ENHANCED GROWTH AND DEVELOPMENT OF COMMERCIALLY IMPORTANT DENDROBIUM WHITE ORCHIDS BY MICROPROPAGATION AND HYDROPONICS

SYEDA KAHKASHAN KAZMI[1]*, SABEEN NADEEM[1], AISHA MEHAK[1],AYISHA AMANULLAH[1], BEENA NAQVI[2] AND AMEER AHMED MIRBAHAR[3]

[1]Department of Biotechnology, Jinnah University for Women, Karachi, Pakistan
[2]Pakistan Council of Scientific and Industrial Research Karachi
[3]Department of Botany, Shah Abdul Latif University Khairpur
Corresponding Author's E-mail: kahkashank1@gmail.com

خلاصہ

Orchidaceae600-800نسلوں اور 25,000-35,000انواع کے ساتھ دوسرا بڑا پودے کا خاندان ہے۔ آرکڈ کی خوبصورتی انسانوں کو مسحور کرتی ہے اور دنیا بھر میں ایک ارب ڈالر کی مارکیٹ بناتی ہے۔ اس مطالعے کا مقصد تجارتی لحاظ سے اہم آرکڈ پودوں کی کم وقت میں پیداوار کے لیے کملاگت سے موثر نمو کا طریقہ قائم کرنا تھا۔ آرکڈز کی نشوونما پودوں کے ٹشو کلچر کی بنیاد پر مائکروپروپیگیشن کے ذریعے قائم کی گئی تھی اور ہائیڈروپونک نظام کے ذریعے صحت مند نشوونما کے لیے موزوں پائی گئی تھی۔ اس تحقیق کے تحت آرکڈز کی شوٹنگ کو رپورٹ شدہ میڈیا پر حاصل کیا گیا اور آرکڈز کی جڑوں کو بہتر بنایا گیا۔ آرکڈ جڑوں کی نشوونما کے لیے *0.8mg/l* سے *1mg/l* تک NAA کے مختلف ارتکاز کا تجربہ کیا گیا۔ جڑوں کی سب سے زیادہ تعداد (12) 0.6 mg/l NAA پر حاصل کی گئی جبکہ جڑوں کی سب سے زیادہ لمبائی (*1.8* cm) NAA *0.8mg/l* پر دیکھی گئی۔ اس مطالعے میں آرکڈز کی آبیاری اور نشوونما کے لیے غذائی اجزا کے سپرے کا تجربہ کیا گیا۔ تین مختلف قسم کے غذائیت کے مہلول سپرے جیسے NPK، Hoaglandاور MS میکرو اور مائیکرو کا تجربہ کیا گیا۔ نتائج بتاتے ہیں کہ تین غذائیت کے سپرے میں سے، N 20:20:20PK (نائٹروجن، فاسفورس، اور پوٹاشیم) تجارتی لحاظ سے اہم آرکڈز کے پودوں کی مؤثر نشوونما کے لیے بہترین غذائیت کا سپرے تھا۔ لہذا، یہ پروٹوکول تجارتی لحاظ سے اہم آرکڈ پودوں کے مائکروپروپیگیشن کے لیے سب سے زیادہ موثر پایا گیا۔

Abstract

Orchidaceae is the second largest plant family with between 600-800 genera and 25,000-35,000 species. Thebeauty of the orchidsmesmerizes human beings and creates a billion-dollar market worldwide. The aim of this study was to establish the cost-effective growth method to produce commercially important orchid plants in shorter time duration. The growth and development of orchids were establishedthrough plant tissue culture based micro propagationand were acclimatized for healthy growth througha hydroponic system.Under this study the shooting of orchids was obtained on the reported media androoting of orchids was optimized. Different concentrations of NAA (naphthaleneacetic acid) from 0mg/l to 0.8mg/l were tested for the development of orchid roots. The highest number of roots (12) was obtained at 0.6mg/l NAA whereas highest lengths of roots (1.8cm) were observed at 0.8mg/l NAA. In this study nutrient sprays weretested for the acclimatization and growth of orchids. Three different types of nutrient solutions like NPK, Hoagland and MS Macro and Micro were tested. Results indicate that among three nutrient solutions, NPK 20:20:20 (Nitrogen, Phosphorous, and Potassium) was the best nutrient solution for the effective growth and development of commercially important orchids. Therefore, this protocol was found to be most effective for the micro propagationof commercially important orchid plants.

