In vitro regeneration of three local potato (*Solanum tuberosum* L.) varieties of Bangladesh

A DISSERTATION SUBMITTED TO THE BRAC UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BIOTECHNOLOGY

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Dedicated to…

My teachers, Parents

and all of my family members.

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Fazlima Parveen

(Fazlima Parveen)

September, 2011

Certificate

This is to certify that the research work embodying the results reported in this thesis entitled "In vitro regeneration of three local potato (Solanum tuberosum L.) varieties of Bangladesh." Submitted by Fazlima Parveen, has been carried out under my supervision in the Biotechnology Laboratory, Biotechnology Division, Bangladesh Agricultural Research Institute (BARI). It is further certified that the research work presented here is original and suitable for submission for the partial fulfillment of the degree of Master of Science in Biotechnology, BRAC University, Dhaka.

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CONTENTS

ABSTRACT

The present study was conducted to develop an efficient and reliable protocol for *in vitro* regeneration of potato varieties. Experiments were conducted with a view to standardize *in vitro* regeneration of three local potato varieties, viz. Zaubilati, Shadaguti and Challisha. Newly sprouted potato varieties were collected and surfaces sterilized with 0.1% (w/v) $HgCl_2$ for 1 minute and were cultured on PROP medium for sprout regeneration. Explants, such as, shoot apex, nodal segment, internode and leaf were collected from these *in vitro* grown shoots and the effect of hormonal treatments viz. cytokinins (BAP and Kn) with or without auxins (GA_3, IAA and IBA) were observed for *in vitro* direct shoot regeneration and root induction. For shoot apex explant, the minimum days required for shoot initiation, the highest shoot length, number of leaves per plantlet, and fresh weight of shoot per plantlet were recorded at Shadaguti variety in PROP medium supplemented with 1.5 mg/l BAP. In case of Kn, no single concentration was found best for all regeneration parameters, but the regeneration frequency of potato varieties in PROP with 0.5 mg/l Kn showed better than BAP treatments. For nodal segment explant, PROP medium supplemented with 1.0 mg/l BAP and 0.5 mg/l Kn found the best concentrations for all regeneration parameters of Shadaguti and Zaubilati varieties but without BAP and Kn supplements Challisha showed better response. For internode explant, Shadaguti variety respond better than other varieties, when cultured in PROP medium supplemented with 1.0 mg/l BAP along with GA_3 (0.2 mg/l) and IAA (0.5 mg/l), while Kn along with GA_3 (0.2 mg/l) and IAA (0.5 mg/l) produced only direct root. For leaf explants, all the regeneration parameters of Shadaguti variety showed better response with PROP medium supplemented with 0.5 - 1.0 mg/l BAP along with GA_3 (0.2 mg/l) and IAA (0.5 mg/l). On the other hand, Kn along with GA_3 (0.2 mg/l) and IAA (0.5 mg/l) showed only direct root regeneration. Depending on the above four explants, shoot apex of potato showed better performance than nodal segment explant and the appropriate concentration was 1.5 mg/l BAP and Shadaguti variety showed the best genotype for *in vitro* shoot regeneration. Similar to shoot apex, internode of potato showed better than leaf explant and PROP medium supplemented with 1.0 mg/l BAP +

0.2 mg/l GA_3 + 0.5 mg/l IAA was the best combination while the best genotype was Shadaguti variety for direct *in vitro* shoot regeneration. For root induction, among the four different treatments, ½ PROP medium supplemented with 0.5 mg/l IBA performed the best. Rooted plantlets were established after proper hardening and acclimatization in poly bags containing garden soil, sand and decomposed cow dung at the ratio of 1:1:1. After transplantation 50% plantlets survived in the natural environment.

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Abbreviation

Abbreviation	Full form
BAP	6-benzylaminopurine
cm	Centimeter (s)
°C	Degree Celsius
EDTA	Ethylene diamine tetra acetic acid
et al.	*et alia* = and other people
GA_3	Gibberellic acid
gm	Gram
g/l	Gram per liter
HCl	Hydrochloric acid
$HgCl_2$	Mercuric chloride
IAA	Indole 3 acetic acid
IBA	Indole 3 butyric acid
Kn	Kinetin
mg	Milligram
mg/ l or mg l^{-1}	Milligram per liter
ml	Milliliter
min	Minutes
MS	Murashige and Skoog
N	Normal
NAA	Naphthalene acetic acid
NaOH	Sodium hydroxide
PGR	Plant growth regulator
p^H	Negative logarithm of Hydrogen ion (H^+)
w/v	Weight by volume
2,4-D	2,4-dichlorophenoxy acetic acid

Chapter 1

Introduction

CHAPTER – 1
INTRODUCTION

Potato is the edible tuber of the cultivated plant *Solanum tuberosum* L. of the family *Solanaceae*. It was the major crop for the original Americans. It is now one of the staple foods in Bangladesh. Its history is difficult to trace, partly because the name potato was also used by early writers for the sweet potato (*Ipomoea batatas*) and for other unrelated plants. Spanish explorers are believed to have brought it in the 16th century from Peru to Spain, whence it spread North and West throughout Europe. European settlers brought it to North America probably around 1600 AD. Thus, like the closely related tomato, it was a food crop reintroduced to the New World. Potato was first accepted as a large-scale crop in the British Isles. It became the major food in Ireland during the 18th century and is hence often called Irish potato to distinguish it from the sweet potato. Potato was also important for 20th century Europe, especially for Germany, where it kept the country alive during 2nd world wars (Das and Khair, 2006).

In Bangladesh, potato represents about 53% of the total edible vegetables. In terms of total production it ranks first among the vegetables (BBS, 2007). Potato has a great demand throughout the year, but its production is concentrated during the months of January to March in Bangladesh. During the lean period of vegetables in Bangladesh potato plays a vital role. Popularity of potato is increasing for its various preparations like chips and french fry in different processing industries and fast food. It is the most important non- cereal food crop and fourth in terms of total global food production after maize, wheat and rice (Chakraborty *et al.*, 2000).

1.1 Potato and potato plant

It is not known exactly when potato was introduced in this subcontinent. It is assumed that at the beginning of the 17th century the Portuguese navigators first brought potato to India. The first record about the cultivation of potato in India is seen in an 1847 issues of The Gardening Monthly, a magazine published from London. Initially, potato was cultivated in areas around Calcutta, from there its cultivation spread to Cherapunjee. When Warren Hastings was the Governor (1772-1785), potato

cultivation spread too many provinces of India, including Bombay, through his initiatives (Das and Khair, 2006).

The potato plant is an herbaceous annual, normally propagated by planting pieces of tubers that bear two or three eyes. Nutritionally, the tuber is rich in carbohydrates or starch and is a good source of protein, vitamin C and B, potassium, phosphorus, and iron. Most of the minerals and protein are concentrated in a thin layer beneath the skin, and the skin itself is a source of food fibre (Sharfuddin *et al.*, 1985).

1.2 Varieties

Several hundred varieties of potatoes are grown in the world. These differ in appearance, tuber structure, size, color, time of maturity, cooking & marketing qualities, yield and resistance to pests. A variety that grows well in one area may do poorly in another. Potato varieties that are cultivated in Bangladesh are broadly categorized into two groups, local and high yielding. The so-called local varieties are in fact, not strictly native. In the distant past those were brought to this part of the subcontinent but in the absence of varietals improvement efforts, gradually degenerated and showing poor yield performance. In spite of poor yields, some of the local varieties are still being cultivated because of their taste and cooking qualities (Das and Khair, 2006).

1.2.1 Local varieties

There are about 27 local varieties of potato cultivated in different parts of Bangladesh. They have familiar local names. It is estimated that local varieties were cultivated in about 5, 20,447 acres of land, producing 92,36,838 tons of tubers during 2007-08 (DAE, 2009). The familiar local varieties are (a) Zaubilati - mostly cultivated in Rangpur. The tuber is rounded, reddish. Each tuber weight about 30 g. (b) Shadaguti - primarily cultivated in Rangpur with tubers rounded, yellowish, each is having a weight of about 55 g. (c) Challisha - mostly cultivated in Dhaka and Narayangonj with tubers rounded, reddish, each having a weight of about 30 g. (d) Sheel Bilatee- cultivated in Rangpur with tuber oblong, reddish, each tuber weight about 35 g. (e) Lal Sheel- cultivated in Bogra with tubers rounded, reddish, each having a weight of about 55 g. This variety is also known as Lal Madda and Bograi. (f) Lal Pakri - cultivated widely in Dinajpur, Bogra and Sirajganj districts with tubers reddish and

round, each weighing about 30 g. (g) Du Hajari - mostly cultivated in the Chittagong area. Tubers appear round and pale, each weighing about 25 g. Among other indigenous varieties Ausha, Hagrai, Indurkani, Surzamukhi are notable.

1.2.2 High yielding varieties (HYV)

In the last few decades, several dozens of high yielding varieties (HYV) of potato were brought to Bangladesh and tried experimentally under local conditions before being recommended for general cultivation (B.B.S. 2007). During the 1970s, about 16 varieties were initially selected for cultivation in the country. There are huge amount of potato seeds are imported every year by the Bangladesh Agricultural Development Corporation (BADC) for distribution among farmers. Bangladesh Agricultural Research Institute (BARI) has also established a farm at Debiganj in Panchagar district for production of HYV seed potatoes. Among the high yielding popular varieties the following are notable: Cardinal, Diamant and Kufri Shindhury. Other notable exotic varieties are Patrones, Alpha, Archa, Multa, Ukama, Hira, Maurin, Origo, Dheera, Granola, Asterix, Felsina, Meridian, Courage, Lady Rosetta, Espirit, Remarka, Voyager, Almera, Ultra, Baraka, Jarla, Binela, Ailsa, Provento, Raja, Saikat etc.

In recent years, the Tuber Crops Research Centre of BARI has collected many new varieties of potato from the International Potato Research Centre, Peru, and from other sources. These are being tested under Bangladesh field conditions, to determine whether they can be recommended for cultivation in the country. The Centre has already made good contribution towards the development of some high yielding potato varieties.

1.3 Cultivation

Potato is widely cultivated in all the districts of Bangladesh during winter. For local and high yielding varieties 5, 20,447 hectors of land were used for potato cultivation during 2007-08 (DAE, 2009; Uddin *et al.*, 2010). Well-fertilized, sunny land with sufficient moisture in soil is appropriate for potato plantation. The first fortnight of November is the right time. In certain Northwestern areas, farmers even plant potato in October to harvest the crop early. Virtually all potatoes in this country are planted manually. On the basis of the soil quality and potato variety the spacing between the

seed tubers and the adjacent rows were determined. The seed tuber to tuber spacing range from 15 to 20 cm and row to row range from 45 to 60 cm (BARI recommended). Optimum depth of planting depends on temperature and moisture of the soil, probable weather following planting, and mode of conducting field operations later. If planting is shallow and only about 5 cm deep, the soil must be gradually ridged over the row incidental to cultivation. This ensures that the developing tubers are well covered with soil to protect them from light and pests. Mulching is frequently done over the rows with water hyacinth, straw etc. to preserve the soil moisture and to prevent the growth of weeds.

1.4 Production

As the potato plants become mature and the tubers are fully formed, the leaves become gradually yellowish and then brownish, and finally the plants die. It is always better to harvest the crop after these signs are evident in the field. Most varieties are harvested in this country during February-March. Collection of the tubers is usually done in Bangladesh manually using a spade or other devices. Most farmers preserve potatoes, particularly local varieties, at home indigenously. Consequently, loss due to dehydration, pest attack and infection by pathogenic organisms is substantial. There are 193 cold storages in the country with installed capacity of about 7,40,000 m tons (B.B.S. 2010)

In Bangladesh potato is grown in an area of about 3, 36,740 acres. For this purpose about 1,50,000 m tons of seed potatoes are necessary. Most of the seeds used are not of high quality. The farmers generally use the tubers they keep for their own consumption as seeds. This results in poor yield in the following season.

Table 1: Area and production of potato during 2007-08 to 2008-09

Potato stat. 2007-08	**Potato stat. 2008-09**
Total Area : 5, 20,447 hac.	Total Area : 4,64,035 hac
Totao Production : 92,36,838 ton Yield : 17.75 t/hac.	Totao Prodduction : 68,94018 ton Yield : 14.86 t/hac.

Source: DAE, 2009; (Uddin *et al.*, 2010)

1.5 Uses

In Bangladesh, potato is primarily used as a vegetable, although in many countries of the world it constitutes the staple food and contributes more than 90% of the carbohydrate food source. Millions of tons of potatoes are processed annually in Europe into starch, alcohol, potato meal, flour, dextrose and other products. Some are processed into potato chips, dehydrated mashed potatoes, French fries and canned potatoes. Large quantities of potatoes in the Netherlands, Ireland, Germany and other countries of Europe are grown specifically for manufacture of alcohol, starch, potato meal or flour, and for livestock feeding. Asian countries consume more rice than potato for carbohydrate foods (Das and Khair, 2006).

In Bangladesh, although the principal use of potatoes is to make potato curry along with fish, meat, and eggs, there exists a great diversity in the consumption of potatoes. Notable among potato-based food items are the boiled potato, fried potato, mashed potato, baked potato, potato chop, potato vegetable mix, potato singara, potato chips, french fry etc. In recent years, bakeries and fast food shops have started preparing a wide variety of potato-based food delicacies.

1.6 Pests

The potato plant is attacked by several dozens insect, mite, and nematode pests and under ecological conditions favorable to them, these may inflict heavy damage to the growing crop. The following, however, probably cause most of the damage: cutworm, crickets, leafhoppers, potato tuber worm, aphids, flea beetles, root knot nematode, and golden nematode. Local varieties, particularly the Lal Pakhari, are very susceptible to their attacks. There are instances of 80% damage in the farmer's home stored potatoes in some locations. Among nematodes, the root knot nematode (*Meloidogyne* species) and the golden or cyst nematodes (*Heterodera* species) cause damage to roots and tubers.

1.7 Diseases

Potatoes suffer from various diseases which are classified according to their causal agents, such as virus, bacteria, fungus, and nematodes. Some non-parasitic diseases or physiological diseases caused by environmental factors or physiological deficiencies are also noticeable. From 1845 to 1847 in Northern Europe, absolute demolition of

potato crop in Ireland caused emigration of one and half million Irish people to USA because of Ilesh famine. The famine was caused by a fungal disease called late blight disease. Late blight is the most serious and widespread of all potato diseases. The causal organism is *Phytopthora infestans*, a parasitic fungus. The first signs of the disease are brownish to black lesions on any portions of the plant tops, principally on the leaves. There are also other important fungal diseases of potato are the early blight, black scurf and stem rot. Early blight, caused by *Alternaria solani*, is also a serious disease of potato in Bangladesh. It appears first as dark brown to black spots on the leaves. The spots are usually irregular. Often several spots coalesce to form large patches, resulting in the leaf blight. When the spots are numerous leaves die.

Among the viral diseases, the Mild mosaic, Rugose mosaic, and Latent mosaic diseases are important. Symptoms of mosaic diseases include mottling of leaves with different shades and spots, necrosis and curling. Often, the disease does not exhibit any appreciable symptom. The milder strains of the latent mosaic produce no visible symptoms.

The notable bacterial diseases of potato of this country are the Blackleg, Brown rot, Bacterial wilt, and Ring rot. The Blackleg is caused by *Erwinia atroseptica*, affects growing plants, and tubers in storage; it is so named because the base of the stem becomes shriveled and blackened. Brown rot is caused by *Pseudomonas solanacearum*. This disease causes the leaves to wilt, shrivel, and finally death of the plant. The disease is also seen in many other tropical countries, and besides potato, tomato, brinjal, chilli, tobacco etc. are also affected.

1.8 Prevention

The best way to prevent or reduce the incidence of potato diseases is the use of disease-free seeds. Seed materials must be examined carefully prior to planting, and if required, seeds should be treated by dipping in recommended chemicals. It is always advisable to use certified disease-free seed potato and disinfected seed cutting knives.

1.9 Alternative way of potato production

Potato is usually propagated asexually by mean of tubers. However, with conventional method of vegetative propagation, potatoes are often prone to pathogen such as fungi, bacteria and viruses, thereby resulting in poor quality and yields. Protection against diseases caused by pathogenic organisms like fungi, bacteria and viruses are a major challenge for crop improvement. Moreover, the most important class of genes that has been used by breeders for diseases control is the plant resistance (R) genes. The insertion of R- gene into elite cultivars via traditional breeding can take up to 10-15 years (Janks *et al.*, 2007). This process can be considerable shortened by introducing these genes into crop plants by genetic transformation using biotechnological approaches (Rommans and Kishore, 2000).

Plant tissue culture techniques have been developed as a modern and worldwide accepted concept to improve the quality and quantity of vegetative propagated potato plants. The techniques of the plant tissue culture have been developed as a powerful tool for crop improvement (Razdan and Cocking, 1981; Fish and Jones, 1988; Hossain, 1994) and received wide attention of scientific world (Larkin *et al.,* 1982). Diseases free good quality seeds and pathogen free planting materials are possible to produce through tissue culture (Hossain, 1994).

The regeneration of plant tissue culture technique is an important and essential component of biotechnological research, and required for the genetic manipulation of the plants. The totipotency of a cell or tissue opens up several new contingencies in plant breeding programmes that provide gene manipulation and selection of desirable character. Tissue culture techniques have several advantages over traditional propagation methods. The application of *in vitro* techniques provides unique possibilities for overcoming the barriers of incompatibility existing between remote species and has facilitated rapid introduction of new varieties.

It may be mention here that several attempts have been taken to establish *in vitro* regeneration protocol for potato. During these attempts a wide variety of explants have been used with the application of several growth regulators to regulate plantlets without intervention of callus. For potato regeneration protocol, many scientists reported that direct shoot regeneration of potato from various explants, such as, shoot

apices, leaf discs, nodes and internodes. Hossain *et al.* (2005) studied all of these explants, Juan *et al.* (2004) using leaf explants, Shahpiri *et al.* (2004) used internodes and leaf discs as explants from *in vitro* grown plantlets to investigate the effect of growth regulator concentrations, cultivars and explants on callus induction and shoot regeneration.

Regeneration response *in vitro* is generally species and often genotype specific (Ritchie and Hodges, 1993). Therefore, regeneration conditions and characteristics may vary among genotypes and need to be determined prior to transformation. In potato different approaches so for have been adapted to obtain efficient *in vitro* regeneration system either from petioles with intact leaflets (Shirley *et al.*, 2001), leaves (Ooms *et al.*, 1987, Cearley and Bolyard, 1997; Trujillo *et al.*, 2001; Sarker and Mustafa, 2002; Anderson *et al.*, 2003), tuber discs (Sheerman and Beavan, 1988; Vasquez and Clarence, 2002), and from stem (Visser *et al.*, 1989; Chang *et al.*, 2002) after passing through callus phase. Recently Osusky *et al.*, (2005) reported regeneration of plants from leaf disc tissues during genetic modification of potato.

A number of crop varieties have already been transformed through *Agrobacterium*-mediated system such as cotton, maize, potato, tobacco, rapeseed, raspberry, soybean, pea, tomato, rice etc. (Wambugu, 1999; Dan *et al.*, 2002; Sarker *et al.*, 2009). In 2009, biotech crop such as wheat, Corn, Soybean, potato, tomato were grown by 13.3 million farmers in 25 countries covering 125 million hectares of land and 90% of the beneficiary farmers were resource-poor farmers from developing countries, whose increased income from biotech crops contributed to the alleviation of poverty (James, 2009). This progress can be attributed to the better understanding of the underlying process involve in DNA recombination and the development in molecular genetics, plant transformation and regeneration techniques (Yousuf and Machray, 2008).

Potato is easily regenerated from different explants in MS medium supplemented with different auxin and cytokinin (Yadav and Sticklen, 1995; Cearly and Bolyard, 1997; Rabbani *et al.*, 2001). Improvement of species through genetic engineering depends on *in vitro* regeneration system and high regeneration frequency of plants from tissues and cells. Both callus induction and plants regeneration from explants require the presence of appropriate combination and concentration of plant growth regulators in

the culture media (Eapen *et al.*, 1998; Ehsanpor *et al.*, 2000; Fiegert *et al.*, 2000; Ahan *et al.*, 2001).

Shoot regeneration responses vary with the cultivar but in most cases cytokinin helps to enhance shoot production (Miller *et al.*, 1987; Linden *et al.*, 2006). Zeatine riboside (ZR) is an important and well known growth regulator for potato transformation (Yadav and Sticklen, 1995; Wendt *et al.*, 2001). It reduces callus phage and accelerates bud formation (Beaujean *et al.*, 1998). However, BAP and thidiazoron (TDZ) are also used as growth regulator (Ashari and Villiers, 1998). TDZ a substituted N-phenyl urea (Ricci *et al.*, 2001) has been established as an important regulator for morphogenic responses in a large number of species as well as diverse experimental systems. These responses includes somatic embryogenesis (Saxena *et al.*, 1992; Visser *et al.*, 1992; Murthy *et al.*, 1995), micropropagation (Murch *et al.*, 2000; Fratini and Ruiz, 2002; Jones *et al.*, 2007), regeneration and multiple shoot formation (Eapen *et al.*, 1998; Li *et al.*, 2000; Chengalrayan *et al.*, 2001)

Haque *et al.* (2009) reported *in vitro* callus induction and regeneration from different explants (such as leaf, node, internodes and shoot tip) using various concentrations of 2, 4- D and Kinetin (Kn). Leaf explants appeared to be best callus production when 1.0 mg/l 2, 4- D + 0.25 mg/l Kn was used.

Gustafson *et al.* (2006) evaluated different concentration and combinations of auxin and trans-zeatine with leaf and stem explants for shoot formation. Several growth regulator combinations resulted in higher plant regeneration rates. They reported 59.5% independent putative transformation from the total explants plated. For the confirm transformation rate by PCR, they obtained 47.1% *nptII* positive transformation.

Bakul, (2005) reported the regeneration of potato plantlets and microtuberization of Cardinal, Diamant and Patrones varieties. BAP has found to enhance plantlet regeneration markely. All the varieties showed better response on plantlet regeneration with 1.0-2.0 mg/l BAP alone or 1 mg/l BAP + 0.01-0.1 mg/l NAA. Gong *et al.*, (2005) reported direct shoot regeneration from various explants of potato using silver nitrate.

Jun *et al.,* (2005) reported the effect of auxin; GA_3 and BAP on potato shoot growth and tuberization under *in vitro* condition. The shoot length of potato explants increased with the increasing of concentrations (0.5-10.0 mg/dm^3) of IAA treatment especially with the addition of GA_3 (0.5 mg/dm^3), but was inhibited by BAP (0.5 mg/dm^3).

Ghaffoor *et al.,* (2003) conducted an experiment with three different growth regulators viz. NAA, IAA and IBA with five concentration levels (0, 0.05, 0.15. 0.25 and 0.35 mg/l) on meristem culture of potato for production of virus free plantlets. Maximum plant height, number of nodes and number of leaves per plant was recorded from 0.15 mg/l NAA, 0.35 mg/l IBA and 0.25 mg/l IAA, respectively.

Omidi *el al.* (2003) was observed the effect of cultivar and explants on callus induction in leaf and internodes explants of six potato cultivars (viz. Agria, Cosmos Sante, Con cord, Ajix and Diamant. Cultivars, explants and their interaction on frequency of callus induction were significant on MS medium supplemented with 5.0 mg/l 2, 4 –D and 0.25 mg/l Kn.

Sarker and Mustafa, (2002) reported regeneration of two indigenous potato varieties of Bangladesh; Lal Pakri and Jam Alu. They observed the effects of various combinations of BAP, Kn and GA_3 on multiple shoot regeneration from nodal segments of potato. Different concentrations of BAP and Kn were indicated that the number of shoots and nodes increased with the increase of BAP or Kn up to (1.0-1.5 mg/l.).

Nodal fragments and stem segments were studied by Rabbani *et al.,* (2001) for mass multiplication of healthy stock and successful *in vitro* seed tuber production. The maximum number of shoots (14) was obtained when 2.0 mg/l BAP was applied.

