TISSUE CULTURE-RAISED APPLE ROOTSTOCK IN INDIA – A Success Story

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Citation: APAARI (2021) Tissue Culture-Raised Apple Rootstock in India – A Success Story. Asia-Pacific Association of Agricultural Research Institutions, Bangkok, Thailand. xiv+66 p.

Published by:
Asia-Pacific Association of Agricultural Research Institutions (APAARI)
182 Larn Luang Road, Klong Mahanak Sub-District, Pomprab Sattrupai
Bangkok 10100, Thailand

ISBN: 978 85 99249 50 5

Cover page photo identity: Tissue Culture-raised Apple Tree in Fruiting Stage

Printed: September, 2021
Published at Angkor Publishers Pvt. ltd.

For copies, please contact:
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Acknowledgements

At the outset, authors would like to express sincere gratitude to the Asia-Pacific Association of Agricultural Research Institutions (APAARI) for giving an opportunity to write a Success Story on Tissue Culture Apple Rootstock in India. We are thankful to Dr Rishi Kumar Tyagi, Coordinator, Asia-Pacific Consortium on Agriculture Biotechnology and Bioresources (APCoAB), for his valuable help in finalizing structural outlines, reviewing and critical editing of the document. We are also thankful to Ms Shilpa Datyal of Nishant Biotech, Bilaspur, Himachal Pradesh, for extending all possible assistance in gathering field-related information as well as Ms Ritambhara, PhD Scholar of Amity University, Noida, Uttar Pradesh, for providing figures and collecting literature related to this document. Authors are thankful to their respective organizations Biotech Consortium India Ltd., New Delhi (Public limited company created by the initiative of Department of Biotechnology, Government of India), Amity University, Noida, Uttar Pradesh and also Nishant Biotech, Bilaspur, Himachal Pradesh (HP) for the support provided throughout the preparation of this Success Story.

Finally, we would like to thank all the stakeholders of apple tissue culture who were directly and indirectly involved in the propagation of tissue culture-raised apple rootstock in India, and without whom this success story would not have been possible.

Authors

Foreword

Apples are known as nutritional powerhouse, a super fruit owing to its indispensable health benefits and nutritional value. It is also known to be best source of nutrients, dietary fibre, and various supplements. Apples are incredibly good for overall health, well-being and regular addition of this fruit lowers the risk of chronic diseases like diabetes, heart disease and cancer. Commercially, apple is the most important temperate fruit and is fourth among the most widely produced fruits in the world after banana, orange and grape. India is ranked as world's fifth largest apple producing country and second largest country in acreage. The cultivation of tissue culture-raised apple rootstock has been increasing and providing higher income to farmers. This has given new direction and understanding to apple growers, farmers and entrepreneurs specifically about the high-density plantation of tissue culture-raised apple rootstock. The Department of Biotechnology (DBT) in India has taken many initiatives for quality control in production of tissue cultured plants by commercial units. DBT has established the National Certification System of Tissue Cultured-Raised Plants (NCS-TCP) and this certification system has encouraged and motivated apple growers/farmers and entrepreneurs to cultivate and produce tissue culture-raised apple rootstock. The Biotechnology Consortium of India Limited (BCIL), New Delhi has been managing the NCS-TCP as its Management Cell since its inception in 2006. As apple rootstock production using tissue culture-raised plantlets has been increasing in India, a further need was felt to compile all the information available on different aspects of raising of apple rootstock through tissue culture and publish as "Success Story on Tissue Culture-Raised Apple Rootstock in India". It is expected that this publication will serve as a case study for most of the apple growing countries of the region and may foster collaborations among the relevant partners in research and development apart from serving as a reference for policy makers.

I must compliment the authors Dr Shiv Kant Shukla and Dr (Mrs) Susmita Shukla for making a meticulous effort to put together information on different aspects of apple cultivation, production and certification of tissue cultured apple and documenting success stories of farmers who have adapted cultivation of tissue-cultured apple. I also thank Dr Rishi Tyagi for conceptualising, reviewing, critical editing and ensuring this important publication.

Ravi Khetarpal
Executive Secretary, APAARI

Preface

Commercialization of plant tissue culture in India was closely witnessed by the lead author Dr Shiv Kant Shukla during his professional career for over last two decades (from year 2006-till date) as the Head of a Commercial Plant Tissue Culture Facility (CPTCF) at Raipur, Chhattisgarh, India, and Principal Investigator of Management Cell of National Certification System for Tissue Culture Raised Plants (NCS-TCP) at Biotech Consortium India Limited (BCIL). In India, commercialization of plant tissue culture started in late 1980s. It has completed a journey of approximate three decades and has now gained recognition as mature and organized industry. Indian tissue culture industry is a great success in terms of attaining a production capacity of approximate 500 million plants per annum with a production of range of crops which mainly includes banana, sugarcane, potato, pomegranate, orchids and bamboo, etc. While interacting with entrepreneur based at Himachal Pradesh (HP), India, during technical handholding for building capacity towards meeting the national standards/guidelines, it was observed that tissue culture-raised apple rootstock has not been only successfully commercialized but it is also creating a positive impact on agriculture community by enhancing overall production and productivity along with quality of produce. With this, the idea was emerged to document this success in collaboration with industry and academic partner under the active guidance of Asia-Pacific Association of Agricultural Research Institutions (APAARI).

It is noteworthy that Government of India, along with State Governments, is playing key role to promote apple tissue culture though financial and technical support to the stakeholders. Financial assistance for establishing tissue culture laboratory and support to growers under Mission for Integrated Horticulture Development

(MIDH) is the key for promoting this sector. The Department of Biotechnology (DBT), Government of India, has been promoting research and development in the area of plant tissue culture since last 30 years through funding the research projects for developing protocol and establishing Micropropagation Technology Parks, and setting up of NCS-TCP to encourage production and distribution of quality planting materials for the benefit of farmers and also to promote growth of tissue culture industry. This success story of tissue culture-raised apple rootstock in India highlights the success in following three dimensions on:

- Plant tissue culture as revolutionary technology to increase production and maintaining quality.
- Plant tissue culture entrepreneurship for mass production of apple root stock.
- Progressive famers for adopting tissue culture plants towards sustainable and profitable agriculture.

Authors are hopeful to create wide awareness through this success story for large scale adoption of certified quality tissue culture apple plants/rootstock by farming communities particularly in developing countries of Asia-Pacific region to meet the regional and global demands of apple.