Keywords: Mass propagation; Plant growth regulators; Rooting media; Carotenoid; Chlorophyll; Orchids.

Introduction

Orchids have a high reputation among ornamentals and are believed to be some of the most significant global pot plants and cut flowers. In 2022, Orchids were the world's 3955th most traded product, with a total trade of $199 million (OEC, 2024). The pure beauty of orchids has mesmerized people since ancient times. With 600-800 genera and 25,000-35,000 species, Orchidaceae is considered as a second largest family of flowering plants (Arditti, 1992). Orchids are found naturally all around the world except warm deserts and subzero Antarctica. Their greatest diversity is found in tropical and sub-tropical areas (Cardilea et al., 2020).Previous studieshave stated that conventional methods have some limitations because in crossbreeding seed germination is correlated to the efficiency and success of the method (Bartareau, 1995).After a core study of the developmental aspects and germination procedures, it has been proved thatdistant hybrid seeds play a vital role toinitiate an efficient germination system. When hybrid seeds are obtained, anappropriate cultivation technique is needed to keep the population secure and growing (Oliveira et al., 2019).In vitro propagation through plant tissue culture is one of the most crucial breeding methods for orchids, as orchid seeds are difficult to proliferate or propagate in the natural environment. The cost of orchids in Pakistan's local

market is high so it is feasible to make their clones and develop new varieties with the help of plant tissue culture technique. There is a contrast that exists between crossbreeding and selection breeding. Selective breeding uses the natural variants of existing species as the native material for alternativesin the breeding process (Osadchuk, 2020) and (Li et al., 2021).

The term plant tissue culture refers to*in vitro* culture of a plant or part of a plant in a defined synthetic medium, under a meticulous environment (Loyola-Vargas & Ochao-Alejo, 2018). Initially plant tissue culture techniques were developed to understand basic plant biologybut now this tool is used for several important applications. These applications include micro propagation of various plant species (Loyola-Vargas & Ochao-Alejo, 2012). Micro propagation is the clonal propagation of plants in a closed container, under aseptic conditions. Plants are grown on culture media containing nutrients and growth regulators under controlled conditions, which is defined as *in vitro*, meaning 'in glass'. Soil-grown plants, in comparison, are defined as *in vivo* which means 'in the living'or refers to the plants being grown in a natural environmental condition. Micro propagation offers better opportunities and future to produce important plant based phytopharmaceuticals. Hence, over conventional methods of propagation,micro propagation has tremendous advantages (Arditti, 1992).

The hydroponics system is a soilless culture**.**In this system vegetables and fruits grow without soil or in little physical spaces. Plants are grown in water or in low-cost substrates such as coconut husk, rice husk, gravel, coco-peat, charcoal and many more. Many plants grow using this system such as beans, tomatoes, lettuce; strawberries etc (Nalwade & Mote, 2017).In the hydroponics system, plant growers can easily control the supply of nutrients, by adjusting the nutrient solution concentration. Controllingthe nutrient supply affects the salt and water relationship of plants and influences the growth of plants (Caruso *et al.,* 2011). After plants growth under controlled environment there is less chances of pests attack as compared to the plants that grow in the open environment (Stone, 2014). In this study nutrient sprays were tested on orchid plants during the acclimatization stage and coconut peat was used as a support material for the plants.