Doo and Boe, (2001) reported leaf discs regeneration on MS medium with various combinations of IAA and Zeatine riboside (ZR). The medium containing 3.5 mg/l IAA and 4.0 mg/l ZR produced the most plantlets.

Zaman *et al.,* (2001) conducted an experiment with three different auxins viz. NAA, IAA and IBA each at four levels (0, 0.1, 0.5 and 1.0 mg/l) on meristem culture of potato for production of virus free plantlets. Maximum plantlets height (8.3 cm), largest number of nodes/plantlets (8.9) were recorded at 0.5 mg/l of NAA followed by IBA at 1.0 mg/l, whereas reasonably high number of roots/ plantlets (23.7) as well as the earliest microtuber formation (17 days) of IBA followed by NAA at 0.1 mg/l.

Potato improvement through genetic engineering was reported by Davies, (2000). He stated that genetic manipulation can improve potato production against pests, diseases and stresses. Transformation of potato using leaf and stem explants along with the maintenance of transformation on selective regeneration media was reported by Dinu and Pop., (2000).

Jatinder *et al.,* (2000) reported direct plant regeneration from leaf segments for *Agrobacterium-* mediated genetic transformation of four potato cultivars: Kufri Badsha, Kufri Chandramukhi, Kufri Jyoti and Kufri Sindhuri. In this study, *Agrobacterium tumefaciens* strain LBA4404 harboring Ti plasmid *pTOK233* having one reporter gene (*GUS*) and two selectable marker genes (*nptII and hpt*) were used. The kanamycin resistant shoots were tested by *GUS* assay. The blue spots showing beta-glucuronidase enzyme activity confirmed the genetic transformation.

Al - Momani *et al.,* (2000) reported *in vitro* morphogenesis of potato cv. Spunta with different culture media and explants types. Shoot tip segments gave shorter shoot lengths than first, second and third nodal segments on MS medium supplemented with a combination of 0.3 mg/l GA_3, 1.0 mg/l IAA and 3.0 mg/l BA. Shoot tips gave the highest shoot numbers associated with proliferation. The longest internodal length of all the explants was induced on MS medium supplemented with 0.3 mg/l GA_3.

1.10 Objective

Several modern varieties (HYV) are now cultivated in Bangladesh but the traditional varieties still occupy about 35% of the total potato production area (Ilangantileke *et al.,* 2001). The average yield of potato in Bangladesh is several times lower than many European countries.

Although potato is being considered as one of the most popular food crops in Bangladesh, its productivity is hampered due to the attack of viruses, fungi and bacterial diseases. The total loss caused by these diseases is 30 - 100 % during cultivation and 2 - 6 months of storage (Anon. 1992). It has been estimated that as high as 57.2 % loss of yield occurs in Bangladesh due to late blight alone (Ali and Dey, 1994).

It is now a well-known fact that through meristem culture or tissue culture, it is possible to develop virus free potato planting stocks in a mass scale. But it is not yet possible to develop fungal resistant varieties through *in vitro* culture techniques. In recent years, genetic transformation techniques are being used to develop disease resistant varieties in many crop plants. Although there are reports on the genetic transformation of potato (Sheerman and Beavan, 1988; Visser *et al.*, 1989; Wenzler *et al.*, 1989; Voyda and Belknap, 1992) but limited work has been carried out on locally available Bangladeshi varieties. Local varieties are more acceptable in village people and within some urban people for its tastiness. Though the yield of local varieties is lower than HYV, but still it have to be cultivated for people demand. Therefore, more initiative needs to be given to local varieties for various developments such as its quality, productivity and yield through biological approaches. Khatun *et al.*, (2010 and 2011) reported in their experiment that some local varieties, namely, Shadaguti and Zaubilati have the potentially to perform better in salt stress condition. So, for the development of salt tolerant variety, those varieties should be used for transformation study because they already have some tolerance. Before going to transformation work, it needs to develop an efficient regeneration protocol. Therefore, attempts have been made to find out the regeneration ability of three local potato varieties using different explants for future transformation work.

Under the current scenario, the present study was conducted with following objectives.

1. To establish *in vitro* regeneration protocol from various explants.
2. To find out the appropriate concentrations of phytohormone for shoot regeneration.
3. To evaluate several genotypes on their performance.

Chapter 2

Materials and Methods

CHAPTER - 2

MATERIALS AND METHODS

These *in vitro* experiments on potato varieties were carried out at the Laboratory of Biotechnology, Biotechnology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur, during the period from April 2010 to April 2011. The materials and methods used in this investigation are described below under the following heads and sub-heads:

2. Materials

2.1 Plant materials

Potato genotypes, viz. Zaubilati, Shadaguti and Challisha were collected from Tuber Crops Research Centre (TCRC), BARI, Gazipur and used in the investigations.

2.2 Sterilizing agents

In the present investigation, Mercuric Chloride ($HgCl_2$) was used as sterilizing agent. Besides, Tween 20 and 70% ethyl alcohol were added to expedite the sterilization procedures.

2.3 Different culture media

Different culture media used in the present investigation for various purposes were as follows:

2.3.1 Shoot initiation and differentiation media

For shoot initiation, explants were cultured on PROP (Haberlacha *et al.*, 1985) medium supplemented with different concentrations and combinations of various growth regulators. After shoot initiation same medium was used for shoot elongation.

2.3.2 Root induction medium

For root induction, regenerated shoots were cultured on full and half strength of PROP medium and different concentrations of IBA.

2.4 Plant growth regulators

In addition to the inorganic nutrients, it is necessary to add one or more phytohormones mainly cytokinins and auxins into support proper growth and development of tissues and organs. Growth regulators used in this experiment were:

- Cytokinins:

1. 6-Benzyl amino purine (BAP)
2. Kinetin (Kn)

- Auxins:

1. Indole-3-acetic acid (IAA)
2. Indole-3-butyric acid (IBA)
3. Gibberellic acid (GA_3)

2.5 Methods

2.5.1 Explants preparation

The potato tubers were put in the green house at 18^0C. The distinct sign of sprouting was visible within 15-20 days. Newly sprouted shoot buds were collected from three genotypes of potato varieties viz. Zaubilati, Shadaguti and Challisha. These sprouts were taken for initiation of shoots. For experiments, shoot apex, node, internode and leaf were used as explant from *in vitro* grown shoots of potato varieties.

2.5.2 Preparation of PROP (Haberlacha *et al.*, 1985) medium

PROP medium was presented following the composition mentioned in Appendix I and media preparation was carried out the following different steps mentioned below.

2.5.3 Preparation of stock solution

Before media preparation it was needed to prepare the stock solutions for immediate use during work. Separate stock solutions were prepared for macronutrients, micronutrients, vitamins, growth regulators etc. because different constituents were required in different concentrations and were stored at refrigerator.

2.5.3.1 Macronutrients (MS I stock solution, 10X; based on Murashige and Skoog, 1962)

The required quantities of major salts (Appendix I) were weighed and dissolved thoroughly in 750 ml of distilled water in a 1000 ml beaker and final volume was made to 1000 ml by further adding of distilled water. The stock solution of macronutrients was prepared at 10 times of desired concentration with distilled water. Prepared solution was poured into a clean reagent bottle, labeled with marker and stored in a refrigerator at 4 ± 1^{0}C.

2.5.3.2 Micronutrients (MS II stock solution, 100X)

The required quantities of minor salts (Appendix I) were weighed and dissolved thoroughly in 750 ml of distilled water in a 1000 ml beaker and final volume was made to 1000 ml by further adding of distilled water. The stock solution of mironutrients was made upto 100 times higher the strength of that required concentration of the medium in one litre of distilled water as described earlier for the stock solution of macronutrients. Prepared solution was poured into a clean reagent bottle, labeled with marker and stored in a refrigerator at 4 ± 1^{0}C.

2.5.3.3 Iron EDTA (MS III stock solution, 100X)

The required quantities of Na_2EDTA. (Ethylene di-amine tetra acetic acid, di-sodium salt) and $FeSO_4.7H_2O$ (Appendix I) were weighed and dissolved separately in a 500 ml beaker. Then mixed together in a 1000 ml beaker and was heated until yellowish color appears. Final volume was made to 1000 ml by through adding of distilled water. The stock solution of Iron-EDTA was prepared at 100 times of desired concentration with distilled. Prepared solution was also poured into an amber color bottle, labeled with marker and stored in a refrigerator at 4 ± 1^{0}C.

2.5.3.4 Myo inositol (MS IV stock solution, 100X)

The stock solution of myo inositol was prepared 100 times the concentration of their final strength. Required amount of myo inositol (Appendix I) was weighed and dissolved in 250 ml of distilled water in a clean beaker until the salt dissolved completely. The final volume was made up to mark by adding of distilled water. The stock solution was also filtered and stored in refrigerator at 4 ± 1^{0}C.

2.5.3.5 MS stock C (100X)

The stock solution of MS stock C was prepared 100 times the concentration of their final strength. Required amount of Calcium chloride ($CaCl_2.2H_2O$) (Appendix I) was weighed and dissolved in 250 ml of distilled water in a clean beaker until the salt dissolved completely. The final volume was made up to mark by adding of distilled water. The stock solution was also stored in refrigerator at 4 ± 1^0C.

2.5.3.6 MS stock D (100X)

The stock solution of MS stock D was prepared 100 times the concentration of their final strength. Required amount of Potassium hydrogen phosphate (KH_2PO_4) (Appendix I) was weighed and dissolved in 250 ml of distilled water in a clean beaker until the salt dissolved completely. The final volume was made up to mark by adding of distilled water. The stock solution was also stored in refrigerator at 4 ± 1^0C.

2.5.3.7 PROP vitamins (1000X)

The stock solutions of PROP vitamins were prepared 1000 times the concentration of their final strength. Required amount of PROP vitamins (Appendix I) were weighed and dissolved in 250 ml of distilled water in a clean beaker until the vitamins dissolved completely. The final volume was made up to mark by adding of distilled water. The stock solution was also stored in refrigerator at 4 ± 1^0C.

2.6 Preparation of growth regulators (Hormonal stock solution)

Separate stock solution of plant growth regulators (PGRs) was prepared by dissolving the desired quantity of ingredients to the appropriate solvent and made the final volume with distilled water. The plant growth regulators were dissolved in proper solvent as mentioned below:

Growth regulators (Solutes)	Solvent and concentration
BAP	0.1 N HCl
Kn	0.1 N HCl
IAA	0.1 N NaOH
IBA	0.1 N NaOH
GA_3	50% Ethyl alcohol

To prepare the stock solution, 10 mg of solid plant growth regulators were taken in a 100 ml clean beaker and then dissolved in 1 ml of respective solvent mentioned above. Then the volume was made 100 ml by the addition of regulators were then stored in a refrigerator at 8 ± 1^0C for subsequent use.

2.7 Steps followed for the preparation of culture media

For the preparation of 1 liter medium, the following steps were followed:

I. The stock solutions I, II, III and IV were taken into a 1000 ml beaker in volumes of 100, 10, 10 and10 ml respectively.

II. The stock solutions C and D were added into beaker in volumes of 10 ml from each stock solution.

III. Then added 1 ml PROP vitamins from stock solutions.

IV. 30 gm of sucrose was dissolved in the mixture components.

V. The mixture was taken up to 800 ml with further addition of distilled water.

VI. Different required concentrations of hormonal supplements were added to the solution individually.

VII. Finally the whole mixture was taken up to 1000 ml with further addition of distilled water.

VIII. pH of the medium was adjusted to 5.8 by using an analogue pH meter with the help of 0.1 N HCl and 0.1 N NaOH as necessary.

IX. For solid medium 8 gm/l agar was added to the solution and melt in a microwave oven. The melted solution was dispensed into test-tubes.

X. The test tubes were covered with aluminum foil.

XI. The media containing test-tubes were sterilized by at 121^0C for 20 minutes. Then the media were allowed to cool and kept in culture room. After cooling, the media were ready for used to inoculation.

2.8 Stock solution of sterilizing agent

To prepare 0.1% solution, 1 gm of Mercuric Chloride ($HgCl_2$) was taken in a 1 liter reagent bottle and dissolved in 1000 ml double distilled water. Freshly prepared $HgCl_2$ solution was always used for surface sterilization of plant materials.

2.9 Sterilization technique

2.9.1 Glassware sterilization

All the empty glassware, culture vessels, test tubes, petridishes, pipettes etc. and small instruments (forceps, scalpel) needed for sterile dissection were autoclaved at 121°C and 1.1 kg/cm^2 pressure for 45minutes.

2.9.2 Sterilization of plant materials

For surface sterilization, the potato sprouts (explants) were thoroughly washed under running tap water for 30 minutes with jet and trix to remove the surface contamination and washed with distilled water 2-3 times. After that the sprouts were dipped in 99% ethanol plus 2 drop of Tween 20 for 20 second under laminar air flow hood and washed with autoclaved distilled water two times. Finally, sprouts were sterilized with 0.1% (w/v) $HgCl_2$ for 1 minute and then washed for 3-4 times with autoclaved distilled water to remove the traces of mercury inside the laminar airflow hood.

2.9.3 Sterilization of culture media

The conical flasks/ test tubes/ glass jars containing prepared media were autoclaved at 121°C and 1.1 kg/cm^2 pressure for 20 minutes.

2.9.4 Sterilizing of working area

Laminar Air flow Cabinet was usually sterilized by switching on the cabinet and wiping the working surface with 70% ethyl alcohol 30 minutes before starting the work. Before cleaning with ethyl alcohol UV light was used for 20 minutes.

2.9.5 Precautions to ensure aseptic conditions

All inoculation and aseptic manipulations were carried out under laminar air flow cabinet. The instruments like scalpels, forceps, needles etc. were pre-sterilized by autoclaving and subsequent sterilization was done by dipping in 70% ethyl alcohol followed by flaming and cooling method inside the laminar airflow cabinet. While not in used, the instruments were kept the laminar airflow cabinet. Hands were also sterilized by wiping with 70% ethyl alcohol. Other required materials like distilled water, glass plate, pertidishes etc. were sterilizes in an autoclave following method of media sterilization. The neck of the culture vessels were flamed before closing it with

the cap. Aseptic conditions were followed during each and every operation to avoid the contamination of cultures.

2.10 Culture condition

All cultures were incubated in the growth room at 22 ± 2^{0}C with a photoperiod of 16/8 hrs light/dark cycle at 2000-3000 lux light intensity with cool white fluorescent lights. The relative humidity was 60-65%.

2.11 Excision and inoculation of explants

The sterilized sprouts were excised with the help of the sterilized scalpel and forceps. Then the excised sprouts were transferred to sterile test tubes which containing PROP medium for sprout regeneration. The inoculated test tubes were incubated at 22 ± 2^{0}C with a photoperiod of 16/8 hrs light/dark cycle at 2000-3000 lux light intensity with cool white fluorescent lights. The relative humidity was 60-65%. The test tubes were checked daily to note the response and the development of contamination.

2.12 Maintenance of subculture

The cultures were regularly subculture on fresh medium at 4 weeks intervals in test tubes. Observations were recorded every 7 days following inoculation and sub-culturing.

2.13 Root induction

For root induction, regenerated shoots (3-4 cm long) were excised and transferred into different rooting media, viz. full and half strength of PROP medium and different concentrations of IBA.

2.14 Transplantation of *in vitro* derived plantlets

Regenerated plantlets with sufficient roots (3-5 cm in length) were transferred in the soil. Before transfer in the soil, rooted plantlets were kept for 5-7 days into room temperature (30±2^{0}C) for acclimatization. After that, plantlets were taken out from the test tubes, washed carefully under running tap water to remove any traces of agar. Then each plantlet was transferred into small plastic pot containing garden soil, sand and decomposed cow dung at the ratio of 1:1:1 in shade house for proper hardening.

2.15 Experiments

Experiments were conducted during the entire period of time (April 2010 to April 2011). These experiments were observed among the three local potato varieties of Bangladesh. These are given below:

(1) Effect of different concentrations of cytokinin (BAP and Kn) on *in vitro* regeneration from shoot apex explant of potato.

(2) Effect of different concentrations of cytokinin (BAP and Kn) on *in vitro* regeneration from nodal segment of potato.

(3) Effect of different concentrations of BAP and Kn along with Gibberelic acid (GA_3) and Indole acetic acid (IAA) on direct regeneration from internode explant of potato.

(4) Effect of different concentrations of BAP and Kn along with Gibberelic acid (GA_3) and Indole acetic acid (IAA) on direct regeneration from leaf explant of potato.

(5) Effect of different media on rooting of regenerate shoots.

2.16 Details of experimental methodology

2.16.1 Treatments

PROP salts with vitamins supplemented with six different concentrations of BAP and Kn, viz. T_1 (0.0), T_2 (0.5), T_3 (1.0), T_4 (1.5), T_5 (2.0), T_6 (2.5) were tested for *in vitro* regeneration of potato from shoot apex and nodal segment (Experiment 1 & 2) and five different concentration of BAP and Kn viz. T_1 (0.0), T_2 (0.5), T_3 (1.0), T_4 (1.5), T_5 (2.0) from internode and leaf explants (Experiment 3 & 4). Every experiment was treated with different concentrations of hormones followed by the experiments heading (Experiment1-4).

For root induction, four treatments were used. These were: full (T_1) and half (T_2) strength of PROP medium and two concentrations of IBA, viz. T_3 (0.1 mg/l), T_4 (0.5 mg/l) with half strength of PROP medium (Experiment 5).

2.16.2 Explants preparation, excision and inoculation of explants

For the experiment, shoot apex, node, internode and leaf were used as explants from *in vitro* grown plants of three potato varieties, namely, Zaubilati, Shadaguti and Challisha. *In vitro* grown plantlets were considered as source for the explants collection. For shoot apex explant, only the first top part of the plantlet was excised. For node explant, only the first 4-5 nodes from the top of the plantlet excluding shoot apex of the each plantlet were excised. For internode explant, only the first 4-5 internodes from the top of the plantlet excluding shoot apex of the each plantlet were excised. Internode segment was also avoiding the auxiliary bud. For leaf explant, thick, healthy leaf from the upper node of the plant was used. Explants were placed in every test tubes/ petridishes in the above mentioned medium. The inoculated test tubes/ petridishes were incubated at 22 ± 2^0C with a photoperiod of 16/8 hrs light/dark cycle at 2000-3000 lux light intensity with cool white fluorescent lights.

2.16.3 Design of the experiment

The experiments were design in Completely Randomized Design (CRD) with two factor factorial treatment having four replicates with six treatments and three varieties (Experiment 1 & 2) and five treatments for experiment 3 & 4.

The experiment (5) was laid out in single factor Completely Randomized Design (CRD) with six replications with four treatments for one variety.

2.16.4 Data collection

The test tubes were checked daily to note the response and the development of contamination. After 30 and 60 days the following data were recoded:

1. Survival percentage of explants
2. Days to shoot initiation
3. Length of shoot per explant (cm)
4. Number of nodes per explant
5. Number of leaves per explant
6. Fresh weight (mg) of shoot per explant
7. Days to root initiation

8. Number of roots per explant
9. Length of root per explants (cm)
10. Regeneration frequency (%)
11. Number of shoot per explant

Survival percentage of explants

Percentage of explant responded was calculated using the following formula:

$$\% \text{ of explants responded} = \frac{\sum Xi}{N} \times 100$$

Where, $\sum$ = Summation

Xi = Number explants survived

N = Number of explants cultured

Days to shoot initiation

The observation of cultures were started from the 3rd day of inoculation and continued up to 30th day for shooting. Any change or development in culture when observed was recorded as days to proliferation or shoot initiation of cultures.

Length of shoot per explant (cm)

The heights of plantlets were measured from the base of plantlets to the tip.

Number of nodes per explant

The single stem plantlet, the number of nodes was counted from base to tip.

Number of leaves per explant

The single stem plantlet, the number of leaves was counted from base to tip.

Fresh weight (mg) of shoot per explants

Fresh weight of shoot was measured in digital balance in mg.

Number of shoot per explant

Multiple shoots have been found in many cultures, which were counted from 5 days after culture and recorded as number of shoots per culture (Experiments 3 & 4).

Number of roots per explant

The single stem plantlet, the number of roots was counted.

Length of root per explant (cm)

The length of root was mean used from the base of plantlet to the tip of root.

Regeneration frequency (%)

Regeneration percent of explants (explant producing shoot, explant producing root, explant producing both shoot and root) were counted after 60 days of culture. Regeneration frequency (%) was calculated using the following formula:

$$\text{Regeneration frequency (\%)} = \frac{\sum \text{Xi}}{\text{N}} \times 100$$

Where, $\sum$ = Summation

Xi = Number explants regenerated

N = Number of explants cultured

2.16.5 Statistical analysis

The results were analyzed using MSTAT-C (version 2.10) and CropStat package computer program (version 2.10). Differences among the mean were compared following LSD test at 5% and 1% level of significant. The analysis of variance for different characters was performed and means were compared by the Least Significant Difference (LSD) (Gomez and Gomez, 1984).

Chapter 3

Results

CHAPTER - 3
RESULTS

In the present study, explants such as, shoot apex, nodal segment, internode and leaf were collected from *in vitro* grown shoots of three local potato varieties, viz. Zaubilati, Shadaguti and Challisha. The effects of hormonal treatments, viz. cytokinins and auxins were observed on *in vitro* shoot regeneration and root induction. It was also observed which explants regenerate direct shoot proliferation without callusing and which genotype performed better for *in vitro* shoot regeneration. The results obtained from each of the experiments are described in details under the following heads:

3.1 Effect of different concentrations of BAP and Kn on *in vitro* regeneration from shoot apex explant of potato

Shoot apex explant of three potato varieties were cultured on PROP medium supplemented with different concentrations (0, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) of cytokinin (BAP and Kn). Data were recorded 30 days after inoculation in respect to parameters and were statistically analyzed. The results are shown in Table 2 - 3 and Figs.1 - 5. The analysis of variance of data has been presented in Table 4 – 5.

3.1.1 Survival percentage of explant

Responsive explants were observed in different concentrations of BAP and Kn in shoot apices explants of potato varieties. The maximum explants responded (100%) was recorded under all treatments of BAP concentrations as well as Kn. It was also found that the maximum explants responded in interaction effect of variety and cytokinin (BAP and Kn) concentrations (Table 2 – 3).

Table 2. *In vitro* regeneration response from shoot apex explant of three potato varieties toward various BAP concentrations.

Variety	Concentrat -ion of BAP (mg/l)	Responsive explant (%)	Days to shoot initiation	Shoot length (cm) / plantlet	No. of nodes / plantlet	No. of leaves / plantlet	Fresh weight(mg) of shoot / plantlet
Zaubilati	0.0	100	5.25	4.68	6.75	6.75	27.35
	0.5	100	4.75	5.30	5.75	6.25	28.08
	1.0	100	4.50	6.85	6.00	7.00	34.45
	1.5	100	3.75	5.65	7.25	8.50	37.70
	2.0	100	5.50	5.25	4.25	6.50	25.03
	2.5	100	5.75	4.38	4.75	5.75	23.18
Shadaguti	0.0	100	5.25	4.50	4.25	5.25	16.48
	0.5	100	4.75	7.18	4.50	6.25	24.75
	1.0	100	3.75	7.98	5.50	7.50	36.05
	1.5	100	2.75	8.30	6.00	8.75	39.83
	2.0	100	3.75	5.73	5.50	6.25	33.18
	2.5	100	4.50	6.98	5.00	6.00	31.58
Challisha	0.0	100	5.25	7.55	7.25	8.25	32.20
	0.5	100	5.75	6.20	5.00	5.50	18.45
	1.0	100	5.00	5.53	4.50	3.75	16.63
	1.5	100	4.50	5.53	4.25	3.50	20.50
	2.0	100	5.50	4.58	3.00	2.50	13.53
	2.5	100	6.25	4.78	3.25	2.75	16.05
LSD (0.01)	-	-	NS	1.12	1.43	1.81	5.08
CV (%)	-	-	13.28	9.98	14.64	16.11	10.18

Significantly at 1% level of probability, NS = Non-significant

Data collected 30 days after inoculation.

