STUDIES ON ALTERNATIVE SOURCES IN *IN-VITRO* CULTURE OF POTATO (*Solanum tuberosum* L.)

By
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(J-18-M-601)

Thesis submitted to Faculty of Postgraduate Studies
in partial fulfillment of requirements
for the degree of

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HORTICULTURE (VEGETABLE SCIENCE)

Division of Vegetable Science and Floriculture

Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu
Main Campus, Chatha, Jammu-180009

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CERTIFICATE-I

This is to certify that the thesis entitled **"Studies on Alternative Sources in *In-vitro* Culture of Potato (*Solanum tuberosum* L.)"** submitted in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture Horticulture (Vegetable Science)** to the **Faculty of Post-Graduate Studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu** is a record of bonafide research carried out by **Ms. Anamika Walia (Registration No. J-18-M-601)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma. The help and assistance received during the course of investigation have been duly acknowledged.

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ABSTRACT

Title of Thesis	: Studies on Alternative Sources in *In-vitro* Culture of Potato (*Solanum tuberosum* L.)
Name of the Student	: Anamika Walia
Registration No.	: J-18-M-601
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Name & Designation of Major Advisor	: Dr. Anil Bhushan Assistant Professor
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The present investigation entitled "Studies on Alternative Sources in *In-vitro* Culture of Potato (*Solanum tuberosum* L.)" was conducted during the year 2019-20 with the objectives (i) To find out alternative gelling agents for low cost medium (ii) To find out alternative carbon sources for low cost medium and (iii) To assess feasibility of natural indirect light as alternative source for *in vitro* culture. Two explants from variety Kufri Girdhari *viz.*, sprouts and nodal segments were used for the experiment after sterilization with NaOCl (2%). For shoot induction, ten different concentrations of BAP (0.0, 0.25, 0.5, 0.75, 1.0 and 2.0 mg/l) and kinetin (0.0, 0.25, 0.5 0.75 and 1.0 mg/l) were used. Amongst all the treatments, significantly superior response with good quality compact, dark green primordia was observed in treatment T_4- MS + 0.75 mg/l BAP manifested in terms of early days to shoot induction in sprout explants (9.58) as well as nodal segment explants (7.43) and higher percentage response in case of sprout explants (87.06 %) as well as nodal segment explants (85.76 %). For shoot multiplication, thirteen different concentrations of BAP (0.0, 0.25, 0.5, 0.75, 1.0, 1.5 & 2.0 mg/l) alone and in combination with kinetin (0.25 mg/l) were used. Amongst all the treatments significantly superior response in terms of number of shoots per explant (15.50), average length of shoots (8.13 cm) and internodal length (1.12 cm) was recorded from treatment T_{10} - MS + 0.75 mg/l BAP + 0.25 mg/l kinetin. For induction of rooting, healthy *in vitro* multiplied shoots were cultured on MS medium supplemented with different concentrations of IBA (0.0, 0.25, 0.5, 1.0, 1.5 and 2.0 mg/l). Amongst all the treatments, T_4 - MS + 1.0 mg/l IBA recorded superior responses in terms of percentage rooting (97.50 %), number of roots per shoot (8.50), average root length (6.97 cm), number of leaves per plantlet (15.79) and plant height (14.83 cm). The *in vitro* raised single shoots obtained from shoot multiplication stage were used during experiments on alternative gelling agents, carbon sources and light sources.

For studies on alternative gelling agents five different combinations of Agar and Isabgol viz. 100 % Isabgol, 75% Isabgol + 25 % Agar, 50 % Isabgol + 50 % Agar, 25% Isabgol + 75 % Agar and 100 % Agar were used on optimised medium for shoot multiplication. The results revealed that the maximum number of shoots per explant was found in treatment T_5- 100 % Agar (15.50) followed by T_2 - 75% Isabgol + 25 % Agar (13.05). The highest average shoot length (8.20 cm) was found in treatment T_2 - 75% Isabgol + 25 % Agar.

In the study on alternative carbon source, *viz.* common table sugar, the results were at par or even better as compared to standard carbon source *viz.* sucrose. The results revealed maximum number of shoots (15.50), longer shoot length (8.13 cm) and longer internodal distance (1.12 cm) were observed in sucrose. The number of nodes per plantlet was maximum (9.02) in common table sugar. The percentage rooting response (98.17) and average root length (7.10 cm) was higher in common table sugar.

Amongst the alternative light sources, natural indirect light was better alternative in terms of number of shoots (15.98), average shoot length (9.85 cm) and internodal distance (1.42 cm) as compared to fluorescent light. The natural indirect light also gave higher percentage rooting response (97.57) with higher response in terms of number of roots per shoot (9.20), average root length (7.03 cm) and plant height (15.36 cm).

Signature of Major Advisor **Signature of Student**

ABBREVIATIONS

MS medium	:	Murashige & Skoog's medium
BAP	:	6-Benzylaminopurine
BA	:	6-Benzyladenine
GA_3	:	Gibberellic acid
IAA	:	Indole-3-acetic acid
IBA	:	Indole-3-butyric acid
NAA	:	1-Napthaleneacetic acid
TDZ	:	Thidiazuron
2, 4-D	:	2, 4-Dicholorophenoxyacetic acid
%	:	Percentage
SE(d)	:	Standard error of Difference
C.V.	:	Coefficient of Variation
C.D.	:	Critical Difference

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INTRODUCTION

CHAPTER-I

INTRODUCTION

Potato (*Solanum tuberosum* L.) is a self-pollinating crop belonging to the family Solanaceae and cultivated as a tuber crop. It is the fourth most important food security crop in the world after rice, wheat and maize (Haverkort *et al*., 2009). It is a native of Tropical South American region where it grows wild and presents the widest diversity. The areas of origin and domestication of the cultivated potatoes is in high plateaus of Peru-Bolivia (Andes region). It is widely cultivated in about 150 countries (Basera *et al.,* 2018) and is considered as the most important non-cereal food crop in the world (Bamberg *et al.,* 2016).

It is universally assented that vegetables are cheaper sources of proteins, vitamins, carbohydrates and minerals. Potato is eulogized as 'King of Vegetables or Poor man's friend or Food for Future'. It has greatest dry matter and protein per unit growing area when compared with cereals. It is a nutrient dense food thus; it has a key role in preventing malnutrition in impoverished areas and contributing to health where food is ample. On an average, potato tubers contain 77% water, 20% carbohydrates and less than 3% of proteins, dietary fibers, minerals, vitamins and other compounds (Zaheer and Akhtar, 2016).

India occupies the prime position in vegetable production and is the second largest producer of vegetables in the world after China (Sundhariaya, 2018). It is the most cultivated vegetable in the country in terms of area, production and productivity. India is the world's second largest producer of potato, after China. Together both countries contribute 38% to the world's total potato production. Major potato producing states are Uttar Pradesh, West Bengal, Bihar, Gujrat, Madhya Pradesh and Punjab. Uttar Pradesh and West Bengal contribute more than 50% of the total potato production. The area under potato cultivation in India is 21.8 lakh hectares with an annual production of 52.5 million tonnes (Anonymous, 2019). In erstwhile state of Jammu and Kashmir, it is cultivated in an area of 6.91 thousand hectares with an annual production of 197.87 thousand metric tonnes. (Anonymous, 2019).

Potato can be propagated vegetatively, mainly through tubers and sexually through botanical seeds (true potato seed). In potato cultivation, seed tubers alone account for 40 to 50% of production costs (Kumar *et al*., 2007). The availability of disease free quality seed/ planting material in sufficient quantity is considered the most important factor for obtaining good yields. In spite of spending a huge amount of money, most of the seeds obtained are non-certified, impure and contaminated by various pathogens (Hussain *et al.,* 2003). Various researchers have reported losses in potato production ranging from 40% to 90% due to insect-pests and diseases (fungal, bacterial and viral). At present, the central seed production agencies of India and states are able to meet only 20-25% requirement of quality seed potatoes. For bridging this wide gap, large scale integration of conventional and innovative methods like micropropagation at commercial level are needed for producing enough quantity of healthy seed tubers in minimum duration (Pandey, 2006).

Plant biotechnology offers opportunities for producing disease free and healthy potato seed tubers *via* clonal propagation for large scale production/multiplication of elite and healthy microtubers. Micropropagation is a potential biotechnological tool that has become commercially viable method of *in vitro* clonal propagation of potato. Bench level protocols are available for *in vitro* shoot multiplication, but high cost involved in production of *in vitro* cultures is a major bottleneck in up-scaling such useful technology at a larger scale. The lack of budget, limited resources and high recurrent costs of consumables for media and lack of awareness has also restricted the usage of this technology. The available alternative includes cost-effective technology for the *in vitro* clonal propagation of disease free potatoes.

The principal components of plant tissue culture media are inorganic nutrients (Macro- and Micronutrients), carbon sources (Sucrose), organic supplements (Vitamins), growth regulators and agar. The cost of the culture nutrient medium is one of the major factors contributing to the high cost of production as this requires expensive chemicals. The most expensive and extensively used component of solid or semisolid nutrient media for *in vitro* culture is agar (Pierik, 1989). It is most frequently used as a solidifying agent in the tissue culture media due to its colloidal and polysaccharidic nature (Lobban and Wynne, 1981). Agar can be substituted by various low cost gelling agents like isabgol, Sago, Corn starch etc. The use of isabgol, a cheap gelling agent, for plant tissue culture medium has been established (Babbar and Jain, 1998) and it has also been reported that isabgol's mucilage is colloidal and polysaccharidic in nature just like agar (Laidlaw and Percival, 1950).

In addition to the gelling agents, the carbon sources such as grade sucrose that is often used in the micropropagation of plants at laboratory contribute towards high production costs (Demo *et al.*, 2008). The commercial grade sugar can replace analytical grade sucrose, with no significant change in the frequency of shoot formation in banana (Ganapathi *et al.*, 1995). Kodym and Zapata-Arias (2001) reported success in reducing the cost of tissue culture banana trees up to 90% by replacing market sugar with grade sucrose.

Light also plays an important role on growth and development of the *in vitro* culture plants. The artificial lighting costs account for 65% of total electricity bill and it also generates heat that has to be reduced by air conditioning that further adds to the electrical load. On the contrary, natural light has also been used in potato tissue cultures in India and Cuba where acceptable cultures were produced with considerable saving of energy (Alix *et al.*, 2001).

The development of a cost-effective technology using low cost media constituents will be the best alternative for *in vitro* propagation of disease free potatoes and rapid multiplication of the elite genotypes. Keeping the above considerations in view, the present investigation entitled **"Studies on Alternative Sources in *In-vitro* Culture of Potato (*Solanum tuberosum* L.)"** was planned with the following objectives:

- To find out alternative gelling agents for low cost medium.
- To find out alternative carbon sources for low cost medium.
- To assess feasibility of natural indirect light as alternative source for *in vitro* culture.

Chapter-II

REVIEW OF LITERATURE

CHAPTER-II

REVIEW OF LITERATURE

A number of studies have been made with respect to *in vitro* multiplication of potato. The literature on various aspects of the present investigation is reviewed under the following sub-heads:-

2.1 Micropropagation

- 2.1.1 Effect of different explants
- 2.1.2 Surface sterilization of explants
- 2.1.3 *In vitro* shoot multiplication
- 2.1.4 *In vitro* rooting
- 2.1.5 Microtuberization
- 2.1.6 Hardening and establishment of the regenerated plant

2.2 Gelling Agents

2.3 Carbon Sources

2.4 Light Sources

2.1 Micropropagation

Micropropagation is a potential biotechnological tool that has become a commercially viable method of *in vitro* clonal propagation of a wide range of herbaceous and woody plants (Garcia *et al.*, 2010). Micropropagation is the practice of rapidly multiplying stock plant material to produce many progeny plants, using modern plant tissue culture methods. The work carried out under *in vitro* propagation is reviewed as under:

2.1.1 Effect of different explants

Nagib *et al.* (2003) obtained virus free clones of four widely cultivated varieties of potato by using apical meristem as an explant. Substantial yield increase was observed in meristem derived plants over their source plants.

Fatima *et al.* (2005) studied effect of explant sources for microtuber induction in two potato varieties. The results showed that nodal explant was better for shoot formation (100%), shoot length, number of microtubers (2.16) and microtuber weight

(164.50 mg/tuber) while shoot tip explant gave significantly more number of leaves (6.61) and higher root formation (81.37%).

Hoque (2010) used three different explants (nodal segment, sprout, and shoot apex) and among these nodal cutting showed the highest per cent (94%) for microtuber formation and average weight of microtuber, whereas lowest response (78%) was visible in the case of sprout explant for microtuber explant.

Liljana *et al.* (2012) used two different explants (nodal segments and sprouts) for studying the effect of PGR's on *in vitro* microtuber formation and growth. Among these nodal segments with MS + 2mg/l BAP + 1 mg/l NAA showed 13.33% microtuberization with better microtuber formation.

Sharma *et al.* (2015) concluded that nodal explants of potato cultivars (Diamont, 1533 and Kufri Badshah) gave maximum shoot regeneration when cultured on MS Basal medium supplemented with BAP 1 mg/l.

Shahriyar *et al.* (2015) investigated that shoot regeneration was observed from both shoot tips and nodal explants obtained from field grown plants. Maximum number of shoot per culture (17) and highest average length of shoot (5 cm) was observed in cultures where shoot tips were used as explants.

Mohapatra *et al.* (2016) studied the effects of growth regulators in different explants (sprouts and shoot tips) of cultivar Kufri Frysona. The maximum survival percentage was noticed in shoot tips (100%) whereas it was 40% in sprouts.

Salem and Hassanein (2017) obtained high frequency of microtuberization (90%) when sprouts of potato tubers were used as explant.

Serine *et al.* (2020) used potato meristem as explant to establish a protocol for virus free mini tubers production and it was concluded that virus free mini tubers can be grown by meristem tip culture and higher yield with better grade of potato can be obtained.

2.1.2 Surface sterilization of explants

Gopal *et al*. (1998) recommended mixture of $HgCl_2$ (0.1%) and Sodium Lauryl Sulphate (0.1%) for 5 minutes for disinfection of single nodal sections from sprouts of potato cultivars.

Badoni and Chauhan (2010) compared two important sterilants sodium hypochlorite and mercuric chloride with different time durations (2, 5 and 8 minutes) for sprout explants. Results showed that amongst the two sterilants sodium hypochlorite (8 minutes) was found better for controlling the infection and it did not have any adverse effect on explants even in long duration. Sodium hypochlorite (NaOCl) for 8 minute was selected for suitable sterilization chemical after 5 minute of savlon wash, 30-second dip in ethanol and at last washed with double distilled water

Hoque (2010) have described sterilization treatment for *Solanum tuberosum*, which includes the surface sterilization by dipping in 0.5 $HgCl_2$ solution for 3-5 minute and then washed 6-7 times with autoclaved distilled water.

Yasmin *et al*. (2011) used dissected segments of sprouts as the experimental plant material which were surface sterilized with 10% commercial bleach containing three drops of polyoxyethylene sorbitan monolaurate (Tween-20) for 10 minutes.

Lilijna *et al*. (2012) reported that surface sterilization with mercuric chloride (0.1%) after 2 minutes ethanol dip gave best results for controlling infection in two potato cultivars i.e. Arija and Andrea.

Bhuiyan (2013) described that in all three varieties of potato (Espirit, Lady Rosetta and Meridian), the percentage of survived explants was the maximum in surface sterilization with 0.1% $HgCl_2$ for 4 minutes when cultured on MS basal medium.

Mohapatra *et al*. (2018) investigated various sterilization treatments on *in vitro* establishment of potato cultivar Kufri Pukhraj. The sterilization treatment bavistin (0.2%) + streptocycline (0.4%) for 45 minutes followed by $HgCl_2$ (0.1%) for 60 seconds was found to give best results.

Kajla *et al.* (2020) found that surface sterilization with 0.2% Bavistin + 0.4% Streptocyclin for 45 minutes followed by 0.1% $HgCl_2$ treatment for 55 seconds was optimum for *in vitro* culture establishment and maximum survival percentage for variety Kufri Lima.

2.1.3 *In vitro* shoot multiplication

Rabbani *et al*. (2001) studied the effects of different concentrations of GA_3 and BAP on *in vitro* multiplication of potato variety "Desiree". The results showed that maximum shoot length (8.96 cm) was obtained when 4 mg/l GA_3 was applied whereas maximum number of shoots (14) was obtained when 2 mg/l BAP was applied.

Shah Zaman *et al*. (2001) found that the highest stem length and the largest single nodes in potato can be achieved in MS medium containing 0.5 mg/l NAA.

Shibli *et al*. (2001) sub-cultured *in vitro* shoots of potato cultivar "Spunta" in liquid MS medium containing 0.0, 0.5, 1.0, 1.5 and 2 mg/l BA and kinetin. They observed a significant reduction in stem and inter-nodal length by increasing BA & kinetin concentration in medium. BA up-to 1.0 & 1.5 mg/l resulted in an increase in number of proliferating shoots & nodes per culture flask.

Rashid *et al.* (2005) used various concentrations of growth regulators to study effect on shoot regeneration. It was observed that BAP played important role in shoot regeneration. Also MS + 2 mg/l BAP and IAA 0.5 mg/l gave maximum shoot regeneration.

Uddin (2006) concluded that the highest number of shoot were formed on MS basal medium supplemented with combination of 3 mg/l BA + 2 mg/l kinetin whereas 2 mg/l BA alone produced only 80 % shoots and 2 mg/l kinetin alone produced 100% shoots. The induction of multiple shoots from each bud was highest (3.0) in combination of 2 mg/l kinetin + 1 mg/l BA while 2.0 mg/l BA produced only 1.37 shoots.

Khadiga *et al.* (2009) investigated different concentrations of TDZ and BA alone or in combinations with NAA. The highest number (5.4 shoots/ explant) of shoot per explant (nodal explant) were obtained when cultured on MS medium supplemented with 3.0 mg/l TDZ in combination with 0.1 mg/l NAA.

Badoni and Chauhan (2010) studied the effects of various growth regulators on meristem tip development and *in vitro* multiplication of potato cultivar "Kufri Himalini". Different combinations of growth regulators (GA_3, NAA and kinetin) were used for study. Results showed that lower concentration of Auxin (0.01 mg/l NAA) with Gibberellic acid (0.25 mg/l) was the best for development of complete plantlets from meristem tips.

Hoque (2010) supplemented MS medium with different concentrations of kinetin (1, 2, 3 and 4 mg/l). It was noticed that minimum days required for shoot regeneration was 5 days in MS+ 4 mg/l kinetin. Also number of shoots per explant was highest (9.28) in MS+ 4 mg/l kinetin.

Molla *et al.* (2011) studied the effect of different concentrations of BAP (6), TDZ (6) and Zeatin riboside (8). Among these Zeatin riboside (5 mg/l) was excellent for direct shoot induction. Also 3 mg/l BAP and 0.3 mg/l TDZ showed good performance for shoot induction from internode explants.

Munir *et al.* (2011) studied the effects of three growth regulators (2, 4-D, BAP and zeatin) on callus culturing. Maximum callus regeneration (90%) was observed at 2.5 mg/l 2, 4-D while minimum growth was seen at 1.5 mg/l zeatin and 1 mg/l BAP.

Bhuiyan (2013) used different concentrations of BAP (0.5-2.5 mg/l) and kinetin (0.5-2.5 mg/l) separately to see their effect on multiple shoot regeneration. The maximum number of shoots per shoot apex and nodal segment was formed at 1 mg/l BAP + MS medium. Also concentrations of GA_3 in combination with BAP or kinetin were tested and combination MS medium with 1 mg/l BAP + 1 mg/l GA_3 gave best results.

Ahmad *et al.* (2014) used various combinations of BAP, kinetin and NAA to study their effects on direct multiple shoot regeneration. Results showed that the MS medium supplemented with 2.0 mg/l BAP in combination with 0.1 mg/l kinetin produced maximum number of shoots (13.10).

Sharma *et al*. (2015) investigated the effect of growth regulators on *in vitro* micropropagation of three potato cultivars cultured on MS basal medium supplemented with different hormonal combinations (BAP & GA_3). Different concentrations used were BAP (0.5, 1 mg/l) alone and in combination with GA_3 (0.2

mg/l). It was concluded that MS + BAP 1 mg/l gave maximum shoot regeneration on all three potato cultivars.

Hamza *et al.* (2017) used various concentrations of NAA (0, 0.1 & 0.2 mg/l) individually or in combination with BA or kinetin at different concentrations (0.0, 0.1, 0.2 & 0.4 mg/l). Results indicated that the highest multiplications aspects were obtained by MS medium containing 0.2 mg/l NAA + 0.2 mg/l kinetin.

Mohapatra *et al.* (2018) sub-cultured sprouted explants of cultivar "Kufri Pukhraj" on MS media supplemented with various growth regulators i.e. BAP and kinetin alone and in combination with NAA, IBA, IAA and GA_3. The results showed that maximum shoot induction (61.5 ± 0.84) was observed in medium MS + BAP 0.25 mg/l. Also the maximum number of shoots (21.3) was observed on medium MS + BAP 0.25 mg/l + 0.01 mg/l IAA.

Hazare *et al.* (2021) concluded that best results for shoot initiation were found in MS medium supplemented with 1.5 mg/l BAP + 3.0 mg/l NAA for Gudiene variety whereas 1.0 mg/l BAP and 2.0 mg/l NAA produced more shoots in Belete variety. The combined effect of BAP and Kinetin did not produce any additional positive effect for shoot multiplication.

2.1.4 *In vitro* rooting

Kaur *et al.* (2000) observed upto 99% rooting of the plantlets in liquid half strength MS medium with IBA at 1.0 mg/l in four potato varieties i.e. Kufri Badshah, Kufri Chandramukhi, Kufri Jyoti and Kufri Sindhuri.

Sarkar and Mustafa (2002) found best response for root induction when MS medium was supplemented with 1.0 mg/l IAA.

Sanavy and Modarres (2003) used different concentrations of NAA and BAP for root induction and they studied that application of BAP and NAA decreased shooting and rooting of single nodes.