Authors

Abbreviations & Acronyms

°C	Degree Celsius
ACLSV	Apple Chlorotic Leaf Spot Virus
AMD	Apple Mosaic Disease
APAARI	Asia-Pacific Association of Agricultural Research Institutions
APCoAB	Asia-Pacific Consortium on Agriculture Biotechnology and Bioresources
ApMV	Apple Mosaic Virus
ASGV	Apple Stem Grooving Virus
ASPV	Apple Stem Pitting Virus
ASSVd	Apple Scar Skin Viroid
ATLs	Accredited Test Laboratories
AVT	AV Thomas and Company Limited, Kerala
BAP	6-Benzylamenopurine
BCIL	Biotech Consortium India Limited
CAGR	Compound Annual Growth Rate
CPTCF	Commercial Plant Tissue Culture Facility
cm	Centimeter
DBT	Department of Biotechnology
ELISA	Enzyme-Linked Immunosorbent Assay
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	FAO Statistical Database
g	Gram
GA3	Gibberellic Acid
ha	Hectares
HDP	High Density Plantation
HEPA	High Efficiency Particulate Air
HP	Himachal Pradesh
IAA	Indole-3-acetic Acid
IBA	Indole-3-butyric Acid

IHBT	Institute of Himalayan Bioresource Technology
INR	Indian Rupees
J&K	Jammu & Kashmir
kg	Kilogram
LDL	Low Density Lipoproteins
m	Meter
mcg	Microgram
MIDH	Mission for Integrated Development of Horticulture
msl	Mean Sea Level
Mg	Magnesium
mg	milligram
ml	Millilitre
MS	Murashige and Skoog
MT	Metric Tonne
NAA	Naphthalene Acetic Acid
NCL	National Chemical Laboratory, Pune
NCS-TCP	National Certification System for Tissue Culture Raised Plants
PNRSV	Prunus Necrotic Ringspot Virus
PVP	Polyvinylpyrrolidone
QMS	Quality management system
RDI	Reference Daily Intake
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SOPs	Standard Operating Procedures
TAE	Tris-Acetate EDTA
TC	Tissue Culture
TCCA	Tissue Culture Certification Agency
TCPF	Tissue Culture Production Facility
TDZ	Thidiazuron
UK	United Kingdom
USA	United States of America
USD	US Dollar

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Executive Summary

Apple ranks fourth in production globally after banana, orange and grape. Commercially, it is the most important temperate fruit. India is ranked as fifth largest apple producing country in the world and second in terms of total area under apple cultivation. Apples are mostly consumed fresh but a small part of the production is processed as juices, jellies, canned slices and other items. Apples are known as best source of nutrients, dietary fibre, and a scope of different supplements along with medicinal property.

Asian countries, for example, India, Indonesia, Iran, Japan, Malaysia, Pakistan, Philippines, Sri Lanka, Thailand, Viet Nam, Bangladesh, Nepal, Republic of Korea and Myanmar are contributing significantly to the total fruit basket of the Asia-Pacific region. Apple farming in India is mostly practiced in scattered hilly regions of Himachal Pradesh (HP), Jammu and Kashmir (J&K), Uttarakhand and Arunachal Pradesh. India recorded approximately 2.32 million tonnes apple production in the year 2019 with globally 5th position, moved from 11th position as was reported in year 2001 (1.23 million tonnes), surpassing Italy, France, Iran, Russia, Argentina and Germany. This growth is encouraging which is broadly attributed to adopting modern agro-techniques, quality planting material specially raised through tissue culture, certification of tissue culture-raised plants and implementing recommended package and practices by growers under high density plantation.

Major constraint of traditional cultivation of apple is the infection caused by various pests and viruses, while pests can be managed through various ways but it is very difficult to eliminate virus infection from the plants. Plant tissue culture has played an important role in the production of healthy, disease-free plants with rapid multiplication of scions and rootstocks with desirable traits. During the last a few decades, apple tissue culture propagation has been used in many countries, especially using new rootstock. In India, tissue culture technology for producing rootstock of apple has been commercialized successfully. Tissue culture commercialization coupled with robust quality management system in the form of NCS-TCP has played key role in acceptance of the tissue cultured rootstock at the level of growers in the country. This success story covers apple business, global and Indian scenario, constrains in traditional cultivation, advantage of tissue culture-raised apple rootstock and its impact in the farmers' fields.

1. Introduction

1.1 Apple

Commercially, apple is the most important temperate fruit and it ranks fourth after banana, orange and grape among the most widely produced fruits in the world. China, United States of America (USA) and Turkey are the top three countries in the production of apple. India is ranked as world's fifth largest apple producing country and second largest country in terms of area under cultivation. In Asia-Pacific region, India ranks second in production of apple after China (Moore, 2020) and is known for economic returns, high nutritive value and popularity (Mir *et al.,* 2018). Apple originated in the Middle East more than 4000 years ago. Spreading across Europe to France, the fruit arrived in England at the time of the Norman Conquest in year 1066. It was introduced into India by the British in the Kullu Valley of Himachal Pradesh (HP) as far back as in the year 1865, while the coloured 'Delicious' cultivars of apple were introduced to Shimla hills of HP in year 1917. The apple cultivar 'Ambri', is considered to be indigenous to Kashmir, had been grown long before introductions were made from western countries. Apple can be grown at altitudes 1,500-2,700 m above mean sea level (msl) in the Himalayan range. This region experiences 1,000-1,500 hours of chilling in a year. The temperature during the growing season is around 21-24 ^{0}C. For optimum growth and fruiting, apple trees need 100-125 cm of annual rainfall, evenly distributed during the growing season. Excessive rains and fog near the fruit maturity period result in poor fruit quality with improper colour development and fungal spots on its surface. Loamy soils, rich in organic matter with pH 5.5 to 6.5 and having adequate drainage and aeration are suitable for cultivation. Areas exposed to high velocity of winds are not desirable for apple cultivation.

Mostly apples are consumed fresh but a small part of the production is processed into juices, jellies, canned slices and other items. Over 700 accessions of apple, introduced from USA, Russia, United Kingdom (UK), Canada, Germany, Israel, Netherlands, Australia, Switzerland, Italy and Denmark have been tried and tested during the last 50 years in India (Pandita *et al.*, 2016). In more recent times, improved spur types and standard colour mutants with 20-50% higher yield potential are favoured (Table 1).

