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EFFECT OF NaH_2PO_4 ON DIFFERENTIATION OF *IN VITRO PROPAGATED* BIRD NEST FERN (*ASPLENIUM NIDUS* L.)

MARIAM RAZIQ[1,2*], SYEDA KAHKASHAN KAZMI[1,3], ASMA NASIB[1,4], FAHEEDA SOOMRO[1,5] AND SAIFULLAH KHAN[1]

[1]*Biotechnology Wing, International Center for Chemical and Biological Sciences (ICCBS), University of Karachi*
[2]*Department of Biomedical Engineering, Sir Syed University of Engineering and Technology, Karachi*
[3]*Department of Biotechnology, Jinnah University for Women*
[4]*DHA Tissue Culture and Research Lab.*
[5]*Begum Nusrat Bhutto Women University Sukkur*
[*]*Corresponding author's email: mariam.raziq@yahoo.com*

Abstract

Nutrients are sources of nourishment and survival for plants. Salt stress changes the morphological, physiological and biochemical responses of plants. Bird nest fern (*Asplenium nidus)* is typically an exotic plant and belongs to the fern family. Spores of *Asplenium nidus* were used as an explant for *In vitro* propagation. After sterilization, the spores of *Asplenium nidus* were sprinkled over on MS initiation media of different strengths (1/2MS, 1/4 MS, 3/4 MS and Full MS). It was experiential that varying strength of nutrient components of the media has shown markedly influence. It was found that 90% highest number of green prothalli, larger in size were germinated on 1/2 strength MS media. Prothalli shifted onto multiplication media which correspond 1/2 strength MS medium supplemented with different growth regulators BAP (1-4 mg/L) and NAA (0.5-0.1 mg/L), 3% sugar and 0.25% w/v phytagel. It is observed that the F7 media showed an intense effect on the multiplication of plants, Plantlets multiplied vigorously were transferred onto differentiation media. *In vitro* differentiation of *Asplenium nidus* plantlets were optimized on differentiation media, MF media which corresponds with half-strength MS media supplemented with NaH_2PO_4, and MK medium supplemented with KH_2PO_4 containing BAP (2mg/L) and NAA (0.5mg/L), 3% sugar and 0.25 w/v solidifying agent (phytagel) was incorporated in each combination at pH 5.75, Media containing NaH_2PO_4 showed different degrees of differentiation as compared to the media correspond with KH2PO4. The effect of liquid and solid supporting media on the rapid growth of micro propagated fern species *Asplenium nidus* was also studied about the accessibility of nutrients and water in the culture medium. In this study, two different types of media were used each restrain half-strength MS media containing growth regulators BAP (2mg/L), NAA (0.5mg/L) and IBA (2mg/L) respectively, along with the addition of 200 mg/L NaH_2PO_4. When shoots attained a sufficient length and become differentiated, then plants were transferred into auxin-rich media. Experiments were conducted to study the effect of different types and levels of auxins in rooting media. Differentiated plantlets were consequently shifted on medium for proliferation of root. Field trials experiments were designed and plantlets of *Asplenium nidus* were acclimatized in the greenhouse.

Key words: *Asplenium nidus*, Bird Nest Fern, Prothalli, MS media, Liquid media, NaH_2PO_4, KH_2PO_4.

Introduction

Bird nest fern (*Asplenium nidus*) is the type of fern which have been traditionally used for indoor decoration but their outdoor uses are also increasing. It is found as pantropical epiphytic plant colonizes trees, rock countenances and stones in damp, tropical rainforests. The lustrous green, dainty, tongue-like fronds have wavy edges and a noticeable, practically dark midrib. They emerge from a thickly bushy crown in an outspread manner, fairly taking after a bird's home. It requires warmth and sufficient humidity (Bertrand *et al.*, 1999). *Asplenium nidus* has great medicinal and ornamental values, as described in economic significance (Khan *et al.*, 2008). A German researcher Baron Justus von Liebig, in the nineteenth century revealed that supplemented elements are fundamental for not, in reality, to establish that various components are significant for plantation. These are fundamental because a plant denied of any of these components would stop to exist." composed the expression "law of the base, "which expresses that" plants will utilize fundamental elements respect to one another, and the component that is in briefest stockpile about the rest will decide how well the plant utilizes the other supplement components (Tucker, 1999).

