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Utilization of Biotechnological and Agronomic Options for Unlocking the Yield Potential of Sweetpotato (Ipomoea batatas (L) Lam.)

Naher, Mst. Kamrun

University of Rajshahi

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Utilization of Biotechnological and Agronomic Options for Unlocking the Yield Potential of Sweetpotato (*Ipomoea batatas* (L) Lam.)

Ph.D Thesis

A Dissertation Submitted in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Botany, University of Rajshahi.

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উদ্ভিদ বিজ্ঞান বিভাগ
রাজশাহী বিশ্ববিদ্যালয়
রাজশাহী-৬২০৫

DEPARTMENT OF BOTANY
Rajshahi University
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Certificate

It is our pleasure to certify that this dissertation entitled: **Utilization of Biotechnological and Agronomic Options for Unlocking the Yield Potential of Sweetpotato (*Ipomoea batatas* (L) Lam.)**, prepared by Mst. Kamrun Naher, Ph.D Fellow in Botany (Session 2006-2007) at the Biotechnology and Microbiology Laboratory, Deptment of Botany University of Rajshahi, Rajshahi, Bangladesh. It is her original work and she prepared the dissertation under our supervision. We also certify that we have gone through the draft and final version of the dissertation carefully and found it satisfactory for submission to the Depertment of Botany, University of Rajshahi. The dissertation is recommended and forwarded to the University of Rajshahi through the Deptment of Botany for necessary formalities leading to its acceptance in partial fulfillment of the requirements for the doctoral degree. The work or part of it has not been submitted before as candidature for any other degree.

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My Sons

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&

Md. Yousha Ebadi Nuha

DECLARATION

I do hereby declare that the Ph.D thesis entitled "Utilization of Biotechnological and Agronomic Options for Unlocking the Yield Potential of Sweetpotato (*Ipomoea batatas* (L) Lam.)" has been carried out by me in the Biotechnology and Microbiology Laboratory under the guidance and supervison of Professors Late Dr. M. Anisuzzaman and Dr. Farzana Ashrafi Neela, Department of Botany, University of Rajshahi is now submitted as a thesis towards the partial fulfillment for the degree of Doctor of Philosophy in the Department of Botany, University of Rajshahi, Rajahahi, Bangladesh. I also declare that, I have never submitted the thesis or any part of this thesis for any degree or diploma elsewhere. This is the original work of mine.

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ABSTRACT

Sweetpotato (*Ipomoea batatas* (L) Lam.) is one of the world's highest yielding crops hold promise to combat nutrition and food deficiency especially in the developing countries. This work aims to apply biotechnological tools and agronomic practices such as meristem culture to produce pathogen eleminated plant meterials, callus induction for inducing somaclonal variation, nitrogen, and nitrogen in combination with potassium application for incrising tuber yield. Four cultivars viz. BARI-1 (Tripti), BARI-2 (Kamalasundari), BARI-3 (Daulatpuri) and BARI sweeet potato -4 were used for these experiments. Shoot tips of 3-4 weeks old field grown vines were used for meristem isolation. Explants were surface sterilized with 0.1% mercuric chloride for 6 minutes. MS liquid medium supplemented with 2.0 mg/l Kinetin+0.5 mg/l GA_3 and 4% sucrose was found most effective for the primary establishment of isolated apical meristem. For shoot and root development from primarily established apical meristem application of 2.5 mg/l Kinetin+0.5 mg/l GA_3 in MS medium was found to be the best. For virus testing, grafting method offeres very low cost options compared to molecular methods. It does not require any special equipment or chemicals. Therefore, plantlets derived from meristem-tips were assayed with virus sensitive indicator plants *Ipomoea sestosa* using grafting method. The assay revealed most of the mericlones are virus-frees. Virus-tested mericlones were further micropropagated and transferred to nursery for further multiplication. Growing mericlones showed some disease symptoms in the second generation indicating re-infection possibly by viral vectore. Use of 3.0 mg/l Kinetin+0.5 mg/l GA_3 and 30% sucrose in MS medium was the most effective for micropropagation of meristem-derived plantlets. Among the studied three explants (internode, root and leaf disc), both internode and root were found good for producing high amount of embryogenic callus. Among the studied different treatments combination of growth regulators, three treatments viz. 2 mg/l 2,4-D+0.5 mg/l NAA, 4 mg/l BAP+0.5 mg/l NAA and 2.0 mg/l TDZ were found very effective in producing embryogenic callus. For plant regeneration from callus 0.5 mg/l BAP+2.0 mg/l 2,4-D was most effective for both internode and root derived callus but for leaves it was 2.0 mg/l zeatin,

showing explants-media interaction. The successful acclimatization of the *in vitro* raised plantlets provides the reproducibility of the developed protocol of using biotechnological techniques. Several somaclones were planted in the field to check their morphological and tuber yield variation. Upon planting in the field condition, several callus-derived plants showed significant variation in their morphological traits. Among these, some somaclones showed better performance in terms of vine length, leaf area and fresh weight of tuberous root. Nevertheless, some were very similar to the mother plants while others are inferior. Among these, some somaclones of BARI-1 showed better performance in terms of vine length, leaf area and fresh weight of tuberous root. On the other hand somaclones of BARI-3 showed poor performance for the same traits. Effect of nitrogen fertilizer has also been studied as one of the main agronomic practices which might affect sweetpotato yield. To test the effect of potassium and nitrogen fertilizer, both conventional vine and disease-free mericlone were tested in this experiment. It was observed that nitrogen fertilizer as urea significantly influences in all vegetative growth traits in both mericlone originated and traditional planting materials. A further increase was observed when both potassium and nitrogen are used combinedly. Tuberous root yields of both the mericlone-derived and traditional planting material were significantly higher than no zero fertilizer treatment groups and peaked at 160 kg/ha urea and 180 kg/ha potash. Fertilizer application positively influenced tuber length and tuber diameter. However, there was no difference in the number of tubers. It was observed that mericlone gave higher tuber yield compared to conventional planting materials in identical fertilizer level. It was revealed that, use of mericlone and the optimal fertilizer can increase up to 70% tuberous yield. From the above study, the results revealed that among all fertilizer doses of NK, 160N+180K treatment gave the highest gross margin and benefit cost ratio (BCR) in both situation Mericlone and conventional. On the other hand, Mericlone plant source of BARI -1 is the best for high yield production of sweet potato. So, treatment Nitrogen and potassium (NK), 160N kg/ha +180K kg/ha and Mericlone plant source of BARI -1 are economically viable for our farmers.

ABBREVIATIONS

The following abbreviations have been used throughout the text:

%	:	Percentage
μ	:	Micron
μM	:	Micro mole
0.1 N	:	0.1 Normal
2, 4-D	:	2, 4- diclorophenoxy acetic acid
ANOVA	:	Analysis of varince
BAP	:	6-benzylaminopurine
cm	:	Centimeter
E	:	East
e.g.	:	Exempli gratia, for example
et al.	:	et alii and others
etc.	:	et cetra, and the rest
Fig./s	:	Figure/ Figures
GA_3	:	Gibberellic acid
gm	:	Gram (s)
ha	:	Hactare
$HgCl_2$	:	Marcuric Chloride
HPT	:	Hygromycine phosphotransferase
hr(s)	:	Hour(s)
i.e.	:	id est=which to say in other words
IAA	:	Indole-3-acetic acid
IBA	:	Indole-3-butaric acid
Kg/ha	:	killogram/hectare
KIN	:	Kinetin (6-furfural amino purine)
LSD	:	Least significant difference
m	:	meter
mg	:	Milligram
mg/l	:	Milligram/litre
ml	:	Milliliter
mm(s)	:	Millimeter (s)

MS	:	Murashige and Skoog (1962) medium
N	:	North
Na_2-EDTA	:	Sodium salt of ferric ethylene diamine tetra acetate
NAA	:	α-naphthalene acetic add
NaOH	:	Sodium hydroxaide
No.	:	Number
Ppm	:	parts per million
Sec	:	Second (s)
Sp	:	Species
T-DNA	:	Transfer DNA
TDZ	:	Thidiazuron
mn(s)	:	Minute (s)
V/v	:	Volume by volume
Viz	:	Namely
wt.	:	Weight
BCR	:	benefit cost ratio

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CHAPTER-1
INTRODUCTION

1.1 General Introduction

Significant part of global population including those from Bangladesh is suffering from serious malnutrition. Vitamin A deficiency (VAD) is a major public health problem among young children in many countries including Bangladesh. Sweetpotato (*Ipomoea batatas* (L.) Lam.) is one of the world's highest yielding crops with higher food value and total food production per unit area exceeding that of rice. Pro-vitamin A rich orange-fleshed sweetpotato (OFSP) can address inadequate vitamin A intakes and food security. The diversified of OFSP thus need to be developed to cater for the country's growing urban population.

Due to its high yield relative to both land and labor, its capacity to grow in nutrient-poor soils, and its high content of carbohydrates and vitamins, especially vitamin A, sweetpotato holds the promise to combat malnutrition and economic gain. Having major economic importance in Bangladesh, it ranks fourth among the food crops after rice, potato and wheat and seventh in the world in terms of total production (FAOSTAT, 2012). Sweet potato occupies the fourth place in acreage after rice, wheat and potato and covered 32,000 hectares of cultivated area of Bangladesh with an annual production of 98438 kg/ha (FAOSTAT, 2012). According to the FAOSTAT statistics the annual sweet potato production in the world was about 106 million tones (FAOSTAT, 2012). However, sweetpotato has minor contributon to the country’s food basket.

1.2 Needs of sweetpotato research for food security and fermers’ well-being

Although sweetpotato ranks the fifth most important food crop in the developing world (after rice, wheat, maize, and white potato), it has been relatively neglected by the global agricultural research community. Recent statistics reveals that there has been a significant increase in agricultural production in

Bangladesh over the last four decades, the production of rice has increased by about 3.5% per year over the last decade, wheat has also experienced a significant increase of 5.5% per year over the same period. But the production of minor grains declined by 0.93%, pulses by 3.72% and sweetpotato by 2.8% over the same period (Alam, 2005). Consequently, the country became more dependent on imports of maize and pulses. It is, therefore, necessary to explore possibilities of import substitution and export promotion through diversification of crop agriculture. Country has substantial potential for diversifying crop agriculture through the expansion of secondary crops. However, the production of maize and potato has experienced a respectable growth rate during the last decade. The tremendous importances of roots and tubers as a source of income and food for poor people of rural and urban area are often overlooked in the debate about improving food security and eradicating poverty in developing countries. Hopefully, the analyses of the International Potato Center (CIP) and the International Food Policy Research Institute (IFPRI, 2000) are helping to give these crops appropriate consideration in future deliberations about the global food system at the national and international levels and thereby improve efforts to ensure access to sufficient food and income for all people (IFPRI, 2000; CIP, 2000b). IMPACT simulations (IFPRI's International Model for Policy Analysis of Agricultural Commodities and Trade) indicate that roots and tubers will play economically important and increasingly diversified roles in developing-country food systems over the next two decades (Scott *et al*., 2000).

A field study conducted by United Nation's Centre for Alleviation of Poverty through Secondary Crops (UNESCAP-CAPSA) in 12 districts of Bangladesh on secondary crops suggests that both financial and economic returns to production of sweetpotato is positive. The financial incentive for production of sweetpotato was examined by calculating Nominal Protection Co-efficient (NPC), Nominal Rate of Protection (NRP), Effective Protection Co-efficient (EPC) and Effective Rate of Protection (ERP). Results showed that there are reasons for protecting these crops for import substitution (Alam, 2005). The

calculated Simpson Index of diversity for the allocation of land to different crops revealed crop diversification is slow over time in Bangladesh (0.42 in 1989-1990 compared to 0.43 in 2001-2002) (Alam, 2004). Thus breeding and agronomic research for the generation of improved technologies in the production of sweetpotato should receive high priority.

By 2020, it is envisioned that the environmentally sound production of a diversified range of high quality, competitive products for food, feed, and industry will integrate sweetpotato into emerging markets. Sweetpotato adaptation to marginal environments, its contribution to household food security, higher nutritional value than any major food items, low production cost and its great flexibility in mixed farming systems, make it an important component of a targeted strategy for improving the welfare of the rural poor and linking smallholder farmers to emerging markets (Scott *et al*., 2000).

Although the projections for future production and use of sweetpotato in developing countries including Bangladesh are realistic, they are by no means guaranteed due to a variety of technical constrains. To minimize or overcome these constraints will require improved germplasm including more technically and economically efficient procedures for producing sweetpotato. In addition to these concerns, a recent review of current research on roots and tubers in the CGIAR and CIP identified the following commodity-specific priorities (Scott *et al*. 2000).

Concerted international efforts are under way to increase dry matter content and yield, exploit national and international germplasm for appropriate postharvest characteristics, including starch quality and pre-beta carotene content, and systematically support national efforts to foster greater product development for sweetpotato by small and medium-scale farmers and entrepreneurs. This is because sweetpotato cultivation is often concentrated in the poorest growing areas and among farmers with limited-resources.

CIP's recent analysis indicates that in the near future, sweetpotato will contribute more to the global food system as a source of starch and animal feed in Asia. It will also grow in importance as a source of vitamin A (CIP, 2000a). So, varietal improvement is very important in achieving increased production, considering the importance of this crop.

At the international level, CIP has had a research program of global sweetpotato improvement since the mid 1980s. AVRDC and IITA did some sweetpotato research even prior to this but later divested of their sweetpotato improvement programmes (Fuglie *et al*.,1999). In Bangladesh sweetpotato improvement programme is mainly confined on selections based on field trials of exotic germplasm and local landraces to quest for weevil and virus resistance, improved nutritional quality and increased dry matter content. Research is also conducted using various agronomic practices such as application of different fertilizers, irrigation, planting and harvesting schedule adjustment, planting density etc in order to increase yield and quality (Hossain *et al*., 1987; Siddique *et al*., 1988; BARI, 2001; BARI, 2002; Islam *et al*., 2002; BARI, 2003).

Given the fragile balance between the population, food production and economic growth, government policy seeks to ensure the country's continued ability to sustain food self-sufficiency. Increases in agricultural productivity and agro-enterprise development are key elements in the government's strategy to increase the incomes of rural households and to facilitate further expansion of overall economy. It is, therefore, necessary to find out ways to shift from rice monoculture to diverse agriculture through the increased production and utilization of secondary crops, like sweetpotato to improve incomes and food security particularly among the poorer segments of the rural population.

1.3. Biology of sweetpotato

1.3.1. Origin and evolution

Sweetpotato (*Ipomoea batatas* (L.) Lam.) was originally domesticated in tropical America more than 5000 years ago (Austin, 1978; Austin, 1988; Yen,

1982). The exact center of origin and domestication of the sweetpotato *has* not been well defined, neither has the wild ancestor of this species been found. Based on the numerical analysis of key morphological characters of sweetpotato and the wild *Ipomoea* species, Austin (1988) postulated that sweetpotato originated in the region between the Yucatán Peninsula of Mexico and the Orinoco River in Venezuela, within which the four major American taxa of the batata group are distributed. Recent reports using molecular markers to assess diversity have found the highest diversity in Central America, supporting the hypothesis that Central America is the primary center of diversity and most likely the center of origin of sweetpotato (Zhang *et al.,* 2000).The sweetpotato *was* one of the first plant introductions from the Americas into Europe from the voyage of Columbus in 1492. From Europe this crop was taken by the Portuguese explorers of the sixteenth century to Africa, India, Southeast Asia and the East Indies (‘batata line’ of dispersal). Direct transfer of Mexican sweet potatoes occurred by Spanish trading galleons between Mexico and the Philippines in the 16th century (camote line). There is a third line of prehistoric transmission: the introduction of the sweetpotato to the Pacific islands prior to the era of European exploration (Yen, 1982). Fossil carbonized storage roots of sweetpotato found in northern New Zealand have been dated back some 1000 years (Yen, 1991).

Rossel *et al*., (2001) analyzed seventy-six sweetpotato cultivars from Peru-Ecuador, Mexico, the Philippines and eight Oceania countries using amplified fragment length polymorphism (AFLP). Multidimensional scaling (MDS) and analysis of molecular variance (AMOVA) revealed wide genetic variation in the Oceania gene pool, greater than that of Peru-Ecuador. There was a significant sweetpotato “gene flow” from Mexico to Oceania. Their results suggest that Peru-Ecuador may not be the source of the Oceania germplasm. Oceania sweetpotato probably came from Mesoamerica through non human dispersal.

1.3.2. Botanical Description

Sweetpotato (*Ipomoea batatas* L.) is a dicotyledonous, perennial plant that are cropped as annuals to produces edible tuberous roots. It belongs to the

morning glory family *Convolvulaceae*, and genus *Ipomoea* that is thought to contain over 500 species with ploidy levels ranging from 2x to 6x (Ozias-Akins and Jarret, 1994). Sweetpotato is the only *Ipomoea* species of economic importance as a food crop. It has both 4x and 6x forms (2n = 4x = 60 or 2n = 6x = 90) and probably originates from a cross between the ancestors of *I. trifida* and another wild *Ipomoea* sp., in Central or Northern South America, at least 5000 years ago (Zhang *et al.,* 2000).

Sweetpotatoes are top herbaceous, drying back to ground each year. Stems forming a running vine up to 4 m long, usually prostrate and slender, with milky juice, lateral stem-branches arising from the short stem and usually not branched Leaves ovate-cordate, borne on long petioles, palmately veined, angular or lobed, depending on variety, green or purplish.

Sweetpotato produces complete flowers of white or pale violet colour with a compound, superior pistil and 5 independent stamens attached to the trumpet-shaped corolla, borne singly or in cymes on short peduncles. Seeds are produced in round pods with 1-4 seeds per pod. Seeds are flattened, hard-coated, and angular. Sweetpotatoes set few viable seed. Many genotypes do not flower easily, and some not at all. It is possible to enhance flowering by trellising vines or by grafting to other *Ipomoea* spp. Defective pollen, self compatibility, self-incompatibility, cross-compatibility, and cross incompatibility all occur in sweetpotato (Clark and Moyer, 1988). Due to problematic seed production and compatibility, sweetpotatoes are produced through vegetative propagation.

Sweetpotato plants produce primary fibrous roots, pencil roots and storage roots. Storage roots are edible. Storage roots are attached to the stem by a stalk of thinner root, which is usually initiated at the stem node just below the soil line. Skin color of storage roots ranges from white to brown to red-orange. Flesh color of storage roots can be red-orange, orange, yellow or white. The flesh can be either soft or firm.

1.4. Sweetpotato cultivars in Bangladesh

Sweetpotato introduced to India and as well as in Bangladesh by the Portuguese during the 16th century (Naskar *et al*., 1999). A large number of sweetpotato genotypes are cultivated in different parts of Bangladesh. They are mostly popular local cultivars having almost no varietal purity. They are usually grouped as white or purple type depending on their skin colour of storage root. Most of the existing cultivars are from clonal selection and self incompatible. A number of cultivars have been obtained through selection and introduction of exotic germplasm or deliberate hybridization (Varma *et al*., 1993).

International Potato Centre (CIP) maintains world's largest gene bank of sweetpotato germplasm having 5,526 cultivated accessions from 57 countries, 2,589 of them from Latin America, Peru alone contributed 1,099 accessions (Zhang *et al*., 2000). A number of sweetpotato germplasm is now available in Tuber Crop Research Center, Bangladesh Agricultural Research Institute, BARI, Joydepur (Hossain and Sultana, 1997). The principal sources of genetic material are CIP, Asian Vegetable Research and Development Centre (AVRDC), Taiwan and the collections of native material by the BARI.

In search of new improved sweetpotato varieties with value added traits, trials are being carried out in different agroecological environments of the country. Some of them are found having good potential as follows: BARI-1 (Tripti), BARI-2 (Kamalasundari), BARI-3 (Daulatpuri), BARI-4, BARI-5, BARI-6 BARI-7, BARI SP-40025, BARI SP-412, BARI SP-434, BARI SP-454 etc. A number of CIP sweetpotato lines viz. Lalkuthi, Kalomegh, D/II/44, 440025, 490074-2 are primarily selected for advanced yield trial and subsequent regional yield trial under the collaboration of BARI and CIP's South and West Asia region programme (CIP, 2000b; BARI, 2002; 2003). Most of them give high yield and have desirable characteristics for varietal improvement.

After successive trials high yielding BARI-1 (Tripti), BARI-2 (Kamala Sundhuri), BARI-3 (Daulatpuri), BARI-4, BARI-5, BARI-6 and BARI-7 have already been released and recommended for commercial cultivation under different

agroecological conditions in the country by the Tuber Crop Research Center (BARI, 2002, 2003).

1.5. Propagation and cultivation

Sweetpotato can be propagated by means of sprouts from tubers or by means of vine cuttings. Healthy tubers of 20 to 50 g should be planted 3 cm deep. The use of sprouts derived from tubers for direct planting of sweetpotato is, however, not recommended as a general practice, because it usually results in low yields compared to stem cuttings. Vine cuttings are the usual method of propagating sweet potato. It is better than using sprouts from tubers for several, reasons. Firstly, plants derived from vine cuttings are free from soil-born diseases. Secondly, by propagating with vine cuttings, the entire tuber harvest can be saved for consumption or marketing instead of reserving some of it for planting purposes. Thirdly, vine cuttings yield better than sprouts, and produce tubers of more uniform size and shape. In the use of vine cuttings, apical cuttings are preferred to those from the middle and basal portion of the stem. However, where the planting material is in short supply, middle and basal portion of the vine cuttings can be used with little decrease in expected yield. Tuber yield tend to increase with increase in the length of the vine cuttings used, and a length of about 30 cm is recommended. Cuttings of greater length than this tend to be wasteful of planting material, while shorter cuttings establish more slowly, and give poorer yields. Various strategies can be adopted to ensure an adequate supply of cuttings at planting time, including nurseries, sprout from storage roots and successive planting.

1.6. Yield-limiting pests and diseases

In Bangladesh, the economically important diseases are viruses, *Fusarium* root and stem rot, *Fusarium* surface rot, soil rot, *Rhizopus* soft rot, scurf, and most devastating sweetpotato weevils. Whiteflies are primarily responsible for virus transmission from infected plant to the healthy one. Generally, any single type of virus causes a little damage but Co-infections of SPCSV with SPFMV and/or SPMMV may result in viral synergism leading to development of severe

symptoms and significant yield losses than infections with each of the viruses alone, although the actual occurrence of viral synergism should be verified in each case. Rotting during post harvest can be prevented or reduced through maintaining proper temperature and humidity. Most of the field disease can be prevented by planting of disease free planting material. It is especially true for viral diseases, since no resistant line is yet developed among Bangladeshi sweetpotato cultivars (BARI, 2003). Some common disease symptoms are presented in Table-1.2 as reported by various investigators.

Table-1.1: Pests and diseases of sweetpotato

Disease	Causal agent	Infected portion	Symptoms
Viral Diseases			
1. Feathery mottle virus (SPFMV)	Sweetpotato Feathery mottle virus (SPFMV)	Leaves	Symptoms appeared as faint to distinct chlorotic patterns on the leaves, sometimes with pigmented borders. Some infected cultivars express internal (internal cork) or external (russet crack) necrosis on the roots. Infected plants may also be symptomless.
2. Mild mottle virus	Sweetpotato mild mottle virus (SPMMV)	Whole plants	Leaf mottling, vein chlorosis, dwarfing and poor growth.
3. Leaf curl	Sweetpotato leaf curl virus (SPLCV)	Leaves and stems	Symptoms included leaf curling, leaf chlorosis, and yellow mottle. Several cultivars reacted with a transient leaf curl that showed only during a short period of time.
4. Sweet potato latent virus	Sweet potato latent virus (SPLV)	Leaves	Symptoms appears as latent or mild chlorosis in sweet potato
5. Cucumber mosaic virus	Cucumber mosaic virus (CMV)	Leaves	Stunting, chlorosis and yellowing of plants. CMV apparently only infects sweet potato plants previously infected with SPFMV.
6. Chlorotic Stunt	Sweetpotato chlorotic stunt virus (SPCSV)	Leaves	Very mild mosaic in old and new leaves

Insect			
1. Sweetpotato weevil	*Cylas formicarius* (Fab.)	Stems and tubers	Roots become porous and dry up, toxic sesquiterpenes produced by the roots in response to weevil feeding impart a bitter taste to the storage roots. Roots are completely destroyed within very short period of time in post harvest storage. Vines are also destroyed in the field.
2. Sweetpotato whitefly	*Bemisia tabaci*	Leaves	Minor injury of leaves
Fungal Diseases			
1. Black rot	*Ceratocystis fimbriata*	Young leaves stem and roots	Circular, almost black spots appear on roots. Affected and adjacent areas have a bitter taste. Small, black lesions often completely girdle underground stems. The fungi fruits abundantly in storage, which helps separate black rot from other storage rots.
2. Stem rot or wilt disease	*Fusarium oxysporum* f. *batatas*	Leaves stem and roots	Leaves become yellow along with discolouration of vascular bundles, stunted growth.
3. Scurf	*Monilochaetes infuscans*	Storage roots (post harvest)	Scurf appears as light brown blotches on the outside of roots. These areas may be small or run together to form large, irregular patches. Although superficial, only skin deep, infection reduces grade and causes undue shrinkage in storage.
4. Soft rot	*Rhizopus stolonifer*	Storage roots (post harvest)	General rotting of storage root leads to complete destruction of infected root after harvesting.
5. Circular spot	*Sclerotium rolfsii*	Storage roots (post harvest)	Lesions of round and up to one inch in diameter are formed. The skin in the center of the spot may crack as roots dry in storage. Circular spots seldom progress in size during storage.
6. Cercospora leaf spot	*Cercospora batatae*	Leaves	Symptoms appear as dark brown leaf spot with a light shade at the centre.

Bacterial Diseases			
1. Soil Rot	*Streptomyces ipomoea*	Storage roots	Rough, scabby pits or shallow surface lesions found on root surface.
2. Stem and Root Rot	*Erwinia chrysanthemi*	Whole plant including storage roots	Dark brown to black lesions are found on stems with dark streaking in the vascular tissue. Infected areas inside storage are light brown and watery. Storage root infection and vine infections often occur independent of each other.

Ref. BARI, 2003; Gutiérrez *et al*., 2003; Mukasa *et al*., 2003

1.7. Nutritional value and uses

Sweet potatoes are rich in starch, which are small in size, resembles corn starch granules in shape rather than tuber starch granules. Quality of sweetpotato is mostly dependent on its sugar content. It varies from 3-6% with cultivars and conditions. Mostly sucrose and a few reducing sugars are present. Maltose, mannose, galactose and pentoses occur only in traces. Sugar percentage in sweetpotato tubers increases during storage and cooking.
Although, the main nutritional importance of sweetpotato is in the starch content of the root, sweetpotato also contains appreciable amount of vitamin A, B and C. Some of the sweetpotato cultivars contain carotene as high as 5.4-20 mg/100g. The carotene content varies with cultivars; yellow- and orange-fleshed cultivars are rich in carotene, which is a precursor of vitamin A (Varma *et al.,* 1993). Leaves contain about 3-2 per cent protein on fresh weight and 12-6 per cent on dry weight basis. The leaves are also rich in carotene and vitamin C. The green tops contain 4% protein and are also a good source of Ca, P and Fe. The most important constituents of fresh tubers of sweetpotato are carbohydrate, protein, fat, sugar, appreciable amount of vitamin A, B and C and some minerals. Table 1.1 shows the average chemical composition of fresh tuber and vine. Note that, chemical composition may vary with cultivars.

Sweetpotato is an important crop in many parts of the world. The storage roots of sweetpotato serve as staple food, animal feed and to a limited extent as a

raw material for industrial purposes as a starch source and for alcohol production. In Japan dehydrated sweetpotato is ground into flour, which is cooked for human consumption. Sweetpotato starch is used for the manufacture of adhesives, textile and paper sizing and in the confectionery and baking industries. In most parts of the tropics, sweetpotato is consumed boiled, baked, roasted or filed. Preparation practices vary according to the location. In Ethiopia, roots are boiled unpeeled or roasted unpeeled in the ash of a fire before being eaten, or less commonly, the sweetpotato is boiled or fried with other vegetables or root crops. In Taiwan most sweet potatoes are eaten boiled or boiled and mixed with white rice. The filed chips are packaged and sold as a snack. For some areas in Asia, the snack types of sweetpotato cultivars were bred for maximum yield and respond to high management inputs. The snack type of sweetpotato used in Japan and Taiwan is also important, particularly in urban areas. The tender leaves are used as a vegetable in Africa, Indonesia and the Philippines. The vines are widely used as a fodder for livestock. Sweetpotato has some medicinal values also. The root is considered laxative. In Malaya, a drink is prepared from the root is given to allay thirst in fever. Tops and tender shoots are used in poultices and leaves as a maturative cataplasm. In Ghana, the leaves are ground with salt and applied to whitlow.

Table-1.2 : Average Chemical Composition of Fresh Tuber and Vine

Constituent	Tuber (%)	Vine (%)
Moisture	70	80.7
Carbohydrates	27	9.7
Proteins	1.5-2.0	4.2
Fats	0.2	0.8
Fibers	1.0	2.4
Sugars	3.0-6.0	-
Minerals (mg/100g)		
Calcium	46	360
Phosphorus	49	60
Iron	0.8	10
Magnesium	24	-
Potassium	373	-
Sodium	13	-
Chlorine	85	-
Sulphur	26	-
Vitamins (mg/100g)		
Thiamine	0.08	0.07
Riboflavin	0.04	0.24
Niacin	0.70	1.70
Vitamin C	24.0	27.0
Carotene	0-18.0	4.0
Energy (K cal)	120	63

Ref: Varma *et al.,* 1993; Thompson *et al.*,2006

1.8. Research in sweetpotato improvement

With the hight necessecity of sweetpotato to combat hunger and malnutrition, improvement programs should be taken up on short- and long-term basis using various breeding methods such as collection, conservation, evaluation, and use of genetic resources, development of new technologies to enlarge genetic variation; development of breeding lines with highly general and specific combining abilities; establishment of efficient selection methods; development of simple evaluation techniques for quality etc. These breeding programmes can be accelerated efficiently using various biotechnological tools.

1.8.1. Conventional Breeding

The strategy can be carried out for conventional breeding of sweetpotato such as interspecific and intervarietal hybridization, population improvement through open pollination, polycross, polyploid, and mutation breeding, selection for various value added traits such as selection for early maturity, drought resistance, from clonal population. Progress has been made in the improvement of sweet potato by using conventional breeding methods for transfer of resistance to diseases (Clark, 1986), nematodes (Jones and Dukes, 1980) and insects (Jones and Cuthbert, 1973), and also for increasing protein content and nutritional quality (Walter *et al*., 1984). Selection is found to be efficient for improvement of Indonesian sweetpotato cultivars (Jusuf, 2003). By using polycross, selection for particular characteristics, such as yield, appearance, and disease and insect resistance can be combined and crossed. Using this technique some orange-fleshed sweetpotato lines having good amount of dry matter content has developed in South Africa (Laurie, 2001). Nevertheless, the selection process is time- consuming and requires a high number of individuals and improved breeding systems, because of the hexaploid status of sweet potato. In addition, breeding efforts may be hampered by the fact that incompatibility and sterility (Martin, 1968, 1970; Martin and Jones, 1971) can also occur between or within species of sweet potato.

CIP's study with commercial processors indicates that dry-matter increases of 25 percent or more will place sweetpotato in a highly competitive position against major sources of cereal-based starch and animal feed. Breeding for high dry-matter content in sweetpotatoes has been very successful at CIP. Improved germplasm that produces sweetpotatoes with 38 percent dry matter (versus the typical dry matter level below 30 percent) and disease resistance has been distributed and is now used in national breeding programs or evaluated on its own (CIP, 2000a).

1.8.2. Biotechnology

Due to the biological complexities of sweetpotato (as it is a perennial-polyploid and vegetatively propagated crop), sexual hybridization strategies have not been very effective in developing improved cultivars because of various constrains like male sterility, incompatibility, low pollen viability, and reduced stigmatic receptivity etc. Beside these, there is also low heritable natural resistance to sweetpotato weevils in its germplasm pool. Therefore, biotechnological tools, such as gene transfer, are very attractive in sweetpotato improvement, as they enable direct introduction of desirable genes from other sources into preadapted cultivars. Selection using DNA markers accelerates conventional breeding programmes, and DNA fingerprinting facilitates improved germplasm collection activities and management of gene banks (Prakash, 1994).

Meristem culture provides clean and healthy planting materials. Somatic embryogenesis has also been achieved in sweetpotato tissue culture. Artificial seeds have produced through encapsulation of somatic embryo in gels. Control of sweetpotato feathery mottled virus (SPFMV) and sweetpotato weevil is the most important strategy of CIP in order to increase production and quality. But it is difficult to control these pests by using chemical, mechanical, or biological strategies, thus calling for alternative strategies. Recently, soyabean trypsin inhibitor gene has, which encode serin-type proteinase inhibitors (proteins), have been cloned and transferred into sweetpotato to confer resistance against weevil (Cipriani *et al.,* 1999). Similarly, resistance against sweetpotato feathery mottle virus has achieved through transgene expression of rice cysteine proteinase inhibitors (Cipriani *et al*., 2001). Improved virus detection techniques such as ELISA and RT-PCR have been developed to ensure planting materials free from viruses. Marker assisted selection can accelerate breeding programme through efficient selection. Fortunately, the first amplified fragment length polymorphism (AFLP) linkage map was developed for an important African traditional cultivar (CIP, 2000a).

1.9. Justification of the current work

Although sweetpotato has tremendous potential for food security, nutrition and overall well-being of growers, its productivity is limited by a number of both biotic and abiotic constrains. Viral disease is considered as one of the most important cause of yield loss and cultivar decline. Among the 11 well-recognized sweetpotato viruses, sweetpotato feathery mottle virus (SPFMV; Potyvirus) has a pervasive distribution while the others are localized to one or more geographic areas (Moyer & Salazar, 1989; Kreuze et al., 2000; Mukasa, 2004). Diverse strains of SPFMV coupled with its ubiquitous nature hindered the identification of many other viruses. In Bangladesh, at least five sweetpotato viruses have been reported (BARI, 2003). Multiple infection and synergism are common in sweetpotato. Sweetpotato viral disease (SPVD), caused by the synergistic interaction of sweetpotato feathery mottle virus and sweetpotato chlorotic stunt virus reduce yield by up to 98% (Mwanga et al., 2002). Besides this, chlorotic dwarf, Camote Kulot and some other complex infections exist (Di Feo et al., 2000; Salazar & Fuentes, 2000). Virtually all sweetpotatoes grown from non virus-tested materials revealed the presence of one or more viruses within them (Moyer & Salazar, 1989).

Accumulation of viruses and diseases occur in sweetpotato through the adventitious, root-to-sprout propagation method used in commercial production. In most regions including Asia and United States, next season’s sweet potato crop is produced by using adventitious sprouts of 'seed' roots saved from the previous crop. Saved 'seed' roots of sweetpotato plants that became infected with viruses and during the growing season produce virus-infected adventitious sprouts. Continuous use of virus-infected planting material may lead to cultivar decline. Moreover, some of the viruses have insect vectors which increase the rate of reinfection in growing season on availability of local inoculum and favorable environment. Overtime, the entire population of a given clonal variety could be infected with the disease. Even without visible symptom, infected plants exhibit reduced growth and yield performance, and could spread the disease to non-target varieties. SPVD epidemics have been, in many cases,

associated with the disappearance of a former elite cultivar (Gibson et al., 1997). Yield loss due to viral diseases was estimated to be 15 to 48% in China, 34% to 97% in Egypt (Salazar & Fuentes, 2000) 50% or more in Israel (Milgram et al., 1996) and 80 to 98% in East Africa (Mwanga et al., 2002; Wambugu, 2003). Quality was also affected by alterations in the shape and skin color of storage roots. The lack of resistant genotypes makes clean planting material as the only immediate straightforward solution to increase the yield and to maintain the production areas. Controlling sweetpotato viral diseases is one of the top research priorities of CIP adopting virus-free seed program to reduce this production constraint in developing countries (Zhang & Salazar, 2000). Current research have demonstrated significant benefits in yield and quality using pathogen tested planting material when compared to farmers' traditional non-tested material (Carey et al., 1999; Fuglie et al., 1999; Zhang & Salazar, 2000; Carroll et al., 2004).

