Fruit, Vegetable and Cereal Science and Biotechnology ©2012 Global Science Books

Sweet Potato: Gains through Biotechnology

Archana Mukherjee* • **Samir Kanti Naskar** •
Korada Rajasekhara Rao • **Ramesh Chandra Ray**

Regional Centre of Central Tuber Crops Research Institute, Bhubaneswar – 751 019, India

Corresponding author: * **archanapsm2@rediffmail.com**

ABSTRACT

Gains through application of modern plant biotechnological tools in any horticultural crop are enormous and sweet potato is no exception. Recently biotechnological work in sweet potato has gained momentum in many national and international laboratories. Techniques like micropropagation through axillary shoot proliferation, organogenesis, embryogenesis and artificial seeds have led rapid propagation and reduction of diseases. Cryostorage and DNA finger printing techniques provide safer conservation and rapid characterization of vast genetic resources efficiently with minimum inputs. On the other hand, genetic engineering coupled with tissue culture technology is redesigning the crops to make it more productive. Development of transgenic sweet potato for resistance to weevil, feathery mottle virus and fungal diseases have been reported in international and national laboratories. Genetic engineering for higher protein content are also found to be quite successful in sweet potato. *In vitro* methodologies have also been developed for faster screening and evaluation of large collections of sweet potato for tolerance to salinity stress. Paclobutrazol (PBZ) and $CaCl_2$ mediated submergence tolerance was also reported in sweet potato. Days are ahead to have nutritionally enriched, disease, pest and salt tolerant sweet potato as source of food, nutrition security and economic sustainability in sweet potato growing countries around the world.

Keywords: sweet potato (*Ipomoea batatas* L.), micropropagation, *in-vitro* storage, DNA markers, genetic transformation, enhanced nutritional value, resistance to biotic and abiotic stresses

Abbreviations: **AFLP**, amplified fragment length polymorphism; **BA**, 6-benzyl adenine; **CAT**, catalase; **CIP**, International Potato Center; **GPX**, guaiacol peroxidase; **ISSR**, inter-simple sequence repeat; **PBZ**, paclobutrazol; **PCA**, principal component analysis; **PEG**, polyethelene glycol; **RAPD**, random amplified polymorphic DNA; **RFLP**, restriction fragment length polymorphism; **SOD**, super oxide dismutase; **SPFMV**, *Sweet potato feathery mottle virus*; **SSR**, simple sequence repeat; **TDZ**, thidiazuron; **UPGMA**, unweighted pair group method with arithmetic mean; **2,4-D**, 2,4-dichlorophenoxyacetic acid; **2,4,5-T**, 2,4,5-trichlorophenoxyacetic acid

CONTENTS

INTRODUCTION

Sweet potato (*Ipomoea batatas* L.) ranks seventh most important food crop of the world. The advantages of sweet potato are well documented (Woolfe 1993). This crop is gaining importance not only for high dry matter production per unit area per unit time but also as cheapest source of anti-oxidants. Being rich in β-carotene and anthocyanin orange as well as purple fleshed sweet potato are most suited as biofortified crop to combat malnutrition in many developing nations (Mukherjee *et al.* 2008, 2009a). Despite a big reservoir of food, energy and nutrition, sweet potato faces a few biological drawbacks. Propagation is vegetative by tuber or vine cuttings, which limits the production of sufficient quantity of healthy planting materials throughout the year. Clonal maintenance of genetic resources in field gene bank makes the crop more vulnerable to biotic and abiotic stresses. *In vitro* propagation and storage to a great extent can help to overcome these limitations (Mukherjee 1999a).

The crop is nutritionally low in protein. Productivity of the crop is severely affected due to weevil as major pest menace and feathery mottle virus disease. Sweet potato weevil (*Cylas formicarius* F.) is the most destructive one, especially in the tropics (Horton and Ewell 1991). This insect feeds on roots in the field and in storage, reducing the quality and marketable yield of sweet potato. Weevil is difficult to be controlled in the tropics because it continuously reproduces throughout the year. Although the sweet potato weevil damage is the most important constraint, damage brought about by plant diseases such as plant virus caused by *Sweet potato feathery mottle virus* (SPFMV) cannot be negated. The most effective management strategy to minimize losses includes the use of resistant varieties. However, there is very little source of resistance to weevil and SPFMV in the sweet potato germplasm. Although there is extensive genetic variability in sweet potato, it is hexaploid and thus, difficult to be improved through conven-

Received: 1 November, 2011. Accepted: 1 November, 2012.

Invited Review

tional breeding (Lowe *et al.* 1994). Further, there are problems of self incompatibility, cross incompatibility as well as instability in hybrid offspring. In this context, biotechnology, especially transgenic research has opened new avenues towards the solution of agri horticultural problems (Mukherjee 2003). Genetically or metabolically engineered sweet potato with higher nutrient contents and resistance to weevil, other diseases and pests will have more commercial importance than any other long duration starch based crop.

Though sweet potato is relatively resistant to moisture stress because of its deep-root system, it is highly sensitive to salinity and water logging. In recent years, such abiotic stresses are becoming predominant as a consequence of global warming (Mukherjee 2011).

Blending the resistance traits of traditional varieties through modern plant breeding will ensure that new varieties are bred to suit specific requirements of local conditions. Salinity and flooding are important factors affecting sweet potato production (Lin *et al.* 2008; Mukherjee *et al.* 2009a). Development of stress tolerant lines of sweet potato will have greater agricultural implications to enhance the productivity and for promoting food and nutritional security (Mukherjee *et al.* 2009a, 2009b).

One of the most effective ways to overcome salinity problems is to identify and grow salt tolerant plants or varieties. Selection and development of suitable genotypes for this purpose requires an efficient screening method (Mukherjee *et al.* 2009a). *In vitro* technology offers a meaningful tool for characterizing salt tolerant plants and also for quick evaluation of germplasm against salt stress under controlled conditions with limited time and space (Gosal and Bajaj 1984). Hydroponics, axillary bud or shoot apex culture has been found to be an effective method for testing and selecting salt tolerant genotypes (Mukherjee *et al*. 1994; Martinez *et al*. 1996; Mukherjee 2001, 2002a). Abiotic stresses like salinity and submergence stress triggers a wide range of plant responses starting from change in morpho-physio-biochemical properties including antioxidative systems. Such studies are important in developing breeding strategies to confer stress tolerance to any crop species.

In sweet potato, $CaCl_2$ and PBZ mediated flooding tolerance through enhanced antioxidative system have been studied (Kuan-Hung *et al.* 2006; Lin *et al.* 2008). Similarly NaCl mediated *in vitro* and *in vivo* large scale screening and evaluation have enabled to identify sweet potato genotypes with salt tolerance traits (Mukherjee 2005; Mukherjee *et al.* 2007, 2009a).

This review reflects the work carried out on *in vitro* propagation, storage, marker based characterization, genetic transformation, tolerance to biotic and abiotic stresses as well as future strategies towards agro biotechnological development of sweet potato as source of food, nutrition security and economic sustainability.

MICROPROPAGATION

Micropropagation is an efficient technique for rapid propagation from small sample. It enables multiplication of plants round-the-year. As plants can be freed from diseases and pests under controlled conditions, *in vitro* propagules minimize the quarantine regulations and ensure safe exchange of genetic resources (Mukherjee 1999a, 1999b). Production of 'true to type' plants at a rapid rate, compared to conventional techniques is the most useful application of micropropagation technique.

Micropropagation technique can be achieved through axillary bud development, adventitious organogenesis or through somatic embryogenesis and artificial seeds. Each technique has its own merits and demerits, but the propagation in all cases involves three basic steps (i) establishment of aseptic culture, (ii) rapid multiplication and (iii) establishment of plants in field. Micropropagation techniques are also ecofriendly. To optimize *in vitro* propagation technique, studies on micropropagation of tuber crops through all possible modes of *in vitro* propagation have been carried out at Central Tuber Crops Research Institute, India and elsewhere. Various micropropagation techniques used in sweet potato are as follows.

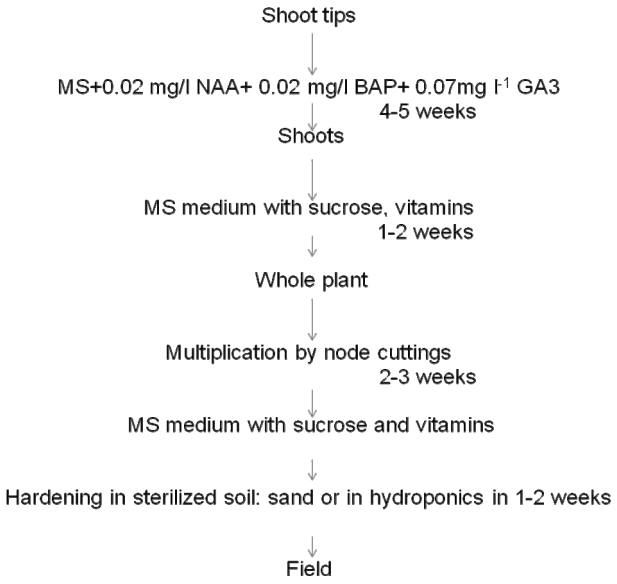

Fig. 1 Shoot tip culture and micropropagation in sweet potato.

Shoot tip, meristem and axillary bud culture

Sweet potato genetic resources are often affected by virus diseases. Important among them are sweet potato feathery mottle virus (SPFMV) diseases. Spread of diseases through vegetative propagation affects their productivity and storage of genetic resources. Elimination of virus infection is thus the most essential step prior to rapid multiplication of plants. Normally, the apical meristem (0.3-0.5 mm) of the virus-infected plant is almost disease free. Regeneration from meristem is the only method to recover healthy plants from virus infected material. Meristem or shoot tip culture techniques coupled with thermotherapy and chemotherapy can eliminate viruses. The protocols of shoot tip culture and micropropagation to suit their cultivars have been standardized. In general, MS salts (Murashige and Skoog 1962), MS vitamins, inositol (100 mg l^{-1}), sucrose (3%) and agar (0.8%) at 5.7-5.8 pH are used as basic medium for *in vitro* culture of sweet potato (Mukherjee 1999a). Shoot tip cultures revealed that vitamins have profound effects on proliferation and propagation of shoot tip explants of sweet potato (Hossain and Sultana 1997).

Protocols to produce shoot tip culture to eliminate viruses in sweet potato have been developed (Mori 1971; Alconero *et al.* 1975; Henderson *et al.* 1984; Love *et al.* 1987; Lizzaraga *et al.* 1992). Procedure for rapid multiplication of diseases free plants was developed for more than 200 genetic resources through shoot tip and axillary bud cultures (Mukherjee *et al.* 1994, Mukherjee 1999a) is presented (**Fig. 1**). Shoot tips (0.3-0.4 mm) can be developed into shoots and whole plant by successive culturing in MS medium supplemented with 0.02 mg l^{-1} NAA, 0.02 mg l^{-1} BA and 0.7 mg l^{-1} GA_3 for 4-5 weeks and on transfer to MS medium without growth regulators (Mukherjee 1999a; Mukherjee 2001).

Whole plants from field sources also can be multiplied by culturing nodes (4-5 mm) on MS medium supplemented with NAA (0.5 mg l^{-1}), BA (1 mg l^{-1}) and GA_3 (0.05-0.1 mg l^{-1}).

Virus elimination techniques have been used routinely for the production of disease-free sweet potato planting material. Disease-free material is used for commercial planting of sweet potato in South Africa, and has been an efficient way of suppressing the level of disease present in the field (Van Zijl and Botha 1998).

Somatic embryogenesis

In vitro techniques like meristem culture, shoot tip culture and axillary bud culture are used to efficiently clean and multiply vegetatively propagated crops. On the other hand, *in vitro* somatic embryogenesis offers a better option for propagation and storage.

