ROLE OF PHYTOHORMONE ON *IN VITRO* PLANT REGENERATION IN SWEET POTATO *(Ipomoea batatas* L.*)*

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CERTIFICATE

This is to certify that thesis entitled, **"ROLE OF PHYTOHORMONE ON *IN VITRO* REGENERATION IN SWEET POTATO *(Ipomoea batatas* L.*)"*** *submitted to the Faculty of* **AGRICULTURE,** *Sher-e-Bangla Agricultural University, Dhaka-1207, in partial fulfillment of the requirements for the degree of* **MASTER OF SCIENCE** *in* **BIOTECHNOLOGY**, *embodies the result of a piece of bona fide research work carried out by* **MD. ZIAUL HASAN SOUROV**, *Registration No.* **13-05434** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged. .

Dated:
Dhaka, Bangladesh

_____

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Dedicated
To
My Beloved Family

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(*Ipomoea batatas* L.)

BY

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ABSTRACT

An experiment was conducted to develop *in vitro* regeneration protocol in sweet potato at the Department of Biotechnology, Sher-e-Bangla Agricultural University, Dhaka. Four Sweet potato varieties *viz* Beni, Cogane, Kokei and Bari12 were used. The phytohormones BA, IBA and 2,4-D were applied to observe the potentiality of each hormone on plantlet production. Meristem was used as explant for callus induction and subsequent plantlet regeneration. The cultivar Cogane took minimum days for shoot regeneration from callus in the treatment 0.5 mg/l of 2,4-D. The highest number of shoots was noticed by the variety Kokei in the same treatment. The treatment 0.5 mg/l of 2,4-D showed best response on callus induction and the variety Kokei was more responsive in plantlet regeneration from meristamatic tissue. The node and shoot tip were used for direct plantlet regeneration on MS media supplemented with different concentration of BA and IBA either individually or in both combination. The treatment 1.0 mg/l of BA resulted the highest length of shoot 7.50 cm, 8.25 cm, 7.90 cm and 8.25 cm in the variety Beni, Cogane, Kokei and BARI 12 respectively. The highest percent of node development was recorded in the variety Kokei. The combined effect of BA and IBA was evaluated for plantlet regeneration and it was noticed that the treatment 1.0 mg/l BA + 2.0 mg/l IBA took minimum days for shoot initiation. The variety BARI 12 and Kokei showed highest number of node in the treatment 1.0 mg/l + 1.5 mg/l IBA. Healthy, robust and good quality plantlets were regenerated in all the four varieties. Among them, the variety Kokei showed the best performance in all the parameter under studies. More than 80% plantlet survived in net house and in open field condition. An efficient protocol has been developed for *in vitro* regeneration of sweet Potato which can be used for large scale commercial purpose of plantlet production.

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ABBREVIATIONS AND ACRONYMS

Agril.	:	Agriculture
Biol.	:	Biological
cm	:	Centimeter
CRD	:	Completely Randomized Design
DMRT	:	Duncan's Multiple Range Test
Conc.	:	Concentration
DAI	:	Days After Inoculation
et al.	:	And others (at elli)
FAO	:	Food and Agricultural Organization
IASC	:	International Aloe Science Council
g/L	:	Gram per litre
BAP	:	6- Benzyl Amino Purine
BA	:	Benzyladenine
KIN	:	Kinetine
IAA	:	Indole acetic acid
IBA	:	Indole butyric acid
NAA	:	*a*- Napthalene acetic acid
2, 4-D	:	2,4- Dichlorophenoxy acetic acid
Int.	:	International
2-ip	:	2-isopentenyladenine
J.	:	Journal
Mol.	:	Molecular
mg/L	:	Milligram per litre
µM	:	Micromole
MS	:	Murashige and Skoog
PGRs	:	Plant Growth Regulators
Res.	:	Research
Sci.	:	Science
TDZ	:	Thidiazuron
PVP	:	Polyvinylpyrrolidone
PLB	:	Protocorm-like bodies
CV	:	Co-efficient of Variation
^{o}C	:	Degree Celsius
etc.	:	Etcetera

CHAPTER I

INTRODUCTION

The Sweet Potato or *Ipomoea batatas* L. is a dicotyledonous plant that belongs to the bindweed or morning glory family, convolvulaceae. Its large, starchy, Sweet-tasting, tuberous roots are a root vegetable. The young leaves and shoots are sometimes eaten as greens. The Sweet Potato is not closely related to the common Potato (*Solanum tuberosum*). The Sweet Potato, especially the orange variety, is often called a "yam" in parts of North America, but it is also entirely unrelated to true yams. (Gibson *et al.*, 1998) The plant is a herbaceous perennial vine, bearing alternate heart-shaped or palmately lobed leaves and medium-sized sympetalous flowers. The edible tuberous root is long and tapered, with a smooth skin whose color ranges among yellow, orange, red, brown, purple and beige. Its flesh ranges from beige through white, red, pink, violet, yellow, orange, and purple (Sihachakr *et al.*, 1987).

It grows best at an average temperature of 24°C (75°F), abundant sunshine and warm nights. Annual rainfalls of 750–1,000 mm (30–39 in) are considered most suitable, with a minimum of 500 mm (20 inch) in the growing season. The crop is sensitive to drought at the tuber initiation stage 50–60 days after planting, and it is not tolerant to water-logging, as it may cause tuber rots and reduce growth of storage roots if aeration is poor. Depending on the cultivar and conditions, tuberous roots mature in two to nine months. With care, early-maturing cultivars can be grown as an annual summer crop in temperate areas, such as the Eastern United States and China. Sweet Potatoes rarely flower when the daylight is longer than 11 hours, as is normal outside of the tropics. They are mostly propagated by stem or root cuttings or by adventitious shoots called "slips" that grow out from the tuberous roots during storage. True seeds are used for breeding only (Sefasi *et al.*, 2012).

Sweet Potatoes are grown on a variety of soils, but well-drained, light- and medium-textured soils with a pH range of 4.5–7.0 are more favorable for the plant. They can be grown in poor soils with little fertilizer. However, Sweet Potatoes are very sensitive to aluminum toxicity and will die about six weeks after planting if lime is not applied at planting in this type of soil.

Cultivation of this crop has increased over the years. In 2018, the global production of Sweet Potatoes amounted to approximately 92 million metric tons. Most of the production comes from China (70,963,630 metric tons) and other Asian countries, including Indonesia, Japan and Korea. In Bangladesh, the area under Sweet Potato production was 25,739 ha in 2018 while it was 40874 ha in 2000. Sweet Potato can play an important role in the context of food security in Bangladesh (Hossain and Siddique, 1985). The total production of Sweet Potato in Bangladesh increased from 92,479 to 154,370 MT in 2000 to 2018, respectively. This is due to adoption of modern cultivation practices by the farmers. Sweet Potato is one of the most important food crops in terms of caloric value per cultivated area (Scott *et al.,* 1992).

Sweet Potato is remarkable because of its high yield, palatability and crude protein content. Orange-fleshed Sweet Potato varieties are rich in beta-carotene, while purple-fleshed ones are high in anthocyanin. These two important antioxidants thought to prevent chronic heart diseases and cancer (Teow *et al.*, 2007). Increased availability of beta-carotene (Pro-vitamin A) and crude protein content is good for nutrition and health (Ukom *et al.*, 2009). Sweet Potato is a highly nutritious food crop which gives better and faster production under diverse agroecological conditions with less input and that has immense potential to combat malnutrition and poverty (CIP, 2008). Despite the added advantages of Sweet Potato production and nutritional benefits, its productivity is highly affected by various biotic and abiotic stress factors (Gong *et al.*, 2005).

Sweet Potato also has the potential for use as a biomass species for methane and ethanol production and has therefore gained interest as a possible alternative to fossil fuels (Iese *et al.* 2018).

Sweet Potato is hexaploid (6x=90), which makes it difficult to form seeds. Additionally, it is difficult to cultivate Sweet Potato under the temperate climate conditions. Accordingly, it is necessary to secure a disease free stock and to overcome the limitations of traditional breeding. However, previous investigations conducted to enable plant regeneration and transformation of Sweet Potato via somatic embryogenesis using diverse explants like anther lateral bud and apical meristem of Sweet Potato have not been successful, in part because of its low frequency of somatic

embryogenesis. In contrast, the majority of successful plant regeneration results were obtained by using apical meristem tissue culture which is different from embryogenesis, and being exploited for plant transformation (Makenzi *et al.*, 2018).

Sweet Potato productivity is however, limited by a number of both biotic and abiotic constraints. Viral disease is the most important limiting factor for Sweet Potato production. Worldwide, up to 20 different viruses have been identified to infect Sweet Potato. Among them, Sweet Potato feathery mottle virus (SPFMV, genus *Potyvirus*, family *Potyviridae*) is found in all Sweet Potato growing areas while the others are localized to one or more geographic areas.

The dual infection of Sweet Potato feathery mottle virus (SPFMV) and Sweet Potato chlorotic stunt virus (SPCSV), is the most severe disease affecting the crop and can cause 56-98% yield loss (Gibson *et al.*, 1998).

Control of Sweet Potato feathery mottled virus (SPFMV) and Sweet Potato weevil is the most important strategy of International Potato Center (CIP) in order to increase production and quality. But it is difficult to control these pests by using pesticides or other chemicals, thus calling for alternatives. Meristem-tip culture allows plants to be freed from other pathogens including viroides, mycoplasmas, bacteria and fungi. Several reasons such as absence of plasmodesmata in the meristematic domes, faster cell division, competition between synthesis of nucleoproteins for cellular division and viral replication and presence of inhibitor substances make meristem tip useful source to obtain virus free plantlets (Gibson *et al.*, 1998).

The Sweet Potato is normally propagated by vine cutting. The use of storage root and vine cuttings as a method for vegetative propagation is the cause for the accumulation of viruses from generation to generation which could result in declining of root yield and loss of superior genotypes. Aphids and whitefly vectors are the main causes of virus transmission (Moyer and Salazar, 1989; Gibson, 2004). This conventional system is also characterized by low multiplication rate, wastage of a large quantity of food material, absence of uniformity, risk of catching diseases. A regular supply of clean materials for planting is therefore necessary for sustainable production (Far *et al.*, 2009).

Plant tissue culture is a modern technique mainly on plant cell culture. This technique has been used in the multiplication of "good clones" of agricultural crops and medicinal plants. The use of *in vitro* culture techniques can ensure speedy multiplication of valuable varieties (Dewir *et al.*, 2016) and the possibility to obtain biological material free from pathogens in large-scale. A balance between auxin and cytokinin determines the *in vitro* regeneration of plants grown in artificial medium. Generally, cytokinin helps in shoot proliferation and auxin helps in callus formation and rooting of proliferated shoots. The presence of auxin in defined combinations with cytokinins in the culture medium is also necessary to obtain adventitious shoot formation (Dolinski *et al.*, 2013). However, the requirement of cytokinin and auxin depends on the plant species, genotype, explant type and culture conditions. Requirement of growth regulators depends on physiological condition also. The best media with optimum growth regulator, growth condition and suitable explants are needed to be standardized for large scale.

The success in production of the healthy or disease free planting material of Sweet Potato is dependent on the reliability and efficiency of the regeneration protocol used. Development of tissue culture regeneration protocol helps to reduce disease severity and hence produce healthy Sweet Potato plants which can be used as planting method. Therefore, the present study was undertaken with the following objectives.

The specific goals were:

a) Callus induction protocol development from meristem culture
b) Investigation of the effect of phytohormone on Sweet Potato regeneration
c) *In vitro* regeneration protocol establishment
d) Virus free plantlet production in Sweet Potato

CHAPTER II

REVIEW OF LITERATURE

In vitro micropropagation of *Ipomoea batata* was investigated through the present study. Regeneration of plant from culture media of explants followed by genetic stability of plantlets seems to be meager. However, available information in this regards were reviewed and presented in this sections.

Plant tissue culture is the newest route in the science of cell biology. Tissue culture is the process of regeneration in an artificial nutrient medium under aseptic condition. The idea of plant tissue culture originated from the cell theory that was formulated by Schwann in 1839.Development of new organized structures i.e. organs from the old one through tissue culture is done by two ways: direct and indirect. Emergence of adventitious organs directly from explants is known as direct method. Indirect is the process of regeneration new organs through shoot and root formation (Ali *et al.*, 2012).

The influences of cytokinins (6-benzyladenine [BA], kinetin, thidiazuron, and zeatin), gibberellic acid, phenylacetic acid, indole-3-butyric acid, silver nitrate, and oxalic acid, culture duration, and medium salt strength were investigated. Optimal proliferation was observed when shoots were cultured on half-strength MS medium that was supplemented with 2 mg L-1 BA and 100 mg L-1 oxalic acid for 2 wk, followed by culture on half-strength MS medium without plant growth regulators for 4 wk. In this medium, the greatest number of shoots (3.1) and total number of nodes (16.1) per explant were observed. All (100%) of the regenerated shoots were rooted and acclimatized, with a survival rate of 96% when transferred and no morphological abnormalities. (Akin *et al.*, 2018).

Manipulation of the composition and ratio of plant growth regulators (PGRs) is often the primary approach used for optimization of *in vitro* micropropagation methods (Shukla *et al.*, 2012).

Different combinations of growth regulators were used for *in vitro* regeneration of Sweet Potato. BAP (6- purine) and KIN (Kinetin) were used for *in vitro* shoot regeneration and IBA (Indole-6-Butyric Acid) with NAA (Naphthalene Acetic Acid) were used for *in vitro* root regeneration. The highest percentage of shoot initiation (91.30), the minimum number of days (9.00) for shoot initiation, the highest number of shoot/plantlet (11.00) and the highest shoot length (4.38 cm) was observed when nodal segment explants cultured on MS medium supplemented with benzylamino BAP 1.5 mg/l + KIN 0.1 mg/l. On the other hand, the highest root initiation percentage (94.12), the minimum number of days (6.00) for root initiation, the highest number of root/plantlet (9.33) and the highest root length (11.13 cm) was observed in the MS medium supplemented with IBA 0.5 mg/l + NAA 0.1 mg/l. Regenerated plantlets were acclimatized for 5 days and adopted in soil by 6-7 days of transplantation (Parvin *et al.,* 2018).