Plant meristem culture is a unique technique to free away various pathogens including viruses, viroides, mycoplasma, bacteria and fungi (Razdan, 1996). Meristems are frequently devoid of systemic pathogen due to the absence of differentiated conducting tissues. In addition, the use of planting material derived from pre-existing meristems has been proposed to reduce the amount of variation among the propagules and to retain genetic integrity (Villordon & LaBonte, 1996). Therefore, its application may help to slow down the process of cultivar decline due to accumulation of viruses and mutations. Reports have been published on successful meristem culture and virus indexing in sweet potato and other crops in two decades (Frison & Ng, 1981; Dagnino et al., 1991; Alam et al., 2004). Nevertheless, sweet potato improvement through virus-indexed mericlones is important for unlocking yield potential of diversified elite genotypes grown under various agro-ecological zones and cultural practices by using disease-free and uniform propagules.

Virus detection is a routine work for virus-free planting material production and safe movement of germplasm. Serology or other molecular diagnoses are expensive for many developing countries. *Ipomoea setosa* is a nearly universal

sensitive indicator plant for sweet potato viruses which is used for graft transmitted virus detection. Current international guidelines document that graft-indexing successfully reveals most sweetpotato viruses (Moyer et al., 1989; Laurie et al., 2000; Loebenstein et al., 2003; Mukasa et al., 2003). Moreover, SPFMV often is present at a concentration below the limit of detection by ELISA (Winter et al., 1992; Vetten et al., 1996; Aritua et al., 1998; Gibson et al., 1998; Karyeija et al., 2000) and in that cases SPFMV can be detected only by grafting onto I. setosa instead of serological assay (Gutiérrez et al., 2003). Therefore, research institutes and seed enterprisers of the developing countries could benefit from using this technique for routine monitoring of planting materials without employing highly skilled manpower in an inexpensive way. Here it has been shown the effectiveness of graft transmitted virus-indexing system of mericlones and their field management as a means of suitable and low cost protocol for producing virus-free sweetpotato planting material for subtropical and warm temperate environmental conditions where viral diseases are very frequent.

Most cultivated sweet potato do not sets fertile seeds. Therefore, creating variation for breeding is difficult. In this case, creating variation in vegetative tissue is the only option. In plant breeding tissue culture in conventional micro propagation has resulted to a large extent in clonal fidelity, it has become increasingly clear that under the appropriate culture conditions, a great deal of genetic variability can be recovered in regenerated plants. If cultures are established from explants that did not contain a pre-organizedmeristem, or if cultures are maintained as callus prior to plant regeneration, the regenerated plants are quite variable. Reorganization of this spontaneous variation inherent in long-term culture led to the use of cell culture for mutagenesis and selection of genetic variants and for direct recovery of novel genotypes from cell cultures via somaclonal variation. Indications of somaclonal variation in several crop plants have stimulated interest inapplication of this method for crop improvement. Therefore, protocol has been developed for callus induction and plant regeneration from different tissues as a potential tool to create variations.

There is fair a body of literature on the use of inorganic fertilizers on sweet potato, although the information is limited compared with that for other staple crops in the tropics, such as rice and maize. Sweet potato consumes considerable amounts of K, and responses to K fertilizers are generally recorded (de Geus, 1973). Thus, Potassium is considered to be the most important nutrient in the production of sweet potato. Potassium promotes the efficient use of nitrogen and assists in water uptake. A number of studies showed that application of potassium fertilizer significantly increase tuberous yield. High potassium maintains the general health of sweet potato plants; potassium improves nutrient value, taste, color, and texture and disease resistance of crops. Potassium also affects the number, size, quality and the unit weight of tuberous roots produced, while the minimum levels of K suggested for healthy growth and yield are twice those recommended for N, although three times as much may be applied and occasionally even more (Degras, 2003). Sweet potato yield is significantly depressed if K is deficient, but eliminating P does not seem to affect the yield as the crop is well adapted to low levels of available P on account of its mycorrhizal association which makes P available to it. In Japan, it was estimated that a tuberous yield of 13 t/ha, removes about 70 kg N/ha, 20 kg P_2O_5/ha and 110 kg K_2O/ha from the soil depending on the variety, crop duration and agro-climatic region (Degras, 2003). Comparisons of this kind are rare for tropical areas but figures given by IFA (1991) depicted the particular importance of both K especially in the root and N in the leaves. Common recommendation in most countries is 35-65 kg N, 50-100 kg P2O5 and80-170 kg K2O per hectare (IFA, 1991). In the Hubei province of China, the optimum K rate varied from 150-300kg K2O/ha (Jian-wei et al., 2001). Whereas in India, the mean optimum requirement was put at 120 kg K2O/ha, while the maximum was 160 kg K2O/ha with a yield response of 6.7 t/ha (Trehan et al., 2009). Sweet potato response to applied K is considerably influenced by the variety grown (Trehan, 2007). The varietal response to applied K is often related to its yield potential and the number of large sized tubers it can produce. Generally, rapid bulking varieties producing

large sized tubers respond more to K than do the varieties with smalltubers (Trehan & Grewal, 1990).

Sweet potato has also high N requirements, but can produce reasonable yields in soils of poor fertility (Hill et al, 1990). This may be partly caused by its capacity to fix atmospheric N through association with symbiotic, non-nodulating bacteria (Yoneyama et al, 1998). However, cultivar differences of this trait are very large. It has been estimated that in some cultivars as much as 40% of the N uptake may be derived from di-nitrogen (Yoneyama et al, 1998). In literature, a very wide range of N fertilizer requirements has been reported for sweet potato (Hill, 1984; Hartemink et al 2000; Ankumah et al 2003). These are much dependent on the cultivar, soil type and climatic conditions (O'Sullivan et al, 1997). Thus it important to determine the effects of potassium and nitrogen fertilizer in locally cultivated varieties.

1.10. Aim and Objectives

Meristem culture and micropropagation are useful in producing disease-free elite clone in a massive scale. Clean seed derived from mericlones may be economically feasible for growers compared to conventional propagation methods. Testing different doses of nitrogen and potassium fertilizer would optimize the root yield in a cost effective manner. Callus culture followed by plant regeneration offers somaclonal variation and a way of genetic transformation.

Therefore, the present investigation has been undertaken considering the following objectives:

1. Development of protocol for meristem culture and subsequent *in vitro* multiplication.
2. Testing of the meristem-derived plantlets for virus through grafting method as a low cost means for producing diseases-free planting material.
3. Acclimatization and transplantation of in vitro grown mericlone plantlets and assessment of their field performance.

4. Establishment of protocol for plant regeneration through callus culture as a potential tool for generating somaclonal variation.
5. Field performance evaluation of several plants regenerated from callus for possible somaclonal variation.
6. Agronomic performance of some selected local sweetopotato varieties in response to nitrogen and potassium fertilizer for possible increase in tuber yield.

CHAPTER-2
REVIEW OF LITERATURE

Sweetpotato is a short duration and secondary crop and well adapted to the climatic condition of Bangladesh. The expansion of secondary crops will promote the diversification of the rural economy, stimulate growth in the non-farm sector, raise the income and wages of the people and enable the supply of low-cost food to improve nutritional status and food security of the poor. Thus expanded production of sweetpotato is likely to reduce the incidence of poverty in the country. In recent years Bangladesh has achieved self-sufficiency in rice production. This has expanded the scope of agricultural diversification in the country and farmers can now produce more secondary crops such as sweetpotato to diversify their food basket, as the country has substantial potential for diversifying crop agriculture through the expansion of secondary crops. It is well-fitted to the Government's policy for diversifying crop agriculture; farmers should be encouraged to produce more sweetpotato through area expansion and adoption of improved technologies in the process of production and consumption. There are, however, several constraints to increasing production of sweetpotato in Bangladesh including low yield rate, poor knowledge of its nutritional value and limited industrial use. Thus research and development activities on a technological front and appropriate policy support from the government can help to utilize the full potential of sweetpotato and increase their production and consumption in future.

2.1. Biotechnological approaches for improving sweetpotato

The improvement of yield potential of cultivated crop like sweetpotato has been obtained through conventional breeding program, some progress of about (20-25%) has also been possible through improved cultural practices. However, conventional breeding has potential limitation for sweetpotato improvement (Prakash, 1994). Recent advances in the field of genetics and genomics provide a more unified understanding of the biology of plants. The application of plant biotechnology techniques, in conjunction with conventional plant

breeding and good crop management, can play a major role in ensuring more food productivity and adequate nutrition. Crop improvement facilitated by modern biotechnology is one the most significant developments in plant biotechnology research and development.

Plant biotechnology refers to all kinf od artificial manipulation made on a plant, or, only on a part of a plant. Plant biotechnology is one such technology that has been regarded as part of the “sustainable productivity equation” in agriculture (Cohen, 2001). Its present applications in agriculture include conventional breeding, tissue culture and micropropagation, molecular breeding or marker-assisted selection, plant disease diagnostics, genetic engineering and the production of GM crops, and the “omics” sciences. Recent advances in the field of biotechnology as well as molecular genetic manipulation of the plants during the recent years have been provided convincing evidence of the usefulness of these powerful technologies in complementing and supplementing plant breeding program for genetic as well as nutritional improvement of important crop species (Datta, 2001). The most promising applications of modern biotechnology are to improve resistance and nutritional quality of foods crop. Since the conventional interventions have not been quite successful in eliminating those constrains, the genetic engineering approach have been reported on the incorporation of disease resistance as well as quality through biotechnology.

Plant cell, tissue and organ culture have developed rapidly and become a major biotechnological tool in agriculture, forestry and industry. For many years tissue culture has been applied by means of micropropagation, pathogen elimination and germplasm conservation (Love *et al.*, 1989; Hossain and Sultana, 1997; Sulaiman *et al.*, 1998; Fugile *et al.*, 1999; Alam et al 2012). However, some of these techniques are still being refined and improved. Intermediate level techniques such as *in vitro* tuberization system (Correa *et al.* 2003;) and embryo and anther culture are having some direct application on germplasms improvement, conservation and distribution. Enhanced resistance

through intrinsic genetic constituents manipulated by genetic engineering may be a preferable solution to reduce crop loss from various biotic and abiotic stresses. There are many reports on such genetic transformation of sweetpotato that have enormous potential to improve sweetpotato production (Cipriani *et al*., 1999; Okada *et al*., 2000; Cipriani *et al*., 2001; Herman *et al*., 2003). Thus, biotechnological approaches by mean of cell culture and genetic manipulation in sweetpotato may contribute a sustainable solution in enhancing food security and nutritional disorders in the developing countries.

2.2. Meristem Culture

In vegetatively propagated plants, once systematically infected with a viral disease, the pathogen is passed from one vegetative generation to next. Infected planting material is the most common source of sweetpotato viruses. Sprouts taken from diseased roots spread virus infection from one production cycle to the next. In addition, viruses can be disseminated through insect vectors (Clark and Moyer, 1988).

The first report of a suspected viral disease of sweetpotato in Eastern Africa was in 1939 from Ituri province of the Democratic Republic of Congo and then in 1944 from Uganda (Hansford, 1944). Later, sweetpotato viral diseases were reported in the present Kenya, Tanzania, Rwanda, Burundi, Malawi and Transvaal province of South Africa (Sheffield, 1957). Bioassays (host range, symptoms and vector transmission) were largely used to explain the aetiology of the various diseases. Since then many virus and virus-like diseases of sweetpotato have been described from several parts of the world (Moyer and Salazar, 1989). Advances in microscopy, serology and molecular biology techniques have resulted in better methods for virus detection, identification and characterization. Worldwide, up to 20 different viruses have been described to infect sweetpotato (Loebenstein *et al*., 2003), but only 11 of them are currently recognized by the International Committee of Taxonomy for Viruses (Mukasa, 2004). Among them Sweetpotato feathery mottle virus (SPFMV, genus *Potyvirus*, family *Potyviridae*) is found in all regions of

sweetpotato production while the others are localized to one or more geographic areas (Moyer and Salazer, 1989; Kreuze *et al*., 2000). There are many strains of SPFMV and coupled with its ubiquitous nature it has hindered the identification of many other viruses (Moyer *et al*., 1989). In Bangladesh at least five sweetpotato viruses have been found so far (BARI, 2002; 2003).

Sweetpotato virus disease (SPVD) is the name used to describe a range of severe symptoms in sweetpotato comprising overall plant stunting, leaf narrowing and distortion, chlorosis, mosaic or vein-clearing (Gibson *et al*., 1998). Mixed infections of SPFMV and SPCSV may result in viral synergism leading to development of severe symptoms and significant yield losses. When two or more viruses co-infect a plant they may influence each other in several ways. They compete for host resources but, however, there are few reports indicating that unrelated viruses suffer a disadvantage during mixed infection (Poolpol and Inouye, 1986). Often, one virus may assist a second, co-infecting virus, leading to increased titres and more severe symptoms and this phenomenon is referred to as viral synergism (Pruss *et al*., 1997). In some cases, the two viruses may benefit from the co-infection (Fondong *et al*., 2000). Data from different parts of the world reported the yield loss is range from 25 to 98% (Ngeve and Bouwkamp,1991; Fugile *et al*., 1999; Milgram *et al*., 1996; Sulaiman *et al*., 1998). Such variation might be due to cultivars and diverse type of trials (Ngeve and Bouwkamp, 1991).

Use of pathogen tested planting material leads to increased yield and economic profit when compared to virus-infected plants (Zhang, 1995; Zhang and Salazar, 2000; CGIAR, 2005). Quality was also affected by alterations in the shape and color of the skin of sweetpotato storage roots (Clark, *et al*., 2002). Virus-free sweet potatoes are increasing yields in China; in Shandong Province alone, yields increased by 30 percent and improved annual production by $145 million (CGIAR, 2005). Viral diseases are important constraints for sweetpotato production also in Bangladesh, as our farmers do not use virus free planting as they need for their cultivation. The major limitation to sweetpotato production in

Bangladesh like many developing countries is the lack of adequate quality planting material.

The meristem is a dome of actively dividing cells, about 0.1 mm in diameter and 0.25 mm long is generally virus-free, even in an infected plant. Such knowledge of the gradient of virus distribution in shoot tips encourages to obtain virus-free plants from infected individuals of *Dahlia* through meristem culture. Morel and Martin (1952) observed that many viruses were unable to infect the apical or axillary meristem of a systematically infected plant and that a virus free plant could be produced if a small (0.1-0.3mm) explants of meristematic tissue was propagated *in vitro* and virus free plant genetically identical to the mother plant. They cultured meristem tips excised from infected *Dahlia* and obtained disease free plants. Since then the advances made in elimination of virus by tissue culture have been such that this approach has now also become a popular horticultural practices.

Meristem-tip culture has also enabled plants to be freed from other pathogens including viroides, mycoplasmas, bacteria and fungi (Razdan, 1996). Production of virus free through meristem culture technique has been reported in many crops (Alconero *et al*., 1975; Razdan, 1996). Today, no definitive explanation can be given to understand this virus eradication (Wang and Charles, 1991). Various explanations have been given: absence of plasmodesm in the meristematic domes, competition between synthesis of nucleoproteins for cellular division and viral replication, inhibitor substances, absence of enzymes preiral replication, inhibitor substances, absence of enzymes present only in the cells of the meristematic zones, and suppression by excision of small meristematic domes.

2.2.1. Culture media for meristem culture

A variety of basal media have been used to initiate sweetpotato meristem culture. These include the MS (Murashige and Skoog, 1962) medium, modified Knop's medium as used by Nielsen (1960), Neergaard's medium as used by Stone (1963) modified White's basal medium as used by Dougall and

Shimbayashi (1960), dialute Hoagland's medium, Gamborg's B5 medium (Gamborg *et al*., 1968). Comparing the Knop's medium, and Neergaard's medium which had been used previously for growing meristem tips, and two MS and White's medium which were developed for tobacco callus, for their effects on growth and development of sweetpotato meristem Elliott (1969) reported that survival rate of meristem tips and rate of growth was consistently best with the MS medium. Beside this, some workers modified MS medium for sweetpotato meristem culture. Alconero *et al*. (1975) used modified MS medium, Doods *et al*. (1991) and Hossain and Sultana (1997) concluded that MS medium supplemented with different vitamins proved to be better for *in vitro* proliferation and propagation of Bangladeshi sweetpotato cultivars

Among growth regulators, generally a small amount of auxin or cytokinin, or both, is beneficial for meristem culture. Among auxins IAA and NAA are widely used for sweetpotato (Elliott, 1969). Of these, NAA is considered more effective due to better stability. Although 2,4-D promote callusing, some workers used it for planlet development from meristem either singly or in combination with BA and GA_3 (Hettiarachchi, 1988). In angiosperms shoot tip is not autonomous for auxin, probably synthesized by the second pair of youngest leaf primordia (Shabde and Murashige, 1977). Those explants requiring only auxin, or cytokinin, probably have a high level of other growth regulator not required by them endogenously in their meristem (Razdan, 1996).Among cytokinins kinetin singly or in combination with other auxin is widely used (Hossain and Sultana, 1997; Verma *et al*., 2004). Suitability of GA_3 is reported for plant regeneration from excised sweetpotato meristem is reported by many workers (Dagnino *et al*., 1991; Torres *et al*., 1996). Differential requirements of plant growth regulators in culture media is due to a wide genetic variation among different sweetpotato cultivars grown worldwide (Hossain and Sultana, 1997; Hossain *et al*., 1999).

Meristem were rescued and cultured on filter paper bridge above a liquid medium. Walkey and Cooper (1976) were able to free *Nicotiana rustica* from

cucumber mosaic and alfalfa mosaic viruses by culturing meristem for 45 days at 32°C rather then at 22°C. A rapid replication of virus particle was probable made possible by the loss of a resistance factor from the plant cells during their high temperature incubation (Razdan, 1996).

2.2.2. Explants factors for meristem culture

The size of the explant is critical for virus elimination since the most systemic viral diseases pathogen often establish a gradient in plant tissue. According to Stone (1963) only shoot tips between 0.2 and 0.5 mm most frequently produce virus free carnation plants. Tomato meristem with 0.3 - 0.5mm length was found effective size for virus elimination using shoot tips collected from infected plants (Alam *et al*., 2004). Lizárraga *et al*. (1999) removed the leaves around the growth point (the apical dome) until only the cupule and two or three foliar primordia are left, measuring between 0.2 and 0.6 mm long for virus eradication in sweetpotato.

Meristem should preferably be taken from actively growing buds. Such explant is especially important for carnation and *Chrysanthemum*. Meristem tips taken from terminal bud were observed to give better results than those from axillary buds. These could be ascribed to the differences in optimum levels of the endogenous hormone for growth response or ascribed to the difference in other factors which affect endogenous levels of the growth hormone. So for high efficiency sweetpotato meristem culture may be initiated in a particular season.

2.3. Micropropagation for large-scale plantlet production

Micropropagation allows the massive multiplication of desired variety. For sweetpotato, this is mainly used for clonal seed production and for germplasm maintenance. It also minimizes the risk of accidentally introducing plant quarantine pests along with the host plant material; in particular, cryptic pathogens such as viruses which pose a special risk during distribution and transfer of germplasms through out the world (Moyer *et al*., 1989). Pathogen-tested *in vitro* plantlets are used as starting material for this purpose. It has all

the advantages of multiplying specific pathogen tested (SPT) stocks and breaking the cycle of traditional storage root or vine born soil organism (Sulaiman *et al*., 1998; CTA, 1998; Carey *et al*., 1999; Fugile *et al*., 1999; Zhang and Salazer, 2000). The propagation methods used are aimed at producing a large number of plantlets in the shortest time possible. In China and South Africa micropropagation of pathogen-tested planting materials has been started from last decades with the standardization of mass propagation techniques (Fugile *et al*., 1999; Hautea and Escaler, 2004).

Researchers from home and abroad standardize the technique of mass propagation using node cutting from meristem derived plantlets. Single node cuttings with leaves are cut from the *in vitro* plantlets and are inoculated on solid agar (Dodds *et al*, 1991; Dagnino *et al*., 1991; Hettiarchchi, 1988; Love *et al.*, 1989; Hossain and Sultana, 1997). There is another micropropagation methods; the liquid shaken culture. This type of culture yields relatively higher proportions of plantlets (Torres *et al.*, 1996). Here nodal cuttings, each having 3-4 nodes, are obtained and inoculated on liquid media and shaken gently (Espinoza *et al.*, 1984).

2.4. Callus induction and plant regeneration

Callus (plural *calluses* or *calli*) is a mass of unorganized parenchyma cells derived from plant tissue (explants). In nature, callus develops by infection of microorganism from wounds due to its stimulation by endogenous growth hormones, the auxins and cytokinins. However, it has been artificially developed by adopting tissue culture techniques. Callus formation is governed by the source of explant, nutritional composition of medium and environmental factors (Dubey, 2002). Its information takes place under the influence of exogenously supplied growth regulators present in the nutrient medium. The type of growth regulator requirement and its concentration in the medium depends strongly on the genotype and endogenous hormone content of an explant (Chawla, 2001). These requirements can be put into three categories (a) auxin alone, (b) cytokinin alone and (c) both auxin and cytokinin. Callus

tissue from different plant species may be different in structure and growth habit: white or coloured, soft (watery) or hard, friable (easy to separate into cells) or compact. Physical growth factors (light, temperature etc.) are also important for callus formation. The effect of light on callus formation is depend on the plant species; light may be required in some cases and darkness in other cases.

Although callus is an abnormal type of growth, it has biological potential to develop normal shoot, root and embryoid, ultimately forming a plant. A somatic embryo is an embryo derived from a somatic cell, other than zygote, usually on culture *in vitro* and the process is known as somatic embryogenesis (Singh, 2000). Plant regeneration via somatic embryogenesis is a complex phenomenon. Plant growth regulators play the most important role in these processes and exogenously supplied auxin is required in appropriate concentration for the induction of somatic embryogenesis from callus or explant. Generally media containing 2,4-dichloro phenoxy acetic acid, 2,4,5-trichloro phenoxy acetic acid and picloram are used as embryo induction medium (Ramawat, 2000). Reduced nitrogen in the embryo development media also suppress embryo development. Beneficial effect of silver nitrate and certain amino acids like prolin, aperagin and glutamine has been reported (Bohorova *et al*., 1995; Otani and Shimada, 1996; Carvalho *et al*., 1997). Cytokinins except zeatin, suppress embryogenesis. The success of regeneration in any crop depends upon the type of medium used in each phase of culture from callus initiation to maintenance during regeneration (Shrivastav and Chawala, 2001).

As a sexually sterile plant, sweetpotato can be benefited from callus culture and subsequent plant regeneration through a number of ways including the exploitation of somaclonal variation, somatic hybridization and genetic transformation. Regeneration via callus induction/somatic embryogenesis is highly desirable as the process regularly affords high multiplication rates and can effectively be maintained for a long time (Sihachakr *et al*., 1997).

Suspensions of small cell aggregates with high organogenic potentials can be established from embryogenic callus. Callus induction and regeneration from different explants have been studied in sweetpotato. (Sihachakr and Ducreux, 1987; Chee and Cantlifffe, 1988; Schultheis *et al*., 1990; Bieniek *et al*., 1995; Otani *et al*., 1996; Otani and Shimada, 1996; Sihachakr *et al*., 1997; Dhir *et al.*, 1998; Padmanabhan *et al*., 1998a; Padmanabhan *et al*., 1998b; Hossain *et al.*, 1999; Aloufa, 2002). Sweetpotato offers a variety of explants to choose for callus induction.

Somatic embryogenesis can be initiated in sweetpotato from anther- derived callus (Tsay and Lin, 1973), leaf, shoot tip, stem and root explants (Liu and Cantliffe, 1984; Liu and Cantliffe, 1987; Hossain *et al*., 1999) and lateral buds in particular (Cavalcante-Alves *et al*., 1993). Bieniek *et al*. (1995) and Liu *et al*. (1999) found an efficient *in vitro* plant regeneration system characterized by rapid and continuous production of somatic embryos using shoot tip explants. However, a variety of somatic embryos develops in culture and varies greatly in their developmental patterns and their ability to convert to plantlets in sweetpotato (Padamanabhan *et al*., 1998a).

MS medium (Murashige and Skoog, 1962) is widely used for callus induction and plantlet regeneration with or without a little modification (Hossain *et al*., 1999; Aloufa, 2002). Tsay and Lin (1973) inoculated sweet potato anthers with pollen mother cells on Blaydes medium (Blaydes, 1966). Somatic embryos were also obtained on MS medium using shoot apices of sweet potato, varying eight kinds of auxins (Otani and Shimada, 1996).

Various workers develop systematic protocols for callus induction followed by plant regeneration (Chee and Cantliffe, 1988; Otani and Shimada, 1996; Dhir *et al*., 1998; Padamanabhan *et al*., 1998b; Liu *et al*., 1999; Aloufa, 2002). Generally, explants are inoculated in MS medium supplemented with 2,4-D, NAA or BAP and 3% sucrose in light or dark condition, after certain stage of development calli are formed into somatic embryos and leading to new

plantlets. It is important to note that, nothing is ideal for cultural condition as *in vitro* responses of sweetpotato is highly genotype dependent because of their diversified mode of distribution and domestication from their origin (Cavalcante-Alves *et al.*, 1993; Zhang, 1995; Sihachakr *et al*., 1997). Plant Regeneration is ultimate goal to callus culture. In sweetpotato, induction of somatic embryogenesis and formation of embryos, as well as their maturation occurred in the presence of high levels of auxins (Chee and Cantliffe, 1988; Sihachakr *et al*., 1997). Generally, for ontogenesis of embryos, the compact embryogenic calli are subcultured in high level of 2,4-D or NAA and low level of Kin or BAP and exposed to light. After 3-4 weeks, the embryogenic calli gave rise to globular embryos, these lead to new plantlets. The embryogenic calli and their ability to regenerate plants can be maintained constantly for over 4 years (Sihachakr *et al*., 1997).

Among growth regulators, auxin appears to play a role not only in those sequences that precede embryo formation, but also in subsequent morphogenic events in embryo development (Zimmerman, 1993). Exogenously applied synthetic auxin such as 2,4-D stimulates the accumulation of endogenous indole acetic acid. This maintains the proliferative state of the callus and prevents subsequent embryo formation. Transferring the tissue to a 2,4-D-free medium results in a decline in total IAA, to levels low enough to set up an internal gradient for initiation and maintenance of polarized growth and subsequent embryo development, functional shoot meristem, premature conversion, extended cell divisions in the shoot apex, precocious vacuolation in the shoot cortex, or aberrant cotyledons (Ammirato, 1985; Nickle and Yeung 1993). Nickle and Yeung (1993) found that loss of conversion capacity in carrot somatic embryos was due to abnormal development of the shoot apical meristem followed by abortion of the meristem. Thus by selecting and manipulating of the proper growth regulator(s) and developmental stage efficient plant regeneration form callus can be achieved. For successful genetic transformation and to generate somaclonal variability, induction and establishment of fast growing embryogenic calli are prerequisite. During cell

division in artificial condition, different types of abnormalities occur in the genetic constituents which ultimately contributed to the regenerated plants (Bairu *et al* 2011). Plant derived for tissue culture has been variously referred as somacolones or callicones or protocolones and variations displayed by such plants are simply called somacolonal variation. Somaclonal variation commonly appears in plant after tissue culture involving a callus state (Bairu et al 2011)

2.5. Agronomic practices for increasing yield

Various agronomic practices affect the yield of sweetpotato. Application of fertilizers is one of them, on which the production cost is badly involved. Optimizing N fertilizer applications is critical for commercial production. While some level of N fertilization is almost always necessary to obtain optimum root yield (Walker and Woodson, 1987) and protein concentration (Purcell et al., 1982), excessive N can reduce yield (Villagarcia et al., 1998). Extreme moisture at high N fertility levels has also resulted in decreased root yield and quality (Constantin *et al*., 1974). Variations in soil and climatic characteristics and genotypic differences in N uptake and utilization efficiencies make identifying this optimum N balance extremely complicated. Sweetpotato yield responses to N application vary considerably and are often inconsistent or contradictory (Bellinder and Morse, 1982;). Large field-to-field differences in crude protein content and other quality factors of sweetpotato also exist (Purcell *et al*., 1978; Constantin *et al*., 1984). The difficulty in determining optimum N rates for sweetpotato production often results in low N use efficiency (NUE; percentage of applied N removed in the harvested portion of the crop), which can lead to excess fertilizer N remaining in the environment and having the potential to impact water quality (Marti and Mills, 2002). This fact combined with the cost of N fertilizer dictate a necessity for high NUE. Villagarcia *et al*., (1998) alluded to the importance of NUE in sweetpotato production when they defined the ideal sweetpotato plant as "one that is efficient in acquisition of N and its use to maintain photosynthetic activity when the substrate supply of N is low, yet one that retains its capacity to initiate storage roots and partition photoassimilates to them when substrate supply is

high." It is documented that N uptake and assimilation rates differ among sweetpotato cultivars (Walker and Woodson, 1987; Villagarcia *et al*., 1998; Hill *et al*., 1990); however, these differences often do not translate into differences in NUE (Marti and Mills, 2002). It has been reported that several sweetpotato cultivars are capables of producing high yield without N fertilizer on N-deficient soils (Hill *et al.,* 1990). Beauregard is such a cultivar widely adopted in USA. Therefore, it is important to identify the optimum N rate and time of application for our country's sweetpotato production and to de-termine the effect of N rate and timing on root yield.

Potassium is another importent fertilizer which greately affects the yield of sweetpotato. Potassium encourage the formation of stearch in the tuberus root (Anon, 1940). It is the most important nutrient in sweet potato production as its application increases yield by the formation of larger sized tubers. Potassium also affects the number, size, quality and the unit weight of tuberous roots produced, while the minimum levels of K suggested for healthy growth and yield are twice those recommended for N, although three times as much may be applied and occasionally even more (Degras, 2003). De genus (1973) stated that sweet potato consumes considerable amounts of K, and responses to K fertilizers are generally recorded. According to Scott and Ogle (1952) total) total uptake of potash was about twice than that of nitrogen and about five times that of phosphate during the growing period of sweetpotato. It is also stated that sweetpotato had high requirement for potash, but a very low phosphate requirement. Morita (1969) reported that heavy application of nitrogen and potassium stimulated vine growth which tended to depress tuber formation, but yields of larger size tubers were increased, because tuber thickening increases when vine growth continued vigorously.

CHAPTER – 3

MATERIALS AND METHODS

Part- 1: Tissue Culture

3.1. Materials

3.1.1. Plant materials

Four popular sweetpotato [*Ipomoea batatas* (L.) Lam.] Cultivars of Bangladesh namely, BARI-1 (Tripti), BARI-2 (Kamalasundari), BARI-3 (Daulatpuri) and BARI Sweet Potaato-4 were used in this study. Sweetpotato vines were collected from Tuber Crop Research Center, Bangladesh Agricultural Research Institute, BARI, Joydebpur and Tuber Crop Research sub Center, Bogra, and maintained in the Botanical garden, University of Rajshahi for collecting explants. Shoot tips were used as primary explant collected from 3-4 weeks old field grown twigs. Nodal segments were collected from *in vitro* grown meristem-derived plantlets for massive plantlets propagation and micro-storage root formation. Internode, petiole and leaf segments of meristem-derived plantlets were used for direct shoot differentiation; Internode, root and leaf disc were used for callus induction, subsequent plant regeneration. For grafting experiments, *Ipomoea setosa* seeds were collected from the botanic garden, University of Rajshahi.

3.1.2. Chemicals

The following chemical compounds were used in the present investigation:

1. **Plant growth regulators:** Different kinds of plant growth regulators and additives were used for this experiment. These includes: indole – 3 acetic acid (IAA), indole – 3 butyric acid (IBA), napthalene acetic acid (NAA), 2,4-dichlorophenoxy acetic acid (2,4-D), 6-Benzale amino purine (BAP), 6-furfural amino purine (Kin), thidiazuron (TDZ), gibberellic acid (GA3)
2. **Sterilant and surfactant:** $HgCl_2$ was used as surface sterilizing agent and Tween-80 and savlon (an antiseptic, ACI Pharma, Bangladesh) were used as detergent and surfactant.

3. **Nutrient Basal salts:** For plants nutrient basal salts were used which content both macro, micro and vitamins.

Macronutrients: $MgSO_4.7H_20$, KH_2PO_4, $NaH_2PO_4.H_2O$, KNO_3, NH_4NO_3, $CaCl_2$. $2H_20$, $(NH_4)_2.SO_4$,

Micronutrients: H_3BO_3, $MnSO_4$. H_20, $ZnSO_4.7H_20$, $NaMoO_4$. $2H_20$, $CuSO_4$. $5H_20$, $CoCl_2.6H_20$, KI, $FeSO_4.7H_20$, Na_2. EDTA.

Vitamins: Thiamine HCL, Pyridoxine HCL, Nicotinic acid, Myo-Inositol.

All chemical compounds including macro and micro nutrients, organic acid and inorganic acids, sugar, agar, KOH, $HgCl_2$, ethanol etc. used in the present study were the reagent grade products of either BDH, England or MERCK, India. The vitamins, amino acids and different growth regulators and antibiotics were the products of Sigma Chemical Company U.S.A and Phytotech (USA).

3.1.3. Other Materials

The culture vessels such as test tube (150 × 25mm), bottle (12 × 5mm), conical flask (250ml, 1000ml), measuring cylinders, glass rods, beakers, pipette, pipette pumps, paraflim, cotton plugs, rubber bands, filter paper, aluminium foils, forceps, fire box, marker pen, spirit lamp, needle, sharp blade, sterio-microscope, electronic balance, autoclave, pH meter, magnetic stirrer, laminar airflow machine etc. were also used in the present experiment.

3.2. Methods

Experimental methods were used for the experiments discussed under the following heads:

3.2.1. Media Used for Tissue Culture and Transformation

In the present experiments different culture media with various growth regulators were used for various purposes. A control experiment using MS medium supplemented with 3% sucrose without growth regulators was also included whenever necessary.

3.2.1.1. Meristem culture

- MS (Murashige and Skoog, 1962) medium either liquid (without agar) or semisolid (with 0.75% agar) containing different concentrations and combinations of plant growth regulators (GA_3, Kin, NAA and BAP) and sucrose, glucose, commercial table sugar as carbon sources.

3.2.1.2. Callus induction and regeneration

- MS semisolid medium supplemented with different concentrations and combinations of 2,4-D, NAA, BAP, Kin and TDZ as growth regulators, and 3% sucrose as carbon source.
- MS semisolid medium supplemented with different concentrations and combinations of 2,4-D, Kin, NAA, BAP and zeatin. 3% sucrose was used for carbon source.

3.2.2. Preparation of Stock Solution for Culture Media

In the first step of the preparation of culture medium, stock solutions were made. Various constituents of the respective nutrient medium were prepared into stock solutions for ready use during the preparation of media for different experiments. As different constituents were required in different concentrations, stock solutions of macro- nutrients, micro- nutrients, organic compound (vitamin and amino acids) and growth regulators were prepared separately.