Embryos being the product of somatic embryogenesis, is more or less similar to a zygotic embryo in a seed comprising embryogenic shoot and root tissue and this bipolar entity allows it to develop into a small plant. Besides the high rate of multiplication, somatic embryogenesis has opened up the possibility of production of artificial seeds by encapsulating the embryos in protective nutrient coating. However, the efficacy of such techniques greatly depends on high reproducibility, homogeneity and genetic stability of the embryogenic regeneration system (Prakash 1994; Mukherjee 1999a).

Regeneration through oraganogenesis or somatic embryogenesis has been developed in different cultivars of sweet potato by culturing leaf, shoot and root explants on MS medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D), kinetin, Picolinic acid, thidiazuron and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Though high frequency regeneration protocols have been developed, most of the results are specific to certain genotypes. Optimization of the concentration of 2,4-D or 2,4-D and kinetin or addition of thidiazuron or picolinic acid could enhance the regeneration response (Mukherjee *et al.* 1991a; Chee and Cantliffe 1992; Mukherjee *et al.* 1993; Desamero *et al.* 1994; Gosukonda *et al.* 1995; Mukherjee *et al.* 1998a; Mukherjee 2002a; Mukherjee and Debata 2002). Use of 2,4,5-T was reported to be more effective than 2,4-D (Almazrooei *et al.* 1997). Exploitation of somatic embryogenesis protocol for genetic transformation and artificial seeding depends on the homogeneity and genetic stability of the regeneration system. Studies on these aspects have been carried out in India by Mukherjee (1999a, 1999b) and Mukherjee *et al.* (2003a). Liu *et al* (2001) developed a system for embryogenic suspension cultures for a wide range of sweet potato genotypes especially for commercial cultivars. Use of β-glucuronidase (GUS) as a reporter optimizes *Agrobacterium*-mediated transformation parameters for sweet potato embryogenic callus (Xing 2007). Plant regeneration and transformation in sweet potato has been obtained by culture of leaf segments (Chen 2010).

Plants can be regenerated through callusing and somatic embryogenesis from stem, leaf, petiole and root explants on subsequent cultures to 2,4-D or 2,4-D+BA (0.5 to 2 mg l^{-1}) supplemented MS to plain MS medium (Mukherjee *et al.* 1998a, 1998b, 2001, 2003). Embryogenic response is observed to be influenced by genotype, growth regulators as well as by explants. Variation in embryogenic response among the genotypes with different growth regulators was quite pronounced. Positive influence of 2,4-D alone in genotype S132 and with BA in other genotypes like 8570 and 8516 have been recorded during embryogenesis. Experiments with different explants exhibited better embryogenic response with leaf and root as compared to petiole and stem explants which required BA along with 2,4-D. Enhanced and synchronous embryogenic response has also been recorded in those 4 different genotypes with addition of NaCl (5 g l^{-1}) or L-proline (700 mg l^{-1}) in 2,4-D (0.2 mg l^{-1})-supplemented MS medium (Mukherjee 1999b). Though the embryos could germinate in plain MS medium, different media combinations were also tested to enhance the rate of germination. Rate of germination was found to be influenced more with embryo induction medium (Mukherjee *et al.* 2003b).

Artificial seeds

Enacapsulated somatic embryos or artificial seeds have been considered to be the novel substitutes for true seeds of clonal crops. Artificial seeds, also known as synthetic seeds

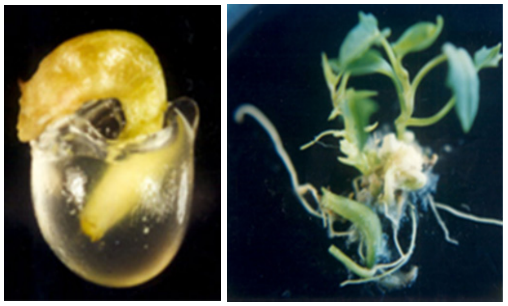

Fig. 2 Encapsulated somatic embryo (artificial seeds) of sweet potato germinating after storage. Source: Mukherjee 2002a.

are good quality somatic embryos or propagules enclosed in protective nutrient coating. Artificial seed technology provides a rapid, low volume, cost competitive propagation method. *In vitro* somatic embryogenesis facilitates the production of 'artificial seeds', provided synchronization in development and germination of embryos is achieved (Mukherjee 1999a, 2002a, 2002b).

Production of artificial seeds in sweet potato has been attempted by Chee and Cantliffe (1992). Scientists at the University of Florida have developed a system to produce artificial seeds of the sweet potato cultivar white star encapsulated in gels (Prakash 1994).

Addition of stress inducing chemicals in regenerating medium was also found to be quite effective to enhance the rate of multiplication significantly and to yield hardy somatic embryos (Mukherjee 2002a). Enhanced and synchronous embryogenic response registered in four different genotypes with addition of NaCl or L-proline in 2,4-D supplemented MS medium may favour artificial seeding in sweet potato (Mukherjee 2002a; Mukherjee *et al.* 2003b). The hardy embryos of sweet potato produced in NaCl medium could be stored at 8°C with protective alginate covering. Embryos encapsulated with Na-alginate (artificial seeds) could be stored for 30 days and can grow into plants in culture medium and also on direct sowing (**Fig. 2**) in sterilized soil (Mukherjee 2002a). Hardy non capsulated somatic embryos could be stored and germinate in *vivo* and *in vitro* (Mukherjee and Debata 2002). However, percentage germination of encapsulated embryos was much higher than non capsulated embryos *in vivo* and *in vitro*. Yield and quality of storage roots developed from artificial seed propagated plants were found to be at par with source plants. Analyses of isozyme and random amplified polymorphic DNA (RAPD) profiles confirmed the genetic homology of the plants raised from artificial seeds with source plants (Mukherjee 2002a; Mukherjee *et al.* 2003a). Thus, production of artificial seeds can be a novel substitute for true seeds for propagation of vegetatively propagated crops like sweet potato.

Organogenic regeneration

Regeneration can also be achieved by adventitious budding on the cultured explants and also from callus tissues lacking preformed meristems. This mode of regeneration especially direct shoot organogenesis is gaining importance for its application in genetic transformation studies for the production of transgenic plants. Shoot organogenesis is found successful in sweet potato directly or through tuberization from *in vitro* roots induced from leaf explants when cultured in plain MS medium at 12 h photoperiod (Mukherjee *et al.* 1993).

The National Aeronautics and Space Administration (NASA) has chosen sweet potato as one of eight crops to be grown for long term space missions. United States Agency for International Development (USAID), USDA, NASA and scientists at Tuskegee University (USA) have developed a tissue culture system that enabled them to quickly produce large numbers of adventitious sweet potato plants

(Prakash 1994).

Significant enhancement in the organogenic regeneration frequency was achieved with supplementation of NaCl (200 mg l^{-1}) or proline (100 mg l^{-1}). Sodium chloride (200 mg l^{-1}) was found to be most responsive to give 40-84% explant response and produced 6.5-7.5 shoots per 50 mg of callus (Mukherjee 1999b; Mukherjee and Debata 2001). The highest percentage of shoot induction (30.9%) was obtained on MS medium containing 10 mg l^{-1} kanamycin, 1.0 mg l^{-1} ABA and 1 mg l^{-1} GA_3 (Xing 2008).

In vitro tuberization

In vitro tubers are less vulnerable to adverse environments compared to *in vitro* plants. Transit and handling are also easy for tubers as excellent means for propagation.

In sweet potato *in vitro* tubers have developed from internodes, fibrous roots and leaf explants. Tuber formation from internode segments in presence of kinetin and auxin is reported by Houndonoughbo (1989), from fibrous root at 8 per cent sucrose with 1 or 5 mg l^{-1} BA by Aboul and Bouwkamp (1990) and from leaf explant in MS medium supplemented with or without NAA (0.2 mg l^{-1}) at 12 h photoperiod by Mukherjee *et al.* (1996, 1999b) and Mukherjee and Debata (2001). Menzel *et al.* (1980) reported that growth retardants stimulate tuberization under unfavorable environmental conditions. Vecchio *et al.* (1994) reported that *in vitro* tuberization is also genotype and medium dependent.

Field establishment of micropropagated plants

Field establishment necessitates hardening of *in vitro* grown plants so that they can tolerate stress and adapt to natural environment. Plantlets can be hardened in sterilized soil and sand mixture (1:1) in moist chamber for 72 h and later under shade for 4-6 weeks before transferring to the field (**Fig. 3**). Regenerated plants of sweet potato can be hardened in sterilized soil and sand mixture as have been followed for other tuber crops. *In vitro* grown plants of sweet potato can also be hardened in hydroponics prior to their transplantation in soil (Mukherjee *et al.* 1994; Mukherjee 1999a).

A study was conducted to compare growth and yield characteristics between the tissue culture regenerated and conventionally propagated sweet potato cultivars. Conventional propagation method gave highest growth rate, however, the difference in yield between the conventional propagation and tissue culture regenerated plants did not vary significantly. Likewise, virus detection for SPFMV by ELISA established that field plants had a higher virus titre compared to the tissue culture regenerated plants (Ogeema *et al.* 2007). Thus, *in vitro* raised shoot apical meristem culture coupled with heat or chemotherapy are being followed to clean virus infected plants. Field establishment, serodiagonosis and subsequent evaluation will ensure the quality of *in vitro* raised plants.

Monitoring for variation and contamination of micropropagated plants

The objectives of *in vitro* propagation cannot be achieved fully unless it assures stability of the rate of propagation as well as genetic stability of the propagules (Mukherjee 1999b; Mukherjee *et al.* 2003a). Chances of getting genetically variant regenerants are less when it is developed directly from the explant in contrast to those obtained through a callus phase. It is evident from in *vitro* regeneration studies that genetic changes are occurring during regeneration and are transmitted to the regenerants and their progenies. Such heritable variations are known as 'somaclonal variations' which is not desirable for a program laid out with the objectives of 'true to type' propagation. Hence, micro propagated plants need to be ensured that they are disease free and no secondary infection has occurred in the field or in nursery through disease indexing techniques. At this stage, those plants need to be tested for genetic purity to ensure 'true to type' as source or mother plants for maintaining the quality.

Fig. 3 *In vitro* raised hardened sweet potato (genotype CIPSWA-2) plants.

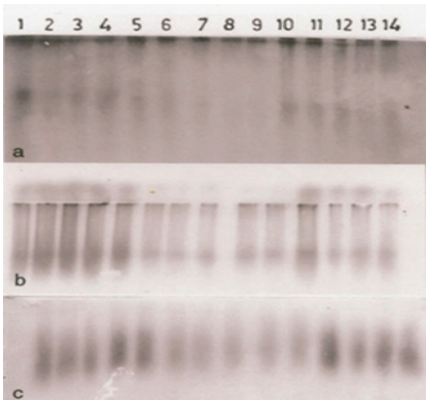

Fig. 4 Isozyme profiles of source plants (1-4) and regenerants (5-14) in sweet potato genotype S132. (a) Esterase; (b) Peroxidase; (c) Acid phosphatase. Source: Mukherjee 2002a.

To asses the phenotypic and genotypic stability, regenerated plants established in soil can be screened with source plant or mother clone for possible phenotypic variations. If variations are observed, variants need to be evaluated further for their cyto-biochemical characteristics to confirm the genetic stability employing cytological, biochemical and molecular marker techniques like isozyme and DNA markers (Mukherjee *et al.* 2001, 2002a, 2002b, 2003a).