Nagib *et al.* (2003) observed high frequency of root formation in combination of GA_3 (0.1 mg/l) and kinetin (0.1 mg/l).

Badoni and Chauhan (2009) used various concentrations of GA_3, NAA and kinetin for rooting of *in vitro* plantlets of potato variety “Kufri Himalini”. The results showed that lower concentrations of auxin (0.01 mg/l NAA) with Gibberelic Acid (0.25 mg/l GA_3) is best for development of complete roots.

Khalafalla *et al.* (2009) in their experiment used regenerated shoots for rooting on MS medium with and without auxin. The longest root (8.8 ± 1.3) was induced from shoot cultured on MS medium lacking growth regulator. The highest number (35 ± 1.3) of roots per shoot was obtained on MS medium supplemented with IBA at 1.0 mg/l.

Bhuiyan (2013) used various concentrations of IAA and IBA for root induction. MS medium supplemented with 0.5 mg/l IAA showed best response for producing roots.

Parveen *et al.* (2014) used half strength of PROP medium supplemented with 0.5 mg/l IBA for rooting of *in vitro* grown shoots.

Shahriyar *et al.* (2015) induced rooting in MS medium supplemented with different concentrations (0.5, 1, 1.5 and 2 mg/l) of IAA and kinetin. IAA and kinetin (1.5 + 1.5 mg/l) showed lowest rate of root regeneration (40%) and the average length of the root (1.5 cm). On the contrary MS medium with no hormone showed high rate (96%) of root regeneration and the highest average length of the root (2.5 cm).

Mohapatra *et al.* (2016) used various concentrations of IBA (0.5- 2.5 mg/l) and NAA (0.5- 2.5 mg/l). From the study it was observed that maximum rooting (100%) occurred in PR_9 (half MS salt supplemented with NAA 2.0 mg/l) with minimum 10.1 days followed by 100% rooting in PR_4 medium having half MS salt + IBA 2.0 mg/l with 11.0 days.

Mohapatra *et al.* (2018) observed best rooting response from half strength MS medium augmented with 2.0 mg/l IBA after 10 days.

Hazare *et al.* (2021) found that the rooting percentage and number of roots/shoots were found best on the MS Medium fortified with 1.0 mg/l IBA + 0.5 mg/l IAA.

2.1.5 Microtuberization

Gopal *et al.* (1998) studied microtuberization in twenty two genotypes of potato under six *in vitro* culture condition and inferred that cultures maintained under a short photoperiod (10 h) and low temperatures (day 20º ± 2º C & night 18º ± 2º C) had both higher yield (255 mg/ plantlet) and greater number (2/plantlet) of microtubers than those maintained under long day and high temperature. Also under short day and low temperature condition and addition of BAP (10 mg/l) increased microtuber yield from 255 mg/plantlet to 645 mg/plantlet and average microtuber weight was also increased from 115 mg to 364 mg.

Nawsheen (2001) concluded that optimal production of microtubers was obtained in MS medium. Best response for cultivar "Desiree" was MS medium supplemented with 6.0 mg/l BA + 6% sucrose and the time taken for microtuberization was 14 days.

Fatima *et al.* (2005) studied the effects of explant source on two potato varieties (PARS-70 and Santa) for microtuber induction at different sucrose levels (3, 6, 9 & 12%) supplemented in MS medium. From the experiment it was concluded that *in vitro* grown shoots and nodal explants proved superior to shoot tips for microtuber induction. Also number of microtubers and microtuber weight was maximum in variety PARS-70 at 6% sucrose concentration.

Hussain *et al.* (2006) compared the tuber induction capacity with different concentrations of CCC (0 to 250 mg/l), BAP (0- 4.0 mg/l) and sucrose (30-120 g/l). Maximum number of tubers (9/flask) were obtained with MS+ 4 mg/l BAP. When medium was supplemented with CCC at 200 mg/l, the number of tubers was maximum (16/flask) but with increase in number, the size of microtuber decreases. Maximum number of tubers (15.6/flask) were produced when 90 g/l sucrose was used. When comparison was taken into consideration between CCC, BAP & sucrose, it was seen that CCC was at par with sucrose.

Aslam and Iqbal (2010) tested varying concentrations of sucrose (4, 6, 8, 10 and 12%) either alone or in combination with kinetin and BA in two potato cultivars "Diamant and Red Norland". Medium MB 8 (5 mg/l BA + 8 % sucrose) in term of induction and mean number of microtubers per single node explants and MB 11 (6 mg/l BA + 7% sucrose) in terms of mean fresh weight per single node provided best results for cultivar “Diamant”. In case of “Red Norland”, medium MK 10 (2mg/l kinetin + 6% sucrose) gave best results in terms of induction, mean number and mean fresh weight of microtubers per multi-node explants.

Badoni and Chauhan (2010) used three different concentrations of BAP (8 mg/l, 10mg/l and 12 mg/l) with MS liquid medium to study effects on microtuberization. In the study, formation and development of microtubers were higher in 10 mg/l BAP concentration.

Imani *et al.* (2010) studied the effects of various concentrations of BAP (0, 12, 15 & 18 mg/l) and sucrose (0, 60 & 80 g/l) on *in vitro* microtuberization of potato. Results indicated that sucrose & BAP had significant effect on microtubers. Sucrose 60 g/l + BAP 15 g/l showed maximum number (5.80) of microtubers whereas, sucrose 60 g/l + BAP 15 g/l showed maximum size of microtuber (0.49 cm).

Aslam *et al*. (2011) tested single node and multi-node explants of two potato varieties (Desiree and Cardinal) for *in vitro* microtuber production. MS media was augmented with varying concentrations of sucrose (4, 6, 8, 10 and 12%) either alone or in combination with BA (4, 5 and 6 mg/l).the results obtained revealed that in case of cultivar “Desiree” medium supplemented with BA 6 mg/l + Sucrose 6% and for cultivar “Cardinal”, supplemented with BA 5 mg/l, Sucrose 8% performed better in terms of induction, mean weight and mean fresh weight per single node explant.

El Nagar and Mekawi (2012) produced microtubers using sub-cultured axillary shoot cuttings on tuberization induction medium. MS medium supplemented with 0.1 mg/l BAP and 30 g/l sucrose was used for induction at 23± 1ºc temperature under complete darkness for 75-90 days.

Saha *et al*. (2013) undertook an experiment to choose most effective sugar concentration in *in vitro* microtuberization. It was seen that percentage of survivability of microtuber producing plants increased with increase of sucrose concentration. For microtuber formation MS medium was supplemented with BAP (5 mg/l), CCC (500 mg/l) and different concentrations of sucrose (5%, 6%, 7%, 8%, 9% and 10%). The highest survival rate (80%) was found in 10% sucrose. The highest number of microtuber (2.17) observed on 10% sucrose. Also, average diameter of tuber/plant was highest in 10% sucrose level.

Mohapatra *et al*. (2018) used nodal segments from well multiplied cultures, for microtuber induction on different mediums. Various concentrations of hormones and sugar were tested and MS medium + 0.01 mg/l BAP + 0.01 mg/l NAA + 0.1 mg/l GA3 + Sugar 80 g/l proved to be the best, resulting in maximum microtuber (3.8) development in 50.1 days with 9.4 mm size and 0.4g of weight.

Meenakshi (2020) used two potato varieties “Kufri Bahar” and “Kufri Surya” for testing *in vitro* tuberization response to MS medium supplemented with three different concentrations of BAP (0.75, 1.5 and 2.25 mg/l). It was concluded that both varieties exhibited a better response when culture were supplemented with 2.25 mg/l of BAP.

2.1.6 Hardening and establishment of the regenerated plant

Obradovic and Sukha (1993) transplanted five week old *in vitro* grown plantlets in pots containing either vermiculite: sand (4:1) or peat moss: sand (4:1) medium. The results indicated that higher yield and number of tubers were obtained with vermiculite: sand medium.

Sanavy and Moeini (2003) studied the effects of four planting beds prepared using Perlite/sand/loamy soil in the ratio of 1:1:1, peat moss/sand in 4:1 ratio, loamy soil/leaf compost/sand 1:1:1 and hull rice/loamy sol in 2:1 ratio. It was investigated that a mixture of peat moss and sand in the ratio of 4:1 proved best for growing plantlets

Uddin (2006) used one part sterile garden soil, one part sand and compost mixture for hardening of rooted plantlets and found that this mixture showed good survival percentage of more than 80%.

Badoni and Chauhan (2010) acclimatized potato plants after rooting under *in vitro* condition. For hardening of plants, they used mixture of soil, sand and vermicompost (2:1:1) and got good results.

Armin *et al*. (2011) studied the effects of four potting mixes on plantlets morphological traits and mini-tuber production were studied in greenhouse. Results showed that Peat moss: sand (4:1) was the best potting mix for plantlets growth and mini tuber production.

Bhushan and Gupta (2017) used mixture of sand, soil and farmyard manure (1:1:1) for transplanting of regenerated plants in the greenhouse for hardening for 6 different genotypes of tomato. All the regenerates exhibited normal characteristics.

Mohapatra *et al.* (2018) studied the effect of different potting mixture on survival percentage of *in vitro* raised plantlets of potato cultivar Kufri Pukhraj. Maximum survival percentage of rooted plantlets was noticed in potting mixture of sand: soil: vermicompost (1:1:1).

2.2 Gelling Agents

The most expensive and extensively used component of solid or semisolid nutrient media for *in vitro* culture is Agar (Pierik, 1989). Prakash (1993) stated that gelling agent, agar which is usually added to increase media viscosity contributes 70% of the media costs. Cheaper agar alternatives which include various types of starch and gums have been investigated in commercial micropropagation (Nagamori and Kobayashi, 2001). Many other options include Isabgol, white flour, laundry starch, semolina, potato starch, rice powder and sago (Prakash *et al.*, 2004). The literature regarding this is reviewed as under-

Bhattacharya *et al.* (1994) reported the efficacy of sago starch and isabgol as gelling agents for the propagation of plantlets of chrysanthemum. Results show that sago and isabgol can be used as substitute for agar in MS medium at 13% (w/v) and 3% (v/v) levels respectively.

Babbar and Jain (1998) have successfully used isabgol as a gelling agent in tissue culture media for *in vitro* shoot formation and rooting in *Syzgium cuminii*. The epicotyl segments excised from the *in vitro* grown seedling when cultured on shoot multiplication medium developed 3 to 6 shoots per explant with an average of 4.1 shoots/ responding explant.

Naik and Sarkar (2001) substituted agar on potato micro-propagated medium with 80 g/l of sago and found that the number of shoots and leaves and root length were significantly higher compared to the agar medium.

Shah *et al.* (2003) evaluated Isabgol as a potential gelling agent for *in vitro* micropropagation of virus free potato. For this purpose they used nine treatments of Isabgol (0, 6, 8, 10, 12, 14, 16, 18 and 20 g/l). From this study it was reported that Isabgol @12g/l was the most suitable concentration and its performance was at par with agar solidified media for *in vitro* micropropagation of virus free potatoes.

Tyagi *et al.* (2008) tested isabgol as alternative gelling agent for *in vitro* culture of *Curcuma longa* L. Non-significant differences were observed between media solidified with isabgol and agar during multiplication. High survival rate of 33-44% was recorded in isabgol-gelled media as compared to 16% survival in case of agar based gelling media.

Mohamed *et al*. (2010) used corn and potato starch as an agar alternative for *Solanum tuberosum* micro-propagation. The highest number of shoots (6.8) was achieved in medium with 50 or 60 g/l of PS + 1 g/l of agar. Media with 50, 60 g/l of PS or 60 g/l of CS and 50 g/l of CS + agar at 1 g/l significantly enhanced the percentage of dry weight. The results suggest that the combination of agar and PS or CS could offer a firm support for plant tissues and could be successfully used for potato micro-propagation.

Tyagi *et al.* (2010) in the experiment used MS medium gelled with 0.8% (w/v), agar (bacteriological grade) 0.25% (w/v), phytagel and 3.5% (w/v) isabgol. It was concluded that up to 12 months, 100% of cultures survived on isabgol-media, which was significantly higher than that on agar-media (79–83%) and on phytagel-media (51–57%). The total cost of medium used for *in vitro* conservation of banana was decreased by 59% by using isabgol as an alternate gelling agent to agar and phytagel.

Khan *et al*. (2012) used two potato varieties (Diamond and Granula) for their study of regeneration on four different combinations of Agar and Isabgol husk (combination 1- 50% + 50%, combination 2- 57% + 43%, combination 3- 71% + 29% and combination 4- 43% + 57%) as gelling agent. The results revealed that culture media with combination 4 gelling agent did not gel properly. Height and health of plantlets grown in agar + Isabgol husk gelled media was almost the same as plantlets grown in agar media.

Venkatasalam *et al.* (2013) developed a highly cost effective *in vitro* mass multiplication protocol for potato at CPRI Shimla. They used four different types of gelling agent's viz. agar (PT), agar (bacteriological), agar (purified) at 7 gm/l concentration and gelrite at 2 gm/l. Better plant height, number of leaves and nodes was seen in medium solidified with gelrite. Thus it was concluded that agar can be replaced with gelrite/ phytagel, though the unit cost of gelrite/ phytagel is more than agar but the quantity of its use for solidifying is much less (25%) and will lead to save 43 to 52% cost on solidifying agent.

Bhushan and Gupta (2017) studied the effects of two gelling agent's viz. isabgol (30 g/l) and sago (130 g/l) as compared to agar on shoot multiplication of six different genotypes of tomato. The results revealed isabgol to be the most suitable gelling agent with maximum shoot multiplication in all genotypes.

Shah *et al.* (2017) established a cost-effective protocol for micropropagation of potato by using Balanga (*Lallemantia royleana*) seeds as gelling agent. After a pilot study, 45 g/l of balanga seeds were selected as suitable concentrations to be used for micropropagation of potato. The results indicated that balanga seeds show parallel even better results than agar to some extent and it is about 32% cheaper than agar.

2.3 Carbon Sources

Sucrose has been reported to be the best carbon and energy source (George, 1993). In spite of widespread use, the cost of refined sucrose is far too high. Sucrose may contribute about 34% of production cost. Thus cheap alternatives to expensive grade sucrose are required to lower the production cost. The literature regarding this is reviewed as under-

Ganapati *et al.* (1995) concluded that commercial grade sugar could replace analytical grade sucrose with no significant change in the frequency of shoot formation in banana cv. 'Basrai' (AAA).

Sharma and Singh (1995) obtained maximum shoots per explants, shoot and root length and roots per shoot on MS medium containing ordinary sugar as compared to MS medium supplemented with analytical grade sucrose in ginger.

Adiga *et al*. (1998) concluded that there was no significant difference in response of *in vitro* cultures of jackfruit for shoot proliferation when carbon source was supplied either as analytical grade sucrose or as market grade table sugar and hence table sugar could be substituted to analytical grade sucrose as it is cheaper cost-wise and easily available in the market.

Prabhakara (1999) determined that commercial grade sugar at 30 g/l was the best alternative for sucrose for *in vitro* propagation of anthurium, which reduced the cost by twelve times that of sucrose. They further opined that among other sources, fructose at very low concentration and glucose at 20 g/l were found to be next best to commercial grade sugar at 30 g/l.

Kodym and Zapata-Arias (2001) tested thirteen different sugars from different countries as an alternative to sucrose in banana micropropagation. Best results were achieved using white and light brown sugars with low electrical conductivity.

Prakash *et al*. (2004) in their experiment concluded that in culturing ginger and turmeric, all other carbohydrate sources (household sugar, double refined sugar and sugar crystals) except sugarcane juice, were found suitable alternatives to laboratory grade sucrose. Also use of common sugar reduced the cost of medium between 78 to 87%.

Raghu *et al*. (2007) concluded that market grade sugar did not affect the proper growth of the *Centella asiatica* and the shoot multiplication rate obtained was not less when compared to the other *in vitro* multiplication trails.

Demo *et al.* (2008) compared laboratory grade sucrose with two types of local commercial table sugar, especially white and brown sugar in potato micropropagation. Three Kenyan cultivars were cultured on full strength of MS medium with combination of sugars and 100% survival rates were seen. It was quantified that table sugar enhanced micropropagation and also lowered input costs by 34 to 51% when compared to analytical grade sucrose.

Nyende (2008) used table sugar as a low cost alternative medium for commercial propagation of potato. Results also showed that table sugar not only enhanced micro-propagation but also significantly lowered the production input costs by 51% when compared with the analytical grade sucrose.

Purohit *et al.* (2009) compared effect of sucrose with three different carbon sources viz sugarcubes, commercial sugar and jaggery in *Wrightia tomentosa*. A higher multiplication rate than the control was obtained on medium containing sugarcubes.

Gitonga *et al.* (2010) substituted sucrose with commercial table sugar and concluded that 97% cost can be reduced by using table sugar as carbon sources.

Venkatasalam *et al*. (2013) used MS medium supplemented with seven types of carbon sources viz. commercial sugar, commercial sugar (sulphur less), sucrose, fructose, dextrose, sugar cubes and galactose at 30 g/l. Among these carbon sources commercial sulphur less sugar significantly enhanced the micro-plant height (10.4 cm) which was also found at par with commercial sugar (10 cm) as well as standard control sucrose (9.5 cm), whereas galactose recorded minimum plant height (2.1 cm). Also sulphur less sugar as well as commercial sugar significantly increased the number of nodes. It was concluded that sucrose can be successfully replaced by any ordinary commercial sugar, which was 5 to 6 times cheaper than sucrose.

Naik *et al.* (2020) concluded that use of common table sugar as a substitute reduces the cost of the medium between 78 to 87 % and also the sugar sold in grocery stores is sufficiently pure for micropropagation.

2.4 Light Sources

Artificial lighting of cultures in the growth rooms is one of the most expensive and inefficient methods in tissue culture technology. Debergh and Read (1991) have reported that lighting costs account for 65% of the total electricity bill, and are one of the highest non-labour costs in a tissue culture laboratory (Dooley,1991). Changing the method of illumination from artificial to natural light is a decisive low cost option in tissue culture (Ahloowalia and Savangikar, 2004). The literature regarding this is reviewed as under-

Zapata-Arias and Kodym (1999) proposed the concept of using sunlight for micropropagation in shoot tip culture of banana cultivar “Grande Naine”. In this experiment culturing in a non-controlled natural light environment was done and the results showed that significantly more shoots were produced by plantlets cultivated in sunlit room (with PPFD up-to 570 µ mol $m^{-2}s^{-1}$, temperature between 25 and 30ºC and photoperiod of 12 to 16 h) than by plantlets under artificial light in a growth chamber with PPFD up-to 65 µ mol $m^{-2}s^{-1}$, temperature between 23 to 29ºC and 16 h photoperiod). The rooted plantlets obtained showed 100% survival rate during acclimatization and further normal development.

Zapata- Arias *et al*. (2001) used tubular skylights to redirect natural light into an interior enclosed room where maximum heat transfer occurred and no cooling was necessary for micropropagation of Potato. Under natural light conditions, micropropagated plantlets were well developed at mean PPFD at 5-13 µ mol $m^{-2}s^{-1}$ and photoperiods of 9-14 hours.

Be *et al.* (2006) compared the growth of Vetiver grass under two environmental conditions i.e. Nethouse conditions (30∓1°C at 11:00 and 31∓1°C at 15:00, under daylight 8-10 h with PAR about 95-125 µ mol $m^{-2}s^{-1}$) and under growth chamber conditions (24 ∓1°C under 12 h daylight with PAR about 30 µ mol $m^{-2}s^{-1}$). The results obtained indicated that there is no statistical difference between the two environmental conditions for propagation, rooting, and survival cluster after ten weeks of acclimatization. From the study it was concluded that under natural light conditions similar quality plantlets to growth chamber conditions can be raised and the protocol may reduce costs by upto 22%.

Arias *et al.* (2014) replaced the conventional micropropagation conditions of maintaining the *in vitro* plants in a controlled room whose temperature varied between 25 and 30°C, its humidity between 60 to 70% and its light intensity of 20,238 to 20,409 for 19 hours; with a room whose roof was made of corrugated plastic sheets that allow partial passage of natural light in potato cultivar Diamant. Under the natural conditions the temperature of this room fluctuated between 14 and 40°C the light intensities were between 20,017 to 20,687 Lux and the humidity between 40 to 90%. From this research no difference were found between yield production of micropropagated plants grown under control and non-controlled conditions. Thus by using this technique we can reduce the total cost of electricity and obtain good quality and quantity of plantlets.

Rehana *et al.* (2018) conducted a study where fluorescent lamps and sunlight were used as lighting source to identify feasibility of using sunlight in plant tissue culture of potato cultivar Cardinal. After 30 days of culture it was observed that all growth factors performed better result in sunlight treatment than those in artificial one. The average height of plantlet at sunlight was 53.33 ± 3.32 mm whereas in artificial light it was 51.67 ± 2.15 mm. The sun-lighted plants were also healthier, vigorous and strong which helped plants to establish in net house faster. By using sunlight, the cost of electricity can be saved and up-to 63% Taka was saved each month by using only sunlight.

Jafar *et al*. (2018) conducted an experiment with nine replications under *in vitro* conditions and four under greenhouse conditions for two varieties (Agria and Savalan). The results indicate that in variety Savalan the plant height is almost similar in both light conditions while a higher plant height was found in florescent light in Agria. Root length, stem diameter, leaf area, number of nodes per plantlet, number of branches per plantlet was higher in natural light in both cultivars. An increase of 23% and 6 % in leaf area, 2.2% and 22.5% in node per plantlet and 18.9% and 17.2 % in fresh weight was found in natural light as compared to florescent light in “Agria and Savalan” respectively. Also higher number of microtubers were produced per plantlet under natural light conditions.

Chapter-III

MATERIALS AND METHODS

CHAPTER-III

MATERIALS AND METHODS

The present investigation entitled **"Studies on Alternative Sources in *In-vitro* Culture of Potato (*Solanum tuberosum* L.)"** was carried out at Plant Tissue Culture Laboratory of the Division of Vegetable Science and Floriculture, SKUAST- Jammu during the year 2019-2020.