Table 3. *In vitro* regeneration response from shoot apex explant of three potato varieties toward various Kn concentrations.

Variety	Concentrat-ion of Kn (mg/l)	Responsive explant (%)	Days to shoot initiation	Shoot length (cm) / plantlet	No. of nodes / plantlet	No. of leaves / plantlet	Fresh weight(mg) of shoot / plantlet
Zaubilati	0.0	100	7.25	8.95	8.25	9.00	68.45
	0.5	100	6.50	10.48	8.50	9.50	81.75
	1.0	100	6.00	6.95	7.50	8.50	42.40
	1.5	100	5.00	6.73	6.00	7.75	58.83
	2.0	100	4.50	6.13	5.25	6.75	32.20
	2.5	100	6.00	6.03	7.75	7.25	58.48
Shadaguti	0.0	100	7.25	11.45	9.50	9.50	45.73
	0.5	100	6.50	11.93	8.50	9.50	53.03
	1.0	100	6.00	9.93	7.00	8.25	48.65
	1.5	100	5.75	10.05	7.00	7.50	42.83
	2.0	100	4.25	9.58	6.75	7.25	36.25
	2.5	100	5.50	5.83	5.75	6.50	32.25
Challisha	0.0	100	7.25	11.38	9.25	9.75	45.85
	0.5	100	7.00	6.40	5.00	5.75	18.48
	1.0	100	6.50	5.58	5.50	6.50	18.55
	1.5	100	5.50	5.48	4.75	6.00	25.78
	2.0	100	4.75	8.03	6.50	7.75	34.05
	2.5	100	6.25	6.85	5.50	7.00	35.23
LSD (0.01)	-	-	NS	1.36	1.59	1.65	5.62
CV (%)	-	-	10.96	8.76	12.19	11.20	6.88

Significantly at 1% level of probability. NS = Non-significant

Data collected 30 days after inoculation.

Table 4. ANOVA of different concentration of BAP on *in vitro* regeneration from shoot apex explant of potato.

Sources of variation	Degrees of freedom	Mean sum of square				
		Days to shoot initiation	Shoot length(cm) / plantlet	Number of nodes / plantlet	Number of leaves / plantlet	Fresh weight(mg) of shoot / plantlet
Variety	2	9.597 **	13.284 **	9.389 **	44.431 **	845.626**
Treatment	5	5.322 **	5.092 **	6.847 **	8.622 **	164.400 **
Variety × Treatment	10	0.747 NS	5.397 **	4.756 *	10.031 **	211.012 **
Error	54	0.407	0.351	0.569	0.917	7.222

** = 1% level of significance, * = 5% level of significance, NS = Non-significant

Table 5. ANOVA of different concentration of Kn on *in vitro* regeneration from shoot apex explant of potato.

Sources of variation	Degrees of freedom	Mean sum of square				
		Days to shoot initiation	Shoot length(cm) / plant	Number of nodes / plant	Number of leaves / plant	Fresh weight(mg) of shoot / plant
Variety	2	0.889 NS	45.691 **	12.347 **	7.681 **	4492.607**
Treatment	5	11.114 **	30.614 **	15.547 **	10.589 **	703.266 **
Variety × Treatment	10	0.239 NS	10.101 **	4.764 **	4.314 **	630.076 **
Error	54	0.431	0.516	0.708	0.759	8.853

** = 1% level of significance, NS = Non-significant

3.1.2 Day requirement for shoot initiation

Time requirement for shoot initiation was significantly influenced by the varieties / genotypes in BAP treatments but not in Kn treatments (Table 4 - 5). In respect to overall response of each variety, early shoot initiation (4.13 days) was found in Shadaguti followed by Zaubilati (4.92 days) in BAP treatment (Fig. 1). Similarly, Shadaguti also showed early shoot initiation (5.88 days) followed by Zaubilati in Kn treatment. The variety, Challisha took the maximum days (5.38 days at BAP and 6.21 days at Kn) for shoot initiation (Fig. 1). Fig. 3 shows the regeneration from sprout in PROP medium in Shadaguti variety.

Different concentrations of cytokinin (BAP and Kn) also markedly influenced day requirement for shoot initiation. BAP (1.5 mg/l) took minimum days (3.67) for shoot initiation where as it was the maximum (5.50 days) in 2.5 mg/l BAP (Fig. 2). On the other hand, 2.0 mg/l Kn took minimum days (4.50) for shoot initiation (Fig. 2).

Remarkable variation was observed in respect of days required for shoot initiation in presence of cytokinin (BAP and Kn) supplementations in the experimental varieties. The maximum days required for shoot initiation (6.25 days) was noted in Challisha with 2.5 mg/l BAP followed by Challisha with 0.5 mg/l BAP and Zaubilati with 2.5 mg/l BAP (5.75 days). Early shoot initiation (2.75 days) was found in Shadaguti with 1.5 mg/l BAP (Table 2). On the other hand, early shoot initiation (4.25 days) at Kn was also found in Shadaguti with 2.0 mg/l followed by Zaubilati (4.50 days) and Challisha (4.75 days) with same concentration. But the maximum day requirement for shoot initiation was found in medium without cytokinin supplement (Table 3).

The results of the study reflected that minimum day requirement for shoot initiation decreased with the increasing of BAP and Kn concentration up to 2.0 mg/l. This indicated that cytokinin (BAP and Kn) enhances cell division and cell elongation.

3.1.3 Shoot length (cm) per plantlet

Significant variation was observed among the varieties regarding length of shoot (Table 4 - 5). The maximum shoot length (6.78 cm) was measured in the variety Shadaguti followed by Challisha (5.69 cm) at BAP treatments (Fig. 1). At Kn treatments, Shadaguti also performed better than other two varieties and its maximum length was measured to be 9.79 cm. The variety Zaubilati produced the minimum length of shoots at BAP (5.35 cm) and in Kn treatment minimum shoot length (7.28

cm) was obtained from Challisha (Fig. 1). The possible reason of varied lengths of shoots might be due to the genotypic influence.

Different levels of cytokinin (BAP and Kn) supplements significantly influenced the length of shoot. The maximum shoot length (6.78 cm) was recorded with 1.0 mg/l BAP concentration and the lowest shoot length (5.18 cm) was measured in 2.0 mg/l BAP. On the other hand, the maximum shoot length (10.59 cm) was recorded in Kn free medium followed by 0.5 mg/l Kn (9.60 cm). The lowest shoot length (6.23 cm) recorded from 2.5 mg/l Kn concentrations (Fig. 2).

The interaction of variety and cytokinin (BAP and Kn) supplementations had significantly influenced the length of shoot per explant (Table 4 - 5). The maximum shoot length (8.30 cm) was measured in Shadaguti with 1.5 mg/l BAP followed by 1.0 mg/l BAP (7.98 cm) (Table 2 and Fig. 4). The lowest shoot length (4.38 cm) was found in Zaubilati with 2.5 mg/l BAP followed by Challisha (4.58 cm) with 2.0 mg/l BAP. On the other hand, Kn showed better result than BAP. The maximum shoot length (11.93 cm) was measured in Shadaguti with 0.5 mg/l Kn followed by Zaubilati (10.48 cm) with 0.5 mg/l Kn (Table 3 and Fig. 5). The lowest shoot length was obtained from Challisha at 1.5 mg/l Kn. Result indicated that higher concentration of Kn had no advantage in case of shoot length. However, in case of BAP the shoot length seems to increase with the increase of BAP concentration up to a limit. With further increase in the BAP amount the effect seems to hamper the elongation.

3.1.4 Number of nodes per plantlet

Significant variation was found among the varieties in respect to number of nodes per shoot (Table 4 - 5). The highest number of nodes per shoot (5.79) was recorded in Zaubilati and the lowest (4.54) in Challisha in BAP treatment (Fig. 1). At Kn treatment, the highest number of nodes per shoot (7.42) was recorded in Shadaguti followed by Zaubilati (7.21), while it was the lowest (6.08) in Challisha (Fig. 1).

Different levels of cytokinin (BAP and Kn) supplements significantly influenced the number of nodes per plantlet. The highest number of nodes per shoot (6.08) was recorded without BAP and the lowest (4.25) was recorded at 2.0 mg/l BAP (Fig. 2). On the other hand, the highest number of nodes per shoot (9.0) was also recorded in absence of Kn which was followed by (7.33) at 0.5 mg/l Kn, and the lowest obtained at 1.5 mg/l Kn (Fig. 2).

Significant variations were found in respect of number of nodes per shoot due to the interaction effect of variety and cytokinin (BAP and Kn) supplementation. The maximum number of nodes per shoot (7.25) was counted in the varieties Zaubilati in presence of 1.5 mg/l BAP and Challisha without BAP concentration (7.25) followed by Shadaguti (6.00) in 1.5 mg/l BAP and also Zaubilati in 1.0 mg/l BAP (Table 2 and Fig. 4). On the other hand, the maximum number of nodes per shoot (9.50) was counted in Shadaguti followed by Challisha (9.25) without Kn concentration (Table 3). The lowest number of nodes per shoot was recorded in Challisha (4.75) with 1.5 mg/l Kn. The result of the present study indicated that the number of nodes increased with the increase of BAP up to (1.0-1.5 mg/l) and decreased with higher concentration (2.0-2.5 mg/l) in two potato varieties Zaubilati and Challisha, but in Challisha BAP had little effect to increase the number of nodes per plantlet.

3.1.5 Number of leaves per plantlet

Significant variations were found among the varieties in respect of number of leaves per plantlet (Table 4 - 5). In BAP treatments, the highest number of leaves per plantlet (6.79) was recorded in Zaubilati followed by Shadaguti (6.67), while it was the lowest (4.38) in Challisha (Fig. 1). On the other hand, the highest number of leaves per plantlet (8.13) was recorded in Zaubilati followed by Shadaguti (8.08) in Kn treatments (Fig. 1).

Different levels of cytokinin (BAP and Kn) supplements significantly influenced the number of leaves per plantlet. The highest number of leaves per plantlet (6.92) was recorded in 1.5 mg/l BAP and the lowest number of leaves per plantlet (4.83) was observed in 2.5 mg/l BAP (Fig. 2). On the other hand, the highest number of leaves per plantlet (9.42) was recorded at Kn free medium followed by 8.25 at 0.5 mg/l Kn (Fig. 2).

Significant variations were found in respect of number of leaves per plantlet due to the interactive effect of variety and cytokinin (BAP and Kn). The maximum number of leaves per plantlet (8.75) was observed in the variety Shadaguti with 1.5 mg/l BAP followed by Zaubilati (8.50) with same level of BAP, which was statistically significant. The lowest number of leaves per plantlet (2.50) was recorded in Challisha with 2.0 mg/l BAP (Table 2). At Kn treatment, the highest number of leaves per plantlet (9.75) was recorded in Challisha without Kn followed by Shadaguti (9.50) at (0- 0.5 mg/l) Kn and also Zaubilati at 0.5 mg/l Kn. On the other hand, the lowest

number of leaves (5.75) was recorded from Challisha at 0.5 mg/l Kn (Table 3). Result also showed that the number of leaves per plantlet decreased with higher concentration of Kn in two varieties, Zaubilati and Shadaguti.

3.1.6 Fresh weight of shoots per plantlet

Significant variation was found among the varieties in respect of fresh weight of shoots. The maximum fresh weight of shoot (30.31 mg) was recorded in the variety Shadaguti in BAP treatments and the minimum fresh weight of shoot (19.56mg) was recorded in Challisha (Fig. 1). On the other hand, the maximum fresh weight of shoot (57.02 mg) was recorded in Zaubilati followed by Shadaguti (43.12 mg) and the minimum fresh weight (29.65 mg) was recorded in Challisha (Fig. 1). Result indicated that Kn performed better than BAP in case of fresh weight.

Different levels of cytokinin (BAP and Kn) supplements significantly influenced the fresh weight of shoot per plantlet. The maximum fresh weight of shoot (32.68 mg) was recorded at 1.5 mg/l BAP concentration (Fig. 2), while at Kn treatment, maximum fresh weight of shoot was recorded without Kn concentration (53.34 mg) followed by 0.5 mg/l Kn (51.08 mg) (Fig. 2). Lesser amount of fresh weight of shoot was found in higher concentrations of both cytokinin treatments.

Statistically significant variation was found in respect of fresh weight of shoot per plantlet in the tested varieties and cytokinin (BAP and Kn) supplementations. The maximum fresh weight of shoot per plantlet (39.83 mg) was recorded in the variety Shadaguti with 1.5 mg/l BAP followed by Zaubilati (37.70 mg) with same level of BAP concentration (Table 2). The minimum fresh weight of shoots per plantlet (13.53 mg) was recorded in Challisha with 2.0 mg/l BAP and at 1.0 mg/l Kn also (18.55 mg).On the other hand, maximum fresh weight of shoot per plant (81.75 mg) was recorded in the variety Zaubilati 0.5 mg/l Kn (Table 3).

The reason for higher fresh weight of shoots might be due to the robust shoot and more number of leaves per plantlet.

Overall response of shoot apex explant of three potato varieties

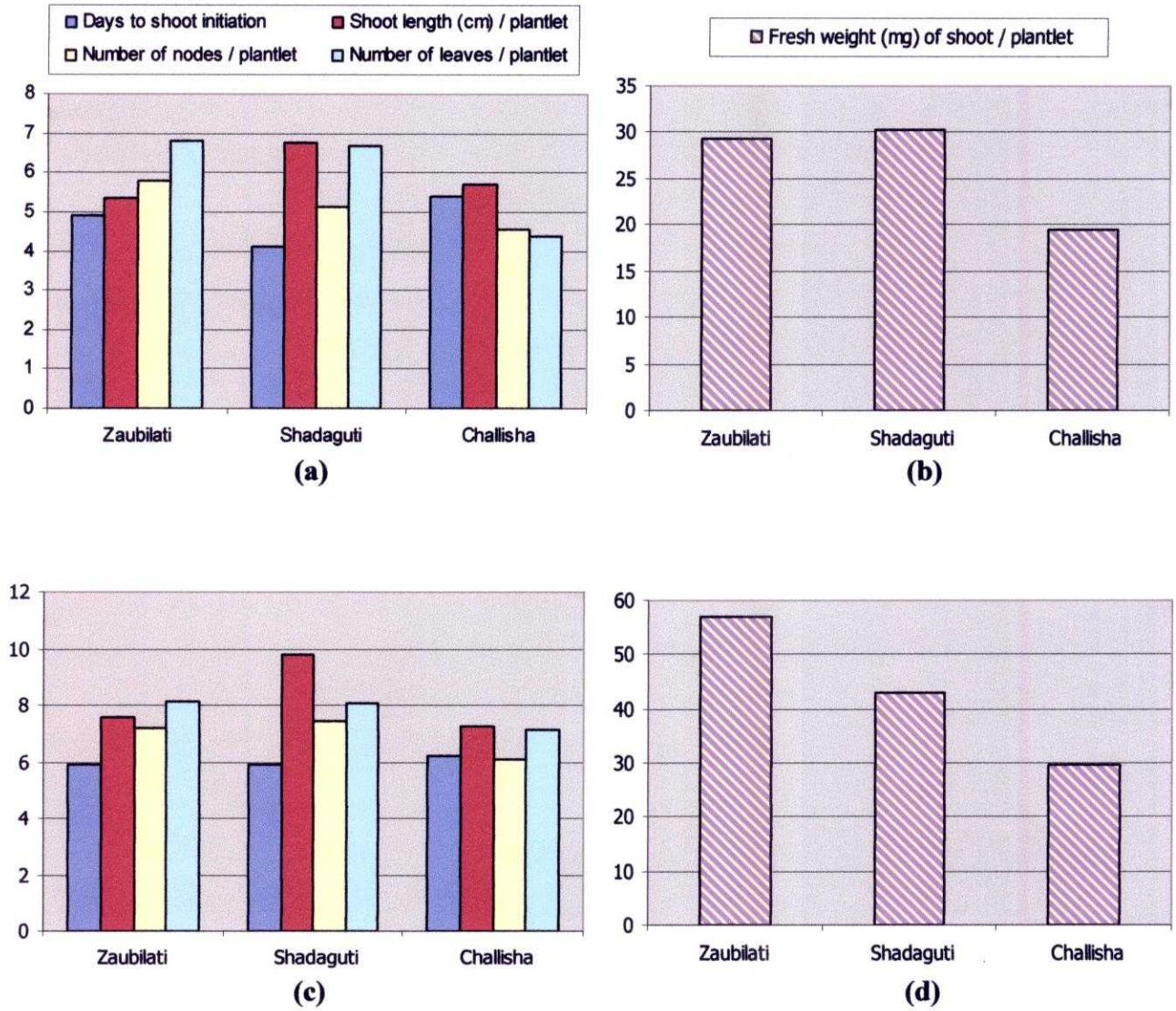

Figure 1. Varietals effect on *in vitro* regeneration response from shoot apex explant of three potato varieties (data collected 30 days after inoculation) in BAP (a-b) and Kn (c-d) treatments.

Overall response of shoot apex explant of three potato varieties

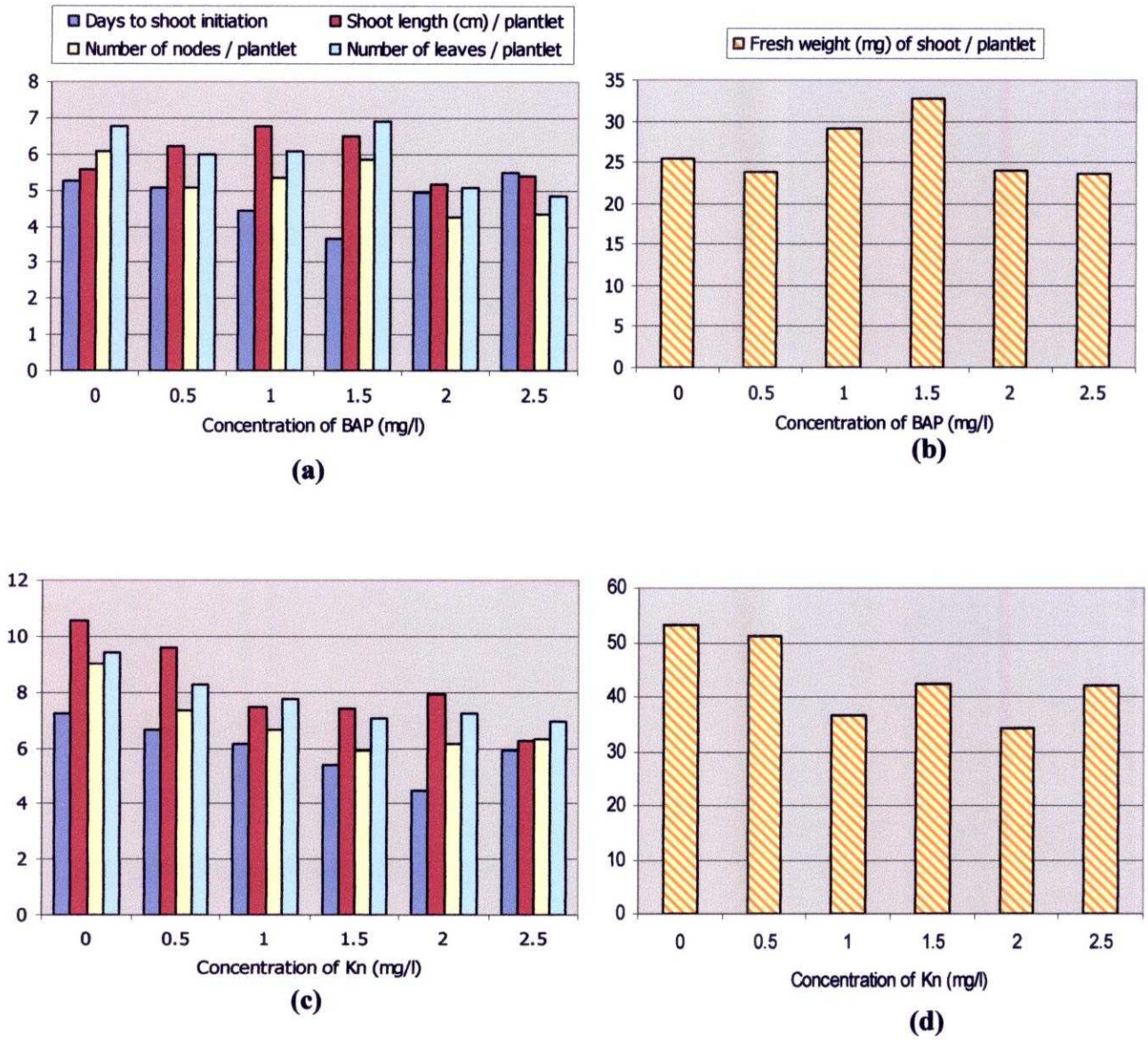

Figure 2. Effect of different concentrations of BAP (a-b) and Kn (c-d) on *in vitro* regeneration from shoot apex explant of three potato varieties (data collected 30 days after inoculation).

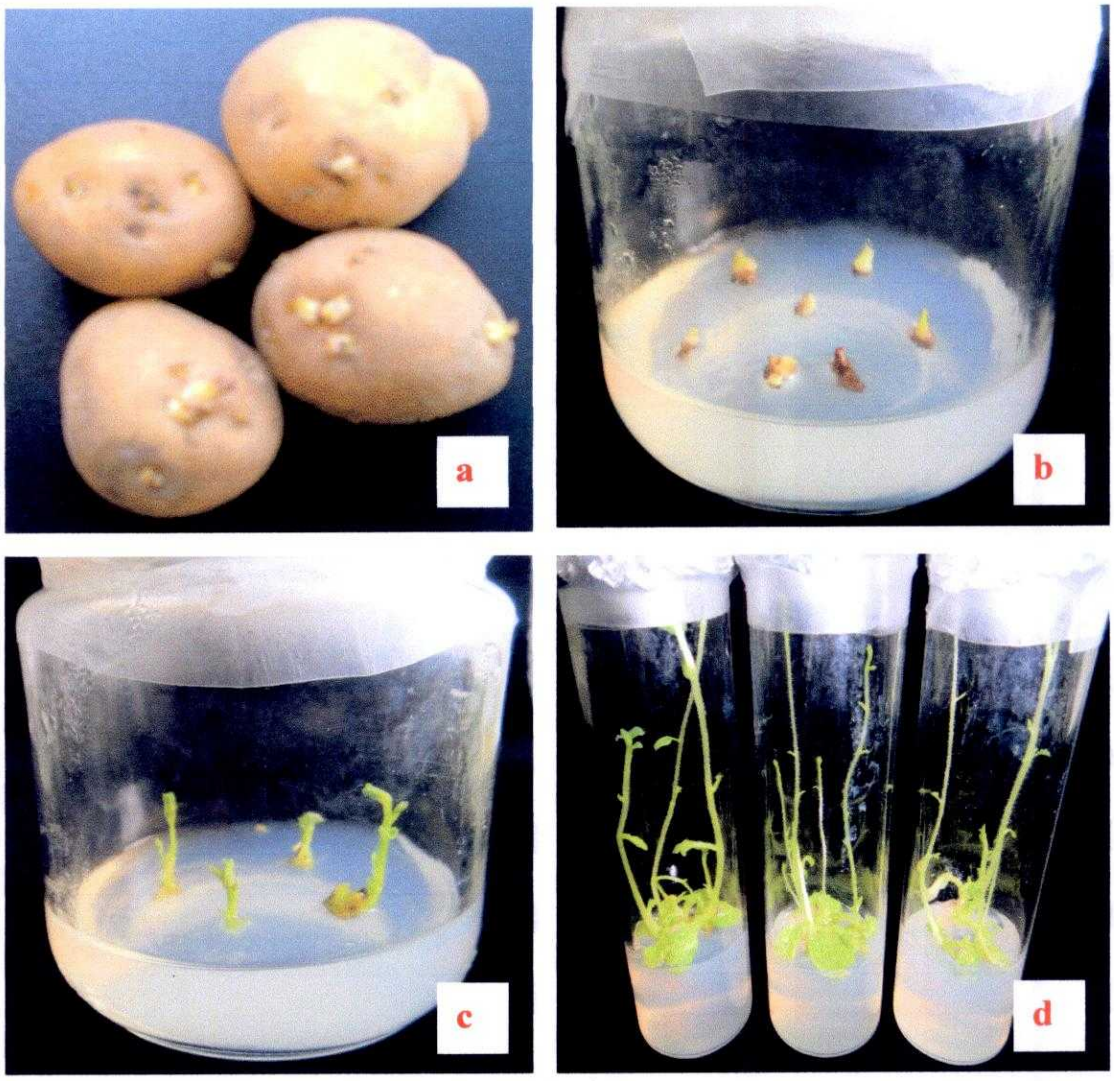

Figure 3. Sprout to plantlet regeneration; a. Sprout of Shadaguti variety, b. Sprout inoculation for regeneration in PROP medium, c. Sprout regeneration 1 week after inoculation, d. Regenerated plantlet (after 4 weeks).