In 2019, Alzahrani reported that salt stress is considered to be one of the vital constraining harvest formations. Globally the salinity effects of the rural area are incidentally increasing more than 20% (Alzahrani *et al*., 2019). According to Miller in 2011 the largest part of grass family members and developed plants are frail to salt stress (Millar *et al*., 2011). The normal plant morpho-physiological and biochemical processes is affected by salt stress which is consider as vital environmental extreme (Kidokoro *et al*., 2009; Jan, *et al.,* 2016.) Proteins are initially accumulated in leaves and roots, and contribute to biomass production before breakdown and redistribution to reproductive plant parts (Ohyama, T. 1983, Basu *et al.,* 2002.)

There are sixteen elements known to be essential for normal plant growth and survival. Many of these elements are the same as those required by humans. In addition, the sixteen substance components are isolated into two primary gatherings' non-mineral supplements and mineral supplements (Tucker, 1999; Fossard, 1981). The non-mineral supplements are hydrogen (H), carbon (C) and oxygen (O), these supplements are found noticeable all around and water is utilized in a cycle called photosynthesis. Plants use energy from the sun to change carbon dioxide (CO2: carbon and oxygen) and water (H2O: hydrogen and oxygen) into plant food (starches and sugar). The other thirteen components needed by the plants are mineral supplements, which they acquire from the dirt.

These are normally isolated into two classes' macronutrients and micronutrients. A macronutrient element was defined as an essential element normally required in a concentration ≥ 0.5mmol/L (Fossard, 1981). Macronutrients are further divided into two groups i.e. primary nutrients and secondary nutrients. Functions of elements in plant metabolism and symptoms are related to their deficiencies. The primary nutrients are phosphorus (P), nitrogen (N), and potassium (K) which are normally present in blended fertilizers in the ratio of 10:10:10. Primary nutrients are consumed in the largest quantity by crops, and consequently are functional at higher rates compared to secondary nutrients as well as micronutrients for their growth and survival. The secondary nutrients magnesium (Mg), calcium (Ca), and sulfur (S) are essential in smaller amounts as compared to the primary nutrients. The most significant nitrogen nutrition mechanism appears to be NO3 assimilation. When N2fixation is insufficient to maintain adequate plant nitrogen supply, such as during a drought or other stressors [1, 8], NO3 assimilation can be used to deliver the additional N needed for plant growth. NO3 assimilation efficiency appears to be a valuable indicator of indirect selection based on its significance and association with yield (Shahzad *et al*., 2019, Khandakera *et al*., 2022). The key source for soil supplementing with calcium and magnesium is known as dolomitic lime (aglime), even though these nutrients are also obtainable from a different range of fertilizer sources. Sulfur is found in fertilizers such as magnesium sulfate and potassium such as gypsum (calcium sulfate) and elemental sulfur. It is also present in sufficient amounts and obtained from the slow decomposition of soil organic matter (Tucker, 1999).

Materials and Methods

Spore induction media: Sterilization of spores were followed by soaking the spores in water containing 0.5% NaOCl and one drop of Tween 20 (Fernandez *et al.,* 2009). After Sterlization the spores of Bird nest fern *(Asplenium nidus)* were sprinkled over1/2 MS initiation media. The formulation of half-strength (½) MS media was described in (Table 1). The initiated spores were incubated in the growth room at 22±2ºC in dark for 6 weeks, data of spores induction was recorded after each week and spores were subcultured on fresh media after 6 weeks.

Multiplication media: A flat, delicate, greenish disc of cells called Prothalli developed from germinated spores. Prothalli were transferred onto multiplication media which correspond half-strength (½) MS media medium (Murashige & Skoog, 1962) containing growth regulators BAP (1-4 mg/L) and NAA (0.5-0.1 mg/L) each respectively (Table 2) with 3% sugar and 0.25% w/v phytagel. Cultures were incubated in the growth room for four weeks at 24±2°C, at 2000 lux for a 16h photoperiod. There were five replicates per formulation. Data were recorded every week to measure the average mean length and height of plants prothalli.

Differentiation media: Plants multiplying vigorously were transferred onto differentiation media. Two different types of media MF and MK were used for *In vitro* differentiation of *Asplenium nidus* as described in (Table 3). MF media correspond with half-strength (½) MS media supplemented with NaH_2PO_4, and MK media supplemented with KH_2PO_4 both media containing growth regulators BAP and NAA with the concentration of 2mg/L and 0.5mg/L respectively, 3% sugar was added in all combinations at pH 5.75, and 0.25 w/v phytagel was added as a Solidifying agent. Cultures were incubated in the growth chamber for four weeks.