Table 1: Important apple cultivars grown in India and their characteristics

1	Spur types	Red spur, Starkrimson, Golden spur, Red chief spur and Oregon spur
2	Colour mutants	Skyline Supreme, Vance Delicious, Top Red
3	Low chilling	Michal, Schlomit
4	Early cultivars	Benoni, Irish Peach, Early Shanburry, Fanny
5	Juice making cultivars	Lord Lambourne, Granny Smith, Allington Pippin
6	Scab resistant cultivars	Co-Op-12, Florina, Firdous, Shirean
7	New Hybrids	Chaubatia Anupam & Chaubatia Princess (Early Shanberry x Red Delicious), Lal Ambri (Red Delicious x Ambri), Sunehari (Ambri x Golden Delicious), Amred (Red Delicious x Ambri)

Source: Ghosh (1999)

1.2 Importance of apple

Apple is considered as best source of nutrients, dietary fibre, and a scope of various supplements along with medicinal property. Because of their changed supplement content, apples may help to forestall a few ailments. Apple is in an assortment of shapes, tones, and enhances and give a range of supplements that can benefit various parts of an individual's well-being. For instance, apple may help lessen the danger of malignancy, stoutness, coronary illness, diabetes, and a few different conditions. Apple contains a scope of cell reinforcements, including quercetin, catechin, phloridzin, chlorogenic corrosive, neurological well-being and dementia. It is significant that most investigations utilized high portions of quercetin that are probably not available in ordinary dietary sources. One medium size apple (6.4 ounces or 182 g) offers the accompanying supplements calories: 95, Carbs: 25 g, Fibre: 4 g, Nutrient C: 14% of the Reference Daily Intake (RDI), Potassium: 6% of the RDI, Nutrient K: 5% of the RDI. Also, a similar serving gives 2–4% of the RDI for manganese, copper, and the nutrients A, E, B1, B2, and B6 (Table 2). Scientists also reported that apples are additionally filling diet, having less energy thick fibre content. Apple is additionally a rich source of polyphenols and fibres proven for various medical advantages such as lower levels of "terrible" LDL cholesterol, fatty oils, absolute cholesterol, lower danger of coronary illness, stroke, and cures neurological disorders, *etc.* One of these polyphenols is the flavonoid epicatechin, which may bring down circulatory strain.

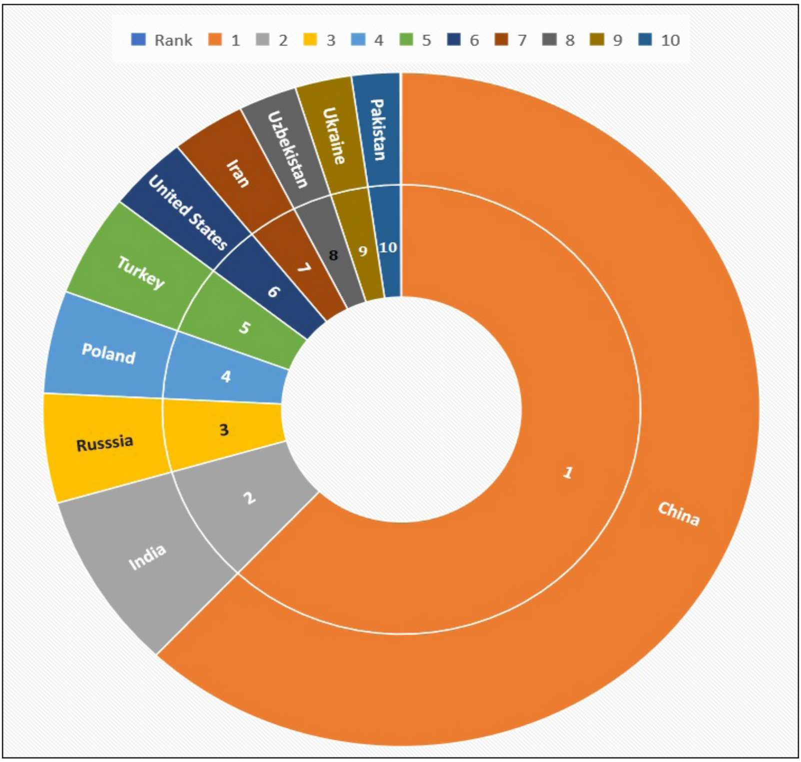
Table 2: Nutritional content of apple

Nutrient	Amount in 1 Apple	Daily Adult Requirement
Energy (Calories)	94.6	1,800-3,000
Carbohydrate (g)	25.1, including 18.9g of sugar	130
Fiber (g)	4.4	22.4-33.6
Calcium(milligrams [mg])	10.9	1,000-1,300
Phosphorus (mg)	20.0	700
Magnesium (mg)	9.1	320-420
Potassium (mg)	195	4,700
Vitamin C (mg)	8.37	75-90
Folate (micrograms [mcg])	5.46	400
Choline (mg)	6.19	425-550
Beta-Carotene (mcg)	49.1	No data
Lutein and Zeaxanthin (mcg)	52.8	No data
Vitamin K (mcg)	4	90-120
Apples also provide Iron, Vitamin A, some B Vitamins and Vitamin E		

Source: Arnarson (2020)

Apple contains gelatine, a sort of fibre that goes about as a prebiotic an excellent source of beneficial microscopic organisms of human gut. It has reported that apples also have defensive impacts against heftiness, type 2 diabetes, and coronary illness. Cell reinforcement rich apples may help in shielding human lungs from oxidative harm. Eating about 15% of apple for every day was connected to a 10% lower danger of this condition (Boyer and Liu, 2004). Apple is among the one of the important food sources, which is not only rich in nutrient contents but also has medicinal importance and gives the feeling of filling. Quality production in large scale is very much essential and also is an important parameter to be considered with respect to food security.

Asian countries such as Bangladesh, Indonesia, Iran, Japan, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Republic of Korea, Sri Lanka, Thailand, and Viet Nam, are contributing significantly to the total fruit basket of the Asia-Pacific region. China, India, Russia, Poland, Turkey, USA, Iran, Uzbekistan, Ukraine and Pakistan are among the top 10 countries regarding harvesting apples in large area in hectares (ha) (Fig. 1). The total area harvested worldwide for apples was 5,293,340 ha in 2016 which declined to 4,933,841 ha in 2017. In 2018, the apple production in China amounted to approximately 39.2 million tonnes (MT). In comparison, the apple production in India was approximately 2.3 MT in 2018. In the 2019/2020 crop year, China was the leading producer of apples worldwide. During that time period, China's apple production amounted to around 41 million MT followed by European Union ranked in second place with about 11.48 million MT of apples. Overall apple production in India has drastically enhanced over the period of past two decades. The area and production of apple in HP and J&K have been increasing over the years. Apple production in India was recorded approximately 2.32 MT in year 2019 with globally 5th position, moved from 11th position reported in year 2001 (1.23 MT) surpassing Italy, France, Iran, Russia, Argentina and Germany (Fig. 2).