In an experiment, plantlets developed from somatic embryos were hardened in hydroponics as per the methodology of Mukherjee *et al.* (1994) and were established in field for comparative evaluation of yield and other cyto-morpho-biochemical characteristics with source plants. Root tip cytology of the regenerants revealed chromosomal homology with source plants (Mukherjee *et al.* 1998a). Random amplified polymorphic DNA (RAPD) studies of regenerants and source plants also showed similarity in banding patterns (**Fig. 4**). No significant variations in yield and other characteristics are observed among the regenerants and between regenerants and source plants, thus implying genetic stability of the regeneration system. Histo-morpho-cytological evaluations of 3-year-old repetitive embryogenic callus cultures have also established cyto-histo-morphological stability of the regeneration system (Mukherjee *et al.* 1998a, 2001, 2003a). Such genetically stable propagules can be used for further multiplication.

Thus, micropropagation, isozyme and DNA marker techniques coupled with conventional breeding can shorten the breeding cycle and can generate sufficient quantity of quality planting materials at will.

CONSERVATION AND STORAGE

Sweet potato has a very high genetic variability and thousands of varieties of sweet potato exist in germplasm collections. As sweet potato is vegetatively propagated, the maintenance of its germplasm in gene banks is a laborious task. Tissue culture assists in the storage of disease-free collections and facilitates easier maintenance and distribution of germplasm (Prakash 1994).

In fact, tools like tissue culture and molecular markers can help to conserve and characterize germplasm of sweet potato efficiently with minimum inputs. Of the different storage protocols, minimal growth culture and cryostorage protocols are the basic tools to develop *in vitro* gene bank for vegetatively propagated crops.

Minimal growth (slow growth) storage is the most direct way of restricting growth and development of *in vitro* materials (plantlets, meristem cultures or callus masses). It can be achieved in different ways like reduced temperature or light and by media manipulation. Media alterations are done by addition of osmotic retardants like mannitol or by growth retardants or by reduced nutritional status with reduced carbon. The advantage of this storage is that cultures can be readily brought back to normal conditions on demand to produce plants (Mukherjee 1999a).

Slow growth culture method has been developed to store sweet potato for 1-2 years by monitoring temperature (18-28°C) or with addition of mannitol (10-30 g l^{-1}) or reduction of sucrose from 30 to 10 g l^{-1} (Nair *et al.* 1994; Mandal and Chandel 1996). Storage at 8°C could extend the culture life for 2-3 years (Dodds 1989).

The International Potato Center (CIP) in Peru, which has the global mandate for sweet potato research, has more than 4,000 accessions in its collection. The Asian Vegetable Research and Development Center (AVRDC) in Taiwan and the Agricultural Research Service of the United States Department of Agriculture (ARS/USDA) also hold extensive collections. Sweet potato germplasm is stored *in vitro* in all these laboratories (Prakash 1994).

Long term storage or cryostorage provides 'indefinite storage' system for organ, tissue or cells at ultra low temperature (at -196°C using liquid nitrogen) by reducing metabolism significantly with no biological deterioration. Long term storage lessens the risks associated with slow growth storage. Despite the genetic stability inherent in organized plant structures such as meristem, there are risk to DNA structure, integrated metabolism and viability under slow growth such as resulting from the oxidative activity of free radicals (Benson 1990). Cryopreservation is safer, economical and space saving method for a long term storage (Kartha 1985; Withers 1987).

In sweet potato, cryopreservation of embryogenic tissue has been attempted by Blakesley *et al.* (1995) and Bhatti *et al.* (1997). Their protocols were based on encapsulation, sucrose pre-treatment and evaporative dehydration prior to freezing. Survival of embryogenic tissue was reported to be 4-38% depending on the genotype.

IN VITRO TOOLS TO RECOVER RARE HYBRIDS

While minimal growth and cryostorage provide safer storage of valuable genetic resources, embryo culture and protoplast culture help in rescuing rare hybrids. Embryo culture is an aid to conventional breeding to overcome the limitations of post fertilization barriers like embryo-endosperm incompatibility and seedling lethality. Embryo rescue technique was developed in sweet potato to recover the hybrids from immature embryos (Mukherjee *et al.* 1991b). Rare hybrids of exotic species of sweet potato and its wild relatives viz. *I. trifida*, *I. cairica*, and *I. murocoides* could be established through *in vitro* embryo culture and seed germination techniques (Mukherjee 1994; Mukherjee and Vimala 1994). An agrobacterium mediated transformation method based on *de novo* organogenesis (callus) has been developed from petioles with leaf for sweet potato (Luo 2006) to recover rare hybrids. Plant regeneration was achieved in protoplasts cultures of sweet potato relatives viz. *I. triloba*, *I. lacunose* and *I. cairica* (Liu *et al.* 1991; Guo *et al.* 2006). Somatic hybrids were also produced by fusing protoplast of sweet potato + *I. triloba* (Liu *et al.* 1994; Wang *et al.* 2003), sweet potato + *I. lacunose* (Liu *et al.* 1998). A significant number of somatic hybrids was produced from sexually incompatible combinations through protoplast fusion of embryogenic suspension cultures of sweet potato and petioles of the relatives (Zhang *et al.* 1999; Zhang *et al.* 2002; Guo *et al.* 2006). Yang *et al.* (2009) obtained storage root bearing somatic hybrid from incompatible combination of sweet potato and *I. triloba.*

Protoplast culture

Isolation of viable protoplast from sweet potato and some of its wild progenitors have been reported by many researchers (Wu and Ma 1979; Eilers *et al.* 1985; Sihachakr and Ducreux 1987; Kobyashi *et al.* 1990; Ozias and Perera 1990; Belarmino *et al.* 1994, 1996). Stem, petiole and leaf explants or callus tissue produced from those explants have been digested either with Cellulase (2%) alone or Cellulase (2%) with Macerozyme (0.05%), Pectolyase (0.3%), Hemicellulase (0.5%) and Driselase (1%) at pH 5.6 to release protoplasts. Belarmino *et al.* (1996) regenerated plants from stem and petiole protoplasts of sweet potato and *Ipomoea lacunosa*. They also reported asymmetric protoplast fusion between sweet potato and *Ipomoea trifida* by electro fusion and polyethelene glycol (PEG) treatment. As they could regenerate only from electrofused protoplast and suggested that PEG might be toxic to the system. They characterized the interspecific hybrids through isozyme analysis. Results of the experiments on protoplast isolation from 4 different genotypes (S 132, 187004.3, 8570 and 8516) and 2 wild progenitors (*I. aquatica* and *I. cairica*) have revealed that young unfolded leaves were better sources for viable protoplast than mature leaves. Protoplasts of young leaves have registered higher density and viability. Enzymes Cellulase (4%) Macerozyme (3%) and Pectolyase (0.08%) were used for cell wall digestion (Mukherjee 1999a).

DNA MARKERS FOR GENETIC RELATEDNESS AND BREEDING

Breeders have used phenotypic markers to follow segregation of genes but most of the agronomic characters are not associated with scorable phenotypic characters. Discovery of restriction enzymes that cleave DNA at sequence-specific sites has evolved the molecular marker technology by splicing any specific segment of DNA and the traits associated with it (Thro *et al.* 1994). Genetic analysis for morphological markers is few (Hahn *et al.* 1989). Application of molecular and biochemical markers may help breeders to tag agronomic traits and to screen those at early stages of growth (Mukherjee 1999a).

The DNA molecular markers exist in every genotypes; their number may vary from hundreds to thousands. They are stable markers and are independent of plants growth stage and external environment. As a result, molecular markers can be scored using DNA from seedlings and plants at any stage of growth in any environment. Interdisciplinary research is required to establish molecular marker associated breeding and selection for the traits of agronomic or market interest.

DNA markers

A variety of molecular techniques have been developed for measuring genetic variability in sweet potato. Restriction fragment length polymorphism (RFLP) markers have been used to assess the relationship between cultivated sweet potato and wild *Ipomoea* species (Prakash 1994). The PCR based fingerprints, such as amplified fragment length polymorphism (AFLP) and RAPD are more discriminatory but

often had much less desirable reproducibility. However, PCR based markers are fast and enables to screen 200 sweet potato varieties in one day. Analysis of preliminary results reveal considerable genetic variation in sweet potato germplasm collected from across the globe but little variation in US sweet potato varieties. The RAPD had been used in cultivar fingerprinting (Connolly *et al.* 1994) and estimation of genetic diversity (Sagredo *et al.* 1998). Huang and Sun (2000) used inter-simple sequence repeat (ISSR) and restriction analysis of chloroplast DNA in the study of genetic relationships of cultivated sweet potato and its wild relatives. The DNA fingerprinting techniques can thus be employed to assess genetic variation and also to identify duplicates in the germplasm collection.

The RAPD analysis was done for 190 Indonesian local cultivars collected from 14 collection sites, along with 23 cultivars from Japan, the Philippines and Papua New Guinea. Fourteen arbitrary 12-mer primers used for the study resulted in 50 polymorphic bands. Analysis of gene diversity suggested that genetic variation in local cultivars is partitioned within regional groups rather than between them. However, a principal component analysis (PCA) based on the genetic similarity indicated weak genetic differentiation between regional groups. Also, cluster analysis indicated that the genetic relationship between regional groups is partially associated with the geographical relationship between collection sites. These results suggest the existence of geographical variations among the Indonesian cultivars. Comparison of gene diversity in each collection site suggested that cultivars in the northern part of Sulawesi Island had the highest degree of diversity among the regional groups analyzed as have been explained by Tanaka *et al.* in 2006.

The DNA fingerprints are also valuable to breeders by enabling them to identify divergent parental lines for hybridization and to monitor somatic hybrids and somaclonal variation. Simple sequence repeats (SSRs) DNA markers are found to be useful in determination of the genetic relationship of sweet potato polycross breeding lines.

The SSRs markers produce easily scorable, unique alleles and/or allele combinations, which make them an ideal system for cultivar identification. Moreover, many have reproducible fingerprints for germplasm management. They were used to analyze the genetic diversity and genetic relationships among sweet potato cultivars. The cultivars consisted of Chinese and Japanese materials, landraces as well as cultivars derived either from hybrid or polycross breeding in Taiwan. The SSR analysis from eight primer pairs revealed a total of 20 alleles of which 17 were polymorphic (85% polymorphism). An average of 2.5 alleles was obtained per SSR primer pair. On average 2.1 alleles per polymorphic SSR locus were amplified. The construction of genetic relationships using unweighted pair group method with arithmetic mean (UPGMA) and PCA demonstrated the capability of simple sequence repeats in sweet potato genotype identification and classification of genetic relationships. The UPGMA clustering and PCA revealed that polycross derived cultivars possessed high levels of genetic diversity and originated from various genetic resources, and suggested the usefulness of polycross breeding strategy in spite of frequent cross-incompatibility. Moreover, high level of genetic variation in polycross breeding lines would assist in obtaining elite sweet potato materials in the future. In addition, most landraces were distantly related to the Chinese and Japanese materials and probably originated from Java and Brahman (Hwang *et al.* 2002).

The DNA molecular markers are also very helpful to identify and create heterotic gene pools but to a lower extent for selection of the best parental combinations within heterotic gene pools. Heterosis is significantly important for polyploid species. Heterosis is larger if parents are recombined which have a high general combining ability. Estimation of the correlations between genetic distances and heterosis under drought and non-drought stress conditions for each: (i) intra-gene pool and (ii) inter-gene pool cross population will allow to make conclusions to which extent SSR markers can be used for gene pool division and cross prediction. In the case of sweetpotato breeding it is specified that the American 'Jewel Gene Pool' and the African 'SPK004 Gene Pool' might have exploitable heterosis in clone families which can improve yields in both regions of the world (Ghislan *et al.* 2004). Such knowledge is useful for the improvement of breeding efficiency.

