*, respectively. The utilization of embryogenic suspension cultures for the isolation of sweet potato protoplasts greatly advanced somatic hybridization between sweet potato and its relatives. Approximately 5,000 interspecific somatic hybrids have been produced from more than 20 sexually incompatible combinations by fusing protoplasts from embryogenic suspension cultures of sweet potato and from petioles of the relatives (Zhang *et al*., 1999; Zhang *et al*., 2002; Guo *et al*., 2006; Yang *et al*., 2009; unpublished data). Yang *et al*. (2009) obtained a storage root-bearing somatic hybrid, designated KT1, between sweet potato cv. Kokei No.14 and *I. triloba* (Fig. 2). Genomic in situ hybridization (GISH) analysis confirmed the presence of chromosomes from both parents and recombinant chromosomes in KT1, and KT1 had significantly higher drought tolerance than its parent Kokei No.14. It has been showed that somatic hybridization through protoplast fusion is an effective tool for overcoming cross-incompatibility and generating useful interspecific somatic hybrids between sweet potato and its relatives. Although it is a promising approach, unfortunately success has not materialized fully and the promise has yet to be realized through release and successful use of new cultivars.

Cell induced mutation and mutant selection

Because genetic resources that exist for many cultivated crops are limited, and traditional breeding methods are less efficient, breeders have explored in vitro selection to cultivate new crop cultivars with stress tolerance, diseases resistance, and high quality. Sweet potato is a clonally propagated crop, and mutational breeding is an important approach for improving this crop. There were several reports on cell induced mutation and mutant selection in sweet potato. Liu *et al*. (1998) obtained regenerated plants from embryogenic suspension cultures of sweet potato cvs. Kokei No.14 and Lizixiang irradiated with 5 Gy to 25 Gy gamma-rays, in which variations in root skin color, root flesh color, and dry matter content were observed. Li *et al*. (2002) suggested that 80 Gy gamma-rays were optimal for irradiation of embryogenic suspension cultures and 30.0% PEG 6000 and 2.0% NaCl could be used as the optimal selection stress for in vitro selection of drought-and salt-tolerant mutants,

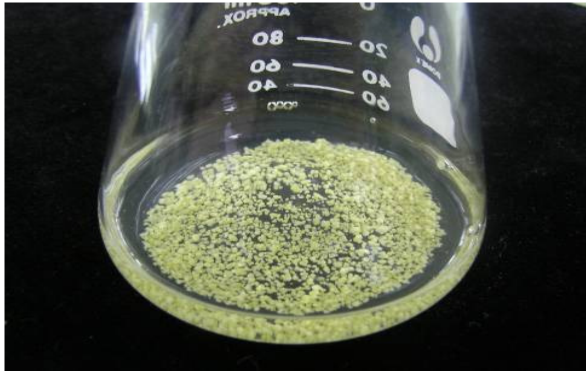

Fig 1. Embryogenic suspension cultures of sweet potato cv. Lizixiang

Fig 2. A storage root-bearing somatic hybrid KT1 (middle) of sweet potato cv. Kokei No. 14 (left) and its relative *I. triloba*.

respectively. Wang *et al*. (2003) obtained drought-tolerant variants from embryogenic suspension cultures of Lizixiang irradiated with 80 Gy gamma-rays by in vitro selection with 30.0% PEG. Embryogenic suspension cultures of sweet potato cv. Lizixiang were irradiated with ion beams $^{12}C^{5+}$ of 0-100 Gy and $^{4}He^{2+}$ of 0-200 Gy, and it was determined that the optimal dose of $^{12}C^{5+}$ and $^{4}He^{2+}$ was 30-50Gy and 50-70 Gy, respectively, based on the survival of the cultures and the frequency of plant regeneration (Wang *et al*., 2005). Using this method, they obtained mutants for leaf shape and root skin color from embryogenic suspension cultures of Lizixiang. Wang *et al*. (2007) reported production of a useful mutant by chronic irradiation of sweet potato. Sweet potato cv. Kokei No.14 was planted in a ^{60}Co gamma field. Shoot apices from the plants irradiated with different doses were cultured according to the method of Liu *et al*. (1993) and regenerated plants. From the regenerated plants, one useful mutant, named Nongdafu 14, was selected, in which the root flesh color changed from light-yellow into orange and carotenoid content of storage roots significantly increased compared to the wild-type (Fig. 3). Applying 0.5% ethylmethanesulphonate (EMS) to the callus of sweet potato cv. Lu 8, Luan *et al*. (2007) obtained salt-tolerant mutants after in vitro selection with 1.2% NaCl. In the study of He *et al*. (2009), embryogenic suspension cultures of sweet potato cv. Lizixiang irradiated with 80 Gy gamma-rays generated salt-tolerant mutants by multi-step selection with 2.0% NaCl. In conclusion, thus several novel sweet potato mutants have been produced through in vitro selection. These mutants are non-chimeras and will be applied in the cloning of important genes as well as in breeding of sweet potato.