3.2.2.1. Stock solution A (I, II& III): Macronutrients

This stock solution was made in such a way that its strength was 10 times more than the final strength of the medium in 500ml distilled water. For this purpose, 10 times the weight of different salts required for 1 liter of medium were weighed accurately. Then the salts were sequentially dissolved one after another in a 500 ml volumetric flask with 350 ml of distilled water. The final volume of the solution was made up to 500 ml by further addition of distilled water. The solution was filtered through Whatman's No. 1 filter paper to remove all the solid contaminants like the dust, cotton etc. and was poured into a clean plastic container. After labeling, the solution was stored in a refrigerator at 4° C for several weeks.

3.2.2.2. Stock solution B (IV & V): Micronutrients

For this constituent of the medium two separate stock solutions were prepared:

(a) This part of the stock solution was made with the micronutrients except $FeSo_4$, $7H_2O$ and Na_2-EDTA. It was made 100 times the final strength of necessary components in 500ml of distilled water as described for the stock solution A. The solution was filtered and stored at 4° C for several weeks.

(b) The second solution was also made 100 times the final strength of $FeSO_4$. $7H_2O$ and Na_2-EDTA in 500 ml distilled water in conical flask and heated slowly at low temperature until the salts dissolved completely. Finally the solution was filtered and stored in refrigerator at 4^oC for several weeks.

3.2.2.3. Stock solution C (VI): vitamin and amino acids

Stock solution C was also made 100 times the final strength of the medium in 500 ml of distilled water as described for stock solution A. The solution was also filtered and stored at 4^oC for several weeks.

3.2.3. Preparation of 1 litter Culture Medium

To prepare of one litter of medium the followings were carried out successively

I. Assembling of the medium components

For the preparation of 1 litter MS medium, 20ml of stock solution-I, 10ml of stock solution-II, 10ml of stock solution-III, 10ml of stock solution IV and 10ml of stock solution MS-V, 10ml of stock solution VI, 10ml of stock solution VII, were added in 1 litter flask containing 500ml distilled water and mixed well.

II. Addition of growth regulators

Stock solutions of growth regulators were added in appropriate concentrations and combinations in above solutions and were mixed well.

III. PH of the medium

In all tests the pH of the medium was adjusted to 5,8 using a digital (TOA, Japan) pH meter with the help of 0.1 N HCl or 0.1N KOH (where necessary) before addition of agar, sugar.

IV. Sucrose and Agar

After the adjustment of pH, sucrose 30 gm/l and Agar 6-6.2 gm/l were added and final volume of the mixture was made 1 litter by adding distilled water. Then the medium was heated for 5 min. in a microwave oven (National, Japan) to melt agar completely.

V. Medium dispensing to culture vessels

The prepared melted medium was disposed into culture vessels such as test tubes (130×25mm) or conical flasks (250ml), through separating funnel. The culture vessels were plugged with cotton-plugs, warped with cheesecloth, which were inserted tightly at the month of culture vessels. The culture vessels were marked to designate specific hormonal supplements.

VI. Sterilization

Finally the culture vessels containing medium were autoclaved at 15 lb / inch^2 pressure and at the temperature of 120-121^{0}C for 20 min to insure sterilization. Then the vessels with the medium were allowed to cool as vertically and then marked with a glass marker pen to indicate specific hormonal supplements and stored in the culture room for ready use.

Part-1 A: Meristem Culture

3.3. Meristem Culture

3.3.1 Meristem isolation and culture

To obtain shoot tips of sweetpotato, vines were grown from fresh storage root. About 3-4 weeks after, when vines were reached a height of 25-50 cm they were considered ready to be harvested. The shoot tips of sweetpotato were excised with the help of sharp blade and collected in a reagent bottle containing distilled water with few drops of Savlon and few drops of tween-20 (poly-oxyethelen sorbitan momolaurate) and quickly brought to laboratory. Then the explants were washed for 2 or 3 times with gradual change of sterile distilled water. The materials were transferred into 250 ml sterilized conical flask. Surface sterilization was carried out by dipping the materials in 0.1% $HgCl_2$ solution with gentle shaking for 3-10 min followed by 3-5 times washing with sterile distilled water inside the running laminar air flow cabinet.

After surface sterilization, shoot tips were laid on the sterile tiles using sterile forceps. Explants were hold in one hand under the sterio-microscope with the help of a pair of forceps and the immature leaves and leaf primordial were snapped with slight pressure from the needle. Then the exposed meristem tips that appeared as a shiny dome were gently isolated with a sharp blade. After deplugging of culture tubes and a single excised meristem tips (0.3mm) was carefully placed on the "M" shaped filter paper bridge of the culture tubes containing liquid MS medium supplemented with different plant growth regulators for primary establishment of isolated meristem (fig-A on Plate-4.1). The neck of the tubes were flamed with sprit lamp and then plugged again.

The inoculated culture tubes were incubated in a growth chamber providing a special culture environment. The tubes were placed on the shelves of a culture environment in the growth chamber. All cultures were grown in the growth chamber illuminated by 40 watts white fluorescent tubes fitted at a distance of 40-30 cm from the culture shelves. The culture was maintained at $25^0 \pm 2^0$C with light intensity varied from 28-34 mol $m^{-2}s^{-1}$. The photoperiod was

maintained 16 hours light and 8 hours dark. The culture tubes were checked regularly to record the morphogenic responses of culture.

3.3.2. Subculture semi-solid medium for shoot and root induction

After 3 to 4 weeks of inoculation of meristem, the primarily established meristem those showed morphogenic responses were removed aseptically from the culture tubes and transferred into fresh MS semisolid medium supplemented with different growth regulators. During inoculation, special care was taken that the explants must be touched the medium equally and do not dip into the medium. The primary established meristem formed shoot and root and well established after 21-28 days of culture initiation.

3.3.3. Virus Indexing Meristem Derived Plantlets

Virus indexing through grafting technique was carried out following IBPGR (1987) hand book. Seeds of the indicator plant (*Ipomoea setosa*) were planted 3-4 weeks before grafting. Then, a branch from the sweet potato plant to be indexed was removed and cut into pieces each containing a node with a fully expanded leaf attached. Wedge-graft each node section on to a separate indicator plant as shown in Plate 4.1a Fig A and B. The grafts were wrapped with 'Parafilm' to prevent desiccation. Scions were protected from direct sunlight by laying a piece of polythene over the graft and the adjoining leaves for 2-3 days. Sweetpotato plants that are to be indexed was produced by *in vitro* nodal propagation of the original meristem cultured plantlet. As advised in that section, additional plantlets should also be maintained in culture to be used as stock plants after the indexing procedure is completed. Each sweetpotato plant that was used for the indexing procedures allowed to establish in soil and to develop until it has a minimum of five nodes with fully expanded leaves. Virus can also be tested by using ELISA method. To compare cost difference between grafting and ELISA, service offered by a company was considere for testing sweetpotato SPFMV and SPCSV using standard NCM-ELISA kit.

3.3.4. Acclimatization and field trials of mericlones

Micropropagated plantlets, with a well-established root system, were washed carefully to remove agar and then transferred to pots containing sterile fertile garden soil. Plantlets (5–8 cm high) were transferred to soil and each pot was enclosed in a polyethylene bag after watering, and maintained in a growth chamber at 24±2°C under 16 h illuminations (45 μM $m^{-2}s^{-1}$) with fluorescent lamps. Bags were progressively opened weekly. After 3 weeks acclimatization had been completed, plantlets were transferred to nursery before transferring to the field.

After 3 weeks of acclimatization, micropropagated plants (R_1) were transferred to experimental plots for field evaluation in two field condition; net house and open field. The field was ploughed well two to three times and during plowing, the soil was pulverized with manure, fertilizer and sand mixed with soil according to need. The bed was prepared after the field was well pulverized. The length of each bed was 3m and width was 1m. The distance between two beds was 50 cm. Before transplantating the plantlets, the field were treated with 1% formaldehyde solution to prevent the soil borne pathogen and covered with polythene sheets for 3-5 days. After 5-6 days, the polythene sheet was removed. The entire field was covered with nylon nets in order to prevent viral vectors (Plate-4.1, fig-G). No insecticides were used for controlling viral vectors in open field condition. All necessary fertilizer applications and other cultural practices were followed. The storage root collected (Plate 4.2,2 Fig D, E, and F) from respective net house and open field R_1 plants were planted next season for producing enough vines for R_2 trial. R_2 generation was tested using planting materials from both open field and net house conditions. Unlike R_1, in R_2 trial traditional planting materials (not tested for viral diseases) were used as control.

3.3.5. Data Recording

3.3.5.1. Data collection for *in vitro* Grown Meristem Derived Plantlets

Data were collected using the following parameters and the methods of data collection are given bellow:

I. **Percentage of responses:** Percentage of explant responded was calculated using following formula:

$$\text{Explants responses (\%)} = \frac{\text{Number of explants formed shoots}}{\text{Total number of explant inoculated}} \times 100$$

II. **Average degree of vigour:** Vigour was calculated from visual observation, using a hypothetical scale range of 0.00-1.00. The scale depicts average growth of the isolated meristems as follows:

Morphogenic response	Value used (individual)	Range of scale (average*)
No growth	0.00	0.00
Poor growth	0.25	0.01-0.25
Moderate growth	0.50	0.26-0.50
Good growth	0.75	0.51-0.75
Excellent growth	1.0	0.76-1.00

*Values are the average of 12 replications.

III. **Number of shoots/explants**: Number of shoots was counted for each culture. After 28 days average no. of shoot number was calculated and noted.

IV. **Shoot length:** After 28 days of culture shoot length was measured in cm scales for each plantlets and average of shoot length was calculated and noted.

V. **Number of roots per shoot:** Number of roots was counted for each plantlet at 28 days of subcultures of 5 randomly selected cultures and then mean value were recorded.

3.3.5.2. Data collection for field Grown Plants

i). Length of main vine (cm)

Length of longest vine was measured in centimeter from the ground level to the tip of the longest vine of an individual plant and the mean value for each treatment was calculated.

ii). Storage/tuberous roots number per plant

Storage/tuberous roots number per plant was counted from selected plants for each plot and recorded the mean Storage roots number.

iii). Root yield per plant

The fresh weight of tuberous roots per plant was measured with electronic balance in gram (gm) from total number of plants and recorded during harvesting.

vi). Incidence of SPFMV, SPMMV and complex infections: The presence and incidence of symptoms of SPFMV, SPMMV and their combinations were assessed by visual observation of the the whole field. The percentage was calculated as:

$$\text{Incidence of viral disease symptoms (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants in the plot or group}} \times 100$$

Part–1 B: Callus Culture and Plant Regeneration

3.4. Callus Culture and Plant Regeneration

3.4.1. Choice of Explants

Explant is a great factor for callus induction. *In vitro* grown sweetpotato plantlets derived through meristem were used for callus induction. For initiation of callus internode, root and leaf segments were used as explants.

3.4.2. Callus induction

To induce callus, internode, root and leaf segments were taken and were cultured in MS medium supplemented with different callus inducing substances and incubated in the light of 25±2°C for 3-6 weeks. MS medium was supplemented with different concentrations of 2, 4-D, NAA, BAP, TDZ and sucrose was used as sources of carbon (30 gm/l) for massive callus induction. After 21 days of incubation the callus induction frequency was estimated. The calli which were compact, friable and white, creamy, greenish, light green, dark green in clour were considered as degree of callus formation.

3.4.3. Subculture of Callus

After callus induction from the explants, the calli were transferred into the fresh medium after every 18-24 days for further proliferation and maintenance. Brown, watery and dead calli were removed during each subculture. Friable, nodular callus or callus having green roundish meristemoid (Plate- 4.3, 4.4) was considered as putative embryogenic calli. These were selected for plant regeneration.

3.4.4. Plant Regeneration

Plantlets were regenerated by transferring the selected calli in MS semisolid medium supplemented with three types of cytokinin BAP, Kin and zeatin and two auxins NAA and 2,4-D in different formulations (Table: 4.15 - 4.17) The culture was incubated at 25±2^{0}C under white light for 16-8 light dark condition. After 3-5 weeks differentiation, of shoots and roots were observed. The number of calli producing shoots and the total number of shoots were counted for each

treatment applied. The shoot from the selected callus was excised and transferred on MS_0 medium for further growth. The plantlets from each of individual callus were further multiplied by node culture.

3.4.5. Field evaluation of regenerated plants

Plantlets regenerated from callus were acclimatized and further multiplied by vine cuttings in soil pots. Four randomly selected plants from each cultivar were chosen for field evaluation. Three cuttings from each line from all four varieties were planted in the field.Plants were named as T_1-T_4, K_1-K_4, D_1-D_4, BS_1-BS_4 in cultivar BARI-1, BARI-2, BARI-3 and BARI-4, respectively. Several morphological parameters were observed for possible arising of somaclonal variation including length of main vine, number of vines, internodes length, leaf areas, and number of tubers per plant, length of tuber per plant, average diameters of tuber, fresh weight of vines/plant, fresh weight of tubers/plant.

3.4.6. Data Recording

3.4.6.1. Data collection for evaluation of callus induction and plant regeneration

Data were collected using the following parameters and the method for data collection is given below:

I) Callus induction frequency:

Explants were cultured in 25mm×150mm culture tubes, 250 ml conical flasks and 200 ml bottles containing media with different concentration of growth regulators for callus induction after required days of culture. Frequency of callus induction was calculated using the following formula.

$$\text{Frequency of callus induction (\%)} = \frac{\text{Number of explants induced callus}}{\text{Total number of explants inoculated}} \times 100$$

ii) Qualitative nature of callus:

Cultured explants that showed callus formation were counted after 20 to 40 days culture. The colour, nature and physical condition of callus varied in respect of explant type and hormonal supplements. So, different symbols were

used to denote different colour, nature and degree of callus formation as given below.

Colour of callus was marked according to the following symbols

Properties	Symbols
Colour of Callus	
White	W
Creamy	Cr
Creamy white	CrW
Greenish	Gr
Light green	LGr
Dark green	DGr
Nature of callus	
Friable	Fr
Spongy	Sp
Loosely compact	Lc
Compact	Com
Nopdular	Nd
Degree of callus growth	
No growth	-
Poor growth	+
Moderate growth	++
Good growth	+++
Massive growth	++++

iii) Shoot Induction: Percentage of explants induced shoots

As mentioned earlier data on different parameters from different treatments of shoot regeneration were recorded after required days of culture. The percentages of explants induced to develop adventitious shoots were calculated using following formula.

$$\text{Explants induced shoot (\%)} = \frac{\text{Number of culture induced shoot}}{\text{Total number of explants inoculated}} \times 100$$

vi) Shoot Induction: Mean number of shoots per callus

Number of shoots per explant was computed after required days of culture. Mean number of adventitious shoots per explants was calculated using following formula:

$$\overline{X} = \frac{\sum Xi}{N}$$

$\overline{X}$ = Average number of shoots

Σ = Summation

Xi = Total number of shoots
N = Number of observation happy

All shoot regenerated from callus produced sufficient amount of roots, so no parameters for root formation was used.

3.4.6.2. Data for field evaluation of plants regenerated from callus

Data were taken for yield contributing character and yield. Four sample plants were selected randomly and tagged initially for data collection from each plot. Data on length of main vine, number of vines, vine internodes length per plant, and leaf areas per plant were recorded 100 days after planting. Data on number of tubers per plant, length of tuber per plant, average diameters of tuber, fresh weight of vines per plant, and fresh weight of tuberous roots per plant were recorded on harvest time (BARI-1, BARI-2, BARI-3 at 140 days and BARI sweetpotato- 4 at 120 days after planting). Data were collected and mean value of each treatment was calculated.

I). Length of main vine

Length of longest vine was measured in centimeter from the ground level to the tip of the longest vine of an individual plant and the mean value for each treatment was calculated.

ii). Number of vines per plant

Total number of vines was counted from selected plants and the mean value for each treatment was calculated.

iii). Vine internodes length

The internodes length was measured in centimeter (cm) and the mean internodes length was determined.

vi). Leaf areas

The leaf area of plant was measured in centimeter (cm^2) and the mean plant leaf area was determined and recorded.

v). Number of tuberous roots per plant

Number of tuberous roots per plant was counted for each plot and recorded the mean tuberous roots number.

vii). Length of tuberous roots per plant

The tuberous roots length was measured in centimeter (cm) lines and the mean tuberous roots length was determined.

viii). Average diameters of tuberous roots

Average diameter of tuberous roots was measured by slide calipers in centimeter.

xi). Fresh weight of vines per plant

The fresh weight of vines per plant was measured with electronic balance in gram (gm) at the harvesting time. The mean fresh weight of vines per plant was determined and recorded.

xi). Fresh weight of tuberous roots per plant

The fresh weight of tuberous roots per plant was measured with electric balance in gram (gm) from total number of plants and recorded during harvesting.

3.4.7. Statistical analysis

The collected data on various parameters were statistically analyzed using MSTAT statistical package program. The mean for all the treatments was calculated and analysed of variance for all the characters were performed by F-variance test. The significance of differences between pairs of treatment means was evaluated by the least significance difference (LSD) test at 5% level of probability.

Part–2: Agronomy

3.5. Effects of nitrogen alone and nitrogen in combination with potassium fertilizer on yield related characters of sweetpotato

In this section, agronomic performance of some selected local sweetopotato varieties in response to nitrogen fertilizer (Experiment-1) and nitrogen in combination with potassium fertilizer (Experiment-2) for possible increase in tuber yield.

3.5.1. Treatments of the experiment

In this experiment, the effects of four levels of nitrogen (as urea) alone and four selected combination of nitrogen and muriate of potash (as potassium chloride, MP) fertilizers were tested with four varieties of sweet potato. Both as meristem derived plants as well as conventional seedlings were tested for response to the nitrogen and potassium. The present experiment considered of two factors, namely, fertilizer and varieties. Fertilizers were located in the main plot and Varieties in the sub plote.

Experiment -1

Main plot: It comprised four levels of nitrogen.

i) N_o - 0 kg N/ha (0 kg urea/ha)
ii) N_1- 90 kg N/ha (100 kg urea/ha)
iii) N_2- 160 kg N/ha (150 kg urea/ha)
iv) N_3-190 kg N/ha (200 kg urea/ha)

Sub plote: It comprised four varieties of sweet potato.

i) V1 – BARI-1 (Tripti)
ii) V2 - BARI-2 (Kamalasunduri)
iii) V3 - BARI-1 (Daulatpuri)
iv) V4- BARI Sweet Potato-4

Experiment -2

Main plot: four selected combination of nitrogen and potassium

i) NK_o - 0 kg Urea/ha + 0 kg MP/ha)

ii) NK_1 - 90 kg Urea/ha + 117 kg MP/ha)

iii) NK_2- 160 kg Urea/ha + 180 kg MP/ha)

iv) NK_3 -190 kg Urea/ha + 217 kg MP/ha)

Sub plote: It comprised four varieties of sweet potato,

i) V1 - BARI-1 (Tripti)

ii) V2 - BARI-2 (Kamalasunduri)

iii) V3 - BARI-3 (Daulatpuri)

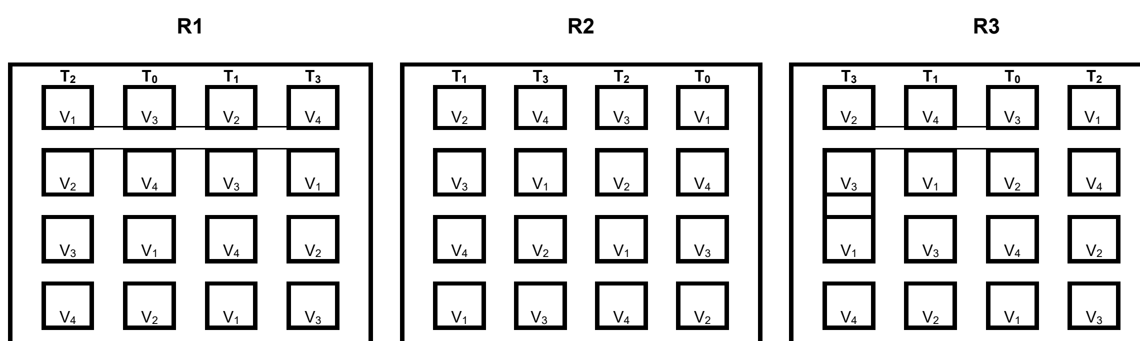
iv) V4- BARI Sweet Potato-4

3.5.2. Experimental Design and Layout

The experiment was laid out in the split plot design with three replications (Fig 3.1 for experiment-1 and 3.2 for experiment-2). The experimental land was first divided into three blocks each containing 16 plots. Thus, there were 48 unit plots of 1.8 m x .90 m in size and using row to row spacing of 60 cm and plant to plant spacing of 30 cm. Treatments were assigned at random to 16 plots of each block. The space between blocks was I m and between the plots was 50 cm. Each plot comprised 3 rows and three vines were planted in each row. The layout of the experimental plot was made on 12 November 2010.

Experiment-1

Main Plot Layout:



Unit plots size-1.80m×.90m

Row to row spacing-60cm

Plant to plant spacing-30 cm

Unit plots to Unit plots –50 cm

Space between replication to replication -1m

Total experimental area–135.11 m2

Where

R=Replication

T=treatment

T_0=Control

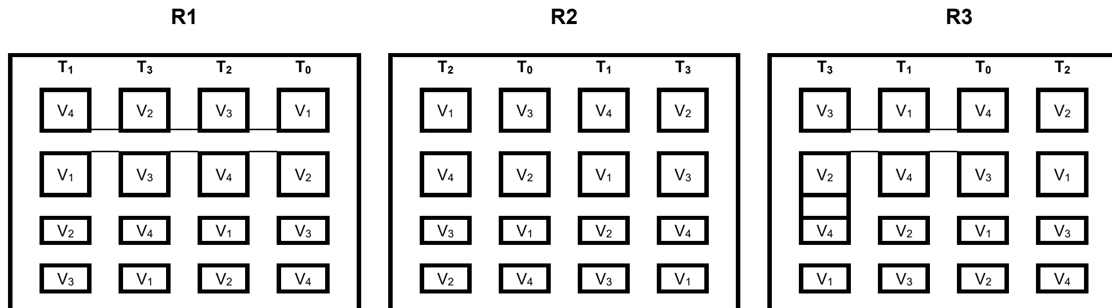
T_1=90kg /ha

T_2=160kg /ha

T_3=190kg /ha

Experiment-2

Main Plot Layout:



Unit plots size-1.80m×.90m

Row to row spacing-60cm

Plant to plant spacing-30 cm

Unit plots to Unit plots –50 cm

Space between replication to replication -1m

Total experimental area–135.11 m2

Where

R=Replication

T=treatment

T_0=Control

T_1=0 kg Urea t/ha + 0 kg MP/ha)

T_2=160 kg Urea t/ha + 180 kg MP/ha

T_3=190 kg Urea t/ha + 217 kg MP/ha)

3.5.3. Experimental Site and Period

The present research work related to the response of four sweet potato varieties to different levels of nitrogen (as urea) alone, and nitrogen and potash combinedly were conducted at the experiment field Department of Botany at University of Rajshahi campus during the period from the November, 2010 to April 2011 with the objective of selecting suitable variety and optimum dose of nitrogen for cultivation at farmers' level. The experiment site was located at $24^0$17'- $24^0$31' N Latitude and $88^0$28'-$88^0$43' E longitude. It is about 20 meter above the sea level. The land selected for the experiments was medium high with adequate irrigation and drainage facilities.

3.5.4. Characteristics of soil

The soil of the experimental plot was sandy loam in texture belonging to the High Barind Tract. The selected plot was high land and kept fallow during the previous season. Soil characteristics of the experimental site at a depth 0-30 cm were analyzed at the "Humboldt Soil Testing Laboratory", Department of Soil Sience, Bangladesh Agricultural University, and Mymensingh. The amount of organic matter, total nitrogen (N), phosphorus (P), potassium (K), sulphur and other nutrient elements were recorded (Table 1.4.)

Table 3.1. Nutrient status of initial soil prior to fertilization

Soil Properties	P^H	OM	Ca	Mg	K	Total N%	P	S	B	Cu	Zn	Mn
			meq/ 100g				$\mu g g^{-1}$					
Analytical result	5.7	1.45	14.0	1.55	0.29	0.083	10.7	11.1	0.18	2.81	0.83	6.9
Critical level			2.0	0.8	0.20	-	14	14	0.2	1	2	5

3.5.5. Climate

The location where the experiments were conducted was under subtropical climate characterized by heavy rainfall in Kharif season (April to September) and scarsity in the Rabi season (October to March). No rainfall was ovsered during the month February. The average maximum temperature during the period of experiment (Month: November 2010 to April 2011) was 28.89^0 C and the average minimum temperature was 15.38° C (Appendix-2).

3.5.6. Land preparation

Land preparation is a very key factor to achieve higher yield of any crop. The nature and extent of land preparation depends on the soil type and cropping system of sweet potato. The land was prepared two weeks before of planting. The land was ploughed and cross-ploughed several times with bullock driven country plough followed by laddering to obtain a good tilth.The land was prepared by giving four ploughings followed by four ladderings. The weeds and stubble's were removed after each laddering. Large clods were broken into pieces by hand mallet. Finally the soil was pulverized and levelled.

3.5.7. Preparation of planting materials

The vine cuttings collected from the Bangladesh Agricultural Research Institute (BARI) on November 9, 2010 were cured for three days under shade.

3.5.8. Planting of vine cuttings

The vine cuttings were planted in the experimental plots in the afternoon of 12 November, 2010 at a spacing of 60 cm x 30 cm (Rashid and Mannan, 1986) one third of the total length of the vine cuttings was put under the soil was followed by light irrigation by watering can.

3.5.9. Intercultural operations

3.5.9.1. Gap filling

Gap filling, whenever necessary was done with vine cuttings planted in the surrounding areas of experimental plots as border plants.

3.5.9.2. Weeding an earthing up.

The first weeding was done 30 days after planting followed by first earthing- up, and the second earthing up was done 60 days. After second weeding, top dressing of fertilizer was done. To obtain optimum yield from the tuber crop it is essential to make the crop free from weed. For a good economic return one weeding between 20-25 days after transplanting emergence should be sufficient.

3.5.9.3. Level of irrigation

Irrigation is the proper material for the appropriate growth of sweet potato. Generally this irrigation was applied in this experiment field three times. Irrigation depends on the soil condition or on the land condition. However there were three irrigations were applied in the research field. The planted vines were irrigated lightly by watering can on the same day of plantation and later on it was continued as and when needed till the establishment of vine cuttings. Thereby each plot was irrigated in two times by watering can. One was done 30 days after planting and second was done 60 days after planting. Light spading was done in irrigated plots when soil started form crust on the upper layer.

3.5.9.4. Vine lifting

Vine lifting was done at one week interval after planting' to avoid formation of roots at the nodes of vine trailing on the soil surface.

3.5.9.5. Plant protection

Miral 100 EC (0.03%) was applied to the soil against soil insects such as cutworms, ants, termites, weevils etc. during land preparation. So the experimental field was almost free from disease and insects. Only hairy caterpillars and thrips were observed to attack leaves during middle of the growth period and were controlled by the application of Nogos 100 EC at a concentration of 0.05%. A few weeks before harvest, some rats attacked the underground tuber. They were controlled successfully by the application of rodenticide Zinc phosphate.

3.5.10. Harvesting

Harvesting is the main stage for the production of crops. Useable experimental varieties were transplanted at the same time with three replications, but all the varieties were not matured at the same time. BARI SP- 4 variety was harvested at full maturity 120 days after planting on 12th March 2010 and BARI SP -1, BARI SP- 2, BARI SP- 3 were harvested at full maturity 140 days after planting on 2nd April 2010. Maturity was understood by the sign of drying of latex to white colour in cut tubers. Harvesting was done by cutting off the basal portion of vines with the help of sickle and digging out the tuberous roots with spade.

3.5.11. Collection of data

To evaluate the effects of nitrogen fertilizer alone or in combination with potassium, data were collected for yield and various yield contributing characters. Five sample plants were selected randomly and tagged initially for data collection from each plot. Data were collected and mean value of each treatment was calculated.

3.5.11.1. Length of main vine

Length of main vine was recorded from selected five on 40 days and on 120 days. Length of longest vine was measured in centimeter (cm) from the ground level to the tip of the longest vine of an individal plant and the mean value for each treatment was calculated.

3.5.11.2. Number of vines per plant

Total number of vines was counted from selected plant on 40 days and 120 days and the mean value for each treatment was calculated.

3.5.11.3. Vine internodes length

The internodes length was measured in centimeter (cm) and the mean internodes length was determined and recorded.

3.5.11.4. Leaf areas

The leaf area of plant was measured in centimeter (cm^2) and the mean plant leaf area was determined and recorded.

3.5.11.5. Fresh weight of vines per plant

The fresh weight of vines per plant was measured with electric balance in gram (gm) from 5 selected sample plants at the duration of harvesting time. The mean fresh weight of vines per plant was determined and recorded.

3.5.11.6. Fresh weight of tuberous roots per plant

The fresh weight of tuberous roots per plant was measured with electric balance in gram (gm) from total number of plants and recorded during harvesting.

3.5.11.7. Number of tuberous roots per plant

Number of tuberous roots per plant was counted from 5 selected plants for each plot and recorded the mean tuberous roots number.

3.5.11.8. Length of tuberous roots per plant

The tuberous roots length was measured in centimeter (cm) from 5 selected sample plants of each treatment and the mean tuberous roots length was determined and recorded

3.5.11.9. Average diameters of tuberous roots

Average diameter of tuberous roots was measured by slide calipers in centimeter from 5 selected sample plants of each treatment.

3.5.11.10. Weight of vines pre plot (kg)

Weight of vines pre plot were converted into vines yield per hectare in kilogram (Kg)

3.5.11.11. Yield of vines per plot (t/ha)

Vines per plot were converted into vines yield per hectare in ton.

3.5.11.12. Weight of tuberous roots pre plot (kg)

Weight of tuberous roots pre plot were converted into yield per hectare in kilogram (kg).

3.5.11.13. Yield of tuberous roots per plot (t/ha)

Tuberous roots per plot were converted into yield per hectare in ton.

3.5.12. Statistical analysis

The collected data on various parameters were statistically analyzed using CropStat 7.2 statistical package program. The mean for all the treatments was calculated and analysed of variance for all the characters were performed by F-variance test. The significance of differences between pairs of treatment means was evaluated by the least significance difference (LSD) test at 5% level of probability.

3.5.13. Economic Analysis

Economic analysis was done in order to compare the profitability of the varieties and treatment combinations**.** The analysis in details was done and given in Table 4.27. The following parameters were assessed.

Variable costs: Variable costs are that type of cost which are expenses to buying of different materials (inputs, labour wages utilities etc) related to production. It may change with the change of volume of production.

Gross return: Gross return are those return which comes from selling all outputs (as for example seed and Stover yield that means biological yield).

Gross Margin: Gross margin means reduction of variable cost or cost of production from gross return called gross margin.

Gross margin = gross return - variable cost

Benefit cost ratio: The ratio between gross return and variable cost is called benefit cost ratio.

Supervisor Late Professor Dr. M. anisuzzaman and Ph.D Fellow Mst. Kamrun Naher.

CHAPTER-4

RESULTS

In this work, four sweetpotato varieties (BARI-1, BARI-2, BARI-3, and BARI Sweet Potato-4) were used for as plant materials for improvement of sweetpotato, using integrated biotechnological approaches and by agronomic field trials. The present investigation has been carried out under three major parts: (i) meristem culture for the production of disease-free stock plants and rapid micropropagation for their propagation (ii) callus induction and plant regeneration for develpoping a protocol as a mean of generating somaclonal variation (iii) field evaluation of regenerated plants for possible somaclonal variation (iv) application of various level of nitrogen fertilizer sololy and in combination with potassium for further yield increase in meristem-derived as well as conventional varieties. Details of the observation and results of the experiments are described under the following four heads:

4.1. Part-1: A. Meristem Culture

4.1.1. Surface sterilization of shoot tip explants

Surface sterilization is the first to optimize in order to establish a meristem from field grown plants in *in vitro* condition. Standardization for surface sterilization was carried out by a trial and error experiments. In the present study, for primary establishment of apical meristem, shoot tips were collected from premature vines from 28-35 days old field grown sweetpotato plants. Shoot tips were surface sterilized by 0.1% $HgCl_2$ solution at different time duration viz. 3, 4, 5, 6, 7, 8, 9 and 10 minutes. The effects of different treatment duration on surface sterilization of meristems are summarized in Table- 4.1 When the shoot tips were treated in 0.1% $HgCl_2$ solution for 2 and 5 minutes, contamination was occurred. More than 90% of explants were found contamination free with healthy tissue when the shoot tips were treated in 0.1% $HgCl_2$ solution for 6-7 minutes. When the explants treated for long time (8-10 minutes), no contamination found but partial or complete tissue killing was observed. So, for surface sterilization of shoot tips treatment with 0.1% $HgCl_2$ solution for 6 minutes duration period was found most effective. No varietal difference was observed to sensitivity to $HgCl_2$.

Table: 4.1. Effect of different time's duration of 0.1% $HgCl_2$ treatments for surface sterilization of shoot tip explants of sweetpotato.

Treatment period of 0.1% $HgCl_2$ in min.	Total no. of cultures	Contamination (%)		Degree of tissue killing	Explants survived (%)
		After 5 days	After 10 days		
3	60	12	18	-	30
4	60	23	33	-	55
5	60	28	46	-	76
6	60	NC	NC	-	85
7	60	NC	NC	+	75
8	60	NC	NC	+ +	55
10	60	NC	NC	+ + +	35

NC = No contamination
-- = No tissue killing
+ = Partial tissue killing
+ + = Moderate tissue killing
+ + + = complete tissue killing

4.1.2. Primary establishment of apical meristem

Under a dissecting microscope apical vegetative meristems were cut as 0.3 mm form the shoot tips following IBPGR (1987) technical handbook. It has been recommended that the leaves around the growth point (the apical dome) should be removed until only the cupule and two or three foliar primordia are left. This is about 0.2-0.6 mm long and suitable for virus eradication in sweetpotato (Lizárraga *et al.*, 1999) According to Stone (1963) only shoot tips between 0.2 and 0.5 mm most frequently produce virus free carnation plants. The explants smaller than 0.2 mm cannot survive and those larger than 0.7 produce plants that still contain mottle virus. After isolation the apical meristems were placed quickly on "M" shaped filter paper bridge in culture tubes containing liquid MS medium supplemented with various concentrations and combinations of different plant growth regulators (Fig-A on Plate 4.1.1). The isolation of the meristematic zone under aseptic conditions and its culture in an adequate nutritive medium allow plantlet development with a differentiation pattern similar to that of a conventional plant.

Proper growths of the isolated meristem require manipulation of plant growth regulators. In MS_0 liquid medium (devoid of any growth regulator), only 16% meristems of BARI-1 and BARI-2 were able to develop shoots, whereas only 10% was observed in cv. BARI-3. Average vigour was poor (0.18 - 0.31) regardless of genotype. Therefore, it was assessed the requirement of plant growth regulators for quick responses of apical meristem. The resulta are described in the following sections. Upon proper growth regulator and nutrient, the cultured apical meristems showed their initial growth response by increasing in size and became greenish in color (i.e. change in vigour). They continued their growth and development of shoot. This condition of the meristem was supposed to be primary established (Fig-B on Plate 4.1.1).