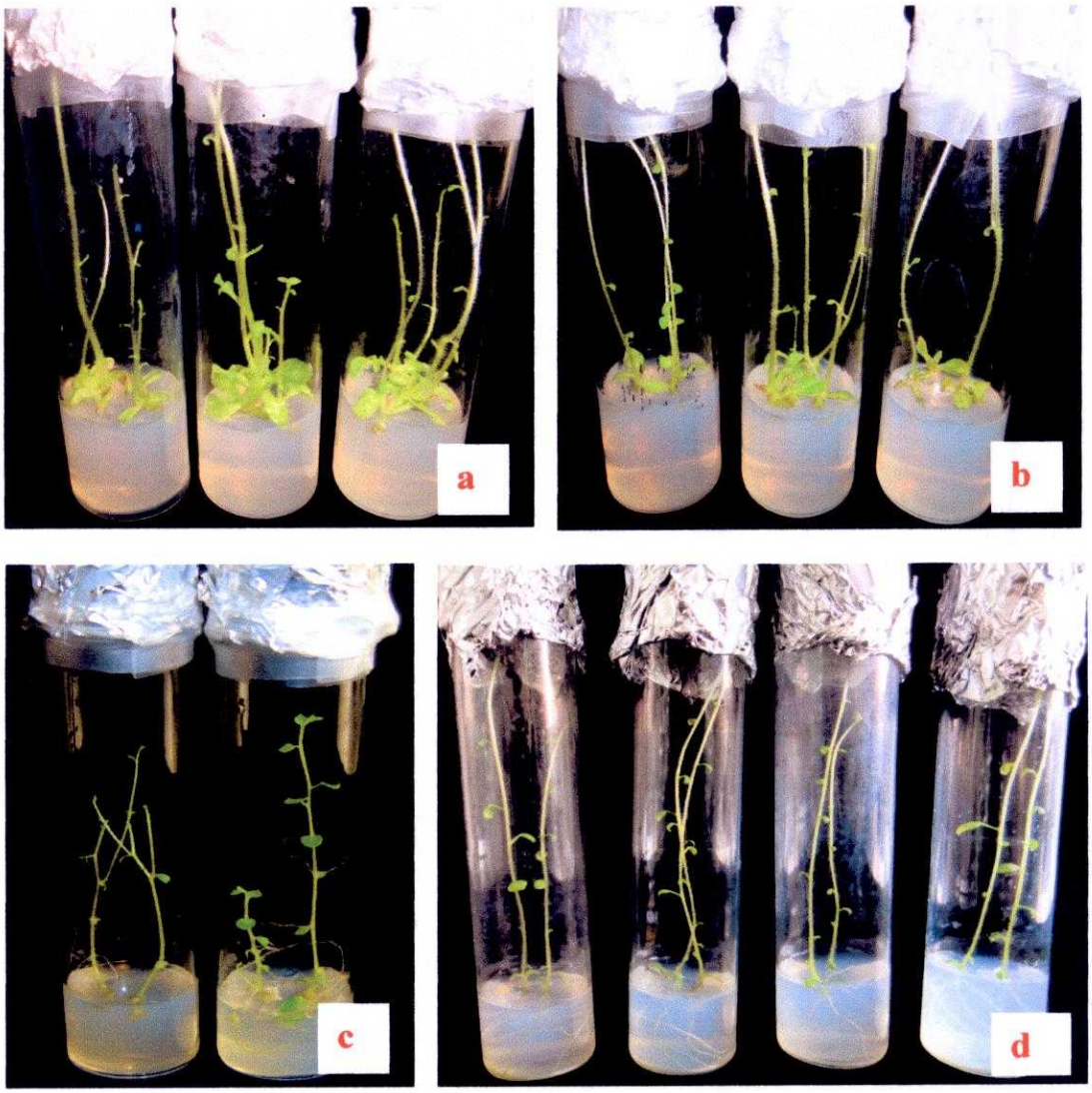

Figure 4. Shoot apex regeneration from Shadaguti (a-b), Zaubilati (c) and Challisha (d); a. PROP with 1.5 mg/l BAP, b. PROP with 1.0 mg/l BAP, c. PROP with 2.0 mg/l BAP and d. absence of BAP; (Photographs taken 30 days after inoculation).

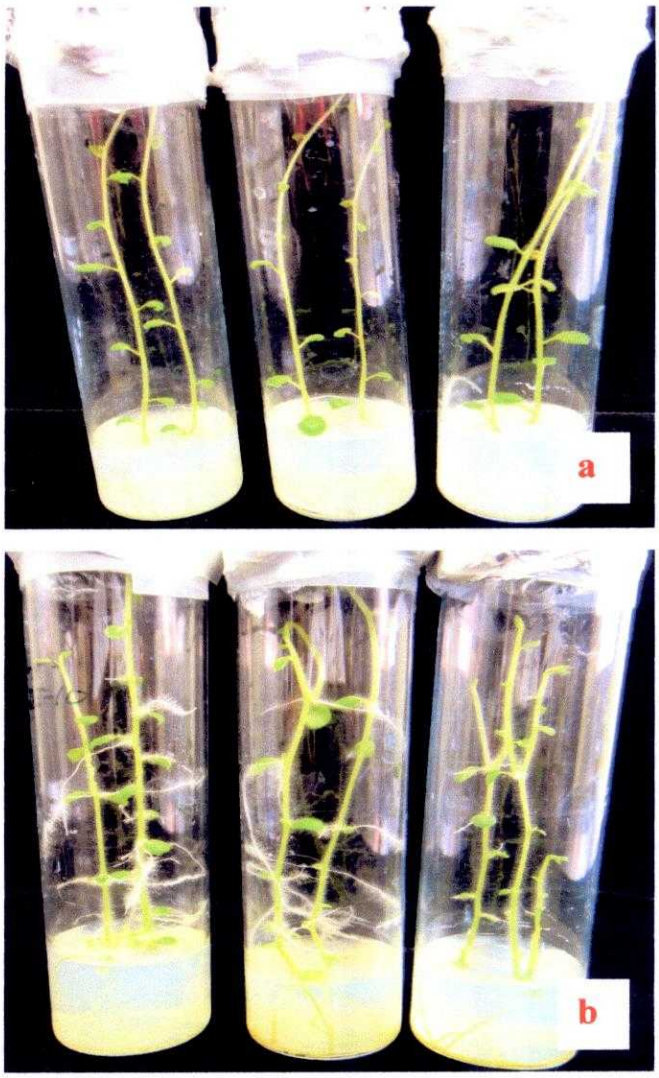

Figure 5. Shoot apex regeneration in PROP with 0.5 mg/l Kn; a. Shadaguti variety and b. Zaubilati variety; (Photographs taken 30 days after inoculation).

3.2 Effect of different concentrations of BAP and Kn on *in vitro* regeneration from nodal segment of potato

Nodal segment of three potato varieties were cultured on PROP medium supplemented with different concentrations (0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) of cytokinins (BAP and Kn). Data were recorded 30 days after inoculation on different parameters and were statistically analyzed. The results are shown in Tables 6 - 7 and Figs. 6 - 9. The analysis of variance of data has been presented in Table 8 and 9.

3.2.1 Survival percentage of explants

Nodal segment of potato varieties showed no significant variation in response to different concentrations of BAP and Kn. The maximum response (100%) was recorded under all treatments of BAP concentrations as well as Kn. It was also found that the maximum explants responded in all varieties with supplementation of various cytokinin like BAP and Kn (Table 6 and 7).

3.2.2 Day requirement for shoot initiation

The days required for shoot initiation was not significantly influenced by the varieties (Table 8 and 9). Early shoot initiation (6.08 days) was found in Shadaguti followed by Zaubilati (6.25 days) in BAP treatment (Fig. 6). On the other hand, Shadaguti also showed early shoot initiation in 6.21 days at Kn treatment (Fig. 6). The variety, Challisha took the highest time (6.33 days at BAP and 6.50 days at Kn) for shoot initiation.

Different concentrations of cytokinin (BAP and Kn) also markedly influenced day requirement for shoot initiation. BAP (1.0 mg/l) took minimum days (5.0) for shoot initiation whereas it was the maximum (7.0 days) in BAP devoid medium (Fig. 7). On the other hand, 1.5 mg/l Kn took minimum days (4.58) for shoot initiation.

Remarkable variation was observed in respect of days required for shoot initiation among the varieties and cytokinin (BAP and Kn) concentrations. The maximum days required for shoot initiation (7.50 days) was noted in Challisha with 2.5 mg/l BAP followed by Zaubilati (7.0 days) with 2.0 mg/l BAP and also without BAP concentration. Early shoot initiation (4.75 days) was found in Shadaguti with 1.0 mg/l BAP (Table 6).

Table 6. *In vitro* regeneration response from nodal segment of three potato varieties toward various BAP concentrations.

Variety	Concentration BAP (mg/l)	Responsive explant (%)	Days to shoot initiation	Shoot length (cm) / plantlet	No. of nodes / plantlet	No. of leaves / plantlet	Fresh weight(mg) of shoot / plantlet
Zaubilati	0.0	100	7.00	8.30	7.00	7.50	32.53
	0.5	100	6.50	5.96	6.00	6.50	27.80
	1.0	100	5.00	5.93	5.00	7.75	43.73
	1.5	100	5.50	6.65	4.75	6.75	32.88
	2.0	100	7.00	5.25	4.00	5.25	26.08
	2.5	100	6.50	4.73	3.25	4.75	17.48
Shadaguti	0.0	100	7.00	5.20	5.50	6.25	13.25
	0.5	100	5.75	5.62	4.75	5.75	15.13
	1.0	100	4.75	7.68	5.00	6.00	27.83
	1.5	100	6.00	6.22	6.00	6.25	13.50
	2.0	100	6.25	5.43	5.25	6.0	13.13
	2.5	100	6.75	5.02	4.50	5.75	19.68
Challisha	0.0	100	7.00	8.78	7.50	7.75	21.68
	0.5	100	6.50	6.50	5.75	5.00	15.25
	1.0	100	5.25	6.18	5.00	4.75	18.03
	1.5	100	5.50	6.25	4.25	3.50	14.50
	2.0	100	6.25	5.23	4.0	3.00	12.55
	2.5	100	7.50	4.45	3.75	4.00	12.45
LSD (0.01)	-	-	NS	0.82	1.04	1.56	3.85
CV (%)	-	-	11.26	7.10	10.82	14.52	9.71

Significantly at 1% level of probability, NS = Non-significant

Data collected 30 days after inoculation.

Table 7. *In vitro* regeneration response from nodal segment of three potato varieties toward various Kn concentrations.

Variety	Concentration Kn (mg/l)	Responsive explant (%)	Days to shoot initiation	Shoot length (cm) / plantlet	No. of nodes / plantlet	No. of leaves / plantlet	Fresh weight(mg) of shoot / plantlet
Zaubilati	0.0	100	7.50	6.45	5.50	6.75	35.55
	0.5	100	6.50	7.45	9.00	10.25	54.68
	1.0	100	5.25	6.80	8.50	10.00	36.75
	1.5	100	4.50	6.45	7.50	9.00	36.40
	2.0	100	6.75	5.53	5.50	7.00	34.18
	2.5	100	7.00	5.25	5.00	6.00	34.50
Shadaguti	0.0	100	7.50	10.95	8.50	8.75	44.40
	0.5	100	6.50	12.68	9.00	9.00	57.50
	1.0	100	5.50	9.45	7.25	7.50	33.98
	1.5	100	4.50	7.23	5.75	6.50	24.95
	2.0	100	5.75	7.78	6.50	7.50	23.58
	2.5	100	7.50	5.75	4.75	5.50	31.95
Challisha	0.0	100	7.50	8.70	7.00	8.25	35.73
	0.5	100	6.50	10.33	7.50	9.00	37.73
	1.0	100	5.75	6.63	5.00	5.75	25.70
	1.5	100	4.75	8.43	5.75	7.50	29.25
	2.0	100	6.75	7.38	5.25	6.25	22.38
	2.5	100	7.75	6.88	5.50	6.50	25.00
LSD (0.01)	-	-	NS	1.14	1.58	1.47	NS
CV (%)	-	-	9.45	7.73	12.67	10.19	22.04

Significantly at 1% level of probability, NS = Non-significant

Data collected 30 days after inoculation.

Table 8. ANOVA of different concentrations of BAP on *in vitro* direct regeneration from nodal segment of potato varieties.

Sources of variation	Degrees of freedom	Mean sum of square				
		Days to shoot initiation	Shoot length(cm) / plant	Number of nodes / plant	Number of leaves / plant	Fresh weight(mg) of shoot / plant
Variety	2	0.389 NS	0.892 *	0.181 NS	21.097 **	1505.023**
Treatment	5	7.122 **	11.012 **	11.281 **	9.181 **	282.639 **
Variety × Treatment	10	0.556 NS	3.830 **	2.564 **	3.481 **	103.665 **
Error	54	0.491	0.186	0.301	0.681	4.144

** = 1% level of significance, * = 5% level of significance, NS = Non-significant

Table 9. ANOVA of different concentrations of Kn on *in vitro* direct regeneration from nodal segment of potato varieties.

Sources of variation	Degrees of freedom	Mean sum of square				
		Days to shoot initiation	Shoot length(cm) / plant	Number of nodes / plant	Number of leaves / plant	Fresh weight(mg) of shoot / plant
Variety	2	0.597 NS	43.469 **	6.514**	5.931 **	562.188 **
Treatment	5	15.181 **	25.838 **	16.714 **	15.489 **	855.352 **
Variety × Treatment	10	0.331 NS	5.338 **	4.897 **	5.531 **	105.833NS
Error	54	0.356	0.361	0.699	0.602	58.385

** = 1% level of significance, NS = Non-significant

On the other hand, in presence of Kn treatment early shoot initiation (4.50 days) was also found in Shadaguti and Zaubilati with 1.5 mg/l Kn followed by Challisha (4.75 days) with same concentration. But the maximum day requirement for shoot initiation was found in medium without cytokinin supplements (Table 7). The results of this study reflected that days required for shoot initiation took minimum days with the increasing BAP and Kn concentrations. This indicated that BAP and Kn enhances cell division and cell elongation.

3.2.3 Shoot length (cm) per plantlet

Significant variation was observed among the varieties regarding length of shoot (Table 8 and 9). The maximum length (6.23 cm) was measured in the variety Challisha at BAP treatment and the variety Shadaguti produced the minimum length (5.86 cm) of shoots (Fig. 6). At Kn treatment, the maximum length (8.97 cm) was measured in the variety Shadaguti and the minimum length (6.32 cm) was measured in Zaubilati (Fig. 6).

Different levels of cytokinin (BAP and Kn) concentration significantly influenced the length of shoot. The maximum shoot length (7.43cm) was recorded without BAP concentration and the lowest shoot length (4.73 cm) was measured in 2.5 mg/l BAP (Fig. 7). On the other hand, the maximum shoot length (10.15 cm) was recorded with 0.5 mg/l Kn, which is better than BAP treatment. In 2.5 mg/l Kn showed the lowest shoot length (5.96 cm) than other concentrations (Fig. 7).

The interaction of variety and cytokinin (BAP and Kn) concentration had significantly influenced the length of shoot per explant. The maximum shoot length (8.78 cm) was measured in Challisha without BAP concentration (Table 6 and Fig. 8). The lowest length (4.45 cm) was found in Challisha with 2.5 mg/l BAP followed by Zaubilati (4.73 cm) with same concentration. On the other hand, Kn showed better result than BAP. The maximum shoot length (12.68 cm) was measured in Shadaguti with 0.5 mg/l Kn followed by without Kn medium (10.95 cm) (Table 7 and Fig. 9). Zaubilati also showed the lowest shoot length (5.25 cm) at 2.5 mg/l Kn concentration. The result also indicated that higher concentrations (2.5 mg/l) of BAP decreased shoot length.

3.2.4 Number of nodes per plantlet

Significant variation was found among the varieties in respect to number of nodes per shoot in Kn treatment but not in BAP treatment (Table 8 and 9). The highest number of nodes per shoot (5.17) was recorded in Shadaguti followed by Challisha (5.04) at BAP treatment (Fig. 6). At Kn treatment, the highest number of nodes per shoot (6.96) was recorded in Shadaguti followed by Zaubilati (6.83) (Fig. 6).

Different levels of cytokinin (BAP and Kn) concentrations significantly influenced the number of nodes per plantlet. The highest number of nodes per shoot (6.67) was recorded at BAP free medium and the lowest (3.83) was recorded at 2.5 mg/l BAP. On the other hand, the highest number of nodes per shoot (8.50) was recorded at 0.5 mg/l Kn and the lowest (5.08) was recorded at 2.5 mg/l Kn (Fig. 7).

Different variations were found in respect of number of nodes per shoot in different variety and cytokinins (BAP and Kn). The maximum number of nodes per shoot (7.50) was counted in the variety Challisha at BAP devoid medium followed by Zaubilati (7.0) at the same treatment (Table 6), while the lowest number of nodes (3.25) was recorded in Zaubilati with 2.5 mg/l BAP. On the other hand, the maximum number of nodes per shoot (9.0) was counted in Shadaguti and Zaubilati with 0.5 mg/l Kn and the lowest (4.75) was obtained from Shadaguti at 2.5 mg/l Kn (Table 7 and Fig. 9). Result also indicated that higher concentration of BAP and Kn did not give any advantage in case of nodes per plantlet.

3.2.5 Number of leaves per plantlet

Remarkable variations were found among the varieties in respect to number of leaves per plantlet. At BAP treatments the highest number of leaves per shoot (6.42) was recorded in Zaubilati followed by Shadaguti (6.0), while it was the lowest (4.63) in Challisha (Fig. 6). On the other hand, the highest number of leaves per shoot (8.17) was recorded in Zaubilati followed by Shadaguti (7.13) also at Kn treatment (Fig. 6).

Different levels of cytokinin (BAP and Kn) concentrations significantly influenced the number of leaves per plantlet. The highest number of leaves per plantlet (7.08) was recorded in without BAP medium and the lowest number of leaves per plant (4.75) was observed in 2.0 mg/l BAP (Fig. 7). On the other hand, the highest number of leaves per plantlet (9.42) was recorded at 0.5 mg/l Kn and the lowest number of leaves per plant (6.0) was observed in 2.5 mg/l Kn (Fig. 7).

Distinct variations were found in respect of number of leaves per plantlet due to the interaction effect of variety and cytokinin (BAP and Kn) concentrations. The maximum number of leaves per shoot (7.75) was observed in the variety Zaubilati with 1.0 mg/l BAP followed by Challisha (7.75) at without BAP. The lowest number of leaves per plantlet (3.0) was recorded in Challisha with 2.0 mg/l BAP (Table 6). At Kn treatment, the highest number of leaves per plantlet (10.25) was recorded in Zaubilati with 0.5 mg/l Kn followed by Shadaguti (9.0) and Challisha (9.0) with same level of BAP. On the other hand, the lowest number of leaves (5.50) was recorded in Shadaguti at 2.5 mg/l Kn (Table 7).The result of the present study revealed that number of leaves per shoot decreased with the increasing of BAP and Kn concentration.

3.2.6 Fresh weight of shoot per plantlet

Significant variation was found among the varieties in respect of fresh weight of shoot. The maximum fresh weight of shoot (30.08 mg) was recorded in the variety Zaubilati. The minimum fresh weight of shoot (15.74 mg) was recorded in Challisha at BAP treatment (Fig. 6). On the other hand, at Kn treatment, the maximum fresh weight of shoot (38.68 mg) was recorded in Zaubilati followed by Shadaguti (36.06 mg) (Fig. 6).

Different levels of cytokinin (BAP and Kn) concentration significantly influenced the fresh weight of shoot per plantlet. The maximum fresh weight of shoot (29.86 mg) was recorded at 1.0 mg/l BAP concentration and the minimum (16.53 mg) was recorded from 2.5 mg/l BAP (Fig. 7). At Kn treatment, the maximum fresh weight of shoot (49.97 mg) was recorded at 0.5 mg/l Kn concentration. The minimum fresh weight of shoot (26.71 mg) was found in 2.0 mg/l Kn concentration (Fig. 7).

Distinct variations were found in respect of fresh weight of shoot per plantlet due to the interaction effect of variety and cytokinin (BAP and Kn) concentration. The maximum fresh weight of shoot per plantlet (43.73 mg) was recorded in the variety Zaubilati with 1.0 mg/l BAP followed by Shadaguti (27.83 mg) with same level of BAP concentration, while the lowest (12.45 mg) was obtained from Challisha at 2.5 mg/l BAP (Table 6). On the other hand, the maximum fresh weight of shoot per plant (57.50 mg) was recorded in the variety Shadaguti with 0.5 mg/l Kn followed by Zaubilati (54.68 mg) with same level of Kn concentration. The minimum fresh weight of shoots per plant (22.38 mg) was recorded in Challisha with 2.0 mg/l Kn (Table 7).

Result indicated that higher concentration of Kn decrease fresh weight of shoot per plantlet.

3.2.7 Decision from above experiments

Considering the responses of two potato explants from three potato varieties, it may be concluded that, Shadaguti variety is found the best genotype for *in vitro* regeneration and it also found that, shoot apex of potato varieties showed better regeneration such as, early shoot initiation, long shoot length, maximum number of nodes, leaves and maximum fresh weight of shoot than the nodal segment of potato. The appropriate concentration of BAP for shoot regeneration found to be 1.5 mg/l BAP for shoot apex explant and for nodal segment application of BAP had no better advantage. Only PROP medium was enough for shoot regeneration. In case of Kn concentration, the appropriate concentration for shoot regeneration was 0.5 mg/l Kn for nodal segment but in shoot apex explant application of Kn had no advantage. Result also indicated that Kn treatment performed better than BAP treatment in all the parameters evaluated for shoot regeneration.