How molecular markers can improve genotypes in a molecular breeding programme

Molecular markers help in detecting QTLs. A QTL is a position in a chromosome that contains one or more polygenes and involved in determination of a quantitative trait. It is impossible to know the inheritance pattern of individual polygenes. As a result these genes cannot be mapped using conventional breeding programme. QTL mapping involves testing DNA markers throughout the genome for the likelihood that they are associated with a QTL. Individuals in a mapping population are analysed in terms of DNA marker genotype and phenotype of interest. For each DNA marker the individual are split into classes according to marker genotype. Mean and variance parameters of phenotype of interest are calculated and compared among the marker genotype classes. A significant difference between the DNA marker and trait of interest indicates a linkage between DNA marker and a trait of interest i.e. DNA marker is probably linked to a QTL controlling the phenotype of interest. The mapping population used for mapping DNA markers are F_2, F_3, RILs, NILs, and Double Haploids. After identification of QTLs, these will be introgressed in other genotypes of interest and improve them. Molecular markers can be used for negative selection i.e. for elimination of undesirable gene in segregating population. Molecular markers are very useful for plant variety protection i.e., in unequivocal identification of plant varieties. Molecular markers would enable an effective selection of horizontal resistance even in the presence of vertical resistance gene, which is not possible by conventional approaches. This may be expected to reduce the risk of vertifolia effect i.e. disease epidemics due to breakdown of vertical resistance.

GENETIC TRANSFORMATION

To transfer a cell or an organism with insertion of foreign DNA carrying gene of interest (GOI) or specific trait is known as genetic transformation. Genetic modification of plants using recombinant DNA techniques holds the promise of increased crop productivity, product quality and reduced dependence on chemical inputs for pest control (Asano *et al.* 1991). In general, methods used for genetic transformation are either by physical method or by biological method of transformation. The physical method includes particle bombardment, microinjection and electroporation. Biological transformation occurs by co-cultivation with the bacterial vector *A. tumefaciens* or *A. rhizogenes*. The whole plant can be regenerated from the transformed cell (Mukherjee 1999a).

Thus, to develop transgenic plants, it is essential to have reliable methods for efficient production of plants in tissue culture. There have been reports of regeneration from a variety of explant sources and genotypes but with varying degrees of success. Shoot organogenesis has been observed from stem internodes, leaves and roots (Carswell and Locy 1984). Regeneration has also been induced directly from adventitious roots from shoot cultures (Ozias-Akins and Perera 1990), and from *de novo* roots formed in tissue culture (Carswell and Locy 1984). Somatic embryogenesis has been induced from stem, petiole, leaf and root tissues (Liu and Cantliffe 1984; Chee and Cantliffe 1988). It is also possible to regenerate sweetpotato from protoplasts (Sihachakr and Ducreux 1987). Regeneration from storage roots has also been demonstrated (Hwang *et al.* 1983; Newell *et al.* 1995). Plants have also regenerated from leaf, stem, petiole and root explants (Mukherjee *et al.* 2001, 2002).

In sweet potato, approaches for producing transgenic plants include the electroporation of protoplasts (Nishiguchi *et al.* 1992), particle bombardment (Prakash and Varadarajan 1992), *Agrobacterium rhizogenes*-mediated transformation (Otani *et al.* 1993), and *A. tumefaciens* mediated transformation (Prakash and Varadarajan 1991). However, these procedures have been found very genotype dependant, and often difficult to reproduce (Lowe *et al.* 1994). The first report of reproducible transformation and regeneration was achieved using pieces of storage root tissue inoculated with the *A. tumefaciens* strain LBA4404 (Newell *et al.* 1995). In that report, the plasmid vector contained the *uid*A gene encoding β–glucuronidase (GUS) (Jefferson 1987) and the wild type neomycin phosphotransferase II gene (*npt*II) (Yenofsky *et al.* 1990). Regeneration and *Agrobacterium*-mediated putative transformation have also been reported in India (Mukherjee 2006). Genetic transformation of embryogenic suspension cultures of sweet potato cultivars Xu55-2was obtained by utilizing the *A. tumefaciens* strain EHA105 that contain the binary vector PBIM 19/SBD2 with SBD2 and neomycin phosphotransferase (NPTII) gene (Xing 2008). Zhai and Liu (2003) produced transgenics of cv. 'Lizixiang' from ECS using *A. tumefaciens* strain A208SE with binary vector pR0A93, *gus A* and *npt II* genes. Similar transformation was reported with *A. tumefaciens* strain LBA4404 with binary vector ApBinh, *oryzacystatin-I* and *nptII* genes (Jiang *et al.* 2004). Luo *et al.* 2006 developed a *de novo* system through callus organogenesis from sweet potato leaves. Zhang *et al.* (2009) developed transgenics showing functional expression of *bar* genes selected with 0.5 mg l^{-1} phosphinithricin. Transgenic research is being continued to have improved nutritional quality as well as disease and pest resistant sweet potato.

Improvement of nutritional quality

The most useful applications of genetic engineering in sweet potato may be in the improvement of nutritional quality traits. As a result of the high yield per hectare, sweet potato rates very high in protein production and has the highest recorded net protein utilization among major food crops (Prakash 1994).

Molecular cloning and nucleotide sequence of cDNA for sporamin, the major soluble protein of sweet potato tuber, was done by Hattori *et al.* (1985, 1990). Otani *et al.* (1993) developed *Agrobacterium rhizogenes*-mediated transformation in sweet potato. However, like other plant proteins, sweet potato protein is deficient in many essential amino acids. To address this problem, research at Tuskegee University attempted to introduce a synthetic storage protein gene that codes for essential amino acids. Theoretically, the nutritional quality of the 'artificial storage protein' is similar to that of milk or egg protein. As the leaf tips of sweet potato are also consumed as a green vegetable, targeting of improved protein gene expression to young leaves would also be nutritionally beneficial (Prakash 1994). Garcia *et al.* (1995) established the methodology for regeneration and genetic transformation of sweet potato for storage protein (sporamin) in two cultivars. Egnin and Prakash (1997) developed transgenic sweet potato expressing a synthetic storage protein gene for high levels of total protein and essential amino acids. Storage roots of transgenic lines had 9% protein over control (2.25%) on dry weight basis. At Tuskegee University, Alabama, USA, South African sweet potato cultivars are being transformed with a gene to increase essential amino acid levels in the tubers (Egnin *et al.* 1999). With other sweet potato cultivars, this research has shown to be extremely successful with transgenic sweet potatoes containing up to five times the normal protein levels.

Disease resistance

There are several fungal, bacterial and viral diseases which infect sweet potato crop. As sweet potato is grown primarily as a subsistence crop in most developing countries, chemical control of these diseases is not widely practised. Frequent replanting with virus free stock is also no enduring solution as warm climates lead to a high reinfection rate. Development of cultivars resistant to diseases is a viable option.

Viral disease in sweet potato is mainly due to white fly transmitted sweet potato chlorotic stunt virus and aphid-transmitted sweet potato feathery mottle virus. The infection causes chlorosis, deformed leaves and severe stunting and can reduce yield by over 90% (Gibson *et al.,* 1997). Mwanga *et al.* (2003) developed a linkage map and identified markers linked to resistant genes of both these viral diseases. They identified an AFLP marker *spcsv1*, which explained 70% of variation in gene resistant to sweet potato chlorotic stunt virus and one RAPD marker *spfmv1* which explained 72% of variation in sweet potato feathery mottle virus resistant gene.

The transfer of a *cecropin* gene from the giant silk moth has already been achieved in tobacco and potato and the resulting transgenic plants have reportedly demonstrated measurable resistance to bacteria and fungi. Synthetic versions of this gene, with improved stability and activity, are being introduced into elite cultivars of sweet potato at Tuskegee University. Transgenics for fungal resistance was also developed by genetic transformation with chitinase and glucanase genes (Walls *et al.* 1996). *Sweet potato feathery mottle virus* (SPFMV) is a major problem that causes 'russet crack' disease and affects sweet potato production, particularly in Africa. Efforts are underway to develop resistance to the feathery mottle virus using the coat protein gene and antisense RNA genes. Research at the US agrochemical company Monsanto and Tuskegee University aimed to develop transgenic sweet potato plants using these genes. Cuban and Chinese scientists have also cloned the coat protein gene for SPFMV to develop virus resistant plants (Prakash 1994).

By integration and expression of genes assessed through Southern, Northern and Western analysis, Maingi *et al.* (1996) could confer resistance to *Sweet potato feathery mottle virus* (SPFMV) in sweet potato through genetic transformation. They developed an efficient transformation system using *A. tumefaciens* strain containing marker for SPFMV coat protein genes.

Plant transformation through *Agrobacterium* using SPFMV coat protein gene demonstrated that only 1.3% plantlet formation can be derived from petiole explant. Plant regeneration of sweet potato is genotype-dependent. 'Jewel' was the best genotype of sweet potato for *in vitro* regeneration and plant transformation.

Transgenic approaches have been developed for sweet potato cultivars important to resource poor farmers in Africa. Transgenic sweet potato resistant to SPFMV has been produced at KARI in a program funded by the United States Agency for International Development (USAID), and Monsanto (Wambugu 1999).

Pest resistance

Among the major insect pest problems of sweetpotato, sweetpotato weevil (*Cylas formicarius* F.) is the most destructive one, especially in the tropics (Horton and Ewell 1991). Production losses due to this insect attack reach 60-100% in certain areas. The sweet potato weevil feeds on stored roots, thereby reducing their quality and yield; secondary compounds produced by roots in response to weevil attack make even slightly damaged roots inedible. Unfortunately, very little resistance to weevil can be found in sweet potato germplasm. Hence transgenic resistance is most desirable to combat pest problem. Similarly, pests like stem nematodes can decrease the yield substantially. Marker assisted screening can screen out the susceptible lines and effectively select the potential resistant lines before the phenotypic expression. Zhou *et al.* (2005) and Jiang *et al.* (2007) developed one RAPD marker linked to stem nema-

tode resistant gene at a genetic distance of over 17cM. Jie *et al.* (2008, 2009) developed a sequence characterized amplified region (SCAR) marker at a genetic distance of 14.2 cM and two AFLP markers (6.9 cM and 11.1cM) linked to this resistance gene. Li *et al.,* 2008 also developed two sequence related amplification polymorphism (SRAP) markers linked to the resistant gene with genetic distance of 4.86 cM and 4.17 cM, respectively.

Garcia *et al.* (1995) have developed transgenic sweet potato plants resistant to pest (*Cylas formicarius*). The expression of Bt genes was detected by Western Blot using polyclonal antibodies. Cipriani and Golmirzaie (1996) reported transformation of sweet potato cultivars using *Agrobacterium* tumefaciens and *A. rhizogenes* for insect resistance.

TOLERANCE TO ABIOTIC STRESSES

Dudal and Purnell (1986) reported that salinity affects 7% (1000 Mha) of the world's land surface which is one of the main limiting constraints to global agriculture. Along with 76 Mha area is affected by secondary salinity (Oldeman *et al*. 1991). In those areas, plant growth is severely affected by salinity through water deficit and other salt specific damages (Munns and Termaat 1986) or oxidative stresses (Hernandez *et al.* 1995). It is claimed that abiotic stress is the primary cause of crop loss, reducing average yields of most of the crops by more than 50% (Wang *et al.* 2003). This proportion will rise with increasing irregularity of climate and frequency of extreme climatic events (Mukherjee 2011). Diversity in species, varieties and practices has permitted agriculture to withstand moderate change in climate over the past 10,000 years.

Research has significantly advanced in developing hardier crop varieties through conventional breeding methods, and the application of tools from molecular biology is speeding the process. Rice researchers have succeeded to identify a rice gene (Sub1A) that allows plants to survive when completely submerged for up to two weeks, while most rice survives underwater for only three days (Russell 2007). Unlike rice, sweet potato is not being subjected to intense research for salinity and submergence tolerance. However, the large scale *in vitro* screening and antioxidative system studied to impart submergence and salinity tolerance in sweet potato are discussed here.