In a study, a reproducible and highly efficient protocol for *in vitro* plant regeneration of six Kenyan farmer preferred SweetPotato, Enaironi, KEMB 36, KSP36, Mugande, Kalamb Nyerere, SPK 013 and SPK004 through direct shoot organogenesis from stem internodes explants was developed. The results revealed that Kalamb nyerere had the highest number of adventitious bud; for light (5.33 and 4.33) and dark (8.00 and 5.00) induction condition for all TDZ hormone level (0.25 mg/l and 0.15 mg/l). When explants incubated in 0.10 mg/l NAA the regeneration frequencies were the highest at 83.33% (Jewel) and 96.67% (Kalamb nyerere) for adventitious buds recovered from light and darkness respectively. This was the optimal auxin concentration which gave the maximum regeneration frequency with adventitious buds recovered from the dark. The best Kenyan SweetPotato genotypes for direct shoot organogenesis were Kalamb nyerere, Kemb 36 and SPK 004. The protocol presented in this work is suitable for improvement of SweetPotato genotypes through tissue culture methods and or genetic transformation. (Makenzi *et al.,* 2018).

Calli were successfully formed in Sweet Potato in almost all media containing 2,4-Dichlorophenoxyacetic acid (2,4-D) with the concentration of 1, 2, 3 and 4 mg l and BAP (concentration: 0.5 and 1 mg l), but the medium of MS + 2 mg l 2,4-D + 0.5 mg l BAP resulted in the highest number of induced calli per treatment (mean=11.36), with the percentage of responsive explants standing at around 96%. The higher the concentration of PEG, the lower the

number of surviving calli. At 20% PEG, only 54.42% calli survived. There were five plants successfully regenerated from the survived calli at 20% PEG, using MS medium containing 1.5 mg l BAP (Mau, 2019).

Frequency of shoot proliferation was maximum at 2.5 mg/l BAP and 0.5mg/l Kn and the number of shoot was 22-25 per explant. It took 26 days for shoot induction and 30 days for root induction. Multiplication rate in the treatment with BAP 0.5 mg/l which showed 2 plantlets were lowest. Among the BAP-2,4 D formulations, maximum multiplication was observed at BAP 1.0 and 0.5 mg/l 2,4 D where the number of shoots was 15. Numerous adventitious shoot primordia were observed near the basal portion of the shoot cluster. Augmentation of MS-medium with 4.5 mg/l BAP recorded the highest number of shoots and leaves (8.0 and 15.50 respectively). Shoot lets were highly rooted on half strength of B5 medium supplemented with 1.0 mg/l NAA. The maximum percentage of acclimatization, hardening and rhizomes production of *in vitro* derived plants in greenhouse was 80–100% (Mohamed *et al.*, 2007).

The aim of this study is to understand the effect of different auxin/cytokinin ratios on indirect shoot organogenesis of this plant. According to our results, the maximum callus induction frequency (100%) was obtained on Murashige and Skoog (MS) medium supplemented with 0.5 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) plus 0.05 mg/l 6-benzylaminopurine (BAP) from petiole segments. For shoot induction purpose, the yellow-brownish, friable, organogenic calli were inoculated on shoot induction medium. On MS medium supplemented with 1.5 mg/l BAP and 0.15 mg/l Indole-3-butyric acid (IBA), 96.66% of the petiole-derived calli responded with an average number of 3.56 shoots per culture. The highest root formation frequency (96.66%), root number (5.5), and root length (4.83 cm) were achieved on MS medium containing 2.0 mg/l IBA plus 0.1 mg/l Naphthaleneacetic acid (NAA). The rooted shoots were successfully transferred to field condition and the substrate with the mixture of cocopeat and perlite (1:1) had the highest survival rate (96.66%). This is the first report of an effective *in vitro* organogenesis protocol for *F. religiosa* by indirect shoot organogenesis through axenic seedling derived petiole explants, which can be efficiently employed for conservation of this important medicinal plant species as well as the utilization of active biomolecules (Vettorazzi *et al.*, 2017).

Plant growth regulator is indispensable material in culture media, and is important to the plant tissue induction, organ differentiation and growth. Therefore, the effects of seven plant growth regulators such as BA, TDZ, KT, NAA, IBA, IAA and GA_3 with different concentrations on the leaf regeneration, subculture of shoots and rooting were studied in *Ziziphus jujuba* cv. 'Dongzao'. The results indicated that the efficiency of TDZ was significantly higher than that of BA in the induction of adventitious bud from leaf. Leaves should be first induced on MS medium supplemented with TDZ (1.0 mg/l) and IBA (0.1 mg/l) for 28 days, and then transferred to medium MS+IBA 0.1 mg/l+GA_3 0.05 mg/l. In this way, the regeneration rate reached 92.45%. MS+BA 1.0 mg/l+KT 0.5 mg/l+IBA 0.1 mg/l was suitable for subculture of shoots, with the multiplication coefficient of 3.64. Supplements of GA_3 at 0.5 mg/l in the medium could significantly increase the elongation of shoots. The efficiency of IAA was best among that of IAA, IBA and NAA in the induction of rooting. The regenerated plantlets rooted well in 1/2 MS medium plus IAA (1.5 mg/l), with rooting percentage of 95.3% (Zhou *et al.*, 2009).

Plant regeneration from calli is possible by de novo organogenesis or somatic embryogenesis. Callus cultures also facilitate the amplification of limiting plant material. plant regeneration from calli permits the isolation of rare somaclonal variants which result either from an existing genetic variability in somatic cells or from the induction of mutations, chromosome aberrations, and epigenetic changes by the *in vitro* applied environmental stimuli, including growth factors added to the cultured cells (Woolf *et al.*, 1991).

The rate of node formation increased consistently over the six weeks of each culture reaching a number that prompted subculture or acclimatization for the case of the final subculture. Nodes are points of shoot development hence crucial during multiplication since one node represents one new plantlet (Sihachakr *et al.*, 1997). Tissue culture of Sweet Potato is mainly through direct organogenesis, through the use of nodal cuttings thus the higher the number of nodes the higher the number of plantlets. Variety KEMB 36 had better culture efficiency compared to Tainurey, having realised a regeneration index of 7 nodes per plantlet on the low cost medium and 4 nodes per plantlet on the conventional medium. This means that the variety is the most suitable for

adoption into farm systems since high number of planting materials can be realised. KEMB 36 had a smaller inter-nodal length compared to Tainurey and this may be one of the qualities that made it produce more nodes compared to Tainurey. The small intermodal length was, however, a challenge when excising plantlets into nodal cuttings for introduction into culture medium during multiplication. The *in vitro* plantlets cultured on the low cost medium had well developed leaves with the same morphology as the mother stock plants. Variety KEMB 36 had superior performance in terms of leaf formation on both the low cost and the conventional media compared to Tainurey (Ogeroy *et al.*,2012).

Root initiation in explants (axillary buds) of *Chrysanthemum morifoliums* on MS medium containing different concentrations and combinations of growth regulators. The best initiation with well differentiated micro shoots was achieved when the cultures were transferred to MS medium fortified with the various concentrations of auxins. Out of the different treatments tried 0.5 mg/1 NAA induced maximum rooting (88.66%) followed by control (79 %) as compared to 1.0 mg/1 NAA (Verma *et al.,* 2012).

The effect of different hormone combinations and type of explant on shoot regeneration of Sweet Potato was evaluated in order to optimize the regeneration protocol. Explants in the form of stem sections, leaf discs, apical shoots and axillary buds derived from *in vitro* stock plant cultures were cultured on Murashige and Skoog (MS) media supplemented with 36 combinations of naphthalene acetic acid (NAA) (0, 0.01, 0.1, 0.2, 0.5, and 1 mg/l) and 6-benzylaminopurine (BAP) (0, 0.01, 0.1, 0.2, 0.5 and 1 mg/l). The highest percentage of shoot regeneration was obtained when apical shoot explants (31%) and axillary bud explants (22%) were cultivated on MS supplemented with 0.01 mg/l NAA + 1 mg/l BAP. Leaf discs and stem section explants produced highly recalcitrant callus that did not regenerate into shoots in shoot induction medium (SIM). Callus from apical shoots explants cultured on SIM developed into shoots. The shoots rooted readily on root induction medium (RIM) and then in hormone free MS medium. Regenerated plants appeared normal and showed a 100% survival rate when transferred to soil. The regeneration protocol described in this study will be used in a plant transformation protocol to produce transgenic Sweet Potato with broad virus resistance (Sivparsad *et al.,* 2012).

Axillary bud shoot proliferation and somatic embryogenesis was observed in *Dendranthemx grandiflnra* (Ramat.) Kitamura cv. Palisade White on modified MS medium supplemented with 1 mg/1 naphthalene acetic acid (NAA) or 2,4- dichlorophenoxyacetic acid (2,4-D), 0.1 mg/1 BA, 200 mg/1casein hydrosylate (CH) and 290 mg/1 proline. Proliferation rate of 3.2 microshoots axillary bud was obtained in MS medium supplemented with BA (0.1 mg/1) was used in combination with GA_3 (0.5 mg/1). The number of roots per shoot was higher using IBA (0.5 mg/1), but IAA (2 mg/1) promoted longer roots. Leaf explants were most responsive; demonstrating the highest percentage of embryogenesis (97.9%), followed by petiole and internode's stem explants (56.3 and 35.1%, respectively). The number of somatic embryos per embryogenic explant was highest on leaf explants. However, the best conversion rate (53.8%) of somatic embryos to plantlets was observed from petiole explants. For this reason, they reported petiole explants to be most suitable type for plant regeneration of chrysanthemum cv. Palisade White through somatic embryogenesis (Keresa *et al.,* 2012).

Root initiation was observed in explants (axillary buds) of *Chrysanthemum morifoliums* on MS medium containing different concentrations and combinations of growth regulators. The best initiation with well differentiated micro shoots was achieved when the cultures were transferred to MS medium fortified with the various concentrations of auxins. Out of the different treatments tried 0.5 mg/1 NAA induced maximum rooting (88.66%) followed by control (79 %) as compared to 1.0 mg/1 NAA (Verma *et al.,* 2012).

Somatic embryogenesis from *in vivo* grown leaf explants of Chrysanthemum cv. Euro was incubated on Murashige and Skoog (MS) medium supplemented with 2.0 mg/l 2,4-dichlorophenoxyacetic acid and 2.0 mg/l kinetin, yielding the highest mean number of embryos (5.97) per explant after 5 weeks of culture. MS medium was observed to be the more effective in promoting the proliferation of somatic embryos than half-strength Murashige and Skoog medium (Kim *et al.*, 2013).

Nodal explants was cultured from *in vitro* grown pyrethrum on Murashige and Skoog (MS) media supplemented with different concentrations of cytokininis, isopenyladenine (2iP),

Benzylaminopurine (BAP), kinetin (KIN), Thidiazuron (TDZ), cysteine, 100 mg/1 Inositol, 2% sucrose. Rooting was evaluated using half strength MS media supplemented with Indole-3-butyric acid (IBA), Indole-3-acetic acid (IAA), 1-napthyleneaceic acid (NAA) and Dicholorophenoxy-acetic acid (2,4-D). Media without growth regulators was used as controls. Results showed that there were significant differences among cytokinins and auxins levels for the number and length of microshoots and roots, respectively. BAP at 40 pM gave the highest mean shoot number of 15.98 i 0.68 and the highest mean shoot length of 1.32 i 0.06 cm (Christian *et al.*, 2013).

Different concentrations and combinations of growth regulators was used to improve root organogenesis and micropropagation in *Chrysanthemum morifolium* (Ramat) cv. Hwiparam. Stem explants were cultured on 3 full strength basal MS (Murashige and Skoog, 1962), SH (Schenk and Hiberlandt, 1976) and B5 (Gamborg *et. al.,* 1972) medium. The best medium for root regeneration was investigated at 4 different concentrations (l/4, 1/z, 1 and 2).The best type of medium for root regeneration and growth was SH medium. The results showed, half strength of SH (1/ZSH) to be best for the number of root per explant (4.3) and root length (31.4 cm) (Soo *et al.*, 2014).

It is very difficult to induce direct shoot regeneration from petiole explants. Only 3.1% of shoots' regeneration occurred on petiole explants with 6.0mg l–1 silver nitrate (Figure 1E), which was not significant. Our results showed that different explants had different responses to silver nitrate; silver nitrate was more suitable to lamina explants than stem and petiole explants. This suggests that the promotive function of silver nitrate on shoot regeneration has organ-specific sensitivity. There was a significant effect of culture time on direct shoot regeneration on basal MS medium supplemented with 1.0mg l–1 NAA and different concentrations of silver nitrate. With the culture time increasing, the frequency of shoot regeneration from various explants of Sweet Potato also increased. Of all the explants tested, stem explants produced high frequency shoot regeneration and were the first to show shoot initiation. Direct shoot regeneration from stems occurred after 20 days of incubation, after 40 days from leaf explants and 60 days from petiole explants. The *in vitro* regeneration of adventitious shoots is an essential base for most methods of genetic transformation (dewir *et al.*, 2016).