Effect of NaH_2PO_4 on Prothalli differentiation: Five different types of media were used to optimize the differentiation media as described in (Table 4). All five media correspond to half-strength MS media with varying concentrations of NaH_2PO_4, containing growth regulators BAP and NAA with the concentration of 2mg/L and 0.5mg/L, 3% sugar was added in all combinations at pH 5.75, 0.25 g w/v phytagel was added as supporting material. Cultures were incubated in the growth chamber for four weeks. Initially, a layer of liquid media was added suspended above the solid media to facilitate the fertilization process. Data of the experiment was recorded every week in terms of the average number of plants, number of leaves, average length, height and width of plants.

Effect of nutrient accessibility on the rapid growth of *In vitro* propagated *Asplenium nidus* plantlets: The effect of liquid and solid media on the rapid growth of micro propagated fern species *Asplenium nidus* was studied about the accessibility of nutrients and water in the culture medium. In this study, two different types of media were used each restrain half-strength (½) MS media containing growth regulators BAP, NAA and IBA with the concentration of 2mg/L, 0.5mg/L and 2mg/L respectively, along with the addition of 200 mg/L NaH_2PO_4. Sugar was added at 3% w/v and pH was maintained at 5.75. Two supporting materials phytagel 0.25g/L and washed clean surgical cotton was also used to study their effects on the growth and propagation of *Asplenium nidus.* Phytagel was replaced by cotton in a liquid media to study the effect on growth.

Table 1. Initiation media for spore induction.

	Full MS	3/4 MS	1/2 MS	1/4 MS
MS Macro (ml)	50	37.5	25	12.5
MS Micro (ml)	1	0.75	0.5	0.25
MS Vitamins (ml)	1	0.75	0.5	0.25
Fe-EDTA (ml)	5	5	5	5
Inositol (gm)	0.1	0.1	0.1	0.1
Sugar(gm)	25	25	25	25
Phytagel (gm)	2.5	2.5	2.5	2.5

Table 2. Multiplication Media formulated with different concentrations of BAP and NAA.

Media Code	BAP (mg/L)	NAA (mg/L)
F1	0.0	0.0
F2	1.0	0.1
F3	2.0	0.1
F4	3.0	0.1
F5	4.0	0.1
F6	1.0	0.5
F7	2.0	0.5
F8	3.0	0.5
F9	4.0	0.5

Table 3. Effect of NaH_2PO_4 and KH_2PO_4 on plantlets differentiation.

Media code	NaH_2PO_4 (mg/L)	KH_2PO_4 (mg/L)
Mo	0	0
MK	0	160
MF	160	0

Table 4 Different concentration Na H_2PO_4 on differentiation of *Asplenium nidus.*

Media code	NaH_2PO_4 (mg/L)
MF0	0
MF1	150
MF2	175
MF3	200
MF4	225

Optimization of salt supplemented media: Several factors like salt concentration of the basal medium, type and concentration of auxins and cytokinins affect the *In vitro* propagation of plants. However, in some species, different supplements were additionally required for differentiation and growth of *In vitro* propagated plants as in the case of fern species. To investigate the effect of two different salts on differentiation of *Asplenium nidus* gametophytes, NaH_2PO_4 (Sodium dihydrogen phosphate) 160mg/L and KH_2PO_4 (Potassium dihydrogen phosphate) 160 mg/L were used as a salt supplement along with 1/2 MS basal medium.

Optimization of root proliferation media: Differentiated plantlets were accordingly shifted on solid medium for root proliferation. Half-strength (½) MS media with various combinations of growth regulators (cytokinin and auxins). In this study cytokinin, BAP 2mg/l and three different types of auxins IBA, IAA and NAA were used in different concentrations 0.5mg/l to 3.0mg/l. 0.5mg/l respectively, along with NaH_2PO_4 200 mg/L, to optimize the root proliferation media. Carbohydrates source was 3%, pH 5.75 was adjusted, and 0.25 w/v phytagel added as supporting material.

All data generated based on the number of roots induced and increment in length over six weeks, this study aimed to optimize the suitable media for root induction.

Acclimatization: Field trials experiments were conducted to select the best supporting material for acclimatization of *Asplenium nidus*.

Statistical Analysis

Statistical analysis of data was generated in which the significant difference is measured by statistical tool analysis of variance using Minitab software version 11 to analyze the difference in prothalli induction, multiplication, differentiation of *In vitro* propagated plantlets of *Asplenium nidus*.