Area under apple cultivation

Fig. 1: Top 10 countries depicting relative area under cultivation of apple

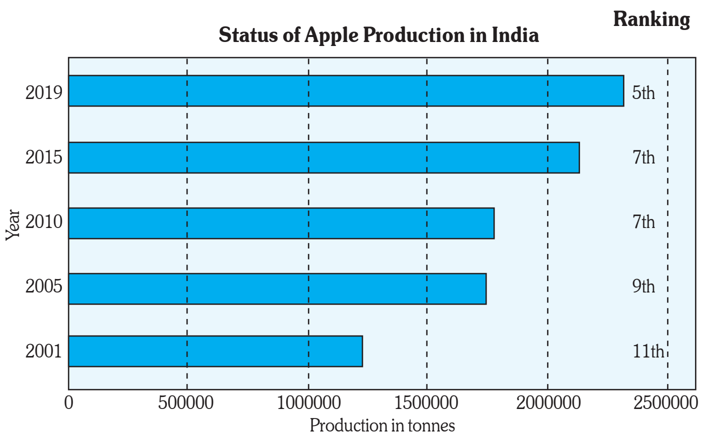


Fig. 2: Apple production in India in past two decades (2001-2019)

1.3 Supply and demand of apple in India

India has witnessed voluminous increase in horticulture production over the past few years. Significant progress has been made in area expansion resulting in higher production of apple. Over the last decade, area under horticulture grew by about 3% per annum and annual production increased by 5.4%. During 2016-17, production of horticulture crops was about 295.2 million tonnes from an area of 24.9 million ha (Horticulture Statistics at a Glance, 2017). Apple has wider consumer acceptability in India both as a fresh table fruit and value-added processed products. It is mostly grown in the J&K, HP, Uttarakhand, Arunachal Pradesh and Nagaland. In HP, apple is the most important crop accounting for about 90% of the total horticultural produce. Whereas J&K and HP accounted for 76% of the area and 94% of the production of the crop in the country in 2001-02. In 2019, from the total area of 307 ha, a total of 2371 MT production of apple was reported from India. The delicious group of cultivars pre-dominates the apple market (Table 3). Areas covered under delicious cultivars are: 83% of the area under apple in HP, 45% in J&K and 30% in Uttarakhand.

Table 3: Important apple cultivars produced in different states of India

S.No.	Category	Varieties
1	Clonal rootstocks	M 9, M 26, M7, MM 106, MM 11
2	Scab resistant	Prima, Priscilla, Sir Prize, Jonafree, Florina, Macfree, Nova Easy Grow, Coop 12, Coop 13 (Redfree), Nova Mac, Liberty, Freedom, Firdous, Shireen
3	Hybrids	Ambrich (Richared x Ambri), LalAmbri (Red Delicious x Ambri), Sunehari (Ambri x Golden Delicious), Chaubattia Princess, Chaubattia Anupam (Early Shanburry x Red Delicious), Ambred (Red Delicious x Ambri), Ambroyal (Starking Delicious x Ambri)
4	Low Chilling	Michal, Tamma, Vered, Neomi, Tropical Beauty, Parlin's Beauty, Schlomit, Anna
5	Pollinizing	Tydeman's Early, Red Gold, Golden Delicious, McIntosh, Lord Lambourne, Winter Banana, Granny Smith, Starkspur Golden, Golden Spur

Source: National Horticulture Board (2018)

In HP, monoculture of a few cultivars has shown negative impact on the apple industry particularly in case of Royal Delicious, Red Delicious and Richared. Serious problems of apple scab disease and outbreak of premature leaf fall and infestation of red spider mite are causing great concern. Uttarakhand, mainly the Kumaon hills division, have the unique advantage of early harvest of apple, due to cultivation of early maturing varieties, namely, Early Shanburry, Fanny and Benoni. J&K, HP and Uttarakhand are majorly involved in production of various promising cultivars of apple (Table 4).

Table 4: Promising cultivars in major apple producing regions of India

States/UT	Jammu & Kashmir	Himanchal Pradesh	Uttarakhand
Varieties	Benoni, Irish Peach, Cox's Orange Pippin, Ambri, White dotted Red, American Apirouge, Red Delicious, Golden Delicious	Tydeman's Early, Mollies Delicious, Starkrimson, Starking Delicious, Red delicious, Richared, Granny-Smith, Red Spur, Top Red, Red Chief, Oregon Spur, Golden Spur, Michal, Schlomit	Early Shanburry, Chaubattia Princess, Fanny Benoni, Red Delicious, Starking Delicious, Rymer, Buckingham

Source: Ghosh (1999)

1.4 Constraints in enhancing production and productivity of apple in India

The main cultivars of apple are Red Delicious, Royal Delicious and Golden Delicious, which account for over 80% of the apple area in India. These cultivars were first introduced in the 1960s from the northern Indian States, namely, J&K and HP. Traditional orchards found near the monasteries comprise of all seedling trees. The scientific knowledge of clonal propagation of deciduous fruits particularly apples was generated after introducing the apple in India. However, the country took some time to establish its own nursery industry for commercial cultivation of apple. It was only in the late 1980s that apple production had risen to the level of major commercial importance as a result of a better road network and development of an export market. The most serious pest which affects apple orchards is the Woolly Aphid (*Eriosoma lanigerum*), Stem and

Twig Borer (*Oberea posticata*). This problem is pronounced in poorly managed and older orchards. Fungi, bacteria, viruses, and viroid are other severe threat to the long-term survival of apple trees.

The major challenges are production of disease-free apple plants, creating awareness about the advance agriculture techniques, skilling of growers/grower groups, trade facilitation, supply capacity and integration of current knowledge and production through quality management systems. Apple production in countries like China, USA, Turkey, Chile and India are growing continuously. Apples turned pricier in the year 2020 in HP and rising consumer demand amidst COVID-19 pandemic. This is due to the fact that people are consuming more fruits during the pandemic times and also a slight drop in import of apples from countries like Chile, New Zealand and the USA, are keeping the prices high. The high demand has brought in a greater number of buyers in year 2020 to HP as compared to previous years (Jayan and Kulkarni, 2020). Traditional cultivation has the issue of low productivity due to various disease and poor-quality planting material.