Well-developed roots with strong connections on the plantlets are desirable for hardening. The two Sweet Potato varieties exhibited varying root formation patterns with Tainurey producing more roots on the low cost medium than on the conventional medium while KEMB 36 had significantly higher number of roots on the conventional medium compared to the low cost medium. This is an indication of genotype-dependent response to tissue culture by the two varieties. This was reinforced by the fact that KEMB 36 produced more roots on the conventional medium compared to Tainurey while on the low cost medium the latter had more roots. Six weeks after the second subculture plantlets were removed from the growth room and transplanted on acclimatization medium containing rice husks and red soil in the ratio 1:2. The acclimatization medium was dispensed in rectangular trays, which were then put in a hardening chamber made of transparent polythene sheet. Survival of the plants was monitored over three weeks, after which surviving plants were transferred onto soil (Ogeroy *et al.*, 2015).

The highest percentage shoot regeneration of 31% was obtained from apical shoot explants generated on 0.01 mg/l NAA + 1 mg/l BAP . Responsive axillary bud explants showed 22% shoot regeneration, and also on 0.01 mg/l NAA + 1 mg/l BAP. Therefore, the hormone concentrations of 0.01 mg/l NAA and 1 mg/l BAP was shown to be the best combination in the regeneration of shoots. When apical shoot and axillary bud explants were cultured in NAA concentrations of 0.2 mg/l or higher, only callus derived roots were produced. Leaf discs and stem section explants produced only highly resistant callus with a compact structure that did not regenerate into shoots. 1 mg/l BAP interacts with 0.01 mg/l NAA in a synergistic manner to promote optimal shoot regeneration via the organogenetic pattern of plant regeneration. However, when the concentration of NAA in the medium was increased to 0.2 mg/l, this synergy ceased and callus regenerated roots instead of shoots. Therefore, results indicated that concentrations of NAA in the lower range of 0.01 to 0.1 mg/l was critical to the development of shoots and concentrations exceeding this threshold resulted in root formation. The distinctive limit of NAA needed to promote shoot regeneration is probably due to the endogenous concentrations of auxin present in this specific genotype (Sivparsad *et al.*, 2012).

In vitro propagation of chrysanthemum was done to have potential for fast multiplication of superior genotypes, allowing the exploitation of maximum genetic gain achieved in the breeding program. Callus induction from leaf explant in MS medium containing 1.5 mg/1 2, 4-D was found to be 100 % from petal explant it was found to be 100 % in MS medium containing 2.0 mg/1 2, 4-D. The best friable calli were subjected to suspension culture in MS media supplemented with 1.0 mg/1 BAP for somatic embryos. All calli in suspension gave rise to somatic embryos, which were regenerated in MS media supplemented with various concentration of BAP. The regenerated plantlets were elongated on MS media supplemented with 0.1 mg/1 BAP + 2.0 mg/l kinetin and rooted on MS basal medium containing IBA (0.1 mg/1). (Mani *et al.,* 2011).

CHAPTER III

MATERIALS AND METHODS

3.1 Time and location of the experiment

The research was carried out in Biotechnology Laboratory of the Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207 from the period of July, 2019 to June, 2020.

3.2 Experimental materials

3.2.1 Source of materials

The planting materials of Sweet Potato (*Ipomoea batatas (L.)* Lam were collected from the central research field of Sher-e-Bangla Agricultural University.

Plate 1. Planting materials of Sweet Potato in central research field of SAU

3.2.2 Plant materials

Healthy shoot tips of Sweet Potato of about 1-3 cm in length were collected and excised from donor plants. Fresh, healthy and disease free vines of Sweet Potato were harvested in a beaker filled with water. The explant were washed thoroughly with running tap water for removing soil. Young shoot tips were trimmed to size of 1-3 cm for further use as explants. Four Sweet Potato varieties viz. Beni, Cogane, Kokei and BARI Sweet Potato-12 were used as experimental material. Those Sweet Potato genotypes were supplied by the Department of Agricultural Botany, Sher-e-Bangla Agricultural University, Dhaka.

3.2.3 Instruments

Metal instruments viz., forceps, scalpel, needles, spatulas and aluminum foils tissue, cotton, plastic caps etc. were used as instruments and Erlenmeyer flasks, culture bottles, flat bottom flasks, pipettes, petridishes, beaker and measuring cylinders (25 ml, 50 ml, 100 ml, 500 ml and 1000 ml etc) were used as glassware. In all the experiments the borosil glassware was used given priority. The glassware were first rinsed with the liquid cleaners and then washed thoroughly with tap water before the detergent (trix) was removed completely. Then set up to autoclave for sterilization.

3.2.4 Culture media

The degree of success in tissue culture is mainly related to the choice of nutritional components and growth regulators. Presence of plant growth regulators plays a significant role in a successful regeneration of any plant species. Media for tissue culture should contain all major and minor elements, vitamins and growth regulators which are essential for normal plant growth. Explants were inoculated onto media composed of basal MS medium supplemented with different concentrations of phytohormones. Hormones were added separately to different media according to the requirements. All the glassware and instruments were first rinsed with the liquid detergent (Trix) and washed thoroughly with tap water until the detergent was removed complete. Then the glassware and instruments were sterilized in an autoclave at a temperature of 121°C and at 1.06 kg/cm^2 (15 PSI) pressure for 30 minutes.

3.3 Preparation of stock solutions

The first step in the preparation of the medium was the preparation of stock solutions. As different ingredients were required in different concentrations, separate stock solutions for macronutrients, micronutrients, vitamins, growth hormones etc, were used.

3.3.1 Stock solution "A" (Macronutrients)

Stock solution of macronutrients was prepared up to 10 times the concentration of the final medium in 1000 ml of distilled water (dw). Ten times the weight of the salts required per litre of the medium were weighed properly and dissolved by using a magnetic stirrer in about 750 ml of distilled water and then made up to 1000 ml by further addition of distilled water (dw). To make the solution free from all sorts of solid contaminants, it was filtered through Whatman no. I filter paper. Then it was poured into a plastic container, labeled with marker and stored in a refrigerator at 4°C for later use.

3.3.2 Stock solution "B" (Micronutrients)

The stock solution of micronutrients was made up to 100 times the final strength of necessary constituents of the medium in 1000 ml of distilled water (dw) as described for the stock solution of macronutrients. The stock solution was filtered, labeled and stored in a refrigerator at 4°C.

3.3.3 Stock solution "C" (Iron sources)

This was prepared at 100 times the final strength of Fe_2SO4 and Na_2EDTA in 100 ml of distilled water and chelated by heating on a heater cum magnetic stirrer. Then the volume was made up to 1000 ml by further addition of distilled water. Finally the stock solution was filtered and stored in a refrigerator at 4°C.

3.3.4 Stock solution "D" (Vitamins)

Each of the desired ingredients except myo-inositol were taken at 10 folds (100x) of their final strength in a measuring cylinder and dissolved in 750 ml of distilled water. Then the final volume was made up to 1000 ml by further addition of distilled water. The solution was dispensed into 10 ml aliquots and stored at 20°C. Myo-inositol was used directly at the time of media preparation.

3.3.5 Stock solution "E" (Hormone)

The first step of the preparation of the medium was the preparation of hormone stock solutions. To expedite the preparation of the medium separate stock solutions for growth regulators were prepared and used. Growth regulators and concentrations used in for *in vitro* regeneration of are presented below:

1. 2,4-D (0.5, 1.0, 1.5 and 2.0 mg/l) for callus induction
2. BA (0.5, 1.0, 1.5 and 2.0 mg/l) for shoot induction
3. IBA (0.5, 1.0, 1.5 and 2.0 mg/l) for root induction
4. BA (1 mg/l) combined with IBA (0.5, 1.0, 1.5 and 2.0 mg/l)

3.4 Preparation of the stock solution of hormones

To prepare the above hormonal supplements, they were dissolved in proper solvent as shown against each of them below. Generally, cytokinins were dissolved in few drops of basic solutions (1N NaOH) and auxins were dissolved in few drops of basic solutions (1N NaOH) or 100% ethyl alcohol.

Hormone (solute)	Solvents used
BA	1N NaoH
IBA	100% ethyl alcohol
2,4-D	1N NaOH

In present experiment, the stock solution of hormones were prepared by general procedure. 10 ml of 100% ethyl alcohol or 1N NaOH solvent and 100 mg of solid hormone was placed in a small beaker and then dissolved with the addition of sterile distilled water using a measuring cylinder and the volume was made up to 100 ml. The prepared hormone solution was then labelled and stored at 4±1°C for use upto two months.

Culture media was prepared from stock solution. It was done in the following process:

1st step: 800 ml sterilized distilled water was taken in a 1L glass beaker,

2nd step: 100 ml Stock solution-01(specific modified stock-01 in case of specific treatment) was added and the rest stock solutions were added at a quantity of 10 ml each. Then the solution is mixed with the help of a magnetic stirrer.
3rd step: The solution volume was brought up to 1L with autoclaved distilled water.
4th step: 30 gm of sucrose was added in the mixture.
5th step: pH of the MS media was fixed at 5.8 with pH meter by adding NaOH or HCl.
6th step: At last 8 gm agar was put together with the prepared medium and heated for some minutes in oven for liquification.

Readymade MS powder was also used for culture media preparation. The following steps were followed when used in readymade MS powder (Duchefa, Netherlands)
Step I. Seven hundred ml of sterile distilled water was poured into 1000 ml beaker.
Step II. Five gm of MS media (readymade) and 30 gm of sucrose was added and gently stirred to dissolve these ingredients completely with the help of a hot plate magnetic stirrer.
Step III. Different concentrations of hormonal supplements were added to the solution either in single or in combinations as required and mixed well.
Step IV. The volume was made up to 1000 ml with addition of sterile distilled water.
Step V. The pH was adjusted at 5.8.
Step VI. Eight gram agar was added to the mixture and heated for 10 minutes in an electric oven for melting of agar.
Step VII. Required volume of hot medium was dispensed into culture vessels. After dispensing and proper cooling of the medium, the culture vessels were plugged with cork and marked with different codes with the help of a glass marker to indicate specific hormonal combinations.

3.5 Steam heat sterilization of media (Autoclaving)

For sterilization the culture medium was poured in culture bottles and then autoclaving was carried out at a temperature of 121°C for 20 minutes at 1.06 kg/cm^2 (15 PSI) pressure. After autoclaving the media were stored in at 23±2 °C for several hours to make it ready for inoculation with explants.

3.6 Sterilization of culture room and transfer area

In the beginning, the culture room was sprayed with formaldehyde and then the room was kept closed for 3 days. After that the room was cleaned through gently washing the floors, walls and rakes with detergent. This is followed by careful wiping them with 70% ethanol. This process of sterilization of culture room was repeated at regular intervals. The transfer area was also cleaned with detergent and also sterilized twice in a month by 70% ethanol. Laminar airflow cabinet was usually sterilized by switching UV ray to kills the microbes inside the laminar airflow. It switches on 30 minutes before working in empty condition and for 20 minutes with all the instruments. The working surface was wiping with 70% ethanol before starting the transfer work.

3.7 Preparation of explants and sterilization

To clean the explants, the vines were carefully trimmed with regular scissors to a minimum size without damaging any other tissue and washed with running water for 1 hours. The vines were immersed in Mancoseb fungicide (80%) for 15 minutes and rinsed with sterile water for 3 times and then soaked in ethanol (70%, v/v) for 1 min and washed with distilled water for 2 times and immediately subjected to a surface disinfecting treatment in 50% (v/v) $HgCl_2$ plus Tween-20 (0.1%) for 15 min, then rinsed three times in sterile distilled water. Shoot tips were then transferred into Petri dishes as explant ready for inoculation.

3.8 Meristem isolation and inoculation of meristem in culture media

For inoculation, the workers hands and forearms were washed thoroughly with soap or antiseptic and repeatedly sprayed with 70% ethanol during the period of work. Prior to use, the surface of the laminar flow bench was swabbed down with 70% ethanol and the interior sprayed with same alcohol. All glassware, instruments and media were steam- sterilized in the autoclave. During the course of work, small instruments in use were placed in a beaker containing 70% ethanol and were flamed repeatedly using a spirit burner. Explants were transferred to large sterile glass petridish or glass plate with the help of sterile forceps under strict aseptic conditions.

To isolate the meristem from the explants, the outer leaves from each shoot was removed. Thus the apex is exposed. Then, the ultimate apex was cut off with the help of scalpel and transferred only those less than 1 mm in length. Then the culture was incubated under 16 hours light at 25˚C. as soon as the growing single leafy shoot or multiple shoots obtained from single shoot tip or

meristem, they were transferred to hormone free medium to develop roots. The mouth of culture vial was flamed before and after positioning of the explants on the medium. This was carried out under sterile condition in laminar air flow cabinet. After vertically inoculating the explants singly in culture bottle, the mouth of bottle was quickly flamed and capped tightly. After proper labeling, mentioning media code, date of inoculation etc. the bottles was transferred to growth room. The temperature was maintained at 25±2 °C with an air conditioner and light intensity varied from 2000–3000 Lux. White fluorescent lights were used for growth of the culture. The photoperiod was generally maintained at 16 hours and 70% relative humidity (RH) for better growth and development. Some explants became black in color within 6-7 days after inoculation. To control blackening the blackish tissues on the explants were removed and the explants were transferred to similar fresh medium. It was repeated each of 10 days interval for about one month to minimize further blackening of the tissue.

3.9 Incubation

The culture vials then transferred to culture racks and allowed to grow in controlled environment. The culture bottles were transferred to growth chamber with a photoperiod of 16 h light and 8 h of dark at 25ºC ± 2 ºC with 3000-4000 lux light intensity and 70% relative humidity (RH) incubated for 3 months.