4.1.2.1. Effect of kinetin on primary establishment of apical meristem

For primary establishment of apical meristem of four different sweetpotato varieties were cultured in MS liquid medium supplemented with 1.0-4.0 mg/l Kin. The initial growth responses and average degree of vigour of apical meristem are presented in Table-4.2. Responses of apical meristem were started from 8-21 days after inoculation. Among the different concentrations of kinetin, it was found that 2.5 mg/l kinetin was better than the others. Highest 58% meristem with an average vigour of 0.73 of cv. BARI-1 responded in this medium after only 8 days followed by 51% with an average vigour of 0.65 in 2.0 mg/l containing medium. In cultivar BARI- 4, 56% meristem responded with an average vigour of 0.66 in 2.5 mg/l kinetin, followed by BARI-2 (51%) and BARI-3 (49%). Where as minimum response (24%) shown in medium containing 4.0 mg/l. in BARI-3.

4.1.2.2. Effect of BAP on primary establishment of apical meristem

In the medium containing 1.0 mg/l BAP maximum frequency of response (23%) with an average vigour of 0.35 was found followed by 16% in 0.5 mg/l and 2.0 mg/l BAP in the cultivar BARI-1. This lower frequency and vigour also observed in other three cultivars. In all concentrations of BAP callus was formed. Callus

growth was concomitant with low shoot growth. This suggests that increased BAP concentration enhance callus growth.

4.1.2.3. Effect of GA_3 on primary establishment of apical meristem

To test the effects of GA_3, meristems were also cultured in MS liquid medium supplemented with 0.5-4.0 mg/l GA_3. The responses were found different from each others Table-4.3. About 56% apical meristems of cv. BARI-1 responded after in 7 days of inoculation with an average vigour of 0.65 in 2.0 mg/l GA_3 containing medium. Where as, only 20% meristem of cultivar BARI-3 responded with an average vigour of 0.26 after 10 days of inoculation.

4.1.2.4. Effect of Kin-GA_3 combinations

The results of different combinations of Kin and GA_3 are presented in Table 4.4. Among them 2.0 mg/l Kin+ 0.5 mg/l GA_3 was found most suitable. In this combination 66% excised meristem responded with an average vigour of 0.94 in cultivar BARI-1 followed by BARI-4 (61%, average vigour 0.90), whereas, BARI-2 (58%, average vigour 0.86) and BARI-3 (55%, average vigour 0.84) ranks third and fourth respectively. Medium having 2.5 mg/l Kin+ 0.5 mg/l GA_3 was also found satisfactory. Most of the excised meristems responded earlier then any other media used.

4.1.2.5. Effect of Kin-NAA combinations

.Highest 47% excised meristems developed in medium containing 2.0 mg/l Kin and 0.1 mg/l NAA with an average vigour of 0.51 in cultivar BARI-1 are presented in Table-4.5. This medium found most appropriate among Kin+NAA combinations regardless of genotype. Root initiation was observed along with primary shoot initiation in most of the culture incubated with reduced vigour. So these combinations are not suitable for efficient meristem establishment since root induction is not a primary objective of meristem derived stock production.

In conclusion, 2.0 mg/l Kin +0.5 mg/l GA_3 was most effective for primary establishment of apical meristem in studied genotypes and among the four cultivars BARI-1 was most responsive.

4.1.3. Shoot and root development

The tiny shoots developed from meristem were transferred in semisolid MS medium containing kinetin and GA_3 either singly or in combinations using different concentrations. For further massive propagation of meristem-derived plantlets it is necessary to shoot and root development. Two parameters on shoot induction (number of shoot/explants and mean length of shoot) and one parameter for root induction (number of roots/culture), were considered for standardization of suitable media composition, MS_0 medium was also tested as control. Virtually, 100% shoots produced roots in all treatments applied in all cultivars. Data of these parameters from different treatments were recorded after 3 weeks of inoculation. These results are presented under following heads:

4.1.3.1. Effect of kinetin or GA_3 or combinations

The primary established meristems were transferred in MS semi solid medium supplemented with kinetin and GA_3 either singly or in different concentrations and combinations including control medium (MS_0) for observing further shoot and root development. The summarized results of this experiment are shown in Table 4.6 and Plate 4.1.1.

BARI-1 produced maximum number (4.11± 0.09 cm) of shoots/explants in 2.5 mg/l Kin+0.5 mg/l GA_3 containing medium; whereas, the minimum number (1.23±0.10cm) of shoots/explants was observed when the explants cultured in MS_0 medium. The highest mean shoot length (4.37±0.20 cm) was found when cultured with 2.5 mg/l Kin+0.5 mg/l GA_3 containing medium. On the other hand the lowest shoot growth (2.82±0.48 cm) was observed when it was cultured in MS_0 medium. BARI-2 also responded similarly. The interms of the number of shoots/explants (2.61+0.10 cm) and as well as highest mean length of shoots (3.73+0.44 cm).

Where as, the lowest number of shoots/explants and lowest mean length of shoot was found when cultured on MS_0 medium. Both the BARI-3 (2.52±0.12 cm) and BARI-4 (3.13±0.05 cm) was showed the similar response.

All the cultivars produced root between a ranges of (1.12±0.07 cm) to (5.91±0.25 cm) in four cultivars studied; although high frequency root is not an objective in this stage.

4.1.4. Test of virus elimination in meristem derived plantlets

Meristem-derived plants were labeled micropropagated and acclimatized in soil pots. One or two plants originated from each mericlone were used as scuion for grafting experiment. The remaining plants were maintained in vitro. Most of the field samples, induced virus symptoms on *Ipomoea setosa* after grafting. The majority of symptoms recorded on *I. setosa* grafts of samples from all trials in the study included: small chlorotic spots, large veinal chlorosis, small veinal chlorosis, crinkling, and cupping with rugosity (Figure B, D, E, and F on Plate 4.2). Symptoms on *I. setosa* usually appear within 2-4 weeks. On the other hand more than 60% of the mericlones isolated from studied cultivars grafted showed no disease symptoms on *I. setosa*.

4.1.5. *In vitro* mass multiplication of virus-free meristem derived plantlets

Virus tested meristem derived plantlets were selected for massive micropropagation of clone plant stock. In these stages Kin, Kin+GA_3 and Kin+NAA were used in different concentrations and combinations. Two parameters on shoot induction (number of shoot/explants and mean length of the longest shoot) and two parameters for root induction (Frequency of root formation and No. of roots/shoot) were considered for standardization of suitable media composition. Data of these parameters from different treatments were recorded after 30 days of inoculation. The summarized results of this experiment are shown in Table 4.7 on Plate 4.1.1 and the results are presented under following heads:

4.1.5.1. Effect of Kin, Kin-GA_3 and Kin-NAA in different concentrations

The proliferated nodal explants excised from *in vitro* grown cultures were sub cultured in MS semi-solid medium supplemented with different formulation of either Kin or GA_3 singly and combined. In cv. BARI-1 maximum number of shoots/explants (6.21±0.05) was found in 3.0 mg/l Kin+0.5 mg/l GA_3 containing medium. Complementary, higher number shoots/explants was found (5.46±0.25) in 2.5 mg/l Kin+0.5 mg/l GA_3. Where as the minimum number (2.46±0.20) of shoots/explants was observed when the explants cultured on 3.0 mg/l Kin+1.0 mg/l NAA containing medium. Highest mean shoot length (8.21±0.16) was found when cultured in 2.5-mg/l Kin+0.5 mg/l GA_3 containing medium. On the other hand the lowest shoot growth was observed when it cultured on 3.0 mg/l Kin+1.0 mg/l NAA containing medium.

In BARI-2, highest mean number of shoots/explants (5.22±0.09) was noted in 3.0 mg/l Kin+0.5 mg/l GA_3 containing medium. Where as the lowest number of shoots/explants was found when cultured on medium having 3.0 mg/l Kin+ 1.0 mg/l NAA. In the medium supplemented with 3.0 mg/l Kin+0.5 mg/l GA_3 highest mean length of shoots (7.36±0.15 cm) was observed. On the other hand the lowest shoot growth was observed in the medium as for BARI-1. Other cultivars also responded similarly.

For root induction maximum number of root obtained in the medium containing 3.0 mg/l Kin +1.0 mg/l NAA regardless of cultivars. BARI-1 produces maximum number of roots (25.41±0.10) followed by BARI-3 (24.09±0.25), BARI-2 (20.12±0.15) and BARI-4 (19.56±0.87). For high frequency of root formation combination of 3.0 mg/l Kin +1.0 mg/l NAA was most effective, but these combinations produces relatively less number of shoots and shoot growth were also poor.

In conclusion, although the combination 3.0 mg/l Kin+0.5 mg/l $GA_{3.}$ produced relatively less number of roots but it was found sufficient for acclimatization and establishment in soil. So for high frequency micropropagation of meristem derived plantlets of described four cultivars MS medium supplemented with 3.0 mg/l Kin+0.5 mg/l GA_3 is recommended.

4.1.6. Acclimatization of mersitem-derived plants

Plantlets, with a well-developed root system, were acclimatized for 15-20 days (as described in Materials and Methods), and were transferred to nursery for further growth. More than 75% of these micropropagated plantlets successfully established and showed new leaf development under soil condition. Cutting from these plants were used for the field experiment.

4.1.7. Field trial of Meristem derived plants (R_1 and R_2)

The results are presented in Table 4.8. Comparing micropropagated (R1) managed either in net house or in open field, no marked variation was observed them for yield related characters. From the analysis of variances, it further reveals that root yield of R_2 generation of net house-managed planting material was better than both the open field and control plants. Other morphological characters for them were also more vigorous than both the open field and control plants. No varietal differences were observed for changing the field conditions. Viral disease incidence in R_1 net house plants was very low whereas, for the same in open field condition it was around 7%. Similar trends were observed when tubers of both conditions were planted for vine production for R_2 trial (data not shown). The incidence was found higher for both the conditions in R_2 generations than R_1. SPFMV reinfection rate was 3-8% and 6-13% in net house and open field condition, respectively. Among the symptoms (SPFMV, SPMMV and complex infections) the incidence of SPFMV was found highest.

Table: 4.2. Effect of different concentrations of cytokinin (Kin and BAP) in MS medium for primary establishment of apical meristems isolated from 25-30 days old-field grown plants.

Treatment		Variety	BARI-1	BARI-2	BARI-3	BARI SP-4
MS_0 (control)		Days to response	10-21	12-21	13-21	12-21
		Growth Response (%)	16	16	10	12
		Average degree of vigour	0. 31	0.23	0.18	0.25
Kin (mg/l)	1.0	Days to response	9-21	9-21	9-21	9-21
		Growth Response (%)	37	31	27	34
		Average degree of vigour	0.37	0.33	0.28	0.34
	1.5	Days to response	9-21	9-21	9-21	9-21
		Growth Response (%)	45	41	39	43
		Average degree of vigour	0.46	0.34	0.34	0.43
	2.0	Days to response	8-21	8-21	8-21	8-21
		Growth Response (%)	51	46	45	50
		Average degree of vigour	0.65	0.56	0.54	0.61
	2.5	Days to response	8-21	8-21	8-21	8-21
		Growth Response (%)	58	51	49	56
		Average degree of vigour	0.73	0.63	0.61	0.66
	3.0	Days to response	8-21	8-21	8-21	8-21
		Growth Response (%)	44	40	36	41
		Average degree of vigour	0.59	0.48	0.45	0.56
	4.0	Days to response	10-21	10-21	10-21	10-21
		Growth Response (%)	31	31	24	30
		Average degree of vigour	0.45	0.38	0.34	0.40
BAP (mg/l)	0.1	Days to response	10-21	10-21	10-21	10-21
		Growth Response (%)	12	7	7	10
		Average degree of vigour	0.29	0.14	0.14	0.25
	0.5	Days to response	10-21	10-21	0.16	10-21
		Growth Response (%)	16	12	12	14
		Average degree of vigour	0.31	0.21	0.26	0.29
	1.0	Days to response	9-21	9-21	9-21	9-21
		Growth Response (%)	23	11	15	17
		Average degree of vigour	0.35	0.28	0.25	0.31
	2.0	Days to response	9-21	9-21	9-21	9-21
		Growth Response (%)	16	11	12	14
		Average degree of vigour	0.29	0.25	0.24	0.28

Table: 4.3. Effect of different concentrations of Gibberelin (GA_3) in MS medium for primary establishment of apical meristems isolated from 25-30 days old-field grown plants.

Treatment		Variety	BARI-1	BARI-2	BARI-3	BARI SP-4
GA_3 (mg/l)	0.5	Days to response	9-21	10-21	10-21	10-21
		Growth Response (%)	30	24	20	26
		Average degree of vigour	0.35	0.28	0.26	0.31
	1.0	Days to response	9-21	9-21	9-21	9-21
		Growth Response (%)	38	32	36	35
		Average degree of vigour	0.45	0.37	0.37	0.40
	1.5	Days to response	9-21	9-21	8-21	9-21
		Growth Response (%)	45	38	35	43
		Average degree of vigour	0.56	0.45	0.43	0.51
	2.0	Days to response	7-21	7-21	7-21	7-21
		Growth Response (%)	56	50	46	52
		Average degree of vigour	0.65	0.58	0.54	0.60
	3.0	Days to response	7-21	7-21	7-21	7-21
		Growth Response (%)	50	41	34	45
		Average degree of vigour	0.61	0.50	0.47	0.54
	4.0	Days to response	8-21	8-21	9-21	9-21
		Growth Response (%)	41	34	34	36
		Average degree of vigour	0.46	0.40	0.36	0.42

Table: 4.4. Effect of different concentrations and combinations of kinetin and Gibberelin (Kin+GA_3) in MS medium for primary establishment of apical meristems isolated from 25-30 days old-field grown plants.

Treatment (Kin+ GA_3) mg/l \ Variety		BARI-1	BARI-2	BARI-3	BARI SP-4
1.0+0.1	Days to response	9-21	8-21	9-21	9-21
	Growth Response (%)	36	28	30	33
	Average degree of vigour	0.46	0.31	0.30	0.39
1.0+0.5	Days to response	9-21	8-21	8-21	8-21
	Growth Response (%)	43	36	34	39
	Average degree of vigour	0.66	0.54	0.51	0.61
1.5+0.5	Days to response	9-21	8-21	9-21	8-21
	Growth Response (%)	56	50	46	53
	Average degree of vigour	0.74	0.65	0.61	0.70
2.0+0.5	Days to response	7-21	7-21	7-21	7-21
	Growth Response (%)	66	58	55	61
	Average degree of vigour	0.94	0.86	0.84	0.90
2.5+0.5	Days to response	7-21	7-21	7-21	7-21
	Growth Response (%)	63	55	54	60
	Average degree of vigour	0.88	0.81	0.77	0.84
3.0+0.5	Days to response	9-21	8-21	8-21	8-21
	Growth Response (%)	50	46	51	56
	Average degree of vigour	0.82	0.61	0.61	0.66
4.0+0.5	Days to response	9-21	9-21	9-21	9-21
	Growth Response (%)	38	28	30	32
	Average degree of vigour	0.54	0.40	0.38	0.48

Table: 4.5. Effect of different concentrations and combinations of cytokinine and auxin (Kin+NAA) in MS medium for primary establishment of apical meristems isolated from 25-30 days old-field grown plants.

Treatment (Kin+NAA) mg/l	**Variety**	**BARI-1**	**BARI-2**	**BARI-3**	**BARI SP-4**
1.0+0.1	Days to response	9-21	9-21	9-21	9-21
	Growth Response (%)	31	26	24	29
	Average degree of vigour	0.29	0.25	0.22	0.26
1.0+0.5	Days to response	8-21	8-21	8-21	8-21
	Growth Response (%)	39	32	29	35
	Average degree of vigour	0.39	0.31	0.24	0.34
2.0+0.1	Days to response	8-21	8-21	8-21	8-21
	Growth Response (%)	47	39	36	43
	Average degree of vigour	0.51	0.42	0.38	0.46
2.0+0.5	Days to response	8-21	8-21	8-21	8-21
	Growth Response (%)	41	36	35	41
	Average degree of vigour	0.41	0.34	0.33	0.37
3.0+0.1	Days to response	9-21	9-21	9-21	9-21
	Growth Response (%)	36	31	27	32
	Average degree of vigour	0.35	0.30	0.27	0.30
3.0+0.5	Days to response	9-21	9-21	9-21	9-21
	Growth Response (%)	29	25	24	25
	Average degree of vigour	0.32	0.24	0.22	0.30

Table: 4.6. Effect of different concentrations and combinations of Kin and GA_3 in MS medium on shoot and root development from developed primary meristem: number and length (cm) of shoot, and root number. Data were recorded at 21 days after inoculation.

Treatment		Variety	BARI-1 ($\bar{X}$ ±SE)	BARI-2 ($\bar{X}$ ±SE)	BARI-3 ($\bar{X}$ ±SE)	BARI SP-4 ($\bar{X}$ ±SE)
MS0 (control)		Number of shoot	1.23 ±0.10	1.14±0.10	1.10 ±0.10	1.16± 0.15
		Number of root	1.91±0.38	1.89±0.35	1.12±0.07	1.83±0.65
		Shoot length	2.82±0.48	2.50±0.08	2.49± 0.48	2.71± 0.15
Kin	2.5 mg/l	Number of shoot	2.81±0.22	1.61±0.16	1.41±0.23	2.25 ±0.22
		Number of root	3.81±0.22	2.6±0.30	2.06±0.20	3.26±0.15
		Shoot length	3.16±0.30	2.62±0.15	2.38± 0.25	2.89± 0.23
	3.5 mg/l	Number of shoot	2.21 ±0.40	1.54±0.15	1.56 ±0.20	1.85 ±0.25
		Number of root	4.02±0.23	3.50±0.80	3.12±0.35	3.86±0.30
		Shoot length	2.92±0.16	2.51±0.25	1.93±0.30	2.76± 0.35
GA_3	2.0 mg/l	Number of shoot	2.12 ±0.50	1.31±0.20	1.12 ±0.10	1.72 ±0.20
		Number of root	4.21±2.40	3.71±2.50	3.26±1.78	4.09±1.40
		Shoot length	3.54±0.32	2.96±0.35	2.78±0.30	3.18±0.80
	3.0 mg/l	Number of shoot	1.81±0.15	1.31±0.09	1.28 ±0.31	1.51 ±0.25
		Number of root	5.26 ±2.30	3.83±1.60	3.34 ±2.41	4.41±1.28
		Shoot length	3.13±0.70	2.82±0.56	2.74±0.79	2.92±0.20
Kin + GA_3	2.0+0.5 (mg/l)	Number of shoot	3.11 ±0.15	2.26 ±0.10	2.31 ±0.15	2.90 ±0.98
		Number of root	3.71±2.30	3.76±1.12	3.801±2.18	4.31 ±1.10
		Shoot length	3.92±0.98	3.65±0.78	3.20± 0.58	2.91± 0.91
	2.5+0.5 (mg/l)	Number of shoot	4.11 ±0.09	2.61±0.10	2.52 ±0.12	3.13 ±0.05
		Number of root	4.31±1.15	5.91±0.16	3.71±1.12	4.81±1.08
		Shoot length	4.37±0.20	3.73±0.25	3.09± 0.50	4.13± 0.12
	3.0+1.0 (mg/l)	Number of shoot	2.82 ±0.05	2.21 ±0.15	1.90 ±0.12	2.16±0.10
		Number of root	3.31±1.10	3.91±0.16	2.70±1.22	4.81±1.09
		Shoot length	3.45±0.98	2.92±0.40	2.75± 0.70	3.02± 0.95

Table: 4.7. Effect of different concentrations of Kin, Kin+GA_3 and Kin+NAA in MS semi-solid medium for micropropagation of four varieties of sweetpotato using nodal segment from meristem derived plantlets. Data were recorded 30 days after inoculation.

Treatment		Varieties	BARI-1 ($\bar{X}$ ±SE)	BARI-2 ($\bar{X}$ ±SE)	BARI-3 ($\bar{X}$ ±SE)	BARI-4 ($\bar{X}$ ±SE)
KIN (mg/l)	2.5	Shoot Number	4.76±0.14	3.31±0.16	3.20±0.10	3.60±0.10
		Root Number	15.24±0.15	15.01±0.12	13.21±0.18	12.46±0.07
		Shoot length	5.24±0.35	4.92±0.14	4.63±0.15	5.20±0.16
	3.0	Shoot Number	5.34±0.10	4.356±0.01	3.96±0.45	4.97±0.20
		Root Number	18.06±0.55	17.36±0.30	15.24±0.22	14.92±0.05
		Shoot length	6.53±0.15	5.41±0.12	5.08±0.70	5.89±0.12
	4.0	Shoot Number	4.26±0.18	3.32±0.17	3.06±0.10	3.66±0.15
		Root Number	17.52±0.87	13.21±0.09	13.20±0.07	16.51±0.49
		Shoot length	5.32±0.45	4.26±0.88	4.20±1.10	5.10±0.15
Kin + GA_3 (mg/l)	2.5+0.5	Shoot Number	5.46±0.25	4.44±0.10	3.86±0.20	4.98±0.12
		Root Number	14.06±0.12	12.24±1.06	12.86±0.18	14.38±1.05
		Shoot length	7.51±0.09	6.26±0.14	5.18±0.98	7.04±0.27
	3.0+0.5	Shoot Number	6.21±0.05	5.22±0.09	4.94±0.12	5.54±0.20
		Root Number	17.20±0.10	15.54±0.15	13.81±0.05	15.76±0.02
		Shoot length	8.21±0.16	7.36±0.15	6.51±0.01	7.91±0.94
	3.0+1.0	Shoot Number	4.96±0.25	3.88±0.89	3.58±0.06	4.12±0.09
		Root Number	16.31±0.05	12.52±0.17	10.56±0.08	14.76±0.00
		Shoot length	6.99±0.05	5.00±1.07	5.24±0.15	6.26±0.12
Kin + NAA (mg/l)	2.5+0.5	Shoot Number	3.24±0.00	2.52±0.03	2.41±0.10	3.16±0.98
		Root Number	20.33±0.06	17.51±1.15	17.31±0.15	18.02±1.05
		Shoot length	4.22±0.09	3.26±0.17	3.48±0.10	3.71±0.10
	3.0+0.5	Shoot Number	3.92±0.20	2.76±0.19	2.81±0.18	3.26±0.12
		Root Number	23.13±0.10	20.31±0.35	19.56±0.15	18.55±0.07
		Shoot length	3.95±0.98	2.90±0.95	2.32±0.88	3.46±0.90
	3.0+1.0	Shoot Number	2.46±0.20	2.21±0.12	2.16±0.09	2.31±0.25
		Root Number	25.41±0.10	24.09±0.25	20.12±0.15	19.56±1.87
		Shoot length	3.51±0.01	2.95±1.85	3.22±0.10	3.30±0.75

Table 4.8. Field evaluation of R_1 and R_2 plants for yield components and disease incidence. (Note: No control was used in R_1. R_2 trial was conducted in open field condition, vines were obtained either from net house or from open field and compared with traditional cuttings).

Cultivar / Parameter	Plant material source	BARI-1		BARI-2		BARI-3		BAR I SP-4	
		R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2
Length of main vine (cm)	Net house	73.3±0.89	117.8±0.67	78.3±1.2	112.06±0.76	70.5±1.04	82.2±1.48	68.2±0.44	190.9±0.62
	Open field	70.7±1.67	116.7±1.07	76.0±1.00	111.8±0.49	64.7±1.76	81.1±0.82	63.3±1.20	189.2±0.68
	Control	-	115.1±1.25	-	111.2±0.43	-	80.4±1.16	-	189.6±0.37
Storage root no./plant ($\bar{x}$ ±SE)	Net house	1.67±0.33	2.00±0.47	2.33±0.67	3.67±0.27	3.67±0.67	4.00±0.00	4.66±0.88	5.33±0.27
	Open field	1.67±0.67	2.00±0.47	2.0±0.57	3.33 ±0.27	3.67±0.88	3.6±0.27	4.33±1.2	5.33±0.72
	Control	-	2.00±0.81	-	3.67±0.54	-	3.66±0.72	-	5.00±0.47
Root yield/plant (g)	Net house	335±2.6	538±1.53	306±1.5	576±1.45	245±1.45	374±1.45	355±1.5	482±2.08
	Open field	334±1.85	531±0.88	310±1.15	570±0.88	238±0.88	369±1.53	352±2.33	481±0.67
	Control	-	529±0.88	-	563±1.85	-	363±1.85	-	476±0.88
SPFMV incidence (%)	Net house	0.0	5.9	0.0	6.1	0.0	3.0	0.0	0.0
	Open field	6.7	11.1	0.0	8.3	7.1	8.3	7.1	13.8
	Control	-	41.7	-	27.8	-	25.0	-	19.4
SPMMV incidence (%)	Net house	0.0	0.0	0.0	3.0	0.0	3.0	0.0	2.7
	Open field	0.0	0.0	6.7	0.0	0.0	0.0	0.0	0.0
	Control	-	2.7	-	5.6	-	13.8	-	0.0
Complex infections (%)	Net house	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Open field	0.0	0.0	0.0	2.7	0.0	6.0	0.0	0.0
	Control	-	8.3		5.6	-	0.0	-	13.8

4.1.8. Comparison of cost between NCM-ELISA and grafting method

Small-scale field trial was conducted (describe in the previous section 4.1.7) to observe the effects of virus elimination and possible reinfection occurred in field. In the following sections, cost of producing disease-tested vines was calculated. To observed actual economic impact, an economic analysis was conducted following bigger field trials describing various agronomic traits (section 4.5).

The typical cost for testing sweetpotato SPFMV and SPCSV using standard NCM-ELISA kit and antisera is 1000 Tk/sample (service hire). However, grafting can be performed using skilled gardener costing 300 Tk/day. A gardener can perform 30-40 grafting operation in a day. Considering 80% success rate, labor cost of per grafted sample could be assumed as less tan 15 Tk. Moreover, the rootstock plant *Ipomoeas setosa* grown as wild plant in Bangladesh, and seeds can be obtained easily. Fair body of literature suggests that grafting method is very sensitive and competitive with serological methods. Thus, grafting method offers low cost option for sweetpotato virus diagnosis.

4.1.9. Cost of micropropagated plants

The described protocol appears to be technologically suitable for micropropagation of sweetpotato. In low cost technology cost reduction is achieved by improving process efficiency and better utilization of resources. Our protocol reports highest multiplication rate (~6x) by node cutting from a single explant. We have analyzed the production cost of 100 complete plants from culture initiation to hardening and field transfer. The detailed cost analysis presented in Table 4.9 shows that at the present market rate the cost of consumables to produce 100 plants does not exceed Taka. 1040.

Table 4.9 Cost estimation of 100 plantlets derived from meristem

Item	Cost (Taka)
0.1% $HgCl_2$ (400 ml)	40
MS basal medium (2L) [Himedia]	320
Sucrose (3%)	5
PGRs. (Kin+GA_3 [Sigma]	40
Agar (0.7%) (2L media)	25
Culture vessel (juice bottle) 20	160
Grafting of mericlones	150
Miscellaneous expenses	300
Total	1,040

Thus, in this experiment cost of virus tested mericlones was estimated as 10.4 Taka/plant. To test the effects of nitrogen and potassium fertilizer, 432 mericlones were required. This can be obtained through vegetative multiplication in the field. By multiplying once 4-6 plants can be obtained easily from a single mericlone. This would reduce cost per plant. At the current market price, cost associated with irrigation, fertilizer and field management would be 500 Taka. Thus, the cost of 432 virus tested plants was 1540 Taka (3.5 Taka/plant).

PLATE-4.1

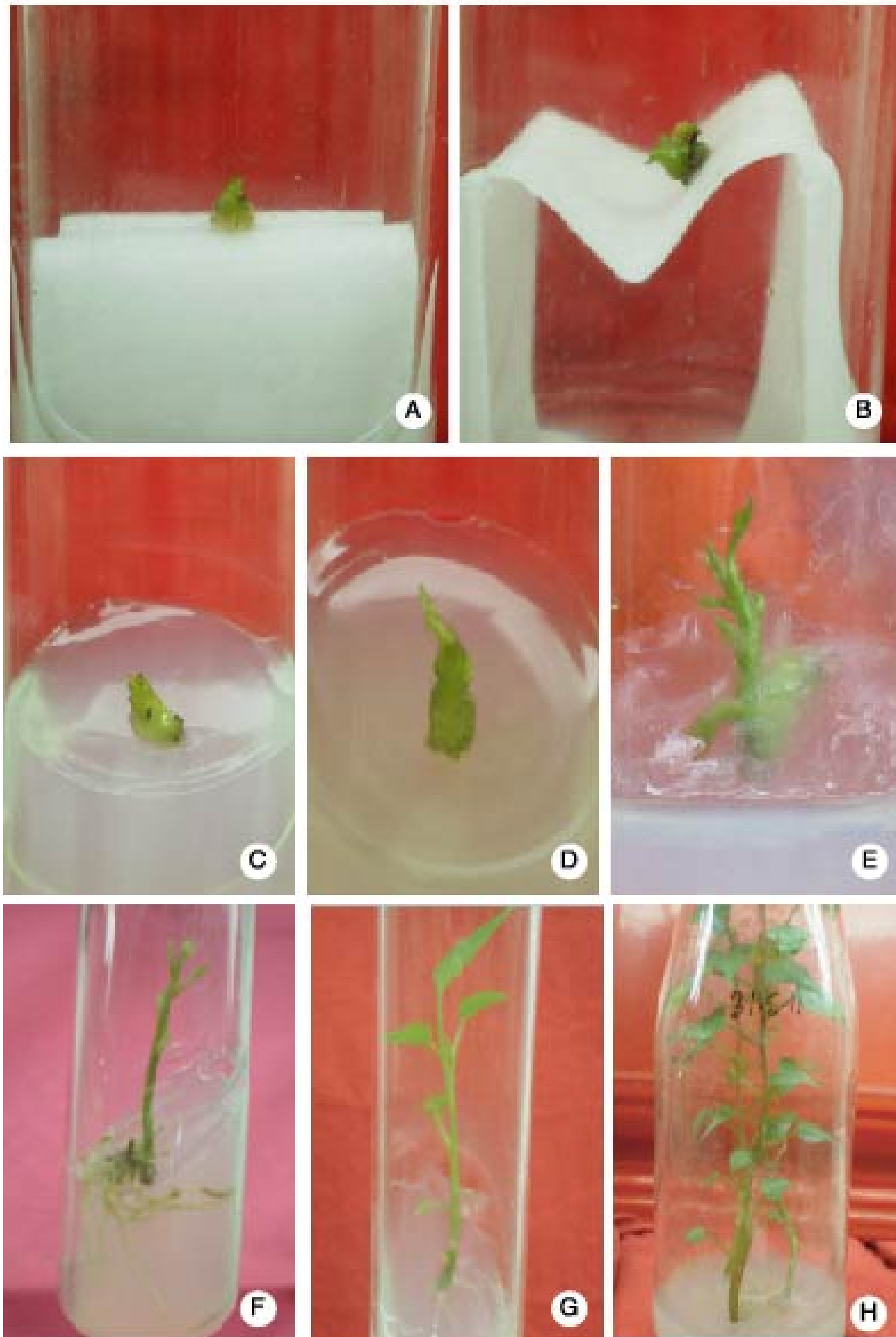


PLATE- 4.1 Meristem culture.

Fig. A: Development of isolated apical meristem (7-days old) on filter paper bridge in liquid medium.
Fig. B Shoot initiation (15-days old) from the isolated meristem in liquid medium.
Fig. C - E: Shoot development from the cultured meristem in semi-solid medium.
Fig. F and G: Development of shoots and root after transferring in semisolid medium.
Fig. H: Mass propagation of meristem derived plantlets.

PLATE-4.2.1

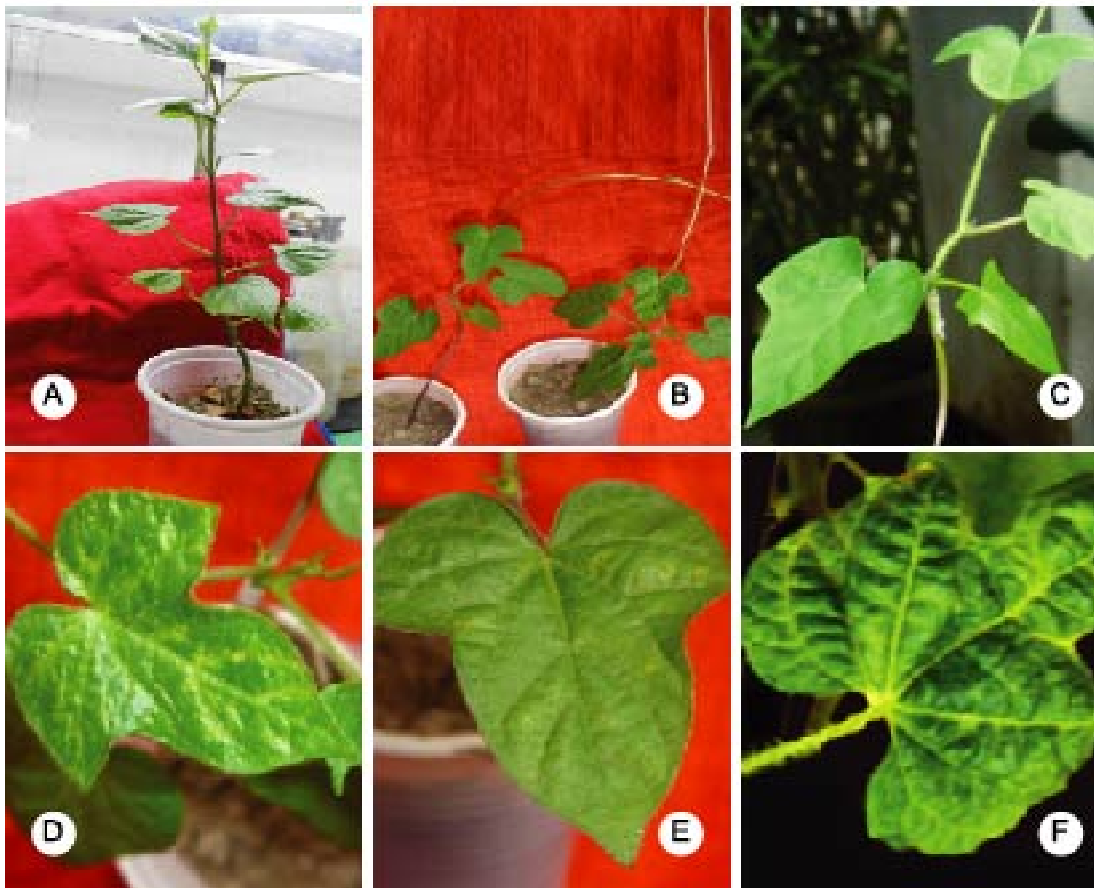


PLATE- 4.2.1 Virus indexing of mericlones through grafting.

Fig. A: Acclimatized meristem-derived plantlets.

Fig. B: Mericlones grafted on Ipomoea setosa stock. Field grown plant grafted on Ipomoea setosa stock showing disease symptoms (Right), while mericlone grafted plants showing no disease symptoms (Left).

Fig. C: Mericlone grafted plants showing no disease symptoms.

Fig. D and F: Field grown plant grafted on Ipomoea setosa stock showing vein chlorosis along with curling on the leaves.

Fig. E. Field grown plant grafted on Ipomoea setosa stock showing chlorotic spots on the leaves.

PLATE-4.2.2

PLATE- 4.2.2 Field establishment of meristem derived plantlets.
Fig.A-C: Plantlets in control, net house and open field after120 days of plantation.
Fig. D-F: Tuber produced in control, net house and open field.