1.5 Possible solutions

In order to have fruits of uniformly good quality, most apple trees are propagated by grafting the scion of desired cultivar (which determines the fruit variety) on selected rootstock. The most common form of grafting carried out was the 'whip and tongue' grafting method and the most common budding method was the 'chip budding' technique. Three years are required for producing a fruit tree *i.e.* one year for growing rootstock linear, one year to establish the transplanted rootstock in nursery field, and one year for graft of the fruit plant to develop. During the period of production, rootstock may become infected with soil-borne or other diseases. Grafting failure can also occur as the buds may die, grow poorly or are damaged by winter mechanical injury.

Clonal rootstocks of M and MM series of cultivars were introduced from East Malling Research Station of UK, and M-9, M-26, M-4, M-7, MM-106 and MM-111 were identified as promising cultivars. Merton 779 was recommended as a commercial rootstock for apple for Kumaon hills of Uttarakhand. Clonal rootstocks were not considered favourable by the apple growers, in general. However, clonal rootstocks were preferred only for high density orchards. *In vitro* protocols of M-9, MM-106 and MM-111 have been developed (Teixeira da Silva *et al.,* 2019). Four rootstocks, namely, EMLA-111 for drought-prone areas, EMLA-7 for sloppy land and EMLA-106 for sloppy and less clayey soil, and EMLA-9 for high density planting on irrigated deep soils, have been recommended.

Tissue culture technique for micropropagation of rootstock has been applied and proven to propagate genetically uniform and disease-free plants. Tissue culture of domesticated apple (*Malus* x *domestica* Borkh.) has a rich and extensive history spanning approximately 60 years (Dobránszki and Teixeira da Silva, 2010). The *in vitro* methods have been developed for large number of rootstocks and scion cultivars. Several research groups established *in vitro* conditions that were suitable for micropropagation of apple and its rootstocks (Modgil and Thakur, 2017; Rumiyati *et al.,* 2017; Verardo *et al.,* 2017; Vogiatzi *et al.,* 2018). However, it is only a decade ago when *in vitro* rootstock production at large scale has taken up speed worldwide. India has also shown remarkable growth in production of tissue culture-raised apple rootstock. Many promising varieties of apple rootstocks are being produced through tissue culture techniques now-a-days. A representative tissue culture-raised plant and *in vitro* materials are shown as Fig. 3A and 3B.

Fig. 3: (A) Tissue culture-raised field-transplanted plants and (B) *in vitro* shoot cultures of apple

2. Apple Business

The global apple market was on the rise to reach US Dollar (USD) 78.8 billion in 2019, after four years of decline. The total market indicated a moderate expansion from 2007 to 2019; its value increased at an average annual rate of +2.1% over the past 12 years.

Asia-Pacific region leads the global consumption of fresh apples, with a share of 62% of the global market. China is the largest producer of apples in the world. China remains the largest apple-consuming country worldwide, with a consumption rate of 40 million MT, which comprises approximately 48% of the total volume. Moreover, apple consumption in China exceeded the figures by 10x as recorded by the USA (Dublin, 2020).

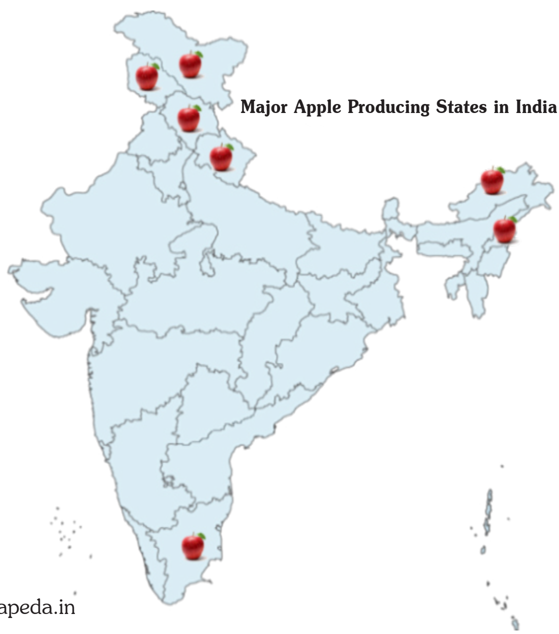
Apple farming in India is mostly practiced in hilly regions of HP, J&K and Uttarakhand. Within these states, the apple-growing areas are scattered, making exchange of knowledge among farmers difficult. Further, due to the difficult terrain and less economic development of these areas, transportation and a farmer-friendly marketing networks are yet to be developed. Producers' knowledge about the mainstream market where their produce is to be sold is heavily limited by these factors, leading them to depend on middlemen who procure the produce at rates much lower than those prevailing in the market. Moreover, high cost of transportation, unpredictable weather conditions and other related issues have led to apple farming becoming an increasingly difficult occupation for people in hilly regions. In order to make the apple farming more profitable, adoption of new technology like tissue culture rootstock is very important along with addressing other local issues as mentioned above.

India's apple imports have grown at a Compound Annual Growth Rate (CAGR) of 32.91% in the last 10 years because domestic production has failed to keep pace with the demand of a rising population. Major apple producing states and production, geographic locations of apple producing states in India, and global apple production are presented in Table 5, Fig. 4 and Fig. 5, respectively.

Table 5: State-wise production of apple (2018)

S.No.	State/Union Territory	Production ('000 MT)
1	Arunachal Pradesh	7.35
2	Himachal Pradesh	446.57
3	Jammu & Kashmir	1808.33
4	Nagaland	1.99
5	Tamil Nadu	0.01
6	Uttarakhand	58.66
	Total	**2326.90**

Source: National Horticulture Board (2018)