Result and Discussion

Induction of spores: After sterilization, the spores were sprinkled over initiation media. It was observed that varying strength of nutrient components of the media have shown markedly influence. The inoculated spores of *Asplenium nidus* in different strengths of MS-basal media became bloated and germinated. After 15-20 days, of incubation, the heart-shaped prothalli and gametophyte clumping which was 1cm in size were observed. It was found that 90% highest number of green prothalli, large were germinated on 1/2 strength MS media, 60% Prothalli germinated on 1/4 strength MS media, 80% prothalli were germinated on 3/4 strength MS media in which few of the prothalli turned brownish and died after 90 days. 70-80% prothalli smaller in size was germinated on full strength MS media (Fig. 3b). To optimize the media, a series of experiments were designed in which 1/4, 1/2, 3/4 and full-strength MS-Media were compared. Analysis of variance revealed a highly significant difference among the prothalli induction media (p<0.001). It was observed that spore germination frequency was lower in full strength MS media than 1/2 strength MS media, the large green clumps of prothalli germinated on 1/2 strength MS media. (Fig. 3c) However, with full and 3/4 strength MS media the spores were germinated into clumps of prothalli which were small in size as compared to1/2 strength MS media. The number of prothalli that developed per spore varied significantly. Lower the concentration of salt in the media increases the prothalli formation and size of the prothallus. On 1/2 MS the large clumps of prothalli were germinated, this variation between different strengths of media could be attributed to the variation in salt strength.

Multiplication of prothalli: Micropropagation has turned into a consistent and usual methodology for extensive rapid plant propagation, which depends on plant cell, tissue and organ culture on distinct tissue culture media under aseptic conditions (Filiz *et al.*, 2009). In this stage of study to test the promontory effect of cytokinin and auxin on shoot multiplication, germination of spores into prothalli, they were transferred on multiplication media (Table 2). In the series of investigations, three different types of parameters were assessed on 10 different media formulations. There were five replicates for each formulation. Data were recorded each week for the average mean length, width and height of plants prothalli.

Analysis of variance revealed a highly significant difference among multiplication media (p<0.001). It was observed that minimal growth was recorded at F_1 media which was control, whereas in F1-F5 media it was observed that on raising the concentration of BAP the increase in the number of axillary shoots and the length, width and height of shoots per prothalli was also increased up till 0.57cm, 0.55 cm and0.57 cm respectively. Moreover, in F6 media the increase in the number of plantlets was also noticed. The F6 media contain BAP 1.0 mg/L and NAA 0.5 mg/L (Table 5). It was also observed in F6 media that the concentration of NAA corresponded with the increase in the number of plantlets but there is no significant increase in length, width and height of axillary shoots (Khan *et al.*, 2008). However, it was experiential that in F8-F9 media where further increase in the concentration of BAP, along with NAA 0.5 mg/L was taken place. It was concluded that prothalli started to show browning which confers the detrimental effect of the dose (Higuchi & Amaki, 1989).

Whereas F7 media showed an intense effect on the multiplication of plants. It was also observed that the significant increase in the multiplication of plants along with an increase in length, width and height of plants and formation of shoot buds proved to be optimal in F7 media which contains BAP 2.0 mg/L and NAA 0.5 mg/L.

In 1999 Bertrand *et al*., stated that for the survival of plants the medium should be consummate by auxins and cytokinins and it is also influenced by the ratio and concentration of growth hormones. According to Fernandez & Revilla in 2003, it is affirmed in various reports that the growth and multiplication rate of plants were directly influenced by the suppression, enhancement and availability of growth regulators. It was also observed that the NAA plays a very crucial role in the production of axillary shoots in the *In vitro* propagation of *Asplenium nidus*. It was noticed that when BAP was used in combination with NAA, there was a markedly increment in the number of shoots per prothalli. This increase was also seen in the length of the shoots. Thus, from the result obtained, it can be concluded that the optimum media for maximum shoot proliferation was F7 media containing 2 mg/L BAP and 0.5 mg/L NAA.