Overall response of nodal segment explant of three potato varieties

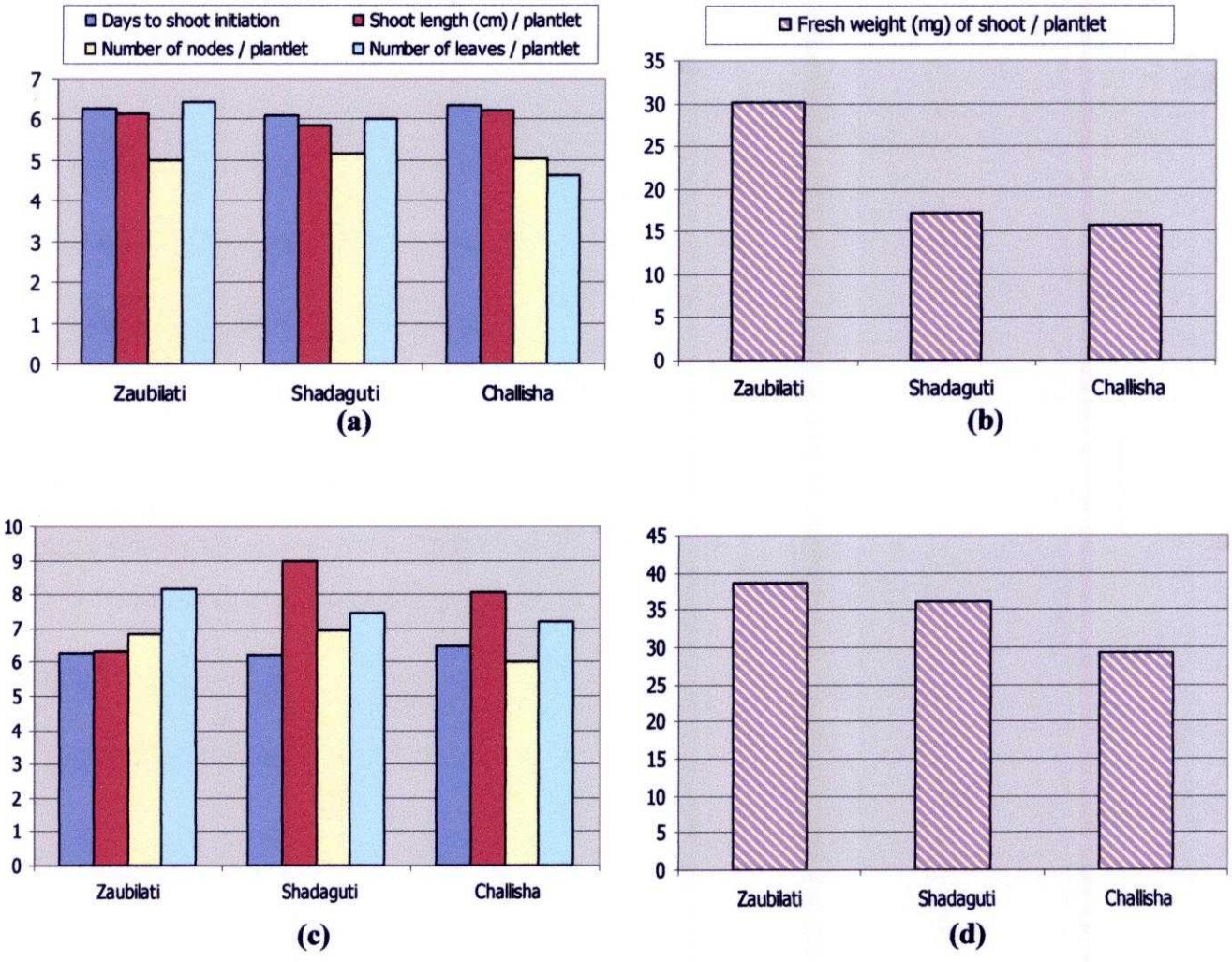

Figure 6. Varietals effect on *in vitro* regeneration from nodal segment explant of three potato varieties (data collected 30 days after inoculation) in BAP (a-b) and Kn (c-d) treatments.

Overall response of nodal segment explant of three potato varieties

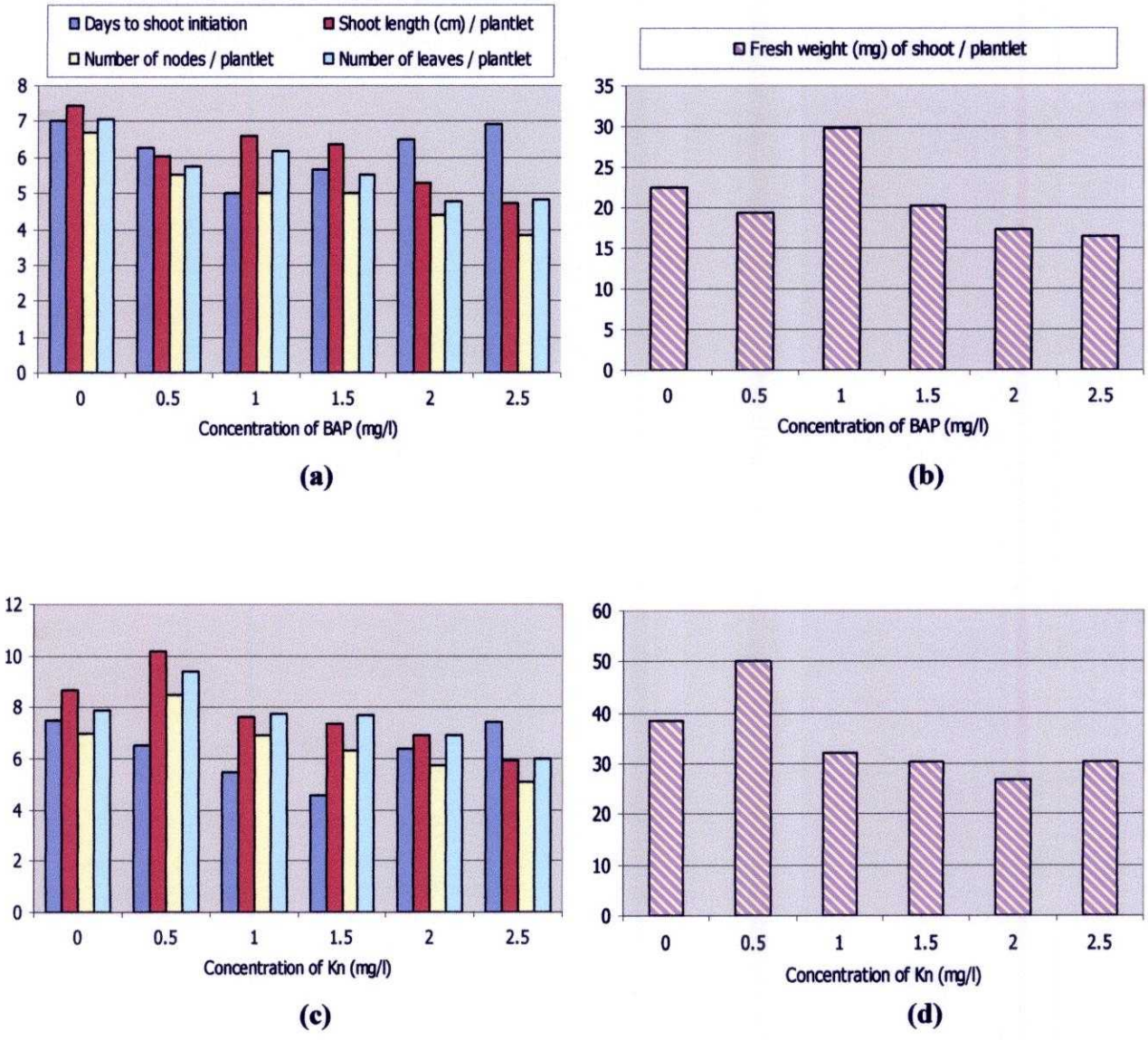

Figure 7. Effect of different concentrations (mg/l) of BAP (a-b) and Kn (c-d) on *in vitro* regeneration from nodal segment explant of three potato varieties (data collected after 30 days of culture).

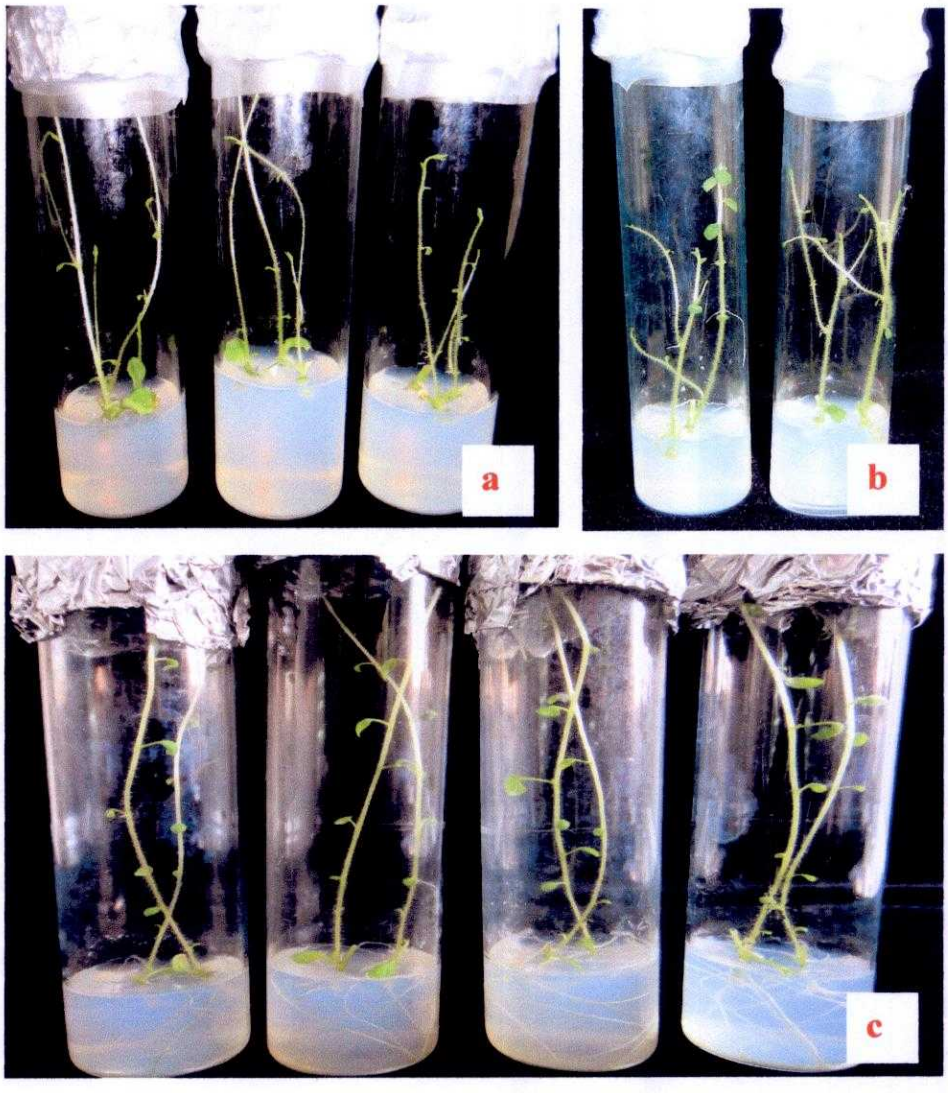

Figure 8. Nodal segment regeneration from Shadaguti (a), Zaubilati (b), Challisha (c); a. PROP with 1.0 mg/l BAP, b. PROP with 1.0 mg/l BAP and c. in absence of BAP; (Photographs taken 30 days after inoculation).

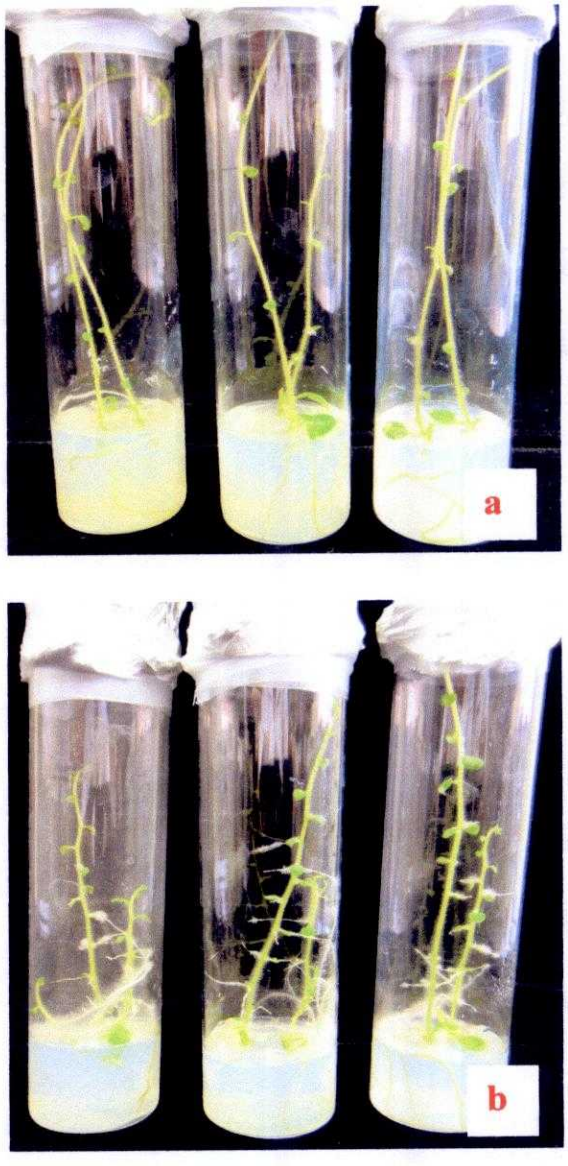

Figure 9. Nodal segment regeneration in PROP with 0.5 mg/l Kn; a. Shadaguti variety and b. Zaubilati variety; (Photographs taken 30 days after inoculation).

3.3 Effect of different concentrations of BAP and Kn along with auxins (GA_3 and IAA) on direct regeneration from internode explant of potato.

Internode explants of three potato varieties did not response in PROP medium supplemented with cytokinin (BAP and Kn). So, to achieve regeneration internode explants were cultured on medium supplemented with auxins, such as, GA_3 and IAA. Different concentrations of BAP and Kn along with GA_3 (0.2 mg/l) and IAA (0.5 mg/l) based on Iqbal *et al.* (2005) and Sarker and Mustafa (2002) were used in this study. Data of different parameters regarding direct regeneration and growth behavior were obtained 60 days after inoculation and statistically analyzed. The results are shown in Tables 10 - 11 and Figs. 10 - 13 and the analysis of variance of data has been presented in Tables 12 - 13.

3.3.1 Day requirement for shoot initiation

Time requirement for shoot initiation was significantly influenced by the varieties / genotype at BAP treatment (Tables 12 - 13). While considering the overall response early shoot initiation (27.81 days) was found in Shadaguti (Fig. 10). However, Kn didn't show any good response because Shadaguti took 42.50 days for shoot initiation (Fig. 10).

Different concentrations of BAP and Kn also markedly influenced the day requirement for shoot initiation. BAP 1.5 mg/l took minimum days (27.75) for shoot initiation whereas, it was the maximum (30.92 days) in 0.5 mg/l BAP followed by 2.0 mg/l BAP (30.50 days) (Fig. 11). In Kn treatment, minimum days (40.2 days) was observed in 1.5 mg/l Kn and the maximum days observed in 2.0 mg/l Kn concentration (Fig. 11).

Remarkable variation was observed in respect of days required for shoot initiation within varieties in different cytokinins (BAP and Kn) along with GA_3 and IAA (Table 12 and 13). The maximum day requirement for shoot initiation (33.5 days) was noted in Challisha with 0.5 mg/l BAP followed by 32.0 days in the same variety with 0.2 mg/l BAP. Early shoot initiation was found (26.50 days) in Shadaguti with 1.5 mg/l BAP (Table 10) followed by 0.5 mg/l BAP (28.0 days) in same variety.

Table 10. *In vitro* regeneration response from internode explant of three potato varieties toward various BAP concentrations along with GA_3 (0.2 mg/l) and IAA (0.5 mg/l).

Variety	Concentrations (mg/l)			Days to shoot initiation	Percentage of explant producing shoot	Percentage of explant producing root	Percentage of explant producing both shoot and root	Number of multiple shoots / explant
	BAP	GA_3	IAA					
Zaubilati	0.0	0.0	0.0	-	-	-	-	-
	0.5	0.2	0.5	31.50	70 (57.08)	10 (13.92)	10 (13.92)	2.04
	1.0	0.2	0.5	31.25	70 (60.51)	25 (29.72)	20 (23.40)	2.85
	1.5	0.2	0.5	28.25	60 (51.31)	20 (26.55)	15 (20.23)	2.81
	2.0	0.2	0.5	30.50	50 (44.98)	20 (20.25)	20 (20.25)	2.38
Shadaguti	0.0	0.0	0.0	-	-	-	-	-
	0.5	0.2	0.5	27.75	90 (76.05)	70 (60.51)	65 (57.35)	3.20
	1.0	0.2	0.5	28.00	100 (88.68)	70 (60.51)	70 (60.51)	3.55
	1.5	0.2	0.5	26.50	95 (82.36)	25 (26.57)	25 (26.57)	4.59
	2.0	0.2	0.5	29.00	100 (88.68)	25 (29.72)	25 (25.72)	4.70
Challisha	0.0	0.0	0.0	-	-	-	-	-
	0.5	0.2	0.5	33.50	60 (51.03)	25 (29.72)	25 (29.72)	1.99
	1.0	0.2	0.5	30.25	65 (53.91)	35 (35.77)	25 (29.72)	1.64
	1.5	0.2	0.5	28.50	65 (53.91)	35 (36.05)	35 (36.05)	2.17
	2.0	0.2	0.5	32.00	60 (51.03)	60 (51.03)	50 (44.98)	1.60
LSD (0.01)	-	-	-	NS	NS	26.65	-	0.77
LSD (0.05)	-	-	-	-	-	-	21.71	-
CV (%)	-	-	-	5.37	17.01	39.58	46.29	19.11

Significantly at 1% and 5% level of probability, NS = Non-significant.

Data collected 60 days after inoculation. Parentheses indicate the arcsin transformed value.

Table 11. *In vitro* regeneration response from internode explant of three potato varieties toward various Kn concentrations along with GA_3 (0.2 mg/l) and IAA (0.5 mg/l).

Variety	Concentrations (mg/l)			Days to shoot initiation	Percentage of explant producing shoot	Percentage of explant producing root	Percentage of explant producing both shoot and root	Number of multiple shoots / explant
	Kn	GA_3	IAA					
Zaubilati	0.0	0.0	0.0	-	-	-	-	-
	0.5	0.2	0.5	44	5 (7.59)	55 (47.86)	5 (7.59)	0.25
	1.0	0.2	0.5	44	5 (7.59)	60 (51.03)	5 (7.59)	0.25
	1.5	0.2	0.5	-	- (1.28)	70 (57.08)	- (1.28)	-
	2.0	0.2	0.5	-	- (1.28)	50 (44.98)	- (1.28)	-
Shadaguti	0.0	0.0	0.0	-	-	-	-	-
	0.5	0.2	0.5	44.33	15 (20.23)	70 (57.08)	15 (20.23)	0.75
	1.0	0.2	0.5	39	15 (20.23)	80 (66.56)	15 (20.23)	0.75
	1.5	0.2	0.5	42.5	25 (29.72)	90 (76.05)	20 (26.55)	1.38
	2.0	0.2	0.5	45	10 (13.92)	65 (53.91)	10 (13.92)	0.50
Challisha	0.0	0.0	0.0	-	-	-	-	-
	0.5	0.2	0.5	41	10 (13.92)	60 (50.75)	10 (13.92)	0.50
	1.0	0.2	0.5	40	10 (10.76)	60 (51.03)	10 (10.76)	0.25
	1.5	0.2	0.5	41.5	10 (13.92)	70 (57.08)	10 (13.92)	0.50
	2.0	0.2	0.5	43	15 (17.08)	55 (47.86)	15 (17.08)	0.50
LSD (0.01)	-	-	-	NS	NS	NS	NS	NS
CV (%)	-	-	-	97.57	99.24	16.34	100.26	103.28

Significantly at 1% level of probability, NS = Non-significant.

Data collected 60 days after inoculation. Parentheses indicate the arcsin transformed value.

Table 12. ANOVA of different concentrations of BAP along with GA_3 and IAA on *in vitro* direct regeneration from internode explant of potato.

Sources of variation	Degrees of freedom	Mean sum of square				
		Days to shoot initiation	Explant producing shoot (%)	Explant producing root (%)	Explant producing both shoot and root (%)	Average No. of multiple shoots / explant
Variety	2	46.938 **	5120.21 **	2003.25 **	2390.68 **	19.520 **
Treatment	3	23.722 **	106.640 NS	314.814 NS	218.694 NS	1.294 **
Variety × Treatment	6	3.993 NS	118.308 NS	807.354 **	665.199 *	0.919 *
Error	36	2.556	115.938	192.192	229.188	0.285

** = 1% level of significance, * = 5% level of significance, NS = Non-significant

Table 13. ANOVA of different concentrations of Kn along with GA_3 and IAA on *in vitro* direct regeneration from internode explant of potato.

Sources of variation	Degrees of freedom	Mean sum of square				
		Days to shoot initiation	Explant producing shoot (%)	Explant producing root (%)	Explant producing both shoot and root (%)	Average No. of multiple shoots / explant
Variety	2	3381.521 **	1108.07 **	833.726 **	1011.41 **	2.078 **
Treatment	3	122.910 NS	38.8003 NS	474.345 **	26.5803 NS	0.186 NS
Variety × Treatment	6	237.243 NS	105.428 NS	46.5310 NS	79.8616 NS	0.259 NS
Error	36	358.118	169.751	81.0465	166.411	0.234

** = 1% level of significance, NS = Non-significant

On the other hand, Kn concentration did not show better response among the three tested potato varieties. Moreover, shoot initiation response was lower than BAP treatment, as it took longer time. The maximum days (39.0) for shoot initiation were found at Shadaguti with 1.0 mg/l Kn. In Zaubilati it took long time (44 days) for shoot initiation in 0.5 mg/l and 1.0 mg/l Kn concentrations but no response in 1.5 and 2.0 mg/l Kn (Table 11). The results of this study reflected that PROP medium supplemented with cytokinin and auxin (GA_3 and IAA) enhanced shoot initiation.

3.3.2 Percentage of explants producing shoots

Percentage of explants producing shoots significantly varied among the different varieties. The maximum responding explant (96.25%) were from Shadaguti variety followed by Zaubilati and Challisha (62.50% both) at BAP treatments (Fig. 10). On the other hand, the maximum response of explants producing shoots (16.25%) found in Shadaguti variety at Kn treatments which was lower than BAP treatments (Fig. 10). Percentage of explants producing shoot significantly varied in response of different concentrations of BAP and Kn along with GA_3 (0.2 mg/l) and IAA (0.5 mg/l) in internode explant. The maximum explants producing shoot (78.33%) was recorded at 1.0 mg/l BAP + 0.2 mg/l GA_3 + 0.5 mg/l IAA followed by 0.5 mg/l and 1.5 mg/l BAP concentration (73.33%) and 70% in 2.0 mg/l BAP + 0.2 mg/l GA_3 + 0.5 mg/l IAA, but without BAP no shoot was observed (Fig. 11). On the other hand, Kn did not show good regeneration. The maximum explants producing shoots (only 11.66 %) found at 1.5 mg/l Kn (Fig. 11).

When considering each hormonal combination individually with each variety striking variation was observed. The maximum explants producing shoot (100%) was observed in 1.0 mg/l BAP and 2.0 mg/l BAP along with GA_3 (0.2 mg/l) and IAA (0.5 mg/l) at Shadaguti variety followed by 1.5 mg/l BAP (95%) in the same variety (Fig. 12). The minimum response (50%) was observed in 2.0 mg/l BAP at Zaubilati (Table 10). On the other hand, in Kn treatment only 25% explants producing shoot was observed in 1.5 mg/l Kn at Shadaguti variety. Zaubilati showed the lowest response (5%) at 0.5 and 1.0 mg/l Kn but, 1.5 and 2.0 mg/l of Kn did not give any shoot (Table 11).

3.3.3 Percentage of explants producing root

A significant portion of the treated explants produced roots. Percentage of explants producing root significantly varied among the different varieties. The maximum explants producing root was recorded at Shadaguti in both BAP (47.50%) and Kn treatments (76.25 %) and the minimum rooting response was observed at Zaubilati in both the treatments (Fig. 10).

Percentage of explants producing root significantly varied at different concentrations of BAP and Kn along with GA_3 (0.2mg/l) and IAA (0.5mg/l) in internodes explants. The maximum explants producing roots (43.33%) and (76.66%) were observed in 1.0 mg/l BAP and 1.5mg/l Kn concentrations respectively. For root initiation, Kn performed better than BAP but in shoot initiation, Kn did not showed any good response. The minimum response of explants producing roots, 26.66% and 56.66 % were observed in 1.5 mg/l BAP and 2.0 mg/l Kn, respectively (Fig. 11).