Submergence tolerance

In an attempt to understand responses to salinity and flooding stress, many enzymes induced by salt stress and flooding have been identified and characterized (Pinheiro *et al.* 1997). Plants receiving paclobutrazol (PBZ) that are under environmental stress have significantly higher antioxidative system levels compared to plants under non stressed conditions (Lurie *et al.* 1994; Paliyath and Fletcher 1995).

Changes of antioxidants and antioxidative enzymes in the flooding stressed sweet potato leaf as affected by PBZ treatment at 24 h prior to flooding was studied by Lin *et al.* (2006). Their results showed that under flooding stress conditions, the level of antioxidative system is linked to PBZ treatment. Pre-treating with PBZ may increase levels of various components of antioxidative systems after exposure to different durations of flooding and drainage, thus inducing flooding tolerance. The PBZ exhibited an important function of enhancing the restoration of leaf oxidative damage under flooding stress after the pre-application of 0.5 mg per plant. These findings will have greater significance for farming in frequently flooded areas.

Similarly, pretreating with $CaCl_2$ could enhance tolerance to flooding stress by enhancing reduced glutathione (GSH). In an experiment, three sweet potato varieties, 'Taoyuan 2', 'Simon 1' and 'Sushu 18', were pretreated with four levels of $CaCl_2$ (0, 60, 120 and 180 kg ha^{-1}) weekly for 50 days from planting before being subjected to non-flooding (control) and flooding conditions. The studied genotypes exhibited unique abilities and specificities through the antioxidative systems in response to flooding stress. The level of activity of the antioxidative system in sweet potato leaves was related to $CaCl_2$ pretreatment during flooding. The ascorbate peroxidase, superoxide dismutase, glutathione reductase, reduced ascorbate, total ascorbate, reduced glutathione and malondialdehyde contents of the three sweet potato varieties under flooding stress significantly increased because of pretreatment with 60 and 120 kg ha^{-1} of $CaCl_2$. Moreover, pretreatment with 60 and 120 kg ha^{-1} $CaCl_2$ enhanced the flooding tolerance of all three sweet potato varieties and mitigated the effects of flooding stress. This information would help for further genetic and physiological studies in sweet potato flood or submergence tolerance (Lin *et al.* 2008; Mukherjee *et al*. 2009b).

Salinity tolerance

Work on salinity tolerance in sweet potato reported certain degree of salinity tolerance in some varieties and also in some collections introduced into India from International Potato Center (CIP), Lima, Peru (Ekanayake and Dodds 1993; Ahmed *et al*. 1999; Mukherjee 2001). *In vitro* method resulted in development of NaCl resistant variant cells tolerant to 1% (w v^{-1}) NaCl for three passages (Salgado *et al.* 1985). Drought tolerant and salinity tolerant mutants were obtained from irradiated embryogenic suspension culture (ECS) by selecting with 30% PEG (Wang *et al.* 2003) and 2% NaCl (He *et al.* 2009), respectively.

In India, information on salinity tolerance is virtually confined to the work carried out at Regional Centre of Central Tuber Crops Research Institute, Bhubaneswar, Orissa (Mukherjee 1999b, 2001, 2002a, 2005; Mukherjee *et al.* 2006, 2009a, 2009b and Dasgupta *et al.* 2008).

In vitro propagation studies revealed enhanced rate of propagation of sweet potato with supplementation of NaCl in culture medium (Mukherjee 1999b, 2001, 2002a, 2002b). Though rate of propagation in all possible routes of *in vitro* propagation was higher in NaCl supplemented medium, level of NaCl tolerance was quite high in case of embryogenic regeneration pathway. Despite the variation in degree of tolerance, all the tested genotypes responded to embryogenesis even with 10 g l^{-1} NaCl supplementation. Regenerated plants recorded high rate (90-100%) of survival in the field. The embryos produced with NaCl supplementation were quite hardy and could tolerate low temperature (8°C) stress during storage without the protective covering of costly sodium alginate (Mukherjee and Debata 2002).

The hydroponic cultures and NaCl mediated *in vitro* protocols developed (Mukherjee *et al*., 1994; Mukherjee 1999b; Mukherjee 2001; Mukherjee 2002a, 2002b; Dasgupta *et al.* 2002; Mukherjee *et al.* 2006) in sweet potato and other tuber crops facilitated faster screening of large collection. Isoenzymes and RAPD marker studies carried out in sweet potato (Mukherjee 2002a; Mukherjee *et al.* 2003a) hastened their characterization.

The varied growth response, morpho-physio-biochemical changes studied under *in vitro* NaCl mediated stress and under *in situ* saline conditions (Mukherjee *et al.* 2009a) is discussed to isolate salt tolerant sweet potato.

Effect of NaCl on rooting and shoot bud development *in vitro*

Roots are among the first organs affected by salt stress and are most sensitive. A well marked difference was noticed in rooting response in all tested genotypes (**Fig. 5**). Delayed rooting response (4-6 days) was observed at higher doses of NaCl (24 dSm^{-1}) as compared to the control (2-4 days). Based on rooting response and minimum percentage of leaf necrosis (**Fig. 5**) at higher dose of NaCl (24.0 dSm^{-1}), 15 genotypes out of 47, were selected and evaluated subsequently under *in vitro* and *in vivo* conditions.

A wide variation in the studied parameters was ob-

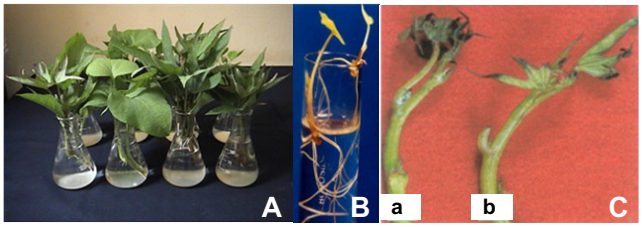

Fig. 5 Screening of genetic resources of sweet potato for salinity tolerance. (**A**) Screening in hydroponics. (**B**) Sweet potato accessions (ST-14) grown in hydroponics culture with NaCl (24.0 dSm^{-1}). (**C**) Necrosis under salt stress in susceptible (a) [90/606] and tolerant (b) [Gouri] accessions. Source: Mukherjee 2005

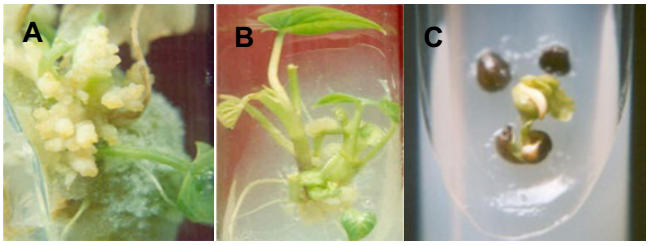

Fig. 6 Induction of stress tolerance in 'ST-14' through *in vitro* cultures. (**A**) Somatic embryogenesis and regeneration from salt-tolerant callus lines. (**B**) Axillary shoot proliferation in NaCl stress. (**C**) Seeds germinated in NaCl conditions. Source: Mukherjee 2005.

served among the genotypes tested with respect to different levels of salinity (Mukherjee 2005). The analysis of variance for growth parameters *in vitro* indicated that there were significant effects of NaCl treatment, genotypes and NaCl × genotypes interaction for all the studied parameters. The results revealed that the growth parameters decreased significantly with increase in salinity stress. Plantlet growth was reduced remarkably in all the genotypes under 0.5 and 1.0% NaCl stress, compared with their control. Particularly, survivability of the explants was reduced to a great extent under salinity. Out of 15 genotypes tested, nine could survive at 0.5% NaCl. However at higher (1.0% NaCl) stress, growth was completely suppressed and only six genotypes viz., 'CIP 420027', 'ST 13', 'Gouri', 'ST 14', 'CIP 187017.1' and 'SB-198/115' could survive, while rest of the genotypes necrosed and ultimately died in between 2-3 weeks of inoculation. At relatively higher stress (1.0% NaCl), no shoots emerged even after 21 days of culture and the explant consequently necrosed in nine genotypes (Dasgupta *et al.* 2008).

In vitro screening and induction of stress tolerance showed varied responses of tested genotypes cultured on MS medium with growth regulators and NaCl (**Fig. 6**). Amongst responded genotypes under 1.0% NaCl, shoot length was decreased by 12.98 to 75.46%. The reduction in leaf number was reported at 0.5% NaCl and the same was pronounced at 1.0% NaCl stress over stress free control. The six genotypes, which produced shoots at higher salt stress could develop roots even when cultured at 1.0% NaCl-mediated stress.

Effect of sodium chloride on antioxidative enzymes

Antioxidative enzyme activities increase in diverse environmental stress situations (Mittler 2002), as a response related to reactive oxygen species (ROS) detoxification. Among the many enzymes regulating the intracellular level of H_2O_2, peroxidases and catalases are considered to be most important. Such studies have been carried out in sweet potato under NaCl stress.

Stress induced increase in CAT (catalase) and GPX (guaiacol peroxidase) activities was reported in sweet potato (Dasgupta *et al*. 2008). Leaf SOD (super oxide dismutase) activity was greater in salt stressed plantlets than that in controls. The CAT activity in leaves of control and salt stressed plants varied significantly. The increase in CAT activity (μ mg^{-1} protein) under NaCl stress over control was reported in the range of 60-70% increase in tolerant genotypes than 30-40% increase in susceptible genotypes. A similar pattern was observed for GPX activity, which also increased significantly in all the tested genotypes under induced salt stress condition. At higher stress, tolerant genotypes exhibited 40-60% increase in GPX over control. Rout and Shaw (2001) suggested CAT and GPX as the most important H_2O_2 scavenging enzymes leading to salt tolerance in aquatic macrophytes. In sweet potato, it could be presumed that CAT is more important H_2O_2 scavenging enzyme in leaves than GPX.

Fig. 7 Promising sweet potato genotypes tolerant to salt stress (EC 6.0-8.0 dsm^{-1}). (**A**) ST-13: 85-90 mg anthocyanin 100 g^{-1}. (**B**) ST-14: 14 mg β carotene 100 g^{-1}. (**C**) CIPSWA-2: 6 mg β-carotene 100 g^{-1}. (**D**) Gouri: 5.2 mg β carotene 100 g^{-1}. Source: Mukherjee *et al*. 2009a.

In situ evaluation of *in vitro* screened sweet potato

In situ evaluation is the most practical approach to validate the results of *in vitro* screening. Fifteen out of 47 genotypes selected *in vitro* showed varied yield and biochemical responses when grown under *in situ* salt stress (6-8.0 dSm^{-1}) in farmers' field of coastal Kendrapara district of Orissa state in India (Mukherjee *et al.* 2006, 2007). The yield ranged from 5.2 t ha^{-1} to 14.99 t ha^{-1} (**Table 1**). The highest yield was recorded by 'CIPSWA-2' (14.99 t ha^{-1}) followed by 'Gouri' (14.80 t ha^{-1}). The genotypes viz. 'ST-13', 'ST-14', '420027' and 'SB 198/115' gave reasonably higher yield under salt stress (6-8.0 dSm^{-1}). Salinity induced biochemical changes showed decrease in reducing sugar and total soluble sugar in the range of 7.31-28.38% and 9.91-31.10% respectively in the tested genotypes. The protein content was observed to be decreasing 9.54-46.49% due to salinity stress. Increasing level of proline accumulation was observed in all the tested genotypes under stress. However, extent of increase was more than 78% in 6 genotypes viz. '420027', 'ST 14', 'ST13', 'CIPSWA 2', 'Gouri' and 'SB-198/115'. Nitrate reductase activity under stress was also high. Isozyme and DNA polymorphisms were quite pronounced in these tolerant genotypes under *in situ* salt stress (Mukherjee *et al.* 2007). Of the identified salt tolerant six genotypes, five are rich in carotene and ST13 is rich in anthocyanin. Carotene and anthocyanin contents (**Table 2** and **Fig. 7**) of the identified salt tolerant genotypes reported to be as good as tubers grown over non stress conditions (Mukherjee *et al.* 2009a). These identified salt tolerant biofortified sweet potato can play pivotal role towards food and nutrition security in coastal areas around the globe (**Table 1**).