3.1 Materials

3.1.1 Planting material

The genotype used in present investigation was 'Kufri Girdhari', a late blight resistant variety, developed at Central Potato Research Institute (CPRI), Shimla. The tubers were obtained from Central Potato Research Institute (CPRI) for experimental purposes.

Explants: Two explants *i.e.* newly developing sprouts and nodal segments were used as explants for the present study.

3.1.2 Chemicals

All the chemicals used in the present study were of analytical grade and were procured from Himedia Labs. India Limited.

3.1.3 Glassware

Glassware used during the course of investigation for culturing included test tubes (25 x 150 mm size) and conical flasks (150 ml) of Borosil (Borosil India Limited, Bombay). Reagent bottles (125 ml) were used to store the stock solution. The pipettes, measuring cylinders, volumetric flasks, beakers etc. were also used in the medium preparation. Autoclaved petri plates, wrapped in aluminium foil were used while sub-culturing.

3.2 Methodology

3.2.1 Preparation of glassware

Glassware was soaked in diluted sulfuric acid for 24 hours followed by prolonged dipping in tap water. Glassware was then washed with detergent Cedepol, which was later removed by thorough washing with tap water. Finally, the glassware was rinsed with distilled water and dried in an oven at 140-160^{o} C for 2-3 hours for subsequent use.

3.2.2 Sterilization of equipments

The scalpels, forceps, petri-plates, etc. were first wiped with 70 percent ethanol and then were wrapped in aluminium foil and autoclaved at 121^{o}C for 15-20 minutes. Laminar airflow chamber was always switched on before use and was surface sterilized by UV light for 15- 30 minutes. The floor and sides of the laminar air flow hood were thoroughly wiped with cotton wool dipped in spirit. The entire operation of inoculation and sub-culturing was carried inside the laminar air flow cabinet under gas flame. All the apparatus *i.e.* forceps, scalpels, needles; scissors were wiped with spirit and were kept dipped in spirit inside the laminar airflow and frequently sterilized over the flame before each operation.

3.2.3 Maintaining aseptic conditions:

To maintain aseptic conditions, all inoculations were carried out under the laminar air flow hood (Saveer, India Limited). The laminar hood was cleaned with 70 percent ethanol spray and hands were washed with antimicrobial hand wash (Hicare®, ACI Limited). The UV light of laminar hood was on for 15 minutes to 30 minutes for sterilization. The instruments (forceps, scalpel, Petri-dish etc.) were sterilized by using a gas flame to prevent air borne bacteria and immersed into absolute alcohol during the inoculation. The flask and Petri-dish covers were flamed twice, once after opening and again before closing them. All contaminants and old bacterial cultures were discarded after autoclaving to maintain bio-safety procedure.

3.2.4 Media preparation

3.2.4.1 Preparation of media- The basal medium as given by Murashige and Skoog (1962) with different modifications depending upon the experiment was used. The medium was prepared by mixing the stock solutions as given in Table 1. Plant bio-regulators (BAP, kinetin and IBA) were added, depending upon the experiment. The various combinations of solidifying agents' *viz.* Agar and Isabgol and carbon sources *viz.* sucrose and common table sugar were added depending on experiments. The pH of the medium was adjusted to 5.7±1 prior to adding gelling agents and carbon sources, with the help of Systronic digital pH meter (Saveer India Limited), using 0.1 N HCl or 0.1 N NaOH. Agar was added while boiling the medium over microwave oven or hot plate whereas Isabgol was dissolved at room temperature. Approximately, 10-15 ml and 25-30 ml of boiled medium was poured into the culture tubes and culture flasks, respectively, which were then plugged with cotton plugs made from non-absorbent cotton wrapped in muslin cloth.

Table 1: Composition of Murashige and Skoog Medium (1962)

Stock solution	Chemicals	In medium (mg/l)	In stock solution (g/l)
A	NH_4NO_3	1650 mg	165 g
	KNO_3	1900 mg	190 g
B	$MgSO_4 \cdot 7H_2O$	370 mg	37 g
	$MnSO_4 \cdot 4H_2O$	16.9 mg	1.69 g
	$ZnSO_4 \cdot 7H_2O$	8.6 mg	0.86 g
	$CuSO_4 \cdot 5H_2O$	0.025 mg	0.002 g
C	$CaCl_2 \cdot 2H_2O$	440 mg	44 g
	KI	0.83 mg	0.083 g
	$CoCl_2 \cdot 6H_2O$	0.025 mg	0.002 g
D	KH_2PO_4	170 mg	17 g
	H_3BO_3	6.2 mg	0.62 g
	$Na_2MoO_4 \cdot 2H_2O$	0.25 mg	0.025 g
E	Na_2EDTA	37.3 mg	3.73 g
	$FeSO_4 \cdot 7H_2O$	27.8 mg	2.78 g
F	Glycine (Amino acid)	2.0 mg	0.20 g
	Pyridoxine HCl (Vit. B6)	0.5 mg	0.05g
	Thiamine HCl (Vit. B1)	0.1 mg	0.01 g
	Nicotinic acid	0.5 mg	0.05 g

These are 100x, the final solution and 10 ml of each stock solution was used for preparing one litre of the culture medium.

3.2.4.2 Stock solutions of growth regulators

Hormone stocks were prepared by adding hormones in 1:1 concentration in double distilled water *i.e.* 50 mg of hormone in 50 ml of double distilled water. As the hormones are insoluble in water they were dissolved in suitable solvents (BAP, IBA in 1N NaOH and kinetin in 1N HCl) before preparation of stock solution.

3.2.4.3 Media autoclaving

Fixed volume of hot media was dispensed into culture vessels (conical flasks and test tubes). The culture vessels were plugged with non-absorbent cotton plugs and covered with aluminium foil and marked with the help of glass marker to indicate specific hormonal supplements. The culture vessels were then autoclaved at 15 lbs/sq. inch at 121ºC or 1.05 kg/cm^2 pressure for 15-20 minutes (culture tubes) and 20-25 minutes (culture flasks). The culture tubes/ flasks were allowed to solidify at room temperature overnight before use.

3.2.4.4 Incubation

The cultures were incubated in the culture room at 25±2^{o}C and uniform light from 3000-3500 lux with cool florescent tubes over a photoperiod of 16 and 8 hours of light and dark period, respectively. However, for feasibility studies of natural light for *in vitro* culture, the cultures were kept under natural room temperature conditions over a photoperiod of 12-14 hours of light and 12-10 hours of dark period, respectively.

3.2.5 Micropropagation studies in potato

3.2.5.1 Surface sterilization of explants

The explants, *viz.* sprouts and nodal segments (excised from tubers grown in pots) were first washed with running water to remove the debris. Few drops of Tween-20 were added and washing under running water was continued for 15- 20 minutes, followed by soaking in 0.2 percent Bavistin for 25-30 minutes. For removing residues to avoid the scorching effect of fungicides, explants were then washed with double distilled water for 3-4 times. Explants were then washed with 70 percent ethanol for 30 seconds and then washed with sterile water. The explants were then brought to laminar air flow chamber and surface sterilized with 2 % Sodium hypochlorite for the interval of 5 minutes followed by washing with sterilized double distilled water for 5-6 times to remove the traces of Sodium hypochlorite.

3.2.5.2 Standardization of medium for shoot induction

Two explants *i.e.* sprouts and nodal segments were put on MS media with various concentrations of BAP and kinetin to study the shoot induction response and subsequent elongation. Different media used for shoot induction response contains MS medium supplemented with different concentrations of BAP and kinetin which were-

T_1	MS basal
T_2	MS + 0.25 mg/l BAP + 0.0 mg/l kinetin
T_3	MS + 0.50 mg/l BAP + 0.0 mg/l kinetin
T_4	MS + 0.75 mg/l BAP + 0.0 mg/l kinetin
T_5	MS + 1.0 mg/l BAP + 0.0 mg/l kinetin
T_6	MS + 2.0 mg/l BAP + 0.0 mg/l kinetin
T_7	MS + 0.0 mg/l BAP + 0.25 mg/l kinetin
T_8	MS + 0.0 mg/l BAP + 0.50 mg/l kinetin
T_9	MS + 0.0 mg/l BAP + 0.75 mg/l kinetin
T_{10}	MS + 0.0 mg/l BAP + 1.0 mg/l kinetin

Five explants were used in each treatment and each treatment was replicated thrice. After 4 weeks, data was recorded on percentage response (%) and days to shoot induction. The individual shoots were further used for shoot multiplication experiments.

3.2.5.3 Standardization of medium for shoot multiplication

The single shoots obtained from 2^{nd} sub-culturing were put on MS medium with different concentrations of BAP alone or in combination with kinetin. The various combinations and concentrations of BAP and kinetin tested for shoot multiplication which were-

T_1	MS basal
T_2	MS + 0.25 mg/l BAP + 0.0 mg/l kinetin
T_3	MS + 0.50 mg/l BAP + 0.0 mg/l kinetin
T_4	MS + 0.75 mg/l BAP + 0.0 mg/l kinetin
T_5	MS + 1.0 mg/l BAP + 0.0 mg/l kinetin
T_6	MS + 1.5 mg/l BAP + 0.0 mg/l kinetin
T_7	MS + 2.0 mg/l BAP + 0.0 mg/l kinetin
T_8	MS + 0.25 mg/l BAP + 0.25 mg/l kinetin
T_9	MS + 0.50 mg/l BAP + 0.25 mg/l kinetin
T_{10}	MS + 0.75 mg/l BAP + 0.25 mg/l kinetin
T_{11}	MS + 1.0 mg/l BAP + 0.25 mg/l kinetin
T_{12}	MS + 1.5 mg/l BAP + 0.25 mg/l kinetin
T_{13}	MS + 2.0 mg/l BAP + 0.25 mg/l kinetin

Five explants were used in each treatment and each treatment was replicated thrice. Data on days to multiple shoot formation, number of shoots per explant, shoot length (cm); number of nodes per plantlet and internodal length (cm) was recorded after 4 weeks of subculture before transferring to the rooting medium.

3.2.5.4 Standardization of medium for root induction:

To induce rooting response, MS basal medium alone or in combination with various concentrations of IBA were used. The various concentrations of IBA are given as under-

T_1	MS basal
T_2	0.25 mg/l IBA
T_3	0.5 mg/l IBA
T_4	1.0 mg/l IBA
T_5	1.5 mg/l IBA
T_6	2.0 mg/l IBA

Data on days to root induction, number of roots per shoot, root length (cm), number of leaves per plantlet and plant height (cm) was recorded after 2 weeks. After that, the plantlets were kept under controlled environment for acclimatization/hardening.

3.2.5.5 Acclimatization of *in vitro* plantlets

Hardening is required to achieve adaptation of the regenerated plantlets to the natural environment. The regenerated plantlets were carefully removed from the rooting media using forceps. The agar/isabgol attached to their root part was gently washed with running water to make sure that the entire gelling agent was removed completely to avoid any contamination. Further the roots were dipped for 2 to 3 minutes in Bavistin (1 mg/l) solution to reduce the soil borne diseases. The plants were then transferred in pots containing sterilized potting mixture soil, vermicompost and cocopeat (1:1:1). Perforated plastic bags were taken to cover the potted plantlets. Humidity was maintained inside the bag through regular spraying of water to protect the hardening plants from moisture stress and for the quick acclimatization. Plantlets were kept inside the acclimatization/hardening room for 15 days. During these 15 days, the moisture inside the bags was maintained constantly.

- **Procedure used for *in vitro* micropropagation of potato cultivar 'Kufri Girdhari'**

Potato explants (sprouts and nodal segments) were taken and cut into segments of 1-2 cm

↓

Washing in running tap water

↓

Washing with 2-3 drops of Tween 20 for 15-20 min

↓

Treatments of 0.2% Bavistin for 25-30 min

↓

Washing 3-4 times with double distilled water

↓

Washing with 70% ethanol for 30 seconds

↓

Sterilization of explants (LAF)

↓

Surface sterilization with 2 % Sodium Hypochlorite for 5 minutes

↓

Washing with sterilized double distilled water (4-6 times)

↓

Inoculation on establishment media

↓ (On 21st day)

Transfer to multiplication media

↓ (On 28th day)

Transfer to rooting media

↓

Hardening (Green House)

↓

Transfer to field

3.3 Effect of low cost alternative sources on *in vitro* culture

The *in vitro* raised plantlets were used for further experiments with alternative gelling agent combinations, common table sugar and natural light. Single shoots were used as explants to study the feasibility of using various low cost alternatives as per different experiments.

3.3.1 Effect of low cost gelling agents

Isabgol was used as a gelling agent along with Agar for this experiment as it shows good gelling capacity and stability. Agar used for experiment was of AR grade from Himedia Labs Limited and concentration of 8 g/l was used as standard. Isabgol used for experiment was procured from The Sidhpur Sat-Isabgol Factory Ltd. and already refined concentration of 30 g/l was used as standard. Isabgol was added to the MS medium when it was luke warm. Over heating rendered the medium rubbery which lead to difficulty in dispensing of the medium. For this experiment different combinations of Isabgol alone and with Agar were assessed for identifying the alternative to expensive gelling agent. The various combinations of gelling agents are given as under:

$\mathbf{T_1}$	100% Isabgol	(30 g/l)
$\mathbf{T_2}$	75% Isabgol + 25% Agar	(22.5 g/l + 2 g/l)
$\mathbf{T_3}$	50% Isabgol + 50% Agar	(15 g/l + 4 g/l)
$\mathbf{T_4}$	25% Isabgol + 75% Agar	(7.5 g/l + 6 g/l)
$\mathbf{T_5}$	100% Agar	(8 g/l)

The data was recorded on the basis of observations averaged from 10 test tubes/ flasks per treatment and each treatment was replicated three times in this experiment.

3.3.2 Effect of low cost carbon sources

Common table sugar was used for finding replacement for expensive AR grade sucrose. For this experiment AR grade sucrose (30 g/l) from Himedia Labs Limited was used. For finding replacement for AR grade sucrose, common table sugar (30 g/l) from Trust Classic Sugar, Simbhaoli Sugars Limited was used. The concentrations used for the experiments were-

T_1	Sucrose	(30 g/l)
T_2	Common table sugar	(30 g/l)

The data was recorded on the basis of observations averaged from 10 test tubes/ flasks per treatment and each treatment was replicated three times for this experiment.

3.3.3 Effect of alternative light sources

In place of artificial illumination through fluorescence tubes, the possibility of natural indirect light was explored as a cheap illumination source. The cultures were kept in two types of culture conditions with respect to source of light. In case of natural light conditions the cultures were kept near a big clear glass window providing natural indirect light. The average light intensity in natural indirect light ranged from 4200-4500 Lux and photoperiod time of approximately 11- 12 hours light and 12 hour dark. The average temperature ranged from 26.0 ^{o}C to 30.5 ^{o}C and relative humidity ranged from 40.5 % to 44.0 %. On the other hand, in artificial light conditions the cultures were kept under fluorescent light with average light intensity 3200-3500 Lux and photoperiod time of 16 hours light and 8 hour dark. The average temperature ranged from 23.15 ^{o}C to 26.5 ^{o}C and relative humidity ranged from 38.0 % to 40.5 %. The culture flasks and tubes were arranged at same distance in culture rack so as to provide uniform light to all the cultures. The data was recorded on the basis of observations averaged from 10 test tubes/ flasks per treatment and each treatment was replicated three times for this experiment.

3.3.4 Observations recorded:

Data was recorded on the basis of observations averaged from 10 flasks/ test tubes per treatment replicated three times.

3.3.4.1 Culture establishment (After 4 weeks of culture)

3.3.4.1.1 Percent response

$$\text{Percent response} = \frac{\text{Number of explants showing response}}{\text{Total number of explants cultured}} \times 100$$

3.3.4.1.2 Days to shoot induction

Days on which first appearance of shoot from the day of transfer of explants to shoot induction medium in each treatment was counted and expressed as days taken for shoot induction.

3.3.4.2 Shoot multiplication (After 4 weeks of culture)

3.3.4.2.1 Days to multiple shoot formation

Days on which first appearance of multiple shoot from the day of transfer of explant to shoot multiplication medium in each treatment was counted and expressed as days taken for shoot multiplication.

3.3.4.2.2 Number of shoots per explant

The number of shoots regenerated per explant after 2 inoculations or sub-culturing were counted and recorded.

3.3.4.2.3 Average shoot length (cm)

The representative plantlets were taken randomly from each replication under each treatment and the average shoot length was measured from its base to the tip and expressed in centimetre.

3.3.4.2.4 Number of nodes per plantlet

The representative plantlets were taken randomly from each replication under each treatment and the number of nodes per plantlet was noted down.

3.3.4.2.5 Internodal length (cm)

The representative plantlets were taken randomly from each replication under each treatment and the length between each internode was measured and expressed in centimetre.

3.3.4.3 Rooting response (After 2 weeks of culture)

3.3.4.3.1 Percent response

$$\text{Rooting response (\%)} = \frac{\text{Number of shoots rooted on root induction medium}}{\text{Total number of shoots placed on root induction medium}} \times 100$$

3.3.4.3.2 Number of roots per plantlet-

Random plantlets were taken out from the agar/ isabgol medium at 15 days after inoculation and the roots were washed in running water to remove the agar/ isabgol media. The average number of roots arising from the base of the shoots were counted and recorded.

3.3.4.3.3 Average root length (cm)

After separation of the roots from the shoots, its average length was measured and recorded in centimetre.

3.3.4.3.4 Number of leaves per plantlet

The total number of leaves per plantlet were counted and recorded.

3.3.4.3.5 Plant height (cm)

The plant height measured from the shoot tip till the root tip and expressed in centimetres.

3.4 Data and Statistical Analysis

The data related to various characters were recorded in replicated form using complete randomized design (CRD). Data were analyzed statistically with one factor analysis using OPSTAT software. To judge the significant difference between means of two treatments, the critical difference (C.D.) was used. The recorded data were subjected to statistical analysis to estimate the parameters as per procedure given by Rangaswamy (1995).

Coefficient of variation: Coefficient of variation for these morphometric characteristics was calculated as described by Panse and Sukhatme (1989), using formula-

$$\text{C.V. (\%)} = \frac{\sigma}{\overline{X}} \times 100$$

Where,

σ = Standard deviation of various observations and

$\overline{X}$ = Mean of the observations

3.4.1 Analysis of variance

The analysis of variance for the experimental design Completely Randomized design (CRD) under laboratory conditions in the culture vessels is based on the model given:-

$$Y_{ij} = u + t_i + e_{ij}$$

$$(i = 1, 2, 3 \ldots\ldots t)$$

$$(j = 1, 2, 3 \ldots\ldots r)$$

Where,

Y_{ij} = The observations on j^{th} plot of i^{th} treatment

U = General mean effect

t_i = Effect due to i^{th} treatment

e_{ij} = Residual effect

ANOVA Table

Source of Variation	**df**	**SS**	**MSS**	**F (cal)**	**F (tab) @ 5%**
Treatment	(t-1)	SST	MST	MST/MSE	----
Error	t (r-1)	ESE	MSE		
Total	r (t-1)				

The test of significance for important traits was carried out as standard error of mean as:-

$$SE_{(m)} = \frac{\pm\sqrt{2MSE}}{r}$$

Where, MSE = Error mean sum of square or error variance

r = No. of replications

The critical difference (C.D.) was calculated for treatment comparison @ 5% level significance as:-

$$C.D. = \frac{\pm\sqrt{2MSE}}{r} \times \text{t at error degree of freedom}$$

Chapter-IV

RESULTS

CHAPTER-IV

EXPERIMENTAL RESULTS

The present investigation entitled **"Studies on Alternative Sources in *In Vitro* Culture of Potato (*Solanum tuberosum* L.)"** was carried out at the Plant Tissue Culture laboratory of the Division of Vegetable Science & Floriculture, SKUAST- Jammu during the year 2019-20. The results of various experiments conducted under the present investigation are given as under:

4.1 Standardization of Micropropagation Protocol

Experiments on standardization of micropropagation protocol of potato cultivar 'Kufri Girdhari' were carried out using MS medium supplemented with different concentrations of BAP alone and in combination with kinetin. Two explants *viz*. sprouts and nodal segments were used for establishing cultures as seen in Plate 1 (A) and (B). The detailed results are given as under:

4.1.1 Effect of different concentrations of BAP and kinetin on shoot induction from sprout explants

For shoot induction using sprouts as explant, MS medium supplemented with different concentrations of BAP (0.25, 0.50, 0.75, 1.0 and 2.0 mg/l) and kinetin (0.25, 0.50, 0.75 and 1.0 mg/l) was used. Amongst all the treatments, the significantly higher percentage response was recorded in T_4 - MS medium + 0.75 mg/l BAP (87.08) and was followed by T_6 - MS medium + 2.0 mg/l BAP (85.53) and T_5 - MS medium + 1.0 mg/l BAP (83.83) as mentioned in Table 2 and Figure 1. However, lowest percentage response (60.44) was recorded in T_1 - MS basal medium.

Significantly minimum number of days to shoot induction was observed in T_4 - MS medium + 0.75 mg/l BAP (9.58) and was followed by T_5 - MS medium + 1.0 mg/l BAP (11.33) and T_3 - MS medium + 0.50 mg/l BAP (12.48) whereas maximum number of days to shoot induction (15.03) was recorded in T_1 - MS basal medium as mentioned in Table 2 and Figure 2.

Among all the treatments, best results were obtained in treatment T_4 - MS medium + 0.75 mg/l BAP with good quality compact, dark green shoot primordia and fibrous hairy roots besides superior percentage response and early shoot induction as seen in Plate 2 (A) and (C).

4.1.2 Effect of different concentrations of BAP and kinetin on shoot induction from nodal segment explants

For shoot induction using nodal segments as explant, different concentrations of BAP (0.25, 0.50, 0.75, 1.0 and 2.0 mg/l) and kinetin (0.25, 0.50, 0.75 and 1.0 mg/l) were used. Amongst all the treatments, the significantly higher percentage response for shoot induction (85.76) was recorded in T_4 - MS medium + 0.75 mg/l BAP which was followed by T_5 - MS medium + 1.0 mg/l BAP (82.10) and T_6 - MS medium + 2.0 mg/l BAP (79.66) as given in Table 3 and Figure 1. The lowest percentage response for shoot induction (55.43) was recorded in T_1 - MS basal medium.

