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"Field project on

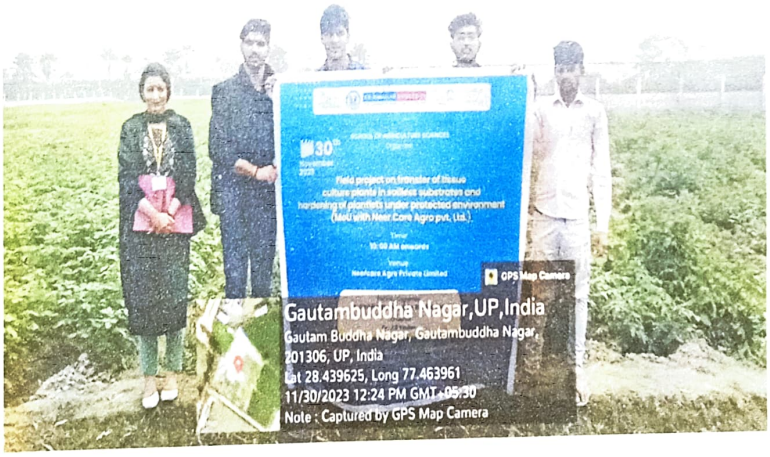
Transfer of tissue culture plants in soil less substrates and hardening of plantlets under protected environment at Neer Care Agro Pvt Ltd."

School Name: School of Agricultural Sciences

Programme Code - 82

Project Guide: Dr. Ambika Bhandari and Rabiya Basri

Venue: Neer Care Agro Pvt. Ltd.



DECLARATION

We undersigned hereby declare that this field project report represents work carried out by us. We also declare that we have adhered to all the principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/ data / fact /source in our submission. We understand that any violation of the above will lead to disciplinary action by the Institute. The findings in this report are based on the sampling / survey /data collection / recording during the field survey held on *date* at *place* under the guidance of *faculty name, designation, school name.*

Sr.No	*Roll No*	*Student Name*	*Program Name*	*Venue*	*Date from*	*Date to*	*Signature*
1	Jyoti Rawat	2113820006	*Bsc (Hons.)Agriculture*	*Neer Care Agro Pvt Ltd*	*30-11- 2023*	*30 -12-2023*	Jyoti
2	Sakshi Singh	2113820017	*Bsc (Hons.)Agriculture*	*Neer Care Agro Pvt Ltd*	*30-11- 2023*	*30 -12-2023*	Sakshi
3	Yogesh	2113820014	*Bsc (Hons.)Agriculture*	*Neer Care Agro Pvt Ltd*	*30-11- 2023*	*30 -12-2023*	Yogesh
4	Anushka	2113820015	*Bsc (Hons.)Agriculture*	*Neer Care Agro Pvt Ltd*	*30-11- 2023*	*30 -12-2023*	Anushka

Verified by; Dr. Ambika

Signature : Ambika

Date: 31-01-23

CERTIFICATE

This is to certify that

Sr. No.	Student's Name	Roll Number	Place of Work	Duration of Field Project
1	Jyoti Rawat	2113820006	*Neer Care Agro Pvt Ltd*	1 month
2	Sakshi Singh	2113820017	*Neer Care Agro Pvt Ltd*	1 month
3	Yogesh	2113820014	*Neer Care Agro Pvt Ltd*	1 month
4	Anushka	2113820015	*Neer Care Agro Pvt Ltd*	1 month

*enrolled in programme name (program code) has / have satisfactorily completed the project title "**Field project on transfer of tissue culture plants in soil less substrates and hardening of plantlets under protected environment at Neer Care Agro Pvt Ltd.**"under the guidance of Dr.Ambika , Assistant Professor "Fruit Science " of School of Agricultural Sciences .This project work represents their original work and the references given in the present report are authentic.*

Guide Name: *Dr Ambika Bhandari, Dr.Rabiya Basri*

Dean Name: *Dr.J .S Yadav*

Department: *SOAS*

School Name : *School of Agricultural Sciences*

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I. Introduction

A controlled environment and a suitable culture medium can be utilized to generate an entire plant from small tissue or plant cells through the process of tissue culture. The resulting plants, known as tissue-culture propagated plants, are exact replicas of the parent plant and exhibit identical characteristics. If the parent plant is a high-yielding variety, the propagated plants will also possess the same high-yielding traits. Tissue culture methods are currently effective for numerous plant species.

The concept of totipotency, proposed by German Botanist Haberlandt in 1902, refers to the ability of a single cell to develop into a fully grown plant. Tissue culture involves placing a portion or tissue of the plant in a nutrient-rich medium to encourage the growth of shoots and roots. Once developed, plantlets are hardened and transplanted into soil. Tissue culture allows for large-scale production of high-quality planting material for economically significant species.