Differentiation of *Asplenium nidus* fronds: Several factors such as salt concentration of the basal medium, type and concentration of auxins and cytokinins are affected on *In vitro* propagation of plants, whereas in some species different supplements were additionally required for differentiation and growth as in the case of fern species. In order to investigate the effect of two different salts on differentiation of *Asplenium nidu* gametophytes, NaH_2PO_4 (Sodium dihydrogen phosphate) 160mg/L and KH_2PO_4 (Potassium dihydrogen phosphate) 160 mg/L were used as salt supplements along with 1/2 MS basal medium. Three different types of media Mo, MF and MK (Table 3) were used for the differentiation of prothalli. In this experiment, it was observed that Bird nest fern *(Asplenium nidus)* is one of the species of fern which required NaH_2PO_4, (Sodium dihydrogen phosphate) to promote the differentiation process. The data was analyzed by taking the mean of 5 replications per treatment on three different types of media whereas, Mo media which is control showed 3% differentiation in plantlets, In MK media 63% plantlets were differentiated into fron.ds, whereas on MF media showed a significant increase in the average number of differentiated plantlets i.e. 90% as shown in (Fig. 4b, c).

In various studies, it is concluded that the stress due to different concentrations of salt-affected on behavior and responses of plants. According to Maggio (2002) these morphological, physiological and biochemical changes of plants are evident on the molecular level by gene expression pattern (Behzad, 2008).

Liu *et al.*, (2006) conducted a study to investigate the effect of salt stress and water stress at the cellular level in this study *In vitro* propagated calli cultures were used to look into the physiological and biochemical mechanism affected by environmental stress. (Jantaro *et al.*, 2003).

Therefore, it is analyzed that the type and concentration of salt significantly affect the differentiation of the *Asplenium nidus* frond. In comparison between two different salts, the media containingNaH_2PO_4 showed a significant increase in differentiation process as 90% of plantlets become differentiated whereas the media containing KH_2PO_4 showed differentiation in 60% plantlets which is lower than NaH_2PO_4, on the other hand, media devoid of salt supplements showed 3% differentiation this indicating the importance of salt supplement in the differentiation of *Asplenium nidus* fronds by Sharma & Thorpe, (1989).

Table 5. Response of *In vitro propagated Asplenium nidus* on Multiplication media.

Media codes	Average length ±SE(cm)	Average width ± SE(cm)	Average height ± SE(cm)
F1	0.23 ± 0.063	0.27 ± 0.029	0.30 ± 0.026
F2	0.47 ± 0.030	0.42 ± 0.024	0.43 ± 0.053
F3	0.53 ± 0.041	0.45 ± 0.023	0.52 ± 0.038
F4	0.56 ± 0.020	0.51 ± 0.041	0.56 ± 0.030
F5	0.57 ± 0.026	0.55 ± 0.095	0.57 ± 0.023
F6	0.59 ± 0.028	0.60± 0.054	0.61 ± 0.018
F7	**0.80 ± 0.011**	**0.82 ± 0.039**	**0.83 ± 0.007**
F8	0.71 ± 0.041	0.70 ± 0.033	0.73 ± 0.008
F9	0.63 ± 0.036	0.63 ± 0.026	0.66 ± 0.017

Table 6. Effect of NaH_2PO_4 on differentiation of *In vitro* propagated *Asplenium nidus* plantlets.

Media codes*	The average number of plants ± SE (cm)	The average number of leaves ± SE (cm)	The average length of plants ± SE (cm)	The average width of plant ± SE (cm)	The average height of plant ± SE (cm)
MF0	2.065 ± 0.200	2.255 ± 0.122	1.516 ± 0.023	1.568 ± 0.017	1.341 ± 0.026
MF1	2.296 ± 0.180	2.736 ± 0.129	1.549 ± 0.022	1.661 ± 0.016	1.529 ± 0.029
MF2	2.337 ± 0.214	2.878 ± 0.144	1.561 ± 0.028	1.612 ± 0.021	1.476 ± 0.028
MF3,	**2.625 ± 0.103**	**3.047 ± 0.136**	**1.618 ± 0.051**	**1.720 ± 0.015**	**1.617 ± 0.082**
MF4	2.201 ± 0.256	2.946 ± 0.143	1.555 ± 0.027	1.655 ± 0.016	1.449 ± 0.024

To optimize the differentiation media different concentrations of NaH_2PO_4 were used. A set of experiments containing 5 different types of combinations in replication were analyzed, which correspond to MF0, MF1, MF2 MF3 and MF4 (Table 4). After 4 weeks of the incubation period, it has been experiential that axillary shoots emerged from the green masses of cell profuse to the differentiation of fronds, after induction on the differentiation media. Analysis of variance showed significant differences amongst differentiation media (p<0.001). It was observed that all media containing NaH_2PO_4 illustrate diverse degrees of differentiation as shown in (Table 6) which reflects that NaH2PO4 acts as a vital element in the differentiation stage of *Asplenium nidus*. The expansion in axillary shoots was also experiential up to a certain concentration of NaH_2PO_4 200 mg/L at its maximum at the same time as the abridged growth was also observed greater than this concentration (Table 6). Kyte & Kleyn (1996) stated that the growth and maturation of plants lead to an esteemed level of differentiation of shoot buds which depend upon the increased level of NaH_2PO_4.