Cloning of genes

Recently, high importance has been attached to the cloning of agronomically important genes from sweet potato in China. Liu *et al*. (2006) and Zhai and Liu (2009) reported that the cDNA (*IbMIP*-1) encoding myo inositol-1-phosphate synthase (MIPS) was cloned from a sweet potato mutant Nongda 601 resistant to stem nematodes, with a length of 1530 bp and encoding 510 amino acids. Sequence analysis indicated that *IbMIP*-1 had over 90% identity to *MIPS* genes from *Ricinus communis*, *Nicotiana tabacum*, *Sesamum indicum*, *Glycine max*, *Populus trichocarpa*, *Vigna radiata*, *Phaseolus vulgaris* and other plants. Real-time quantitative PCR analysis showed that the expression of *IbMIPS*-1 gene was induced by sweet potato stem nematodes, suggesting that this gene might be related to nematode resistance. Using salt-tolerant or high carotenoid content mutants of sweet potato, we have constructed cDNA libraries and cloned several genes related to salt tolerance and carotenoid biosynthesis, and are focusing on their functional characterization (Yang *et al*., 2007). Zhou *et al*. (2010) isolated an anthocyanidin synthase (ANS) gene from purple-fleshed sweet potato cv. Yamakawamurasaki, designated *IbANS*, with a 1,086 bp open reading frame (ORF) encoding a 362-amino acid polypeptide. In five cultivars of sweet potato, *IbANS* expression was strongly associated with anthocyanin accumulation and it was deduced that this gene could be associated with anthocyanin biosynthesis. *IbANS* gene was also cloned from purple-fleshed sweet potato cv. Yuzi 263 (Liu *et al*., 2010). Xu *et al*. (2010) reported cloning and characterization of the Rubisco activase gene from sweet potato. Although several genes have been isolated from sweet potato, their functions have not been analyzed in detail. It is more important for these genes to be utilized for the improvement of sweet potato.

Genetic transformation and transgenic plant production

Genetic transformation is a promising tool that can enhance improvement of sweet potato by enabling the introduction of desirable and commercially important traits into known genotypes, without altering their existing, highly selected genetic background. *Agrobacterium*-mediated transformation is a reliable, efficient and rapid gene transfer technique in sweet potato. Zhai and Liu (2003) obtained 33 GUS-positive plants from 1,304 inoculated cell aggregates from embryogenic suspension cultures of sweet potato cv. Lizixiang using *A. tumefaciens* strain A208SE harboring the binary vector pROA93 with *gusA* and *npt*Ⅱ genes. A few transgenic plants also were produced in this manner using *A. tumefaciens* strain LBA4404 with the binary vector pBinh with *oryzacystatin*-Ⅰ (*OC*Ⅰ) and *npt*Ⅱ genes (Jiang *et al*., 2004). Luo *et al*. (2006) developed a rapid genetic transformation system based on de novo (via callus) organogenesis from sweet potato leaves. Xing and colleagues (2008) introduced an engineered tandem-repeat starch-binding domain (*SBD*2) into sweet potato cv. Xu 55-2 and found that *SBD*2 expression in transgenic plants affected granule morphology without altering the primary structure of the constituent starch molecules. We succeeded in efficient *A. tumefaciens*-mediated transformation using embryogenic suspension cultures of sweet potato cv. Lizixiang (Yu *et al*., 2007). Addition of 30 mg/l acetosyringone (AS) to the co-cultivation medium significantly increased transformation

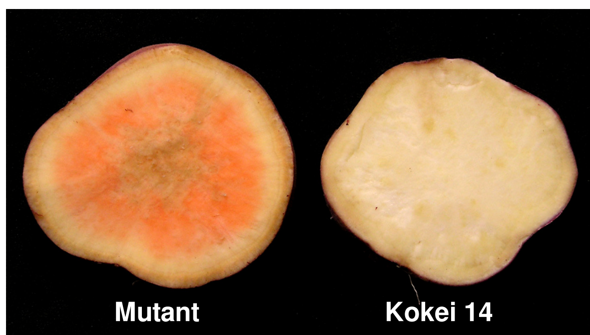

Fig 3. The mutant Nongdafu 14 (left) and its wild-type cv. Kokei No.14.