4.2. Part-1. B: Callus Induction and Plant Regeneration

4.2.1. Callus Induction

Most sweetpotato varieties are sterile; therefore creating variation is difficult for breeding purpose. This experiment was conducted to develop a protocol for callus induction and subsequent plant regeneration as a tool of generating somaclonal variation. This section describes the results of callus induction and subsequent plant regeneration from inter nodal, root and leaf disc explants from meristem derived *in vitro* grown plantlets. The explants were cultured on MS semi solid media supplemented with different concentrations of either 2,4-D, BAP, TDZ, NAA alone or in combinations of NAA with BAP and 2,4-D in order to find out a suitable culture medium for development of maximum embryogenic callus induction. Percentages of explants induced callus were noted after 2nd–3rd weeks of inoculation. The results are shown in Table 4.8-4.13 and described in the following sections.

4.2.1.1. Callus induction from internode explants

For embryogenic callus induction from internode explants, different growth regulators such as 2,4-D, BAP and NAA either singly or in combination with each other were used. Beside these different concentrations of TDZ were also tested. Data were recorded after 21 days after culture.

4.2.1.2. Effect of 2, 4-D on callus induction

Internode segments were inoculated in different concentrations of 2, 4-D ranged from 0.5 – 5.0 mg/l. The results are shown in Table 4.10. The highest frequency of call induction was observed in BARI-1 (87.98%) in MS medium supplemented with 3.0 mg/l of 2, 4-D. The second highest (85.15%) result was shown in the medium having 2.5 mg/l 2,4-D. On the other hand lowest callus formation (60.25%) was found in medium with 0.5 mg/l 2, 4-D. callus indction in other cultivars was also good in the 3.0 mg/l 2,4-D (80.78, 79.88 and 86.55% in BARI-2 BARI-3 and BARI-4, respectively). In all the cases the morphological nature of the calli was varied from creamy to creamy white in colour and friable to spongy in nature. The growth rate of calli was noticeable.

4.2.1.3. Effect of different 2, 4-D-NAA combinations

MS medium supplemented with different concentration 2, 4-D with NAA were applied to induce callus from internode explants. The results of the 2, 4-D+NAA media formulation are presented in the Table 4.10. The highest percentage of callus induction was observed in in BARI-1, (80.10%) in 2.0 mg/l 2, 4-D+0.5 mg/l NAA. Other cultivars also performed best in the same combination. Morphological nature of the calli was varied from creamy to creamy white in colour and friable in nature. The growth of calli was moderate irrespective of cultivars.

4.2.1.4. Effect of different concentrations of TDZ

MS medium supplemented with different concentration of TDZ was tested for callus induction internode explants. The results are shown in Table 4.10**.** Among all the concentrations, TDZ in 2.0 mg/l formulation maximum explants (86.05%) induced callus in BARI-1. Other cultivars also performed best in the same combination. Most of the calli were dark green in colour with sporadic emerging somatic embryonic structure was observed.

4.2.1.5. Effect of BAP and BAP-NAA combinations

As shown in Table-4.11, BAP was found moderately effective for callus formation in high frequency in studied four cultivars. Highest (89.95%) callus formation was observed in concentration 4.0 mg/l in cv. BARI-1. Similar results were noted in case of other cultivars. For cultivars BARI-2, BARI-3 and BARI SP-4 83.89%, 80.98%, and 84.96%, respectively were observed. Lowest percentage was found in high concentrations of BAP (>5.5 mg/l) irrespective of cultivars. Most of the calli were dark green in colour and compact in nature.

NAA was found to produce appreciable amount of callus in sweetpotato as presented in Table 4.9. In BARI-1, maximum 71.85% callus was induced from internode explants in MS medium containing 5.0 mg/l NAA, followed by 66.79% in 6.0 mg/l. Whereas, 54.45% was obtained in 2.0 mg/l of NAA. In BARI-2, highest frequency of callus induction (65.50%) was observed in 5.0 mg/l NAA containing MS medium; on the other hand only 48.98% was recorded in

7.0mg/l NAA. BARI-3 and BARI SP-4 also produced maximum callus (62.99% and 66.07% respectively) in the same medium as in BARI-2. Among the studied all experiments highest frequency of callus induction was obtained in BAP (4.0 mg/l) + NAA (0.5mg/l NAA) combinations in all studied cultivars. The figures were 95.09%, 86.86%, 85.97% and 90.75% for BARI-1, BARI-2, BARI-3 and BARI-4, respectively. In addition to this, 4.5 mg/l BAP+0.5mg/l NAA combination was also found satisfactory. The results further reveal that increasing concentration of BAP had negative effect on callus induction. The calli were creamy in colour and friable and nodular in structure.

4.2.2. Callus induction from root explants

The single effect of 2,4-D, NAA, BAP and TDZ, and combined effects of 2,4-D+NAA and BAP+NAA were used for callus induction from root explants of *in vitro* grown plants. The results are presented in Table- 4.12 - 4.13. Data were recorded after 21 days after culture. The effects are presented as picture in Plate-4.3

All the growth regulators tested produced callus with different induction frequencies. Highest callus induction was observed in 2.5 mg/l of 2,4-D treated BARI-1 roots explant (89.65%). When combined with NAA callus induction was little reduces (45.67% in BARI-1 in 0.5 mg/l 2,4-D + 0.1 mg/l NAA).

Frequency of callus produced in TDZ-treated explants was similar to that of 2, 4-D. In BARI-1, 85.87% callus induction was observed in 2.0 mg/l TDZ.

Similar trend was also observed in all the cultivars. The growth of calli was moderate irrespective of cultivars. Like internode explants, the calli induced from root was also found in good structure and colour.

3.0 mg/l of BAP resulted in significant callus induction. BARI-1 produced the maximum (86.97%) amount of callus in this media. Similar trend was observed in other cultivars. However, they were not relatively less responsive to callus induction.

Effect of different concentration of NAA on callus induction was shown in Table 4.13. Significant number of explants was turned into callus upon culturing on various level of NAA. Highest induction was observed in 5.0mg/l concentration. Similarly, BAP-NAA combinations were also produced sufficient callus. Almost all calli were greenish and friable, some were nodular.

4.2.3. Callus induction from leaf disc explants

As above, the single effect of 2, 4-D, NAA, BAP and TDZ, and combination effects of 2,4-D+NAA and BAP+NAA were tested. 2, 4-D, NAA and BAP either singly or in combination with each other were used for callus induction from leaf disc explants of *in vitro* grown plants. Beside this, TDZ singly was also employed in different concentrations. Data were recorded after 21 days after culture. The results are presented at Table- 4.14 - 4.15 and the effects are presented as picture in Plate-4.5.

4.2.3.1 Effect of 2, 4-D

Leaves of *in vitro* grown plantlets were cut into pieces and inoculated in different concentrations of 2, 4-D ranged from 0.5 – 5.0mg/l. The results are shown in Table 4.14 Among all the concentrations, in BARI-1 maximum (77.56%) callus induction was obtained in medium supplemented with 2.5mg/l of 2,4-D. Complimentary, higher (75.10%) was in 2.0mg/l of 2,4-D concentration. On the other hand minimum (49.85%) callus initiation observed when cultured on the medium having in 0.5 mg/l 2,4-D. Cultivar BARI-4, BARI-2 and BARI-3 also showed highest response (74.89%, 72.88% and 70.05% respectively) in 2.5 mg/l of 2,4-D. The calli were creamy to creamy white in colour and friable, some were spongy in nature. The growth of calli was good.

4.2.3.2 Effect of 2,4-D with NAA

MS medium supplemented with 2,4-D and NAA in different combinations were applied to induce callus from leaf disc explants. The results are tabulated in Table 4.14 In BARI-1, the maximum (79.95%) of the callus induction was noted in the medium supplemented with 2.0 mg/l 2,4-D + 0.5 mg/l NAA, A minimum frequency of 51.58 % callus induction was obtained when it were cultured on

medium containing 0.5 mg/l 2,4-D + 0.1 mg/l NAA. BARI-2, BARI-3 and BARI-4 also produced maximum amount of callus (71.05%, 64.15% and 76.95% respectively) in the 2.0 mg/l 2, 4-D + 0.5 mg/l NAA. The calli were almost greenish in colour and friable in nature. The growth of calli was moderate irrespective of cultivars.

4.2.3.3. Effect of TDZ

TDZ found to produce rapid callus formation in four studied sweetpotato cultivars. In BARI-1, the maximum (88.99%) of the explants formed callus in the medium containing 2.0 mg/l mg/l TDZ followed by (79.75%) in 3.0 mg/l. BARI-2, BARI-3 and BARI-4 also produced maximum callus 82.98%, 79.82% and 84.98% respectively in the 2.0 mg/l TDZ. Increasing concentrations of TDZ decreased callus formation. Most of the calli formed in intermediate concentration of TDZ were creamy to light green and friable, while some nodular calli were also found. On the other hand calli developed in high concentration of TDZ were mostly light green and firm.

4.2.3.4 Effect of BAP

Highest (85.10%) callus induction was noted in the medium supplemented with 3.0 mg/l BAP in BARI-1. BARI-2, BARI-3 and BARI-4 also responded similarly with a callus induction frequency of (80.25%), (77.66%) and (81.89%0), respectively. Lowest percentage was found in high concentrations of BAP (>5.5 mg/l) irrespective of cultivars. Most of the calli produced from leaf disc in BAP containing medium were creamy white to creamy and friable in nature, although some spongy non-embryogenic calli were also produced.

4.2.3.5 Effect of different concentrations of NAA on callus induction

Leaves of *in vitro* grown plantlets were inoculated in MS medium with different concentrations of NAA ranged from 2.0-7.0 mg/l. The results are presented in Table 4.15. BARI-1 showed maximum explants (70.05%) to form callus in medium having 4.0 mg/l of NAA. Complimentary, higher (64.98%) callus was developed when it cultured on the medium having 3.0 mg/l NAA. On the other hand minimum (39.90%) explants induced callus in 7.0mg/l formulation. In case

of Cultivar BARI-2, BARI-3 and BARI-4 highest frequency (61.85%, 59.78% and 66.98% respectively) was observed in 4.0 mg/l NAA. The calli were mostly light green in colour and friable in nature with good growth.

4.2.3.6 Effect of different BAP-NAA combinations

MS medium supplemented with different combinations of BAP and NAA was also tested for callus induction from leaf disc in all of four sweetpotato cultivars. The results are shown in Table 4.15. In BARI-1, the maximum (81.75%) callus formation was observed in the medium containing 4.0 mg/l BAP + 0.5mg/l NAA. A minimum frequency (60.00 %) of callus induction was obtained when it were cultured on the medium containing 6.0 mg/l BAP + 0.5 mg/l NAA. BARI-2, BARI-1. BARI-3 and BARI-4 also produced maximum callus (76.99%, 74.12% and 81.05% respectively) in the 4.00 mg/l BAP + 0.5 mg/l NAA. The calli were almost creamy to light green and friable. The growth of calli was good irrespective of cultivars.

From the overall experimentation it can be concluded that, although percentage of callus induction were mostly above 75% in suitable media mentioned earlier, a varied degree of variation was observed in nature, colour and texture that reflects on further embryogenesis and subsequent plant regeneration. In this respect, 2 mg/l 2,4-D+0.5 mg/l NAA, 4 mg/l BAP+0.5 mg/l NAA and 2.0 mg/l TDZ produced good amount of friable/granular calli regardless of cultivars. Most of the calli formed embryoid or meristemoid like structure. Among the explants internode was found suitable than root and leaves and BARI-1 was most responsive to callus induction followed by BARI-4, BARI-2 and BARI-3.

Table 4.10. Effect of various concentration of 2.4-D, singly and in combinations with NAA and TDZ in MS medium for callus induction (%) along with its morphological nature induced from internodes of *in vitro* grown plants of four sweetpotato cultivars. Data were recorded 21 days after inoculation.

	BARI-1				BARI-2				BARI-3				BARI SP-4			
Plant growth regulator	Frequency (%)	Colour	Nature	Degree of growth	Frequency	Colour	Nature	Degree of Growth	Frequency	Colour	Nature	Degree of Growth	Frequency	Colour	Nature	Degree of Growth
2, 4-D (mg/l)																
0.5	59.96	CrW	Sp	+	57.95	CrW	Sp	+	54.98	CrW	Sp	+	58.94	Cr	Sp	+
1.0	69.85	CrW	Sp	++	64.10	CrW	Sp	+	60.56	CrW	Sp	+	69.05	Cr	Sp	+
2.0	74.22	Cr	Fr	++	69.85	CrW	Sp	++	68.15	CrW	Fr	++	71.89	CrW	Fr	++
2.5	85.15	Cr	Fr	+++	79.95	Cr	Fr	+++	77.10	Cr	Fr	+++	81.95	CrW	Fr	+++
3.0	87.98	Cr	Fr	+++	80.78	Cr	Fr	+++	79.88	Cr	Fr	+++	86.55	CrW	Fr	+++
3.5	70.91	Cr	Fr	++	64.86	Cr	Fr	++	62.89	CrW	Sp	++	68.58	Cr	Sp	+++
4.0	67.35	CrW	Sp	+	61.98	CrW	Sp	+	58.96	CrW	Sp	++	64.86	Cr	Sp	+
5.0	60.40	CrW	Sp	+	57.88	CrW	Sp	+	55.95	CrW	Sp	+	58.97	Cr	Sp	+
2, 4-D+NAA(mg/l)																
0.5+0.1	64.90	CrW	Fr	+	60.94	CrW	Fr	+	57.78	CrW	Fr	+	62.90	CrW	Fr	+
1.0+0.5	67.99	Gr	Fr	+	64.10	Gr	Fr	+	60.17	Gr	Fr	+	65.35	Gr	Fr	+
2.0+0.5	80.10	Gr	Fr	++	74.98	Gr	Fr	++	72.80	Gr	Fr	++	75.72	Gr	Fr	++
2.5+0.5	64.87	Gr	Fr	+++	60.00	Gr	Fr	++	56.95	Gr	Fr	++	61.89	Gr	Fr	+++
3.0+0.5	60.10	Gr	Fr	+	54.96	Gr	Fr	+	52.72	Gr	Fr	+	57.95	Gr	Fr	+
TDZ(mg/l)																
0.5	60.55	DGr	Com	++	50.40	DGr	Com	++	44.87	LGr	Com	++	54.85	LGr	Com	++
1.0	71.30	DGr	Com	+++	62.99	Gr	Com	+++	57.99	LGr	Com	+++	67.78	LGr	Com	+++
2.0	86.05	DGr	Com	++++	81.00	Gr	Com	++++	75.92	DGr	Com	+++	82.55	DGr	Com	++++
3.0	64.95	DGr	Com	+++	61.25	DGr	Com	+++	56.06	DGr	Com	++	60.79	DGr	Com	+++
5.0	44.88	DGr	Com	++	42.95	DGr	Com	++	39.89	DGr	Com	+	46.96	DGr	Com	++

DGr = Dark green
LGr = Light green
Gr = Greenish
Cr = Creamy
CrW = Creamy white

Com = Compact
Sp = Spongy
Fr = Friable
Lc = Loosely compact

+ = Poor growth
++ = Moderate growth
+++ = Good growth
++++ = Massive growth

Table 4.11. Effect of various concentrations of BAP and NAA and their combinations in MS medium for callus induction (%) along with its morphological nature induced from internodes of *in vitro* grown plants of four sweetpotato cultivars. Data were recorded 21 days after inoculation.

	BARI-1				BARI-2				BARI-3				BARI SP-4			
Plant growth regulator	Frequency (%)	Colour	Nature	Degree of growth	Frequency	Colour	Nature	Degree of Growth	Frequency	Colour	Nature	Degree of Growth	Frequency	Colour	Nature	Degree of Growth
BAP (mg/l)																
1.0	65.99	DGr	Com	+	60.10	DGr	Com	+	57.86	DGr	Com	+	62.75	DGr	Com	+
2.0	72.10	DGr	Com	+	63.94	DGr	Com	+	58.98	DGr	Com	+	68.89	DGr	Com	+
3.0	82.97	DGr	Com	++	74.85	DGr	Com	++	71.66	DGr	Com	++	75.98	DGr	Com	++
4.0	89.95	DGr	Com	+++	81.90	DGr	Com	+++	77.87	DGr	Com	+++	79.92	DGr	Com	+++
4.5	86.91	DGr	Com	+++	83.89	DGr	Com	+++	80.98	DGr	Com	+++	84.96	DGr	Com	+++
5.0	74.85	DGr	Com	++++	67.75	DGr	Com	+++	65.58	DGr	Com	+++	70.99	DGr	Com	+++
5.5	67.65	DGr	Com	++++	59.79	DGr	Com	++	57.85	DGr	Com	+++	63.88	DGr	Com	++++
6.0	60.25	DGr	Com	++	55.33	DGr	Com	++	53.99	DGr	Com	++	57.65	DGr	Com	++
NAA(mg/l)																
2.0	54.45	Gr	Fr	+	50.59	Gr	Fr	+	46.89	Gr	Fr	+	50.10	Gr	Fr	+
3.0	60.10	Gr	Fr	++	52.79	Gr	Fr	++	50.00	Gr	Fr	++	55.70	Gr	Fr	++
4.0	66.09	Gr	Fr	+++	60.75	Gr	Fr	+++	59.70	Gr	Fr	+++	40.45	Gr	Fr	+++
5.0	71.85	Gr	Fr	++++	65.50	Gr	Fr	+++	62.99	Gr	Fr	++++	66.07	Gr	Fr	++++
6.0	66.79	Gr	Fr	+++	61.89	Gr	Fr	++	58.90	Gr	Fr	++	65.90	Gr	Fr	++
7.0	57.90	Gr	Fr	++	48.98	Gr	Fr	++	46.89	Gr	Fr	+	50.10	Gr	Fr	++
BAP+NAA(mg/l)																
2.0+0.1	70.20	LGr	Fr	++	62.98	LGr	Fr	+	60.55	LGr	Fr	+	65.95	LGr	Fr	++
3.0+0.5	74.98	Cr	Fr	+++	64.35	LGr	Fr	++	61.89	LGr	Fr	++	70.89	Cr	Fr	+++
4.0+0.5	95.09	Cr	Nd	++++	86.86	Cr	Nd	++++	85.97	Cr	Nd	++++	90.75	Cr	Nd	+++
4.5+0.5	81.10	Cr	Nd	++++	76.12	Cr	Nd	+++	72.90	Cr	Fr	+++	76.85	Cr	Nd	++++
5.0+0.5	75.79	Cr	Nd	++++	70.20	Cr	Fr	+++	66.96	Cr	Fr	++	72.92	Cr	Nd	++++
6.0+0.5	62.08	LGr	Fr	+++	55.95	LGr	Fr	++	52.98	LGr	Fr	+	60.00	LGr	Fr	++

DGr = Dark green
LGr = Light green
Gr = Greenish
Cr = Creamy
CrW = Creamy white

Com = Compact
Sp = Spongy
Fr = Friable
Lc = Loosely compact
Nd = Not detected

+ = Poor growth
++ = Moderate growth
+++ = Good growth
++++ = Massive growth

Table 4.12. Effect of various concentration of 2.4-D and TDZ singly and 2,4-D along with NAA in MS medium for callus induction (%) along with its morphological nature induced from root of *in vitro* grown plants of four sweetpotato cultivars. Data were recorded 21 days after inoculation.

	BARI-1				BARI-2				BARI-3				BARI SP-4			
Plant growth regulator	Frequency (%)	Colour	Nature	Degree of growth	Frequency	Colour	Nature	Degree of Growth	Frequency	Colour	Nature	Degree of Growth	Frequency	Colour	Nature	Degree of Growth
2, 4-D (mg/l)																
0.5	50.78	CrW	Fr	+	46.80	CrW	Fr	+	44.80	CrW	Fr	+	50.30	CrW	Fr	+
1.0	69.98	Cr	Fr	++	64.55	Cr	Fr	++	61.86	Cr	Fr	++	67.82	Cr	Fr	++
2.0	81.97	Cr	Fr	++	72.70	Cr	Fr	++	70.37	Cr	Fr	++	78.93	Cr	Fr	++
2.5	89.65	Cr	Fr	++++	84.95	Cr	Fr	++++	81.90	Cr	Fr	++++	87.85	Cr	Fr	++++
3.0	74.94	Cr	Fr	++++	65.67	Cr	Fr	++++	61.46	Cr	Fr	++++	69.90	Cr	Fr	++++
3.5	62.78	Cr	Fr	+++	58.82	Cr	Fr	++	53.56	Cr	Com	+++	60.83	Cr	Fr	+++
4.0	54.95	Cr	Com	++	48.70	Cr	Com	++	45.90	Cr	Com	++	51.98	Cr	Com	++
5.0	50.25	CrW	Com	+	45.20	CrW	Com	+	42.71	CrW	Com	+	46.80	CrW	Com	++
2, 4-D+NAA(mg/l)																
0.5+0.1	45.67	Cr	Fr	+	40.60	Cr	Fr	+	39.10	Cr	Fr	+	42.73	Cr	Fr	+
1.0+0.5	61.89	Cr	Fr	++	56.05	Cr	Fr	++	51.69	Cr	Fr	++	60.10	Cr	Fr	++
2.0+0.5	69.56	Cr	Fr	++	35.95	Cr	Fr	++++	60.93	Cr	Fr	+++	67.33	Cr	Fr	+++
2.5+0.5	58.78	Cr	Fr	+++	51.86	Cr	Fr	+++	50.73	Cr	Fr	+++	54.97	Cr	Fr	+++
3.0+0.5	44.90	Cr	Fr	+	40.83	Cr	Fr	+	40.82	Cr	Fr	+	41.61	Cr	Fr	+
TDZ(mg/l)																
0.5	64.77	LGR	Fr	++	60.32	LGr	Fr	++	57.90	LGr	Fr	++	62.83	LGr	Fr	+
1.0	79.56	Cr	Fr	++++	63.92	Cr	Fr	+++	61.99	Cr	Fr	+++	67.90	Cr	Fr	++
2.0	85.87	Cr	Fr	++++	81.99	Cr	Fr	+++	80.90	Cr	Fr	++++	84.97	Cr	Fr	++++
3.0	71.69	Cr	Fr	+++	64.85	Cr	Fr	++	62.80	Cr	Fr	++	68.89	Cr	Fr	+++
5.0	54.40	LGr	Fr	+	45.90	LGr	Fr	+	45.30	LGr	Fr	+	50.90	LGr	Fr	+

DGr = Dark green
LGr = Light green
Gr = Greenish
Cr = Creamy
CrW = Creamy white

Com = Compact
Sp = Spongy
Fr = Friable
Lc = Loosely compact

+ = Poor growth
++ = Moderate growth
+++ = Good growth
++++ = Massive growth

Table 4.13. Effect of various concentrations of BAP and NAA and their combinations in MS medium for callus induction (%) along with its morphological nature induced from root of *in vitro* grown plants of four sweetpotato cultivars. Data were recorded 21 days after inoculation.

	BARI-1				BARI-2				BARI-3				BARI SP-4			
Plant growth regulator	Frequency (%)	Colour	Nature	Degree of growth	Frequency	Colour	Nature	Degree of Growth	Frequency	Colour	Nature	Degree of Growth	Frequency	Colour	Nature	Degree of Growth
BAP (mg/l)																
1.0	70.50	Cr	Lc	+	64.70	Cr	Lc	++	61.91	Cr	Lc	+	41.65	Cr	Lc	++
2.0	76.95	Cr	Lc	++	71.87	Cr	Lc	++	68.89	Cr	Lc	++	46.90	Cr	Lc	++
3.0	86.97	Cr	Fr	+++	80.15	Cr	Lc	+++	75.45	Cr	Fr	+++	66.80	Cr	Lc	+++
4.0	79.96	Cr	Fr	++++	75.99	Cr	Lc	++++	72.90	Cr	Fr	++++	74.96	Cr	Lc	++++
4.5	71.80	LGr	Fr	++++	67.87	Cr	Lc	++++	64.95	Cr	Fr	++++	83.89	Cr	Lc	++++
5.0	59.85	LGr	Lc	+++	54.23	Cr	Lc	+++	49.77	Cr	Fr	+++	77.00	Cr	Lc	+++
5.5	55.92	LGr	Lc	+++	49.68	Cr	Lc	++	47.85	Cr	Lc	+++	69.75	Cr	Lc	+++
6.0	50.00	LGr	Lc	++	44.88	Cr	Lc	++	41.87	Cr	Lc	++	56.59	Cr	Lc	++
NAA(mg/l)																
2.0	54.25	CrW	Lc	+	52.90	CrW	Lc	+	49.67	CrW	Lc	+	53.83	CrW	Lc	+
3.0	69.98	CrW	Lc	++	57.57	CrW	Lc	++	54.93	CrW	Lc	++	58.60	CrW	Lc	++
4.0	64.59	CrW	Lc	+++	64.59	CrW	Lc	+++	60.12	CrW	Lc	++++	67.86	CrW	Fr	+++
5.0	71.80	CrW	Lc	++++	54.90	CrW	Lc	++++	52.86	CrW	Lc	+++	59.89	CrW	Lc	++++
6.0	66.90	CrW	Lc	++	50.48	CrW	Lc	++	46.78	CrW	Lc	++	51.80	CrW	Lc	+++
7.0	54.92	CrW	Lc	+	47.96	CrW	Lc	+	42.92	CrW	Lc	+	44.98	CrW	Lc	+
BAP+NAA(mg/l)																
2.0+0.1	65.85	Gr	Fr	++	60.10	Gr	Fr	+	56.89	Gr	Fr	+	62.79	Gr	Fr	++
3.0+0.5	69.75	Gr	Fr	+++	66.15	Gr	Fr	++	59.85	Gr	Fr	++	68.88	Gr	Fr	+++
4.0+0.5	64.70	Gr	Fr	++++	59.80	Gr	Fr	++++	54.75	Gr	Fr	++++	61.79	Gr	Fr	+++
4.5+0.5	58.98	Gr	Fr	+++	53.83	Gr	Fr	+++	51.16	Gr	Fr	+++	54.97	Gr	Fr	++++
5.0+0.5	49.82	Gr	Fr	++	46.87	Gr	Fr	+++	45.85	Gr	Fr	++	50.00	Gr	Fr	++++
6.0+0.5	47.99	Gr	Fr	++	44.17	Gr	Fr	++	40.33	Gr	Fr	+	44.65	Gr	Fr	++

DGr = Dark green
LGr = Light green
Gr = Greenish
Cr = Creamy
CrW = Creamy white

Com = Compact
Sp = Spongy
Fr = Friable
Lc = Loosely compact

+ = Poor growth
++ = Moderate growth
+++ = Good growth
++++ = Massive growth

Table 4.14. Effect of various concentration of 2.4-D and TDZ and 2,4-D in combinations with NAA in MS for callus induction (%) along with its morphological nature induced from leaf disc of *in vitro* grown plants of four sweetpotato cultivars. Data were recorded 21 days after inoculation.

	BARI-1				BARI-2				BARI-3				BARI SP-4			
Plant growth regulator	Frequency (%)	Colour	Nature	Degree of growth	Frequency	Colour	Nature	Degree of Growth	Frequency	Colour	Nature	Degree of Growth	Frequency	Colour	Nature	Degree of Growth
2, 4-D (mg/l)																
0.5	49.85	CrW	Sp	+	44.90	CrW	Sp	+	39.63	CrW	Sp	+	47.80	CrW	Sp	+
1.0	77.90	CrW	Sp	++	60.87	CrW	Sp	+	57.76	CrW	Sp	+	65.20	CrW	Sp	+
2.0	75.10	CrW	Fr	++	69.85	CrW	Sp	++	65.97	CrW	Fr	++	71.99	CrW	Fr	++
2.5	77.56	CrW	Fr	+++	72.88	CrW	Fr	+++	70.05	CrW	Fr	+++	74.89	CrW	Fr	+++
3.0	71.85	CrW	Fr	+++	67.87	CrW	Fr	+++	63.80	CrW	Fr	+++	69.85	CrW	Fr	+++
3.5	66.87	CrW	Fr	++	63.99	CrW	Fr	++	60.05	CrW	Sp	++	61.95	CrW	Sp	+++
4.0	59.85	CrW	Sp	+	55.75	CrW	Sp	+	51.65	CrW	Sp	++	68.00	CrW	Sp	+
5.0	55.75	CrW	Sp	+	51.76	CrW	Sp	+	47.16	CrW	Sp	+	52.78	CrW	Sp	+
2, 4-D+NAA(mg/l)																
0.5+0.1	51.58	Cr	Fr	+	45.50	Cr	Sp	+	41.70	CrW	Sp	+	47.15	CrW	Sp	+
1.0+0.5	61.85	Gr	Fr	+	56.59	Gr	Fr	+	51.90	Gr	Fr	+	59.75	Gr	Fr	+
2.0+0.5	79.95	Gr	Fr	+++	71.05	Gr	Fr	+++	64.15	Gr	Fr	+++	76.95	Gr	Fr	+++
2.5+0.5	74.96	Gr	Fr	+++	67.69	Gr	Fr	+++	64.90	Gr	Fr	++	70.85	Gr	Fr	+++
3.0+0.5	71.99	Gr	Fr	+	60.50	Gr	Fr	+	52.05	Gr	Fr	+	69.01	Gr	Fr	+
TDZ(mg/l)																
0.5	59.84	Cr	Fr	+	64.70	LGr	Fr	+	60.98	LGr	Fr	++	65.98	Cr	Fr	+
1.0	74.89	Cr	Fr	++	70.98	Cr	Fr	++	67.76	Cr	Fr	+++	71.63	Cr	Nd	++
2.0	88.99	Cr	Fr	++++	82.98	Cr	Fr	++++	79.82	Cr	Fr	+++	84.98	Cr	Nd	+++
3.0	79.75	Cr	Fr	+++	76.08	Cr	Fr	++	71.72	Cr	Fr	++	77.85	Cr	Nd	++
5.0	54.50	LGr	Fr	+	50.10	LGr	Fr	+	47.89	LGr	Fr	+	52.75	LGr	Fr	+

DGr = Dark green
LGr = Light green
Gr = Greenish
Cr = Creamy
CrW = Creamy white

Com = Compact
Sp = Spongy
Fr = Friable
Lc = Loosely compact
Nd = Not detected

+ = Poor growth
++ = Moderate growth
+++ = Good growth
++++ = Massive growth

Table 4.15. Effect of various concentrations of BAP and NAA and their combinations in MS medium for callus induction (%) along with its morphological nature induced from internodes of *in vitro* grown plants of four sweetpotato cultivars. Data were recorded 21 days after inoculation.

BAP (mg/l)																
1.0	71.83	CrW	Sp	++	66.89	CrW	Sp	++	62.99	CrW	Sp	++	68.90	CrW	Sp	++
2.0	81.02	CrW	Fr	+++	74.89	CrW	Fr	+++	70.88	CrW	Fr	+++	76.95	CrW	Fr	+++
3.0	85.10	Cr	Fr	++++	80.25	Cr	Fr	++++	77.66	CrW	Fr	++++	81.89	CrW	Fr	++++
4.0	73.99	Cr	Fr	++++	66.89	Cr	Fr	++++	64.56	Cr	Fr	++++	69.75	CrW	Fr	++++
4.5	68.30	Cr	Fr	++++	62.50	Cr	Fr	+++	59.98	Cr	Fr	++	64.60	Cr	Fr	++
5.0	60.44	Cr	Fr	++	55.87	Cr	Fr	++	54.79	Cr	Fr	++	57.86	Cr	Fr	++
5.5	56.87	Cr	Fr	++	52.80	Cr	Fr	+	50.10	Cr	Fr	++	54.98	Cr	Fr	+
6.0	55.10	Cr	Fr	+	48.75	Cr	Fr	+	44.72	Cr	Fr	+	50.90	Cr	Fr	+
NAA(mg/l)																
2.0	60.10	LGr	Sp	++	54.95	LGr	Sp	++	50.05	LGr	Sp	++	57.90	LGr	Sp	++
3.0	64.98	LGr	Fr	++	59.71	LGr	Sp	++	56.12	LGr	Sp	++	62.00	LGr	Fr	+++
4.0	70.05	LGr	Fr	+++	61.85	LGr	Fr	+++	59.78	LGr	Fr	+++	66.98	LGr	Fr	+++
5.0	64.00	LGr	Fr	+++	56.83	LGr	Fr	+++	53.90	LGr	Fr	+++	69.87	LGr	Fr	++
6.0	48.98	LGr	Fr	++	44.85	LGr	Fr	++	41.99	LGr	Fr	++	45.50	LGr	Fr	++
7.0	39.90	LGr	Sp	+	38.60	LGr	Sp	+	34.85	LGr	Fr	+	40.00	LGr	Fr	+
BAP+NAA(mg/l)																
2.0+0.1	69.86	LGr	Fr	+	66.17	LGr	Fr	+	63.86	LGr	Fr	+	67.50	LGr	Fr	+
3.0+0.5	77.90	LGr	Fr	+++	70.80	LGr	Fr	++	67.78	LGr	Fr	++	74.88	Cr	Fr	++
4.0+0.5	81.75	Cr	Fr	+++	76.99	Cr	Fr	+++	74.12	Cr	Fr	+++	81.05	Cr	Fr	+++
4.5+0.5	72.79	Cr	Fr	++	67.95	Cr	Fr	++	63.15	Cr	Fr	++	69.88	Cr	Fr	+++
5.0+0.5	64.95	LGr	Fr	++	60.00	Cr	Fr	++	55.95	Cr	Fr	++	62.70	Cr	Fr	++
6.0+0.5	60.00	LGr	Fr	+	53.45	LGr	Fr	+	50.00	LGr	Fr	+	57.87	LGr	Fr	+

DGr = Dark green
LGr = Light green
Gr = Greenish
Cr = Creamy
CrW = Creamy white

Com = Compact
Sp = Spongy
Fr = Friable
Lc = Loosely compact

+ = Poor growth
++ = Moderate growth
+++ = Good growth
++++ = Massive growth

4.2.4. Plant Regeneration

After formation of callus form different explants (internode, root and leaf disc) those were sub cultured in fresh medium after 18-24 days interval. Watery, spongy, very compact, brown or dead calli were discarded regularly during every subculture. Friable, nodular callus or callus having green roundish meristemoid was considered as putative embryogenic calli. These were selected for furthur evaluation of regeneration potentiality. Medium supplemented with different combinations and concentrations of Kin+2,4-D, Kin+NAA, BAP+2,4-D, BAP+NAA were tested for plant regeneration. Beside this different doses of zeatin were also tested as sole agent. The results were shown in Table- 4.16 - 4.18 and Plate- 4.3 - 4.5 and are described in the following sections.

4.2.4.1 Plant regeneration from internode derived callus

The highest (62.56%) calli found to differentiate into shoots and roots in the medium containing 0.5 mg/l BAP+2.0 mg/l 2,4-D, followed by (57.88%) in 0.1 mg/l BAP+1.0 mg/l NAA, and (51.98%) in 0.1 mg/l Kin+0.5 mg/l 2,4-D in cv. BARI-1,. Whereas, only (14.70%) callus regenerated shoots in 0.1 mg/l BAP+0.5 mg/l 2,4-D supplemented medium. The highest mean number of shoots (6.7±0.10) was obtained in medium containing 0.5 mg/l BAP+2.0 mg/l 2,4-D, and lowest mean number of shoots per culture was (2.8±0.33) recorded in medium containing 0.5 mg/l Kin+1.0 mg/l NAA.

In cv. BARI-2, highest (56.65%) calli regenerated into shoot when they were cultured in medium supplemented with 0.5 mg/l BAP+2.0 mg/l 2,4-D. Whereas, only (13.75%) callus regenerated into shoots in 0.1 mg/l BAP+0.1 mg/l NAA supplemented medium. The highest mean number of shoots was (6.2±0.850) in medium containing 0.5 mg/l BAP+2.0 mg/l 2,4-D. The lowest mean number of shoot per culture was (2.4±0.25) in media having 0.5 mg/l KIN+1.0 mg/l NAA.