As far as earliness of response is concerned, best response was observed in T_4 - MS medium + 0.75 mg/l BAP in terms of minimum days to shoot induction (7.43) which was followed by T_5 - MS medium + 1.0 mg/l BAP (7.88) and T_6 - MS medium + 2.0 mg/l BAP (8.02) whereas T_1 - MS Basal medium recorded maximum number of days to shoot induction (12.13) as given in Table 3 and Figure 2.

Amongst all the treatments, superior response was obtained in T_4 – MS medium + 0.75 mg/l BAP with good quality, sturdy and dark green single shoot with three leaves besides superior percentage response and early shoot induction as seen in Plate 2 (B) and (D).

4.1.3 Effect of different concentrations of BAP and Kinetin on shoot multiplication

Effect of different concentrations of BAP (0.25, 0.50, 0.75, 1.0, 1.5 and 2.0 mg/l) in combination with kinetin (0.25 mg/l) on shoot multiplication was recorded after four weeks from culture of *in vitro* single shoots and the data has been given in Table 4 and Figure 3.

From the data, it was revealed that amongst all the treatments, superior response in terms of number of shoots per explant (15.50) was observed in T_{10} - MS medium + 0.75 mg/l BAP + 0.25 mg/l kinetin followed by T_{11} - MS medium + 1.0

Table 2: Effect of different concentrations of BAP and kinetin on shoot induction from sprouts

Treatment	Hormonal concentrations (mg/l) (BAP + kinetin)	Days to shoot induction	Percentage response (%)	Morphogenetic response
T_1	MS Basal	15.03	60.44	Light green shoot primordia, No root formation
T_2	0.25 + 00	13.31	64.96	Light green shoot primordia, no root formation
T_3	0.50 + 00	12.48	73.97	Light green shoot primordia, hairy fibrous root growth
T_4	0.75 + 00	9.58	87.08	Dark Green shoot primordia, hairy fibrous root growth
T_5	1.0 + 00	11.33	83.83	Dark Green shoot primordia, hairy fibrous roots
T_6	2.0 + 00	11.41	85.53	Light Green shoot primordia, hairy fibrous root growth
T_7	0.0 + 0.25	12.80	68.83	Light green shoot primordia, no root formation
T_8	0.0 + 0.50	13.54	71.43	Light green shoot primordia, no root formation
T_9	0.0 + 0.75	14.25	72.57	Dark green shoot primordia, hairy fibrous root growth
T_{10}	0.0 + 1.0	13.77	72.40	Dark green shoot primordia, hairy fibrous root growth
SE(d)±		0.46	0.73	
C.D. (5%)		0.97	1.54	
C.V. (%)		4.45	1.21	

Table 3: Effect of different concentrations of BAP and kinetin on shoot induction from nodal segments

Treatment	Hormonal concentrations (mg/l) (BAP + kinetin)	Days to shoot induction	Percentage response (%)	Morphogenetic response
T_1	MS Basal	12.13	55.43	Green Small single shoot growth with two leaves
T_2	0.25 + 00	10.32	68.63	Green Small single shoot growth with two leaves
T_3	0.50 + 00	8.80	77.10	Dark green Single shoot with three leaves and two fine roots near base
T_4	0.75 + 00	7.43	85.76	Dark green Single shoot with three leaves and four fine roots near base
T_5	1.0 + 00	7.88	82.10	Dark green Single shoot with three leaves and four fine roots near base
T_6	2.0 + 00	8.02	79.66	Green Single shoot with three leaves and two fine roots near base
T_7	0.0 + 0.25	10.42	79.00	Dark green single shoot with three leaves
T_8	0.0 + 0.50	10.10	72.30	Green Single shoot with two leaves and two fine roots near base
T_9	0.0 + 0.75	9.58	68.76	Dark green single shoot with three leaves
T_{10}	0.0 + 1.0	9.31	67.06	Dark green single shoot with two leaves
SE(d) ± **C.D. (5%)** **C.V. (%)**		0.24 0.50 3.12	0.95 1.98 1.56	

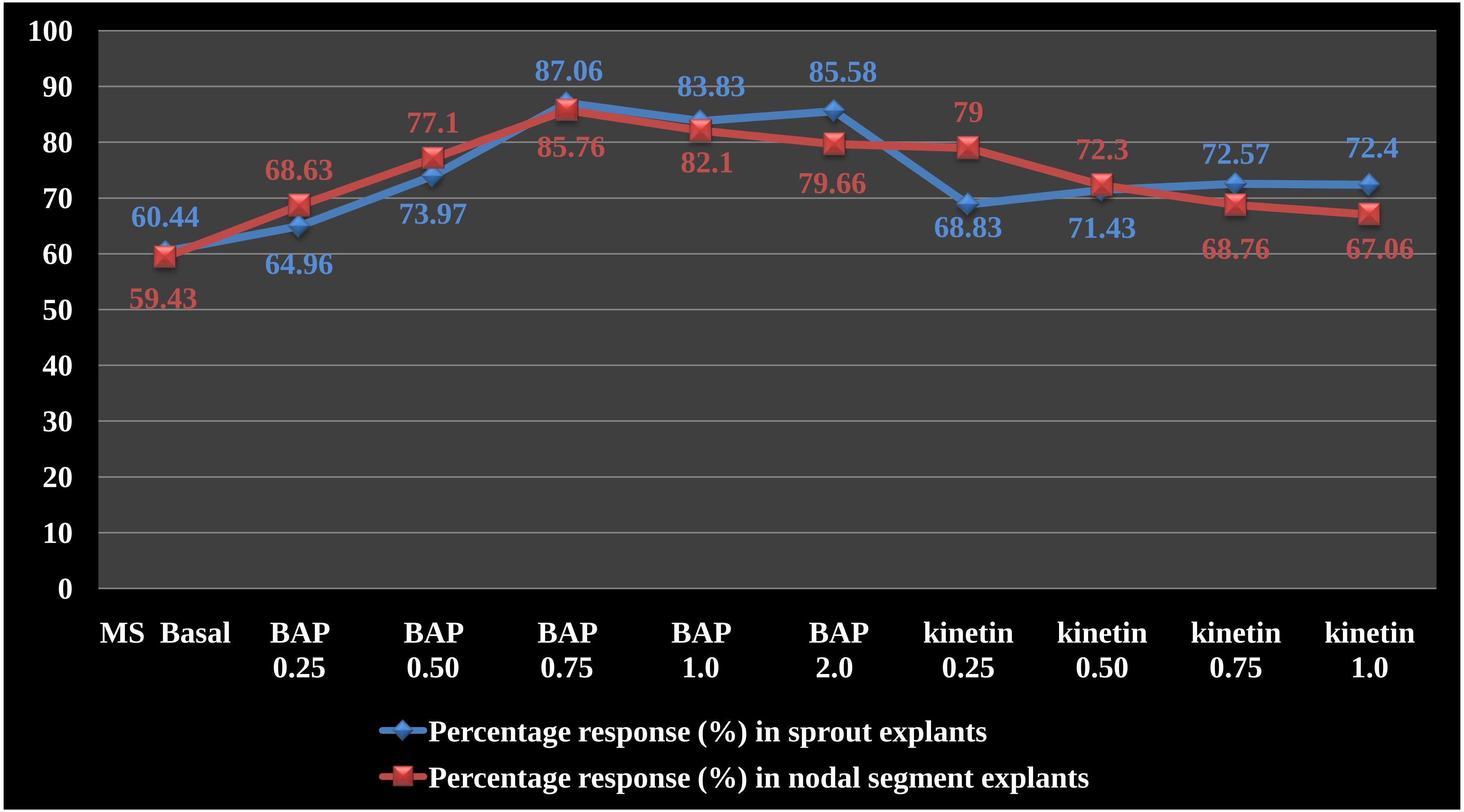


Figure1: Effect of different concentrations of BAP and kinetin on percentage response in sprout and nodal segment explants

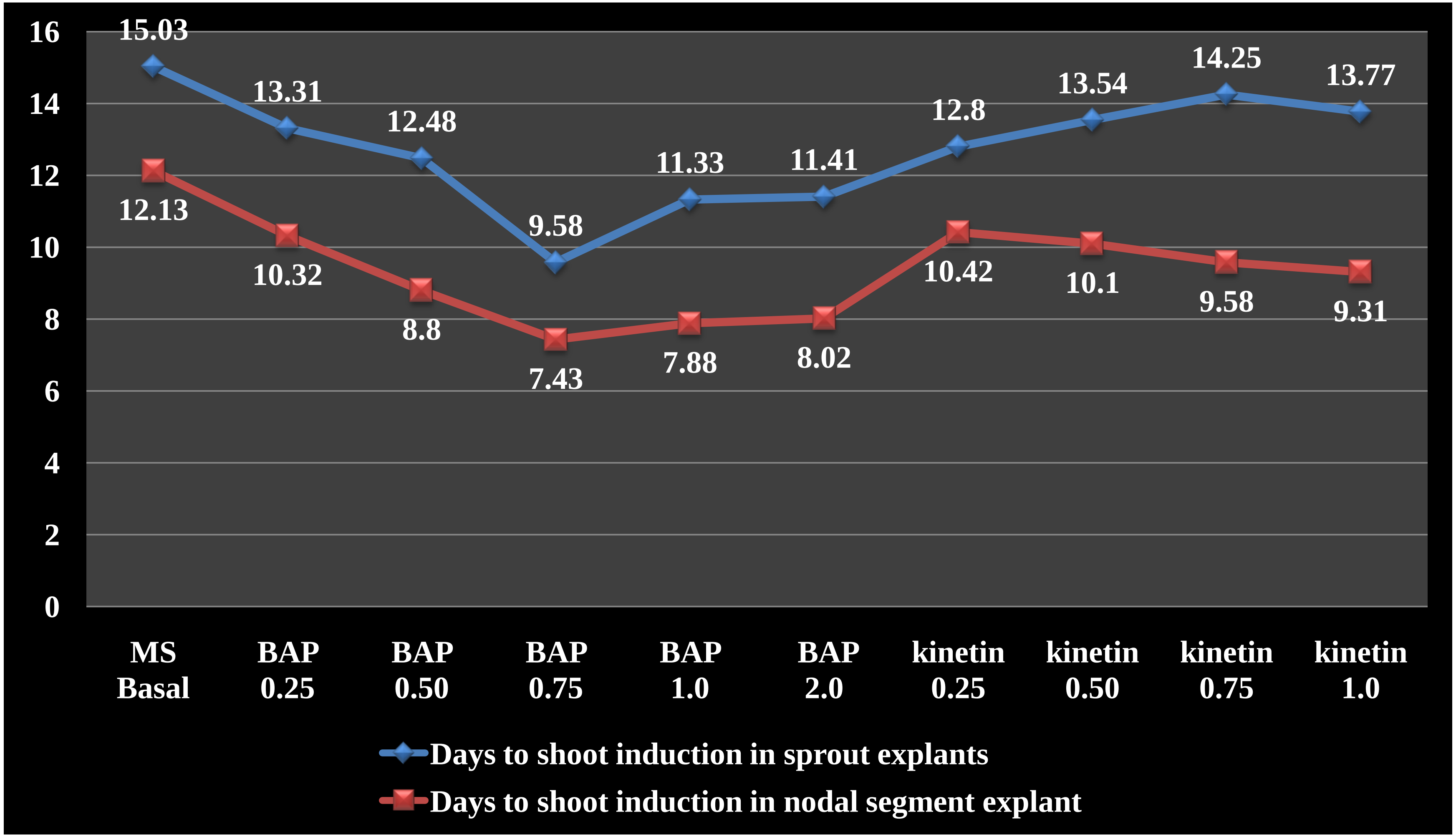


Figure 2: Effect of different concentrations of BAP and kinetin on days to shoot induction in sprout and nodal segment explants

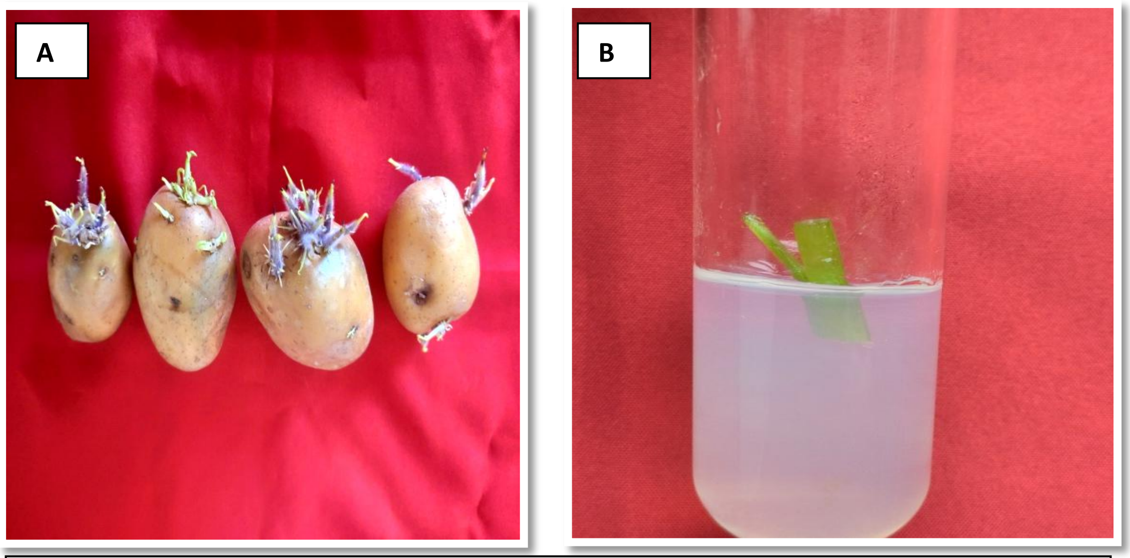


Plate 1: (A) Sprouts and (B) nodal segment as explant for *in vitro* micropropagation

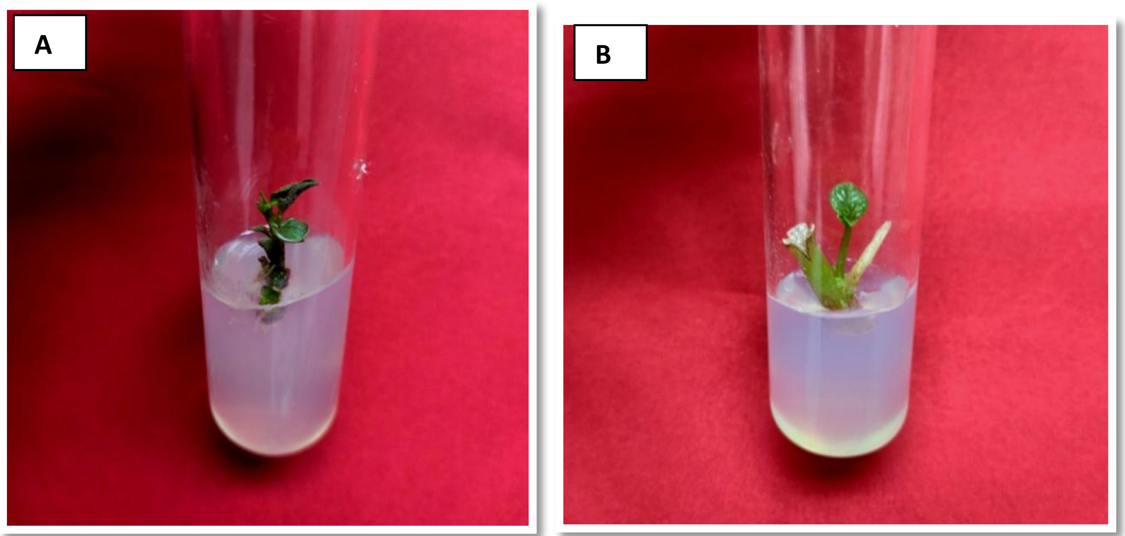


Plate 2 (A) (B): Single shoot induction at BAP 0.75 mg/l on (A) sprout explant (B) nodal segment explant

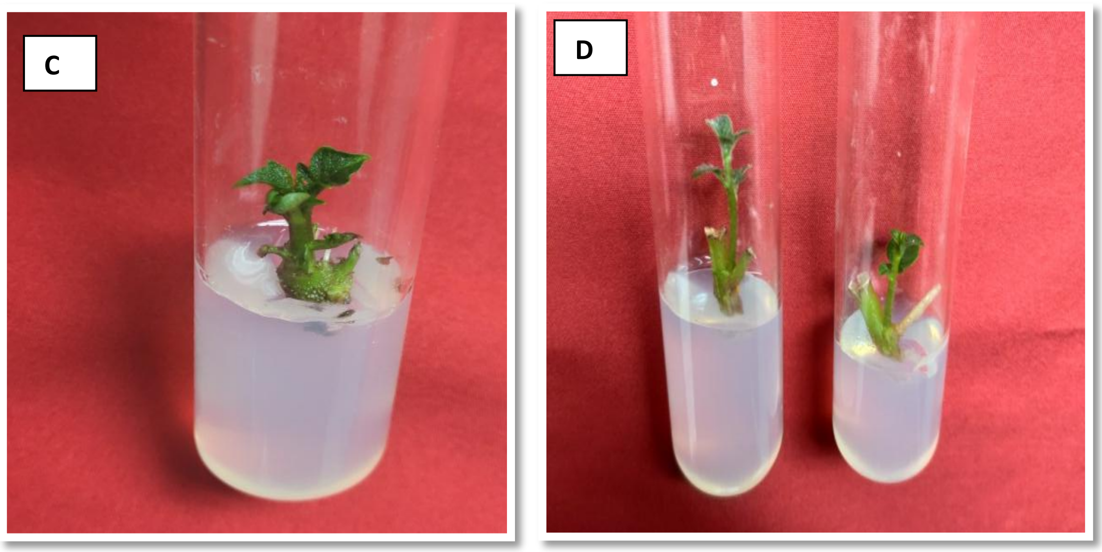


Plate 2 (C) (D): Shoot growth at BAP 0.75 mg/l on (C) sprout explant (D) nodal segments

Table 4: Effect of different concentrations of BAP and kinetin on shoot multiplication

Treatment	Hormonal concentrations (mg/l) (BAP + kinetin)	Number of shoots per explant	Average shoot length (cm)	Number of nodes per plantlet	Internodal length (cm)
T_1	MS Basal	4.50	4.11	4.33	0.97
T_2	0.25 + 00	6.40	4.20	4.18	0.98
T_3	0.5 + 00	5.87	5.21	5.39	0.98
T_4	0.75 + 00	5.67	4.20	4.50	1.04
T_5	1.0 + 00	5.83	4.13	4.23	0.96
T_6	1.5 + 00	7.43	5.53	5.33	0.96
T_7	2.0 + 00	11.83	7.33	8.54	0.84
T_8	0.25 + 0.25	6.50	7.32	7.83	1.11
T_9	0.5 + 0.25	8.96	7.90	8.04	1.02
T_{10}	0.75 + 0.25	15.50	8.13	8.37	1.12
T_{11}	1.0 + 0.25	14.66	7.83	7.80	1.07
T_{12}	1.5 + 0.25	10.70	7.37	7.77	0.92
T_{13}	2.0 + 0.25	5.42	7.07	7.88	0.95
SE(d) ±		0.51	0.34	0.35	0.07
C.D. (5%)		1.06	0.70	0.72	0.15
C.V. (%)		4.48	5.68	5.27	6.02