Nowadays, the growth of orchids is more than an industry; it has become a worldwide business(Griesbach, 2002). Orchid plants are marketed in the form of potted plants as well as in the form of cut flowers. Thailand, Taiwan, UK, New Zealand, Brazil, Japan, Italy are the largest exporter of potted orchids while these plants are mainly imported into United States. The orchid industry contributes extensively to the economy of various countrieswith various orchid varieties, such as Dendrobium, Phalaenopsis, Oncidium and Cymbidium,these orchidsare supplied worldwide (Chugh *et al.,* 2009). The World's most commercially important industries are creating a multibillion-dollar market by orchid production. Orchids are a group of highly fascinating plants with commercial and medicinal qualities that have been used for the medication in many parts of the world for a variety of disorders (Bastin&Jeyachandran, 2015). Now, with the application of agricultural biotechnology, orchids are the most attractive and significant plants that can be cloned through tissue culture technique. Dendrobium, accounts for around 80% of the overall tissue cultured orchids.

Orchids are among the finest of flowering plants and have a very high price in overseas markets. Today's orchid industry relies on micropropagation, as orchid plants don't develop spontaneously, and their seeds are deficient in a functional endosperm. In tissue culture, the appropriate growth and protection of orchid plantlets need a peripheral supply of hormones under controlled aseptic conditions (Mohan Raj, 2018). During the last 45 years, not only hasthe expeditious and extensive production of orchids been achieved, but the ex-situ conservation of orchid plants has also been carried out by using tissue culture techniques. Various methods have been employed for the extensive production of several orchid plants by in vitro culture of different plantlets; it may include tips of roots and shoots, buds, rhizomes, and flower stalk nodes. For micropropagation, revival from genetically engineered mother plants is worthwhile compared to seed culture, because of the year-round availability of mother plants. Now it's important because of the immense consistency of micro propagated plants; the future marketplace of orchid plants would rely on micro propagated plants and not on vegetatively propagated plants (Chugh et al., 2009). In this study, micropropagation of industrially important Dendrobium white orchids was conducted**.** The shooting was achieved on the reported media by Khan et al. (2010). Rooting media for the growth of the orchid was optimized by using different concentrations of NAA plant growth regulator. After the successful micropropagation of orchids, the nutrient sprays were applied to plantlets for acclimatization and healthy growth to obtain good flower yield in the greenhouse.

Materials and Methods

***Initiation of Explant*:** For initiation of orchids, the procedure of induced pollination was used. The petals of a Dendrobium white were gently opened, and the white shaft-like petal column was pinpointed at its middle point. The

column was lifted softly and analyzed for the anther cap at the bottom of the upper end. Sterilized toothpicks were used to move the hatch aside for easy access to pollen inside. After three weeks, the pods were separated from the plant and washed three times withfour drops of Tween 20. Pods were further sterilized with a 20% bleach solution, and then washed three times with autoclaved distilled water. Finally, a longitudinal cut was made in a petri plate with a scalpel and the fertilized embryo was removed with the scalpel and sprinkled on ½ MS media jars. Then, jars were placed for 25 days in a growth room at 25±2°Cwith a 6/8-hour photoperiod.

***Multiplication*:** After absolute initiationon half MS Medium (Murashige & Skoog, 1962) as shown in Fig. 1 the germinated plants ofDendrobium white were shifted on multiplication media for in vitro multiplication experiment.The media used for multiplication was reported by Khan *et al*., (2010).

***Rooting*:** After 25 days of initiation, shoots of chrysanthemum were separated from initiated cultures and transferred on hormonal combination medium with ½ MS as basal medium along with different concentrations of BAP from 0 mg/L to 4 mg/L, for in vitro multiplication. Rooting was easily obtained on ½ MS media along with 0.5gm/l activated charcoal in 25 days. After three months of initiation and multiplication process, the plants of orchids were dissociated from multiplication cultures and then shifted on rooting experimental media with MS as a basal medium along with different concentrations of NAA (0mg/l, 0.2mg/l, 0.4mg/l, 0.6mg/l and 0.8mg/l) along with 0.2gm/l activated charcoal for 30 days.