3.10 Sub-culturing and maintaining of proliferating shoots

Initial sub-culturing was done after 30 days when the explants had produced some shoots. For sub-culturing, the entire samples of *in vitro* shoot were cut into small pieces. Shoots were excised in aseptic condition with help of sterile scalpel blade and sterile forceps and transferred to new MS media which was supplemented with same concentration of growth hormones in order to increase budding frequency. Initial Subculturing of the developed shoot into new media 4(A) explant developed shoot in culture media subcultured from the developed shoot of explant. The observations on development pattern of shoots were made throughout the entire culture period. Data recording was started after 2nd weeks from inoculation, so that each piece would contain about one shoot. Leaf and blackish or brownish basal tissues were removed. Each piece was inoculated into a similar fresh medium. It was practiced at the interval of 20-25 days.

3.11 Rooting of *in vitro* induced shoots

Shoots (3.0 cm) derived from shoot bunches were excised and rooted on medium consisting of MS basal medium supplemented with Indole Butric Acid (IBA) at each (0.5, 1.0, 1.5, and 2.0 mg/l) . All the media used in this study were supplemented with 3% (w/v) sucrose, solidified with 0.8% (w/v) agar and the pH was adjusted to 5.8± 0.1 before autoclaving at 121°C and 15 lb psi for 15 min.

3.12 Sub-experiments

Four sub-experiments were conducted to assess the effect of different concentrations of 2,4-D, BA and IBA on callus induction, shoot proliferation and root induction.

Sub-experiment 1. Effect of 2,4-D on callus induction potentiality in different varieties of Sweet Potato

In this sub-experiment, meristems of Sweet Potato were used as sources material to investigate the effect of 2,4-D for callus induction.

Treatments: 0.5, 1.0, 1.5 and 2.0 mg/l of 2.4-D were used. The experiments were arranged in completely randomized design (CRD) with three replications.

Sub-experiment 2. Effect of BA on shoot induction potentiality in different varieties of Sweet Potato

In this sub-experiment, nodes and shoot tips of Sweet Potato were used as source material to investigate the effect of BA for shoot induction respectively.

Treatments: 0.5, 1.0, 1.5 and 2.0 mg/l of BA were used. The experiments were arranged in completely randomized design (CRD) with three replications.

Sub-experiment 3. Effect of IBA on root induction potentiality in different varieties of Sweet Potato

In this sub-experiment, nodes and shoot tips of Sweet Potato were used as sources material to investigate the effect of IBA on root induction respectively.

Treatments: 0.5, 1.0, 1.5 and 2.0 mg/l of IBA were used. The experiments were arranged in completely randomized design (CRD) with three replications.

Sub-experiment 4. Combined effect of BA and IBA on shoot and root induction potentiality in different varieties of Sweet Potato

In this sub-experiment, *in vitro* regenerated calli, nodes and shoot tips of Sweet Potato were used as sources material to investigate the combined effect of BA and IBA on shoot and root induction.

Treatments: 1.0 mg/l of BA supplemented with 0.5, 1.0, 1.5 and 2.0 mg/l of IBA were used. The experiments were arranged in completely randomized design (CRD) with three replications.

3.13 Acclimatization and establishment of plantlets on soil

In vitro rooted plants were removed from rooting medium and washed to remove adhering gel and transplanted to plastic pots containing autoclaved garden soil and sand at 3:1 ratio. Plants were kept under culture room conditions for 15 days then transferred to green house and placed under shade until growth was observed and then finally in field condition.

3.14 Data collection

The observations on development pattern of shoots and roots were made throughout the entire culture period. Three replicates (single shoot per culture bottle) were used per treartment. Data were recorded after 14, 21 and 28 days of induction on culture media in case of shoot and root proliferation. The following observations were recorded in case of callus, shoot and root transformation under *in vitro* condition.

1) days to callus initiation
2) Weight of callus
3) No. of shoots from callus
4) Days to shoot induction
5) No of shoot
6) Length of shoot (cm)
7) No of leaves/plantlet
8) Days to root induction
9) Lenth of root/plantlet (cm)

3.14.1 Days to callus initiation

Days to callus initiation were calculated by counting the days from explants inoculation to the first initiation of callus.

3.14.2 Weight of callus

The fresh weight of callus (gm) was measured using analytical balance after about 28 days of inoculation.

3.14.3 Number of shoot from callus after 28 days of initiation

After 28 days of initiation of callus, number of shoot from each callus was observed through naked eye.

3.14.4 Days to shoot induction

Days to shoot induction were calculated by counting the days from explants inoculation to the first induction of shoots.

3.14.5 Number of node per explant

Number of node per explant in each variety was recorded after 14, 21 and 28 days after inoculation.

3.14.6 Length of shoot

Shoot length was measured in centimeter (cm) from the base to the top of the explants by a measuring scale. Then the mean was calculated.

3.14.7 No of leaves/plantlet

After 28 days of inoculation, number of leaves per plantlet in each of the variety was recorded and their mean was calculated.

3.14.8 Days to root induction

Days to root induction were calculated by counting the days from explants inoculation to the first induction of roots.

3.14.9 Length of roots

Root length was determined in centimeter (cm) from the base to tip of the roots. Average length of the root was calculated by formula.

3.14.10 Percentage of established plantlets

The percentages of established plantlets were calculated based on the number of plantlets placed in the plastic pots and the number of plants finally survived. The percentages of established plantlet were calculated by using the following formula:

$$Percentage\ of\ established\ plantlets = \frac{\text{number of established plantlets}}{\text{number of plantlets inoculated}} \times 100$$

3.14.11 Calculation of survival rate of plantlet

The survival rate of established plants was calculated based on the number of plantlets placed in the pot and the number of plants finally established or survived by the following equation-

$$Survival\ rate(\%)\ of\ plantlets = \frac{number\ of\ established\ plantlets}{Total\ number\ of\ explants} \times 100$$

3.15 Statistical data analysis

Data recorded for different parameters under study were statistically analyzed to ascertain the significance of the experimental results. The means for all the treatments were calculated and analyses of variance of all the characters were performed through MSTAT C software. Experiment was conducted in laboratory and arranged in Completely Randomized Design (CRD) with three replications. The significant difference between the pair of means was evaluated at 5% level of significance by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

CHAPTER IV

RESULT AND DISCUSSION

Different investigations were made on this experiment under laboratory condition to evaluate the effect of different plant growth regulators on callus induction, shoot and root induction in Sweet Potato. The overall objective of the present study has been to develop a system regeneration in Sweet Potato. Manipulating the relative ratio of different growth regulators has been successfully used in the current investigation. The results of these experiments have been presented and discussed in this chapter with Plates (2-12), Figures (1.1-4.2) and Tables (1-12). Analyses of variance in respect of all the parameters have been presented in Appendices (I-XI).

4.1 Sub-experiment 1. Effect of 2,4-D on callus induction potentiality in different varieties of Sweet Potato

Meristems were used for callus induction in Sweet Potato. The result of the effect of different concentrations of 2,4-D has been presented under following headings with Figure 1.1 and Table (1-3).

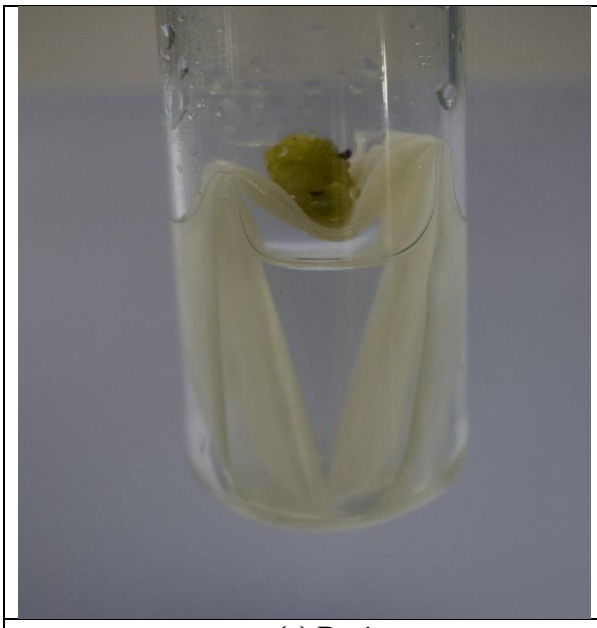
4.1.1 Days to callus initiation

Significant variations were observed among different concentration of 2,4-D on days to callus initiation at 5% level of significance which is presented under table 1. The maximum 15.33 days (V_1), 13.67 days (V_2), 15.67 days (V_3) and 16.33 days (V_4) were recorded in 2,4-D 1.5 mg/l treatment while minimum 10.66 days(V_1), 11.67 days (V_2), 11.00 days (V_3) and 13.33 days (V_4) were recorded in the treatment 2,4-D 0.5 mg/l. Among all the varieties, the Beni variety showed minimum days to callus initiation (10.66). Bhattacharya *et al.* (1990) studied the influence of different growth regulators on the *in vitro* morphogenesis of chrysanthemum. They reported that a combination of 0.1 mg/l IAA and 0.2 mg/l BAP was most appropriate for callus formation.

Table 1. Effect of 2,4-D on days to callus initiation in different varieties of Sweet Potato

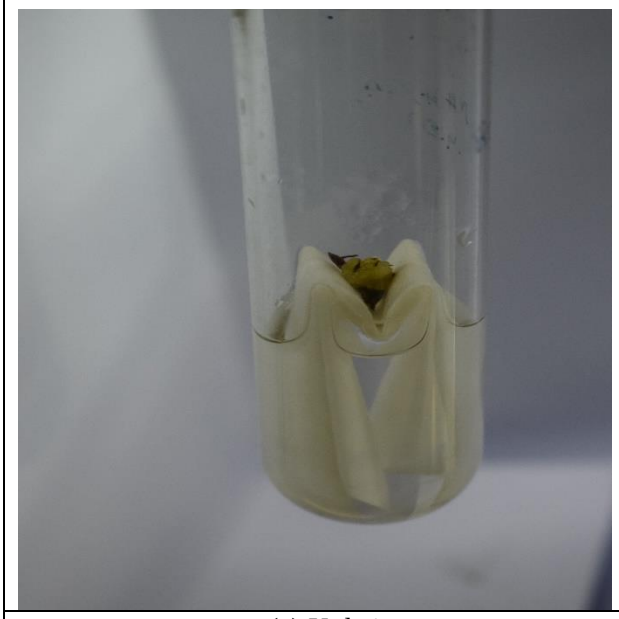
Treatment (2,4-D Concentration)	Days to callus initiation			
	Varieties			
	Beni (V_1)	Cogane (V_2)	Kokei (V_3)	BARI Sweet Potato-12(V_4)
T_1 = 0.5 mg/l	10.66c	11.67c	11.00c	13.33c
T_2 = 1.0 mg/l	12.33b	12.00bc	14.33ab	11.67d
T_3 = 1.5mg/l	15.33a	13.67a	15.67a	16.33a
T_4 = 2.0 mg/l	14.67ab	12.33b	13.33b	14.33b
CV (%)	13.85	11.09	12.05	10.34
LSD $_{(0.05)}$	1.80	1.03	1.56	2.12

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.



(a) Beni

(b) Cogane



(c) Kokei

(d) BARI Sweet Potato-12

Plate 2. Callus initiation in different varieties of Sweet Potato at 28 DAI

4.1.2 Days required for shoot regeneration from callus

Significant variations were observed among different concentrations of 2,4-D on days required for shoot regeneration from callus at 5% level of significance as presented in table 2. The maximum days required for shoot regeneration from callus (45.33 days, 49.67 days, 50.67 days and 50.00 days) at V_1, V_2, V_3 and V_4 respectively were recorded in 2,4-D (2.0, 2.0, 1.5 and 2.0) mg/l treatments respectively while minimum (30.33 days, 25.33 days, 34.33 days and 35.67 days) at V_1, V_2, V_3 and V_4 respectively were recorded in the treatment 2,4-D 0.5 mg/l. It was noticed that the Cogane variety (25.33) required minimum days for shoot regeneration from callus. Mishra *et al.* (2006) obtained 4.43 number of shoots shoots on shoot tip explants in MS medium supplemented with 3.0 mg/1 BA and 0.01 mg/l NAA and Sun *et al.* (2007) also reported best shoot regeneration from leaf explants via callus induction in medium with 0.2 mg/l NAA and 1 mg/1 BA.

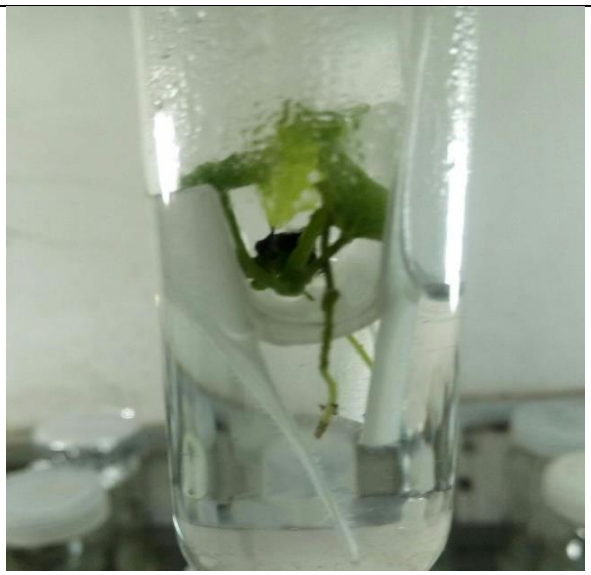
Table 2. Effect of 2,4-D on days required for shoot regeneration from callus

Treatment (2,4-D Concentration)	Days required for shoot regeneration from callus			
	Varieties			
	Beni (V_1)	Cogane (V_2)	Kokei (V_3)	BARI Sweet Potato-12 (V_4)
T_1 = 0.5 mg/l	30.33d	25.33d	34.33c	35.67c
T_2 = 1.0 mg/l	40.67bc	38.00c	39.33b	40.67b
T_3 = 1.5mg/l	42.00b	45.33b	50.67a	48.33ab
T_4 = 2.0 mg/l	45.33a	49.67a	49.67ab	50.00a
CV (%)	13.45	11.45	13.55	10.88
LSD (0.05)	0.32	2.34	2.56	0.32

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.