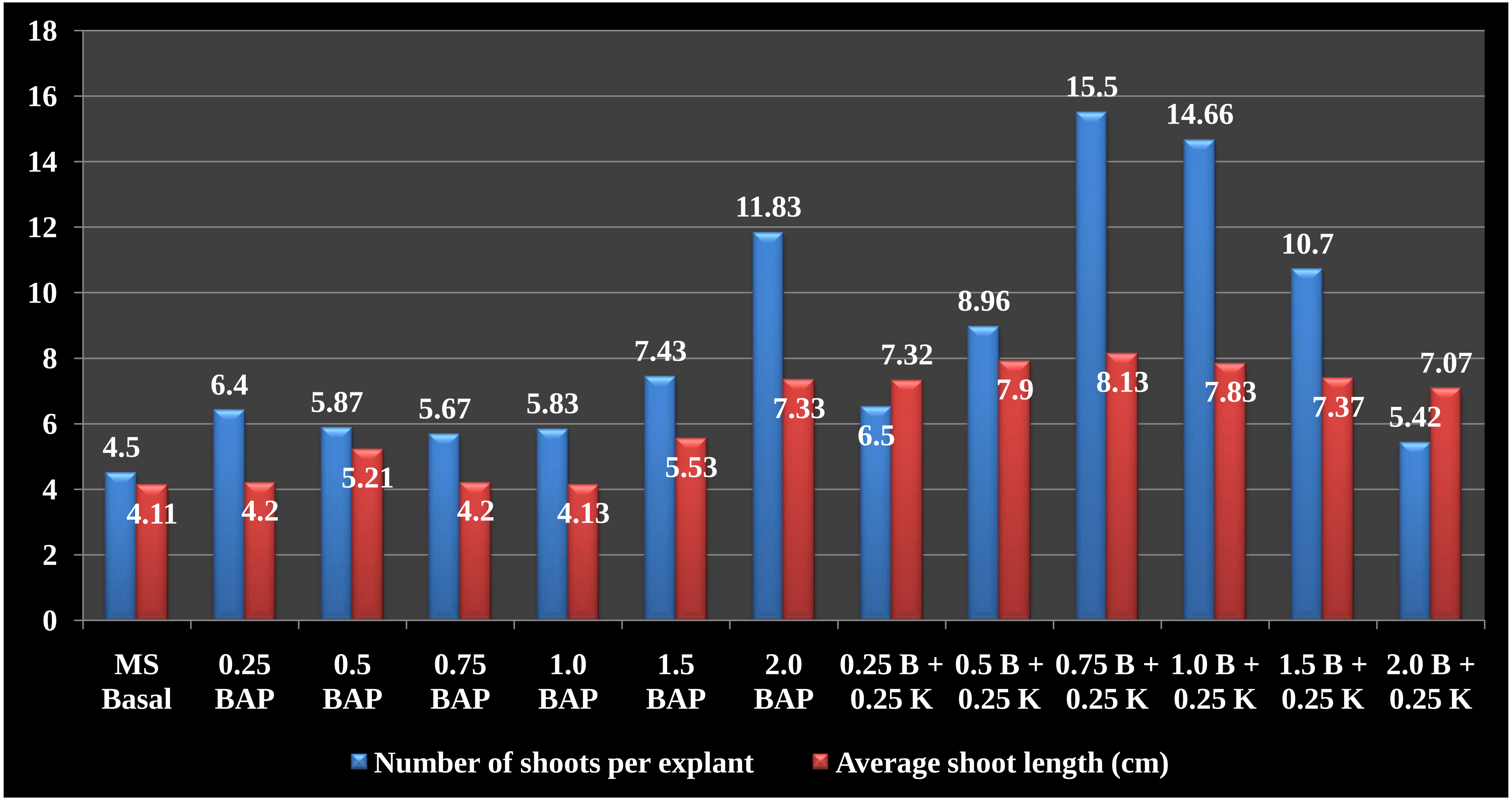
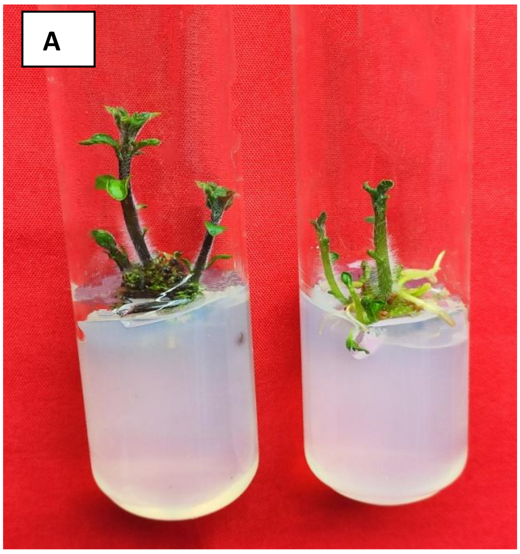


Figure 3: Effect of different concentrations of BAP and kinetin on shoot multiplication



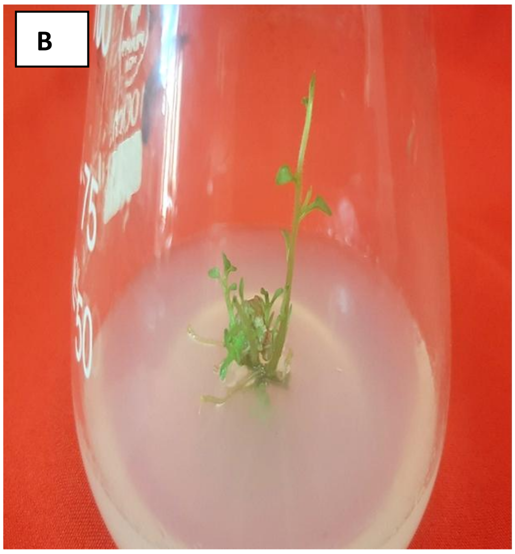
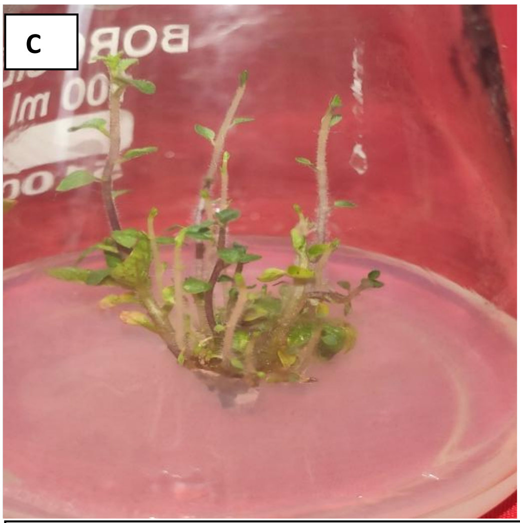


Plate 3 (A) (B): Multiple shoot formation from sprout explants at 0.75 mg/l BAP + 0.25 mg/l kinetin



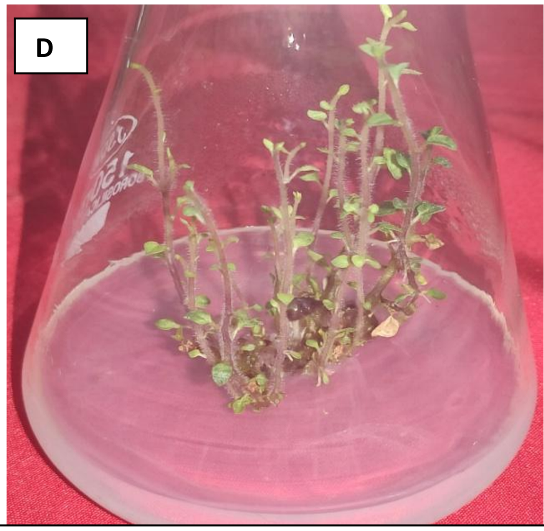


Plate 3 (C) (D): Multiple shoot formation from nodal segment explants at 0.75 mg/l BAP + 0.25 mg/l kinetin

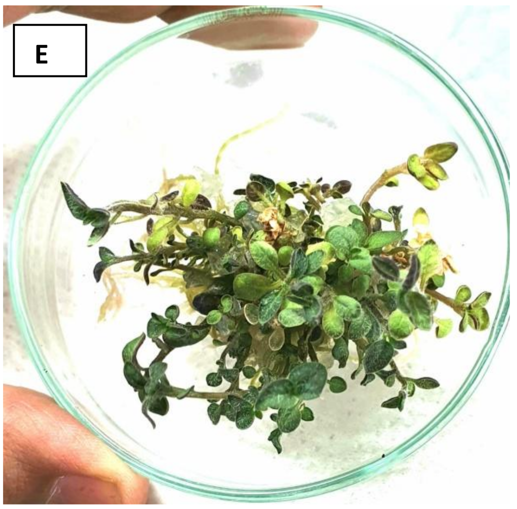


Plate 3 (E): Profuse shoot proliferation of potato

Plate 3 (F): Complete shoot formation for transfer into rooting media

Plate 3 (G): Multiple shoot formation in MS medium supplemented with BAP & kinetin, 1 = 0.5 BAP + 0.25 kinetin; 2 = 2.0 BAP; 3 = 1.0 BAP + 0.25 kinetin; 4 = 0.75 BAP + 0.25 kinetin; 5 = 0.25 BAP + 0.25 kinetin

Plate 3 (H): Cultures showing multiple shoot formation in best optimized media *i.e.* MS medium + 0.75 mg/l BAP + 0.25 mg/l kinetin

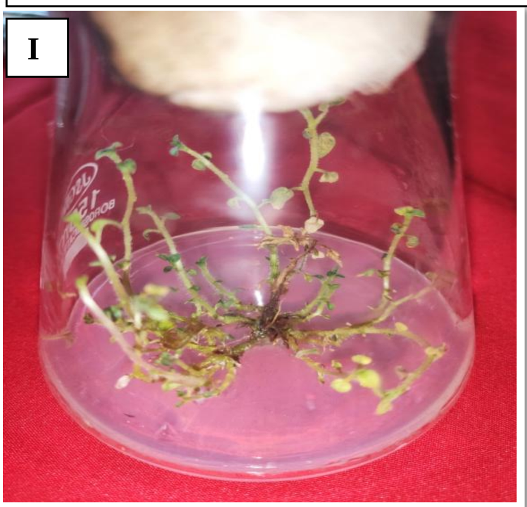


Plate 3 (I): Culture showing multiple shoot formation in MS medium + 1.0 mg/l BAP + 0.25 mg/l kinetin

mg/l BAP + 0.25 mg/l kinetin (14.66) and T_7 - MS medium + 2.0 mg/l BAP (11.83) whereas minimum number of shoots per explant (4.50) was observed in T_1 - MS basal medium.

The maximum shoot length (8.13 cm) was observed in T_{10} - MS medium + 0.75 mg/l BAP + 0.25 mg/l kinetin followed by T_9 - MS medium + 0.50 mg/l BAP + 0.25 mg/l kinetin (7.90 cm) and T_{11} - MS medium + 1.0 mg/l BAP + 0.25 mg/l kinetin (7.83 cm). The minimum shoot length (4.11 cm) was recorded in T_1 - MS Basal medium.

The results further revealed that the maximum number of nodes per plantlet (8.54) was observed in T_7 - MS medium + 2.0 mg/l BAP followed by T_{10} - MS medium + 0.75 mg/l BAP + 0.25 mg/l kinetin (8.37) and T_9 - MS medium + 0.50 mg/l BAP + 0.25 mg/l kinetin (8.04). The minimum number of nodes per plantlet (4.18) was observed in T_2 - MS medium + 0.25 mg/l BAP.

The longest internodal length (1.12 cm) was observed in T_{10} - MS medium + 0.75 mg/l BAP + 0.25 mg/l kinetin followed by T_8 - MS medium + 0.25 mg/l BAP + 0.25 mg/l kinetin (1.11 cm) and T_{11} - MS medium + 1.0 mg/l BAP + 0.25 mg/l kinetin (1.07 cm) whereas, shortest internodal length (0.84 cm) was observed in T_7 - MS medium + 2.0 mg/l BAP.

Amongst all the treatments, best response was obtained in T_{10} - MS medium + 0.75 mg/l BAP + 0.25 mg/l kinetin with sturdy and dark green multiple shoots besides better shoot length and number of internodes as visible in Plate- 3 (A) (B) (C) and (D). During shoot multiplication stage a few microtubers have also been formed especially at higher concentrations of BAP (2.0 mg/l) alone or in combination with kinetin (0.25 mg/l) as visible in Plate 5 (A) (B) and (C).

4.1.4 Effect of different concentrations of IBA for root induction

For induction of rooting, healthy *in vitro* multiplied shoots (3-4 cm) obtained after shoot multiplication, were cultured on MS medium supplemented with different concentrations of IBA (0.0, 0.25, 0.5, 1.0, 1.5 and 2.0 mg/l). From the Table 5 and Figure 4, it can be observed that the overall percentage rooting response in all treatments ranged between 83.13 to 97.50 per cent.

Significantly superior percentage rooting response (97.50) was observed in T_4 - MS medium + 1.0 mg/l IBA and was followed by T_3 - MS + 0.5 mg/l IBA (95.13) and T_2 - MS medium+ 0.25 mg/l IBA (88.65) whereas treatment T_6 – MS + 2.0 mg/l IBA recorded minimum percentage rooting response (83.13).

The maximum number of roots per shoot (8.50) was recorded in T_4 - MS medium + 1.0 mg/l IBA followed by T_3 - MS medium + 0.50 mg/l IBA (8.03) and T_2 - MS medium + IBA 0.25 mg/l (7.83), whereas T_6 - MS + 2.0 mg/l IBA recorded minimum of roots per shoot (5.83) as compared to all other treatments.

As far as root length is concerned, the significantly longer root length (6.97 cm) was observed in T_4 - MS medium + 1.0 mg/l IBA and was followed by T_3 - MS medium + 0.50 mg/l IBA (6.18 cm) and T_1 - MS basal medium (6.12 cm) whereas T_6 – MS medium + 0.50 mg/l IBA recorded shortest average root length (5.06 cm).

The significantly higher number of leaves per plantlet was recorded in T_4 - MS medium + 1.0 mg/l IBA (15.79) and was followed by T_3 - MS medium + 0.50 mg/l IBA (13.50) and T_2 - MS medium + 0.25 mg/l IBA (10.50). Treatment T_1 - MS basal media recorded minimum number of leaves per plantlet (6.13) as compared to all other treatments.

Data on increase in plant height was also recorded along with the rooting parameters which revealed maximum plant height in T_4 - MS medium + 1.0 mg/l IBA (14.83 cm) which was significantly superior to the other treatments and was followed by T_3 - MS medium + 0.50 mg/l IBA (13.03 cm) and T_2 - MS medium + 0.25 mg/l IBA (11.47 cm). However, T_6 - MS medium + 2.0 mg/l IBA recorded minimum plant height (8.53 cm).

Amongst all the treatments, best results were obtained in T_4 - MS medium + 1.0 mg/l IBA with long, thick and creamish white roots besides superior percentage rooting response and number of roots per shoots as visible in Plate 4 (A) (B) and (C).

4.2 Effect of low cost gelling agents

In an endeavour to reduce the cost of medium, different combinations of cost effective alternate gelling agents *viz.,* isabgol and standard gelling agent *viz.,* agar were tried. MS medium supplemented with standardised hormonal concentrations were used along with five different combinations of solidifying agents viz. 100 %

Table 5: Effect of different concentrations of IBA on rooting

Treatments	Hormonal concentrations IBA (mg/l)	Percentage response (%)	Number of roots per shoot	Average root length (cm)	Number of leaves per plantlet	Plant height (cm)
T_1	MS Basal	84.57	6.82	6.12	6.13	8.89
T_2	0.25	88.65	7.83	6.10	10.50	11.47
T_3	0.50	95.13	8.03	6.18	13.50	13.03
T_4	1.00	97.50	8.50	6.97	15.79	14.83
T_5	1.50	87.10	7.50	5.59	7.10	10.42
T_6	2.00	83.13	5.83	5.06	6.76	8.53
SE(d) ±		0.72	0.47	0.31	0.44	0.42
C.D. (5%)		1.59	1.04	0.67	0.97	0.92
C.V. (%)		0.99	4.78	5.21	5.43	5.46

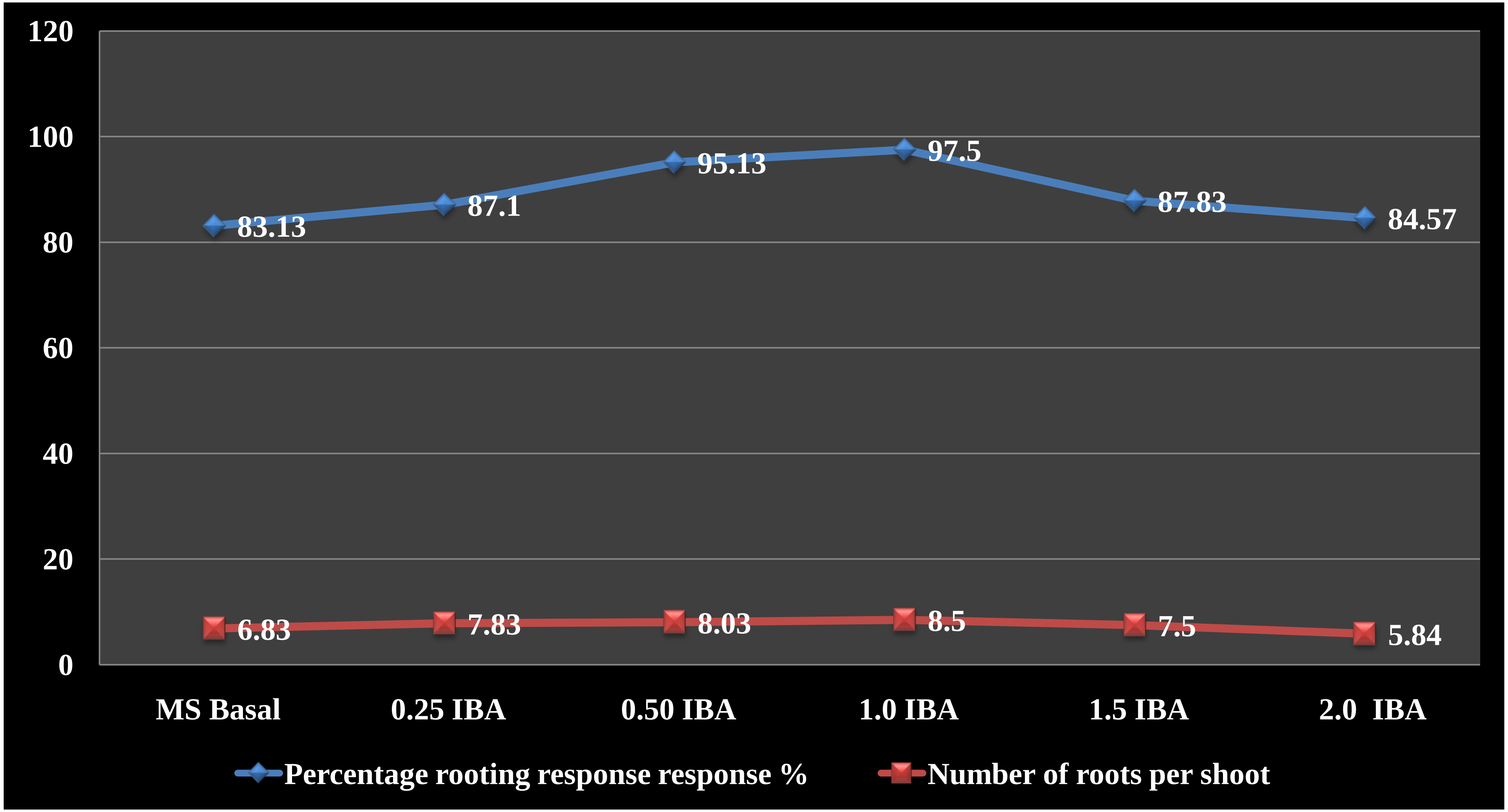
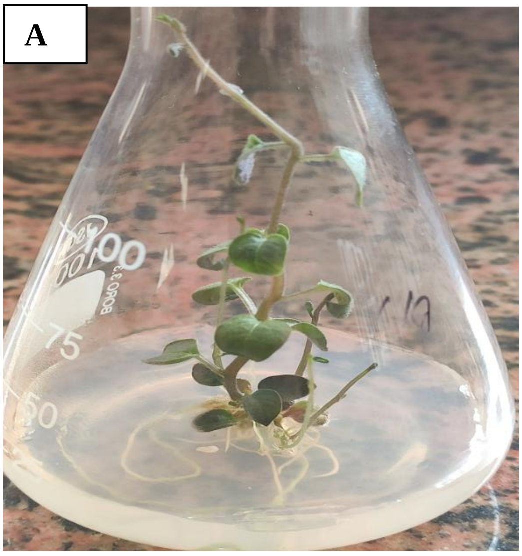


Figure 4: Effect of different concentrations of IBA on rooting



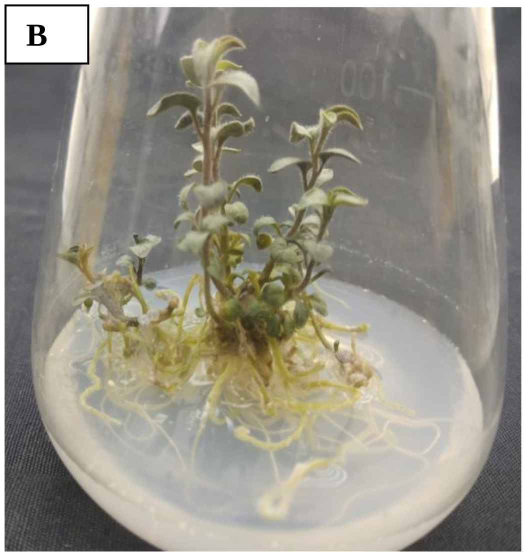


Plate 4 (A): Culture showing rooting in MS basal medium

Plate 4 (B): Culture showing rooting on 0.5 mg/l IBA

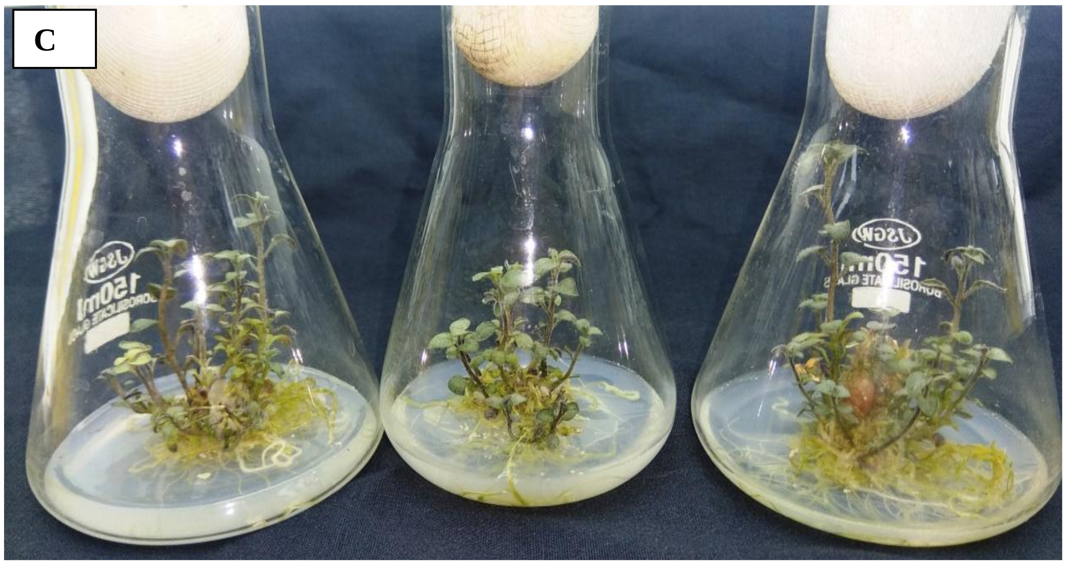


Plate 4 (C): Cultures showing rooting at best optimized media *i.e.* on 1.0 mg/l IBA