***Acclimatization*:** For acclimatization in a growth room,*in-vitro* grown plants were transferred to coco peat first for one month as shown in Fig. 1(G). Then they were placed in coconut husk with below layer of charcoal for nutrient sprays treatment Fig 1(H). Plants were kept under humid and semi-controlled environment in the green house. The sprays of Hoagland (Hoagland & Arnon, 1950), 20:20:20 NPK (Nitrogen, Phosphorous, Potassium) and MS macro and micro solutions of (Murashige & Skoog, 1962) medium were tested three times in a week for two months. All experiments were performed in triplicates.

***Estimation of Chlorophyll and Carotenoid Content*:** The determination of chlorophyll and carotenoid content of orchid plants were performed that were sprayed by three nutrient solutions like Hoagland solution (Hoagland &Arnon, 1950), 20:20:20 NPK (Nitrogen, Phosphorous, and Potassium) and MS macro and micro (Murashige&Skoog, 1962). Chlorophyll and carotenoid contents were estimated by preparing plant extracts of fresh leaves, using 80% acetone. Absorbance of plant extracts were measured using Spectrophotometer at three different wavelengths 480nm, 645nm, and 663nm. Based on the absorbance level total chlorophyll and carotenoid contents were calculated by using Arnon method (Arnon, 1949).