The effects of liquid and solid media on subcultured *Asplenium nidus* showed vigorous shoot regeneration. Plants were also investigated. It was observed that the average number of plants per jar was 16 on liquid medium while the average number of plants per jar were nine on medium containing agar solid media. Whereas the average length of shoot produced on liquid media per jar was 1.4 cm compared with solid media having 0.86 cm. It is obvious that liquid medium showed a significantly high degree of shoot induction and showed the best response towards multiple shoot regeneration. Response of rapid growth of *In vitro* propagated *Asplenium nidus* studied on liquid and solid media was shown in (Fig. 2). Data for each set of the experiment were recorded weekly, analysis of variance revealed a highly significant difference among the solid and liquid supporting media (p<0.001). In liquid medium, supporting material, i.e., cotton fiber shows drastic effects on the augmentation of *Asplenium nidus* plantlets (Fig. 5a,b). Making use of cotton fiber improved the accessibility of nutrients and also has superlative absorptive characteristics for phenolic compounds released by inoculated plantlets. The phenolic and other toxic compounds released by the plants in the cotton fiber system are immediately diluted and converted into a lesser amount of toxic. It has been also reported in the study conducted by Singha in 1982 and Debergh in 1983 that one of the major reasons for enhanced growth of plantlet in a media ensuing with liquid or low concentration of phytagel is due to the excessive accessibility of water and nutrients.

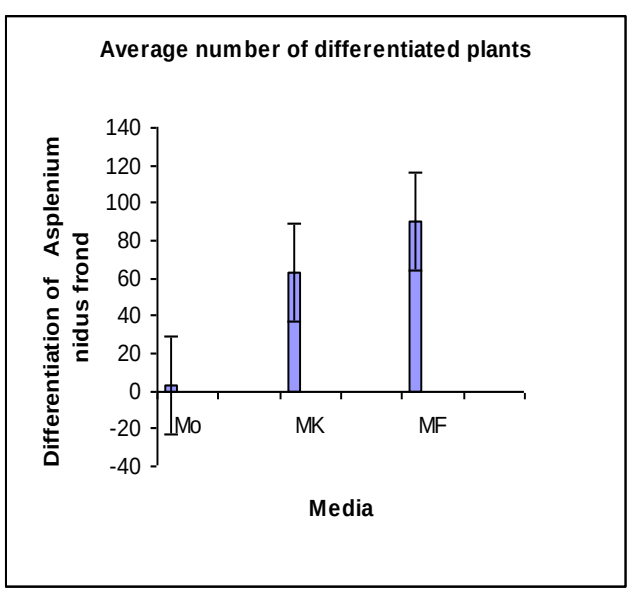

Fig. 1. Effect of salt stress on plantlet differentiation.

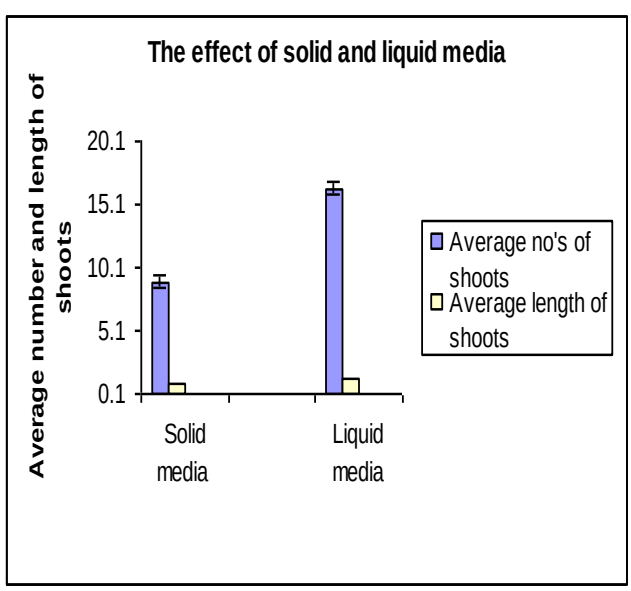

Fig. 2. Effect of solid and liquid media on mass propagation.