Nearly any portion of a plant can initiate plant tissue culture, with shoot tips, particularly meristematic tissue, being ideal for micro propagation or direct shoot regeneration. The physiological condition of the plant affects its responsiveness to tissue culture, so a healthy mother plant without signs of disease or pest infestation is crucial. Shoot tip explants are advantageous due to their high percentage of actively dividing cells. Initiating cultures should rely on high-quality mother plant stock for optimal results.

Cultural conditions for initiating and maintaining plant cells in culture, as well as regenerating complete plants, vary among different plant species. Each variety or clone within a species often possesses specific and distinct cultural requirements.

Soilless agriculture, a method of growing plants without soil using mineral nutrient solutions, can help address challenges in traditional agriculture. Plants can grow with roots in mineral nutrient solutions or in inert mediums like perlite, gravel, rock wool, or coconut husks. With the world's population projected to reach 9.8 billion by 2050, traditional agriculture faces challenges such as ecological destruction, resource scarcity, and unequal food distribution. Soilless techniques like hydroponics, aeroponics, and aquaponics aim to combat these issues.

The total agricultural land increased by 3% from 1958 to 2005, mainly in tropical countries, but decreased by 0.19% between 2005 to 2011. Rapid urbanization, industrialization, and climate change contribute to a drop in per-capita land availability. Soilless techniques can play a role in reducing agricultural water consumption while maintaining or improving economic efficiency, especially in arid and semi-arid regions. This becomes crucial as the world grapples with the challenges of feeding a growing population, with at least 6000 tons of food required, often imported from non-trusted sources in terms of quality standards.

Objectives

1. Provide participants with essential knowledge and skills related to the successful transfer of tissue culture plants to soilless substrates.
2. To develop a systematic hardening-off process to acclimate tissue culture plantlets to external environmental conditions

Classification of soilless culture:

1. Solid media culture
2. Solution culture
3. Aeroponics

Solid media culture:

Soilless media can be in the form of substrates originated from peat moss, bark, coir, compost, rice hulls, vermiculite and perlite. This soil less culture is a mainstream practice in developing countries as normal ground soils are typically discontented in usage for crop production. Hence, the rudimentary characteristics of good soilless media would be easy to acquire, economical, abundant in nature, light weight, possess upright chemical properties and has a satisfactory water retention capabilities. The quality of the growing media must also be greatly maintained to ensure good growth of seedlings. This was because sustainable production of ornamental flower and

other crops would need to compensate decent growing media with sufficient water holding capacity and aeration. The most common incorporated soilless media are coir-dust based substrates and sphagnum peat in which it is among the most preferred and commercialized primary media. This was because it is occasionally acknowledged as substrates or growth media with the most prominent crop production mechanisms for containerized or raised beds with restricted volumes and was appropriate for continuous supply of nutrients through fertilization.

Vertical Farming:

Vertical farming is one such solution that's been implemented around the world. By Vertical Farming, food crops can be cultivated easily in urban areas by planting in vertically stacked layers in order to save space and use minimal energy and water for irrigation. In India, Vertical Farming is at nascent stages, however, there are few startups and agri-tech companies working to revolutionize the field.

Techniques of Vertical Farming:

1. Hydroponics
2. Aeroponics
3. Aquaponics

Hydroponics:

Hydroponics' word has its origin from Greek language where 'hydro' refers to water and 'ponos' refers to labour (Beibel, J.P. 1960).In hydroponic system it is assumed that soil is not necessary for plant growth actually it acts as a source of essential macro and micronutrients that regulate the plant growth and development. Thus, if soil is replaced with a solution having appropriate combination of macro and micro nutrients it is possible to raise a crop to its full maturity.

Types of Hydroponics system:

1. Wick system
2. Nutrient film techniques
3. Water culture or deep water culture (DWC)
4. Drip system
5. Ebb and flow systems

Wick system:

This is simplest hydroponic system requiring no electricity, pump and aerators. Plants are placed in an absorbent medium like coco coir, vermiculite, perlite with a nylon wick running from plant roots into a reservoir of nutrient solution. Water or nutrient solution supplied to plants through capillary action. This system works well for small plants, herbs and spice and doesn't work effectively that needs lot of water.