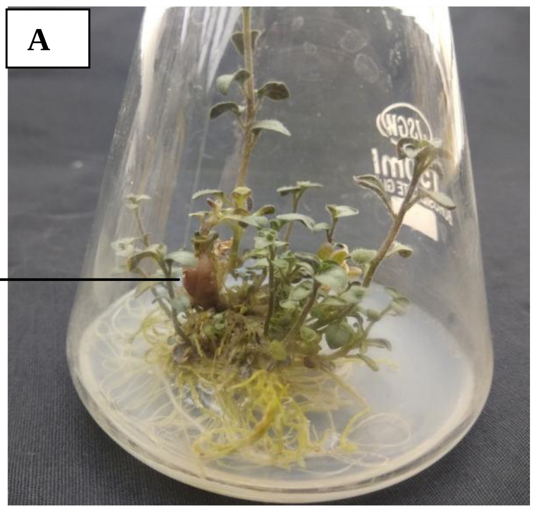


Plate 5 (A): Microtuberisation on MS medium supplemented with 2.0 mg/l BAP + 0.25 mg/l kinetin in sprout explant

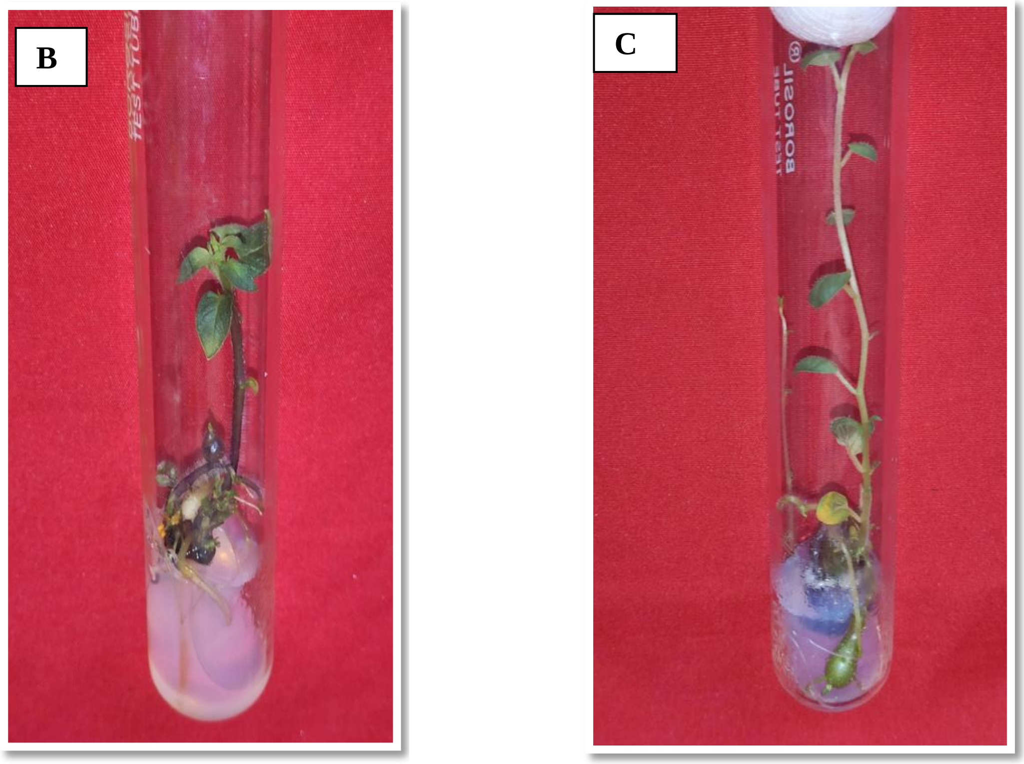


Plate 5 (B) (C): Development of microtubers at 2.0 mg/l BAP + 0.25 mg/l kinetin in nodal segment explants

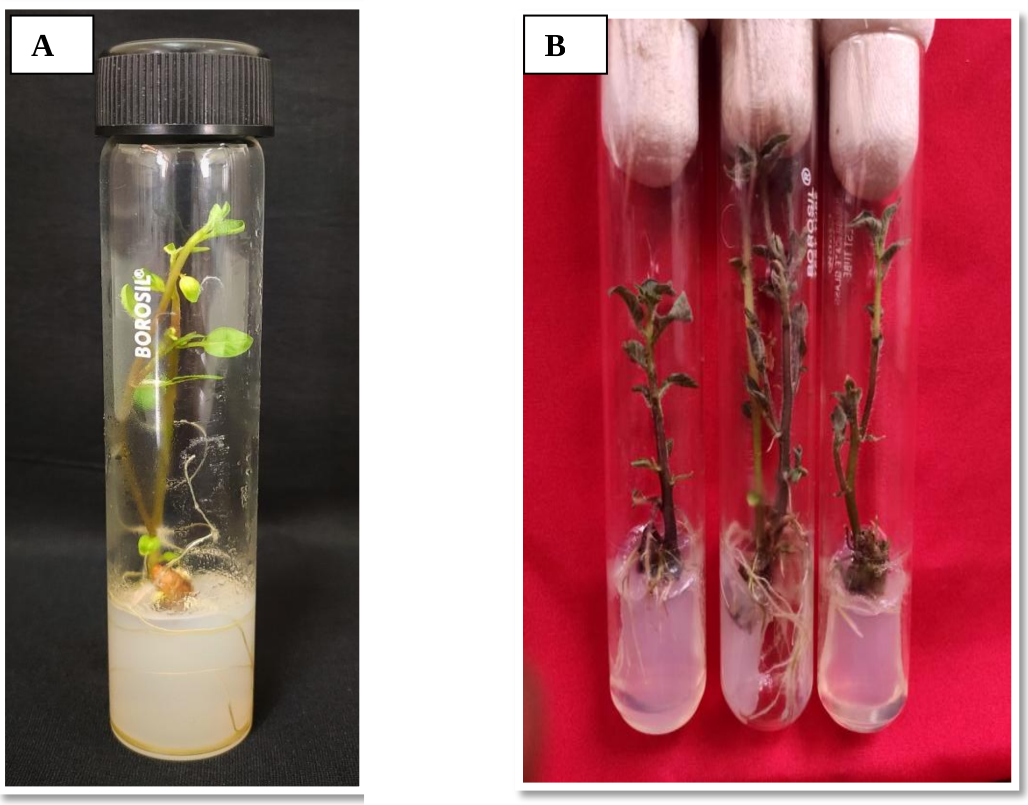


Plate 6 (A) (B): *In vitro* raised rooted plantlets

Isabgol, 75% Isabgol + 25 % Agar, 50 % Isabgol + 50 % Agar, 25% Isabgol + 75 % Agar and 100 % Agar for *in vitro* shoot multiplication and rooting experiments as seen in Plate 7 (A) (B) and (C).

4.2.1 Effect of low cost gelling agents on shoot multiplication

From the data presented in Table 6 and Figure 5, it is revealed that amongst all the different treatment combinations, significantly higher number of shoots per explant were observed in T_5 - 100 % Agar (15.50) which was followed by T_2 - 75% Isabgol + 25 % Agar (13.05) and T_3 - 50 % Isabgol + 50 % Agar (12.17) whereas treatment T_1 - 100 % Isabgol performed lower in terms of number of shoots per explant (10.08).

The average shoot length was highest in T_2 - 75% Isabgol + 25 % Agar (8.20 cm) which was followed by T_5- 100 % Agar (8.13 cm) and T_3 - 50 % Isabgol + 50 % Agar (7.66 cm). The lowest average shoot length was observed in T_1 - 100 % Isabgol (7.42 cm).

In case of number of nodes per plantlet the highest number was observed in T_3 - 50 % Isabgol + 50 % Agar (8.29) followed by T_5 - 100 % Agar (8.04) and T_2 - 75% Isabgol + 25 % Agar (7.90) whereas, lowest number of nodes per plantlet (7.62) were observed in T_4 - 25% Isabgol + 75 % Agar.

As far as the internodal length is concerned longest internodal distance was observed in T_1 - 100 % Isabgol (1.43 cm) followed by T_4- 25% Isabgol + 75 % Agar (1.40 cm) and T_5- 100 % Agar (1.20 cm) whereas shortest internodal distance was observed in T_3- 50 % Isabgol + 50 % Agar (0.92 cm).

4.2.2 Effect of low cost gelling agents on rooting

From the data given in Table 7 and Figure 6, it was revealed that the overall percentage rooting response in all treatments ranged between 93.31 to 97.50 per cent. Superior rooting response (97.50) was observed in T_5 - 100% Agar which was followed by T_2 - 75% Isabgol + 25% Agar (97.30) and T_1 - 100% Isabgol (95.93) whereas treatment T_4 - 25% Isabgol + 75% Agar recorded minimum percentage rooting response (93.31).

The maximum number of roots per shoot (8.93) was recorded in T_2 - 75% Isabgol + 25% Agar which was followed by T_5 - 100% Agar (8.50) and T_1 - 100% Isabgol (8.03), whereas T_3 - 50% Isabgol + 50% Agar recorded minimum of roots per shoot (7.90) as compared to all other treatments.

As far as root length is concerned, longest average root length (6.97 cm) was observed in T_5 - 100% Agar followed by T_2 - 75% Isabgol + 25% Agar (6.87 cm) and T_4 - 25% Isabgol + 75% Agar (6.21 cm) whereas T_3 - 50% Isabgol + 50% Agar recorded shortest average root length (5.70 cm).

Significantly superior response in terms of number of leaves per plantlet was obtained in T_5 - 100% Agar (15.76) which was followed by T_1 - 100% Isabgol (13.95) and T_2 - 75% Isabgol + 25% Agar (13.50). Treatment T_3 - 50% Isabgol + 50% Agar recorded minimum number of leaves per plantlet (10.82) as compared to all other treatments.

Data on plant height was also recorded along with the rooting parameters, which revealed significantly higher plant height in T_5 - 100% Agar (14.83 cm) and was followed by T_2 - 75% Isabgol + 25% Agar (13.40 cm) and T_1 - 100% Isabgol (13.33 cm) whereas, T_3 - 50% Isabgol + 50% Agar recorded minimum plant height (12.72 cm).

4.3 Effect of low cost carbon source

For reduction of cost of medium, a cost-effective carbon source *viz*. common table sugar was used for finding replacement for expensive standard carbon source *viz.* sucrose in this experiment. A comparative study between sucrose and common table sugar was performed. The quantity for both carbon sources was kept same i.e. 30 g/l. The results obtained are given as under-

4.3.1 Effect of low cost carbon source on shoot multiplication

From the data recorded in Table 8 and Figure 7, it can be clearly seen that there is no significant difference in between the observations recorded. The shoots obtained under both treatments are green and sturdy as seen in Plate 8 (A) and (B). Maximum number of shoots was observed in T_1- sucrose (15.50) as compared to T_2- common table sugar (15.39). The average shoot length was higher in T_1-sucrose (8.13 cm) whereas in T_2- common table sugar it was lower (8.09 cm).

Table 6: Effect of different combinations of gelling agent on shoot multiplication

Treatment	Gelling agents	Number of shoots per explant	Average shoot length (cm)	Number of nodes per plantlet	Internodal length (cm)
T_1	100% Isabgol	10.08	7.42	7.86	1.43
T_2	75% Isabgol + 25% Agar	13.05	8.20	7.90	1.24
T_3	50% Isabgol + 50% Agar	12.17	7.66	8.29	0.92
T_4	25% Isabgol + 75% Agar	11.07	7.57	7.62	1.40
T_5	100% Agar	15.50	8.13	8.04	1.20
SE(d) ±		0.57	0.23	0.17	0.07
C.D. (5%)		1.29	0.53	0.39	0.16
C.V. (%)		5.89	3.69	2.60	6.35

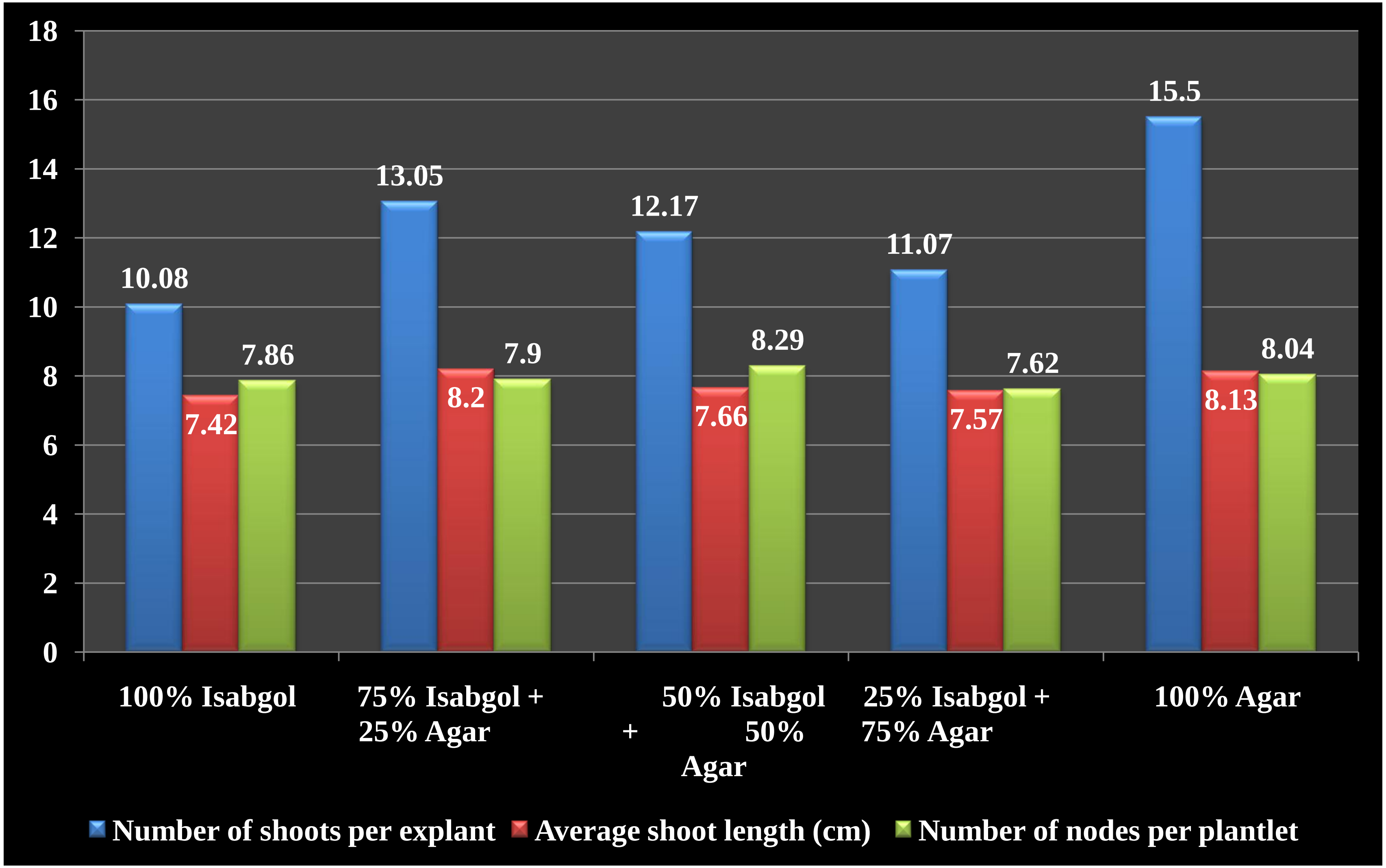


Figure 5: Effect of different combinations of gelling agents on shoot multiplication

Table 7: Effect of different combinations of gelling agents on rooting

Treatments	Gelling agents	Percentage response (%)	Number of roots per shoot	Average root length (cm)	No. of leaves per plantlet	Plant height (cm)
T_1	100% Isabgol	95.93	8.03	6.09	13.95	13.33
T_2	75% Isabgol + 25% Agar	97.30	8.93	6.87	13.50	13.40
T_3	50% Isabgol + 50% Agar	93.60	7.90	5.70	10.82	12.72
T_4	25% Isabgol + 75% Agar	93.31	7.92	6.21	11.18	12.82
T_5	100% Agar	97.50	8.50	6.97	15.76	14.83
SE(d) ±		0.85	0.31	0.39	0.36	0.31
C.D. (5%)		1.92	0.70	0.89	0.81	0.69
C.V. (%)		1.09	4.59	4.67	3.40	2.81

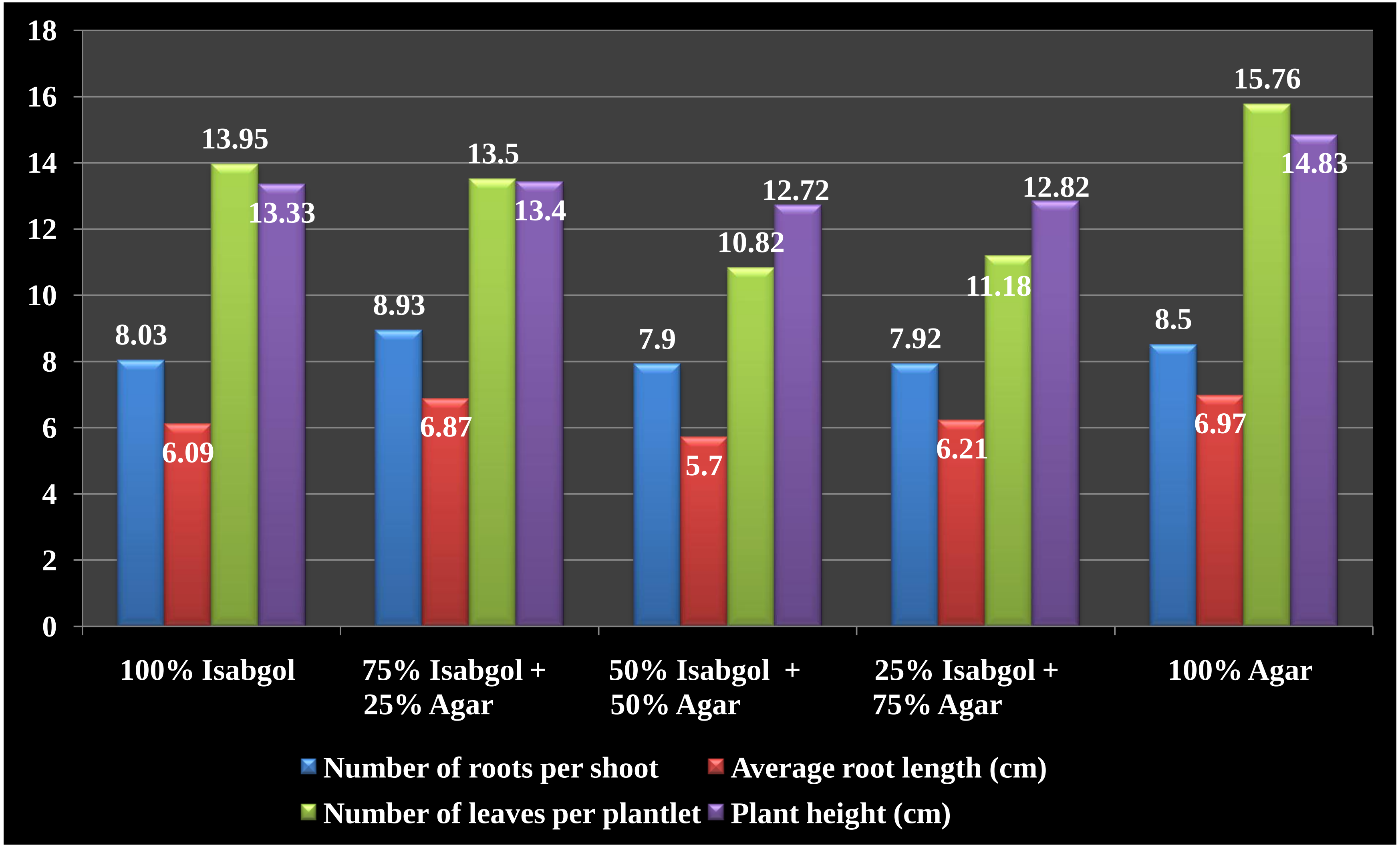


Figure 6: Effect of different combinations of gelling agents on rooting

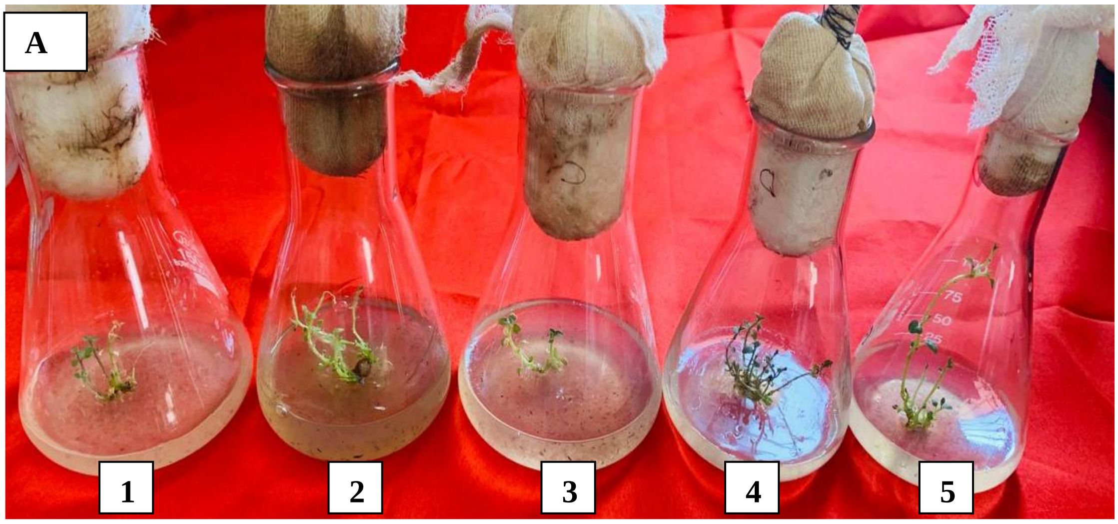


Plate 7 (A): Shoot multiplication using different combinations of Isabgol and Agar
1= 100% Isabgol; 2= 75% Isabgol +25% Agar; 3= 50% Isabgol +50% Agar;
4= 25% Isabgol +75% Agar; 5= 100% Agar

Plate 7(B): Shoot multiplication on MS medium supplemented with gelling agent
T_1- 100% Isabgol

Plate 7 (C): Shoot multiplication in gelling agent combination T_2=75% Isabgol + 25% Agar

The number of nodes per plantlet was higher in T_2- common table sugar (9.02) as compared to T_1- sucrose (8.37). The internodal distance was longer in T_1- sucrose (1.12 cm) as compared to T_2- common table sugar (1.07 cm).