Source: http://apeda.in

Fig. 4: Major apple producing states

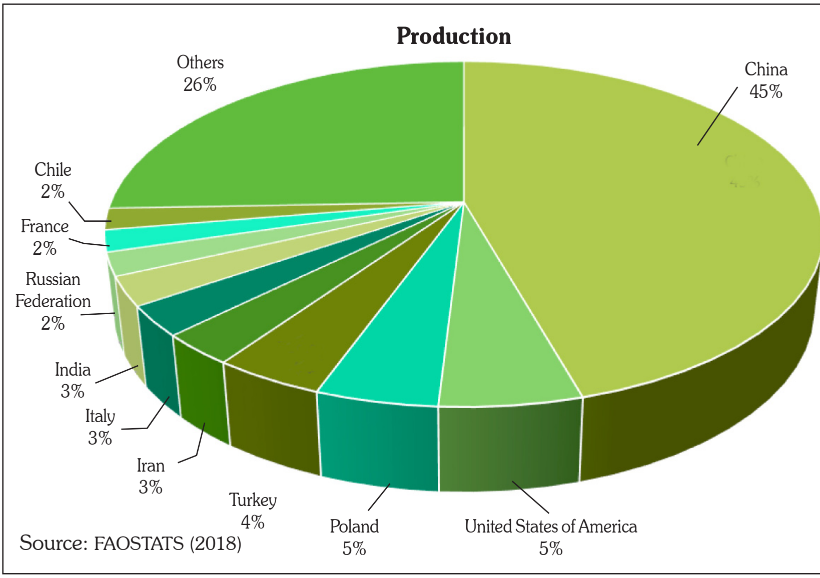
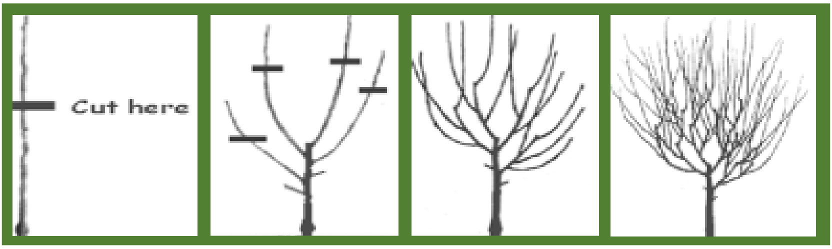


Fig. 5: Apple production in the world

3. Traditional Cultivation and Constraints

The traditional systems of planting, have long juvenile period, are labour intensive and low yielding with poor quality fruits. Whereas high density is easily manageable, has higher yield potential, with better quality fruits and higher returns/unit area. There is enormous scope for improvement of apple productivity through introduction of improved cultivars, replacement and rejuvenation of old and senile orchards. Low productivity of apple orchards can also be attributed to inadequate proportion of pollinizers, lack of pollinators, poor canopy management, and incidence of diseases and pests.

Apple cultivation around the world has undergone radical changes over the last 60 years moving from traditional production systems using widely-spaced large trees to more intensive production systems using closely-spaced smaller trees. In the early 1900s, most commercial apple orchards utilized trees on seedling rootstocks and had large globular shaped tree canopies that allowed cattle to graze under the trees. Over the last 60 years, numerous planting systems for modern orchards have been developed such as dwarfing rootstocks, high density planting, renewal pruning, and use of growth regulators which resulted into higher sustained yields and better fruit quality. Before 1970, almost all maiden trees produced by nurseries were single shoot whips. With the development of high-density orchard systems, several researchers began to study alternative approaches to tree management that involved fewer pruning efforts. Regarding pruning of newly planted trees, the internal quality of nursery trees has become important. It takes five years for bearing fruits after pruning (Fig. 6). Conventionally, apple trees are generally



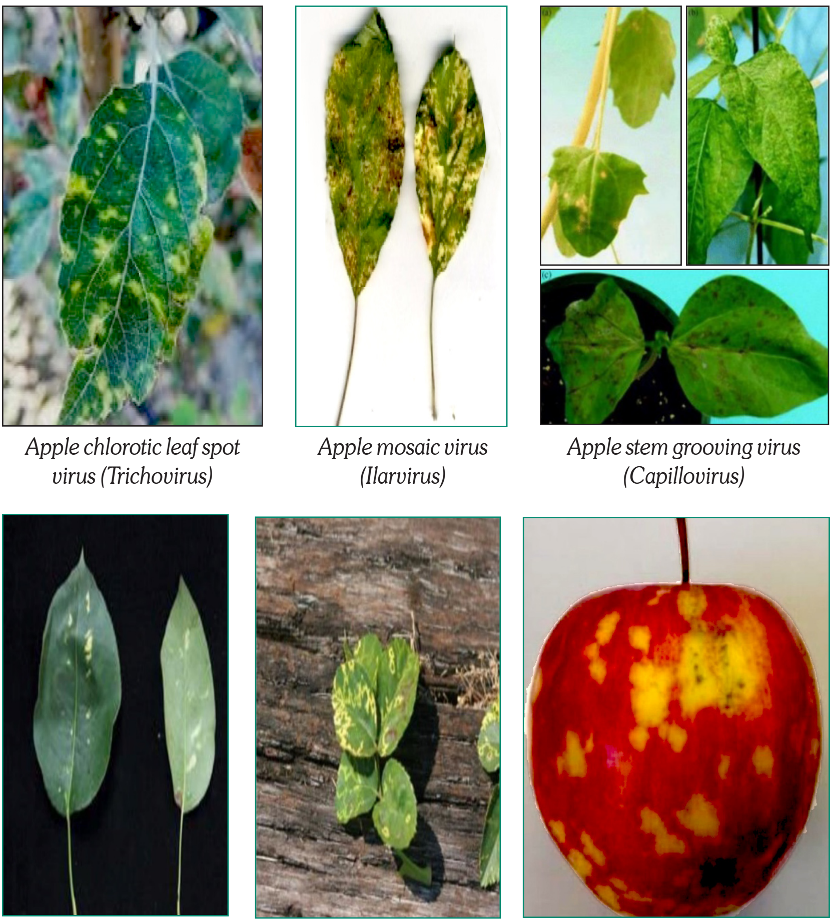
Dormant pruning stages at 1st, 2nd 3rd and 4th year

Fig. 6: Different stages of pruning

propagated by grafting, although wild apples grow readily from seed. Right from the begining, the technique of vegetative propagation was practiced. The rootstocks were propagated through stooling and hardwood cuttings while the scions were collected from mother trees.

Major constraints of traditional cultivation is infection caused through pest and viruses wherein pest can be managed through various ways but it is very difficult to eliminate virus infection. There is an emergent need of producing quality planting materials throughout the year at large scale which are uniform and disease-free. Viral problems need more attention, because virus infection is systemic in nature which passes to the successive generations through the propagating material and cannot be controlled by chemical means, thus, causes a severe decline in health of trees (Hadidi *et al.,* 2001). Apple trees are infected by more than 12 viruses and virus like diseases, leads to significant economic losses. Among various viruses those infecting apple crop, most of the viruses are latent in nature, which do not produce any symptom. However, fungi, bacteria, viruses, and viroids pose severe threats to the long-term survival of perennial woody plants including apple trees. Important viruses and viroids include apple chlorotic leaf spot virus (ACLSV), apple stem grooving virus (ASGV), apple stem pitting virus (ASPV), and apple scar skin viroid (ASSVd). During the course of survey, an incidence of 7-90% of apple mosaic disease (AMD) was recorded in apple orchards in J&K among various commercially grown cultivars. The maximum incidence of AMD was observed in cultivar Golden Delicious. In addition to mosaic, symptoms of chlorosis, necrosis and ring spots were also observed. Most commonly known virus infecting apple trees with symptoms are depicted in Fig. 7.