In response to various concentrations of BAP or Kn along with GA_3 (0.2 mg/l) and IAA (0.5mg/l), the tested varieties showed varied response. The maximum explants producing root (90%) was observed in 1.5 mg/l Kn at Shadaguti variety followed by Zaubilati (70%) and the minimum response of explant producing root (50%) was observed in Zaubilati at 2.0 mg/l Kn concentration (Table 11 and Fig. 13). On the other hand, the highest number of explant producing root (70%) was observed in 0.5 mg/l and 1.0 mg/l BAP at Shadaguti variety. The minimum explants producing root (10%) was observed in 0.5 mg/l BAP at Zaubilati but without cytokinin did not give any root (Table 10).

3.3.4 Percentage of explants producing both shoot and root

Percentage of explants producing both shoot and root simultaneously significantly varied among the varieties in both the treatments. The highest explants producing both shoot and root (46.25%) was observed at Shadaguti variety and in Kn treatment, it was 15% at the same variety (Fig. 10).

The better concentration for explants producing both shoot and root (38.33%) was found in 1.0 mg/l BAP and (0.5- 1.5) mg/l in Kn concentrations (10%) (Fig. 11).

The interactive effect of variety and BAP treatment showed significant variation. The highest explants producing both shoot and root (70%) in 1.0 mg/l BAP at Shadaguti and the lowest 10% was observed in 0.5 mg/l BAP at Zaubilati (Table 10). On the other hand, Kn showed the poor performance in all varieties because the highest

explants producing both shoot and root were only 20% in 1.5 mg/l at Shadaguti and the lowest (5%) was obtained from Zaubilati at 0.5-1.0 mg/l Kn and also no regeneration was noticed in 1.5-2.0 mg/l Kn in Zaubilati variety (Table 11).

3.3.5 Number of multiple shoots per explant

Significant variation was found among the varieties in number of multiple shoots per explants. The highest number of multiple shoots per explants (4.01) was recorded from Shadaguti in BAP treatments and the lowest (1.85) was recorded from Challisha (Fig. 10). On the other hand, in Kn treatments, multiple shoot regeneration were not observed in any of the tested varieties (Table 11 and Fig. 10).

Different levels of BAP significantly influenced the number of multiple shoots per explants. The highest number of multiple shoots per explants (3.19) was recorded in 1.5 mg/l BAP concentration and the lowest (2.41) was recorded in 0.5 mg/l BAP (Fig. 11). On the other hand, there was no significant variation was observed in different concentrations of Kn (Fig. 11).

Distinct variations were found in respect of number of multiple shoots per explants among variety toward various BAP concentrations (Table 10). The maximum number of multiple shoots per explants (4.70) was counted in the variety Shadaguti with 2.0 mg/l BAP followed by 1.5 mg/l BAP (4.59) (Fig. 12) and the lowest (1.60) was observed it Challisha with 2.0 mg/l BAP where, Zaubilati produced 2.38 shoot at the same concentration. It was mentionable here that without cytokinin only auxins did not give any shoot. Result indicates that, variety Shadaguti has high regeneration potential than Challisha and Zaubilati.

Overall response of internode explant of three potato varieties

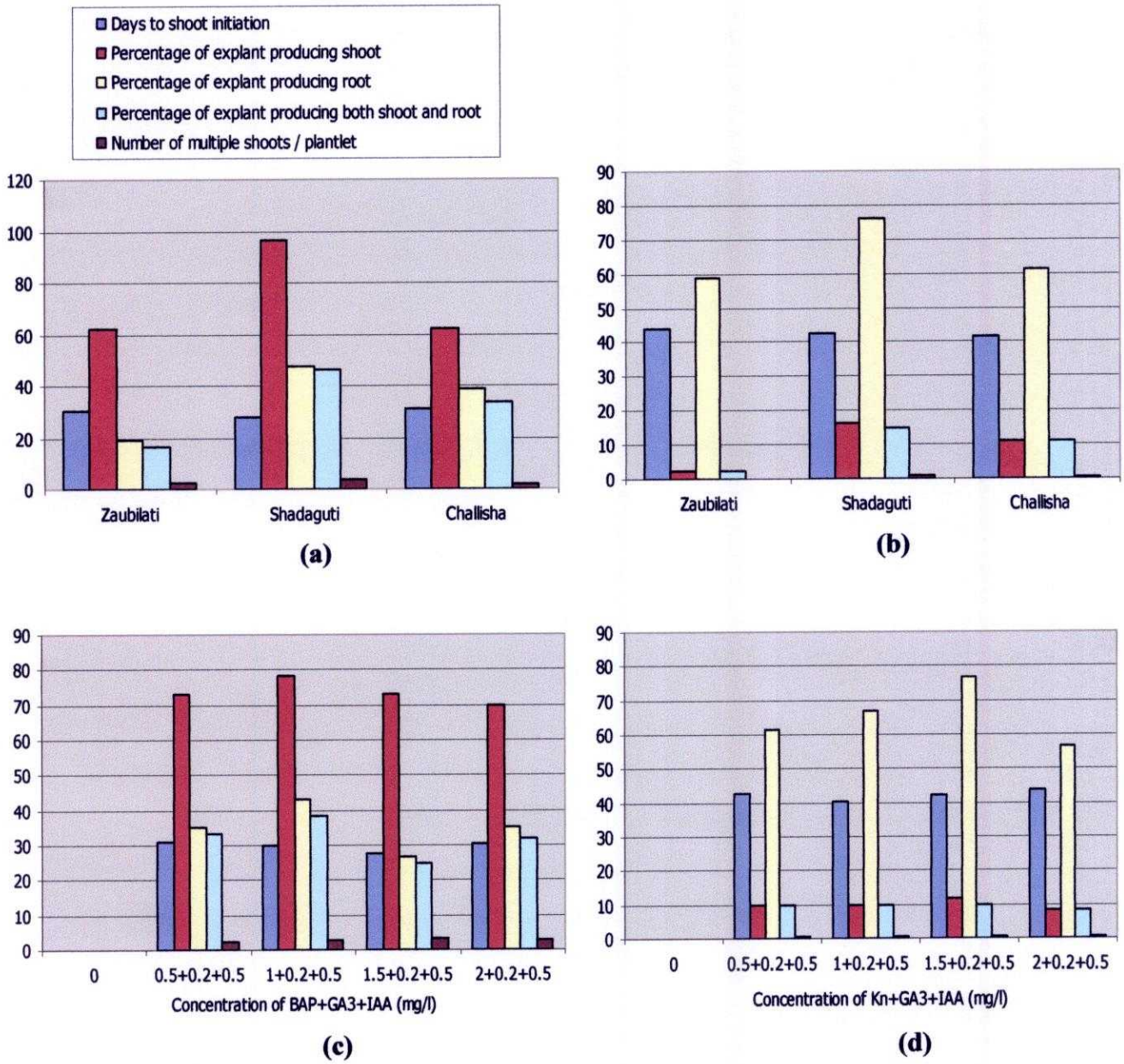

Figure 10 (a-b). *In vitro* direct regeneration response from internode explant of three potato varieties (data collected 60 after inoculation) in BAP (a) and Kn (b) treatments.

Figure 11 (c-d). Effect of different concentrations of BAP (c) and Kn (d) along with GA_3 (0.2 mg/l) and IAA (0.5 mg/l) on *in vitro* direct regeneration from internode explant of three potato varieties (data collected 60 after inoculation).

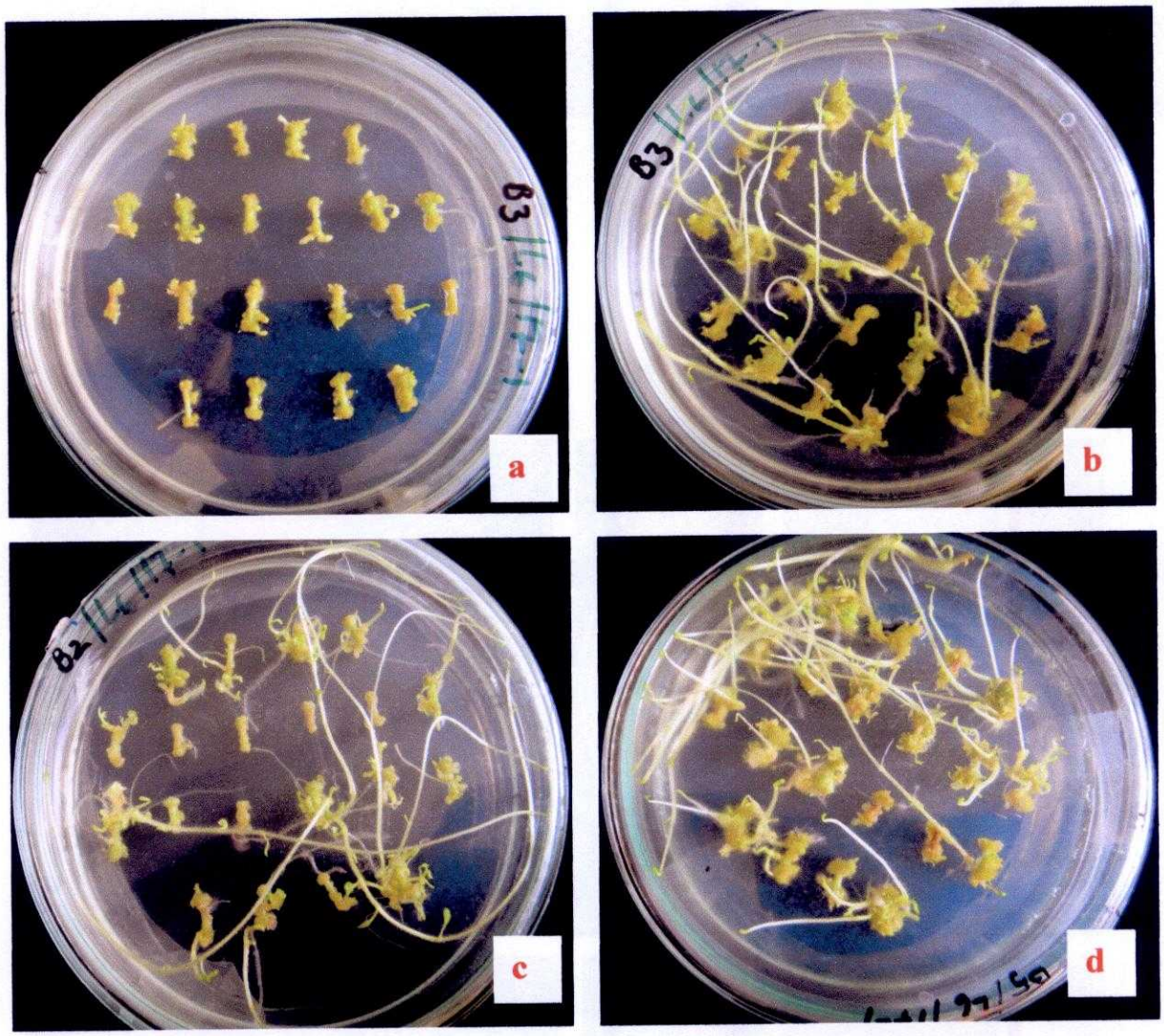

Figure 12. Regeneration from internode explant in PROP medium with 0.2mg/l GA_3 + 0.5mg/l IAA along with different concentrations of BAP in Shadaguti variety; a. 1.0 mg/l BAP (after 4 weeks), b. 1.0 mg/l BAP (after 7 weeks), c. 0.5 mg/l BAP (after 7 weeks), d. 2.0 mg/l BAP (after 7 weeks).

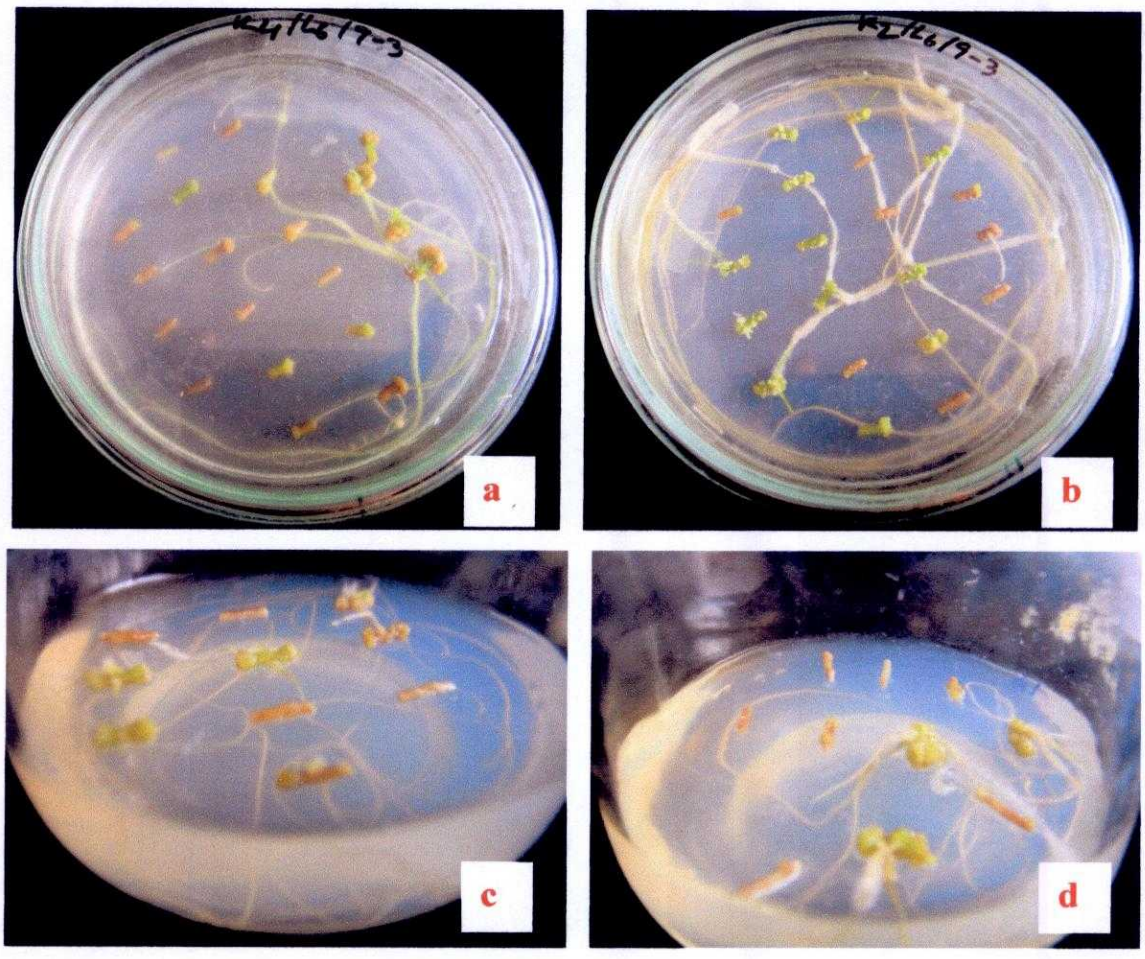

Figure 13. Regeneration from internode explant in PROP medium with 1.5 mg/l Kn + 0.2mg/l GA_3 + 0.5mg/l IAA; a-b. Shadaguti variety after 7 weeks, c-d. Zaubilati variety after 7 weeks.

3.4 Effect of different concentrations of BAP and Kn along with auxins (GA_3 and IAA) on direct regeneration from leaf explant of potato.

Similar to internode, leaf explant of three potato cultivars did not response in PROP medium supplemented with or without cytokinins (BAP and Kn). So, to achieve regeneration leaf explants were cultured on medium supplement with auxins, such as, GA_3 and IAA along with either BAP or Kn. Data of different concentrations of BAP and Kn along with GA_3 (0.2 mg/l) and IAA (0.5 mg/l) was selected for this study based on Iqbal *et al.* (2005) and Sarker and Mustafa (2002). Different parameters regarding direct regeneration and growth behavior were analyzed 60 days after inoculation and statistically evaluated. The results are shown in Tables 14 - 15 and Figs. 14 - 17 and the analysis of variance of data has been presented in Tables 16 - 17.

3.4.1 Day requirement for shoot initiation

The time required for shoot initiation was significantly different among the varieties at BAP treatments. Early shoot initiation (28.94 days) was found in Shadaguti (Fig. 14). However, Kn didn't show good response as Shadaguti and took 42.14 days for shoot initiation (Fig. 14).

Different concentrations of BAP and Kn also markedly influenced the day requirement for shoot initiation from leaf explants. BAP (2.0 mg/l) took minimum days (28.50 days) for shoot initiation whereas it was the maximum (32.0 days) in 0.5 mg/l BAP followed by 1.0 mg/l BAP (30.50 days) (Fig. 15). In Kn treatment, minimum time required (42.40 days) in 1.0 mg/l Kn, while the maximum (46 days) was observed in 2.0 mg/l Kn followed by 0.5 mg/l Kn (45 days) (Fig. 15).

Remarkable variation was observed in respect of days required for shoot initiation in response to different cytokinins (BAP and Kn) along with GA_3 and IAA in the tested varieties (Table 16 and 17). The maximum days required for shoot initiation (33.5 days) was noted in Challisha with 0.5 mg/l BAP followed by 32.25 days in Zaubilati with same concentration of BAP. Early shoot initiation was found (27.25 days) in Shadaguti with 2.0 mg/l BAP (Table 14) followed by 1.5 mg/l BAP (28.5 days) in the same variety and also in Zaubilati with 2.0 mg/l BAP.

Table 14. *In vitro* regeneration response from leaf explant of three potato varieties toward various BAP concentrations along with GA_3 (0.2 mg/l) and IAA (0.5 mg/l).

Variety	Concentrations (mg/l)			Days to shoot initiation	Percentage of explant producing shoot	Percentage of explant producing root	Percentage of explant producing both shoot and root	Number of multiple shoots / explant
	BAP	GA_3	IAA					
Zaubilati	0.0	0.0	0.0	-	-	-	-	-
	0.5	0.2	0.5	32.25	40 (39.22)	10 (13.92)	10 (13.92)	2.38
	1.0	0.2	0.5	30.75	60 (51.03)	25 (29.72)	20 (26.55)	2.69
	1.5	0.2	0.5	29.50	60 (51.03)	10 (10.76)	10 (10.76)	2.87
	2.0	0.2	0.5	28.50	55 (47.86)	10 (10.76)	5 (7.59)	2.04
Shadaguti	0.0	0.0	0.0	-	-	-	-	-
	0.5	0.2	0.5	30.25	60 (51.31)	45 (42.09)	45 (42.09)	2.56
	1.0	0.2	0.5	29.75	85 (69.73)	45 (42.09)	45 (42.09)	3.06
	1.5	0.2	0.5	28.50	80 (66.56)	15 (17.08)	15 (17.08)	3.78
	2.0	0.2	0.5	27.25	90 (76.05)	25 (26.56)	25 (27.56)	3.26
Challisha	0.0	0.0	0.0	-	-	-	-	-
	0.5	0.2	0.5	33.50	50 (44.98)	- (1.28)	- (1.28)	1.75
	1.0	0.2	0.5	31.00	60 (50.75)	30 (32.88)	30 (32.88)	1.49
	1.5	0.2	0.5	30.25	75 (63.67)	40 (38.93)	40 (38.93)	2.31
	2.0	0.2	0.5	29.75	55 (47.86)	35 (35.76)	25 (29.72)	1.54
LSD (0.01)	-	-	-	NS	NS	24.85	22.68	NS
CV (%)	-	-	-	4.54	20.56	51.38	48.89	22.87

Significantly at 1% level of probability, NS = Non-significant. Parentheses indicate the arcsin transformed value; (Data collected 60 days after inoculation).

Table 15. *In vitro* regeneration response from leaf explant of three potato varieties toward various Kn concentrations along with GA_3 (0.2 mg/l) and IAA (0.5 mg/l).

Variety	Concentrations (mg/l)			Days to shoot initiation	Percentage of explant producing shoot	Percentage of explant producing root	Percentage of explant producing both shoot and root	Number of multiple shoots / explant
	Kn	GA_3	IAA					
Zaubilati	0.0	0.0	0.0	-	-	-	-	-
	0.5	0.2	0.5	47	5 (7.59)	35 (36.05)	5 (7.59)	0.25
	1.0	0.2	0.5	46	5 (7.59)	30 (32.88)	5 (7.59)	0.25
	1.5	0.2	0.5	-	- (1.28)	50 (44.98)	- (1.28)	-
	2.0	0.2	0.5	-	- (1.28)	30 (32.88)	- (1.28)	-
Shadaguti	0.0	0.0	0.0	-	-	-	-	-
	0.5	0.2	0.5	43.5	10 (20.23)	55 (47.86)	10 (13.92)	0.50
	1.0	0.2	0.5	40.5	10 (13.92)	70 (60.51)	10 (13.92)	0.75
	1.5	0.2	0.5	42.33	20 (17.08)	60 (51.03)	20 (23.40)	0.88
	2.0	0.2	0.5	-	- (1.28)	50 (44.98)	- (1.28)	-
Challisha	0.0	0.0	0.0	-	-	-	-	-
	0.5	0.2	0.5	46	5 (7.59)	35 (35.76)	5 (7.59)	0.25
	1.0	0.2	0.5	42.5	10 (13.92)	50 (44.98)	10 (13.92)	0.50
	1.5	0.2	0.5	45	5 (7.59)	35 (35.76)	5 (7.59)	0.25
	2.0	0.2	0.5	46	5 (7.59)	45 (41.82)	5 (7.59)	0.25
LSD (0.01)	-	-	-	NS	NS	NS	NS	NS
CV (%)	-	-	-	158.63	135.19	24.18	133.11	160.64

Significantly at 1% level of probability, NS = Non-significant. Parentheses indicate the arcsin transformed value; (Data collected 60 days after inoculation).

Table 16. ANOVA of different concentrations of BAP along with GA_3 and IAA on *in vitro* direct regeneration from leaf explant of potato.

Sources of variation	Degrees of freedom	Mean sum of square				
		Days to shoot initiation	Explant producing shoot (%)	Explant producing root (%)	Explant producing both shoot and root (%)	Average No. of multiple shoots / explant
Variety	2	19.396 **	1509.72 **	1033.15 **	1220.59 **	7.752 **
Treatment	3	27.186 **	543.413 *	562.656 *	524.978 *	1.456 **
Variety × Treatment	6	0.813 NS	147.314 NS	797.517 **	739.019 **	0.325 NS
Error	36	1.868	127.909	167.078	139.125	0.321

** = 1% level of significance, * = 5% level of significance, NS = Non-significant

Table 17. ANOVA of different concentrations of Kn along with GA_3 and IAA on *in vitro* direct regeneration from leaf explant of potato.

Sources of variation	Degrees of freedom	Mean sum of square				
		Days to shoot initiation	Explant producing shoot (%)	Explant producing root (%)	Explant producing both shoot and root (%)	Average No. of multiple shoots / explant
Variety	2	653.896 NS	302.847 NS	928.446 **	302.847 NS	0.661 NS
Treatment	3	444.972 NS	189.626 NS	115.048 NS	171.917 NS	0.366 NS
Variety × Treatment	6	270.285 NS	89.8594 NS	141.069 NS	125.380 NS	0.189 NS
Error	36	406.375	145.303	105.412	140.858	0.269

** = 1% level of significance, NS = Non-significant

On the other hand, Kn concentration did not show good response in any of the three potato varieties. In this experiment, the shoot initiation response is lower than BAP treatments, and took longer duration for shoot formation. The maximum days (47.0 days) for shoot initiation were found in Zaubilati with 0.5 mg/l Kn and the manimum days (40.5 days) for shoot initiation were found in Shadaguti with 1.0 mg/l Kn (Table 15). This study showed that PROP medium supplemented with or without cytokinin give no shoot initiation from potato leaf explants. But addition of auxins (GA_3 and IAA) with cytokinins enhanced shoot initiation.

3.4.2 Percentage of explant producing shoots

Shooting response of leaf explants varied considerably among the varieties. Percentage of explant producing shoots significantly varied among the different varieties at BAP treatments but not significantly varied in Kn treatments (Table 16 and 17). The maximum explants producing shoot (78.75%) was recorded in Shadaguti variety followed by Challisha (60%) at BAP treatment. Though maximum shooting response in Kn treatment was found in Shadaguti, but it was vary low (only 10%). Data presented in figure 14.