Nutrient Film Technique (NFT) system:

NFT was developed in the mid-1960s in England by Dr. Alen Cooper to overcome the shortcomings of ebb and flow system. In this system, water or a nutrient solution circulates throughout the entire system; and enters the growth tray via a water pump without a time control (Domingues *et al.*, 2012). The system is slightly slanted so that nutrient solution runs through roots and down back into a reservoir. Plants are placed in channel or tube with roots dangling in a hydroponic solution. Roots are susceptible to fungal infection because they are constantly immersed in water or nutrient. In this system, many leafy green can easily be grown and commercially most widely used for lettuce production.

Deep water culture system:

In deep water culture, roots of plants is suspended in nutrient rich water and air is provided directly to the roots by an air stone. Hydroponics buckets system is classical example of this system. Plants are placed in net pots and roots are suspended in nutrient solution where they grow quickly in a large mass. It is mandatory to monitor the oxygen and nutrient concentrations, salinity and pH (Domingues *et al.*, 2012) as algae and moulds can grow rapidly in the reservoir. This system work well for larger plants that produce fruits especially cucumber and tomato, grow well in this system.

Drip system:

The drip hydroponic system is widely used method among both home and commercial growers. Water or nutrient solution from the reservoir is provided to individual plant roots in appropriate proportion with the help of pump (Rouphael and Colla, 2005). Plants are usually placed in moderately absorbent growing medium so that the nutrient solution drips slowly. Various crops can be grown systematically with more conservation of water.

Ebb and Flow system:

This is first commercial hydroponic system which works on the principle of flood and drain. Nutrient solution and water from reservoir flooded through a water pump to grow bed until it reaches a certain level and stay there for certain period of time so that it provide nutrients and moisture to plants. Besides, it is possible to grow different kinds of crops but the problem of root rot, algae and mould is very common (Nielsen *et al.*, 2006) therefore, some modified system with filtration unit is required.

Observations

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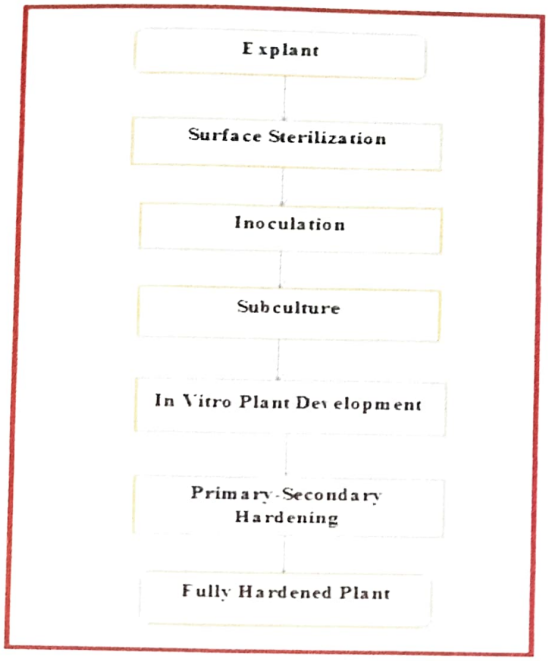
II. Steps of tissue culture