Apple mosaic disease is an economically important disease, which is prevalent throughout the apple cultivating regions of the world, and poses a severe threat to the apple industry. Apple mosaic virus (ApMV), a species of the genus Ilarvirus, family Bromoviridae (Roossinck *et al.*, 2005), is found worldwide. The



Apple chlorotic leaf spot virus (Trichovirus)

Apple mosaic virus (Ilarvirus)

Apple stem grooving virus (Capillovirus)

Apple stem pitting virus (Foveavirus)

Prunus necrotic ringspot virus (Ilarvirus)

Apple scar skin viroid (Apscaviroid)

Fig. 7: Six common viruses and viroid infecting apple plants

virus is economically significant and is easily transmitted by infected propagating material (Mink, 1992), with infection resulting in reduced production and tree decline (Desvignes, 1999). ApMV can also be detected using molecular methods (Hassan *et al.,* 2006; Lenz *et al.,* 2008) and enzyme-linked immunosorbent assay (ELISA) (Torrance and Dolby, 1984), which can serve as a quick screening approach. Apple chlorotic leaf spot trichovirus (ACLSV) is flexuous virus containing a single stranded RNA. Apple chlorotic leaf spot virus (ACLSV) is the type member of the Trichovirus group. Due to its cosmopolitan distribution, ACLSV is considered one of the most economically important viruses affecting apple. Sensitive apple varieties show malformations and size reduction of leaves and chlorotic rings or line patterns, resulting decline of grafted trees, with ACLSV infection. The absence of obvious symptoms on most infected trees increases the risk of unintentional propagation and distribution of ACLSV-infected rootstock. Apple stem pitting virus (ASPV) is a latent virus of pome fruits with worldwide distribution. In susceptible apple cultivars Charden and Spy 227, symptoms may include xylem pitting, leaf epinasty or downward growth, and tree decline. Apple scar skin viroid (ASSVd) causes scar skin or dapple symptoms in apple fruits, especially in China, Japan, South Korea, and India. ASSVd causes serious yield losses in apple and affected fruits are significantly downgraded or unmarketable. Prunus necrotic ringspot virus (PNRSV) causes reduced leaf size and produces diffused chlorotic rings and/or spots. Generally, symptoms of PNRSV appear in the year following infection, and then become symptomless, although some strains cause recurrent symptoms annually. The details of virus types, symptoms and transmission is depicted in Table 6.

Table 6: Common viruses, their symptoms and mode of transmission in apple

Virus	Apple chlorotic leaf spot virus (ACLSV)	Apple mosaic virus (ApMV)	Apple stem grooving virus (Capillovirus)	Apple stem pitting foveavirus (ASPV)	Prunus necrotic ringspot virus (PNRSV)	Apple scar skin viroids (ASSVd)
Details	ACLSV was isolated for the first time from apple trees in the USA after transmission to *Malus platycarpa*. In most commercial apple cultivars the infection generally is latent, but it causes an important disease of apple trees grown on Maruba kaido (*Malus prunifolia* cv. Ringo) rootstocks in Japan.	ApMV is a plant pathogenic virus of the family Bromoviridae. It is named after its symptoms that were first present on apples. ApMV is a positive sense RNA-based virus. Overall, it causes a severe yield reduction and decreased life expectancy of fruit trees.	Apple stem grooving virus is a plant pathogenic virus of the family Betaflexiviridae. The virus is symptomless in most commercial apple and pear cultivars, unless grafted on to sensitive rootstocks resulting in top working disease (taka tsugi byo).	ASPV is one of the most important and widespread virus infecting apples in the world. It possesses a single stranded positive RNA (+ssRNA) genome comprising of approximately 9300 nucleotides.	PNRSV belonging to family Bromoviridae and genus Ilarvirus. is a plant pathogenic virus causing ring spot diseases affecting species of the genus *Prunus*, as well as other species such as rose (*Rosa* spp.) and hops (*Humulus lupulus*). PNRSV is found worldwide due to easy transmission through plant propagation methods and infected seed.	ASSVd was reported as 'Manshu ringo sabi-ka byo' (fruit russet disease of Manchurian apple) or Manchurian apple 'Sabika' disease from Manchuria, China. In 1986, Koganezawa suggested the viroid aetiology of the disease. The viroid may also be latent in some pear cultivars without causing a disease.

(contd...)

Table 6: Common viruses, their symptoms and mode of transmission in apple (contd...)

Virus	**Apple chlorotic leaf spot virus (ACLSV)**	**Apple mosaic virus (ApMV)**	**Apple stem grooving virus (Capillovirus)**	**Apple stem pitting foveavirus (ASPV)**	**Prunus necrotic ringspot virus (PNRSV)**	**Apple scar skin viroids (ASSVd)**
Symp-toms	Growing point/ dieback Leaves: abnormal colors, abnormal forms, abnormal leaf fall, abnormal patterns Stems: canker on woody dieback, discoloration of bark, necrosis whole plant / dwarfing.	Asymptomatic after infection white gray mottling on field maple to yellow chlorotic spots on hawthorn leaves.	Fruit/reduced size leaves/ abnormal colours, abnormal leaf fall, yellowed or dead, distortion whole plant.	Xylem pits epinasty decline vein yellowing leaf red mottling pear necrotic spot fruit stony pits.	Death of buds and roots reduced tree survival and uniformity, and increased susceptibility to winter injury.	Scar skin or dapple symptoms in apple fruits.

(contd...)

Table 6: Common viruses, their symptoms and mode of transmission in apple (contd...)

Virus	Apple chlorotic leaf spot virus (ACLSV)	Apple mosaic virus (ApMV)	Apple stem grooving virus (Capillovirus)	Apple stem pitting foveavirus (ASPV)	Prunus necrotic ringspot virus (PNRSV)	Apple scar skin viroids (ASSVd)
Transmission	ACLSV is disseminated through vegetative propagation, grafting. The virus can be transmitted through mechanical inoculation, sap, seed, during pruning and various other hosts.	The ApMV is generally graft-transmissible as it persists in vegetative propagation material (scion) from infected trees, which constitutes the main source of inoculum for the virus.	Mechanical sap-transmission, contaminated scapel blades, from immature leaves, buds, cambial tissue, and ripe fruit, during grafting *etc.*	Unintentional, careless use of scion budwood collected from infected trees for propagation or top working, and from infected rootstock liners.	Transmitted through plant propagation methods, making spread through tree nursery stock and root grafting in orchards, pollen, seeds, thrips.	The viroid could be transferred to these hosts through agroinoculation, rubbing of dimeric RNA transcripts, dimeric DNA plasmids and through sap inoculation.