The results obtained reveal that the observations are at par with each other and sucrose can be replaced with common table sugar without causing any detrimental effect to the growing explant.

4.3.2 Effect of low cost carbon source on rooting

From the data recorded in Table 9 and Figure 8, it can be clearly seen that there is no significant difference in between the observations recorded. Good quality hairy fibrous roots with creamish white colour were obtained as seen in Plate 8 (C).The percentage rooting response obtained was maximum in T_2- common table sugar (98.17) as compared to T_1- sucrose (97.50).

Maximum number of roots per shoot was observed in T_1- sucrose (8.50) as compared to T_2- common table sugar (8.21). The average root length was higher in T_2- common table sugar (7.10 cm) whereas in T_1- sucrose it was lower (6.97 cm).

The numbers of leaves per plantlet were higher in T_1- sucrose (15.79) as compared to T_2- common table sugar (15.56). As far as plant height is concerned longer plant height was observed in T_1- sucrose (14.83 cm), whereas it was lower in T_2- common table sugar (14.32 cm).

The results reveal that the observations are at par with each other and sucrose can be replaced with common table sugar without causing any detrimental effect on rooting response.

4.4. Effect of alternative light sources

In place of artificial illumination through fluorescence light, the possibility of natural indirect light was explored as a cheap illumination source. The cultures were kept under two culture conditions with respect to light sources as visible in Plate 9 (A) and (B) and the results obtained are given as under-

4.4.1 Effect of alternative light sources on shoot multiplication

From the data recorded in Table 10 and Figure 9, it can be clearly seen that there is not much significant difference between observations recorded. The results revealed that natural indirect light performed even better than artificial illumination through fluorescence tubes. The shoots obtained under T_1- fluorescent light are sturdy, dark green in colour with more number of leaves and nodes per plantlet whereas under T_2- natural indirect light plants are sturdy, green coloured with higher shoot length and internodal length and less number of leaves.

Maximum number of shoots was observed in T_2- natural indirect light (15.98) as compared to T_1- fluorescent light (15.50). The average shoot length was higher in T_2- natural indirect light (9.85 cm) whereas in T_1 - fluorescent light it was lower (8.13 cm).

The number of nodes per plantlet was high in T_1- fluorescent light (8.37) as compared to T_2- natural indirect light (7.80). The internodal distance was longer in T_2- natural indirect light (1.42 cm) as compared to in T_1- fluorescent light (1.12 cm).

From the results obtained, it can be clearly seen that results are comparable with each other and replacing artificial fluorescence light with natural indirect light is feasible and can give better results for shoot multiplication in long term.

4.4.2 Effect of alternative light sources on rooting

From the data recorded in Table 11and Figure 10, it can be clearly seen that there is no significant difference between any observations recorded. The percentage rooting response obtained was maximum in T_2- natural indirect light (97.57) when compared to T_1- fluorescent light (97.10).

Maximum number of roots per shoot was observed in T_2- natural indirect light (9.20) as compared to T_1- fluorescent light (8.50). The average root length was higher in T_2- natural indirect light (7.03 cm) whereas in T_1- fluorescent light it was lower (6.97 cm).

The number of leaves per plantlet was high in T_1- fluorescent light (15.79) as compared to T_{2-} natural indirect light (13.27). As far as plant height is concerned longer plant height was observed in T_2- natural indirect light (15.36 cm), whereas it was lower in T_1- fluorescent light (14.83 cm).

Table 8: Effect of alternative carbon sources on shoot multiplication

Treatments	Number of shoots	Average shoot length (cm)	Number of nodes per plantlet	Internodal length (cm)	Morphogenetic response
T_1- Sucrose	15.50	8.13	8.37	1.12	Shoots green and sturdy, less number of nodes.
T_2- Common Table sugar	15.39	8.09	9.02	1.07	Shoots green and sturdy, more number of nodes.

Table 9: Effect of alternative carbon sources on rooting

Treatments	Percentage response (%)	Number of roots per shoot	Average root length (cm)	Number of leaves per plantlet	Plant height (cm)
T_1- Sucrose	97.50 %	8.50	6.97	15.79	14.83
T_2- Common Table sugar	98.17 %	8.21	7.10	15.56	14.32

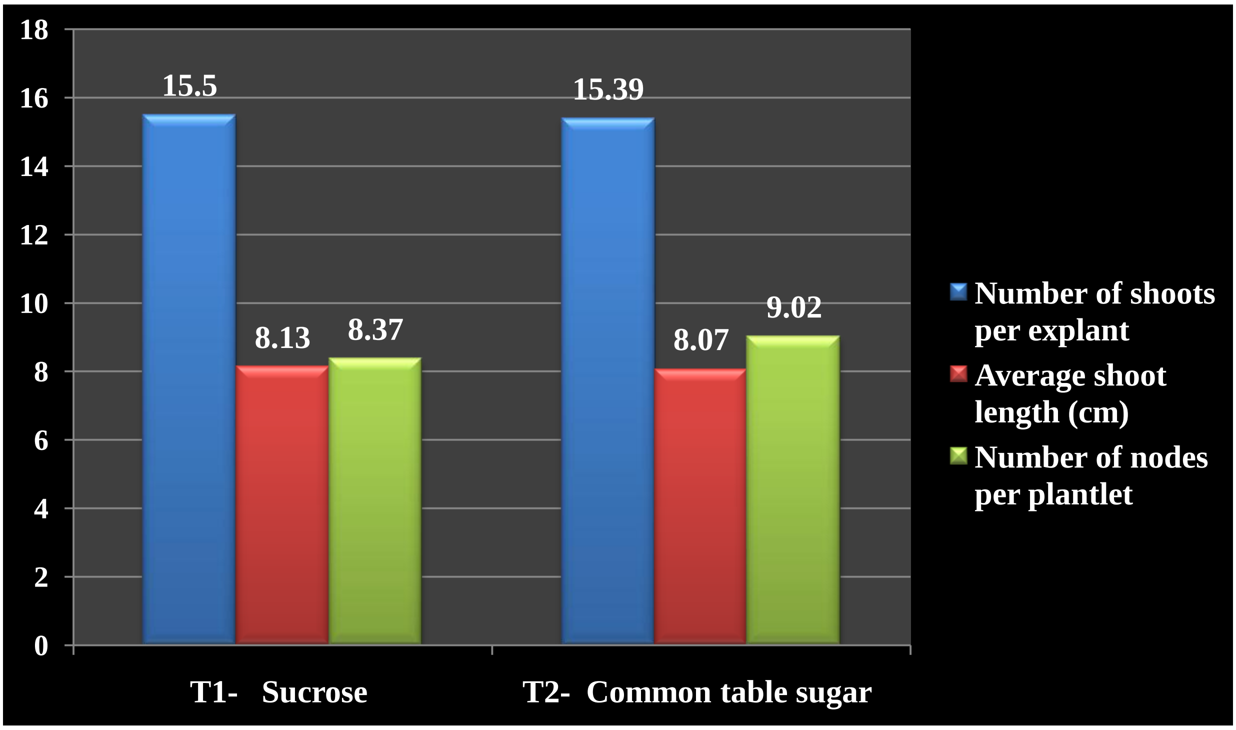


Figure 7: Effect of alternative carbon sources on shoot multiplication

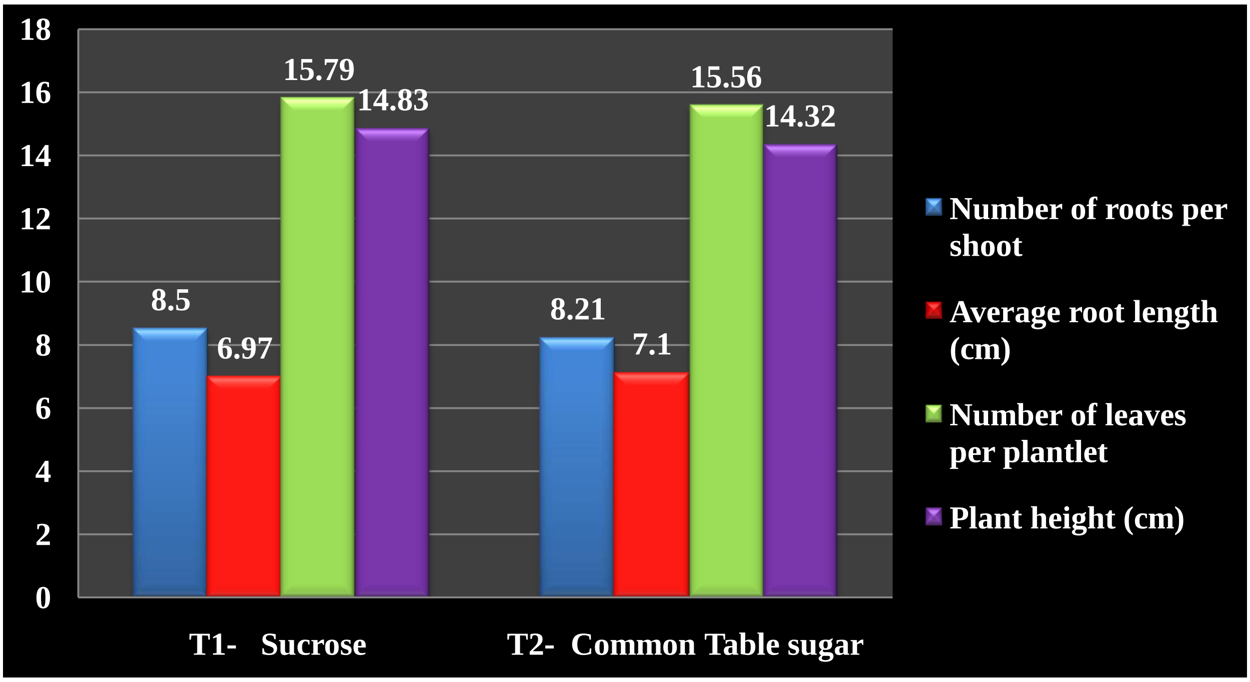


Figure 8: Effect of alternative carbon sources on rooting

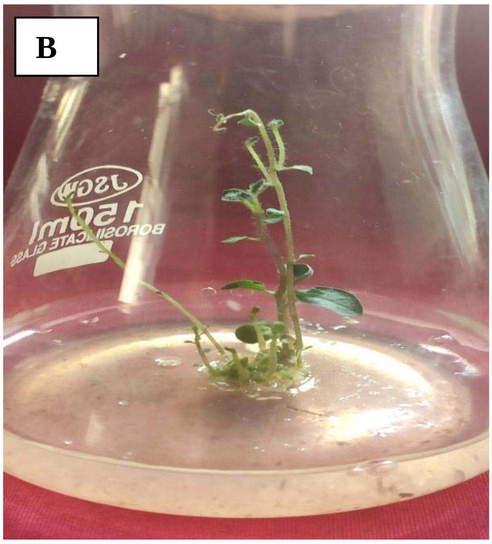


Plate 8 (A): Shoot multiplication in MS medium supplemented with sucrose

Plate 8 (B): Shoot multiplication in MS medium supplemented with common table sugar

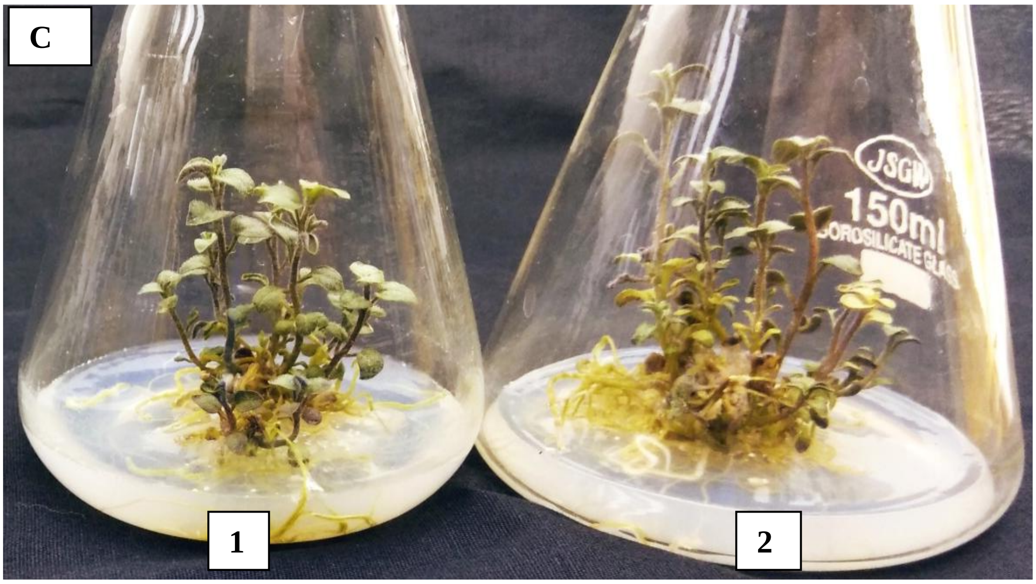


Plate 8 (C): Rooting in MS medium supplemented with (1) sucrose and (2) common table sugar

Table 10: Effect of alternative light sources on shoot multiplication

Treatments	Number of shoots	Average shoot length (cm)	Number of nodes per plantlet	Internodal distance (cm)	Morphogenetic response
T_1- Fluorescent light	15.50	8.13	8.37	1.12	Shoots are comparatively shorter & dark green in colour, leaves bigger in size, more number of nodes
T_2- Natural indirect light	15.98	9.85	7.80	1.42	Shoots are comparatively longer & green in colour, leaves smaller in size, less number of nodes.

Table 11: Effect of alternative light sources on rooting

Treatments	Percentage response (%)	Number of roots per shoot	Average root length (cm)	Number of leaves per plantlet	Plant height (cm)
T_1- Fluorescent light	97.10 %	8.50	6.97	15.79	14.83
T_2- Natural indirect light	97.57 %	9.20	7.03	13.27	15.36

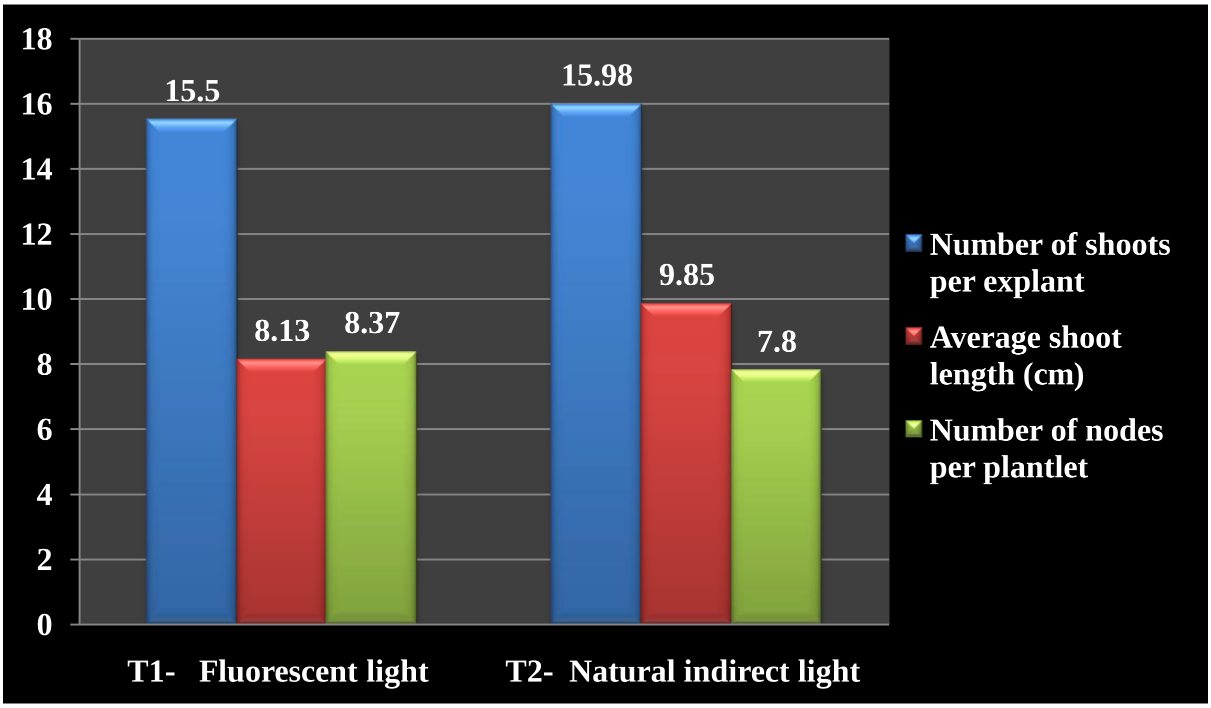


Figure 9: Effect of alternative light sources on shoot multiplication

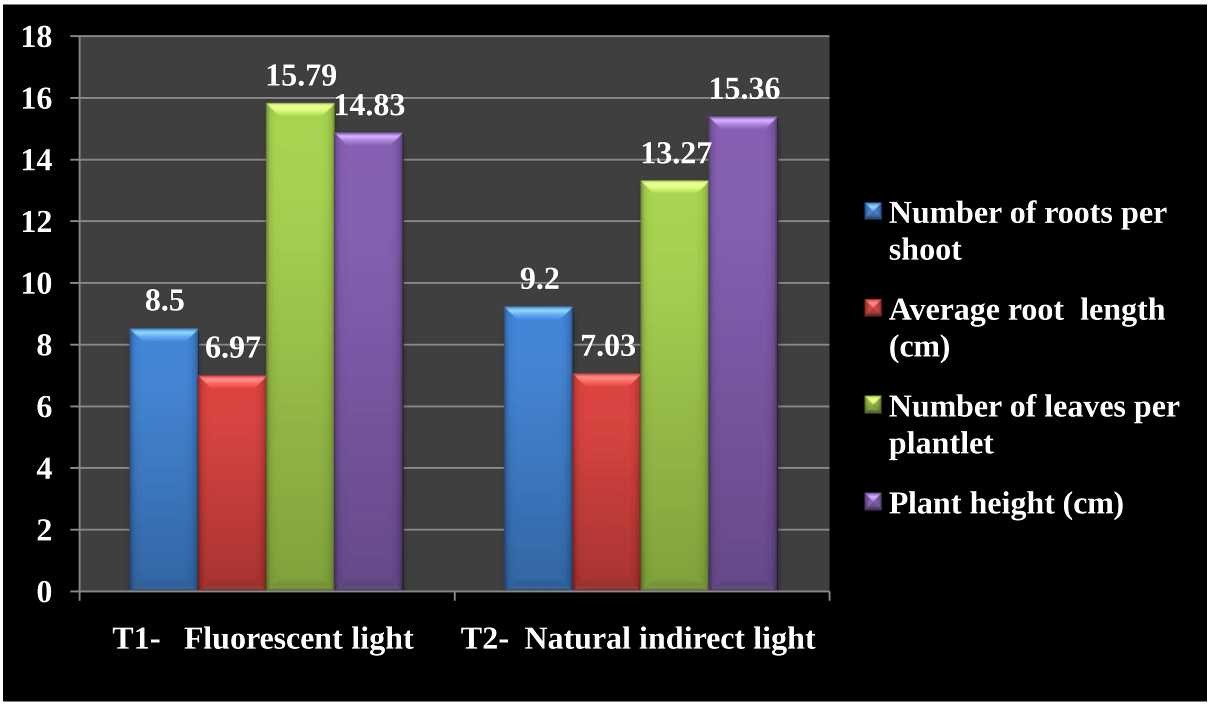


Figure 10: Effect of alternative light sources on rooting

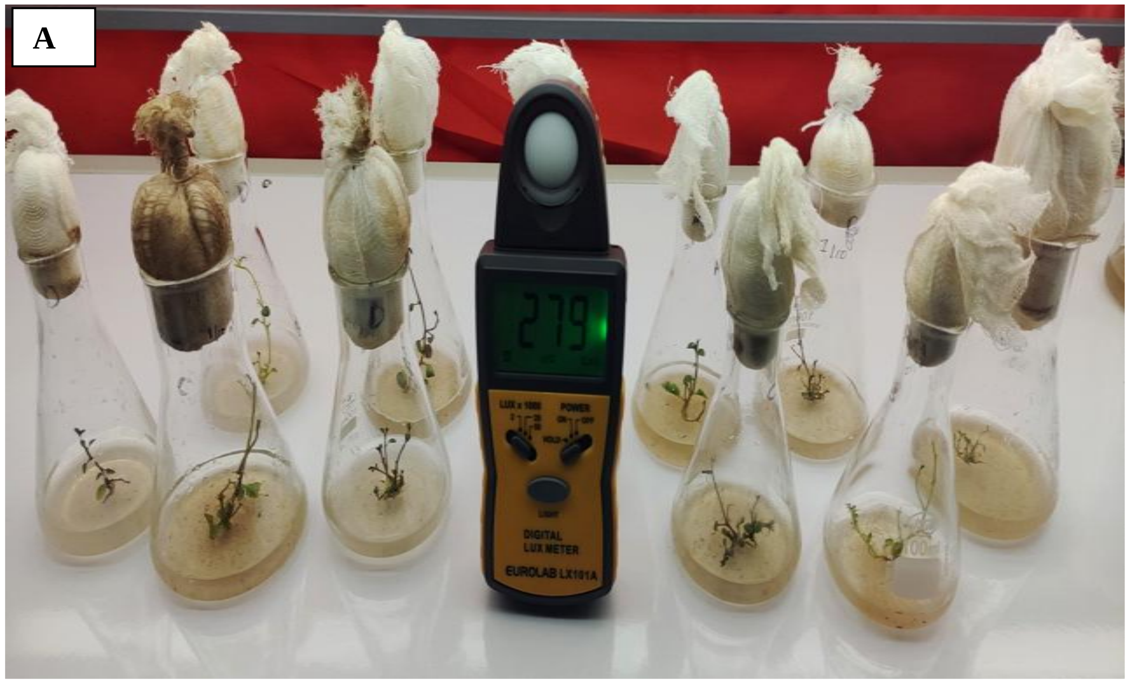


Plate 9 (A): Cultures under artificial fluorescent light conditions

Plate 9 (B): Cultures under natural indirect light conditions

From the results obtained, it can be clearly seen that results are comparable with each other and replacing artificial fluorescence light with natural indirect light is feasible and can give better results for rooting in long term.