In cv. BARI-3, highest (60.12%) calli produced multiple shoot and root in the medium having 0.5 mg/l BAP+2.0 mg/l 2,4-D, followed by (55.85%) in 0.1 mg/l BAP+1.0 mg/l NAA, and (50.10%) in 0.1 mg/l BAP+2.0 mg/l 2,4-D. Whereas, only (14.87%) callus regenerated into shoots in 0.1 mg/l BAP+0.5 mg/l 2,4-D supplemented medium. The highest mean number of shoots (6.4±0.57) was recorded in the medium containing 0.5 mg/l BAP+2.0 mg/l 2,4-D and lowest mean number of shoots per culture was (2.5±0.57) in media having 0.5 mg/l Kin+1.0 mg/l NAA.

In cv. BARI-4, highest (55.50%) calli regenerated into shoots and roots in the medium having 0.5 mg/l BAP+2.0 mg/l 2,4-D, followed by (50.10%) in 0.1 mg/l BAP+1.0 mg/l NAA, and (44.78%) in 0.1 mg/l BAP+2.0 mg/l 2,4-D. Whereas only (10.93%)callus regenerated into shoots in 0.1 mg/l BAP+0.5 mg/l 2,4-D containing medium. The highest mean number of shoots was (6.1±0.45) in the medium containing 0.5 mg/l BAP+2.0 mg/l 2,4-D, and lowest mean number of shoots per culture was (2.1±0.40) in media supplemented with 0.1 mg/l Kin+0.1 mg/l 2,4-D.

In conclusion, 0.5 mg/l BAP+2.0 mg/l 2,4-D is best for regeneration from internode derived callus among the treatments applied. BARI-1 found most responsive genotype for regeneration medium followed by BARI-3, BARI-2 and BARI-4.

4.2.4.2 Plant regeneration from root derived callus

Callus derived from root segment was also tested in the same media for shoot regeneration as those used for internode derived calli. In cv. BARI-1 maximum (60.10%) root derived calli regenerated into shoots when they were cultured on the medium supplemented with 0.5 mg/l BAP+2.0mg/l 2,4-D, followed by 51.25% and 49.85% in medium having 0.1mg/l BAP + 1.0mg/l NAA and 0.1 BAP+2.0mg/l 2,4-D, respectively. Where as only 14.85% shoot regeneration was observed when cultured on 0.1 mg/l BAP+0.5mg/l 2,4-D. Highest mean number of shoots (6.2±0.34) was obtained in 0.5mg/l BAP+2.0mg/l 2,4-D

followed by 5.8±0.50 and 5.8±0.48 in medium supplemented with 0.1 BAP+2.0mg/l 2,4-D and 0.1 mg/l BAP + 1.0mg/l NAA, respectively. Lowest mean number of shoots was recorded in 0.5 mg/l Kin+2.0mg/l 2,4-D.

For BARI-2 (55.87%) callus developed shoot from callus when they were cultured on 0.5mg/l BAP+2.0mg/l 2,4-D containing medium followed by 45.80% in 2.0mg/l zeatin. Minimum number of shoot regeneration (15.00%) occurred from the callus in 0.1mg/l BAP+0.1mg/l NAA containing medium. Highest mean number of shoots (5.6±0.51) was recorded in 0.5 mg/l BAP+2.0mg/l 2,4-D followed by 5.4±0.35 in medium supplemented with 0.1 BAP+2.0mg/l 2,4-D; while lowest mean number (2.3±0.75) of shoots was noted in 0.5mg/l Kin+2.0 mg/l 2,4-D.

In case of cv. BARI-3, (57.97%) shoots regenerated from the callus in 0.5mg/l BAP+2.0 mg/l 2,4-D containing medium higher to 50.15% in 0.1mg/l Kin+0.5 mg/l 2,4-D. Whereas, only 13.72% shoot regeneration obtained from the callus when they cultured on 0.1 mg/l BAP and 0.5 mg/l 2,4-D containing medium.

In respect of highest mean number of shoot (6.1±0.27), 0.5mg/l BAP+2.0 mg/l 2,4-D also found best combination. Lowest number of shoot produced in 0.5 mg/l Kin+ 2.0mgl 2,4-D. In case of cultivar BARI-4, similar results were noted as in BARI-3, here highest regeneration frequency was 53.76% and lowest was 9.55% in medium having 0.5 mg/l BAP + 2.0 mg/l 2,4-D and 0.5 Kin +1.0 mg/l NAA respectively. Highest number of shoots was 5.5±0.45 in medium containing 0.5mg/l BAP+2.0 mg/l 2,4-D.

In conclusion, root-derive callus respond almost similarly as shown in internode derived callus. Distinct variation for regeneration ability was observed among the cultivars. Here BARI-1 also ranks first to regenerate followed by BARI-3, and BARI-4 had lowest frequency and as well as shoot number.

4.2.4.3 Plant regeneration from leaf disc derived callus

In cultivar BARI-1, highest (58.95%) callus developed shoot and root in the medium having 2.0mg/l zeatin, followed by 54.15% in 0.5 mg/l BAP+2.0mg/l 2,4-D. Where as lowest response (14.00%) was recorded in media having 0.1mg/l BAP+0.1mg/l NAA. Highest mean number of shoots (6.5±0.45) obtained in 2.0mg/l zeatin and lowest number of shoots (3.3±0.15) was obtained in 0.01 mg/l Kin+0.1 mg/l NAA. In case of cultivar BARI-2, same results were concluded as in BARI-1. Here also 2.0mg/l zeatin gave highest response frequency (52.58%) as well as highest mean number of shoots (5.8±0.75). Considering response frequency and mean shoot number medium with 0.5 mg/l BAP+2.0mg/l 2,4-D scored second position. On the other hand, lowest frequency (11.12%) was observed in the medium supplemented with 0.5 mg/Kin+1.0 mg/l NAA. Lowest mean number (3.2±0.17) of shoots was obtained in medium supplemented with 0.01mg/l Kin+ 0.1mg/l NAA.

In cultivar BARI-3 and BARI-4, medium having 2.0mg/l zeatin gave highest regeneration frequency 54.70% and 51.00% respectively from leaf disc derived calli. Highest mean number of shoots was also obtained in this formulation, 6.3±1.10 in BARI-3 and 5.7±0.35 in BARI-4. Lowest response was noted in medium with 0.01mg/l Kin+0.1 mg/l NAA. Application of 0.5mg/l BAP+2.0 mg/l 2, 4-D also was found satisfactory for regeneration.

Form above discussion about plant regeneration efficiency on callus from the studied three explants of four cultivars, it reveals that internode was found best explant followed by roots and leaves. In comparing between roots and leaves, the performance of root was near to internodes. Regarding the effective media composition variation was observed among the explants used. Although for both internode and root, 0.5mg/l BAP+2.0mg/l 2,4-D was effective but for leaves it was 2.0mg/l zeatin. Among the four cultivars BARI-1 was found most effective for *in vitro* response others, were found moderate in response. Finally the regenerated plants were acclimatized successfully in soil condition. In these cases, 78% plants were survived after 3 weeks of hardening.

Table 4.16. Effect of different concentrations and combinations of 2,4-D + KIN, 2,4-D + BAP, BAP+NAA and KIN+NAA in MS medium on shoot regeneration from internode derived callus of four sweetpotato cultivars. Data were recorded after 42 days of inoculation for regeneration.

Growth regulator	Morphogenic Response	Cultivars BARI-1	BARI-2	BARI-3	BARI SP-4
KIN+2,4-D					
0.1+0.1	No. of Calli Regenerated Shoot (%)	44.75	37.98	41.80	35.50
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	3.4±0.60	2.8±0.45	3.0±0.25	2.1±0.40
0.1+0.5	No. of Calli Regenerated Shoot (%)	51.98	44.75	47.99	42.86
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.6±0.56	3.8±0.35	4.2±0.55	4.2±0.70
0.2+0.5	No. of Calli Regenerated Shoot (%)	40.00	35.50	37.96	31.80
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	5.4±0.25	4.3±0.25	5.1±0.83	4.1±0.29
0.5+1.0	No. of Calli Regenerated Shoot (%)	30.55	25.78	27.70	23.85
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	3.8±0.90	3.5±0.42	3.5±0.70	3.2±0.72
0.5+2.0	No. of Calli Regenerated Shoot (%)	25.89	19.79	24.80	18.99
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	3.3±0.59	2.7±0.55	3.0±0.65	2.5±0.43
BAP+2,4-D					
0.1+0.5	No. of Calli Regenerated Shoot (%)	14.70	13.75	14.87	10.93
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.7±0.86	4.6±0.10	4.6±0.45	4.2±0.25
0.1+1.0	No. of Calli Regenerated Shoot (%)	40.00	35.30	37.87	33.25
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	5.3±0.50	4.8±0.40	5.2±0.20	4.6±0.10
0.1+2.0	No. of Calli Regenerated Shoot (%)	51.75	46.85	50.10	44.78
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	6.5±0.67	5.6±0.10	6.0±0.17	5.6±0.38
0.5+2.0	No. of Calli Regenerated Shoot (%)	62.56	56.65	60.12	55.50
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	6.7±0.10	6.2±0.85	6.4±0.57	6.1±0.45
0.5+2.5	No. of Calli Regenerated Shoot (%)	44.92	39.70	42.90	37.95
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	6.0±0.35	5.5±0.96	5.8±0.50	5.4±0.10
0.5+3.0	No. of Calli Regenerated Shoot (%)	28.80	25.77	27.70	23.80
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.6±0.51	3.8±0.62	4.2±0.04	3.7±0.15
BAP+NAA					
0.1+0.1	No. of Calli Regenerated Shoot (%)	18.02	13.70	16.18	12.33
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	3.5±0.28	3.1±0.12	3.5±0.50	2.8±0.20
0.1+0.5	No. of Calli Regenerated Shoot (%)	34.85	30.15	31.89	26.95
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.8±0.12	4.4±0.15	4.5±0.58	4.1±0.89
0.1+1.0	No. of Calli Regenerated Shoot (%)	57.88	52.70	55.85	50.10
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	6.2±0.12	5.6±0.12	5.7±0.35	5.2±0.20
0.2+1.0	No. of Calli Regenerated Shoot (%)	40.35	36.08	37.87	33.70
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	5.6±0.17	5.4±0.18	5.5±0.32	5.1±0.50
0.5+1.0	No. of Calli Regenerated Shoot (%)	27.60	23.65	24.89	21.85
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.3±0.90	3.7±0.12	3.8±0.36	3.1±0.34
KIN+NAA					
0.01+0.1	No. of Calli Regenerated Shoot (%)	35.50	30.65	32.50	27.90
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.4±0.15	3.7±0.25	4.1±0.35	3.6±0.52
0.1+0.5	No. of Calli Regenerated Shoot (%)	49.08	40.96	44.87	37.98
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	5.1±0.37	4.6±0.16	4.8±0.25	4.1±0.43
0.1+1.0	No. of Calli Regenerated Shoot (%)	28.15	25.98	26.89	23.85
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.0±0.12	3.4±0.36	3.7±0.15	3.2±0.33
0.5+1.0	No. of Calli Regenerated Shoot (%)	17.89	14.65	15.95	13.86
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	2.8±0.33	2.4±0.25	2.5±0.57	2.3±0.0
Zeatin					
0.5	No. of Calli Regenerated Shoot (%)	25.69	22.87	23.50	15.10
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	3.6±0.55	3.2±0.40	3.5±0.57	3.0±0.50
1.0	No. of Calli Regenerated Shoot (%)	35.00	29.56	32.98	27.78
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.8±0.56	4.6±0.50	4.7±0.15	4.2±0.50
2.0	No. of Calli Regenerated Shoot (%)	51.10	45.10	46.89	43.89
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	5.8±0.25	5.4±0.30	5.6±0.22	4.8±0.15
2.5	No. of Calli Regenerated Shoot (%)	41.85	36.78	38.00	34.88
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	5.6±0.10	5.2±0.50	5.4±0.12	4.8±0.20
3.0	No. of Calli Regenerated Shoot (%)	30.15	27.85	28.90	2.86
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.7±0.120	3.6±0.15	3.7±0.33	3.±0.15

Table 4.17. Effect of different concentrations and combinations of 2,4-D + KIN, 2,4-D +BAP, BAP+NAA and KIN+NAA in MS medium on shoot regeneration from root segment derived callus of four sweetpotato cultivars. Data were recorded after 42 days of inoculation.

Growth regulator	Morphogenic Response	Cultivars			
		BARI-1	BARI-2	BARI-3	BARI SP-4
KIN+2,4-D					
0.1+0.1	No. of Calli Regenerated Shoot (%)	44.50	41.78	37.65	29.98
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	3.1±0.20	2.6±0.45	2.8±0.40	2.5±0.56
0.1+0.5	No. of Calli Regenerated Shoot (%)	50.00	41.98	50.15	37.12
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.5±0.50	3.9±0.20	4.2±0.50	3.8±0.20
0.2+0.5	No. of Calli Regenerated Shoot (%)	40.12	25.86	33.87	30.10
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	5.2±0.16	4.6±0.50	4.9±0.80	4.6±0.14
0.5+1.0	No. of Calli Regenerated Shoot (%)	32.20	22.92	28.57	20.35
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	3.9±0.20	3.3±0.25	3.6±0.10	3.1±0.50
0.5+2.0	No. of Calli Regenerated Shoot (%)	26.09	15.45	20.75	13.85
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	2.8±0.29	2.3±0.75	2.5±0.40	2.2±0.10
BAP+2,4-D					
0.1+0.5	No. of Calli Regenerated Shoot (%)	14.85	12.80	13.72	11.00
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.6±0.20	3.8±0.33	4.2±0.20	3.5±0.30
0.1+1.0	No. of Calli Regenerated Shoot (%)	39.16	32.20	35.15	30.21
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	5.1±0.20	4.6±0.46	4.9±0.20	4.4±0.40
0.1+2.0	No. of Calli Regenerated Shoot (%)	49.87	45.98	47.55	42.50
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	5.8±0.50	5.4±0.35	5.7±0.50	5.4±0.45
0.5+2.0	No. of Calli Regenerated Shoot (%)	59.10	55.87	57.97	53.76
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	6.2±0.34	5.6±0.51	6.1±0.10	5.5±0.45
0.5+2.5	No. of Calli Regenerated Shoot (%)	40.15	36.65	38.59	31.75
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	5.8±0.25	5.4±0.62	5.6±0.50	5.3±0.20
0.5+3.0	No. of Calli Regenerated Shoot (%)	25.18	21.57	23.97	20.00
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.2±0.16	3.8±0.35	4.0±0.81	3.7±0.35
BAP+NAA					
0.1+0.1	No. of Calli Regenerated Shoot (%)	15.00	12.98	14.51	11.08
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	3.5±0.10	3.1±0.25	3.3±0.35	2.9±0.59
0.1+0.5	No. of Calli Regenerated Shoot (%)	25.98	21.50	23.69	18.88
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.9±0.15	4.5±0.27	4.6±0.36	4.3±0.45
0.1+1.0	No. of Calli Regenerated Shoot (%)	51.25	45.35	47.18	42.30
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	5.8±0.48	5.4±0.30	5.6±0.15	5.1±0.42
0.2+1.0	No. of Calli Regenerated Shoot (%)	38.80	32.90	36.00	31.10
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	5.4±0.43	5.1±0.51	5.3±0.51	4.9±0.35
0.5+1.0	No. of Calli Regenerated Shoot (%)	28.17	23.68	25.76	21.58
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	3.7±0.25	32±0.36	3.4±0.5	31±0.25
KIN+NAA					
0.01+0.1	No. of Calli Regenerated Shoot (%)	39.00	33.15	28.93	39.12
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.4±0.20	4.3±0.25	4.2±0.35	4.1±0.60
0.1+0.5	No. of Calli Regenerated Shoot (%)	51.10	46.10	46.46	41.15
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.7±0.41	4.5±0.50	4.6±0.45	4.2±0.40
0.1+1.0	No. of Calli Regenerated Shoot (%)	25.12	18.99	16.95	16.00
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.0±0.42	3.2±0.30	3.7±0.41	3.1±0.25
0.5+1.0	No. of Calli Regenerated Shoot (%)	25.00	22.10	12.98	9.55
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	3.2±0.24	2.8±0.25	3.1±0.34	2.7±0.54
Zeatin					
0.5	No. of Calli Regenerated Shoot (%)	24.55	22.58	23.10	20.14
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	3.6±0.40	3.3±0.65	3.4±0.10	3.0±0.00
1.0	No. of Calli Regenerated Shoot (%)	32.00	28.13	31.21	25.98
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.5±0.44	4.3±0.49	4.4±0.29	4.2±0.30
2.0	No. of Calli Regenerated Shoot (%)	45.80	43.16	46.25	40.18
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	5.8±0.16	5.2±0.47	5.4±0.65	5.1±0.12
2.5	No. of Calli Regenerated Shoot (%)	40.12	36.21	37.09	33.15
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	5.2±0.54	5.0±0.64	5.1±0.47	4.6±0.60
3.0	No. of Calli Regenerated Shoot (%)	28.15	15.25	19.00	21.45
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.3±0.25	4.1±0.21	4.1±0.55	3.9±0.50

Table 4.18. Effect of different concentrations and combinations of 2,4-D + KIN, 2,4-D +BAP, BAP+NAA and KIN+NAA in MS medium on shoot regeneration from leaf disc derived callus of four sweetpotato cultivars. Data were recorded after 42 days of inoculation.

Growth regulator	Morphogenic Response	Cultivars			
		BARI-1	BARI-2	BARI-3	BARI SP-4
KIN+2,4-D					
0.1+0.1	No. of Calli Regenerated Shoot (%)	40.81	37.15	38.78	36.12
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.9±0.10	4.4±0.25	4.6±0.50	4.2±0.18
0.1+0.5	No. of Calli Regenerated Shoot (%)	48.12	43.16	45.98	41.85
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	5.9±0.25	5.5±0.49	5.7±0.47	5.4±0.41
0.2+0.5	No. of Calli Regenerated Shoot (%)	36.05	30.98	33.10	27.99
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.2±0.25	3.8±0.38	4.0±0.29	3.5±0.43
0.5+1.0	No. of Calli Regenerated Shoot (%)	20.15	23.87	26.10	23.00
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	3.9±0.17	3.6±0.24	3.8±0.46	3.3±0.26
0.5+2.0	No. of Calli Regenerated Shoot (%)	24.10	20.00	21.20	18.89
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	3.8±0.30	3.5±0.15	3.7±0.15	3.3±0.37
BAP+2,4-D					
0.1+0.5	No. of Calli Regenerated Shoot (%)	14.12	11.04	12.35	10.06
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.4±0.20	4.1±0.30	4.2±0.50	3.9±0.43
0.1+1.0	No. of Calli Regenerated Shoot (%)	27.12	29.10	25.21	22.96
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.9±0.67	4.4±0.30	4.7±0.39	4.2±0.40
0.1+2.0	No. of Calli Regenerated Shoot (%)	41.21	46.16	38.65	35.73
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	5.7±0.31	5.5±0.48	5.9±0.40	5.4±0.40
0.5+2.0	No. of Calli Regenerated Shoot (%)	54.15	51.18	53.00	49.16
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	6.5±0.70	6.0±0.34	6.1±0.18	5.7±0.17
0.5+2.5	No. of Calli Regenerated Shoot (%)	40.10	36.85	39.18	36.14
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	5.7±0.37	5.4±0.35	5.5±0.10	5.4±0.09
0.5+3.0	No. of Calli Regenerated Shoot (%)	25.17	21.09	23.98	20.12
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.1±0.40	3.7±0.35	3.9±0.37	3.7±0.75
BAP+NAA					
0.1+0.1	No. of Calli Regenerated Shoot (%)	14.00	11.25	13.08	10.00
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	3.9±0.17	3.6±0.15	3.7±0.21	3.7±0.25
0.1+0.5	No. of Calli Regenerated Shoot (%)	27.10	23.15	25.12	20.15
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.6±0.43	3.9±0.75	4.3±0.12	3.9±0.37
0.1+1.0	No. of Calli Regenerated Shoot (%)	51.21	45.50	47.77	42.25
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	5.9±0.65	5.6±0.67	5.7±0.75	5.4±0.51
0.2+1.0	No. of Calli Regenerated Shoot (%)	39.15	33.85	36.14	31.19
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	5.4±0.18	5.1±0.48	5.2±0.10	4.9±0.50
0.5+1.0	No. of Calli Regenerated Shoot (%)	29.06	25.12	26.80	22.99
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	3.9±0.39	3.5±0.34	3.7±0.35	3.4±0.26
KIN+NAA					
0.01+0.1	No. of Calli Regenerated Shoot (%)	30.12	25.06	27.15	23.79
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	3.3±0.15	3.2±0.17	3.1±0.67	2.9±0.67
0.1+0.5	No. of Calli Regenerated Shoot (%)	43.15	38.82	40.75	35.59
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	5.4±1.08	5.1±0.95	5.3±0.55	5.1±1.10
0.1+1.0	No. of Calli Regenerated Shoot (%)	24.86	21.91	23.76	20.87
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.3±0.02	3.9±0.85	4.2±0.77	3.8±0.70
0.5+1.0	No. of Calli Regenerated Shoot (%)	14.95	11.12	13.19	10.55
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	3.6±0.57	3.3±0.56	3.5±0.21	3.2±0.28
Zeatin					
0.5	No. of Calli Regenerated Shoot (%)	31.95	29.25	30.15	28.31
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	3.8±0.25	3.4±0.53	3.6±0.47	3.3±0.32
1.0	No. of Calli Regenerated Shoot (%)	49.12	45.15	47.10	42.12
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	5.4 ±0.75	5.2±0.35	5.3±0.45	5.1±0.37
2.0	No. of Calli Regenerated Shoot (%)	58.95	52.85	54.70	51.00
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	6.5±0.45	5.8±0.75	6.3±1.10	5.7±0.35
2.5	No. of Calli Regenerated Shoot (%)	47.25	43.75	45755	42.89
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	5.9±0.45	5.5±0.41	5.6±0.41	5.3±0.31
3.0	No. of Calli Regenerated Shoot (%)	35.20	31.15	34.50	30.00
	Mean No. of Shoots Per Callus ($\bar{x}$ ±SE)	4.9±0.65	4.7±0.52	4.8±0.15	4.4±0.25

PLATE-4.3

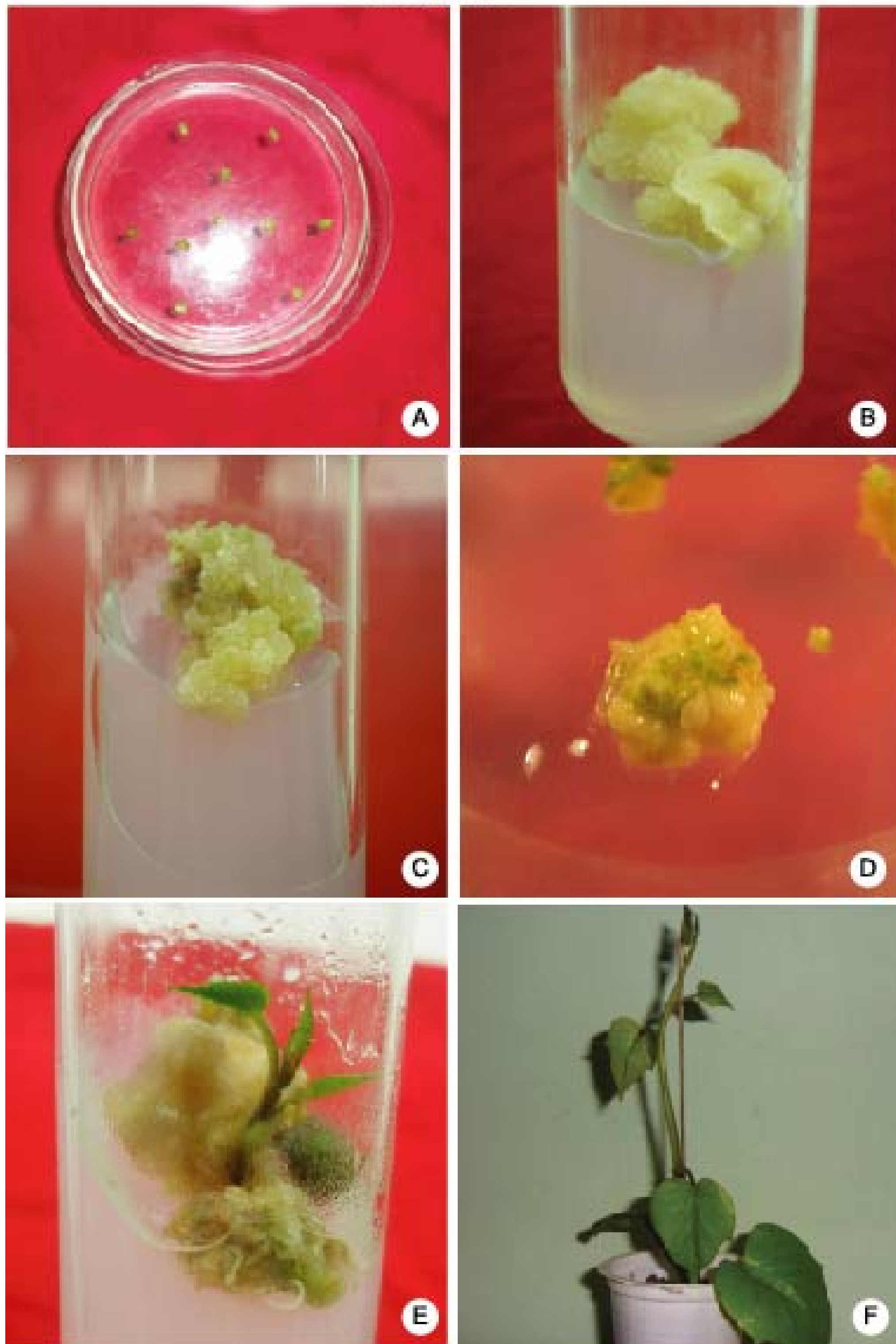


PLATE- 4.3 Callus induction and plant regeneration from internode

Fig. A: Callus initiation from internode explants after 10 days of culture.
Fig. B: Proliferation of callus from (after 21 days of inoculation).
Fig. C: Formation of embryogenic callus.
Fig. D: Appearance of green spots on the callus showing shoots initiation.
Fig. E: Complete plant regeneration from callus
Fig. F: Establishment of regenerated plants in the soil

PLATE-4.4

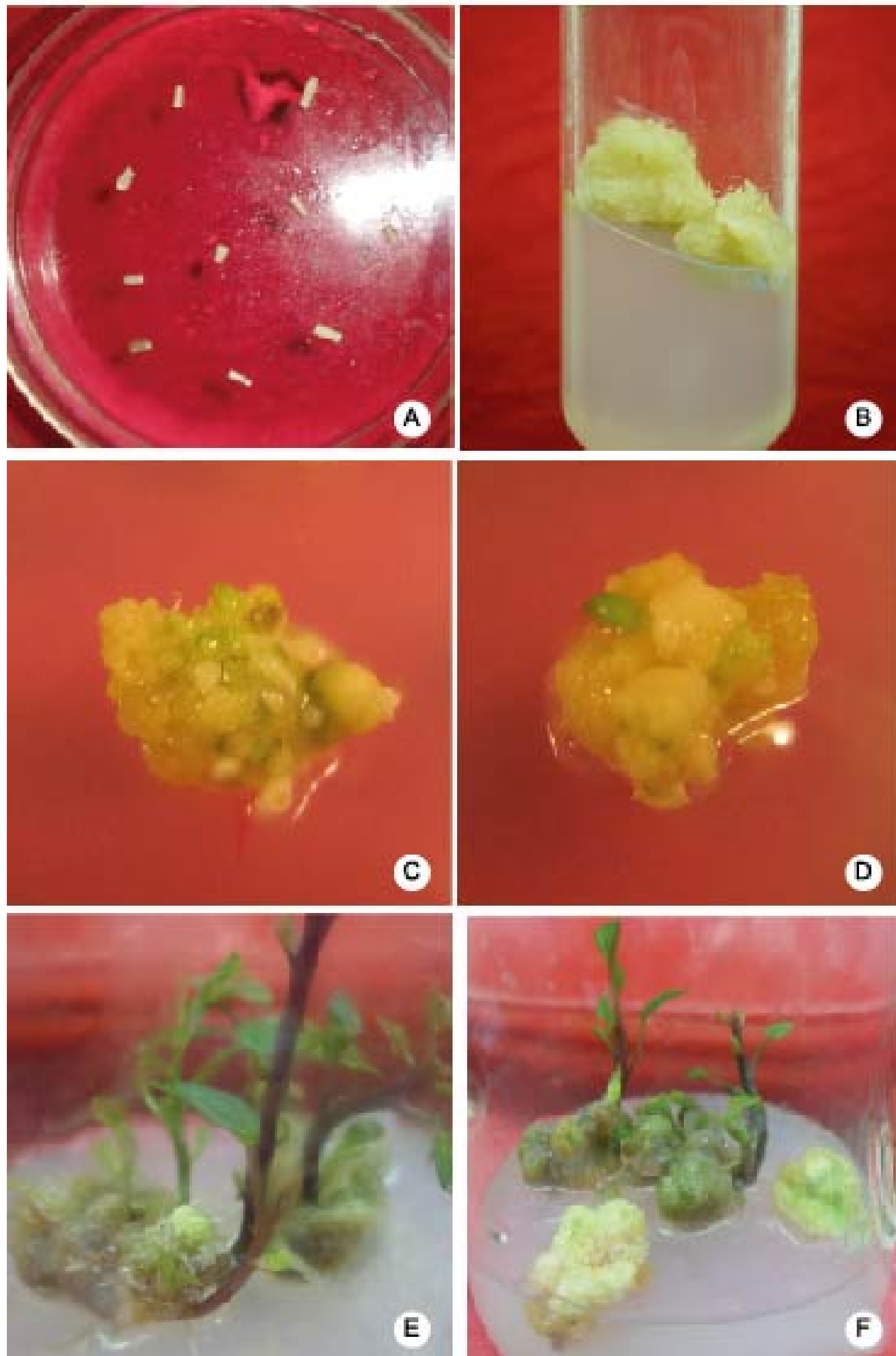


PLATE- 4.4 Callus induction and plant regeneration from root

Fig. A: Callus initiation from in vitro grown root explants.
Fig. B: Callus proliferation in the subcultures.
Fig. C and D: Embryogenic calli showing green spots, the sign of plant regeneration
Fig. E: Plant regeneration from callus.
Fig. F: Regenerated plantlets with well developed roots

PLATE-4.5

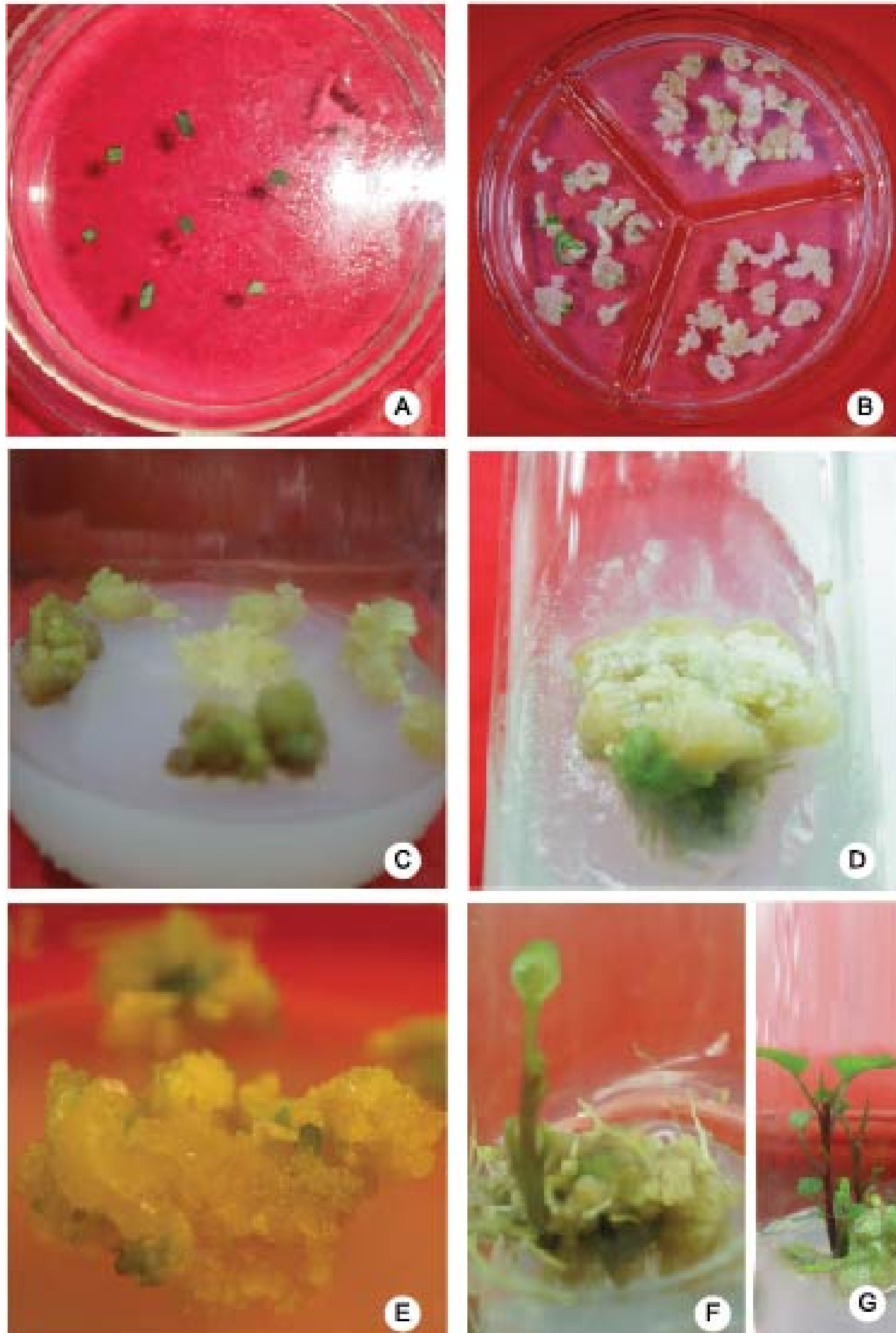


PLATE- 4.5 Callus induction and plant regeneration from leaf disc.

Fig. A: Callus initiation from leaf explants.

Fig. B: Proliferating callus from explants (after 15 days of inoculation).

Fig. C: Growth of callus.

Fig. D and E: Embryogenic cell formation.

Fig. F and G: Shoot and root regeneration from embroyid.

4.2.4.4 Evaluation of regenerated plants for possible somaclonal variation

Acclimatized plants were grown for 4-5 months for full vegetative growth and cuttings were prepared for planting in the field. Four randomly selected plants from each cultivar were chosen for field evaluation. They were named as T_1-T_4, K_1-K_4, D_1-D_4, and BS_1-BS_4 in cultivar BARI-1, BARI-2, BARI-3 and BARI Sweet Potato-4, respectively. Several morphological parameters were observed for possible arising of somaclonal variation including length of main vine, number of vines, internodes length, leaf areas, and number of tubers per plant, length of tuber per plant, average diameters of tuber, fresh weight of vines/plant, fresh weight of tubers/plant. The data were averaged from three replications and presented in the table 4.19-4.22.on plate 4.6

Although most of the somaclones were very similar compared the mother plants, considerable variation was noticed among the regenerated plants compared to controls. Among various somaclones of BARI-1, T_2 plants had the smallest vine and lowest tuber yield (Table 4.19). By contrast, T_3 plants exhibited the longest vine length and tuber yield, which is also higher than control plants.