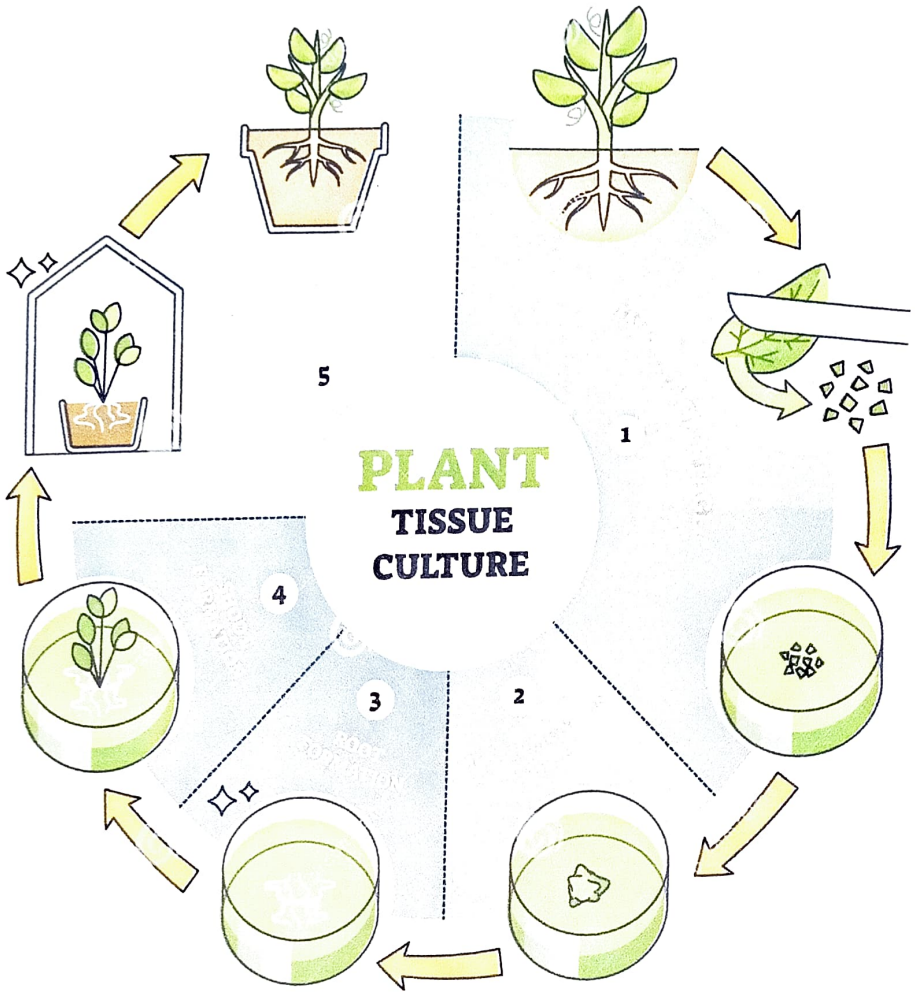


Figure I: Production process of Tissue Culture Plants (TCPs)

1. Stages of Tissue Culture Process

1.1 Preparation of nutrient medium

semi-solid medium is prepared in double distilled water containing macro elements, micro elements, amino acids, vitamins, iron source, carbon source like sucrose and photo-hormones. The medium is heated for dissolving the agar and 25 to 50 ml is dispensed into each wide mouth bottles. The vessels containing culture media are then sealed and sterilized by autoclaving.

1.2 Establishment of aseptic culture

The starting material for the process is normally an actively growing shoot tip of auxiliary or terminal bud or shoot tip of a plant. The process of tissue culture starts from the selection of mother plants having the desired characteristics. Ex-plant preferably the meristematic tissue of the selected mother plant is isolated. The excised tissue/explant is washed with water and then rinsed with disinfectant such as savlon or detol solution followed by a sterile-water wash. The tissue is then dipped in 10% bleach solution for ten minutes for disinfecting the plant tissue material, killing most of the fungal and bacterial organisms. Sterilization process of explants depends on the plant species andtypes of explants.

1.3 Inoculation

Inoculation is carried out under aseptic conditions. In this process explants or micro shoots are transferred on to the sterilized nutrient medium.

Fig II: Inoculation of excised micro shoot

1.4 Development of plants in growth room

After the inoculation of the plant tissue, the bottles are sealed and transferred into growth room to trigger developmental process under diffused light (fluorescent light of 1000-2000 lux) at 25 ± 2°C and 50 to 60% relative humidity. Light and temperature requirements vary from species to species and sometimes during the various stages of development.The cultures are observed daily for growth and any signs of infection/ contamination. Cultures, that do not show good growth or infected, are discarded. The healthy cultures grow into small shoot buds. These are sub- cultured on the fresh medium after 4 weeks. The number of subcultures required is specific to the plant species, which are standardized. The shoots generally develop after 4 weeks. After enough number of shoots is developed in each container (10 to 15), to a minimum height of 2 cm they are transferred to another medium for initiating the process of rooting.

Fig III: Development of plants

1.5 Hardening of micro plants

Due to very high humidity inside the culture vessel and artificial conditions of development. the plantlets are tender and are therefore are not ready for coping up with the filed conditions. The plants removed from the sterile medium are washed and are maintained under intermittent mist or are covered with clean transparent plastic. After 10 to 15 days under high humidity, the plants are transferred to green house and maintained for another 4 to 6 weeks. They are then ready to be transferred to net house or the field. Normally, the tissue culture plants are sold either as ex-agar plantsor hardened plants from the green house.

Fig V : Transfer of tissue culture plants in potrays

1.5.1 : Ex-agar plants

Depending on the parameters such as location/the site of planting, soil quality and the climatic conditions defined by the customer, the ex-agar plant for sale could be *in vitro* rooted plants or only the shoots. When the tissue culture plants are sold at this stage, the plants are washed in sterilized water to remove the agar medium.

The washed plants are sorted into 2 to 3 grades and packed in corrugated plastic boxes lined with sterilized tissue paper as per specifications of the Plant Quarantine Authority, Government of India for exports. The number of plants per box depends on the customer's requirement. Depending on the final destination and the preference of the customer, the plants are treated with specific fungicides and antibiotics to avoid infection.