Table 1 Evaluation for salinity tolerance in sweet potato under *in situ* salt stress (EC: 6-8 dSm^{-1}) at coastal Kendrapara of Orissa State, India.

Genotypes	Yield (t ha^{-1})
SB 198/115	11.21
S30X15	6.38
90/606	5.2
440127	10.25
ST-14	12.25
440038	9.93
108X1	8.61
GOURI	14.80
187017.1	9.11
SP 61	5.95
ST-13	12.49
90/774	6.94
420027	11.85
90/696	6.11
CIPSWA-2	14.99

Source: Mukherjee *et. al.* 2009a

Table 2 Carotene content of identified salt tolerant genotypes grown under *in situ* salt stress (EC: 6-8 dSm^{-1}).

Genotypes	Carotenoids (mg 100 g^{-1})
ST-14	13.2
CIP-SWA 2	6.5
420027	5.9
GOURI	5.2
SB 198/115	4.8

Source: Mukherjee *et. al.* 2009a

FUTURE STRATEGIES

Tuberous root crops like sweet potato have immense potential to meet the challenges of today's food demand. Diversity within its genotypes and wide distribution and potential to adapt to harsh environmental conditions advocates for its further exploitation to conserve and propagate efficiently as well as to develop genetically improved lines.

Considering the limitations of conventional propagation of sweet potato, micropropagation protocols can be taken up for a large scale product. However, unlike other starchy crops, commercialization of micropropagation protocols of existing varieties may not receive attention unless transgenics with delayed post harvest deterioration and value added traits are produced. Production of transgenics with value added traits in experimental level is successful in sweet potato.

Sweet potato with high protein and resistance to weevil and other pests and diseases has a better market to compete with other starchy and vegetable crops. Success in plant regeneration through organogenesis and embryogenesis has made sweet potato more amenable for genetic transformation as compared to other tuber crops. Genetic transformation experiments for production of transgenics resistant to mosaic, fungal diseases and pests as well as transgenics with higher protein content are quite successful. Commercial production of transgenics, however, depends on highly reproducible and genetically stable regeneration system. In India, establishment of repetitive embryogenic callus cultures and regenerants (Mukherjee *et al.* 1998b; Mukherjee *et al.* 2001; Mukherjee 2002a; Mukherjee *et al.* 2003a) is quite promising. Scientists in India and Peru employ tissue culture to maintain the sweet potato germplasm. Scientists at the International Institute for Tropical Agriculture (IITA) in Nigeria, have developed methods to produce virus free sweet potato plants through meristem culture. Considerable research on sweet potato tissue culture has been persuaded in China, Indonesia and many other developing countries. However, there is also every reason to believe that there can be a smooth and sustainable transfer of the technology to the field, and that farmers will be able to benefit from these crop protection transgenic strategies (Wambugu 1999). Farmers can receive transgenic plants in a form that is familiar to them.

The research conducted by International Potato Centre, Peru, Kenyan Agricultural Research Institute, Uganda National Agricultural Research Organization and International Centre for Research on Women showed that the addition of small amount of orange-flesh sweet potato to the family's diet can mitigate vitamin A deficiency in both children and adults.

In India and other developing countries, under privileged and under nourished people live in coastal backward areas. Thus, the results of the studies on salt tolerant sweet potato in India are very promising. Of the six genotypes identified with salt tolerant traits, five are rich in β-carotene (5-14 mg 100 g^{-1}) and one is rich in anthocyanin (85-90 mg 100 g^{-1}). Nutrition experts view that enhancing β-carotene level cannot fight vitamin A deficiency. Their studies indicate that absorption of vitamin A by the human body depends on the diversity of the food consumed. Intake of 'vitamin A rich' naturally occurring food and vegetable crops will be a better and efficient route to combat Vitamin A or nutrition deficiency, In this context salt tolerant biofortified orange and purple flesh sweet potato can play a significant role (Mukherjee *et al.* 2007). The performance of *in vitro* screened salt tolerant genotypes with reasonably good yield coupled with high carotene and anthocyanin contents even under *in situ* salt stress (6-8 dSm^{-1}) have greater impact. In fact, it is a judicious step towards sustainable agriculture for coastal food and nutrition security (Mukherjee *et al.* 2007, 2008). Similarly results on flooding tolerance have greater implication to enhance productivity even on water logged conditions. Future studies of these stress tolerant and sensitive lines would help in augmenting breeding programme and to understand molecular mechanisms of stress tolerance to identify the stress tolerant genes. Once such genes will be identified it will be easier to introgress them in other genotypes with their original background and improve them. Such knowledge would be beneficial for metabolic engineering of sweet potato for multiple uses and multi resistance (Mukherjee 2011).

Salt, submergence (abiotic stresses) and weevil (biotic stress) tolerant sweet potato packed with high energy (194 MJ/ha/day), nutritive value (high β-carotene, anthocyanin, minerals) coupled with high rate of productivity can alone supplement food, feed and industrial requirements under harsh environment. Hence days are ahead to have right choice for sweet potato as source for food, nutrition security and economic sustainability to tackle the problem of food insecurity and food demand for the ever growing population.

ACKNOWLEDGEMENTS

Financial assistance by the Indian Council of Agricultural Research, New Delhi, is gratefully acknowledged.

REFERENCES

Aboul Nasr MH, Bouwkamp JC (1990) *In vitro* formation of storage roots in sweet potato (*Ipomoea batatas*). *Journal of Horticulture Science* **25 (6)**, 627

Ahmed MS, Mannan MA, Bhuiyan MKR, Dayal TR, Ahmed HU (1999) Evaluation of sweet potato germplasm for yield, salinity and other agronomic characters. In: Balagopalan C, Nayar TVR, Sundaresan S, Premkumar T, Lakshmi KR (Eds) *Tropical Tuber Crops*, Oxford IBH, New Delhi, pp 128-136

Alconero R, Santiago AG, Morales F, Rodriguez F (1975) Meristem tip culture and virus indexing of sweet potatoes. *Phytopathology* **65**, 769-773

Almazrooei S, Bhatti MH, Henshaw GG, Taylor NJ, Blakesley D (1997) Optimisation of somatic embryogenesis in fourteen cultivars of sweet potato (*Ipomoea batatas* (L) Lam.). *Plant Cell Reports* **16 (10)**, 710-714

Asano Y, Otsuki Y, Meeusun RL (1991) Insect control with genetically engineered crops. *TibTech* **9**, 197-200

Belarmino MM, Abe T, Sasahara T (1994) Plants regenerated from stem and petiole protoplasts of sweet potato (*Ipomoea batatas*). *Plant Cell, Tissue and Organ Culture* **37 (2)**, 145-150

Belarmino MM, Abe T, Sasahara T (1996) Asymmetric protoplast fusion between sweet potato and its relatives and plant regeneration. *Plant Cell, Tissue and Organ Culture* **46 (3)**, 155-202

Benson EE (1990) Free radical damage in stored plant germplasm, IBPGR, Rome, pp 24-25

Bhatti MH, Percival T, Davey CDM, Henshaw GG, Blakesley D (1997) Cyropreservation of embryogenic tissue of a range of genotypes of sweet potato (*Ipomoea batatas* [L] Lam.) using an encapsulation protocol. *Plant Cell Reports* **16**, 802-806

Blakesley D, Almazrooei S, Henshaw GG (1995) Cryopreservation of embryogenic tissue of a sweet potato (*Ipomoea batatas*): Use of sucrose and dehydration for cryoprotection. *Plant Cell Reports* **15**, 259-263

Carswell GK, Locy RD (1984) Root and shoot initiation by leaf, stem, and storage root explants of sweetpotato. *Plant Cell, Tissue and Organ Culture* **3**, 229-236

Chee RP, Cantliffe DJ (1988) Somatic embryony patterns and plant regeneration in *Ipomoea batatas* Poir. *In Vitro Cellular and Developmental Biology – Plant* **24**, 955-958

Chee RP, Cantliffe DJ (1992) Improved production procedures for somatic embryos of sweet potato for a synthetic seed system. *HortScience* **27 (12)**, 1314-1316

Chen L-Z, Du Z-S, Nishimura Y, Hamaguchi T, Sugita T, Nagata R, Terao H, Tsuzuki E (2010) Approach to establishment of plant regeneration and transformation system in sweet potato (*Ipomoea batatas*) by culture of leaf segments. *Bulletin of Minamikyushu University* **40A**, 59-63

Cipriani G, Golmirzaie A (1996) Genetic engineering for sweet potato improvement. *In Vitro* **32**, 3

Connolly AG, Godwin ID, Cooper M, DeLacy IH (1994) Interpretation of randomly amplified polymorphic DNA marker data for fingerprinting sweet potato (*Ipomoea batatas* L.) genotypes. *Theoretical and Applied Genetics* **88**, 332-336

Dasgupta M, Mukherjee A, Nasker SK, Debata BK (2002) Use of hydroponics for isolation of tolerant lines of sweet potato (*Ipomoea batatas* L.) towards salinity stress. In: Sahoo S, Ramesh DB, Panda PK, Misra VN (Eds) *Proceedings of the National Seminar on Plant Resources Utilization*, December 28-29, 2002, Regional Research Laboratory, Bhubaneswar, Orissa, India, Allied Publishers, New Delhi, pp 143-147

Dasgupta M, Sahoo MR, Kole PC, Mukherjee A (2008) Evaluation of orange fleshed sweet potato (*Ipomoea batatas* L.) genotypes for salt tolerance through shoot apex culture under *in vitro* NaCl mediated salinity stress conditions. *Plant Cell, Tissue and Organ Culture* **94**, 161-170

Desamero NV, Rhodes BB, Decoteau DR, Bridges WC (1994) Picolinic acid-induced direct somatic embryogenesis in sweet potato. *Plant Cell, Tissue and Organ Culture* **37 (2)**, 103-111

Dodds JH (1989) *In vitro* sweet potato germplasm management. In: *Improvement of Sweet Potato (Ipomoea batatas) in Asia*, Workshop on sweet potato Improvement in Asia, Philippines, pp 195-202

Egnin M, Prakash CS (1997) Transgenic sweet potato (*Ipomoea batatas*) expressing a synthetic storage protein gene exhibits high levels of total protein and essential amino acids. *In Vitro Cellular and Developmental Biology – Plant* **33**, 52A

Egnin M, Prakash CS, Walker M, Shireen K, Pace R, Jaynes J, Nakamura K (1999) Enhanced protein content and quality in sweet potato engineered with a synthetic storage protein gene. Paper presented at the *Plant Biology 99, Annual Meeting of the ASPP*, Baltimore, MD, p 34

Eilers RJ, Smith MAL, Skirvin RM (1985) Petiole protoplast isolation from green-house sweet potato (*Ipomoea batatas*). *Journal of Horticulture Science* **20 (3)**, 571-572

Ekanayake IJ, Dodds JH (1993) *In vitro* testing for the effects of salt stress on growth and survival of sweet potato. *Scientia Horticulturae* **55**, 239-248

Garcia R, Mena J, Armas R, Lopez A, Rodriguez J, Zaldua Z, Somonte D, Pimentel E, Garcia M, Gomez M, de la Riva G, Moran R, Selman H G (1995) Transgenic sweet potato plants resistant to pests: field results. *Advances in Modern Biotechnology* **3 (II)**, 72