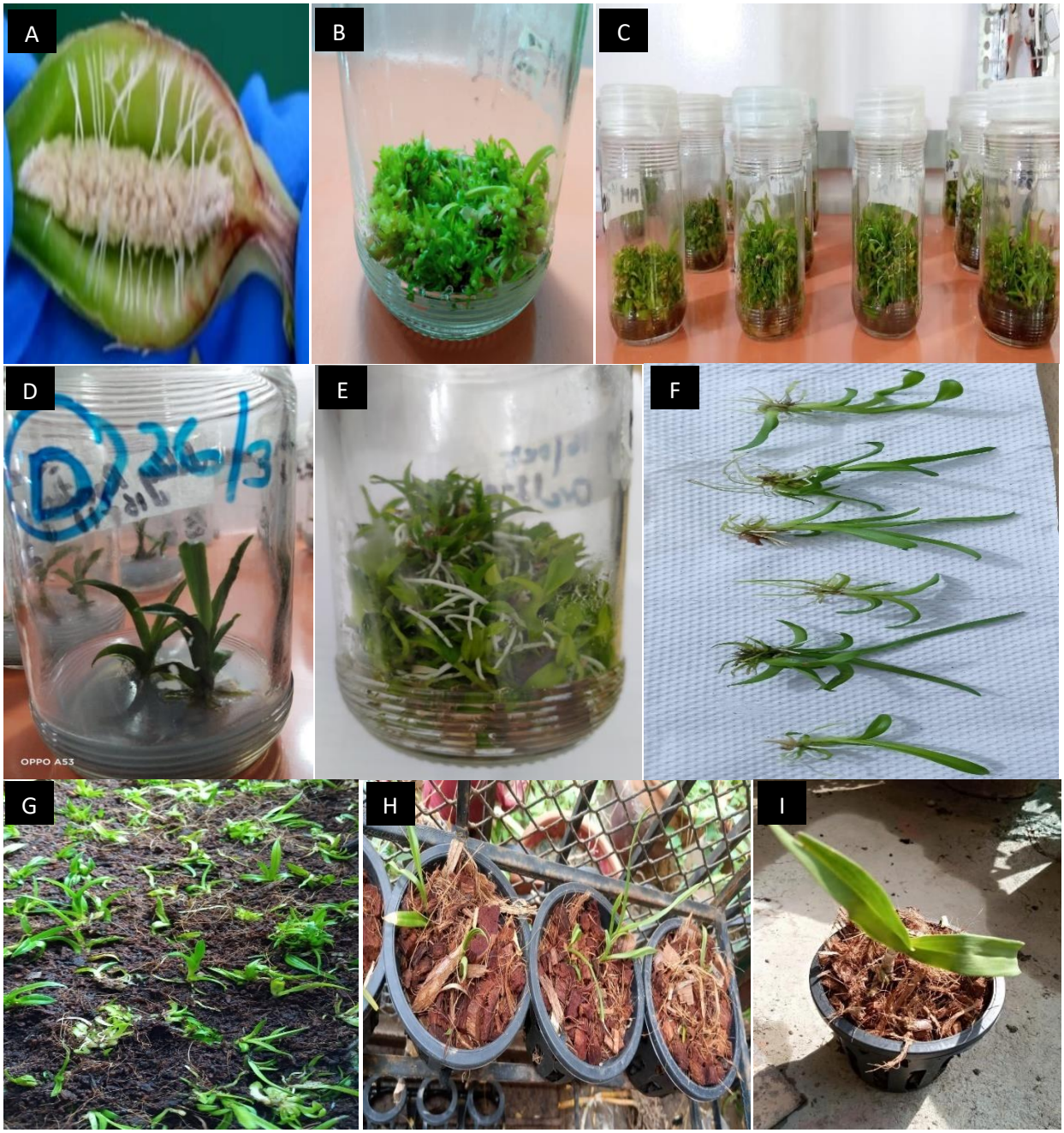
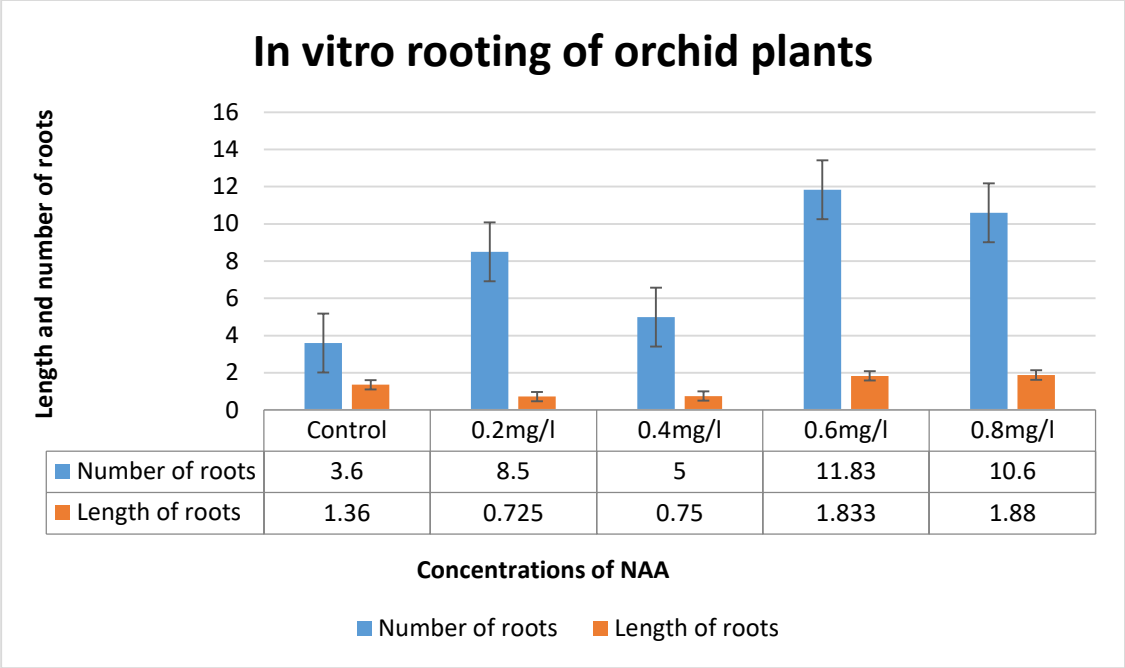
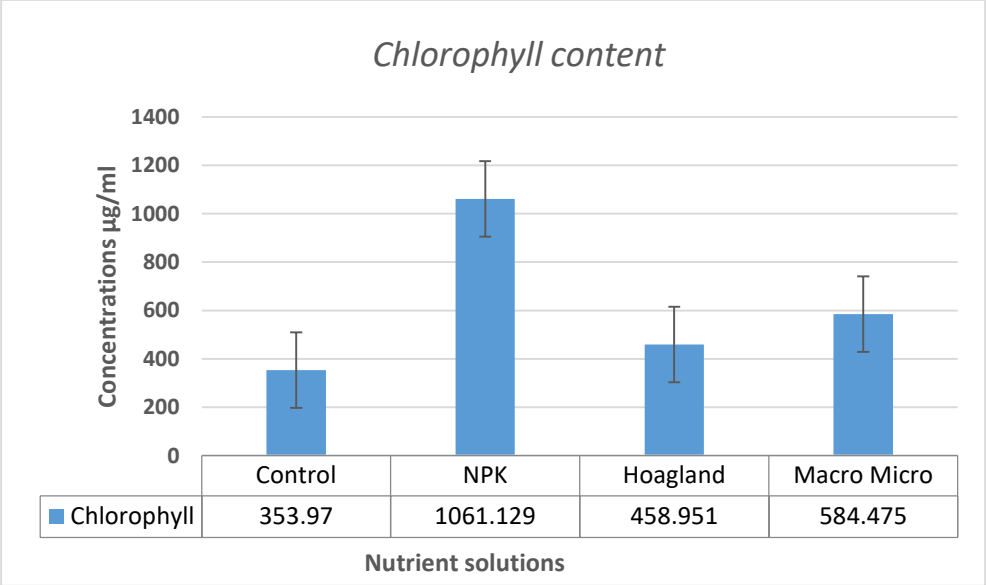