(a) Shoot initiation from meristem in the Beni variety

(b) Plantlet regeneration in the Beni variety on paper bridge from meristamatic callus



(c) Plantlet regeneration in the Kokei variety on paper bridge from meristamatic callus

Plate 3. Shoot regeneration from callus in different Sweet Potato varieties

4.1.3 Weight of callus (gm)

Significant variations were observed among different concentration of 2,4-D on weight of callus at 5% level of significance as represented in figure 1.1. The highest weight of callus (1.59g, 1.56g, 1.54g and 1.53 g) was recorded at V_1, V_2, V_3 and V_4 respectively in 2,4-D 0.5 mg/l treatment at 7 weeks after callus initiation while minimum weight of callus (1.25 g, 1.29 g, 1.31 g and 1.31 g) at V_1, V_2, V_3 and V_4 respectively were recorded in the treatment 2,4-D 1.5 mg/l. It was noticed that the Beni variety showed the highest weight of callus (1.59 g).

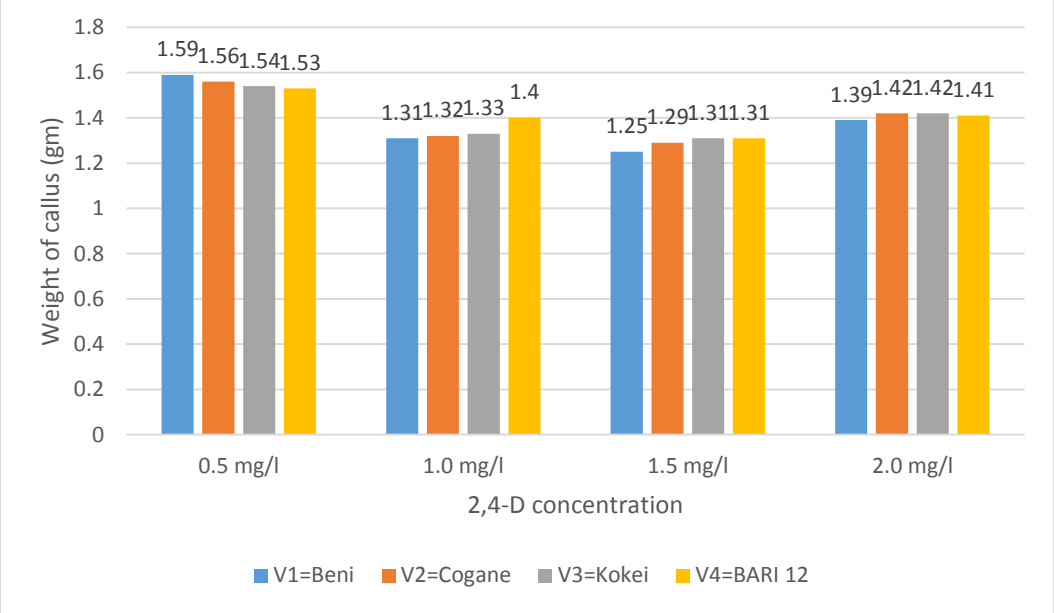


Figure 1.1 Weight of callus in different varieties of Sweet Potato after 7 weeks of callus initiation

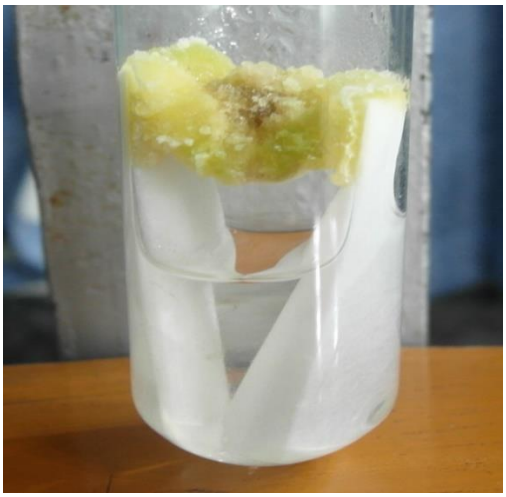


Plate 4. Callus formation from meristem in the paper bridge

4.1.4 Number of shoot from callus at 7 weeks of DAI

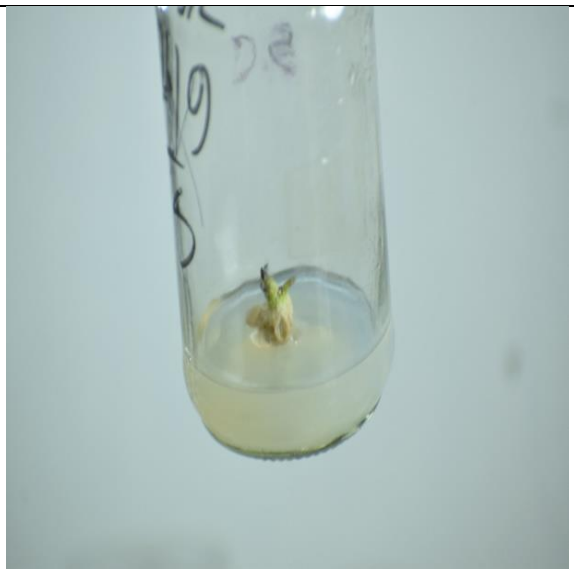
Significant variations were observed among different concentration of 2,4-D on number of shoot from callus after 7 weeks of callus initiation at 5% level of significance as represented in table 3. The highest number of shoot from callus (3.00, 2.67, 4.00 and 2.33) at V_1, V_2, V_3 and V_4 respectively were recorded in 2,4-D 0.5 mg/l treatment while lowest number of shoot from callus (1.67, 1.33, 1.00 and 1.33) at V_1, V_2, V_3 and V_4 respectively were recorded in the treatment 2,4-D 1.5 mg/l. It was noticed that the Kokei variety showed maximum number of callus (4.0). The highest number of shoots per node (2.40 ± 0.11) was obtained on MS medium containing 1.0 mg/l BAP from shoots originally obtained from callus of leaf explants of Awassa-83 (Getu *et al.*, 2012).

Table 3. Effect of 2,4-D on number of shoot from callus at 28 DAI in different varieties of Sweet Potato

Treatment (2,4-D Concentration)	No of shoot from callus after 7 weeks of callus initiation			
	Varieties			
	Beni (V_1)	Cogane (V_2)	Kokei (V_3)	BARI Sweet Potato-12 (V_4)
T_1 = 0.5 mg/l	3.00a	2.67a	4.00a	2.33a
T_2 = 1.0 mg/l	2.33b	2.00ab	1.67bc	2.00b
T_3 = 1.5mg/l	1.67c	1.33c	1.00c	1.33d
T_4 = 2.0 mg/l	2.67bc	2.33b	2.00b	1.67cd
CV (%)	13.86	31.05	11.36	11.74
LSD $_{(0.05)}$	1.09	2.24	1.08	1.08

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.

(a)



(b)

(c)

(d)

Plate 5. Number of shoot per callus in different Sweet Potato varieties after 7 weeks of callus initiation

(a) Number of shoot in Beni variety on 7 weeks after callus initiation

(b) Number of shoot in Cogane variety on 7 weeks after callus initiation

(c) Maximum number of shoot was noticed in Kokei variety on 7 weeks after callus initiation in the treatment 2,4-D 0.5 mg/L

(d) Number of shoot in BARI Sweet Potato-12 variety on 7 weeks after callus initiation

4.2 Sub-experiment 2. Effect of BA on shoot induction potentiality in different varieties of Sweet Potato

The result of the effect of different concentrations of BA has been presented under following headings with Figure 2.1 and Table (4-6). Nodal segments were used as explant.

4.2.1 Days to shoot induction

Significant variations were observed among different concentrations of BA on days to shoot induction at 5% level of significance as represented in table 4. The maximum days to shoot induction (8.67, 10.33, 9.67 and 10.67) was recorded at V_1, V_2, V_3 and V_4 respectively in BA 2.0 mg/l treatment while minimum days to shoot induction (5.67, 7.33, 6.33 and 6.00) was recorded at V_1, V_2, V_3 and V_4 respectively were in the treatment BA 1.0 mg/l. It was noticed that the Beni variety required minimum days (6.67) to shoot initiation. The frequency of shoot regeneration using different concentrations of BAP was influenced by 2,4-D and kinetin used in callus induction medium. Shoot regeneration was observed only on those calli induced on MS medium containing 0.05 mg/l 2,4-D in combination with kinetin (0.5, 1.0, 1.5 mg/l) (Getu *et al.*, 2012).

Table 4. Effect of BA on days to shoot induction in different varieties of Sweet Potato

Treatment (BA Concentration)	Days to shoot induction			
	Varieties			
	Beni (V_1)	Cogane (V_2)	Kokei (V_3)	BARI Sweet Potato-12 (V_4)
T_1 = 0.5 mg/l	6.67bc	8.33bc	7.67b	8.00b
T_2 = 1.0 mg/l	5.67c	7.33c	6.33c	6.00c
T_3 = 1.5mg/l	7.33b	9.00b	7.67ab	9.33ab
T_4 = 2.0 mg/l	8.67a	10.33a	9.67a	10.67a
CV (%)	8.15	8.15	8.08	9.61
LSD (0.05)	1.09	1.09	1.33	1.54

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.

4.2.2 Length of shoot (cm)

Significant variations were observed among different concentrations of BA on length of shoot at 5% level of significance as represented in table 5. The highest length of shoot (7.50 cm, 8.25 cm, 7.90 cm and 8.25 cm) was recorded at V_1, V_2, V_3 and V_4 respectively in BA 1.0 mg/l treatment while minimum length of shoot (5.12 cm, 5.75 cm, 5.03 cm and 6.50 cm) at V_1, V_2, V_3 and V_4 respectively were recorded in the treatment BA 2.0 mg/l. It was noticed that the Cogane and BARI Sweet Potato-12 variety showed the highest length of shoot (8.25 cm). The highest shoot formation frequencies were acquired from calli derived from internode tissue in the MS medium with 1.0 mg/l NAA and 5.0 mg/l BA (Kim *et al.*, 2015).

Table 5. Effect of BA on Length of Shoot in different varieties of Sweet Potato

Treatment (BA Concentration)	Length of shoot (cm)			
	Varieties			
	Beni (V_1)	Cogane (V_2)	Kokei (V_3)	BARI Sweet Potato-12 (V_4)
T_1 = 0.5 mg/l	6.60bc	6.30b	5.92bc	6.90b
T_2 = 1.0 mg/l	7.50a	8.25a	7.90a	8.25a
T_3 = 1.5mg/l	6.80c	6.55c	6.53c	7.50bc
T_4 = 2.0 mg/l	5.12d	5.75d	5.03d	6.50c
CV (%)	3.01	2.77	1.45	3.62
LSD (0.05)	0.32	0.15	1.09	0.20

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.

Plate 6. Highest length of shoot at 28 DAI in BARI Sweet Potato-12 variety in the treatment BA 1.0 mg/l

4.2.3 Number of node per explant

Significant variations were observed among different concentrations of BA on number of node per explant at different DAI at 5% level of significance as represented in table 6. In V_1, the maximum number of shoot (3.60, 7.33 and 9.67) at 14DAI, 21DAI and 28DAI respectively were recorded in BA 1.0 mg/l treatment while minimum number of node (2.33, 4.67 and 6.33) at 14DAI, 21DAI and 28DAI respectively were recorded in the treatment BA 2.0 mg/l. In V_2, the maximum number of node (3.67, 6.67 and 7.60) at 14DAI, 21DAI and 28DAI respectively were recorded in BA 1.0 mg/l treatment while minimum number of node (1.67, 3.33 and 4.33) at 14DAI, 21DAI and 28DAI respectively were recorded in the treatment BA 2.0 mg/l. In V_3, the maximum number of node (3.67, 6.67 and 8.67) at 14DAI, 21DAI and 28DAI respectively were recorded in BA 2.0 mg/l treatment while minimum number of node (1.67, 4.00 and 5.33) at 14DAI, 21DAI and 28DAI respectively were recorded in the treatment BA 2.0 mg/l. In V_4, the maximum number of node (3.33, 6.33 and 8.00) at 14DAI, 21DAI and 28DAI respectively were recorded in BA 2.0 mg/l treatment while minimum number of node (1.33, 2.67 and 4.33) at 14DAI, 21DAI and 28DAI respectively were recorded in the treatment BA 2.0 mg/l. It was noticed that the Beni variety showed the maximum nodes 3.0, 6.0 and 7.67 at 14 days, 21 days and 28 days respectively. The highest percentage of node regeneration from leaf explants was obtained on growth regulators-free

MS medium while the best regeneration from petiole explants was obtained on MS medium supplemented with 1.0 mg/l BAP in both varieties (Getu *et al.*, 2012).

Table 6. Effect of BA on number of node per explant at different days in different varieties of Sweet Potato

(Treatment) BA Concentration	Number of node per explant at different DAI											
	14DAI				21DAI				28DAI			
	V1	V2	V3	V4	V1	V2	V3	V4	V1	V2	V3	V4
T_1 = 0.5 mg/l	3.00a	2.33b	2.33ab	2.33b	6.00b	4.67b	5.33b	4.33b	7.67b	6.33b	7.33b	5.67a
T_2 = 1.0 mg/l	3.67a	3.67a	3.67a	3.33a	7.33a	6.67a	6.67a	6.33a	9.67a	7.67a	8.67a	8.00a
T_3 = 1.5 mg/l	3.00a	2.67ab	2.67ab	2.00bc	6.33ab	4.33bc	5.00b	4.00b	8.67ab	5.67b	6.33b	5.33bc
T_4 = 2.0 mg/l	2.33a	1.67b	1.67b	1.33c	4.67c	3.33c	4.00c	2.67c	6.33c	4.33c	5.33c	4.33c
CV (%)	27.22	22.35	29.57	22.22	11.62	12.15	7.78	11.54	7.14	9.62	8.35	12.12
LSD $_{(0.05)}$	1.53	1.09	0.77	0.94	1.33	1.09	0.77	0.94	1.09	1.09	1.09	1.33

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.