Ghislan M, Gruneberg W, Benavides J, Mwanga R (2004) Application of molecular markers for gene pool division and heterosis estimation under drought stress conditions in sweet potato. *International Potato Centre* (CIP), Peru, pp 34-35

Gibson RW, Mwanga ROM, Kasule S, Mpembe I, Carey EE (1997) Apparent absence of viruses in most symptomless Weld-grown sweet potato in Uganda. *Annals of Applied Biology* **130**, 481-490

Gosal SS, Bajaj YPS (1984) Isolation of sodium chloride resistant cell lines in some grain legumes. *Indian Journal of Experimental Biology* **22**, 209-214

Gosukonda RM, Parobodessai A, Blay E, Prakash CS, Peterson CM (1995) Thidiazuron-induced adventitious shoot regeneration of sweetpotato (*Ipomoea batatas*). *In Vitro Cellular and Developmental Biology – Plant* **31**, 65-71

Guo JM, Liu QC, Zhai H, Wang YP (2006) Regeneration of plants from *Ipomoea cairica* L. protoplasts and production of somatic hybrids between *I. cairica* L. and sweet potato, *I. batatas* (L.) Lam. *Plant Cell, Tissue and Organ Culture* **87**, 321-327

Hahn SK, Isoba JCG, Ikotun T (1989) Resistance breeding in root and tuber crops at the International Institute of Tropical Tuber Agriculture (IITR), Nigeria. *Crop Protection* **8**, 147-168

Hattori T, Nakagawa T, Maeshima M, Nakamura K, Asahi T (1985) Molecular cloning and nucleotide sequence of cDNA for sporamin, the major soluble protein of sweet potato tuberous roots. *Journal of Plant Molecular Biology* **5**, 313-320

Hattori T, Nakagawa S, Nakamura K (1990) High level expression of tuberous root storage protein genes of sweet potato in stems of plantlets grown *in-vitro* on sucrose medium. *Journal of Plant Molecular Biology* **14 (4)**, 595-604

Henderson JHM, Phills BR, Whatley BT (1984) Sweet potato. In: Sharp (Ed) *Handbook of Plant Cell Culture*, Macmillan, New York, pp 302-326

Hernandez JA, Olmos E, Corpas FJ, Sevilla F, del Río LA (1995) Salt induced oxidative stress in chloroplasts of pea plants. *Plant Science* **105**, 151-167

Horton DE, Ewell PT (1991) Sweet potato pest management: A social science perspective. In: Jansson RK, Raman KV (Eds) *Sweet Potato Pest Management: A Global Perspective*, Westview Press, Westview, Boulder, CO, pp 407-427

Hossain MJ, Sultana N (1997) *In vitro* propagation of sweet potato (*Ipomoea batata* L.). *Plant Tissue Culture* **7 (2)**, 89-95

Houndonougbo B (1989) Influence of different concentrations of indole acetic acid, naphthalene acetic acid, 2,4-D and kinetin on callusing and organogenesis *in-vitro* of internode fragments of 2 varieties of sweet potato (*Ipomoea batatas* L. Lam.). *Journal of Agronomy* **9 (7)**, 653-660

Huang JC, Sun M (2000) Genetic diversity and relationships of sweet potato and its wild relatives in *Ipomoea batatas* (Convolvulaceae) as revealed by inter-simple sequence repeat (ISSR) and restriction analysis of chloroplast DNA. *Theoretical and Applied Genetics* **100**, 1050-1060

Hwang Shih Y, Tseng Yu T, Lo Hsiao F (2002) Application of simple sequence repeats in determining the genetic relationships of cultivars used in sweet potato polycross breeding in Taiwan. *Scientia Horticulturae* **19**, 215-224

Hwang LS, Skirvin RM, Casayo J, Bouwkamp J (1983) Adventitious shoot formation from sections of sweet potato grown *in vitro*. *Scientia Horticulturae* **20**, 119-129

Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusions: Glucuronidase as a versatile gene fusion marker in higher plants. *EMBO Journal* **6**, 3901-3907

Jiang SJ, Liu QC, Zhai H, Wu LS, Wang YP (2004) Regeneration of sweet potato transgenic plants with oryzacystatin-I (OCI) gene. *Journal of Agricultural Biotechnology* **12**, 34-37

Jiang L, Ruan L, Zha XD, Wu F, Ma DF, Xie YP, Li XY, Wang Y (2007) A RAPD marker linked to the resistance *Ditylenchus destructor* in sweet potato. *Molecular Plant Breeding* **5**, 655-660

Jie Q, Jiang W, Li H, Zhai H, Ma DF, Xie YP, Liu QC (2008) Inheritance analysis and SCAR marker of the gene for stem nematode resistance in sweet potato, *Ipomoea batatas* (L.) Lam. *Molecular Plant Breeding* **6**, 523-526

Jie Q, Li H, Zhai H, Wang YP, Li Q, Ma DF, Xie YP, Liu QC (2009) Development of AFLP markers linked to stem nematode resistance gene in sweet potato [*Ipomoea batatas* (L.) Lam.]. *Chinese Journal of Agricultural Biotechnology* **6**, 97-101

Kartha KK (1985) *Cryopreservation of Plant Cells and Organs*, CRC Press, Boca Raton, Florida, 324 pp

Kobayashi R, Bouwkamp JC, Sinden SL (1990) Protoplast isolation and culture of some *Ipomoea* species in the section *batatas*. *Journal of Horticulture Science* **25 (9)**, 155

Li AX, Wang QM, Hou FY, Zhang HY, Zhang LM (2008) Two SRAP markers linked to sweet potato stem nematode resistance gene in sweet potato. *Molecular Plant Breeding* **6**, 1204-1208

Lin KH, Chiou YK, Hwang SY, Chen LF, Lo HF (2008) Calcium chloride enhances the antioxidative system of sweet potato (*Ipomoea batatas*) under flooding stress. *Annals of Applied Biology* **152**, 157-168

Lin KR, Tsou CC, Hwang SY, Chen LF, Lo HF (2006) Paclobutrazol pretreatment enhanced flooding tolerance of sweet potato. *Journal of Plant Physiology* **163**, 750-760

Liu JR, Cantliffe DJ (1984) Somatic embryogenesis and plant regeneration in tissue cultures of sweet potato (*Ipomoea batatas* Poir). *Plant Cell Reports* **3**, 112-115

Liu QC, Kokubu T, Sato M (1991) Plant regeneration from *Ipomoea triloba* L. protoplasts. *Japanese Journal of Breeding* **41**, 103-108

Liu QC, Luo JQ, Zhou HY, Lu SY (1993) High frequency somatic embryogenesis and plant regeneration in sweet potato, *Ipomoea batatas* (L.) Lam. *Journal of Agricultural Biotechnology* **1 (1)**, 84-89

Liu QC, Mi KX, Zhou HY, Ma B, Zhai H (1998) Regeneration and identification of interspecific somatic hybrid plants between sweet potato and *Ipomoea lacunosa*. *Acta Agriculturae Boreali Sinica* **24**, 529-535

Lizzaraga R, Panta A, Espinoza NN, Dodds JH (1992) Tissue culture of *Ipomoea batatas*: Micropropagation and maintenance. CIP Research Guide 32, CIP, Lima, Peru, 18 pp

Love SL, Rhodes BB, Moyer JW (1987) Practical manual for handling crop germplasm *in-vitro*. I. Meristem tip culture and virus indexing of sweet potatoes. IBPGR, Rome, Italy, 41 pp

Lowe JM, Hamilton WDO, Newell CA (1994) Genetic transformation in *Ipomoea batatas* (L.) Lam (sweet potato). In: Bajaj YBS (Ed) *Biotechnology in Agriculture and Forestry (Vol 29) Plant Protoplasts and Genetic Engineering*, Springer-Verlag, Berlin, Germany, pp 308-320

Luo HR, Santa-Maria M, Benavides J, Zhang DP, Zhang YZ, Ghislain M (2006) Rapid genetic transformation of sweet potato (*Ipomoea batatas* (L.) Lam.) via organogenesis. *African Journal of Biotechnology* **5**, 1851-1857

Lurie S, Lipsker RZ, Aloni B (1994) Effects of paclobutrazol and chilling temperatures on lipids, antioxidants and ATPase activity of plasma membrane isolated from green bell pepper fruits. *Physiologia Plantarum* **91**, 593-598

Maingi DM, Hinchee M, Lowe JM, Kaniewska MB, Macharia CW, Wambugu FW, Kaniewski W (1996) Transformation of sweet potato to confer resistance to *Sweet potato feathery mottle virus*. *In Vitro Cellular and Developmental Biology – Plant* **32 (4)**, 312

Mandal BB, Chandel KPS (1996) Conservation of genetic diversity in sweet potato and yams using *in-vitro* strategies. In: Kurup GT (Ed) *Tropical Tuber Crops*, Oxford & IBH, New Delhi, India, pp 49-54

Martinez CA, Maestri M, Lani EG (1996) *In vitro* salt tolerance and proline accumulation in andean potato (*Solanum* spp.) differing in frost resistance. *Plant Science* **116**, 177-184

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science* **7**, 405-410

Mori K (1971) Production of virus-free plants by means of meristem culture. *Journal of Agricultural Research Quarterly* **6**, 1-7

Mukherjee A (1994) Somatic embryogenesis in *Ipomoea cairica* a wild relative of sweet potato. *Journal of Root Crops* **20 (2)**, 125-126

Mukherjee A (1999a) Tuber crops. In: Ghosh SP (Ed) *Biotechnology and its Application in Horticulture*, Narosa Publishing House, New Delhi, India, pp 267-294

Mukherjee A (1999b) *In-vitro* propagation studies in sweet potato (*Ipomoea batatas* L). PhD thesis, Utkal University, Orissa, India, 175 pp

Mukherjee A (2001) Effect of NaCl on axillary shoot proliferation in sweet potato. *Annals of Tropical Research* **23 (1)**, 1-10

Mukherjee A (2002a) Effect of NaCl on *in vitro* propagation of sweet potato (*Ipomoea batatas*). *Applied Biochemistry and Biotechnology* **102**, 431-441

Mukherjee A (2002b) Micropropagation of tropical tuber crops. In: (Eds) *Proceedings of the State Level Seminar on Advances in Production of Quality Planting Materials of Horticultural Crops*, September 6-7, 2002, OUAT, Bhubaneswar, Orissa, India. Regional Centre of Central Tuber Crops Research Institute, Orissa, India, pp 60-64

Mukherjee A (2003) Biotechnology - sustainable agro-ecotechnology: Women's concern. In: Khuntia S, Parida A, Mishra Vibhuti N (Eds) *Down-sizing Technology for Rural Development* (Vol 1), Allied Publishers Pvt. Ltd., New Delhi, India, pp 299-304

Mukherjee A (2005) Integrated approach for selection, induction and characterization for development of salt tolerant sweet potato. In: *Final Report (May 2002-April 2005),* Code No. 303073652 [F. No. 8 (46)/2002-Hort.II dated 10th January, 2002, ICAR Cess Fund Scheme], 43 pp

Mukherjee A (2006) Synthetic seeding and genetic transformation studies in sweet potato and taro. Annual Report, CTCRI, Thiruvananthapuram, Kerala, India, pp 56-58

Mukherjee A (2012) Today's thoughts vs. sweet potato (*Ipomoea batatas* L.), its climatic resilience and tolerance to salinity, submergence stresses for food and nutrition security. *Indian Journal of Geo-Marine Sciences* in press

Mukherjee A, Dasgupta M, Debata BK, Naskar SK (2003b) Enhancement of somatic embryogenesis in sweet potato: Effect of growth regulators sodium chloride, proline and maltose. *Indian Journal of Plant Physiology* **Special Issue**, 326-331