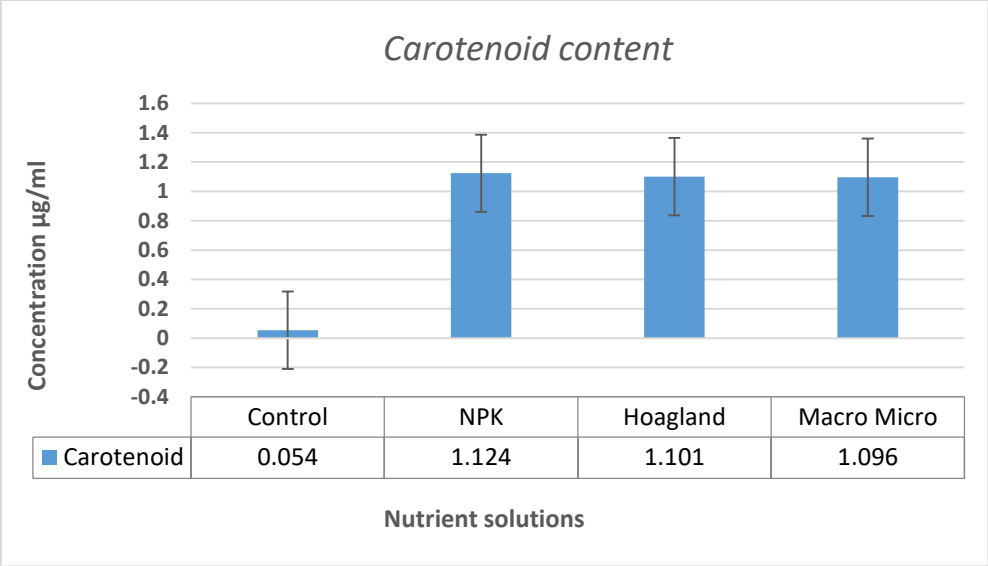
Fig. 1: (A) Pollinated ovules in a seed pod (B) Initiation of orchids (C) Multiplication of orchids (D) Shooting of orchids(E) Rooting of orchid plantlets (F) Orchids ready for acclimatization on coco-peat (G) Acclimatization stage on coco-peat (H)Hydroponics based growth of orchids on initial stage (I) Full growth of orchid plants on hydroponics.



Graph. 1. Number and length of roots at different NAA concentrations.



Graph. 2. Effect of nutrient solutions on chlorophyll content of orchids.



Graph. 3. Effect of nutrient solutions on carotenoid content of orchids.