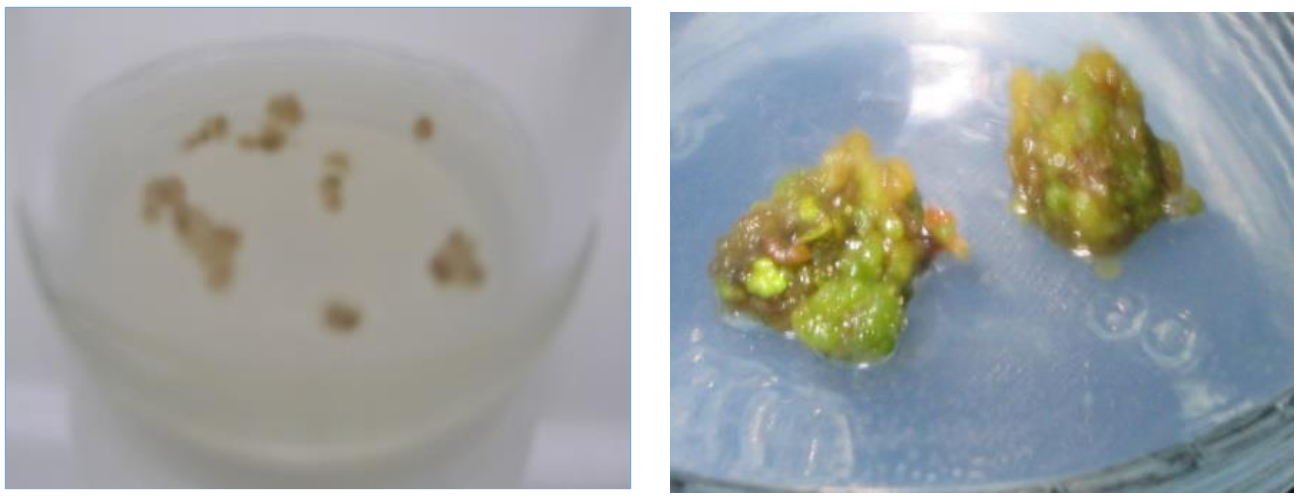

Fig. 3. Inoculation of Explant (A) Spores sprinkled on Induction media (B) Prothalli formation.

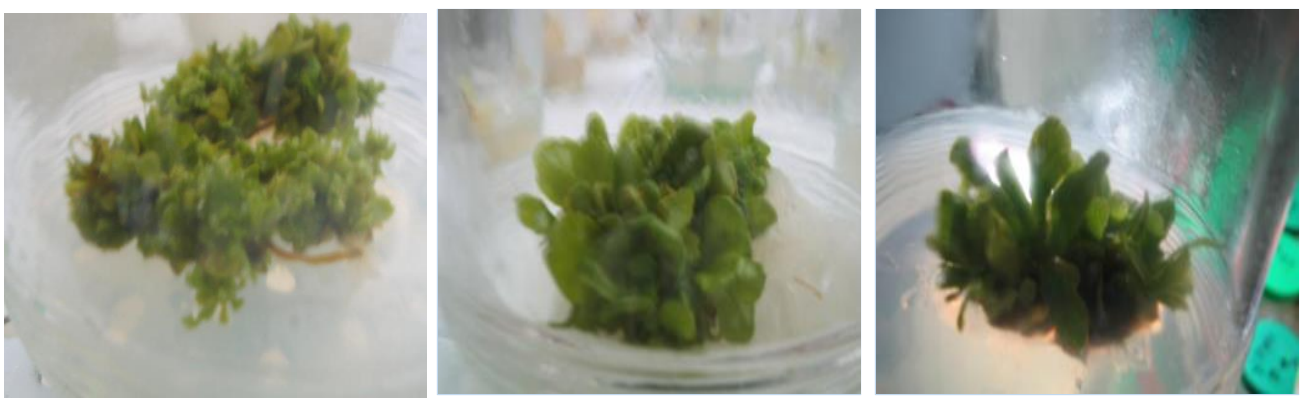

Fig. 4. Multiplication and differentiation of *Asplenium nidus* L. (A) Multiplication of *Asplenium nidus* L. (B) Plantlets becomes Differentiated. (C) Differentiation of *Asplenium nidus* L.