Mukherjee A, Debata BK (2001) Organogenic regeneration in sweet potato: Effects of NaCl, proline and maltose. *Journal of Root Crops* **27 (1)**, 138-144

Mukherjee A, Debata BK (2002) Plant formation from encapsulated and non encapsulated somatic embryos of sweet potato *in vitro* and *in vivo*. *Plant Science Research* **24 (1/2)**, 38-42

Mukherjee A, Debata BK, Naskar SK (1998a) Somatic embryogenesis and chromosomal stability of regenerants in sweet potato and taro. *Journal of Scientific and Industrial Research* **57**, 709-715

Mukherjee A, Debata BK, Naskar SK (1998b) Cytology of callus and regenerated plants of sweet potato (*Ipomoea batatas* L.). *Cytobios* **96**, 109-118

Mukherjee A, Debata BK, Mukherjee PS, Mallick SK (2001) Morpho-histobiochemical characteristics of embryogenic and non embryogenic callus cultures of sweet potato (*Ipomoea batatas* L). *Cytobios* **106**, 113-124

Mukherjee A, Nair NG, Jose JS (1993) Direct shoot regeneration from *in vitro* roots of sweet potato (*Ipomoea batatas* L.). *Journal of Root Crops* **19 (2)**, 127-130

Mukherjee A, Nair NG, Rajendran PG (1994) Use of hydroponics in the hardening of *in vitro* plantlets of sweet potato and cassava. *Journal of Root Crops* **20 (2)**, 123-124

Mukherjee A, Naskar SK, Debata BK, Lakhanpaul S, Bhatt KV (2003a) Cytogenetics of callus cultures and *in vitro* regenerated plants of sweet potato (*Ipomoea batatas* L). *Plant Science Research* **25 (1/2)**, 13-20

Mukherjee A, Naskar SK, Edison S (2007) Salinity tolerance in orange and purple fleshed sweet potato: Impact on coastal food and nutrition security. In: International symposium on management of coastal ecosystem: Technological advancement and livelihood security, ISCAR, Kolkata, India, October 27-30, 2007, 40 pp

Mukherjee A, Naskar SK, Edison S (2009a) Salt tolerant biofortified orange and purple flesh sweet potato: coastal food and nutrition security, Technical Bulletin No. *51*, CTCRI, Thiruvananthapuram, Kerala, India, 36 pp

Mukherjee A, Naskar S K, Edison S, Dasgupta M (2006) Response of orange flesh sweet potato genotypes to salinity stress. In: *14th Triennial Symposium of the International Society for Tropical Root Crops*, Trivandrum, Kerala, 20-26 November, 2006, pp 151-152

Mukherjee A, Naskar SK, Poddar A, Dasgupta M, Sahoo MR, Chand PK (2009b) Submergence and salinity tolerance in sweet potato (*Ipomoea batatas* L.): Progress and prospects. *Plant Science Research* **31 (1/2)**, 1-9

Mukherjee A, Satapathy BS, Naskar SK (2008) Combating vitamin A deficiency with golden food crops: rice, millet and sweet potato. *Sabujima* **16**, 94-97

Mukherjee A, Unnikrishnan M, Nair NG (1991a) Callus induction, embryogenesis and regeneration from sweet potato anther. *Journal of Root Crops* **17**, 302-304

Mukherjee A, Unnikrishnan M, Nair NG (1991b) Growth and morphogenesis of immature embryos of sweet potato (*Ipomoea batatas* L.) *in vitro*. *Plant Cell, Tissue and Organ Culture* **26 (2)**, 97-99

Mukherjee A, Unnikrishnan M, Indira P (1996) *In vitro* tuber induction in sweet potato. In: Kurup GT (Ed) *Tropical Tuber Crops*, Oxford & IBH, New Delhi, India, pp 37-42

Mukherjee A, Vimala B (1994) *In vitro* seed germination and plantlet establishment of two wild relatives of sweet potato (*Ipomoea trifida* and *I. amnicola*). *Current Science* **66 (12)**, 941-942

Munns R, Termaat A (1986) Whole-plant responses to salinity. *Australian Journal of Plant Physiology* **13**, 143-160

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiology of Plant* **15**, 473-479

Mwanga ROM, Odongo B, Turyamureeba G, Alajo A (2003) Release of six sweet potato cultivars ('Naspot 1' to 'Naspot6') in Uganda. *HortScience* **38**, 475-476

Nair NG, Unnikrishnan M, Mukherjee A (1994) Use of *in-vitro* culture in the improvement of tuber crops. In: Chadha KL, Nayar GG (Eds) *Advances in Horticulture* (Vol 8), MPH, New Delhi, India, pp 211-231

Newell CA, Lowe JM, Merryweather A, Rooke LM, Hamilton WDO (1995) Transformation of sweet potato (*Ipomoea batatas* L. Lam.) with *Agrobacterium tumefaciens* and regeneration of plants expressing cowpea trypsin inhibitor and snowdrop lectin. *Plant Science* **107**, 215-227

Nishiguchi M, Uehara Y, Komaki K (1992) Stable transformation of sweet potato by electroporation. *In Vitro Cellular and Developmental Biology – Plant* **28**, 126-152

Oggema JN, Kinyua MG, Ouma JP, Owuoche JO (2007) Agronomic performance of locally adapted sweet potato (*Ipomoea batatas* (L) Lam.) cultivars derived from tissue culture regenerated plants. *African Journal of Biotechnology* **6 (12)**, 1418-1425

Otani M, Mii M, Handa T, Kamada H, Shimada T (1993) Transformation of sweet potato (*Ipomoea batatas* (L.) Lam.) plants by *Agrobacterium rhizogenes*. *Plant Science* **94 (1-2)**, 151-159

Ozias Akins P, Pererra S (1990) Organogenesis in cultured adventitious root segments, and in protoplast-derived callus of sweet potato. *HortScience* **25**, 121

Paliyath G, Fletcher RA (1995) Paclobutrazol treatment alters peroxidase and catalase activities in heat-stressed maize coleoptiles. *Physiology and Molecular Biology of Plants* **1**, 171-178

Pinhero RG, Rao MV, Paliyath G, Murr DP, Fletcher RA (1997) Changes in activities of antioxidant enzymes and their relationship to genetic and paclobutrazol-induced chilling tolerance of maize seedlings. *Plant Physiology* **114**, 695-704

Prakash CS (1994) Sweet potato biotechnology: Progress and potential. *Biotechnology and Development Monitor* **18**, 123-128

Prakash CS, Varadarajan U (1991) Expression of foreign genes in transgenic sweet potatoes. In: *Proceedings of the 3rd International Congress of Plant Molecular Biology*, October 6-12, 1991, Tucson, Arizona

Prakash CS, Varadarajan U (1992) Genetic transformation of sweet potato by particle bombardment. *Plant Cell Reports* **11**, 53-57

Rout NP, Shaw BP (2001) Salt tolerance in aquatic macrophytes: Possible involvement of the antioxidative enzymes. *Plant Science* **160**, 415-423

Russel N (2007) The agricultural impact of global climate change: How can developing-country farmers cope? *Geotimes (Earth)* **52 (4)**, 30-34

Sagredo B, Hinrichsen P, López H, Cubillos A, Muñoz C (1998) Genetic variation of sweet potatoes (*Ipomoea batatas* L.) cultivated in Chile determined by RAPDs. *Euphytica* **101**, 193-198

Salgado GR, Lopez GF, Ochoa AN (1985) NaCl-resistant variant cells isolated from sweet potato cell suspensions. *Plant Cell, Tissue and Organ Culture* **5 (1)**, 3-12

Sihachakr D, Ducreux G (1987) Plant regeneration from protoplast culture of sweet potato (*Ipomoea batatas* L. Lam.). *Plant Cell Reports* **6**, 326-328

Tanaka M, Nakayama H, Takahata Y, Yakub MJ, Somantri IH, Minantyorini KH, Nakatani M (2006) Molecular analysis of the genetic diversity in Indonesian sweet potatoes. *Acta Horticulturae* **75**, 760-768

Thro AM, Bonierbale M, Roca WM, Best R, Henry G (1994) Why, what who and when: An overview of the cassava biotechnology network (CBN) and current cassava biotechnology research worldwide. In: *Second International Scientific Meeting of Cassava Biotechnology Network (CBN)*, 22-26, August, 1994, Indonesia, working doc. No. 150, V-I, CIAT, Colombia

Van Zijl JJB, Botha R (1998) Conservation of plant genes III. In: Adams RP, Adams JE (Eds) *Conservation and Utilization of African Plants*, Missouri

Botanical Garden Press, St. Louis, MO, pp 147-153

Walls R, Egnin M, Prakash CS (1996) Genetic transformation of sweet potato with chitinase and glucanase genes for fungal resistance. In: *World Congress on In-vitro Biology*, June 22-27, 1996, San Francisco, pp 105-107

Wambugu F (1999) Why Africa needs agricultural biotech. *Nature* **400**, 15-16

Wang W, Vinocur B, Altman A (2003) Plant responses to drought, salinity and extreme temperatures towards genetic engineering for stress tolerance. *Planta* **218**, 1-14

Wang YP, Liu QC, Li AX, Zhai H, Zhang SS, Liu BL (2003) *In vitro* selection and identification of drought-tolerant mutants of sweet potato. *Scientia Agriculturae Sinica* **36**, 1000-1005

Withers LA (1987) The low temperature preservation of plant cells, tissue and organ cultures and seed for genetic conservation and improved agricultural practices. In: Grout BWW, Morris GL (Eds) *The Effect of Low Temperatures on Biological Systems*, Arnold Press, London, UK, pp 389-409

Woolfe J (1993) *Sweet Potato: An Untapped Food Resource*, Cambridge University Press, Cambridge, 643 pp

Wu YW, Ma TP (1979) Isolation, culture and callus formation of *Ipomoea batatas* protoplasts. *Acta Botanica Sinica* **21**, 334-338

Xing YJ, Ji Q, Yang Q, Luo YM, Li Q, Wang X (2008) Expression of an engineered tandem-repeat starch-binding domain in sweet potato plant. In: Liu QC (Ed) *Proceedings of the 3rd China-Japan-Korea Workshop on Sweet Potato*, October 12-15, 2008, China Agricultural University Press, Beijing, pp 317-325

Yang YF, Guan SK, Zhai H, He SZ, Liu QC (2009) Development and evaluation of a storage root-bearing sweet potato somatic hybrid between *Ipomoea batatas* (L.) Lam. and *I. triloba* L. *Plant Cell, Tissue and Organ Culture* **99**, 83-89

Yenofsky RL, Fine M, Pellow JW (1990) A mutant neomycin phosphotransferase II gene reduces the resistance of transformants to antibiotic selection pressure. In*: Proceedings of International Academy of Science*, USA, pp 3435-3439

Xing Y-J, Ji Q, Yang Q (2008) Studies on *Agrobacterium*-mediated genetic transformation of embryogenic suspension cultures of sweet potato. *African Journal of Biotechnology* **7 (5)**, 534-540

Zhang BY, Liu QC, Zhai H, Zhou HY (1999) Effective regeneration of interspecific somatic hybrid plants between sweet potato and its kindred species. *Scientia Agriculturae Sinica* **32 (6)**, 23-27

Zhang BY, Liu QC, Zhai H, Zhou HY, Zhang DP, Wang Y (2002) Production of fertile interspecific somatic hybrid plants between sweet potato and its wild relative, *Ipomoea lacunosa*. *Acta Horticulturae* **583**, 81-85

Zhou Z, Wang X, Ma DF, Li HM, Xie YP, Li XY (2005) Identification of RAPD markers linked to stem nematode resistant gene in sweet potato. *Journal of Agricultural Biotechnology* **13**, 549-552