In BARI-2, most of the somaclones showed higher vine length and tuber weight compared to mother plants (Table 4.19). The differences were statistically significant. The differences in other traits were unclear, however. Among the BARI-3 somaclones, most of the somaclones were very similar to the controls. However, D_3 line had the significantly smaller vine lenth and the lowest tuber yield. Among the BARI sweet potato-4 somaclones, BS_3 line produced the highest tuber yield, while BS_2 produced the least tuber weight. Both were statistically different compared to the control plants.

In conclusion, among the tested regenerated plants some somaclones showed better performance, some were very similar to the mother plants while others are inferior. It is important to continue the field trial to observe if the changes are revertable of not.

Table 4.19: Field evaluation of plants regenerated from callus for possible somaclonal variation. Four randomly selected regenerated plants (cultiver BARI-1) were planted in the field. Morphological characters were compard to those of convantional vine cutting control plants. Data were recorded on 100 days after planting and harvest (140 days after planting).

plant ID	Length of main vine (cm)	Numbers of vines / plant	Vine internodes length/ plant(cm)	Leaf areas/plant (cm^{-2})	Number of tubers/ plant (cm)	Length of tuber/plant (cm)	Average diameters of tuber	Fresh weight of vines/plant (gm)	Fresh weight of tubers/ plant (gm)
T_1	132.2 a	18.18 bc	3.60 b c	64.27 bc	5.10 a	12.47 a	4.40 bc	740.2 0 c	550.10 b
T_2	95.22 d	17.14 c	3.66 bc	56.34 c	3 .00 b	11.00 b	4.00 c	690.20 c	480.10 bc
T_3	135 .00 a	23.10 a	4.40 a	72.63 ab	6 .00 a	12.90 a	5.42 a	1050.00 a	811.90 a
T_4	112 .00 c	12.12 d	3.4 0 c	58.30 bc	3.23 b	12.10 ab	3.83 c	650.20 c	420.2 0c
control	122.10 b	21.25 ab	4 .00 ab	80.17 a	5.03 a	13.20 a	4.60 b	920.30 b	730.40 a
LSD at 5% level	5.35	3.26	0.51	14.91	1.28	1.28	0.58	121	124.70
CV	2.38%	9.43%	7.12%	11.94%	15.31%	5.52%	7.00%	7.93%	11.06%

4.20: Field evaluation of plants regenerated from callus for possible somaclonal variation. Four randomly selected regenerated plants (cultiver BARI-2) were planted in the field. Morphological characters were compard to those of convantional vine cutting control plants. Data were recorded on100 days after planting and harvest (140 days after planting).

Plant ID	Length of main vine (cm)	Numbers of vines / plant	Vine internodes length / plant(cm)	Leaf areas/plant(cm2)	Number of tubers / plant(cm)	Length of tuber/plant (cm)	Average diameters of tuber	Fresh weight of vines /plant(gm)	Fresh weight of tubers / plant(gm)
K_1	125.20 b	12.56 b	5.60 ab	80.34 bc	3.3 0bc	11.04 b	4 ab	521.37 ab	450.06c
K_2	145.10 a	14.15 a	6.13 a	94.4 0 a	3.80 a	12.03 a	4.33 a	580.17 a	540.11a
K_3	115.1 0c	12.00 b	5.21 bc	78.14 bc	3.2 0 bc	10.80 b	4 ab	440.21 c	430.17bc
K_4	128.10 b	13.14 ab	5.21 bc	82.22 b	3.60 ab	11.22 b	4.4 a	493.53 bc	480.08b
control	102.18d	12.93 ab	4.8 bc	73.07 c	3.10 c	11.05 b	3.66 b	503.39 abc	460.22bc
LSD 5%	6.10	1.21	0.73	9.24	0.46	0.56	0.52	75.20	65.89
CV	1.81%	4.97 %	4.94 %	4.14%	4.97 %	1.82 %	4.69 %	5.41%	8.58%

For tables means in a coloums with common letter (s) are not significantly different at the 5% level.

Table 4.21: Field evaluation of plants regenerated from callus for possible somaclonal variation. Four randomly selected regenerated plants (cultiver BARI-3) were planted in the field. Morphological characters were compard to those of convantional vine cutting control plants. Data were recorded on 100 days after planting and harvest (140 days after planting).

Plant ID	Length of main vine (cm)	Numbers of vines / plant	Vine internodes length / plant(cm)	Leaf areas/ plant(cm2)	No. of tubers/ plant	Length of tuber/plant(cm)	Average diameters of tuber	Fresh weight of vines /plant(gm)	Fresh weight of tubers / plant(gm)
D_1	78.23 a	12.27 a	2.00 b	50.60 ab	5.00 a	10.10 b	2.69 b	510.40 a	365.1 a
D_2	68.13 bc	11.83 ab	1.50 c	49.03 b	3.66 c	8.93 c	2.46 b	413.40 b	280.7 b
D_3	65.17 c	10.89 b	1.00 d	36.73 c	3.03 d	7.800 d	2.30 b	231.90 c	183.6 c
D_4	71.35 b	12.57 a	1.80 bc	49.55 ab	4.20 bc	10.43 b	2.30 b	380.40 b	270.6 b
control	80.42 a	12.33 a	2.36 a	56.17 a	4.40 ab	11.40 a	3.50 a	520.20 a	360.2 a
LSD	5.59	0.97	0.30	7.11	0.60	0.37	0.57	50.85	41.55
CV	4.09%	4.34%	10.74%	7.81 %	7.93%	2.03%	11.41%	6.57%	7.56%

Table 4.22: Field evaluation of plants regenerated from callus for possible somaclonal variation. Four randomly selected regenerated plants (cultiver BARI Sweet potatp-4) were planted in the field. Morphological characters were compard to those of convantional vine cutting control plants. Data were recorded on 100 days after planting and harvest (120 days after planting).

plant IDS	Length of main vine (cm)	Numbers of vines / plant	Vine internodes length / plant(cm)	Leaf areas/plant (cm2)	Number of tubers / plant (cm)	Length of tuber /plant (cm)	Average diameters of tuber	Fresh weight of vines /plant(gm)	Fresh weight of tubers / plant(gm)
BSP-4_1	113.33 c	16.03 c	4.00 c	63.43 b	4.61 b	11.10 bc	4.76 a	610 b	530.22 b
BSP-4_2	117.81 b	15.213 d	3.60 d	52.816 d	3.50 d	10.80 d	3.74 de	418.76 de	396.66 d
BSP-4_3	125.50 a	19.53 a	4.50 ab	65.39 a	5.20 a	11.14 b	4.63 ab	716.66 a	640.00 a
BSP-4_4	112.03 bc	14.59 e	3.95 dc	49.83 e	3.20 de	9.83 e	4.00 c	430.33 d	383.40 de
control	121.33 a	17.41 b	4.60 a	57.58 c	4.00 c	12.19 a	3.84 d	520.06 c	450.06 c
LSD	4.92	3.10	0.32	6.21	0.48	0.34	65.25	76.68	56.55
CV	2.22 %	9.96%	4.16%	5.71%	6.19%	3.22%	4.37%	6.43%	8.48%

For tables means in a coloums with common letter (s) are not significantly different at the 5% level.

PLATE-4.6

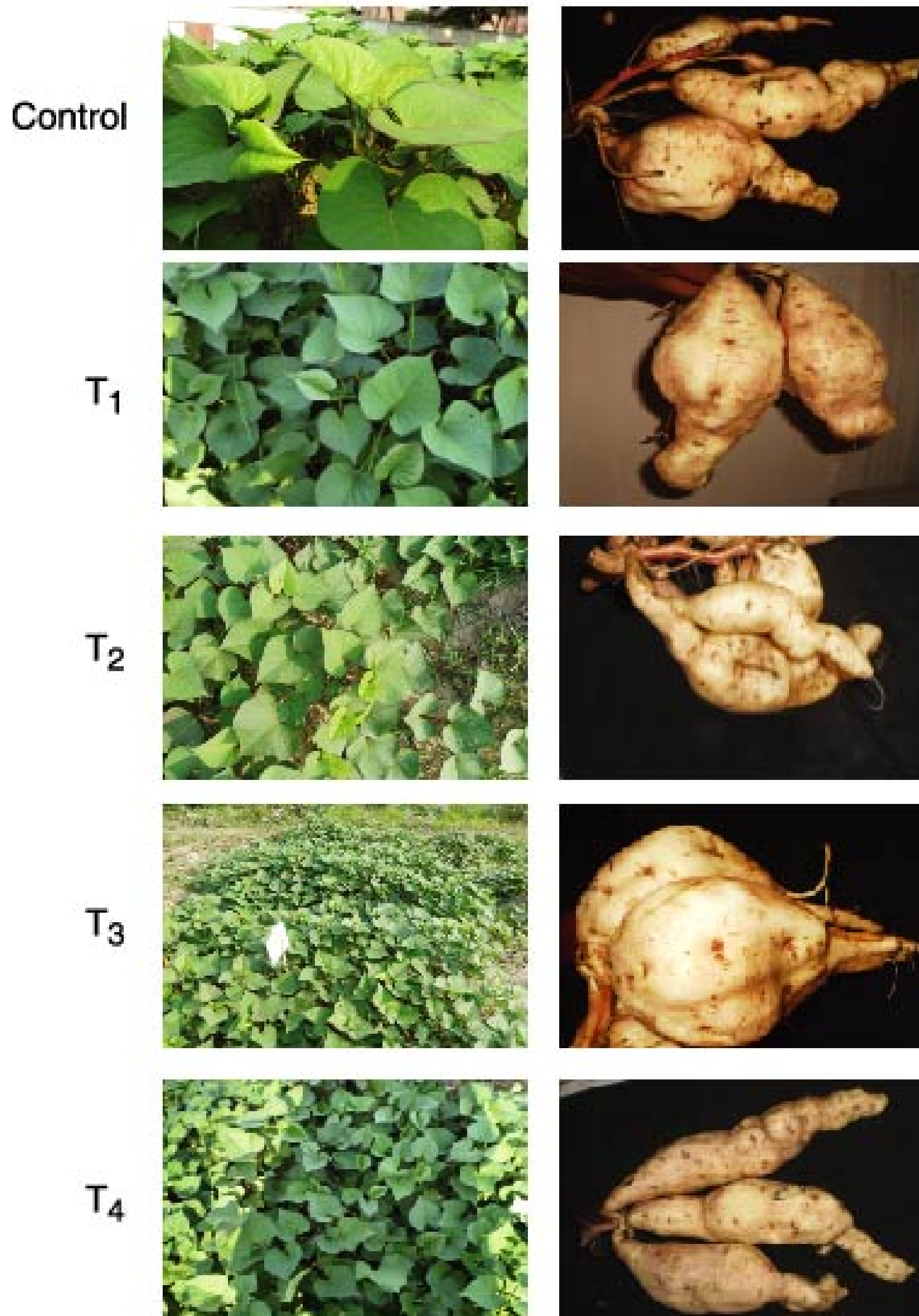


PLATE- 4.6 Showing somaclonal variation for leaves and tuber over different treatments.
Fig. Control: Leaf characterstics and tuber size in control plant.
Fig. (T1-T4):Leaf characterstics and tuber size in four types of somaclonales of BARI- 1

Part -2. Agronomy

Experiment: 1

4.3. Effect of Nitrogen Fertilizer on Yield Related Characters

Sustainable and profitable commercial sweetpotato production requires proper management of nitrogen fertilizer. Inadequate or excessive amounts of nitrogen fertilizer can be detrimental to sweettato crop and can negatively affect on yield potential. In addition, excessive or improperly timed nitrogen fertilizer can result in added expense, leaching and contamination of surface and ground water. Informed decisions that consider crop history and soil type are important factors to consider in determining the proper amount of nitrogen fertilizer that should be applied in sweetpotato production.

4.3.1. Length of main vine

A wide variation was observed in length of main vine of all the treatments and varieties (Table 4.23. Longer vine is associated with the increased internodes length which is about 50% longer in treatment groups. Result indicated that increasing trend of length of main vine by increasing of nitrogen fertilizer up to certain stage. It was found that the highest nitrogen fertilizer was 160kg/ha and lowest was zero fertilizer in the both case. Highest length of main vine in mericlone and conventional was gradually 192.23cm and 170.49cm for cultivar BARI-2 and shortest vine was obtained 110.26cm and 87.2 cm for cultivar BARI-3 respectively. Mericlone was the best performance between mericlone originated and traditional planting materials.

4.3.2. Number of vines per plant

From the analysis of variance it also observed that the effect of nitrogen fertilizer and varietals items were significant (Table-4.23). Excellent growth and improvement indicates of sweet potato with growing trend of number of vines per plant, and it is openly inter related to the yield. The more branches have more leaves .The highest value of number of vain was obtained for nitrogen fertilizer 160kg/ha and lowest was zero nitrogen for both mericlone and conventional plant. Among the varieties BARI-3 gradually produced the highest

number of vine (27.56 and 23.12) and BARI-2 produced the lowest (19.33 and 15.99). Between the varieties mericlone was good.

4.3.3. Vine inter-node length

Result showed small different trend to vine inter node length with increasing of nitrogen fertilizer doses (Table-4.23). Effects of the nitrogen fertilizer and varietals items treatment on vine inter-node length were significantly different to each other, which was increasing by nitrogen fertilizer doses (Table-4.22). The highest treatment was found 160kg/ha and lowest was zero nitrogen for both mericlone and conventional plant. The highest values (6.49 cm and 6.23 cm) were found in BARI-2 and lowest (3.53 cm and 2.87 cm) were found in BARI-3 variety for mericlone and conventional respectively.

4.3.4. Leaf area

It was observed from the analysis of variance that the nitrogen fertilizer and varietal items were also significant (Table-4.23). Excellent growth and maturity of sweet potato is related to increasing trend of photosynthesis, and it is directly related to the yield. The highest leaf area mean was obtained in nitrogen fertilizer 160kg/ha and lowest mean was found from control of fertilizer in both mericlone and conventional respectively. Among the varieties, the best result was found BARI-2 (112.83cm^2 and, 104.97 cm^2) and lowest was found BARI-4, (50.32 cm^2 and 47.36 cm^2) and similar to result of BARI-3 respectively.

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4.3.5. Fresh weight of vines per plant

Nitrogen fertilizer significantly influences vegetative growth traits in both mericlone originated and traditional planting materials (Table 4.24). Fresh weight of the vine was increased with increasing nitrogen fertilizer and peaked at 160 kg/ha. Significant effects of varieties were also found on Fresh weight of vines per plant. Among all the varieties, BARI-1 showed better performance (810.52gm and 743.71gm) and BARI-3 showed comparatively lower (534.55gm and 486.66gm) than others in mericlone and conventional respectively. It was also observed that, mericlone line showed highest result than conventional.

4.3.6. Fresh weight of tuberous rootsous roots per plant

Different nitrogen levels had significant effect on fresh weight of tuberous roots per plant (Table 4.24). Results indicated that increasing trend of fresh weight of tuberous roots per plant by higher level of nitrogen until a certain stage (160kg/h). The high values of fresh weight of tuberous roots per plant were recorded with 662gm and 558.8gm, where lowest values with 456.6gm and 396.3gm in both cases mericolon and conventional line. It is also observed that varietal effects on fresh weight of tuber per plant were significant from each other. Variety BARI-1 showed highest result and BARI-3 showed lowest in this case.

4.3.7. Number of tuberous roots per plant

Result showed that the increasing trend of number of tuberous roots r per plant with increasing the nitrogen levels until a certain stage 160kg/h (Table-4.24). Effect of nitrogen fertilizer on number of tuberous roots per plant is statistically dissimilar to each other. The lowest number of tuber per plant was recorded at zero treatment. Varietal effects were also significant, where variety BARI-1 showed high performance (4.82 and 4.74) and BARI-2 showed lowest performance (4.02 and 4.16) in both mericlone and conventional respectively.

4.3.8. Length of tuberous roots per plant

Result showed that significant effect of nitrogen level on length of tuber (Table-4.24). Treatment 160kg/h also showed high performance (13.73cm and 13.55cm) and zero showed lowest (10.53cm and 10.37cm) than others. Significant effect was also found among the varieties, where high value of length of tuberous roots was found in variety BARI-1 (13.39cm and 13.3cm) and lowest was found in variety BARI-2 (11.53cm and 11.60cm) for both mericlone and conventional respectively.

4.3.9. Average diameter of tuberous roots

Diameter of tuberous roots was increased by different doses of nitrogen fertilizer (Table- 4.24). Nitrogen application positively influenced tuber diameter. The highest nitrogen fertilizer value of diameter of tuberous roots was found for

160kg/ha and lowest value was found for zero fertilizer treatment. Significant effect was observed for the varietals items. The maximum average diameter of tuberous roots in both mericlone and conventional gradually 5.09cm and 4.77cm for cultivar BARI-2 and minimum average diameter of tuberous roots 3.65cm and 3.48cm in cultivar BARI-3.

4.3.10. Weight of tuberous roots per plot and yield of tuberous roots per hectare

Yield of tuberous roots per hectare was calculated on the basis of weight of tuberous roots per plot. The data on weight of tuberous roots per plot have been presented in Tables- (4.24).

Result indicated that increasing trend of the yield by increasing of nitrogen fertilizer up to certain stage. It was observed that the highest yield of tuberous roots (36.98 t/ha) and (31.68t/ha) was found in the treatment of 160 kg N/ha and lowest (22.25 t/ha) and (19.85t/ha) was zero fertilizer in the both case.

The highest yield (35.99 t/ha) and (31.04t/ha) was found in the variety BARI-1 which was statistically similar with variety BARI-2 (35.34 t/ha) and (31.04t/ha) for both mericlone and conventional respectively. BARI-3 showed comparatively lower yield per hectare (25.36t/ha) and (22.01t/ha) than others in mericlones and their local varieties respectively.

Table 4.23: Effects of Nitrogen fertilizers on morphological related characters of sweetpotato under field condition. Data on length of main vine, number of vines, vine internodes length/plant, and leaf areas/plant were recorded 100 days after planting.

Means for Nitrogen (N) Effect

Nitrogen kg/ha	Length of main vine (cm)		Number of vine (cm)		Vine inter node length (cm)		Leaf area (cm2)	
	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional
0	107 d	96.61 d	14.4 d	12 d	3.91 d	3.73 c	59.74 d	56.43 d
90	142 c	125.2 c	20.3 c	17 c	5.03 c	4.59 b	74.22 b	70.76 c
160	172 a	148.7 b	25.2 b	22 b	5.72 a	5.4 ab	82.4 ab	77.51 ab
190	179 b	151.6 a	26.1 a	23 a	5.64 b	5.28 a	83.58 a	78.32 a
SE(±)	1.55	0.72	1.34	0.44	0.36	0.07	1.07	0.65
LSD at 5%	15.32	11.56	5.31	5.36	1.13	1.18	9.14	12.21

Means for Varieties (V) Effect

Variety	Length of main vine (cm)		Number of vine (cm)		Vine inter node length(cm)		Leaf area(cm2)	
	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional
BARI-1	158.73 b	137.2 b	21.7 b	19.4 b	5.34 b	5.07 b	82.14 b	77.73 b
BARI-2	192.23 a	170.49 a	17.42 c	15.57 cd	6.49 a	6.23 a	112.83 a	104.97 a
BARI-3	101.26 d	87.2 d	27.56 a	23.12 a	3.53 d	2.87 d	54.65 c	52.96 c
BARI-4	147.55 c	127.25 c	19.33 d	15.99 c	4.95 c	4.83 c	50.32 d	47.36 d
SE(±)	1.02	2.35	0.37	0.56	0.03	0.055	1.06	0.67
LSD at 5%	13.12	16.45	2.07	0.52	1.50	2.13	3.05	3.06

For tables means in a coloums with common letter (s) are not significantly different at the 5% level.

Table- 4.24: Effects of Nitrogen fertilizers on yield and yield contributing characters of sweetpotato under field condition. Data on number of tubers/plant, length of tuberous roots/plant, average diameters of tuber, fresh weight of vines/plant, and fresh weight of tubers/plant were recorded on harvest time.

Means for Nitrogen (N) Effect

Nitrogen kg/ha	Fresh weights of vines per plant (gm)		Fresh weights of tuberous roots per plant (gm)		Numbers of tuberous roots per plant		Length of tuberous roots (cm)		Average diameters of tuberous roots (cm)		Weight of tuberous roots / plot(kg)		Yield(ton/ha)	
	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional l	Mericlone	Conventional	Mericlone	Conventional
0	482.8 d	460.5 c	414.6 d	357.4 d	4d	3.86 d	10.53 d	10.37 d	3.54 d	3.43 d	3.56 d	3.21 d	22.25 d	19.85 d
90	646.5 c	593.63 b	576.2 c	506.4 c	4.58 c	4.59 b	12.03 c	11.58 c	4.47 c	4.09 c	5.18 b	4.55 c	32.01 c	28.13 c
160	765.45 a	666.41 a	665.8 a	570.4 a	5.24 a	5.02 a	13.73 a	13.55 a	5.15 a	4.8 a	4 c	5.13 a	36.98 a	31.68 a
190	693.85 b	616.93 ab	594.8 b	519.2 b	4.66 b	4.16 b	12.37 b	12.28 b	4.56 b	4.25 b	5.35 a	4.67 b	33.04 b	28.84 b
SE(±)	2.58	1.82	3.86	1.02	0.90	0.46	0.78	0.44	0.75	0.64	0.92	0.77	0.85	0.56
LSD at 5%	20.17	16..20	13.10	19.12	0..34	0..26	2.53	1.16	1.12	0.75	0.50	1..35	11.30	10.78

Means for Varieties (V) Effect

Variety	Fresh weights of vines per plant (gm)		Fresh weights of tuberous roots per plant (gm)		Numbers of tuberous roots per plant		Length of tuberous roots r (cm)		Average diameters of tuberous roots (cm)		Weight of tuberous roots / plot(kg)		Yield(ton/ha)	
	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventionall	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional
aBARI-1	810.52 a	743.71 a	662 a	558.8 a	4.82 a	4.74 b	13.39 a	13.3 a	4.85 b	4.44 b	5.82 a	5.02 a	35.99 a	31.04 a
BARI-2	705.23 b	638.83 b	636.2 b	558 b	4.02 d	4.16 d	11.53 d	11.6 b	5.09 a	4.77 a	5.72 b	5.02 a	35.34 b	31.04 a
BARI-3	534.55 d	486.66 c	456.6 d	396.3 d	4.42 c	4.41 c	11.79 c	11.43 bc	3.65 d	3.48d	4.07 d	3.56 c	25.36 c	22.01 c
BARI-4	552.97 c	468.26 d	496.5 c	433 c	4.55 b	5.16 a	11.95 b	11.44 bc	4.16 c	3.87 c	4.47 c	3.95 b	27.59 b	24.41 b
SE(±)	3.33	1.96	1.32	2.84	0.32	0.24	1.51	0.45	0.83	0.70	1.11	0.85	0.72	1.24
LSD at 5%	12.34	14.12	15.12	12.33	18.33	0.17	0.13	0.39	0.65	0.35	0.40	0.35	2.50	2.45

For tables means in a coloums with common letter (s) are not significantly different at the 5% level.

PLATE-4.7

PLATE- 4.7 Effects of Nitrogen in field level

Fig. A: Overall field condition after 120 days of plantation.

Fig. B: BARI-1 *sweet potato* plants after 140 days of plantation with best tuberous roots.

Fig. C: BARI-3 *sweet potato* plants after 140 days of plantation with lowest tuberous roots.

Experiment: 2

4.4. Effect of Potassium and Nitrogen Fertilizer on Yield Related Characters

Potassium promotes the efficient use of nitrogen and assists in water uptake. A number of studies showed that application of potassium fertilizer significantly increase tuberous yield. High potassium maintains the general health of sweet potato plants; potassium improves nutrient value, taste, color, texture and disease resistance of crops. However, there is a variation in the potassium response across the cultivars tested. Inadequate or excessive amounts of potassium fertilizer can be detrimental to sweet-potato crop and can negatively affect on yield potential. Moreover, excessive applications result in added expense, leaching and contamination of surface and ground water. Therefore, proper amount of potassium fertilizer needs to be applied for sustainable sweetpotato production. In the present experiment, different doses of potassium (as MP) along with nitrogen fertilizers were applied to sweetpotato. Yield and yield related characteristics were assessed to determine the optimum potassium dose. The results are summarized in the Table 4.25, 4.26 and discussed under the following subheads.

4.4.1. Length of main vine

A wide variation was observed in length of main vine among all the treatments and varieties (Table 4.25). Longer vine is associated with the increased internodes length which is about 50% longer in treatment groups. Result indicated that increasing trend of length of main vine was greater in highest combination. Highest length of main vine in mericlone and traditional was 201.11cm and 152.60cm respectively in cultivar BARI-2 and shortest vine was obtained as 112.32cm and 87.04cm for cultivar BARI-3 respectively. Mericlone showed better performance than traditional planting materials.

4.4.2 .Number of vines per plant

From the analysis of variance it also observed that the effect of N+K and varietals items were significant (Table-4.25). Excellent growth and improvement in vine number per plant was observed in different combinaton than contol plot

which leads to better yield performance. The more branches have more leaves .The highest number of vain was obtained 160kg/ha N + 180 kg/h K and lowest was in zero tratment. Among the varieties BARI-3 gradually produced the highest number of vine (36.28 and 29.59) and BARI-2 produced the lowest (24.87 and 19.00). Mericlones showed better performence than its original varieties in all the cultivers.

4.4.3. Vine internode length

Vine internode length was significantly affected by the fertilizer combination used (Table-4.25). Varietals were also significantly different from each other for this trait. Vine inter node length was highest in the higher combnation 160kg/ha N + 180 Kg/h K iand lowest was in control plots irrespective of cultivers. The highest length (5.09cm and 4.93cm) were found in BARI-2 and lowest (3.05cm and 2.75cm) were found in BARI-3 variety for both in mericlone and traditional respectively.

4.4.4. Leaf area

IAnalysis of variance showed that fertilizer combinations and varietal items were also significant (Table-4.25) in respect of this trait. Excellent growth and maturity of sweet potato is dependent on higher photosynthetic rate and this rate is dependent on total leaf area of the plant. The highest leaf area mean was found in the highst combination 160kg/ha N+ 180 kg/h K and lowest mean was found in control in both mericlone and their traditional varities. Among the varieties, the best result was found in BARI-2 (112.36cm^2 and 107.64cm^2) and lowest was found in BARI-4, (53.35cm^2 and 51.04cm^2). Simiral result was also found in BARI-3.

4.4.5. Fresh weight of vines per plant

Use of potassium in combination with nitrogen fertilizer influences the vegetative growth traits significantly in both mericlone originated and traditional planting materials (Table 4.26). Fresh weight of the vine was increased in increased combinations and peaked at 160 kg/ha N+ 180kg/ha K. Varietis also showed signifacant differnece in respect of fresh weight of vines per plant. Among all the

varieties, BARI-1 showed better performance (1013.33gm and 877.50gm) and BARI-3 showed comparatively lower (668.16gm and 565.66gm) than others in mericlones and their local varieties respectively. It was also observed that, mericlone line showed bettet performance than others.

4.4.6. Fresh weight of tuberous roots per plant

Different combinations of nitrogen and potassium had significant effect on fresh weight of tuberous roots per plant (Table 4.26).

Results indicates that fresh weight of t tuberous roots per plant was incrased iwhen nitrogen and potassium was used in a higher dose (160 kg/ha N + 180 kg/ha k). The highest value of fresh weight of tuberous roots per plant was recorded as 832.33gm and 713.50gm, whereas lowest weight was 485.50gm and 382.66gm in mericolon and traditional line respectively. Varietal effects on fresh weight of tuberous roots per plant were also found significant from each other. Variety BARI-1 showed highest performance and BARI-3 showed lowest in this case.

4.4.7. Number of tuberous roots per plant

Result showed that number of tuberous roots per plant was increased significantly in higer combanation of fertilzer (160kg/h N+ 180 kg/h K) used (Table-4.26). A combination effect of fertilizer on number of tuberous roots per plant is statistically dissimilar to each other. The lowest number of tuberous roots per plant was recorded in the control plots. Varietal effects were also significant, where variety BARI-1 showed highest performance (4.93 and 4.78) and BARI-2 showed lowest performance (4.03 and 3.88) in both mericlone and their traditional varieties respectively.

4.4.8. Length of tuberous roots per plant

Result showed that there is a significant effect of nitrogen and potassium on length of tuberous roots (Table-4.26). Treatment 160kg/h N+ 180 kg/h K showed highest performance (15.22cm and 14.12cm) and control plots showed the lowest results (11.49cm and 10.79cm) than others. Significant effect was

also found among the varieties, where highest length of tuberous roots was found in variety BARI-1 (14.75cm and 13.78cm) and lowest was found in variety BARI-2 (12.96cm and 11.83cm) for both mericlone and their traditional varieties respectively.

4.4.9. Average diameters of tuberous roots

Diameter of tuberous roots showed significant difference in different combination of fertilizer (Table- 4.26). Combined use of N+K positively influenced tuber diameter. The highest diameter of tuberous roots was found in 160kg/ha N + 180 Kg/h K and lowest value were found in zero treatment level. Varieties also showed significant difference for this trait. The maximum average diameter of tuberous roots in both type were 5.81cm and 5.24 cm in cultivar BARI-2 and minimum average diameter of tuberous roots 4.47cm and 3.96cm in cultivar BARI-3.

4.4.10. Weight of vines per plot and yield of vines per hectare

Weight of vines per hectare was calculated on the basis of yield of vines per plot. Different combinations of nitrogen and potassium had significant effect on yield of vines per hectare (Table 4.26) and (Fig-4.3, 4.4).

Results indicates that weight of vines per hectare was incrased when nitrogen and potassium was used in a higher dose (160 kg/ha N + 180 kg/ha k). The highest value of weight of vines per hectare were recorded as 53.16t/ha and 46.31t/ha, where as lowest weight were 33.53t/ha and 27.47t/ha in mericolon and traditional line respectively. Varietal effects on weight of vines per hectare were also found significant from each other. Variety BARI-1 showed highest performance and BARI-3 showed lowest in this case.

4.4.11. Weight of tuberous roots per plot and yield of tuberous roots per hectare

Yield of tuberous roots per hectare was calculated on the basis of weight of tuberous roots per plot. The yield of tuberous roots per plot was affected significantly (at 5% level) by different levels of nitrogen and potassium (Table 4.26). The results showed that mericlone gave the highest and significantly

better amount of yield (46.24t/ha) when nitrogen and pottasium was used in a combination of 160kg N+180kg K per hectare. Although, traditional varieties were also produced, highest amount of yield (38.88t/ha) in the same combination. In control plots receiving no nitrogen and potassium produced lowest amount of yield in both the variety.

From the analysis of variance it is also observed that the varietals items are significant in weight of tuberous root. Among the varieties the highest value of fresh weight of tubers was found in variety BARI-1 mericlone (45.00t/ha) and conventional (38.18 t/,ha) which was statistically similar BARI-2 mericlone (44.18t/h) and conventional (36.99t/h) lowest in variety BARI-3 mericlone-(31.46t/ha) and conventional (26.83t/ha). It was also observed that, mericlone line showed bettet performance than others.

Table 4.25: Effects of nitrogen and potassium fertilizers on morphological related characters of sweetpotato under field condition. Data on length of main vine, number of vines, vine internodes length/plant, and leaf areas/plant were recorded (60 and 120days) after planting.

Means for Nitrogen (N) and Potassium (K) Effect

Nitrogen and Potassium kg/ha	Length of main vine (cm)		Number of vine (cm)		Vine internode length(cm)		Leaf area(cm2)	
	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional
0+0	112.95 c	85.78 d	20.30 c	14.45 c	3.39 d	3.15 c	64.73 d	56.00 d
90+117	169.80 b	129.51 c	31.31 b	25.11 b	4.33 b	4.10 b	80.83 b	73.91c
160+180	184.21 a	141.63 a	35.73 a	31.04 a	4.71 a	4.50 a	84.41 a	83.35 a
190+217	169.55 b	131.16 b	31.61 b	25.58 b	4.23 c	4.14 b	75.28 c	76.29 b
SE(±)	1.20	2.65	0.48	0.55	0.04	0.06	1.09	0.79
LSD at 5%	3.51	7.76	1.41	1.61	0.12	0.19	3.18	2.32

Means for Varieties (V) Effect

Variety	Length of main vine (cm)		Number of vine (cm)		Vine inter node length(cm)		Leaf area(cm2)	
	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional
BARI-1	165.99 b	125.77 b	30.55 b	25.12 b	4.06 c	4.01b	84.60 b	76.16 b
BARI-2	201.11 a	152.60 a	24.87 d	19.00 d	5.09 a	4.93 a	112.36 a	107.64 a
BARI-3	112.32 d	87.04 d	36.28 a	29.59 a	3.05 d	2.75 c	54.95 c	54.72 c
BARI-4	157.10 c	122.68 c	27.26 c	22.48 c	4.46 b	4.20 b	53.35 c	51.04 c
SE(±)	1.80	2.95	0.65	0.78	0.06	0.08	1.12	0.95
LSD at 5%	4.21	8.01	1.74	1.87	0.14	0.25	3.91	2.75

For tables means in a coloums with common letter (s) are not significantly different at the 5% level.

Table 4.26: Effects of Nitrogen and potassium fertilizers on yield and yield contributing characters of sweet potato under field condition. Data on number of tubers/plant, length of tuber/plant, average diameters of tuber, fresh weight of vines/plant, and fresh weight of tubers/plant were recorded on (60 and 120days) after planting.