Results and Discussion

Orchid plantlets were successfully initiated from pods developed after induced pollination. Multiplication was achieved on reported media by Khan *et al*., (2010). Multiplied shoots were also elongated on the same media before transferring to rooting experimental media Fig. 1 (A, B, C, D). After successful initiation and multiplication, the media for the rooting was optimized. It was observed that medium containing 0.6mg/l NAA showed highest number of roots (12) among other NAA concentrations. Whereas the highest length of roots (1.8cm) was obtained on medium containing 0.8mg/l NAA concentration as shown in Fig.1 (E, F) and Graph 1.All the plantlets survived after 30 days under nutrient sprays treatment. It was observed that NPK (20:20:20) showed more positive effect on the growth of plants and produced higher chlorophyll and carotenoid contents as compared to the Hoagland and MS macro and micronutrient solutions. Based on absorbance reading on different wavelengths 480nm, 645nm, and 663nm, plants those were sprayed with NPK solution showed the higher concentration of chlorophyll (1061 µg/ml) and carotenoid (1.124 µg/ml) content as shown in Graph 2 and Graph 3.

Micro propagation system can be only beneficial when it is successful in transfer of plants from *in vitro* conditions to *ex vitro* where they can survive under the harsh environmental conditions. In the present study for the efficient micro propagation of orchids, reported media by Khan *et al*., (2010) was used which contains BAP (2.0 mg/l), IBA (1.0 mg/l), IAA (1.0 mg/l), Peptone (1.0 mg/l) and sugar (60 g/l). Fast multiplication and elongation of shoots in early and later stages of plants development was achievedon this media, these results are similar with the findings of (Talukder*et al.*, 2003, Jang *et al.*, 2019) they reported use of plant growth regulators in different concentrations and had notable effect on shoot proliferation of Dendrobium species.For roots development, MS media along with (0-0.8 mg/l) concentrations of NAA plant growth hormone supplemented with 0.2mg/l activated charcoal was used. Maximum roots were obtained on media containing 0.8mg/l NAA concentration. This result is in agreement with one of the previous studies reported by (Arditti & Ernst, 1993) in which they concluded that different concentrations of NAA exert a differential influence on mother plant.In this study using various concentrations of NAA, orchid showed the highest number of roots on MS medium along with 0.6mg/l NAAsupplemented with 0.2 mg/l activated charcoal (Graph 1). While MS as a basal medium containing 0.8mg/l NAA along with activated charcoal 0.2 gm/l, was found to enhance the highest length of roots in orchids (Graph 1). These findings agree with (Paudel & Pant, 2012) whoreported that (0.5mg/l to1.0 mg/l) NAA was found to be the best rooting medium in Esmeralda clarkei Rchb orchids in contrast to Dendrobium nobile where it was noticed that increase in the concentration of NAA up to 3.0 mg/l, badly effect the rooting of plants(Asghar*et al.*,2011).

In the previous literature, effect of nutrient solutions on plants growth and development is explained. Nutrient sprays containthe adequate supply of nutrients which exhibits several benefits for healthy growth of plants (Libia&Fernando, 2012). It is also emphasized that in nutrient sprays, nutrients should be provided according to the plant requirements (Hansen, 1978). In the present study Hoagland solution (Hoagland &Arnon, 1950) iscommercially

available.20:20:20 NPK (Nitrogen, Phosphorous, Potassium) formulation and MS macro and micro solutions (Murashige & Skoog, 1962) were tested and in comparison, among these three nutrient solutions, NPK was found more effective. NPK(20:20:20) treated plants showed thehighest chlorophyll and carotenoid contentwith thehighest survival rate of plants. (Rodrigues *et al.*,2010)reported that NPK application to the plants showed better response in terms of plant growth. Increase in the growth of apricot was reported when nitrogenous fertilizers in combination with potassium and phosphorus(Bajwa& Chadha, 1968). Calcium in hoagland solution was also reported to cause eminence improvement of Oncidium flower (Hsu *et al.*, 2007).

Conclusion

Conclusively, this study reports a highly efficient and reproducible method for the micro propagation and acclimatization of industrially important orchids. This protocol will alsobe helpful for the conservation of plants as well as to support ornamental industry in capturing local market and earning foreign exchange.

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