Percentage of explants producing shoot significantly varied in different concentration of BAP along with GA_3 (0.2mg/l) and IAA (0.5mg/l) in leaf explants but not significantly varied in Kn treatments. The maximum explant producing shoots (71.66%) was recorded in 1.5 mg/l BAP followed by 1.0mg/l BAP (68.33%). On the other hand, Kn did not show good regeneration. The maximum explant producing shoots was only 8.33 % at 1.5mg/l Kn (Fig. 15).

Response of leaf explant of three varieties with respect to various cytokinin (BAP and Kn) supplementation along with other hormones (GA_3 0.2 mg/l and IAA 0.5 mg/l) found to show quite variation. The maximum explants producing shoot (90%) was observed in 2.0 mg/l BAP at Shadaguti variety followed by 1.0 mg/l BAP (85%) in the same variety (Fig. 16). The minimum explants producing shoot (40%) was observed in 0.5 mg/l BAP at Zaubilati (Table 14). On the other hand, in Kn treatments, it was observed that only 20% explants produced shoot in 1.5 mg/l Kn within Shadaguti variety. Zaubilati showed the lowest shooting (5%) in 0.5 and 1.0 mg/l Kn, while higher Kn supplementation fail to regenerate any shoot (Table 15).

3.4.3 Percentage of explants producing root

Percentage of explants producing root significantly varied among different varieties. The maximum explants producing root was recorded at Shadaguti variety in both BAP (32.50%) and Kn treatment (58.75 %) and the minimum rooting response was observed at Zaubilati also in both treatments (Fig. 14).

Percentage of explants producing root significantly varied due to the different concentration of BAP along with GA_3 (0.2mg/l) and IAA (0.5mg/l) but not significantly different in Kn treatment. The maximum explants producing roots 33.33% and 50.0% were observed in 1.0 mg/l BAP and 1.0 mg/l Kn, respectively (Fig. 15). For root initiation, Kn performance was better than BAP but in case of shoot initiation, Kn did not show good response. The minimum explants producing roots observed in 0.5 mg/l BAP (18.33%), where Kn showed the lowest explants producing roots and in 0.5 mg/l and 2.0 mg/l Kn (41.66 %).

Significant variation was found in respect of various varieties producing root in cytokinin (BAP and Kn) concentrations along with GA_3 (0.2 mg/l) and IAA (0.5mg/l) (Table 16 and 17). The maximum explants producing root (45%) was observed in 0.5 - 1.0 mg/l BAP at Shadaguti. The minimum explants producing root (10%) was observed in 0.5 mg/l, 1.5 mg/l and 2.0 mg/l BAP at Zaubilati (Table 14). On the other hand, the maximum explants producing root (70%) was observed in 1.0 mg/l Kn at Shadaguti (Fig. 17) and the minimum explant producing root (35%) was observed in Zaubilati at 0.5 mg/l Kn and Challisha with 0.5 and 1.5 mg/l Kn concentrations (Table 15).

3.4.4 Percentage of explants producing both shoot and root

Percentage of explants producing both shoot and root were significantly varied among the varieties in BAP treatments but not in Kn treatments (Table 16 and 17). The highest explants producing both shoot and root (32.5%) was observed at Shadaguti variety and in Kn treatment it was only 10% at same variety (Fig. 14).

The better concentration for leaf explant producing both shoot and root (31.66%) was found in 1.0 mg/l BAP and among Kn treatment, it was found in 1.5 mg/l Kn (8.33%) (Fig. 15).

The interactive effect of variety and BAP concentration varied significantly; however, Kn did not varied significantly (Table 16 and 17). The maximum explant producing both shoot and root (45%) was observed in 0.5-1.0 mg/l at Shadaguti in BAP

treatment and the lowest 5% was observed in 2.0 mg/l BAP at Zaubilati (Table 14). On the other hand, Kn showed poor performance in all the varieties tested. Because the highest explants producing both shoot and root were only 20% in 1.5 mg/l at Shadaguti and at 2.0 mg/l at Shadaguti and in Zaubilati 1.5-2.0 mg/l Kn did not show any regeneration (Table 15).

3.4.5 Number of multiple shoots per explant

Significant variation was found among the varieties in respect to number of multiple shoots regenerated per explant in BAP treatments but not in Kn treatments (Table 16 and 17). The highest number of multiple shoots per explant (3.16) was recorded in Shadaguti variety at BAP treatments, while the lowest (1.77) was recorded in Challisha (Fig. 14). On the other hand, at Kn treatment, no multiple shoots were observed in among the varieties (Fig. 14). No multiple shoot was also recorded in different concentrations of Kn thus in interactive effect of variety and Kn concentrations (Table 15).

Different level of BAP significantly influenced the number of multiple shoots per explant (Fig. 15). The highest number of multiple shoots per explant (2.99) was recorded in 1.5 mg/l BAP concentration and the lowest (2.23) was recorded in 0.5 mg/l BAP.

Distinct variations were found in respect of number of multiple shoots per explant when analyzed against various BAP concentrations (Table 14). The maximum number of multiple shoots per explants (3.78) was counted in the variety Shadaguti with 1.5 mg/l BAP followed by 2.0 mg/l BAP (3.26) and the lowest (1.49) was observed in Challisha with 1.0 mg/l BAP but without BAP no shoot was regenerated (Table 14 and Fig. 16).

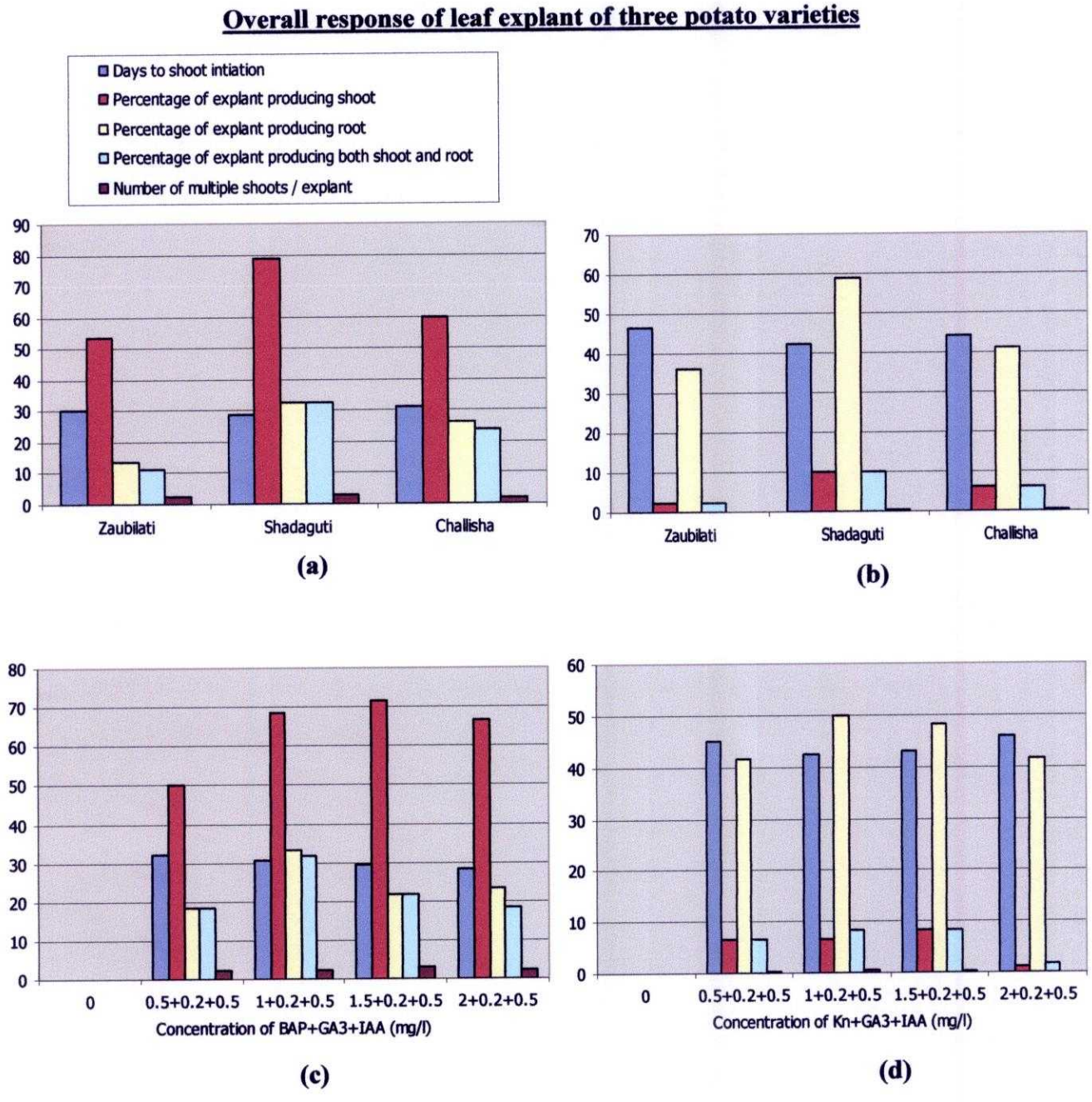

Figure 14 (a-b). *In vitro* direct regeneration from leaf explant of three potato varieties (data collected 60 after inoculation) in BAP (a) and Kn (b) treatments.

Figure 15 (c-d). Effect of different concentrations BAP (c) and Kn (d) along with GA3 (0.2 mg/l) and IAA (0.5 mg/l) on *in vitro* direct regeneration from leaf explant of three potato varieties (data collected 60 after inoculation).

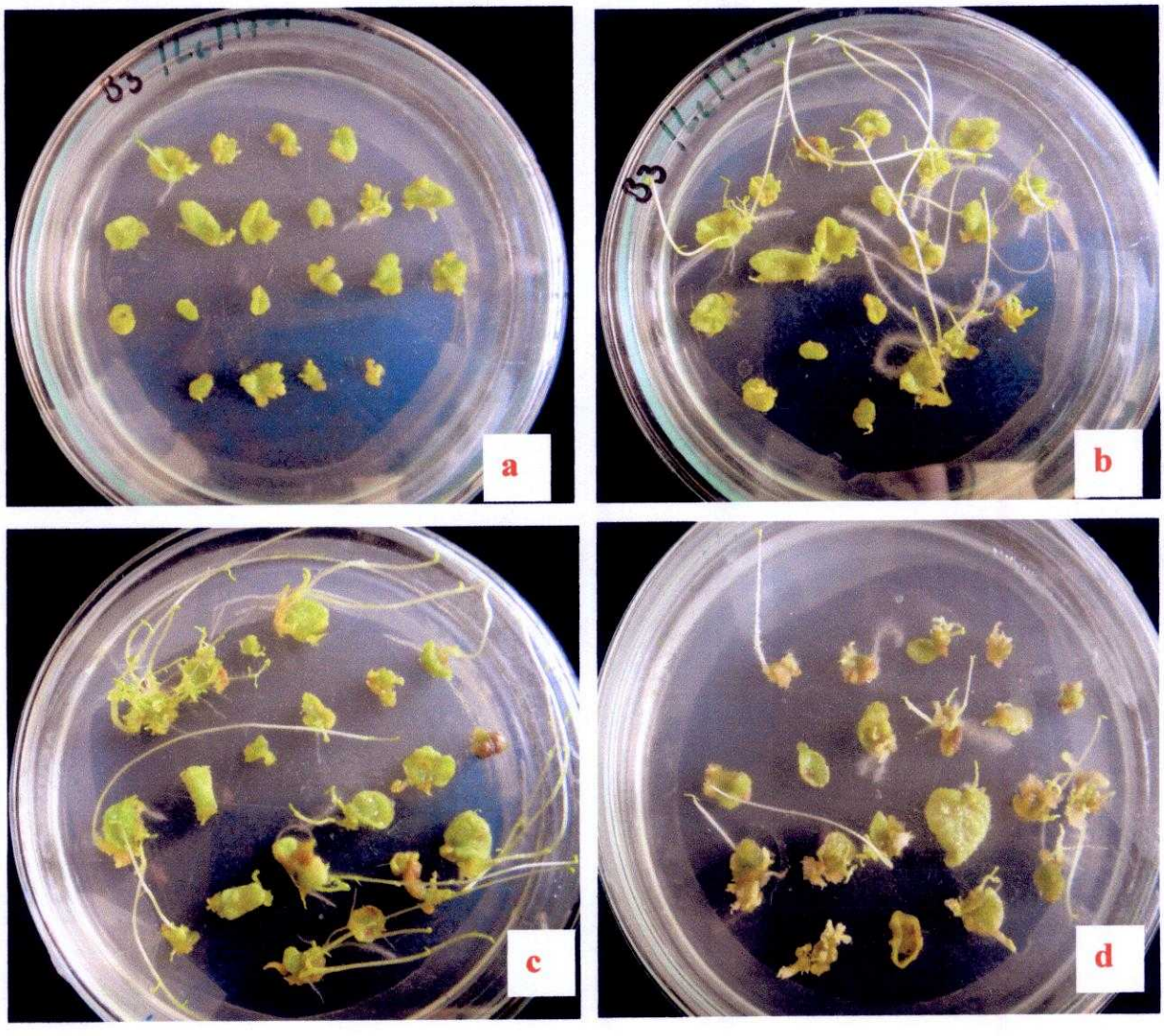

Figure 16. Regeneration from leaf explant in PROP medium with 0.2mg/l GA_3 + 0.5mg/l IAA along with different concentration of BAP in Shadaguti variety; a. 1.0 mg/l BAP (after 4 weeks), b. 1.0 mg/l BAP (after 7 weeks), c. 1.5 mg/l BAP (after 7 weeks), d. 2.0 mg/l BAP (after 7 weeks).

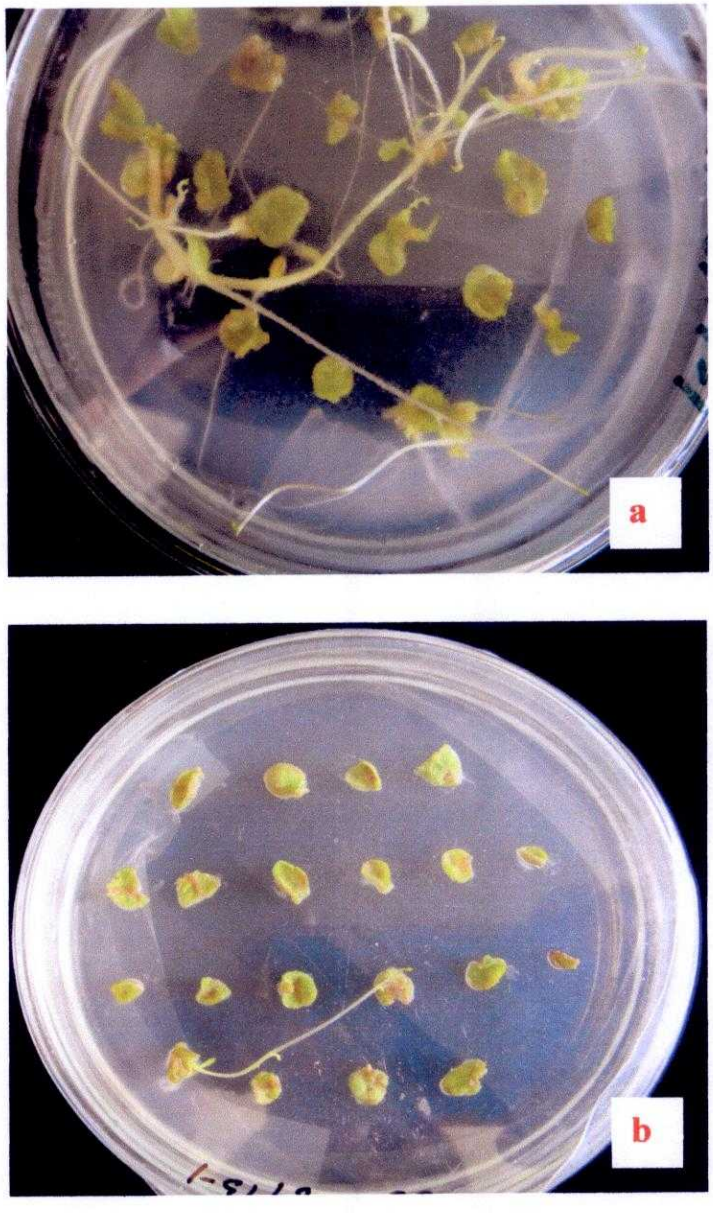

Figure 17. Regeneration from leaf explant in PROP medium with 0.2mg/l GA_3 + 0.5mg/l IAA along with different concentration of Kn in Shadaguti variety; a. 1.0 mg/l Kn (after 7 weeks), b. 1.5 mg/l Kn (after 7 weeks).

3.4.6 Decision from above two experiments

Based on the above two experiments, it may be mentioned that among the three varieties used was in the present study, Shadaguti variety found the best genotype for *in vitro* direct regeneration and it also found that internode explant of potato varieties are showed better shoot regeneration, such as, highest shoot production, explants producing roots and number of multiple shoots than leaf explant of potato.

The appropriate concentration of cytokinin (BAP and Kn) along with GA_3 (0.2mg/l) and IAA (0.5 mg/l) for direct shoot regeneration is 1.0 mg/l BAP for internode explant and 1.5 mg/l BAP for leaf explant. Kn along with GA_3 and IAA did not give more than 20% shoot but it produced direct root regeneration (70%) at 1.0 mg/l Kn concentration in leaf explant of potato. Results revealed that BAP treatments performed better than Kn treatments for direct shoot regeneration.

3.5 Effect of basal media and different concentrations of IBA on rooting of *in vitro* regenerated shoots

In some cases several roots developed spontaneously from *in vitro* grown shoots. But they were found to be inadequate for transplantation. Hence, adequate root induction was necessary for induction of roots, shoots of 3-4 cm in length were excised and cultured on different rooting media, such as, Full PROP, ½ PROP, ½ PROP + 0.1 mg/l IBA, ½ PROP + 0.5 mg/l IBA. In this experiment, only *in vitro* grown shoots of Shadaguti variety were used for root induction. Data were recorded after 30 days of culture on different parameters and were statistically analyzed. Results are presented in Tables 18 -19 and Fig. 18.

3.5.1 Day requirement for root initiation

Days required for root initiation varied to the different types of media. The minimum days (5.50 days) required for root initiation when the explants treated with ½ PROP medium supplemented with 0.5mg/l IBA. On the other hand, the maximum days required for root initiation (9.0 days) in Full PROP medium without IBA (Table 18). This indicated that IBA enhances root induction.

3.5.2 Number of roots per plantlet

Significant variation was observed among the media as to the number of roots per plantlet (Table 19). The maximum number of roots (11.83) per plant was recorded in ½ PROP medium supplemented with 0.5mg/l IBA followed by ½ PROP medium + 0.1mg/l IBA (8.83) (Fig. 18). On the other hand, the lowest number of roots per plantlet (3.67) was noted in ½ PROP medium without IBA (Table 18).

3.5.3 Root length (cm) per plantlet

Significant variation was found in the length of roots due to the different type of media (Table 19). The highest root length (6.33 cm) per plantlet was recorded in ½ PROP medium whereas the lowest (4.53 cm) was in ½ PROP medium supplemented with 0.5mg/l IBA. Result indicated that minimum number of roots was observed the highest length of root (Table 18).

Table 18. Effect of basal media and different concentrations of IBA on rooting of *in vitro* regenerated shoots of Shadaguti variety.

Medium	Days to root initiation	No. of roots / plantlet	Root length (cm) / plantlet	Visual growth of root	Days required for well developed roots
Full PROP	9.0	5.50	5.90	+	18-21
½ PROP	7.5	3.67	6.33	+	18-21
½ PROP + 0.1 mg/l IBA	6.33	8.83	5.95	++	16-18
½ PROP + 0.5 mg/l IBA	5.50	11.83	4.53	+++	12-14
LSD(0.01)	0.695	1.23	0.54	-	-
CV (%)	11.95	20.09	11.56	-	-

Significantly at 1% level of probability. Data collected 30 days after inoculation

+ = Poor, ++ = good, +++ = Very good, - = absent.

Table 19. ANOVA of different media on *in vitro* rooting from regenerate shoots of potato.

Sources of variation	Degrees of freedom	Mean sum of square		
		Days to root initiation	Number of roots / plant	Root length (cm) / plant
Treatment	3	13.8333 **	78.4861 **	3.72597 **
Error	20	0.71667	2.2250	0.431083

** = 1% level of significance

3.5.4 Growth of root

Root growth was visually identified. It was observed that ½ PROP medium supplemented with 0.5mg/l IBA produced huge number of roots as well as healthy and vigorous in growth than other media (Fig. 18d-i).

3.5.5 Days required for well developed roots

Days required for well developed roots counted based on the root establishment and color of the roots. However, it ranged from 12-21 days. The minimum days (12-14) required for well developed roots was noted in ½ PROP medium supplemented with 0.5 mg/l IBA (Fig. 18). In ½ PROP medium supplemented with 0.1mg/l IBA took 16-18 days for well developed roots. On the other hand, the maximum days (18-21) required for well developed roots was needed in ½ PROP medium and Full PROP medium (Table 18). The possible reason for maximum days required for well developed roots because, in this medium root initiation occurred later.

3.6 Transplantation

After proper development plantlets were taken out from the test tubes. Then each plantlet was transferred into soil and kept in shade house. After two to four weeks 50% plantlets were survived. Then plantlets were ready to transplant in the field (Fig. 19).

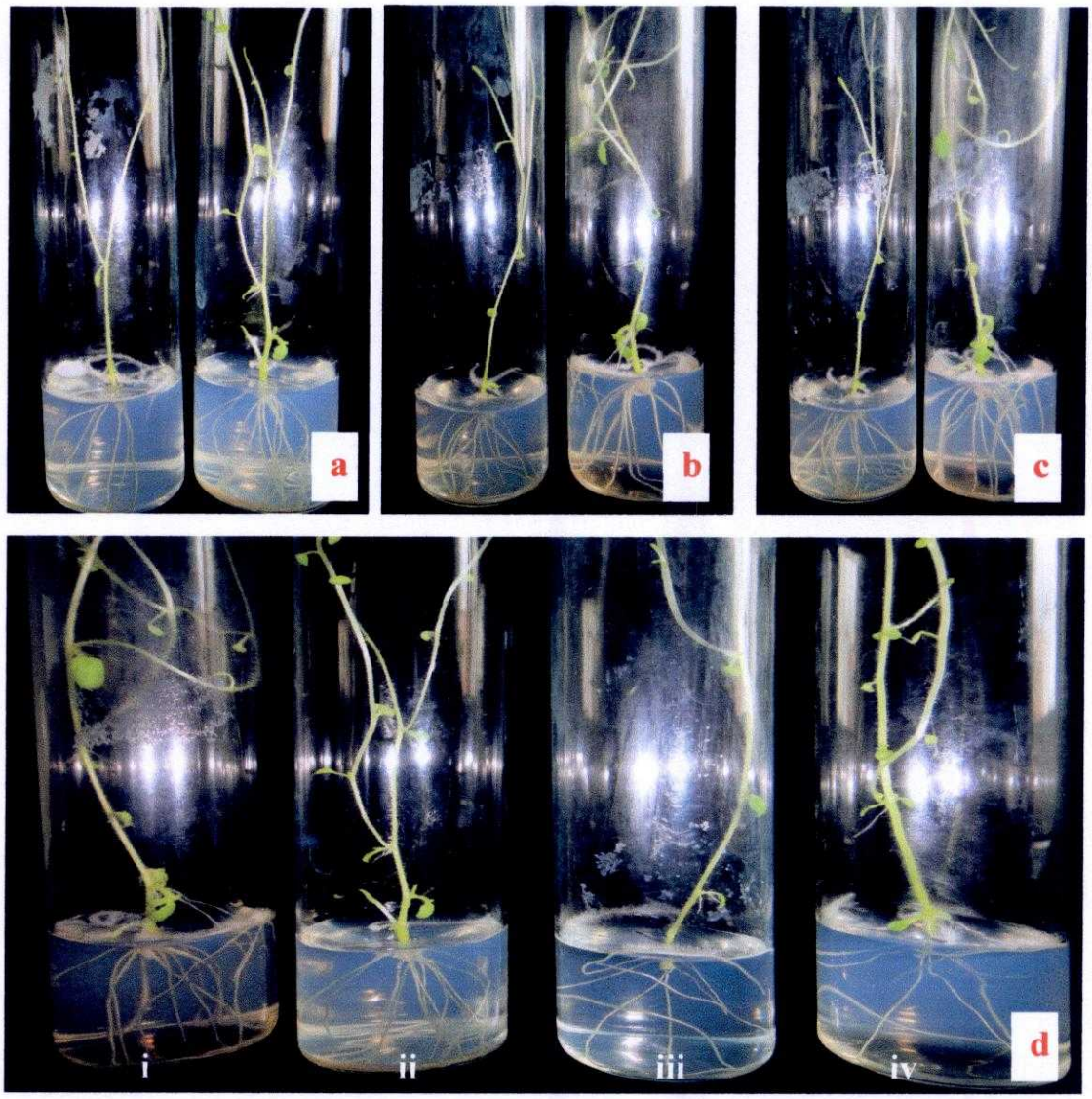

Figure 18. Root formation in Shadaguti variety; a. Single shootlet was rooted in ½ PROP + 0.1 mg/l IBA after 4 weeks, b-c. Single shootlet was rooted in ½ PROP + 0.5 mg/l IBA after 4 weeks, d. Single shootlet was rooted in four different media after 4 weeks; (i = ½ PROP + 0.5 mg/l IBA, ii = ½ PROP + 0.1 mg/l IBA, iii = ½ PROP, iv = Full PROP).