Plate 7. Maximum number of node at 28 DAI in Kokei variety

4.2.4 Number of leaves

Significant variations were observed among different concentrations of BA on number of leaves at 5% level of significance as presented in figure 2.1. The highest number of leaves (5.67, 6.67, 7.33 and 4.33) was recorded at V_1, V_2, V_3 and V_4 respectively in BA 1.0 mg/l treatment while lowest number of leaves (3.33, 3.33, 4 and 2.67) was recorded at V_1, V_2, V_3 and V_4 respectively in the treatment BA 2.0 mg/l. It was noticed that the Kokei variety showed the maximum number of leaves (7.33). The combination of 0.1 mg/l NAA and 2.0 mg/l BA produced the highest regeneration frequencies for the explants of young leaves (Kim *et al.*, 2015).

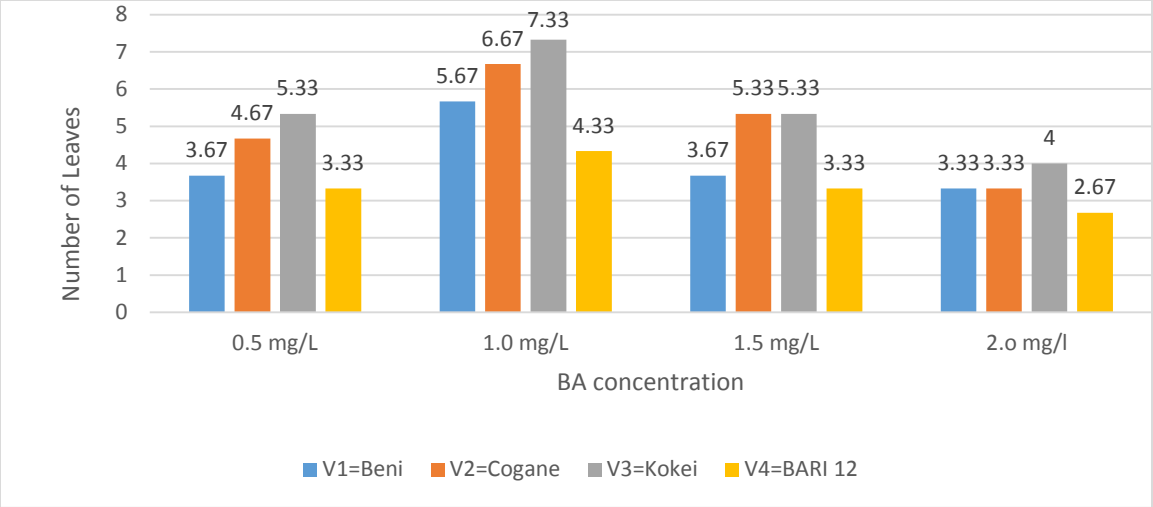


Figure 2.1 Number of leaves in different varieties of Sweet Potato

4.3 Sub-experiment 3. Effect of IBA on root induction potentiality in different varieties of Sweet Potato

The result of the effect of different concentrations of IBA has been presented under following headings with Figure 3.1 and Table 6.

4.3.1 Days to root induction

Significant variations were observed among different concentrations of IBA on days to root induction at 5% level of significance as represented in table 7. The maximum days to root induction (8.67, 9.33, 10.33 and 7.67) was recorded at V_1, V_2, V_3 and V_4 respectively were IBA 0.5 mg/l treatment while minimum days to root induction (6.33, 6.33, 7.67 and 6.33) was recorded at V_1, V_2, V_3 and V_4 respectively were the treatment IBA 1.5 mg/l. It was noticed that all of the Beni, Cogane and BARI SWEET POTATO-12varieties required minimum 6.33 days. Nalini (2012) revealed that minimum 10.33 days were needed for root initiation through shoot tip explants in MS medium supplemented with IBA 1.0 mg/l.

Table 7. Effect of IBA on days to root induction in different varieties of Sweet Potato

Treatment (IBA Concentration)	Days to root induction			
	Varieties			
	Beni (V_1)	Cogane (V_2)	Kokei (V_3)	BARI Sweet Potato-12 (V_4)
T_1 = 0.5 mg/l	8.67a	9.33a	10.33a	7.67a
T_2 = 1.0 mg/l	7.33b	7.67b	8.67ab	7.67a
T_3 = 1.5mg/l	6.33b	6.33c	7.67b	6.33b
T_4 = 2.0 mg/l	7.33b	8.00b	9.33ab	7.33ab
CV (%)	7.78	0.94	10.14	7.96
LSD (0.05)	1.09	0.94	1.72	1.09

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.

4.3.2 Length of root

Significant variations were observed among different concentrations of IBA on length of root at 5% level of significance as represented in figure 3.1. The highest length of root (5.47 cm, 6.2 cm, 6.00 cm and 5.50 cm)) at V_1, V_2, V_3 and V_4 respectively were recorded in IBA 1.5 mg/l treatment while lowest (2.87 cm, 2.53 cm, 2.73 cm and 2.67 cm) at V_1, V_2, V_3 and V_4 respectively were recorded in the treatment IBA 0.5 mg/l. It was noticed that the Kokei variety showed the highest length of root (5.47 cm). Siddique *et al.* (2015) found that *in vitro* rooting in regenerated shoots were best obtained in half-strength MS medium supplemented with 2.0 µM indole-3- butyric acid (IBA).

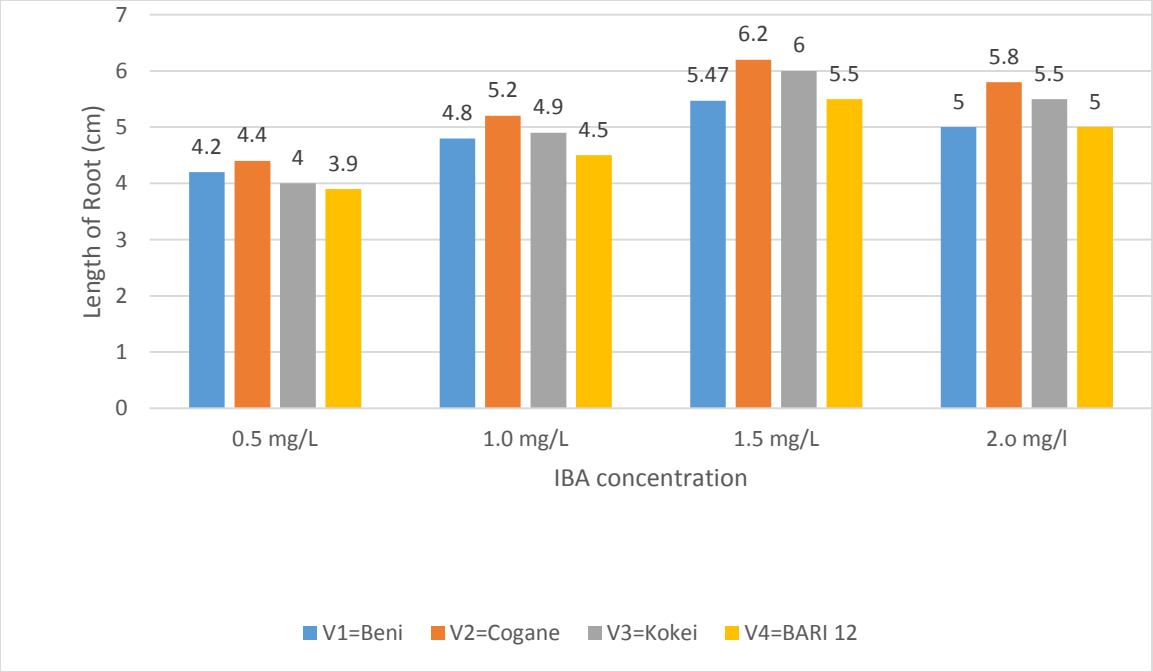


Figure 3.1 Length of root in different varieties of Sweet Potato

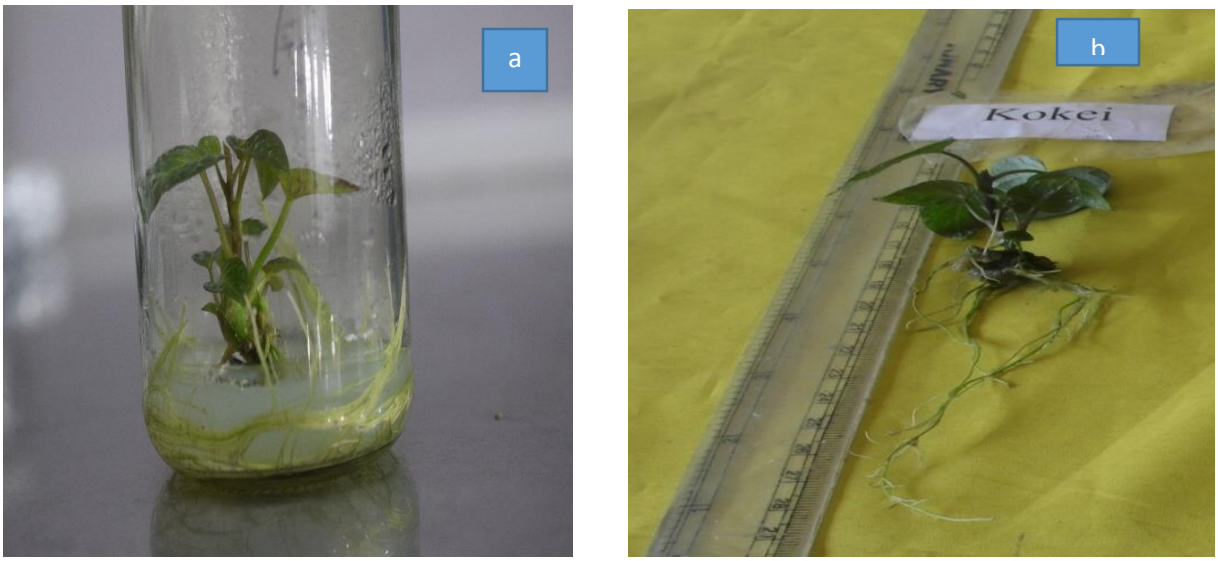


Plate 8. Highest length of root at 28 DAI in Cogane (a) and Kokei (b) variety

4.4 Sub-experiment 4. Combined effect of BA and IBA on shoot and root induction potentiality in different varieties of Sweet Potato

The result of the combined effect of different concentrations of BA and IBA has been presented under following headings with Figure (4.1-4.2) and Table (8-11).

4.4.1 Days to shoot induction

Significant variations were observed among different concentrations of BA and IBA on days to shoot induction at 5% level of significance as represented in table 8. The maximum days to shoot induction (8.33, 8.33, 9.33 and 8.00) at V_1, V_2, V_3 and V_4 respectively were recorded in 1.0 mg/l BA + 2.0 mg/l IBA treatment while minimum days to shoot induction (5.33, 5.67, 7.33 and 5.33) at V_1, V_2, V_3 and V_4 respectively were recorded in the treatment 1.0 mg/l BA + 1.5 mg/l IBA. It was noticed that the BARI Sweet Potato-12 variety showed the minimum days to shoot induction (6.33). Bo *et al.* (2005) obtained adventitious shoots of Chrysanthemum of 93.8% explants on MS media supplemented with 2.0 mg/1 BA and 2.0 mg/1 NAA.

Table 8. Combined effect of BA and IBA on days to shoot induction in different varieties of Sweet Potato

Treatment (IBA Concentration)	Days to shoot induction			
	Varieties			
	Beni (V_1)	Cogane (V_2)	Kokei (V_3)	BARI Sweet Potato-12 (V_4)
T_1= 1.0 mg/l BA + 0.5 mg/l IBA	6.67b	7.67ab	8.33ab	6.33bc
T_2=1.0 mg/l BA + 1.0 mg/l IBA	7.33ab	6.67bc	8.00ab	6.67b
T_3=1.0 mg/l BA + 1.5 mg/l IBA	5.33c	5.67c	7.33b	5.33c
T_4=1.0 mg/l BA + 2.0 mg/l IBA	8.33a	8.33a	9.33a	8.00a
CV (%)	8.35	8.15	10.50	10.74
LSD (0.05)	1.09	1.09	1.63	1.33

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.

4.4.2 Length of shoot (cm)

The result of the experimental finding is presented in figure 4.1. Significant variations were observed among different concentrations of BA and IBA on length of shoot at 5% level of significance. The highest length of shoot (8.10 cm, 7.50 cm, 8.50 cm and 8.90 cm) at V_1, V_2, V_3 and V_4 respectively were recorded in 1.0 mg/l BA + 1.5 mg/l IBA treatment while lowest length of shoot (5.5 cm, 4.7 cm, 5.70 cm and 5.60 cm) at V_1, V_2, V_3 and V_4 respectively were recorded in the treatment 1 mg/l BA + 2 mg/l IBA. It was noticed that the BARI Sweet Potato-12variety showed the highest length of shoot (8.90 cm).