Fig 4. Transgenic plants of sweet potato cv. Lizixiang displaying complete Basta resistance (TB) and the untransformed control plants (CK) one day after spraying with 1,000 mg/l PPT of Basta.

efficiency. Cell aggregates from embryogenic suspension cultures were co-cultivated with the *A. tumefaciens* strain EHA105 harboring a binary vector pCAMBIA1301 with *gusA* and *hpt*Ⅱ genes for three days. Selection on cultures was conducted using 25 mg/l hygromycin (hyg). Cell aggregates of one gram fresh weight produced approximately 500 transgenic plants via somatic embryogenesis. *Agrobacterium tumefaciens* strain EHA105 is strongly recommended for genetic transformation of sweet potato embryogenic suspension cultures, a system that can be extended to other sweet potato cultivars. It is also possible to successfully transform recalcitrant sweet potato cultivars using this system. Zang *et al*. (2009) successfully developed transgenic plants exhibiting functional expression of the *bar* gene using the genetic transformation system established by Yu *et al*. (2007), in which selection was conducted with 0.5 mg/l phosphinothricin (PPT) (Fig. 4). The copy number of integrated *bar* gene ranged from one to three. This study also provides a simple and efficient transformation system for sweet potato using the *bar* gene as a selectable marker gene, which can be combined with other agronomically important genes for the improvement. *OC*Ⅰ, *LOS*5 (low expression of osmotically responsive), *SOS* (salt overly sensitive), and *IbMIPS*-1 genes now also have been introduced to sweet potato cultivars and have generated large numbers of transgenic plants.

Development of molecular markers

Sweet potato is hexaploid with 2n=6x=90. Because of self-incompatibility and a high degree of cross-incompatibility in sweet potato, conventional hybridization has been of limited use for improvement of this crop. Thus, marker-assisted selection techniques will be effective tools for improving diseases resistance, stresses tolerance, and quality in sweet potato. In China, stem nematode is one of the most serious diseases that limits sweet potato production. This disease usually decreases sweet potato yield by 20-50%, and even zero yield can occur in fields seriously infected by stem nematodes. Thus, breeding of sweet potato cultivars resistant to stem nematodes has become especially important. There is a significant negative correlation between stem nematode resistance and other important quality traits, which limits the improvement of these important traits by conventional hybridization (Ma *et al*., 1997). Recently, we developed a mapping population consisting of 202 individuals of a cross between 'Xu 781', a cultivar resistant to stem nematodes, with high starch content and low yield, and 'Xushu 18', which is susceptible to stem nematodes, has moderate starch content and high yield. A genetic linkage map of this population has been constructed, with 3,746 AFLP markers placed in the framework of linkage maps for the two parent cultivars. Using the above mapping population, Zhou *et al*. (2005) and Jiang *et al*. (2007) developed one RAPD marker linked to a stem nematode resistance gene at a genetic distance of over 17 cM. Jie *et al*. (2008, 2009) developed a sequence characterized amplified regions (SCAR) marker (14.2 cM) and two AFLP markers (6.9 cM and 11.1 cM) also linked to this resistance gene. Using the same mapping population, Li *et al*. (2008) developed two sequence-related amplification polymorphism (SRAP) markers linked to this same resistance gene with genetic distances of 4.86 cM and 4.17 cM, respectively.

Future directions

In China, sweet potato is an important food and industrial material crop and will be an alternative source for fuel alcohol production. High yield, high nutritional quality, diseases resistance, and salt and drought tolerances have been the breeding objectives for sweet potato in China. As mentioned above, biotechnology offers great potential for improving these traits. In fact, biotechnology has not been applied successfully to the improvement of sweet potato anywhere in the world. To expedite sweet potato improvement through biotechnology, the following directions for research in omics and biotechnology of this crop in China are proposed:

(1) Somatic hybridization is an alternative method to overcome intra- and inter-specific cross-incompatibility to a large extent. More somatic hybrids should be produced by somatic hybridization to enrich sweet potato germplasm for breeding use.

(2) Cell induced mutation can increase the frequency of useful genetic variation in sweet potato. Novel salt-tolerant, stem nematode-resistant, or high carotenoid content mutants have been developed through in vitro selection. These mutants could be used to isolate agronomically important genes from sweet potato.

(3) *A. tumefaciens*-mediated transformation has been established using embryogenic suspension cultures of sweet potato. Introduction of agronomically important genes to commercial sweet potato cultivars should be conducted in a large scale.

(4) High-density genetic linkage maps should be constructed. Molecular markers tightly linked to agronomicaly important traits or genes can be developed and utilized in the selection of new cultivars. QTLs for high yield and high starch content should be mapped and cloned. A further goal for the

near future should be to obtain the complete sequence of the sweet potato genome.

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