4.5 Economics of low cost alternative sources

4.5.1 Economics of gelling agents

The cost of five different combinations of alternate gelling agents *viz.*, isabgol and standard gelling agent *viz.*, agar was compared as given in table 12. According to our study, the combination 75% Isabgol + 25 % Agar performed better as compared to other combinations. Thus, by usage of this combination we can reduce the cost of gelling agents by 37.0 %. The use of combinations 50 % Isabgol + 50 % Agar and 25% Isabgol + 50 % Agar can reduce the cost of gelling agent by 24.7 % and 14.6 % respectively.

4.5.2 Economics of carbon sources

The cost-effective carbon source *viz*. common table sugar was used as a replacement for expensive standard carbon source *viz.* sucrose as given in Table 13. The cost of common table sugar was compared to the standard carbon source sucrose as required for 1 litre medium. By substitution of sucrose used in conventional tissue culture with common table sugar we can achieve up to 95.6 % cost reduction.

4.5.3 Economics of light sources

The light intensity cost (Fluorescent light) was calculated by using the formula given by Theraja and Theraja (2005).

E= Illuminance (lm/m^2) or (lux)

or, $\varphi/A = L$ A=Area, m^2

or, $\varphi = LA$ L= light intensity, lux

φ=flux, lm

P Lux light intensity comes from = Q watt

φ Lux light intensity comes from = Q/P X φ watt

= R watt (total watt or W_{total})

R watt= W_{total} X 10-3 X h = Z units (Z KWh)

where, h=hour per day

1 unit electricity cost = Y Rupees
Z units electricity cost = Y x Z Rupees = T Rupees

Generally, potato tissue culture needs 3000-3500 lux light intensity to maintain its morphological characters. In the plant tissue culture laboratory growth room has an area of about (10 ×10) sq. feet i.e. (3.048×3.048) m^2 =9.29 m^2. Therefore light intensity cost (artificial light) has been calculated by using the following formula -

A = 9.29 m^2 (3.05 x 3.05)
Φ = LA
Light intensity, L= 3250 lux
3250 lux x 9.29 = 30,192.5 lux

2580 Lux light intensity comes from = 36 watt (energy pack)

30192.5 Lux light intensity comes from= (36/2580) × 30192.5 watt = 419.67 watt (total watt or W_{total})

In case of using artificial light (considering 16 hour lighting period)

1 hour electricity consumption 419.67 watt

16 hours electricity consumption = 419.67 × 16 ×10^{-3} KWh (units) = 6.71 KWh

1 day requires electricity 6.71 KWh/Units

30 days requires electricity = 6.71 KWh/units x 30 = 201.3 Units.

Thus by keeping cultures in the natural indirect light instead of fluorescent light can result in saving of **201.3 Units** per month.

In J&K on an average 1 unit electricity cost is 2-3 Rupees

201.3 units electricity cost is = 201.3 x 3 = 603.9 Rupees

Thus by keeping cultures in the natural indirect light instead of fluorescent light can result in saving **Rupees 604 per month averagely.**

Table 12: Economics of gelling agents

Gelling agent combinations	Approximate price (₹/Kg)	Price per gram	Quantity required for 1 L medium in grams	Cost of gelling agent for 1 L medium (Rupees)	Percentage reduction in cost of gelling agent in medium
100% Isabgol	900	0.75	30 g/l	22.5 ₹	**49.4 %**
75 % Isabgol + 25% Agar	900 6670	0.90 6.67	22.5 g/l + 2 g/l	20.3 + 13.3 = 33.6 ₹	**37.0 %**
50% Isabgol + 50% Agar	900 6670	0.90 6.67	15 g/l + 4 g/l	13.5 + 26.7 = 40.2 ₹	**24.7 %**
25 % Isabgol + 75% Agar	900 6670	0.90 6.67	7.5 g/l + 6 g/l	6.75 + 40.0 = 45.6 ₹	**14.6 %**
100 % Agar	6670	6.67	8 g/l	53.4 ₹	**-**

Table 13: Economics of carbon sources

Carbon Sources	Approxi-mate price (₹ /Kg)	Price per gram	Quantity required for 1 L medium in grams	Cost of carbon source for 1 L medium (Rupees)	Percentage reduction in cost of carbon source in medium
Common table sugar	50	0.05	30 g	1.5 ₹	**95.6 %**
Sucrose	1155	1.15	30 g	34.5 ₹	**-**

Chapter-V

DISCUSSION

DISCUSSION

The results of various experiments conducted under the present investigation entitled **"Studies on Alternative Sources in *In-vitro* Culture of Potato (*Solanum tuberosum* L.)"** are discussed as under:

5.1 Standardization of Micropropagation protocol

5.1.1 Effect of different concentrations of BAP and kinetin on shoot induction

In the present study, two explants *i.e.* sprouts and nodal segments were used for shoot induction. Various workers have used different explants for establishment of fresh cultures with varying degree of success like nodal segments (Sharma *et al*., 2011; Rai *et al*., 2012), leaf explant (Al-Sulaiman, 2011) and shoot/meristems tip culture (Marcela *et al*., 2011; Raza, 2011; Yasmin *et al*., 2011; Mohapatra *et al.*, 2018). However, our results revealed better percentage response in sprout explants whereas nodal segments explants exhibited comparatively early response. The results were in conformity with the work reported by Parvizi *et al*. (2008); Houqe (2010) and Gami *et al*. (2013).

In order to standardize media for shoot induction, explants were cultured on MS medium supplemented with different concentrations of BAP (0.0, 0.25, 0.50, 0.75, 1.0 and 2.0 mg/l) and kinetin (0.0, 0.25, 0.50, 0.75 and 1.0 mg/l). Amongst the array of hormonal treatments used, our best results were obtained on T_4 treatment comprising of MS medium supplemented with 0.75 mg/l BAP. Similar results have been reported by Mohapatra *et al*. (2018) using sprouts explant on MS medium supplemented with BAP 0.25 mg/l whereas Sharma *et al*. (2015) reported highest shoot regeneration response by using nodal segment explants on MS medium supplemented with 1.0 mg/l BAP. It has been well established that shoot regeneration is dependent on optimum auxin: cytokinin ratio (De Ropp, 1955 and Skoog and Miller, 1957) and solanaceous crops are known to have high levels of endogenous auxins which could be the probable reason for high regeneration response on BAP or kinetin. However, superiority of BAP over kinetin for shoot bud induction has also been demonstrated by various workers like Sarker and Mustafa (2002); Hussain *et al.*

(2006); Bhuiyan (2013) and Shahriyar *et al.* (2015). The effectiveness of BAP on shoot induction can be due to the abilities of plant tissue to metabolize the natural hormones more readily than artificial growth regulators or due to the abilities of BAP to induce natural hormones such as Zeatin within tissue and thus works through natural hormones (Zaerr and Mapes, 1982). However, contrary to our findings some researchers like Uddin (2006) and Ahmad *et al.* (2013) etc. obtained maximum shoot regeneration in treatment combinations of BAP and kinetin also.

5.1.2 Effect of different concentrations of BAP and kinetin on shoot multiplication

Experiments on standardizing media for *in vitro* shoot multiplication were conducted using MS basal medium supplemented with different concentrations of BAP (0.0, 0.25, 0.50, 0.75, 1.0, 1.5 and 2.0 mg/l) alone or in combination with kinetin (0.25 mg/l). Our results revealed that among all the treatments, T_{10} treatment comprising of MS medium supplemented with 0.75 mg/l BAP + 0.25 mg/l kinetin, recorded significantly superior response expressed in terms of number of shoots per explant. The synergistic effect of BAP and kinetin for increased shoot multiplication rate and proliferation was also reported on *B. tulda and M. baccifera* (Waikhom and Louis, 2014). The results reveal that a proper balance between two cytokinins may trigger more efficient shoot multiplication response. This may be due to variation in specific level of endogenous hormones as influenced by genotypes and environmental factors. Similar results were obtained by Genene *et* al. (2018) in potato variety Gudenie, on MS medium supplemented with 0.5 mg/l BAP + 2 mg/l kinetin. Ahmad *et al.* (2014) used MS medium supplemented with BAP 2.0 mg/l in combination with 0.1 mg/l kinetin to obtain maximum mean number of shoots. Uddin (2006) also concluded that number of shoots per explant were higher in combined treatment of BA and kinetin (3 mg/l + 2 mg/l). On the contrary, few workers like Rashid *et al.* (2005); Liljana *et al*. (2012) and Mohapatra *et al.* (2018) have also used combinations of auxins and cytokinins to obtain maximum shoot multiplication response. Shah Zaman *et al*. (2001) also exhibited multiple shoot formation using only cytokinins.

During the course of experimentation for multiple shoot formation, a few microtubers have also been formed especially at higher concentrations of BAP (2.0 mg/l) alone or in combination with kinetin (0.25 mg/l) as visible in Plate 5 (A) (B) and (C). Donnelly *et* al. (2003) have reported that growth regulators (BAP, kinetin and NAA) can induce microtubers in potato *in vitro,* but in some cases the effect of growth regulators was found to be genotype dependent (Hussain *et* al. 2006). Similar response was obtained by Meenakshi (2020) using two potato varieties Kufri Bahar and Kufri Surya for testing *in vitro* microtuberization response in MS medium supplemented with three different concentrations of BAP (0.75, 1.5 and 2.25 mg/l) and concluded that both varieties exhibited a better response when cultures were supplemented with 2.25 mg/l BAP.

5.1.3 Effect of different concentrations of IBA on rooting

For induction of rooting, healthy *in vitro* multiplied micro shoots (3-4 cm) obtained were cultured on MS medium supplemented with different concentrations of IBA (0.0, 0.25, 0.5, 1.0, 1.5 and 2.0 mg/l). The beneficial effect of using MS medium supplemented with IBA has already been reported for potato by Khatun *et al*. (2003). Among all the treatments our best results were obtained in treatment T_4 - MS medium supplemented with 1.0 mg/l IBA with significantly superior rooting response and maximum number of roots per shoot. The results further revealed relatively better rooting response in lower concentrations as compared to higher concentrations of IBA. This may be due to the higher level of endogenous auxins present in solanaceous vegetables as reported by Bhushan and Gupta (2017). Similar results have been reported by Kaur *et al.* (2000); Uddin (2006); Khalafalla *et al*. (2009); Khalafalla *et al*. (2010) and Mohapatra *et al.* (2018). However, few researchers also reported good results by using combination of IBA and NAA (Genene *et al*., 2018), IAA (Sarkar and Mustafa, 2002) and MS Basal medium (Khalafalla *et al*., 2009 and Shahriyar *et al.,* 2015) for root induction.

5.2 Effect of low cost gelling agents

Alternative gelling sources can be used successfully for solidification of MS media in potato (Venkatasalam *et al*. 2013). Various workers like Bhattacharya *et al*. (1994); Jain *et al*. (1997); Babbar and Jain (1998); Hussain Shah *et al.* (2003); Bhushan *et al*. (2005); Tyagi *et al*. (2007); Tyagi *et al*. (2008); Agrawal *et al*. (2010); Gour and Kant (2011); Khan *et al*. (2012); Jain and Yadav (2016); Pant (2016) and Saraswathi *et al*. (2016) have quantified the usage of isabgol as an alternative gelling agent in different plant species. Feasibility of isabgol as alternate gelling agent has been reported in ginger (Paul, 2000) and tomato (Bhushan, 2001) and better results were obtained in isabgol as compared to agar gelled medium. However, problems were noticed by previous workers during subsequent sub culturing in isabgol gelled medium due to its stickiness. Moreover, problem of delicate roots was encountered which normally does not occur in standard agar medium. To overcome this problem we used the mixture of agar and isabgol in an appropriate ratio. Therefore, five concentrations of isabgol alone and in combination with agar *viz.* 100 % Isabgol, 75% Isabgol + 25 % Agar, 50 % Isabgol + 50 % Agar, 25% Isabgol + 75 % Agar and 100 % Agar were used to find out the best combination for shoot and root multiplication experiments. Our best results for shoot multiplication were obtained in 100% agar gelled medium followed by 75% Isabgol + 25 % Agar whereas best rooting response was also obtained in combination 75% Isabgol + 25 % Agar. However, the other shoot/root parameters *viz.* average shoot length, internodal length, number of roots per shoots were found to be superior in combination 75% Isabgol + 25 % Agar. Comparable performance of isabgol + agar combination with that of agar for medium solidification and explant growth strongly suggests that isabgol + agar media combination can be used effectively to reduce the cost of gelling agent during media preparation. During media preparation, the husk even after autoclaving remained suspended. So, in order to mix the isabgol husk properly, vigorous shaking of flask was done after autoclaving so as to make the media reasonably transparent and form a proper gradient. It was also observed that stickiness of medium increased with the increase in concentration of isabgol in all the combinations even after autoclaving, but the sticky nature of isabgol gelled medium resulted in no cracking, which is a frequent problem in case of agar solidified media (Khan *et al*., 2012). The results are in agreement with Babbar and Jain (1998) who had very similar observations on *in vitro* culture of *Syzygium cuminii* and *Datura innoxia*. In terms of feasibility and ease of culturing besides better response, combination T_2- 75% Isabgol + 25 % Agar gave the best results and use of this combination can reduce the cost upto 37%.

5.3 Effect of different carbon sources

Carbon sources such as AR grade sucrose which is extensively used during media preparation contribute about 34% of the production cost (Demo *et al*., 2008). This cost can be reduced by identifying the cheap alternatives without causing any adverse effects to micropropagated plants. Thus, in our study we substituted the AR grade sucrose with common table sugar. The use of market sugar instead of sucrose has been reported to reduce the cost of *in vitro* micropropagation of banana, with no significant effect on regeneration compared to sucrose (Agrawal *et al*., 2010). Similar results were obtained in our comparative study which revealed that the number of shoots, average shoot length and internodal length was higher in sucrose whereas the number of nodes per plantlet was higher in common table sugar. For rooting response, the percentage response and average root length was higher in common table sugar whereas number of roots per shoot, number of leaves per plantlet and plant height was higher in sucrose. The quality and performance of plantlets grown on AR grade sucrose was comparable to those grown on common table sugar for both shoot and root multiplication experiments. Our findings are in consistency with Ganapati *et al*. (1995); Sharma and Singh (1995); Adiga *et al*. (1998); Prabhakara (1999); Prakash *et al*. (2004); Raghu *et al.* (2007); Nyende (2008); Purohit *et al.* (2009); Gitonga *et al.* (2010) and Venkatasalam *et al*. (2013).

The common table sugar enhanced proliferation and growth of plantlets similar or better than AR grade sucrose. This may be due to the fact that different carbon sources available in culture medium were easily translocated and assimilated by the explants enhancing cell division thus leading to eventual growth (Demo *et al*., 2008). In spite of the widespread use of sucrose as carbon source, the higher cost is major hindrance for its justified use on commercial and laboratory scale. Our results exhibited that substitution of sucrose used in conventional tissue culture with common table sugar and thereby cost reduction up to 95.6 % can be achieved.

5.4 Effect of different light sources

Light plays an important role on growth and development of the *in vitro* culture plants. To meet the light requirement of *in vitro* culture plants, artificial light sources are used in plant tissue culture laboratory. Artificial lighting costs account for 65% of total electricity bill (Standaet de Metsenaere, 1991) and is one of the highest non-labour costs in a tissue culture laboratory (Dooley, 1991). Therefore, feasibility studies on use of indirect natural light instead of conventional fluorescent tube lights were conducted to reduce the overall cost of production of micropropagated plantlets. Our results revealed better overall response of cultures in indirect natural light manifested in the terms of higher number of shoots, average shoot length and internodal distance whereas the number of nodes per plantlet was higher in fluorescent light. Our findings have been corroborated by similar work reported by various researchers Rehana *et al.* (2018) conducted a similar study where fluorescent light and sunlight were used as lighting source. Better results were observed in cultures kept under sunlight with respect to various growth factors than those in artificial one after 30 days of culture. The sun-lighted plants were also healthier, vigorous and strong which helped plants to establish in net house faster. Jao and Fang (2004) showed that intermittent or pulse light (natural light) promotes the growth of potato plantlet significantly compared to artificial light. Miyashita *et al.* (2005) showed that shoot length of potato increased with increasing FR/PPFD ratio when R/PPFD ratio was 0.1- 0.5. They also showed that shoot length was greatest with R/PPFD ratio of 1.0. The reason for increased response in natural indirect light can be attributed to the presence of the all the spectrums including red light spectrum which may allow the plantlet to grow more vigorously. On the other hand, the red light spectrum is present in a limited volume in artificial light which may result thinner plantlets with spindly shoot as red light is the most important fuel for driving photosynthesis and biomass production of plants (Rehana *et al.,* 2018).

Our results revealed higher percentage rooting response, number of roots per shoot, average root length and plant height in natural indirect light whereas the number of leaves per plantlet was higher in fluorescent light. High percentage response for rooting can be attributed due to presence of ultraviolet radiation present in sunlight (Silva *et al*., 2005). Similar results were obtained by Kwapata *et al*. (1999) in the *in vitro* rooting of *Faidherbia albida*. Workers like Jeong *et al.* (1995); Kozai *et al*. (1997) and Kozai *et al*. (1999) also obtained similar results for rooting response.

Based on our findings during the present investigation, it can be concluded that *in vitro* plants grown in indirect natural light were more healthy, vigorous and hardy as compared to those raised under the artificial light. These findings were in conformity with various workers like Zapata-Arias & Kodym (1999); Zapata- Arias *et al*. (2001); Be *et al.* (2006); Arias *et al.* (2014) and Jafar *et al*. (2018).

Chapter-VI

SUMMARY AND CONCLUSION

CHAPTER-VI

SUMMARY AND CONCLUSION

The present investigation entitled, **"Studies on Alternative Sources in *In-vitro* Culture of Potato (*Solanum tuberosum* L.)"** was carried out at the Division of Vegetable Science and Floriculture, SKUAST-Jammu during the year 2019-20 with the objectives; 1) To find out alternative gelling agents for low cost medium; 2) To find out alternative carbon sources for low cost medium; 3) To assess feasibility of natural indirect light as alternative source for *in vitro* culture.

For standardization of micropropagation protocol we used Kufri Girdhari, a late blight resistant variety, released by CPRI, Shimla. For shoot induction the explants were transferred on MS medium supplemented with BAP (0.25, 0.50, 0.75, 1.0 and 2.0 mg/l) and kinetin (0.25, 0.50, 0.75 and 1.0 mg/l). The highest percentage response was obtained on MS medium supplemented with 0.75 mg/l BAP on sprout explant. As far as earliness of response was concerned, best response was observed in MS medium supplemented with 0.75 mg/l BAP in nodal segment explants. For shoot multiplication different concentrations of BAP (0.25, 0.50, 0.75, 1.0, 1.5 and 2.0 mg/l) in combination with kinetin (0.25 mg/l) were used. The superior response in terms of number of shoots per explant was observed in MS medium supplemented with 0.75 mg/l BAP + 0.25 mg/l kinetin. Microtuberization response was also observed in cultures supplemented with 2.0 mg/l BAP alone and in combination with 0.25 mg/l kinetin. For induction of rooting, healthy *in vitro* multiplied micro shoots (3-4 cm) were cultured on MS medium supplemented with different concentrations of IBA (0.0, 0.25, 0.5, 1.0, 1.5 and 2.0 mg/l). From the study, highest percentage response and maximum number of roots per shoot was obtained on MS medium supplemented with 1.0 mg/l IBA.

In an endeavor to find out the most suitable and cost effective alternate gelling agent combination using isabgol along with standard agar, we tested five different concentrations of Isabgol alone and in combination with Agar. Our studies revealed best shoot multiplication rate in 100% agar gelled medium followed by 75% Isabgol + 25 % Agar whereas best rooting response was recorded in combination 75% Isabgol + 25 % Agar.

Feasibility of cheaper carbon source (common table sugar), instead of costly standard sucrose, was tested for shoot and root multiplication. Our results revealed comparable results with respect to shoot multiplication and considerably better rooting response using common table sugar as carbon source in comparison with standard sucrose.

The effectiveness of natural indirect light instead of artificial fluorescent light on plantlet growth was also tested. The results inferred that higher response for shoot and root multiplication was obtained in natural indirect light as compared to artificial fluorescent light. Also, the cultures kept under natural indirect light were green, healthier, more vigorous and strong which can help plants to establish in field conditions faster. The plantlets kept under natural light conditions also acclimatized well in the *ex vitro* conditions during hardening.

Thus, from the present investigation it can be concluded that in terms of feasibility and ease of culturing besides better response, combination 75% Isabgol + 25 % Agar can be proffered, as this combination can reduce the cost of gelling agents by upto 37%. Common table sugar can be successfully used as an alternative carbon source with cost reduction rate of 95.6 %. Also, use of natural indirect light as source of illumination carries a high potential for micropropagation of potato. All these cost effective technologies can be used at both commercial and laboratory scale.

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CERTIFICATE-IV

Certified that all the necessary corrections as suggested by the external examiner and the advisory committee have been duly incorporated in the thesis entitled **"Studies on Alternative Sources in *In-vitro* Culture of Potato (*Solanum tuberosum* L.)"** submitted by **Ms. Anamika Walia,** Registration No. **J-18-M-601.**

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