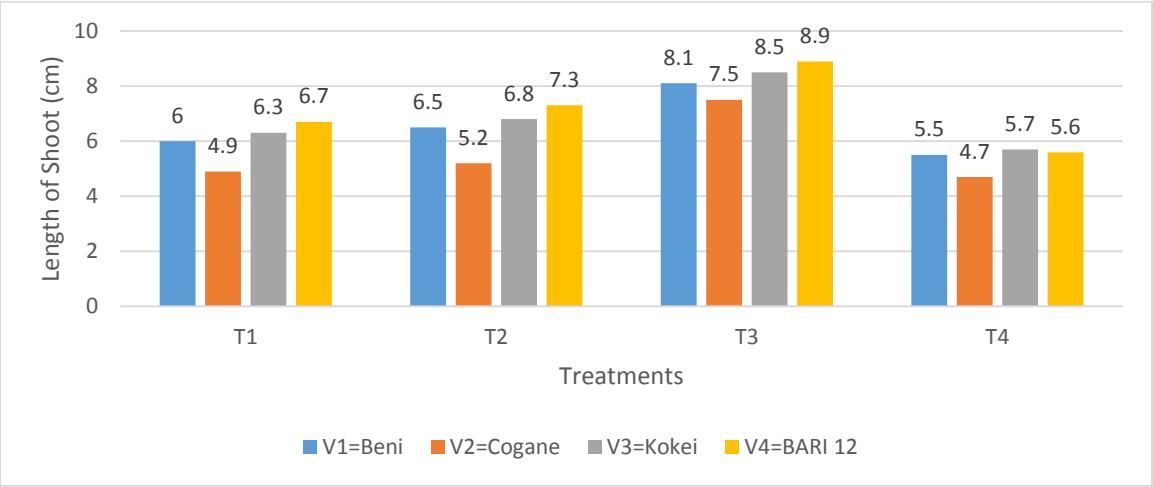


Figure 4.1 Combined effect of BA and IBA on length of shoot in different varieties of Sweet Potato

4.4.3 Number of node per explant

Significant variations were observed among different concentrations of BA and IBA on Number of shoot per explant at different DAI at 5% level of significance. The results were presented in table 9. In the variety Beni (V_1), the maximum number of node (5.00, 8.67 and 10.33) at 14DAI, 21DAI and 28DAI respectively were recorded in 1.0 mg/l BA + 1.5 mg/l IBA treatment while minimum number of node (1.33, 4.33 and 6.67) at 14DAI, 21DAI and 28DAI respectively were recorded in the treatment 1.0 mg/l BA + 2.0 mg/l IBA. In the variety Cogane (V_2), the maximum number of node (3.33, 5.67 and 7.67) at 14DAI, 21DAI and 28DAI respectively were recorded in

2.0 mg/l BA + 1.5 mg/l IBA treatment while minimum number of node (1.33, 3.33 and 4.33) at 14DAI, 21DAI and 28DAI respectively were recorded in the treatment 1.0 mg/l BA + 2.0 mg/l IBA. In the variety Kokei (V_3), the maximum number of node (4.33, 7.33 and 10.33) at 14DAI, 21DAI and 28DAI respectively were recorded in 1.0 mg/l BA + 1.5 mg/l IBA treatment while minimum number of node (1.33, 3.33 and 6.33) at 14DAI, 21DAI and 28DAI respectively were recorded in the treatment 1.0 mg/l BA + 2.0 mg/l IBA. In the variety BARI Sweet Potato-12(V_4), the maximum number of node (4.33, 8.33 and 9.67) at 14DAI, 21DAI and 28DAI respectively were recorded in 1.0 mg/l BA + 1.5 mg/l IBA treatment while minimum number of node (3.00, 5.33 and 6.33) at 14DAI, 21DAI and 28DAI respectively were recorded in the treatment 1.0 mg/l BA + 2.0 mg/l IBA. It was noticed that the Beni variety showed the maximum 5.00, 8.67 and 10.33 number of nodes at 14 days, 21 days and 28 days respectively.

Table 9. Combined effect of BA and IBA on number of node per explant in different varieties of Sweet Potato

Treatments	Number of node per explant											
	14DAI				21DAI				28DAI			
	V1	V2	V3	V4	V1	V2	V3	V4	V1	V2	V3	V4
T1	2.67b	2.00bc	2.67b	2.00c	6.67b	4.67ab	5.33b	6.33bc	8.67b	6.33b	8.33b	7.33bc
T2	3.33b	2.6ab	2.33bc	3.33ab	7.33b	3.67bc	5.33b	6.67b	9.67ab	5.33bc	7.67b	8.00b
T3	5.00a	3.33a	4.33a	4.33a	8.67a	5.67a	7.33a	8.33a	10.33a	7.67a	10.33a	9.67a
T4	1.33c	1.33c	1.33c	3.00bc	4.33c	3.33c	3.33c	5.33c	6.67c	4.33c	6.33c	6.33c
CV (%)	22.93	21.43	21.65	20.38	8.55	21.43	10.83	8.66	6.54	9.76	7.07	9.03
LSD (0.05)	1.33	0.94	1.08	1.21	1.09	1.09	1.08	1.09	1.09	1.09	1.07	1.33

T_1=1.0 mg/l BA + 0.5 mg/l IBA
T_2=1.0 mg/l BA + 1.0 mg/l IBA
T_3=1.0 mg/l BA + 1.5 mg/l IBA
T_4=1.0 mg/l BA + 2.0 mg/l IBA

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference

	
9(a) Mature plantlet of the variety Beni	**9(b) Mature plantlet of the variety Cogane**
	
9(c) Mature plantlet of the variety Kokei	**9(d) Mature plantlet of the variety BARI Sweet Potato-12**

Plate 9. Maximum number of nodes at 28 days in different varieties in the treatment 1.0 mg/l BA + 1.5 mg/l IBA

4.4.4 Number of leaves

Significant variations were observed among different concentrations of BA and IBA on number of leaves at 5% level of significance. The result is shown in table 10. The maximum number of leaves (5.67, 7.00, 6.67 and 6.00) at V_1, V_2, V_3 and V_4 respectively were recorded in 1.0 mg/l BA + 1.5 mg/l IBA treatment while minimum number of leaves (2.00, 2.67, 4.00 and 4.00) at V_1, V_2, V_3 and V_4 respectively were recorded in the treatment 1.0 mg/l BA + 2.0 mg/l IBA. It was noticed that the Cogane variety showed the maximum number of leaves (7.0). Alam *et al.* (2010) found that for optimal micropropagation by node cutting, MS medium supplemented with 3.0mg/l Kn+0.5mg/l GA3 was most effective. More than 75% of these micropropagated plantlets were successfully established and showed new leaf development under soil condition.

Table 10. Combined effect of BA and IBA on number of leaves in different varieties of Sweet Potato

Treatment (IBA Concentration)	Number of leaves			
	Varieties			
	Beni (V_1)	Cogane (V_2)	Kokei (V_3)	BARI Sweet Potato-12 (V_4)
T_1= 1.0 mg/l BA + 0.5 mg/l IBA	3.33b	4.33b	5.67ab	5.00ab
T_2=1.0 mg/l BA + 1.0 mg/l IBA	3.67b	5.33b	5.33b	5.00ab
T_3=1.0 mg/l BA + 1.5 mg/l IBA	5.67a	7.00a	6.67a	6.00a
T_4=1.0 mg/l BA + 2.0 mg/l IBA	2.00c	2.67c	4.00c	4.00b
CV (%)	19.28	14.63	13.05	20.00
LSD (0.05)	1.33	1.33	1.33	1.88

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.

Plate 10. Maximum number of leaves at 28 Days in Cogane variety in the treatment BA 1.0 mg/L + IBA 1.5 mg/l

4.5.5 Days to root induction

Significant variations were observed among different concentrations of BA and IBA on days to root Induction at 5% level of significance. The result is shown in table 11. The maximum days to root induction (9.33, 9.67, 10.00 and 8.67) at V_1, V_2, V_3 and V_4 respectively were recorded in 1 mg/l BA + 2 mg/l IBA treatment while minimum days to root induction (7.33, 6.33, 7.33 and 6.33) at V_1, V_2, V_3 and V_4 respectively were recorded in the treatment 1.0 mg/l BA + 1.5 mg/l IBA. It was noticed that the Beni variety showed the highest weight of callus (1.59 g).

Table 11. Combined effect of BA and IBA on days to root induction in different varieties of Sweet Potato

Treatment (IBA Concentration)	Days to root induction			
	Varieties			
	Beni (V_1)	Cogane (V_2)	Kokei (V_3)	BARI Sweet Potato-12 (V_4)
T_1= 1.0 mg/l BA + 0.5 mg/l IBA	8.67a	7.67b	8.67b	7.33bc
T_2=1.0 mg/l BA + 1.0 mg/l IBA	8.33ab	8.33b	8.67b	7.67ab
T_3=1.0 mg/l BA + 1.5 mg/l IBA	7.33b	6.33c	7.33c	6.33c
T_4=1.0 mg/l BA + 2.0 mg/l IBA	9.33a	9.67a	10.00a	8.67a
CV (%)	6.86	7.22	8.16	7.70
LSD (0.05)	1.09	1.09	1.33	1.08

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.

4.5.6 Length of root

Significant variations were observed among different concentrations of BA and IBA on length of root at 5% level of significance. The highest length of root (9.30 cm, 8.50 cm, 9.90 cm and 8.20 cm) at V_1, V_2, V_3 and V_4 respectively were recorded in 1 mg/l BA + 1.5 mg/l IBA treatment while lowest length of root (6.40 cm, 5.80 cm, 7.0 cm and 5.40 cm) at V_1, V_2, V_3 and V_4 respectively were recorded in the treatment 1 mg/l BA + 2 mg/l IBA. It was noticed that the Kokei variety showed the highest length of root (9.90 cm). Parvin *et al.* (2018) carried out an experiment where the nodal segment explants and different combinations of growth regulators were used for *in vitro* regeneration of Sweet Potato. The highest number of root/plantlet (9.33) and the highest root length (11.13 cm) was observed in the MS medium supplemented with IBA 0.5 mg/l + NAA 0.1 mg/l. The findings of their study seems completely different from our result. This variation may be

because of interaction between genotype, growth regulators and environmental factors. (Sen, J. *et al.*, 2002).

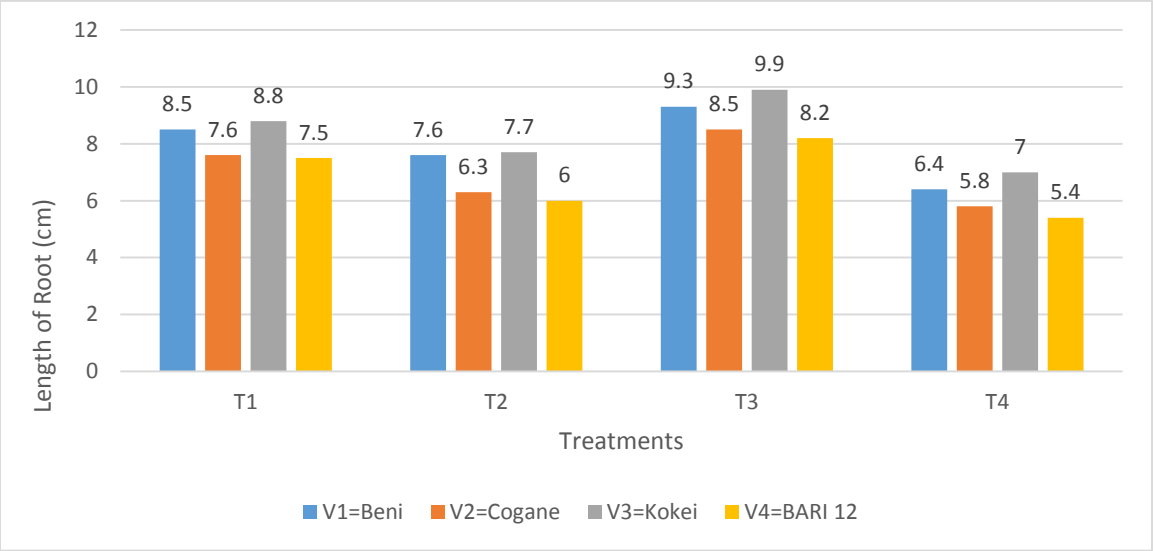


Figure 4.2 Length of root in different varieties of Sweet Potato

Plate 11. Highest length of root at 28 days in different varieties in the treatment BA 1.0 mg/l + IBA 1.5 mg/l

4.5 Sub-experiment 5. Acclimatization and establishment of plantlets on soil

After a satisfactory number of shoot and root development at 7 weeks of culture the individual plantlets were moved from vial carefully without any root damage. The roots were washed with running tap water for removing surplus media. The plantlets were then transplanted into small plastic pot prepared with a standard ratio of cowdung and soil in a shade condition. The plantlets were sprayed occasionally with water for maintaining humidity. At first 30 plants of each variety were hardened in glass house. Among them, 25, 19, 23 and 21 plants survived respectively in Beni, Cogane, Kokei and BARI Sweet Potato-12variety. They were hardened in netting condition and 23, 16, 20 and 18 plants survived respectively in Beni, Cogane, Kokei and BARI Sweet Potato-12variety. So, in glass house survival rate was 83.33%, 63.33%, 76.66% and 70.00% at V_1, V_2, V_3 and V_4 respectively and in netting condition, survival rate was 92.00%, 84.21%, 86.95% and 85.71% at V_1, V_2, V_3 and V_4 respectively. Finally in open atmospheric condition the plants were transplanted in the main field. Getu *et al.* (2012) found that after one month of acclimatization in glasshouse, 90% and 100% of plantlets originally derived from shoots of petiole and leaf of beletech, respectively, survived. Similarly, 80% and 90% of plantlets originally derived from shoots of petiole and leaf of Awassa-83, respectively, survived. So, analyzing the survival rate it can be said that acclimatization potentiality of Sweet Potato was satisfactory.