Means for Nitrogen (N) and Potassium (K) Effect

Nitrogen and potassium kg/ha	Fresh weights of vines per plant (gm)		Fresh weights of tuberous roots per plant (gm)		Numbers of tuberous roots per plant		Length of tuberous roots (cm)		Average diameters of tuberous roots (cm)		Weights of vines per plot (kg)		Weights of vines per plot t/ha		Weight of tuberous roots / plot(kg)		Yield(ton/ha)	
	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional
0+0	603.50 d	494.50 c	485.50 d	382.66 d	3.95 d	3.81 d	11.49 d	10.79 d	4.13 d	3.69 c	5.43 c	4.45 c	33.53 d	27.47 d	4.36 d	3.45 d	26.97 d	21.31 d
90+117	847.66 c	742.50 b	720.33 c	633.33 c	4.63 b	4.40 b	13.84 c	12.51 c	5.35 b	4.76 b	7.63 bc	6.68 b	47.09 b	41.25 bc	6.48 bc	5.78 bc	40.01 c	35.18 c
160+180	956.83 a	833.66 a	832.33 a	713.50 a	5.11 a	4.83 a	15.22 a	14.12 a	6.00 a	5.46 a	8.61 a	7.5 a	53.16 a	46.31 a	7.49 a	6.42 a	46.24 a	38.88 a
190+217	867.33 b	758.50 b	743.50 b	649.62 b	4.31 c	4.36 c	14.17 b	12.81 b	5.32 b	4.83 b	7.81 b	6.83 b	48.19 c	42.14 b	6.69 b	5.84 b	41.30 b	36.09 b
SE(±)	6.57	7.08	6.54	6.56	0.05	0.05	0.09	0.07	0.05	0.04	0.06	0.99	2.44	0.56	0.98	2.03	5.62	4.23
LSD at 5%	19.19	20.67	19.10	19.17	0.15	0.15	0.28	0.20	0.17	0.13	0.18	2.67	2.78	2.33	2.12	2.35	12.10	11.32

Means for Varieties (V) Effect

Variety	Fresh weights of vines per plant (gm)		Fresh weights of tuberous root per plant (gm)		Numbers of tuberous root per plant		Length of tuber (cm)		Average diameters of tuber (cm)		Fresh weights of vines per plot (kg)		Fresh weights of vines per plot (t/ha)		Weight of tuberous root / plot(kg		Yield(ton/ha)	
	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional	Mericlone	Conventional
BARI-1	1013.33 a	877.50 a	810.0 a	687.25 a	4.93 a	4.78 a	14.75 a	13.78 a	5.59 b	5.06 b	9.12 a	7.9 a	56.3 a	48.75 a	7.29 a	6.18 a	45 a	38.18 a
BARI-2	902.50 b	770.16 b	795.33 a	679.33 a	4.03 c	3.88 c	12.96 c	11.83 c	5.81 a	5.24 a	8.12 b	6.93 b	50.14 b	42.79 ab	7.18 a	6.11 a	44.18 b	36.99 b
BARI-3	668.16 d	565.66 d	566.33 c	482.04 c	4.46 b	4.31 b	13.61 b	12.33 b	4.47 d	3.96 d	6.01 c	5.09 c	37.12 cd	31.43 cd	5.09 bc	4.34 bc	31.46 c	26.83 cd
BARI-4	691.33 c	615.83 c	610.00 b	530.50 b	4.58 b	4.43 b	13.39 b	12.28 b	4.93 c	4.48 c	6.22 c	5.54 c	38.41 c	34.21 c	5.49 b	4.85 b	33.88 d	29.47 c
SE(±)	7.02	8.04	6.87	6.65	0.15	0.10	0.25	0.12	0.25	0.18	0.06	1.30	0.10	2.98	0.05	1.20	0.09	2.65
LSD at 5%	20.07	21.75	20.55	19.94	0.45	0.35	0.43	0.33	0.38	0.45	0.50	0.64	2.89	3.79	0.40	0.51	2.44	3.32

For tables means in a coloums with common letter (s) are not significantly different at the 5% level.

PLATE-4.8

PLATE- 4.8 Effect of Nitrogen and potassium in field level.
Fig. Overall field condition after 120 days of plantation.
Fig. B: BARI-1 sweet potato plants after 140 days of plantation with best tuberous roots.
Fig. C: BARI-2 sweet potato plants after 140 days of plantation with similar tuberous roots.
Fig. D: BARI-3 sweet potato plants after 140 days of plantation with lowest tuberous roots.
Fig. E: BARI sweet potato-4 plants after 120 days of plantation with good tuberous roots.

4.5. Economic Analysis

Economic analysis was done to observe the comparative cost and benefit trend of sweet potato cultivation as influenced by meristem derived and conventional varieties plants as well as by the use of fertilizres. For this purpose, chemicals and maintainence cost in the labratory procedure for tissue culture part, net grafting cost, input cost for land preparation, vines transplanting, fertilizer, irrigation and manpower required for all the operations including harvesting of tuberous roots was recorded for per unit plot and then converted into cost per hectare. The price of the sweet potato tuberous roots was determined on market rate basis and vines were determined on farm gate rate basis. The data in this regard have been presented in Table 27.

In case of mericlone plant source of BARI -1, the highest gross margin (320290 Tk /ha) and benefit cost ratio (BCR) (2.65) was obtained from treatment 160N kg/ha + 180K kg/ha under different fertilizer doses in field condition. On the other hand, the lowest gross margin (149870 Tk /ha) and benefit cost ratio (BCR) (1.79) was found in control (Table 4.27) due to low yield production. In case of conventional plant source of BARI -1, the highest gross margin (238050 Tk /ha) and benefit cost ratio (BCR) (2.24) was obtained from Nitrogen and potassium (NK) treatment of 160N kg/ha + 180K kg/ha under different fertilizer doses in field condition. On the other hand, the lowest gross margin (54920 Tk /ha) and benefit cost ratio (BCR) (1.29) was found in control (Table 1) due to low yield production. So, treatment 160N kg/ha + 180K kg/ha is the best for high yield production of BARI -1 in both situation Mericlone and conventional. Here. Also observed that mericlone plant gave highest gross margin (320290 tk /ha) and benefit cost ratio (BCR) (2.65) as compare with gross margin (238050 Tk /ha) and benefit cost ratio (BCR) (2.24) of conventional plant source.

Table 4. 27. Economic performance of BARI SP-1 (as a Meri clone and Conventional plant source) under different fertilizer doses of Nitrogen and Potassium in field condition.

Vine source	Nitrogen + Potassium (Kg/ha)	Yield (t/ha)		Total variable cost (Tk/ha)	Gross return (Tk/ha)	Gross margin (Tk/ha)	Benefit cost ratio(BCR)
		Vine	Tuber	-	-	-	-
Mericlone	0N+0K	33.52	26.97	188200	33520+304550 =338070	149870	1.79
	90N+117K	47.09	40.01	191395	47090+400150 =447240	255845	2.33
	160N+180K	53.15	46.24	193460	53150+ 460600 =513750	320290	2.65
	190N+217K	48.18	41.30	194495	48180+460680 =508860	314365	2.61
Conventional	0N+0K	27.47	21.31	186200	27470 +213650 = 241120	54920	1.29
	90N+117K	41.24	35.18	189395	41240+352700 = 393940	204545	2.07
	160N+180K	46.31	38.88	191460	46310+383200 = 429510	238050	2.24
	190N+217K	42.13	36.09	192495	42130+360900 =403030	210535	2.09

Urea = Tk 16.00 /kg, TSP = Tk 22.00 /kg, MP = Tk 15.00 /kg, Zinc Sulphate = Tk 27.00 /kg, Gypsum = Tk 10.00 /kg, Labour = Tk 250.00/8 per/head, Irrigation = Tk 4500 /ha , = Tk 10000 /plough/ha, vine selling = Tk 1/kg, Tuber= Tk 10/kg, Total cost of meri clone + vine regenerate from tissue culture = TK 3040/ha, cost of vine buying for conventional plant = Tk 1000/ha, Pesticide= TK. 20000/ha, Land hire= TK 40000/ha/annum

Conclusion

From the above study, the results revealed that among all fertilizer doses of NK, 160N+180K treatment gave the highest gross margin and benefit cost ratio (BCR) in both situation Mericlone and conventional. On the other hand, Mericlone plant source of BARI -1 is the best for high yield production of sweet potato. So, treatment Nitrogen and potassium (NK), 160N kg/ha +180K kg/ha and Mericlone plant source of BARI -1 are economically viable for our farmers.

CHAPTER-5
DISCUSSIONS

Roots and tubers have myriad and complex parts to play in feeding the ever increasing population in the coming decades (CIP, 2000b). The sweetpotato is an important food crop that provides carbohydrate and protein to a large sector of the world population and also has potential as a biomass species for methane and ethanol production. Its nutritive value as a food crop is due to richness in calories and vitamins, as well as protein content ranging from 2% to 10% of dry matter. Sweetpotato will contribute more to the global food system as a source of starch and animal feed in Asia (CIP, 2000b). So it demands considerable attention for its improvement.

High male sterility, self- and cross-incompatibility, limited genetic base and the hexaploid nature of sweet potato have resulted in very limited improvement of this plant by classical breeding methods (Lee *et al*., 2002; Lee *et al*., 2003). Therefore, interest has been directed towards the genetic improvement of this crop through novel *in vitro* techniques, such as somaclonal variation (Lee *et al*., 2003), somatic hybridization (Sihachakr and Ducreux, 1987), cytoplasmic recombination (Sihachakr and Ducreux, 1993), and DNA transfer. Nevertheless, the application of such techniques requires the control of plant regeneration from tissue cultures (Sihachakr *et al*., 1997). Therefore, the present work describes meristem culture, callus culture and plant regeneration as a system framework for improvement of sweetpotato using four Bangladeshi popular cultivars viz. BARI-1, BARI-2, BARI-3 and BARI Sweet Patato sweet-4.The obtained results are discussed in the following paragraph with an endeavor to justify them.

5.1. Meristem Culture

5.1.1. Surface Sterilization of the shoot tips

Surface sterilization is prerequisite for initiating any *in vitro* experiment. Many workers used various sterilizing agents in order to obtain contamination free but remain healthy explants. Among these chemical treatments are especially used widely. These include: 1% solution of sodium hypochlorite, 70% ethanol, 0.2%

$HgCl_2$ solution, 1% silver nitrate solution. There are also many other reports on using $HgCl_2$ for surface sterilization of the explants (Das *et al.,* 2001).

It showed that maximum 90% contamination free culture without considerable tissue killing of the explants were obtained when the explants were treated with 0.1% $HgCl_2$ solution for 6 min. Most of the workers described a two-step process; they used 0.525% sodium hypochlorite solution for 10-20 min, prior to dipping in 70% ethanol (Dagnino *et al*., 1991; Verma *et al*., 2004). Even Verma *et al*. (2004) used a coating of Rifampicin (100mg/ml) solution on medium surface in addition to this, to prevent bacterial contamination. Elliott (1969) describes deleterious effect of sodium hypochlorite on shoot apices and used nodal segment for surface sterilization in order to obtain *in vitro* shoot tips for meristem culture. Therefore surface sterilization of shoot tips explants of field grown sweetpotato, 0.1% $HgCl_2$ solution for 6 min. was considered to be most effective and suitable for meristem culture.

5.1.2. Primary culture and establishment of apical meristem

Various growth regulators such as, Kin, BA, GA_3, Kin+NAA and Kin+GA_3 were tested in MS liquid medium for primary establishment of apical meristem form field grown plants. MS liquid medium without any growth regulators was also used as control. Many workers used liquid culture methods for growth of isolated meristem of sweetpotato and other crops (Elliott, 1969; Dodds *et al*., 1991; Alam *et al*., 2004). Among these growth regulators, 2.0 mg/l Kin +0.5 mg/l GA_3 showed the quickest responses (7–21 days) and highest (65%) growth responses were found for apical meristem. In case of growth regulator-free MS, only 10-15% isolated meristem was survived, just remains live without any considerable growth. This suggested essentiality of growth regulators for quick response and high rate establishment of inoculated meristem. This result is more or less similar to the results of others (Love *et al*., 1989; De Andrade and De Andrade, 1995). Nevertheless, use of NAA, IAA, BAP and 2,4-D has also been used Garrido and Casares, 1989; Dagnino *et al*., 1991). A considerable number of isolated meristem died even in the suitable media, probably due to dissection injury (Alam *et al*., 2010; 2012).

5.1.3. Shoot and Root Development from the Meristem

To establish plantlets from primarily established meristem, two growth regulators viz. Kin and GA_3 were used either singly or in combinations with each other in different concentrations for proper shoot and root development. Among the different combination of Kin and GA_3 either singly or in combinations, 2.5 mg/l Kin+0.5mg/l GA_3 was found effective for high number shoot formation. Single use of low concentration of Kin (2mg/l) results high number of shoot and node development and decrease at higher concentration in sweetpotato (Hossain and Sultana, 1997). Combined use of Kin and GA_3 in MS medium was reported good for plantlets development from meristem-tip culture in sweetpotato (Love *et al*., 1989; Verma *et al*., 2004; Verma *et al*., 2005). However, other workers found good results using BAP and GA_3 (Dong, 1986; De Andrade and De Andrade, 1995). Failure of growth in MS_0 proves necessity of growth regulators in this stage of development. Comparing the different varieties, primary meristem of BARI-1 was found more responsive than the others. Considering the all treatments use of 2.5 mg/l Kin+0.5mg/l GA_3 is recommended for proper root and shoot development from primary meristem.

5.1.4. Virus indexing through Grafting Method

Ipomoea. setosa is very sensitive to viruses and is a nearly universal indicator plant for sweetpotato viruses (Tairo and Kullaya, 2004). Grafting method was used by many workers to detect viral diseases in sweetpotato (Love *et al*., 1989. The severity of the virus symptoms and the length of time before they appear on the indicator plants are dependent upon the type and amount of virus present in the scion. In the present investigation, most of the field grown plants grafted showed the most common symptoms of virus infection are spotting, vein clearing (chlorosis), and stunting. The symptoms are most severe on the leaves of the indicator plant nearest to the graft. By contrast, mericlones causes almost no symptoms. Similar results were noted by many workers for virus identification, characterization and detection in mericlones (Moyer *et al*., 1989; Chavi *et al*., 1997). Indexing by using *I. setosa* as indicator plants has both advantages and disadvantages for virus detection. The technique is non-specific and detects nearly all of the viruses that infect sweetpotatoes. However, it is slow, requiring up to several months to complete, and lacks

sensitivity. Sometimes it is necessary to repeat the indexing of negatively indexed sweet potato plants two more times before declaring them free from known viruses (Frison and Ng, 1981). Thus, serological or PCR based methods can provide more precise results. But this technique can provide a low cost process of detection of known viruses (Alam *et al*., 2012).

5.1.5. Mass propagation of virus-free plantlets

Virus-tested clean plantlets were labeled and the nodal segments were used as explants for micropropagation of plantlets. Use of Kin and in combination with GA_3 or NAA were tested for micropropagation. Based on earlier results MS_0 (control) medium was not tested here. Among the different formulations 3.0 mg/l Kin+0.5 mg/l GA_3 was found good for high frequency shoot multiplication. Considering shoot length, use of 2.5 mg/l Kin+0.5 mg/l GA_3 was found most effective for most of the studied cultivars. More or less similar observations are also reported by others (González *et al.,* 1999). Combinations of Kin with NAA for adventitious shoot development were reported by many workers (González *et al.,* 1999). These combinations were associated with higher number of roots as observed by Dagnino *et al.* (1991). For high frequency root formation, combination of 3.0 mg/l Kin + 0.5mg/l NAA was most effective. But this combination produces relatively less number of shoots and shoots growth were also less than 2.0 mg/l Kin. The combination 3.0 mg/l Kin+0.5 mg/l GA_3 also produced relatively less number of roots but it was found sufficient for acclimatization and subsequent establishment in soil. So for high frequency micropropagation of meristem derived plantlets of described four cultivars, MS medium supplemented with 3.0 mg/l Kin+0.5 mg/l GA_3 is recommended.

For micropropagation, BARI-1 found most responsive. Differential responses observed on different sweetpotato varieties is due to their genetic make up towards *in vitro* shoot multiplication and their development since *in vitro* response of sweetpotato is strongly influenced by genotype (Dong, 1986; Chée *et al.,* 1992).

5.2. Callus Culture and Plant Regeneration

5.2.1. Callus Induction

A protocol has been established for callus induction from internode, root and leaf discs explants and their maintenance for further growth. The explants were

collected from *in vitro* grown plantlets developed through meristem culture of four sweetpotato cultivars viz. BARI-1, BARI-2, BARI-3 and BARI-4.

The explants were cultured on MS semisolid medium supplemented with 2,4-D, BAP, TDZ, NAA alone or in combinations of NAA with BAP and 2,4-D. Many factors such as genotype, compositions of the nutrient medium, physiological growth factors such as light, temperature, humidity and endogenous supply of growth regulations are important for callus induction in sweetpotato (Desamero *et al*., 1994; Otani *et al*., 1996; Sihachakr *et al*., 1997; Dhir *et al*., 1998). Many workers observed 2,4-D was the best auxin for callus induction as common as in monocot and even in dicot including sweetpotato (Lu *et al*., 1982; Ho and Vasil, 1983). The responses of callus induction varied greatly with different concentrations and combinations of growth regulator formulations. From the results it was clearly observed that about 90% callus formation occurred when explants were cultured in 2.5 mg/l 2,4-D. Increased concentration of 2,4-D decreased callus production as supported by Chee and Cantliffe (1988). Application of 2,4-D for effective callus induction has been reported by many workers Chee and Cantliffe (1988); Sihachakr *et al*., 1997; Padmanabhan *et al,* 1998a; Wang *et al*., 2000; Aloufa, 2002). 2,4-D in combination with NAA was also used by Dong (1986) for high frequency callus induction.

It showed that, internode, root and leaf explants of BARI-1 showed the better performance to induce callus in 2.5 mg/l 2,4-D + 0.5 mg/l NAA combination than other cultivars. This difference in callus induction may be due to different genotypes.

Role of cytokinin in accelerating embryogenic callus formation has been reported in many other species (Ernst and Oesterhelt, 1984; Nomura and Komamine, 1985). In sweetpotato, application of cytokinin for high frequency embryogenic callus induction is reported by many workers. It showed that, BAP in different concentration produced a good amount of callus having good structure and nature for further embryogenesis. 5.0 mg/l BAP produced maximum callus. About 60% cultures formed callus that indicates the major role of BAP on callusing. This was reported earlier workers (Carswell and Locy, 1984).

Thidizuron (TDZ) showed high cytokinin activity in promoting growth of cytokinin-dependent callus culture (Mok *et al*., 1982). TDZ is not only capable of adventitious and/or axillary shoot production through organogenesis but also somatic embryogenesis in many dicot plant species (Lu, 1993; Murthy *et al*., 1998). In the present investigation application of 2.0 mg/l of TDZ found efficient for high frequency callus formation in four cultivars. This result is similar to Gosukonda *et al*. (1995). Auxin in combination with cytokinine was also produced sufficient amount of callus in sweetpotato. In the present investigation 4.0 mg/l BAP+0.5 mg/l NAA was found efficient for callus induction. This result is more or less similar to the observation of Wang *et al*. (2000); Aloufa, (2002). However 2,4-D in combination with Kin was also reported (Otani and Shimda, 1996; Liu *et al*., 1999).

These results suggest that, nodal segment is found better than root and leaf disc for callus induction. Genotypic difference is clear among the studied cultivars. BARI-1 was most responsive to callus induction followed by BARI-4, BARI-2 and BARI-4; as observed by Hossain *et al*. (1999) for Bangladeshi cultivars.

5.2.2. Plant Regeneration

Calli derived from different sources of explants were subcultured for shoot regeneration in fresh MS semi-solid medium containing different compositions of plant growth regulators such as 2,4-D+Kin, 2,4-D+BAP, BAP+NAA, Kin+NAA and Zeatin at different concentration and combinations. After subculture of callus shoot regeneration started within 20 days of culture, sometimes along with more callus proliferation. Regeneration in sweetpotato has described by many workers (Chee and Cantliffe, 1988; Dhir *et al*., 1998; Sihachakr *et al*., 1997; Padmanabhan *et al.,* 1998b).

Embryogenic calli produced on 2,4-D were further subcultured on 2,4-D in combination with cytokinin for embryo development i.e. regeneration of plantlets. This result has been agreed by Dhir *et al*. (1998). However, this is contrary to Chee and Cantliffe (1988), who got embryogenesis omitting auxin from the medium. Considering all the media formulation 0.5 mg/l BAP+2.0 mg/l 2,4-D was found best for regeneration from internode and root derived callus among the treatments applied. Similar results were reported by so many

workers (Chee and Cantliffe, 1988; Dhir *et al*., 1998 Padmanabhan *et al*., 1998a). Beside this 0.1 mg/l Kin+0.5 mg/l 2,4-D, 0.1 mg/l BAP+1.0 mg/l NAA and 2.0 mg/l zeatin also found efficient for regeneration from callus, as reported by other workers (Otani and Shimada, 1996; Desamero *et al*., 1994; Wang *et al*., 2000; Aloufa; 2002). The superior effect of BA over Kin in shoot organogenesis was also reported by many workers (Azad *et al*. 1997).

Our investigation reveals that leaf discs derived callus is most responsive to zeatin for regeneration. Many workers have described application of zeatin for regeneration (Gonzalez *et al*., 1999;).

Form the study of overall regeneration efficiency of above three explants of four cultivars it can be concluded as follows: internode derived calli has more regeneration potential than those of leaves, while performance roots also produce good and close to internodes irrespective of cultivars. One growth regulator, zeatin affects differentially to develop somatic embryo while effects of other formulations rather liner that adduced that growth regulator is interacting with source of explants. Among the different genotype BARI-1 responds predominantly.

5.2.3. Somaclonal variation

Sweepotato is an asexually propagated crop. Creating genetic variation is therefore difficult. Genetic variability frequently occurs in *in vitro* regenerated plants and is highly valuable for breeding program. Several callus-derived plantlets were planted in soil and observed for any somaclonal variation. The morphological data clearely showed some variation in several clones compared to the controls. The variation occuered in several morphological traits including length of main vine, number of vines, internodes length, leaf areas, and number of tubers per plant, length of tuber per plant, average diameters of tuber, fresh weight of vines/plant, fresh weight of tubers/plant. Only a few reports are available for the plant regeneration from callus, while somacloneal variation is not studied. However, in potato, Rosenberg et al. (2010) showed that meristem clone differed in yield, number and weight of tubers and late blight resistance.

Somaclonal variation appears to result from both pre-existing genetic variation within the explants and variation induced during the tissue culture phase (Evans et al., 1984). In this study, it is possible that they could arose during meristem culture of callus culture. Patricia et al. (2004) reported that 1.65 mM of piclorm and 11.5 mM of 2, 4-D created somaclonal variation in potato. In spite of the causes, the variations found in our studies are statistically significant. However, it is necessary to observe whether these variations could pass through vegetative generations.

5.3. Fertilizer application for increasing yield

5.3. 1. Nirtogen application for increasing yield

The accumulation and distribution of dry matter within plants are important processes determining crop productivity. Insufficient allocation of assimilates to the vegetative organs may give a poor crop, but an excess in nearly in the season may result in high total biomass but with a relatively low proportion used for the production of storage organ.

As noted, during the vegetative growth stage, the foliage is the main sink for assimilates; however, the tubers gradually become the main sink after tuberization is initiated. Chen and Setter (2012) found that if a strong leaf and stem sink capacity is formed before tuber initation, it will compete with developing tubers for photosynthates. Furthrmore, a build-up of assimilates in leaves can decrease the rate of photosynthesis. Dry matter accumulated in tubers comes from the carbohydrates photosynthesized during tuber development and retransferred from leaves and stems.

In our present investigation, nitrogen fertilizer significantly influences in all vegetative growth traits in both mericlone originated and traditional planting materials. Fresh weight of the vine was increased with increased nitrogen fertilizer and peaked at 160 kg/ha. In this rate of fertilizer, the length of main vine was 184.21cm in cultivar BARI-2 compared to 85.78 cm in zero fertilizer application. Hossain (1993) reported that weight of vines per plant varied from variety to variety and the more nitrogen absorption caused vigorous vine growth, which increase weight of vine per plant. Longer vine is associated with the increased internode length which is about 50% longer in treatment groups.

Highest internode length was observed in BARI-2. The differences in traditional and mericlone were minor in case of the morphological characters. Total tuber yields of both the mericlone-derived and traditional planting material were higher with increased nitrogen fertilizer treatments. Nitrogen application positively influenced tuber length and tuber diameter. Zamil *et al.* (2010) reported that number storage roots per plant was increased by 200 kg N/ha. Above this rate it was decreased. Hossain (1993) reported that thickened tubers number increased with increasing N rate. Our results conclude that application of nitrogen fertilizer has positive influence in increasing sweetpotato yield. Yield of tubers per hectare increased with increasing levels of nitrogen as stated by different workers (Zamil *et al.,* 2010).

5.3. 2. Potassium application for increasing yield

Nitrogen encourages sweetpotato plant to produce more foliage. Potassium encourages more root development. Potassium fertilizer had significantly raised the yield by accelerating the transportation of carbohydrates from leaves to storage roots (Shi et al 2002). Nevertheless, foliage growth is required for effective production of photoassimilates to sink them to roots. Consequently, sweet potato like Irish potato and cassava are crops that have high demands for K because leaves, vines, stems and tubers usually remove substantial quantity of K from the soil. Potassium appears to be the most important nutrient in the production of sweet potato as its application increases yield by the formation of larger sized tubers. Potassium also affects the number, size, quality and the unit weight of tuberous roots produced, while the minimum levels of K suggested for healthy growth and yield are twice those recommended for N, although three times as much may be applied and occasionally even more (Degras, 2003). Sweet potato yield is significantly depressed if K is deficient, but eliminating P does not affect the yield as the crop is well adapted to low levels of available P on account of its mycorrhizal association which makes P available to it. In Japan, it was estimated that a tuberous yield of 13 t/ha, removes about 70 kg N/ha, 20 kg P_2O_5/ha and 110 kg K_2O/ha from the soil depending on the variety, crop duration and agro-climatic region (Degras, 2003). Comparisons of this kind are rare for tropical areas but figures given by IFA (1991) depicted the particular importance of both K especially in the root and N in the leaves. Common recommendation in most

countries is 35-65 kg N, 50-100 kg P2O5 and 80-170 kg K2O per hectare (IFA, 1991). In the Hubei province of China, the optimum K rate varied from 150-300kg K_2O/ha (Jian-wei et al., 2001). Whereas in India, the mean optimum requirement was put at 120 kg K_2O/ha, while the maximum was 160 kg K2O/ha with a yield response of 6.7 t/ha (Trehan et al., 2009).Thus, it appears that sweetpotato require both nitrogen and potassium for optimal yield. Nevwertheless the impact fertilizer application on yield can be complex due to several facts. These include presence of preexisting potassium in soils, and ability of sweetpotato to absorbe atmospheric nitrogen through symbiosis or any other means. The present experiment revealed that adequet potassium input significantly increased sweetpotato yield (Table 4.25 and 4.26). The yield increase was about 71% compared to the zero potassium control. The highest values of vegetative growth characters (vine length, internode length, leaf area, fresh weight of vines, number, length and diameter of tuberous root) were recorded by the treatment that received 180 kg/ha potassium. It has been reported that application of potassium fertilizer positively affects the root numbers (Shi et al 2002; Bourke et al 1985). Our observation increment in average roots weight per plant in response to increasing potassium application rate resulted in high total yield. Our results are also in agreement with El-Baky et al (2010), who demonstrated these effects in Egyptian cultivars.

These authors also a positive correlation between increased rate of K and several biochemical parameters including starch content, protein content etc. Thus the effect may be due to the fact that potassium plays an important tole in the promoting synthesis of photosynthates and their transport to roots (Mengel 1987). Increasing roots yield of plants due to increasing potassium application rate can be attributed as reported by Marschner (Marschner, H., 1995) to the crucial role of potassium in the energy status of the plant, translocation and storage of assimilates and maintenance of tissue water relations. Data also indicate that the potassium benefit was greater in meristem-derived vines than in conventional vine cuttings. This might also be attributed to the enhanced capacity of resourse utilization of the healthy plants. Moreover, short drought spells are very common during the growth of sweetpotato. It has been shown that the leaf photosynthesis of sweet potato was limited mainly by the stomatal factors under drought condition (Zhu et al 2012). In this case, the stomatal

effects could be lightened by potassium application with the water use efficiency of leaves decreased but the water productivity increased. Since higher potassium application could cause the non-stomatal limitation to photosynthesis. Therefore, proper management of the potassium fertilization management could increase yield by tuber bulking and alleviating drought as well.

5.4. Potential economic benefits of using meristem-derived clean planting materials and fertilizer applications

The viral disease clean up and utilization of urea and potash fertilizer is hypothesied to improve the yield and quality of sweetpotato and provide economic benefits for the growers.

In the 1980s, average farm yields were significantly below potential yields in China (Shandong province). This was due to disease transmission, especially from viruses, through farmer-saved seed roots may be a significant factor in suppressing yields (Moyer and Salazar 1989). The adoption of tissue culture propagation and ELISA testing methods were developed to produce disease-free mother plants for existing varieties. These clean materials were then multiplied under controlled conditions and distributed to farmers. This approach boosts the sweetpotato yield. Such systems are now being developed in the main sweet potato growing areas of China. The most advanced seed program can be found in Shandong Province, where virus-free seed was first distributed to farmers in 1994. Virus-free seed was targeted to reach 80 percent of the sweet potato area in the province by 1998 (Zhang et al. 1995).

In our experiment, adoption of grafting technique instead of ELISA reduces the production cost of disease-free planting materials. In case of mericlone plant source of BARI -1, the highest gross margin (320290 Tk /ha) and benefit cost ratio (BCR) (2.65) was obtained by applying 160 kg/ha of urea and 180 kg/ha of potash among the tested fertilizer doses in field condition. On the other hand, the lowest gross margin (149870 Tk /ha) and benefit cost ratio (1.79) was found in control due to low yield production. This is due to enhanced resourse utilization ability of the plants and consequent relocation to storage roots increasing the yield. Nonetheless, the final effect depends on other factors also

such as soil properties and climatic conditions. Positive economic benefits of utilizing optimal fertilizers have been shown by some other researchers (Sebastiani et al 2007).Overall, meristem culture followed by disease testing through grafting method produces disease free planting materials. In addition to the optimized fertilizer dose, use of these materials provides significantly higher economic benefits.Kassalia (2011) showed that sweet potato yield has a greater impact in improving profitability and capital inputs had the least impact in reducing profit. Experience, planting material, output transportation to market, adoption of new varieties and fertilizer level positively influenced sweet potato output. There is scale inefficiency and no input was used efficiently. Among factors, fertilizers are underutilized. An increase in scale of sweet potato production and increase in yield can improve efficiency in sweet potato production. Thus, our results showed the way of making economic gain of sweetpotato cultivators.

CHAPTER-6

CONCLUSION AND RECOMMENDATIONS

CONCLUSION

Among various roots and tubers, sweetpotato has tremendous potential for nutrition and proverty alleviation particularly in the developing world. In Bangladesh, average sweetpotato yield is low due to disease incidence, lack of suitable varieties and knowlwdge on the proper use of fertilizers. Initiative for the application of biotechnological approaches for its improvement is scarce. Thus, biotechnological and agronomic approaches such as, meristem culture, somaclonal variation and fertilizer application could improve sweetpotato yield in Bangladesh. In this work, these approaches were tested in four cultivars namely, BARI-1, BARI-2, BARI-3 and BARI Sweet potato-4).

The first objective was to produce disease-free seedling production through meristem culture technique to include them in commercial production. For this, meristems were isolated from shoot tips of 28-35 days old field grown plantsand surface sterilized in 0.1% $HgCl_2$ for 6 min for complete killing of surface pathogens without considerable tissues damage. Among the different concentrations and combinations of plant growth requlators in MS liquid medium, 2.0 mg/l of Kin+0.5 mg/l of GA3 combination with 4% sucrose was themost effective and suitable for primary establishment of isolated apical meristem. For proper root and shoot development from the primary established meristem, semi solid MS medium containing 2.5 mg/l of Kin+0.5 mg/l of GA3 was the most suitable. These microplantlets can be further multiplied by node cutting in 3.0 mg/l Kin+0.5 mg/l GA3 with 3% sucrose in MS medium. Among the studied four cultivars, BARI-1 was found most responsive for micropropagation using meristem culture. Meristem derived plantlets were grafted onto *Ipomoea setisa* to induce any disease symptoms, if present. Most of the mericlones did not induce any disease symptom suggesting the disease clean up capacity of our technique. Moreover, the grafting technique offers low cost method of disease testing compared to NCM-ELISA method.

Plant regeneration from callus offers a unique opportunity of producing somaclonal variants with potential to include then in breeding program. Meristem derived plantlets were used for callus induction and plant regeneration study. Among different media formulation 2 mg/l 2,4-D+0.5 mg/l NAA, 4 mg/l BAP+0.5 mg/l NAA or 2.0 mg/l TDZ in MS medium showed suitable performance for callus induction either from internodes, roots or leaf discs. Internodes were better explant than leaf disc for callus induction, while response of roots was near to internodes. For plant regeneration, 0.5 mg/l BAP+2.0 mg/l 2,4-D was found most effective from internode and root derived callus but for leaves it was 2.0 mg/l zeatin. Among the four cultivars BARI-1 was found most effective for in vitro response. Several somaclones were planted in the field to theck their morphological traits. Among these, some somaclones showed better performance in terms of vine length, leaf area and fresh weight of tuberous root. Nevertheless, some were very similar to the mother plants while others are inferior.

As sweetpotato yield is affected by nitrogen and potassium fertilizers, field trialsl were conducted to unlock yield potential due to lack of these nutrients. Clearely, it was obsered that application of nitrogen and potassium fertilizer resulted in significantly higher tuberous root yield. Followed by a number of trial and errors, 160 kg/ha urea in combination with 180 kg/ha potash is recommended for the best sweetpotato yield. All other parameters related to yield were influenced positively leading the highest yield. In all cases, meristem-derived planting materials exhibited higher tuberous yield. An economic analysis showed that the adoption of meristem-derived plantlets and application of nitrogen and potassium fertilizer increase production cost. However, the net economic return is significantly higher than using conventional planting materials.

RECOMMENDATIONS

From this study the following suggestions or recommendations are advised:

- For using pathogen free clean vine as planting materials, meristem culture derived plants without pathogen should be used for its large scale production.
- For testing pathogen elemination of meristem derived plants, low cost grafting technique using *Ipomoea setosa* is recommended over using of high cost NCM-ELISA method.
- The protocol on meristem isolation and culture developed from this study is recommanded for large scale commercial production of pathogen free clean vine as planting materials.
- As sweetpotato is sexualiay sterile, conventional plant breeding using hybridization technique has limitations for producing variable population for selecting desirable genotypes. Therefore, as an alternative way somaclonal variants can be exploited induced form callus derived plantlets. Our results supported this idea. It was observed that some somaclones showed better performance over the mother clone.
- Among the studied genotypes, BARI-1 is recommended for further experimentaion on tissue culture and fertilizer uses.
- For incrasing tuber production, besides using of meristem derived pathogen free clean planting materials, combined use of urea (160kg/ha) and potassium (180 kg/ha) alongwith other fertilizers is recommended.

CHAPTER-7

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APPENDIX-1

Preparation of Murashige and Skoog (MS) Medium.

Component	Concentration (mg/l)
Major nutrients	
NH_4NO_3	1650.00
KNO_3	1900.00
$MgSO_4.7H_2O$	370.00
$CaCl_2.H_2O$	440.00
KH_2PO_4	170.00
Minor nutrients	
$MnSO_4.4H_2O$	22.30
$ZnSO_4.4H_2O$	8.60
$CuSO_4.5H_2O$	0.025
KI	0.83
$CoCl_2.6H_2O$	0.025
H_3BO_3	6.20
$Na_2MoO_4.2H_2O$	0.25
$FeSO_4.7H_2O$	27.85
Na_2 -EDTA	37.25
Vitamins	
Nicotinic acid	0.50
Pyridoxine HCL	0.50
Thiamine HCL	0.10
Glycine	2.00
Inositol	100.00
Sucrose	30000.00
pH = 5.8	

Ref. Murashige and Skoog (1962)

APPENDIX-2

Weather Condition (During the period of expreriment)

Month: November-December/2010					
Month	Temperature		Average	Rainfall	Humidity
	Highest	Lowest		(mm)	(%)
November	28.88	18.4	24.14	0.10	81
December	25.103	11.868	18.485	1.24	

Month: January-April/2011					
Month	Temperature		Average	Rainfall	Humidity
	Highest	Lowest		(mm)	(%)
January	22.668	8.5806	15.624	0.20	81.226
February	28.261	12.629	20.445	0.00	73.786
March	33.658	18.694	26.176	0.3548	64.452
April	34.777	22.153	28.465	3.11	72.567

Mean temperatur (November 10 - April 11)

Temperature		Average	Rainfall	Humidity
Highest	Lowest		(mm)	(%)
28.89	15.38	22.22	0.84	75.66

Ref. Weather station, Shyampur, Rajshahi.