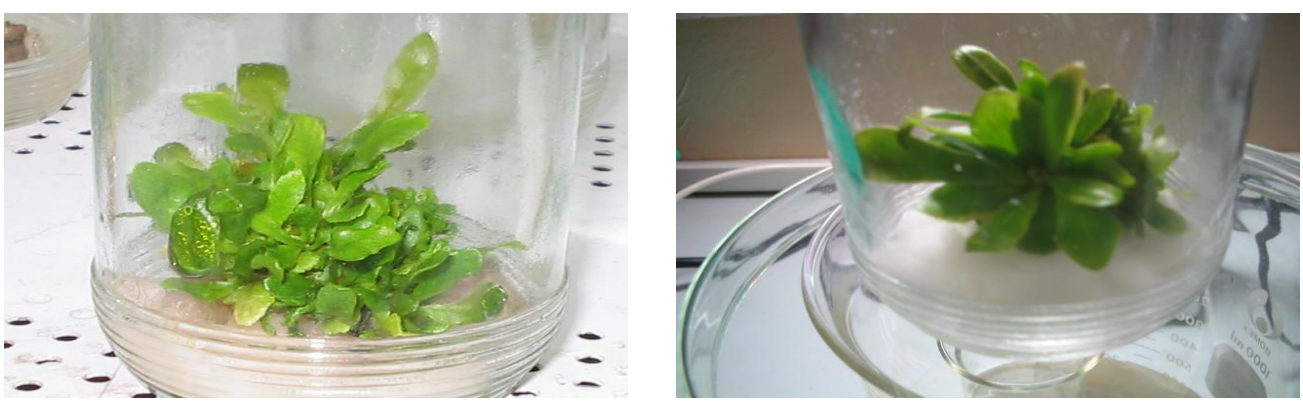

Fig. 5. *In vitro* propagation of *Asplenium nidus* L. plantlets on liquid media. (A) *In vitro propagation* on liquid media. (B) Differentiation of plantlets on liquid media.

Root proliferation: Differentiated plants were consequently transferred on medium for root formation, in these study three different types of auxins IBA, IAA and NAA were used in different concentrations 0.5mg/L to 3.0mg/l. All data was generated on the basis of the number of roots induced and increment in length over a 6 week period the optimum media containing IBA at a concentration of 2.00mg/l which resulted in an overall rich yield of roots with maximum length in a short span. Singh *et al*., (2014) reported that IBA increased interfascicular cambium dedifferentiation, promoted callus development, and generated a large number of cells that differentiated into root primordia and root cells. It is also reported the breakdown of starch and the decrease in levels of amyloplast, which encourage rooting in stem cuttings of duranta golden, are increased at optimal levels of exogenous IBA treatment. When compared to the control treatment, Vitis vinifera generated the highest roots at the optimal IBA concentration. (Shahzad *et al.,* 2019, Khandakera *et al*., 2022) after healthy production of roots the *In vitro* plants were ready to be shifted out in situ for acclimatization.

Conclusion

In this study, it is concluded that the NaH_2PO_4 act as an essential element in the differentiation of Bird nest fern *(Asplenium nidus)* fronds. Tucker (1999) reported about the physiological responses of plants, it is stated that only essential elements in proportion utilized by plantlets and the element that is in minimal supply brought in proportion to the others will instigate how healthy the plant utilizes the supplementary nutrient elements. In this study, the influence of salt stress was perceived by using a varied combination of NaH_2PO_4 which showed the drastic effect on the differentiation of shoot buds and also effect on plant maturation and growth. Different physiological and biochemical progressions in plants are affected by salt stress which results in the alteration of some metabolic pathways (Liu and Van Staden, 2001).

In this study, cotton fiber proved to be an economical supporting medium, in which inoculated plants showed vigorous growth and the maximum number of plantlets. In the comparison between solid and liquid media, it was evident that the cotton fiber showed a considerably high degree of shoot induction and showed the best response towards multiplication due to its association with increases in their organic nitrogen and sugar content, which represents the nutrient assimilation, was preferential in liquid media. The organisation pattern of plantlets are also depends on the growth regulators supply and the contact period between explants and growth regulators, (Fernandez *et al*., 1997b). Thus it was concluded that the subcultured *Asplenium nidus* plantlets showed the maximum number of shoots as well as elongation with cotton fiber in lia quid medium. Whereas the root proliferation was also studied by optimization of rooting media comprises different types and levels of auxins in rooting media. Differentiated plantlets were consequently shifted on medium for proliferation of root. Field trials experiments were designed and plantlets of Bird nest fern *(Asplenium nidus)* were acclimatized in greenhouses.

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