The ex-agar plants are preferred for export or for destinations where hardening facility are available. The plants after being removed from nutrient media should preferably be transplanted within 72 hours.

III.: Hardened plants

The plants are transferred to net pots/ pro tray for acclimatization after they fully develop shoots and roots in the bottles. The rooted plantlets are transferred to pots filled with suitable substrate and are watered. This operation is carried out on an open bench. These pots are then transferred to the green house for 4 to 6 weeks. During this process, they are given fertilizers and treated like plantlets obtained by any other means of propagation. After the plants are acclimatized fully, they are transferred to poly-bags. At this stage the plants are completely hardened and are ready to be planted in the field for cultivation. Hardening units can be set up in sites away from the micropropagation unit.

Fig. VII: Hardening of plants in green house

5. Commercially propagated plants through micro-propagation in India

The plants in each category which are commercially propagatedare as follows

Plant category	Plants
Fruits	Banana, Pineapple, Strawberry
Cash crops	Sugarcane, Potato
Spices	Turmeric, Ginger, Vanilla, Large cardamom, Small Cardamom
Medicinal plants	Aloevera, Geranium, Stevia, Patchouli, Neem
Ornamentals	Gerbera, Carnation, Anthurium,Lily

6. Mitigating Risks of commercial plant tissue culture

The utilization of plant tissue culture for commercial production is limited by two major risks viz., spread of diseases especially those caused by viruses, and variations.

The movement of plants also involves accidental risk of introducing plant disease. Pathogens that are often symptom less, such as viruses, pose a risk. The risk of distribution of inferior micro propagated plants has posed a major threat to the ever-increasing agribusiness industry. In order to prevent these risks, effective testing (indexing) procedures are required prior to bulking up culture for commercial propagation. Standardprocedure should be adopted such as:

- Carefully selection of mother plants
- Ensuring establishment of virus free culture through indexing of 100 % ex plants
- Proper package and practices to be adopted such as limited number of cycles of multiplication, grading of cultures as well as plants, insect, pest monitoring in hardening area etc.

Fig VIII: Geo-tagged pics of the event

Interpretation and Analysis

Comparative concentration ranges of macronutrients (mM) in soil and soilless crop

Nutrient	Soil (mM)	Hydroponics (mM)
N-NO3-	0.5-10	5-20
N-NH4+	0.02-0.05	0.5-2
P(H2PO4-)	0.0005-0.05	0.5-2
K+	0.2-2	5-10
Ca2+	0.5-4	3-6
Mg2+	0.2-2	1-2
S (SO42-)	0.1-2	1.5-4

Epstein 1972; Marschner 1996

Table 4.Comparison B/W Soil Less Vs Soil Crops Productivity/Acre

Vegetables	Hydroponics / acres	Traditional /acres
Tomato	180 tons	5-10 tons
Cauliflower	30,000lb	10-1500lb
Lettuce	21,000lb	9,000lb
Cucumber	28,000lb	7,000lb

Source: Solanki *et al.* 2017

IV. Conclusion and Recommendations

In recent years, soil less cultivation has become increasingly important as a promising strategy for growing a variety of crops. This approach provides the opportunity to grow short-lived crops, such as vegetables, throughout the whole year with comparatively few land and labor requirements. Especially in regions with limited water or soil resources, hydroponic cultivation techniques can open up new approaches to food production. To support this development, cost-effective hydroponic technologies that save labor as well as operating costs through increased automation need to reach the market. On the other hand, hurdles such as the risk of the rapid spread of diseases within the closed systems, as well as contradictions such as the need for fossil energy resources have to be overcome. As long as sustainability is limited by the need for fossil resources, large building structures, technical equipment, any disinfectants and waste materials, the use of hydroponic techniques should always be critically considered in terms of environmental balance and long-term consequences for both the planet's health and ours. When evaluating current research results, there is a lack of comparable sustainability studies. Efforts are needed to obtain clear data, especially regarding the environmental impact of high energy costs, and to find alternatives to fossil energy sources. In order to be able to more accurately assess the future opportunities of soil less techniques, it is necessary to know the advantages and disadvantages of the individual systems, substrates and organisms and to understand their applicability. Not all systems are equally efficient, nor can they be applied in all areas and locations. Similarly, not all plant species are equally suitable for cultivation in soil less systems. Economic efficiencies cannot be neglected here. The substrates used in some cases also have a separate impact on the environment, which must be critically included in the overall consideration. So, in addition to the lighting and nutrient supply, the numerous systems, applications, substrates and organisms and their economic viability and sustainability have to be considered in order to get an understanding of whether and when it is worthwhile to use soil less technique.

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Appendix

Geotagged and Non geotagged Photographs