Table 11. Survival rate of *in vitro* regenerated plantlets of Sweet Potato

Sl No	**Variety**	Hardening in Glass house			Hardening in Netting Condition		
		No. of plantlets transferred in plastic pot	No. of survived plantlets	Survival rate (%)	No. of plantlets transferred	No of seedlings established	Survival rate (%)
1	**V_1 = Beni**	30	25	83.33	25	23	92.00
2	**V_2=Cogane**	30	19	63.33	19	16	84.21
3	**V_3 =Kokei**	30	23	76.66	23	20	86.95
4	**V_4 = BARI Sweet Potato-12**	30	21	70.00	21	18	85.71

Plate 12. Hardening of Kokei variety plantlet in plastic pot

CHAPTER V

SUMMARY AND CONCLUSIONS

The present research was carried out in Biotechnology Laboratory of the Department of Biotechnology, Sher-e-Bangla Agricultural University, Dhaka-1207 from the period of June 2019 to June 2020. The nodal segments of four varieties (V_1=Beni, V_2=Cogane, V_3=Kokei and V_4= BARI Sweet Potato 12) of *Ipomoea batatas (L.)* Lam were used as experimental materials in the present investigation. Four levels of 2,4-D (0.5, 1.0, 1.5 and 2.0 mg/l) were used for callus induction. BA 2.0 mg/l (0.5, 1.0, 1.5 and 2.0 mg/l), was used to study shoot induction potentiality. Four levels of IBA (0.5, 1.0, 1.5 and 2.0) were used to study root induction potentiality. Combined effect of BA and IBA on shoot and root induction potentiality was also investigated in Sweet Potato regeneration. The experiments were arranged in completely randomized design (CRD) with 5 replications.

The maximum 15.33 days (V_1), 13.67 days (V_2), 11.00 days (V_3) and 13.33 days (V_4) were recorded in 2,4-D 1.5 mg/l treatment while minimum 10.66 days(V_1), 11.67 days (V_2), 3.67 days (V_3) and 3.33 days (V_4) were recorded in the treatment 2,4-D 0.5 mg/l. The highest number of shoot from callus (3.00, 2.67, 4.00 and 2.33) at V_1, V_2, V_3 and V_4 respectively were recorded in 2,4-D 0.5 mg/l treatment while lowest number of shoot from callus (1.67, 1.33, 1.00 and 1.33) at V_1, V_2, V_3 and V_4 respectively were recorded in the treatment 2,4-D 1.5 mg/l.

The maximum days to shoot induction (8.67, 10.33, 9.67 and 10.67) at V_1, V_2, V_3 and V_4 respectively were recorded in BA 2.0 mg/l treatment while minimum days to shoot induction (5.67, 7.33, 6.33 and 6.00) at V_1, V_2, V_3 and V_4 respectively were recorded in the treatment BA 1.0 mg/l. The highest length of shoot (4.50 cm, 3.20 cm, 4.27 cm and 3.57 cm) at V_1, V_2, V_3 and V_4 respectively were recorded in BA 1.0 mg/l treatment while minimum length of shoot at V_1, V_2, V_3 and V_4 respectively were recorded in the treatment BA 2.0 mg/l. The highest number of leaves (5.67, 6.67, 7.33 and 4.33) at V_1, V_2, V_3 and V_4 respectively were recorded in BA 1.0 mg/l

treatment while lowest number of leaves (3.33, 3.33, 4 and 2.67) at V_1, V_2, V_3 and V_4 respectively were recorded in the treatment BA 2.0 mg/l.

The maximum days to root induction (8.67, 9.33, 10.33 and 7.67) at V_1, V_2, V_3 and V_4 respectively were recorded in IBA 0.5 mg/l treatment while minimum days to root induction (6.33, 6.33, 7.67 and 6.33) at V_1, V_2, V_3 and V_4 respectively were recorded in the treatment IBA 1.5 mg/l. The highest length of root (5.47 cm, 6.2 cm, 6.00 cm and 5.50 cm)) at V_1, V_2, V_3 and V_4 respectively were recorded in IBA 1.5 mg/l treatment while lowest (2.87 cm, 2.53 cm, 2.73 cm and 2.67 cm) at V_1, V_2, V_3 and V_4 respectively were recorded in the treatment IBA 0.5 mg/l.

The maximum days to shoot induction (8.33, 8.33, 9.33 and 8.00) at V_1, V_2, V_3 and V_4 respectively were recorded in 1.0 mg/l BA + 2.0 mg/l IBA treatment while minimum days to shoot induction (5.33, 5.67, 7.33 and 5.33) at V_1, V_2, V_3 and V_4 respectively were recorded in the treatment 1.0 mg/l BA + 1.5 mg/l IBA. The highest length of shoot (8.10 cm, 7.50 cm, 8.50 cm and 8.90 cm) at V_1, V_2, V_3 and V_4 respectively were recorded in 1 mg/l BA + 1.5 mg/l IBA treatment while lowest length of shoot (5.50 cm, 4.70 cm, 5.70 cm and 5.60 cm) at V_1, V_2, V_3 and V_4 respectively were recorded in the treatment 1 mg/l BA + 2 mg/l IBA. The maximum number of leaves (5.67, 7.00, 6.67 and 6.00) at V_1, V_2, V_3 and V_4 respectively were recorded in 1.0 mg/l BA + 1.5 mg/l IBA treatment while minimum number of leaves (2.00, 2.67, 4.00 and 4.00) at V_1, V_2, V_3 and V_4 respectively were recorded in the treatment 1.0 mg/l BA + 2.0 mg/l IBA.

The maximum days to root induction (9.33, 9.67, 10.00 and 8.67) at V_1, V_2, V_3 and V_4 respectively were recorded in 1 mg/l BA + 2 mg/l IBA treatment while minimum days to root induction (7.33, 6.33, 7.33 and 6.33) at V_1, V_2, V_3 and V_4 respectively were recorded in the treatment 1.0 mg/l BA + 1.5 mg/l IBA. The highest length of root (3.47 cm, 3.33 cm, 3.30 cm and 2.60 cm) at V_1, V_2, V_3 and V_4 respectively were recorded in 1 mg/l BA + 1.5 mg/l IBA treatment while lowest length of root (2.63 cm, 2.50 cm, 2.60 cm and 2.03 cm) at V_1, V_2, V_3 and V_4 respectively were recorded in the treatment 1 mg/l BA + 2 mg/l IBA.

In glass house survival rate was (83.33%, 63.33%, 76.66% and 70.00%) at V_1, V_2, V_3 and V_4 respectively and in netting condition, survival rate was (92.00%, 84.21%, 86.95% and 85.71%) at V_1, V_2, V_3 and V_4 respectively.

So, finally it can be concluded that, a convenient protocol of rapid regeneration of Sweet Potato is established. From the above summary, the results of the present study indicated that Sweet Potato could be successfully micro propagated with 1.0 mg/l BA for rapid shoot regeneration and proliferation and 0.5 mg/l 2,4-D for callus induction. BA 1.0 mg/l with IBA 1.5 mg/l showed the good performance for shoot and root induction and IBA 1.5 mg/l alone comparatively gave the best performance in case of root. Considering the finding of the study MS medium supplemented with 1.0 mg/l BA+1.5 mg/l IBA showed the best response for shoot and root formation.

RECOMMENDATIONS

Based on the summary and conclusions following recommendations can be made:

i. For further study BAP, KIN, NAA and IAA etc. types of cytokinin and auxin can be used for more trial.
ii. Except nodal segment other explants like shoot tip, petiole, leaf and root portion could be practiced for culture.
iii. For callus induction, NAA or other callus induction hormone could be used individually or in combine dose for large number of shoot induction.
iv. Influence of other factors (elicitors, antioxidants) such as ascorbic acid, activated charcoal should be considered.
v. More research can be done with more varieties of Sweet Potato.

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APPENDICES

Appendix I. Analysis of variance (ANOVA) of effect of 2,4-D on days to callus initiation and weight of callus

Source of variance	d.f.	Days to Callus Initiation				Weight of callus			
		Varieties				Varieties			
		Beni	Cogane	Kokei	BARI SWEET POTATO-12	Beni	Cogane	Kokei	BARI Sweet Potato-12
Treatments	3	1.444**	3.194**	7.132**	3.234**	0.069**	0.045**	4.111**	5.873**
Error	8	0.333	0.583	0.202	1.213	0.0008	0.0006	1.416	0.002
Total	11								

**= Significant at 1% level of Probability.

Appendix II. Analysis of variance (ANOVA) of effect of on days required for shoot regeneration from callus

Source of variance	d.f.	days required for shoot regeneration fron callus			
		Varieties			
		Beni	Cogane	Kokei	BARI Sweet Potato-12
Treatments	3	16.333**	9.323**	7.323**	10.898**
Error	8	0.334	0.246	0.076	0.243
Total	11				

**= Significant at 1% level of Probability.

Appendix III. Analysis of variance (ANOVA) of effect of 2,4-D on number of shoot from callus at 28 days

Source of variance	d.f.	Number of shoot from callus at 28 days			
		Varieties			
		Beni	Cogane	Kokei	BARI Sweet Potato-12
Treatments	3	10.333**	9.223**	5.323**	7.898**
Error	8	0.333	0.203	0.003	0.222
Total	11				

**= Significant at 1% level of Probability.

Appendix IV. Analysis of variance (ANOVA) of effect of different concentration of BA on days to shoot initiation and length of shoot

Source of variance	d.f.	Number of shoot from callus at 28 days			
		Varieties			
		Beni	Cogane	Kokei	BARI Sweet Potato -12
Treatments	3	10.333**	9.223**	5.323**	7.898**
Error	8	0.333	0.203	0.003	0.222
Total	11				

**= Significant at 1% level of Probability.

Appendix V. Analysis of variance (ANOVA) of effect of different concentration of BA on number of node per explant at 14 and 21 days

Source of variance	d.f.	NS14				NS21			
		Varieties				Varieties			
		Beni	Cogane	Kokei	BARI SWEET POTATO-12	Beni	Cogane	Kokei	BARI Sweet Potato -12
Treatments	3	2.210**	2.814**	2.950**	2.956**	1.473**	2.780**	8.853**	3.875**
Error	8	0.007	0.011	0.012	0.176	0.150	0.167	0.233	0.203
Total	11								

**= Significant at 1% level of Probability.

Appendix VI. Analysis of variance (ANOVA) of effect of different concentration of BA on number of node per explant at 28 days and number of leaves

Source of variance	d.f.	Number of node per explant				Number of leaves			
		Varieties				Varieties			
		Beni	Cogane	Kokei	BARI SWEET POTATO-12	Beni	Cogane	Kokei	BARI Sweet Potato -12
Treatments	3	2.947**	2.956**	3.197**	5.946**	0.288**	0.821**	1.401**	2.857**
Error	8	0.107	0.739	0.005	0.846	0.077	0.331	0.350	0.395
Total	11								

**= Significant at 1% level of Probability.

Appendix VII. Analysis of variance (ANOVA) of effect of different concentration of IBA on days to root induction and length of root

Source of variance	d.f.	Days to root induction				Length of root			
		Varieties				Varieties			
		Beni	Cogane	Kokei	BARI SWEET POTATO-12	Beni	Cogane	Kokei	BARI Sweet Potato -12
Treatments	3	1.986**	1.978**	2.552**	1.068**	1.475**	2.957**	5.587**	2.068**
Error	8	0.020	0.016	0.012	0.587	0.957	0.896	0.247	0.036
Total	11								

**= Significant at 1% level of Probability.

Appendix VIII. Analysis of variance (ANOVA) of combined effect of different concentration of BA and IBA on days to shoot induction and number of node at 14 days

Source of variance	d.f.	Nays to shoot induction				Number of node at 14 days			
		Varieties				Varieties			
		Beni	Cogane	Kokei	BARI SWEET POTATO-12	Beni	Cogane	Kokei	BARI Sweet Potato -12
Treatments	3	3.967**	2.8956**	1.954**	4.107**	1.056**	2.654**	1.857**	1.058**
Error	8	0.297	0.048	0.108	0.857	1.047	0.587	0.016	0.968
Total	11								

**= Significant at 1% level of Probability.

Appendix IX. Analysis of variance (ANOVA) of combined effect of different concentration of BA and IBA on number of node at 21 days and number of node at 28 days

Source of variance	d.f.	number of node at 21 days				number of node at 28 days			
		Varieties				Varieties			
		Beni	Cogane	Kokei	BARI SWEET POTATO-12	Beni	Cogane	Kokei	BARI Sweet Potato -12
Treatments	3	1.211**	3.814**	1.950**	4.956**	1.473**	5.780**	7.853**	1.875**
Error	8	0.009	0.016	0.017	0.172	0.159	0.162	0.236	0.201
Total	11								

**= Significant at 1% level of Probability.

Appendix X. Analysis of variance (ANOVA) of combined effect of different concentration of BA and IBA on number of leaves

Source of variance	d.f.	Number of leaves			
		Varieties			
		Beni	Cogane	Kokei	BARI Sweet Potato -12
Treatments	3	2.115**	3.853**	5.868**	3.867**
Error	8	0.020	0.026	0.263	0.036
Total	11				

**= Significant at 1% level of Probability.

Appendix XII. Analysis of variance (ANOVA) of combined effect of different concentration of BA and IBA on days to root induction and length of root

Source of variance	d.f.	days to root induction				length of root			
		Varieties				Varieties			
		Beni	Cogane	Kokei	BARI SWEET POTATO-12	Beni	Cogane	Kokei	BARI Sweet Potato -12
Treatments	3	1.473**	2.780**	8.853**	5.174**	2.660**	5.540**	7.760**	4.698**
Error	8	0.150	0.167	0.233	0.295	0.0220	0.220	0.180	0.046
Total	11								

**= Significant at 1% level of Probability.