Figure 19. *Ex vitro* established potato plantlets of Shadaguti variety; a-b. Transplanted potato plantlets after 2 weeks, c-d. Transplanted potato plantlets after 4 weeks.

Chapter 4

Discussion

CHAPTER - 4
DISCUSSION

In the present study *in vitro* response of three locally grown potato varieties were tested with respect to four different explants and various hormonal combinations. Considering the responses of two potato explants, shoot apex and nodal segment from three potato varieties viz. Zaubilati, Shadaguti and Challisha, it may be concluded that, Shadaguti variety found to be the best genotype for *in vitro* regeneration and it also found that, shoot apex explant of potato varieties showed better regeneration with regard to early shoot initiation, long shoot length, maximum number of nodes, leaves and maximum fresh weight of shoot. The appropriate concentration of BAP to produce shoot regeneration in 1.5mg/l BAP from shoot apex explant and for nodal segment application of BAP found to have no advantages. In case of Kn supplementation, the appropriate concentration for shoot regeneration was 0.5 mg/l Kn for nodal segment, while in shoot apex, presence of Kn didn't show any enhancement. Results also indicated that Kn treatment performed better than BAP treatment in all the parameters evaluated for shoot regeneration.

Iqbal *et al.* (2005) reported that shoot apex and nodal segment explants of three potato cultivars viz. Cardinal, Altamash and Diamant were 100% responded in all treatment of BAP (0.0 to 2.0 mg/l). Present study was no exception. But Sarker and Mustafa (2002) reported that the BAP gives better response than Kn in two potato varieties, Lal Pakri and Jam Alu. These variations can be due to variation in potato genotypes.

The results of the study reflected that minimum day requirement for shoot initiation decreased with the increasing of BAP and Kn concentration up to 2.0 mg/l. This indicates that cytokinin (BAP and Kn) enhances cell division and cell elongation. Hossain *et al.* (2005) obtained new shoots in semisolid medium within 3-5 days. In the present study, shoot initiation observed within 2.75 to 6.25 days in BAP treatments and 4.25 to 7.25 days in Kn treatments. The findings of the present study partially supported with the result of Hossain *et al.* (2005) though they used different media.

Result of the present study also indicated that the higher concentration of BAP (up to 1.5 mg/l) was good for higher shoot length but beyond that level, shoot length decreased. This result partially supported with the result of Iqbal *et al.* (2005). They reported that shoot apex explants of three potato cultivars viz. Cardinal, Altamash and Diamant were showed long length at 2.0 mg/l BAP supplementation. This variation observed may be due to the different variety used. The varied length of shoot may be influenced due to the genotypic effect. It has been previously documented that regeneration response *in vitro* is generally species and often genotype specific (Richie and Hodges, 1993).

There were some results which was dissimilar with the previously reported studies. Yousuf *et al.* (1997) observed the highest shoot length (22 cm) in MS medium supplemented with 2.0 mg/l NAA + 0.5 mg/l IBA. Zaman *et al.* (2001) reported that MS medium supplemented with 0.5 mg/l NAA produced the highest shoot length (8.30 cm), the maximum number of nodes per plantlet (7.30) and the highest number of leaves per plantlet (8.90). Ghaffor *et al.* (2003) recorded the maximum length of shoot (7.34 cm), maximum number of nodes per plantlet (9.71) and maximum number of leaves (6.0) from the MS medium supplemented with 0.35 mg/l IBA. Hossain *et al.* (2005) reported that Cardinal gave the highest number of leaves per plant (9.0). They also concluded that the higher concentration of auxin is helpful for the production of higher number of leaves per plant, which is not supported by the present study.

Similar observations were also reported by Sarker and Mustafa (2002) that the number of nodes and number of leaves increased with the increase of BAP up to 1.5 mg/l and decreased with further increase of BAP (2.0 – 4.0 mg/l) in case of nodal segment explant in two potato varieties, Lal Pakri and Jam Alu. The reason for higher fresh weight of shoots might be due to higher length of shoot and number of leaves per plantlet. Hossain *et al.* (2005) reported that liquid media comparatively better for quick growth of plantlet than semisolid media.

For internode and leaf explants, it may be mentioned that among the three varieties used in the present study, Shadaguti variety found to be the best genotype for *in vitro* direct regeneration and it also found that internode explant showed better shoot

regeneration, such as, highest shoot production, explants producing roots and number of multiple shoots than leaf explant.

For direct shoot regeneration, the appropriate concentration of cytokinin (BAP and Kn) along with GA_3 (0.2mg/l) and IAA (0.5 mg/l) found to be 1.0 mg/l BAP for internode explant and 1.5 mg/l BAP for leaf explant. Kn along with GA_3 and IAA did not give more than 20% shoot but it produced direct root regeneration (70%) at 1.0 mg/l Kn concentration in leaf explants of potato. Result revealed that BAP treatment performed better response than Kn treatment for direct shoot regeneration.

Kaburu *et al.* (1990) reported that 25-31 days needed for shoot induction from internode and leaf explants of potato (*Solanum phureeja*) on shoot induction medium which partially supported by the present study.

Wendt *et al.* (2001) stated that internodes of potato cultured in MS medium supplemented with BAP showed higher regeneration rate compare to leaves and this response decreased with higher hormonal doses. This result is supported by the present study. This is also in agreement with both Sarker and Mustafa (2002) and Asma *et al.* (2001) who observed organogenesis in potato with the BAP and GA_3. Martel *et al.* (1992) reported that both BAP and GA_3 are needed in higher concentration for shoot formation. This variation may be due to genotype.

Similar to the present results, Sarker and Mustafa (2002) found that the number of multiple shoots per explants (internode and leaf) increased with the increase of BAP or Kn concentration (along with GA_3) up to 1.0 – 1.5 mg/l and decreased with higher concentration (2.0 – 4.0 mg/l) in two potato varieties Lal Pakri and Jam Alu. Though there is varietals difference, this response seems comparable.

For *in vitro* rooting, the present study reflected that among the different treatments, ½ PROP medium supplemented with 0.5 mg/l IBA performed the best. In this treatment, explants produced 11.83 healthy and vigorous roots and needs minimum days (5.50) for root initiation. Well developed roots were produced within 12 – 14 days in the same treatment.

Ghaffor *et al.* (2003) counted the maximum number of roots (15.14 and 16.0) per plantlet in MS medium supplemented with 0.5 mg/l IBA and MS medium supplemented with 0.35 mg/l NAA, respectively, which supports the present study.

Similar observations were also reported by Shah *et al.* (2002). They reported that higher concentration of growth regulators were best for the production of maximum number of roots per plantlet. Result of the present study, also indicated that high IBA concentration enhanced rooting. As a result maximum number of root obtained in IBA treated media. The results of the present study showed consistency with other studies, where the addition of IBA promotes the induction of roots in several systems including *Artemisia judaica* (Liu *et al.*, 2003), *Bixa orelana* (Neto *et al.,* 2003), *Dioscorea zingiberensis* (Chen *et al.*, 2003) and *Exacum travancorium* (Elangomathavan *et al.*, 2006).

Among the varieties, Shadaguti showed the best response but other two varieties also produced shoots followed by *in vitro* rooting. The plantlets were successfully transplanted in soil. Therefore, the presently evaluated hormonal supplements gives not only a good evaluation of the locally grown popular potato varieties, but also establish a genotype independent regeneration protocol. Therefore, this protocol can be used in future to develop transgenic crops to improve production.

Chapter 5

References

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Appendix

Appendix I. Composition of PROP (Potato Propagation medium)

Macro nutrients (MS I stock solution)	**Amount per liter (g)**
KNO_3	19.0
NH_4NO_3	16.50
$MgSO_4.7H_2O$	3.70
$CaCl_2.2H_2O$	4.40
KH_2PO_4	1.70
Micro nutrients (MS II stock solution)	**Amount per liter (mg)**
$MnSO_4.4H_2O$	22.3
H_3BO_3	6.2
$ZnSO_4.7H_2O$	8.6
$NaMoO_4.2H_2O$	0.25
$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025
KI	0.83
Iron Source (MS III stock solution)	**Amount per liter (mg)**
$FeSO_4.7H_2O$	27.8
$NaMoO_4.2H_2O$	37.3
Organic (MS IV stock solution)	**Amount per liter (mg)**
Myoinositol	100
MS stock C : $CaCl_2.2H_2O$	4.40
MS stock D : KH_2PO_4	1.70
PROP Vitamins	**Amount per liter (mg)**
Glycine	40
Nicotinic acid	10
Pyridoxin.HCl	10
Thiamine.HCl	10
Sucrose	30 g
Agar	8 g

Appendix II. Varietals effect on *in vitro* regeneration from shoot apex explant of potato after 30 days of culture in BAP treatments (Figure. 1).

Variety	Responsive explant (%)	Days to shoot initiation	Shoot length(cm) / plantlet	No. of nodes / plantlet	No. of leaves / plantlet	Fresh weight(mg) of shoot / plantlet
Zaubilati	100	4.92	5.35	5.79	6.79	29.30
Shadaguti	100	4.13	6.78	5.13	6.67	30.31
Challisha	100	5.38	5.69	4.54	4.38	19.56
LSD (0.01)	-	0.49	0.46	0.58	0.74	2.07
CV (%)	-	13.28	9.98	14.64	16.11	10.18

Significantly at 1% level of probability

Appendix III. Varietals effect on *in vitro* regeneration from shoot apex explant of potato after 30 days of culture in Kn treatments (Figure. 1).

Variety	Responsive explant (%)	Days to shoot initiation	Shoot length(cm) / plantlet	No. of nodes / plantlet	No. of leaves / plantlet	Fresh weight(mg) of shoot / plantlet
Zaubilati	100	5.88	7.54	7.21	8.13	57.02
Shadaguti	100	5.88	9.79	7.42	8.08	43.12
Challisha	100	6.21	7.28	6.08	7.13	29.65
LSD (0.01)	-	NS	0.55	0.65	0.67	2.30
CV (%)	-	10.96	8.76	12.19	11.20	6.88

Significantly at 1% level of probability, NS = Non-significant.

Appendix IV. Effect of different concentrations of BAP on *in vitro* regeneration from shoot apex explant of potato after 30 days of culture (Figure. 2).

Concentration of BAP (mg/l)	Responsive explant (%)	Days to shoot initiation	Shoot length(cm) / plantlet	No. of nodes / plantlet	No. of leaves / plantlet	Fresh weight(mg) of shoot / plantlet
0.0	100	5.25	5.58	6.08	6.75	25.34
0.5	100	5.08	6.23	5.08	6.00	23.76
1.0	100	4.42	6.78	5.33	6.08	29.04
1.5	100	3.67	6.49	5.83	6.92	32.68
2.0	100	4.92	5.18	4.25	5.08	23.91
2.5	100	5.50	5.38	4.33	4.83	23.60
LSD(0.01)	-	0.70	0.65	0.82	1.05	2.93
CV (%)	-	13.28	9.98	14.64	16.11	10.18

Significantly at 1% level of probability.

Appendix V. Effect of different concentrations of Kn on *in vitro* regeneration from shoot apex explant of potato after 30 days of culture (Figure. 2).

Concentration of Kn (mg/l)	Responsive explant (%)	Days to shoot initiation	Shoot length(cm) / plantlet	No. of nodes / plantlet	No. of leaves / plantlet	Fresh weight(mg) of shoot / plantlet
0.0	100	7.25	10.59	9.0	9.42	53.34
0.5	100	6.67	9.60	7.33	8.25	51.08
1.0	100	6.17	7.48	6.67	7.75	36.53
1.5	100	5.42	7.42	5.92	7.08	42.48
2.0	100	4.50	7.91	6.17	7.25	34.17
2.5	100	5.92	6.23	6.33	6.92	41.98
LSD(0.01)	-	0.72	078	0.92	0.95	3.25
CV (%)	-	10.96	8.76	12.19	11.20	6.88

Significantly at 1% level of probability.

Appendix VI. Varietals effect on *in vitro* regeneration from nodal segment of potato after 30 days of culture in BAP treatments (Figure. 6).

Variety	Responsive explant (%)	Days to shoot initiation	Shoot length(cm) / plantlet	No. of nodes / plantlet	No. of leaves / plantlet	Fresh weight(mg) of shoot / plantlet
Zaubilati	100	6.25	6.14	5.0	6.42	30.08
Shadaguti	100	6.08	5.86	5.17	6.00	17.08
Challisha	100	6.33	6.23	5.04	4.63	15.74
LSD (0.01)	-	NS	-	NS	0.64	1.57
LSD (0.05)	-	-	0.25	-	-	-
CV (%)	-	11.26	7.10	10.82	14.52	9.71

Significantly at 1% and 5% levels of probability, NS = Non-significant.

Appendix VII. Varietals effect on *in vitro* regeneration from nodal segment of potato after 30 days of culture in Kn treatments (Figure. 6).

Variety	Responsive explant (%)	Days to shoot initiation	Shoot length(cm) / plantlet	No. of nodes / plantlet	No. of leaves / plantlet	Fresh weight(mg) of shoot / plantlet
Zaubilati	100	6.25	6.32	6.83	8.17	38.68
Shadaguti	100	6.21	8.97	6.96	7.46	36.06
Challisha	100	6.50	8.05	6.00	7.21	29.30
LSD (0.01)	-	NS	0.46	0.65	0.60	5.90
CV (%)	-	9.45	7.73	12.67	10.19	22.04

Significantly at 1% level of probability, NS = Non-significant.

Appendix VIII. Effect of different concentration of BAP on *in vitro* regeneration from nodal segment of potato after 30 days of culture (Figure. 7).

Concentration BAP (mg/l)	Responsive explant (%)	Days to shoot initiation	Shoot length(cm) / plantlet	No. of nodes / plantlet	No. of leaves / plantlet	Fresh weight(mg) of shoot / plantlet
0.0	100	7.00	7.43	6.67	7.08	22.48
0.5	100	6.25	6.03	5.50	5.75	19.39
1.0	100	5.00	6.59	5.00	6.17	29.86
1.5	100	5.67	6.37	5.00	5.50	20.29
2.0	100	6.50	5.30	4.42	4.75	17.25
2.5	100	6.92	4.73	3.83	4.83	16.53
LSD(0.01)	-	0.77	0.47	0.60	0.90	2.22
CV (%)	-	11.26	7.10	10.82	14.52	9.71

Significantly at 1% level of probability

Appendix IX. Effect of different concentration of Kn on *in vitro* regeneration from nodal segment of potato after 30 days of culture (Figure. 7).

Concentration Kn (mg/l)	Responsive explant (%)	Days to shoot initiation	Shoot length(cm) / plantlet	No. of nodes / plantlet	No. of leaves / plantlet	Fresh weight(mg) of shoot / plantlet
0.0	100	7.50	8.70	7.00	7.92	38.56
0.5	100	6.50	10.15	8.50	9.42	49.97
1.0	100	5.50	7.63	6.92	7.75	32.14
1.5	100	4.58	7.37	6.33	7.67	30.20
2.0	100	6.42	6.89	5.75	6.92	26.71
2.5	100	7.42	5.96	5.08	6.00	30.48
LSD(0.01)	-	0.65	0.66	0.91	0.85	8.34
CV (%)	-	9.45	7.73	12.67	10.19	22.04

Significantly at 1% level of probability

Appendix X. Varietals performance on *in vitro* direct regeneration from internode explant of potato after 60 days of culture in BAP treatments (Figure. 10).

Variety	Days to shoot initiation	Percentage of explant producing shoot	Percentage of explant producing root	Percentage of explant producing both shoot and root	Number of multiple shoots / explant
Zaubilati	30.38	62.50 (53.47)	18.75 (22.61)	16.25 (19.45)	2.52
Shadaguti	27.81	96.25 (83.94)	47.50 (44.33)	46.25 (43.54)	4.01
Challisha	31.06	62.50 (52.47)	38.75 (38.14)	33.75 (35.12)	1.85
LSD(0.01)	1.54	10.35	13.33	14.55	0.51
CV (%)	5.37	17.01	39.58	46.29	19.11

Significantly at 1% level of probability

Appendix XI. Varietals performance on *in vitro* direct regeneration from internode explant of potato after 60 days of culture in Kn treatments (Figure. 10).

Variety	Days to shoot initiation	Percentage of explant producing shoot	Percentage of explant producing root	Percentage of explant producing both shoot and root	Number of multiple shoots / explant
Zaubilati	44.00	2.50 (4.44)	58.75 (50.24)	2.50 (4.44)	0.13
Shadaguti	42.50	16.25 (21.03)	76.25 (63.40)	15 (20.24)	0.84
Challisha	41.50	11.25 (13.92)	61.25 (51.68)	11.25 (13.92)	0.44
LSD(0.01)	NS	12.52	8.65	12.40	0.47
CV (%)	97.57	99.24	16.34	100.26	103.28

Significantly at 1% level of probability, NS = Non-significant.

Appendix XII. Effect of different concentrations of BAP along with GA_3 (0.2 mg/l) and IAA (0.5 mg/l) on *in vitro* direct regeneration from internode explant of potato after 60 days of culture (Figure. 11).

Concentration of BAP (mg/l)	Days to shoot initiation	Percentage of explant producing shoot	Percentage of explant producing root	Percentage of explant producing both shoot and root	Number of multiple shoots / explant
0.0	-	-	-	-	-
0.5	30.92	73.33 (61.39)	35.0 (34.72	33.33 (33.66)	2.41
1.0	29.83	78.33 (67.70)	43.33 (42.0)	38.33 (37.87)	2.68
1.5	27.75	73.33 (62.53)	26.66 (29.72)	25.0 (27.62)	3.19
2.0	30.50	70 (61.57)	35.0 (33.66)	31.66 (31.65)	2.89
LSD(0.01)	1.78	NS	NS	NS	0.59
CV (%)	5.37	17.01	39.58	46.29	19.11

Significantly at 1% level of probability, NS = Non-significant.

Appendix XIII. Effect of different concentrations of Kn along with GA_3 (0.2 mg/l) and IAA (0.5 mg/l) on *in vitro* direct regeneration from internode explant of potato after 60 days of culture (Figure. 11).

Concentration of Kn (mg/l)	Days to shoot initiation	Percentage of explant producing shoot	Percentage of explant producing root	Percentage of explant producing both shoot and root	Number of multiple shoots / explant
0.0	-	-	-	-	-
0.5	43	10 (13.92)	61.66 (51.89)	10 (13.92)	0.50
1.0	40.2	10 (12.87)	66.66 (56.21)	10 (12.87)	0.42
1.5	42.16	11.66 (14.97)	76.66 (63.40)	10 (13.92)	0.63
2.0	44	8.33 (10.76)	56.66 (48.92)	8.33 (10.76)	0.33
LSD(0.01)	NS	NS	9.99	NS	NS
CV (%)	97.57	99.24	16.34	100.26	103.28

Significantly at 1% level of probability, NS = Non-significant.

Appendix XIV. Varietals performance on *in vitro* direct regeneration from leaf explant of potato after 60 days of culture in BAP treatments (Figure. 14)

Variety	Days to shoot initiation	Percentage of explant producing shoot	Percentage of explant producing root	Percentage of explant producing both shoot and root	Number of multiple shoots / explant
Zaubilati	30.25	53.75 (47.29)	13.75 (16.29)	11.25 (14.71)	2.49
Shadaguti	28.94	78.75 (65.91)	32.50 (31.96)	32.50 (31.96)	3.16
Challisha	31.13	60 (51.82)	26.25 (27.22)	23.75 (25.71)	1.77
LSD(0.01)	1.31	10.87	12.43	11.34	0.55
CV (%)	4.54	20.56	51.38	48.89	22.87

Significantly at 1% level of probability

Appendix XV. Varietals performance on *in vitro* direct regeneration from leaf explant of potato after 60 days of culture in BAP treatments (Figure. 14).

Variety	Days to shoot initiation	Percentage of explant producing shoot	Percentage of explant producing root	Percentage of explant producing both shoot and root	Number of multiple shoots / explant
Zaubilati	46.5	2.50 (4.44)	36.25 (36.70)	2.50 (4.44)	0.13
Shadaguti	42.14	10 (13.13)	58.75 (51.09)	10 (13.13)	0.53
Challisha	44.4	6.25 (9.18)	41.25 (39.58)	6.25 (9.18)	0.31
LSD(0.01)	NS	NS	9.87	NS	NS
CV (%)	158.63	135.19	24.18	133.11	160.64

Significantly at 1% level of probability, NS = Non-significant.

Appendix XVI. Effect of different concentrations of BAP along with GA_3 (0.2 mg/l) and IAA (0.5 mg/l) on *in vitro* direct regeneration from leaf explant of potato after 60 days of culture (Figure. 15).

Concentration of BAP (mg/l)	Days to shoot initiation	Percentage of explant producing shoot	Percentage of explant producing root	Percentage of explant producing both shoot and root	Number of multiple shoots / explant
0.0	-	-	-	-	-
0.5	32	50 (45.17)	18.33 (19.09)	18.33 (19.09)	2.23
1.0	30.5	68.33 (57.17)	33.33 (34.90)	31.66 (33.85)	2.42
1.5	29.42	71.66 (60.42)	21.66 (22.26)	21.66 (22.26)	2.99
2.0	28.5	66.66 (57.26)	23.33 (24.37)	18.33 (21.29)	2.28
LSD(0.01)	1.52	-	-	-	0.63
LSD(0.05)	-	9.36	10.70	9.76	-
CV (%)	4.54	20.56	51.38	48.89	22.87

Significantly at 1% and 5% levels of probability

Appendix XVII. Effect of different concentrations of Kn along with GA_3 (0.2 mg/l) and IAA (0.5 mg/l) on *in vitro* direct regeneration from leaf explant of potato after 60 days of culture (Figure. 15).

Concentration of Kn (mg/l)	Days to shoot initiation	Percentage of explant producing shoot	Percentage of explant producing root	Percentage of explant producing both shoot and root	Number of multiple shoots / explant
0.0	-	-	-	-	-
0.5	45	6.66 (11.81)	41.66 (39.89)	6.66 (11.81)	0.33
1.0	42.4	6.66 (11.81)	50 (46.13)	8.33 (11.81)	0.50
1.5	43	8.33 (8.65)	48.33 (43.93)	8.33 (8.65)	0.38
2.0	46	1.25 (3.38)	41.66 (39.89)	1.66 (3.38)	0.08
LSD(0.01)	NS	NS	NS	NS	NS
CV (%)	158.63	135.19	24.18	133.11	160.64

Significantly at 1% level of probability, NS = Non-significant.