4. Research and Commercialization of Tissue Culture Rootstock of Apple in India

4.1 *In vitro* propagation of apple

Plant tissue culture or micropropagation has been successfully commercially applied to propagate genetically uniform and disease-free apple rootstock. However, there are a wide range of factors that need to be considered for successful *in vitro* propagation such as disinfection of *ex vitro*-derived plant material (explants), genotype, disinfection procedure, type of explants, age and physiological state of the mother plant, physical and chemical conditions applied to *in vitro* cultures (Teixeira da Silva *et al.,* 2019).

In vitro propagation of apple is often hampered by tissue browning caused by oxidation of polyphenolics due to wounding during *in vitro* culture establishment (Dobránszki and Teixeira da Silva, 2010). Reducing tissue browning is possible by collection of explants at appropriate time, adding antioxidants to the culture medium such as ascorbic acid, or polyvinylpyrrolidine (PVP), alone or in combination, or the use of liquid culture or micrografting (Yepes and Aldwinckle, 1994; Modgil *et al.,* 1999; Dobránszki *et al.,* 2000; Kaushal *et al.,* 2005).

Maintaining pH levels while preparing medium in plant tissue culture plays a very important role during shoot proliferation, root induction and organ regeneration of apple plants. Apple cultivars such as Fuji, Golden Delicious, Jonagold, and Gala were initiated in the medium under different pH, was found that the influence of the pH adjustment is variable among apple cultivars. Cultivar Gala appeared as the most sensitive to pH of the medium, whereas

Golden Delicious and M26 showed limited effects of pH of the culture medium. Cultivar Fuji was relatively difficult to root and regenerate, and showed sensitivity to the changes of culture conditions.

4.1.2 Common apple tissue culture protocols

Micropropagation has played an important role in the production of healthy, disease-free plants and in the rapid multiplication of scions and rootstocks with desirable traits. *In vitro* propagation of apple generally includes five stages, as in other plant species: (1) establishment of *in vitro* cultures from *in vivo* plants; (2) shoot regeneration and/or multiplication; (3) rooting of microshoots; (4) acclimatization of *in vitro* plantlets; and (5) the establishments of plant in an *in vivo* environment. The most frequently used explants in Stage 1 are shoot tips or axillary buds. Protocol for tissue culture of apple rootstock for its commercial production is summarized as process and in table in subsequent pages in which Murashige and Skooog (MS) medium is supplemented with different concentrations of phyohormones such as 6-Benzyl-Aminopurine (BAP), Kinetin (Kn), Indole-3-butyric acids (IBA), Naphthalene Acetic Acid (NAA), *etc.* which may vary depending upon varieties/cultivars/source of explants.

Tissue culture protocols for some of the major cultivars reported by researchers are shown in flow chart on next page and described in Table 7 for ready reference for the readers.

Selection of Mother Plants

- Superior clone of mother plant is selected which is disease free and vigrouse (Pre-chilled explants from dormant axillary cuttings).

Explants Establishment

- Surface sterilization of explants is done with suitable disinfectants to eliminate the growth of microbes and inoculated in an optimized media (quick dip of 70% alcohol, followed by 0.1% $HgCl_2$ for 3-5 minutes. Washing with few drops of tween 20 in sterilized distilled water and finally washing with sterilized distilled water. Inoculated aseptically in MS medium supplemented with 2.0 mg/1 BAP.

Indexing of all the successfully established cultures for all the known viruses mentioned at Table 6

Shoot Proliferation

- Intiated cultures are added in multiplication medium with suitable PGRs and adjuvants (2 mg/l BAP + 0.5 mg/l IAA + 1 mg /1 KN). It is subcultured at 4-6 weeks interval up to 12 cycles.

Rooting

- Multiplied shoots are separated and inoculated in an optimized rooting medium supplemented with auxins (MS + 1 mg/l IBA).

Acclimatization

- The rooted plantlets are gently transfered in protrays containing artifical substrates and are transfered to greenhouse for hardening (Cocopeat) for 45 days.

Batch certification by random sampling to ensure supply of virus free root stock

Field Transfer

- The primary hardened plants are transfered in nursery bags/pots containing FYM and processed for secondary hardening in field (70% survival).

Table 7: Tissue culture protocol of major apple rootstock cultivars

Cultivar	Explant	Multiplication Medium	Establishment Rate	Rooting Medium	Hardening Rate	References
M9, MM106 and MM111	Meristem/ axillary/ terminal shoot tips	MS, supplemented with 1 mg/l BAP, 0.5 mg/l GA 3 and 0.1 mg/l IBA	Higher shoot formation was 95.4% for the M9 from terminal shoots, 93.3% for the MM106 from lateral shoots and 81.2% for the MM111 from terminal shoots	MS supplemented with 0.5 mg/l IBA	Rooting ability of the shootlets was higher (62.5-90.3%) for MM106, medium (53.6-66.6%) for M9, and low (12.1-40.0%) for MM111	Mert and Soylu, 2010
M26	Nodal explants	2.0 mg/l BAP, 1.0 mg/l GA3 and 0.2 mg/l IBA/ NAA	14-20% explant establishment	NAA dip for root induction and transferred to NAA free rooting medium for root elongation	90-100%	Modgil and Thakur, 2017

(contd...)

Table 7: Tissue culture protocol of major apple rootstock cultivars (contd...)

Cultivar	Explant	Multiplication Medium	Establishment Rate	Rooting Medium	Hardening Rate	References
M9', 'M27', and 'MM106	Nodal explants	4.4 μM BAP and 2.27 μM TDZ during the shoot multiplication phase, but 8.8 μM BAP+ 1.14 μM TDZ and 2.8 μM (GA3) during the shoot elongation phase	The best shoot growth was observed in the high (2X) concentration mineral treatment	half-strength MS medium containing 5.4 μM IBA and 1.2 μM 2,4-D	The highest percentage (64%) rooting was produced for 'MM106' genotype and the lowest (11%) for 'M9' after 3 months	Amiri and Elahinia, 2011
Malus domestica	nodal explants	MS medium supplemented with BAP (0.1 mg/l) and IBA (0.1 mg/l)	A high frequency of shoot regeneration (85.0%) and differentiation was observed in the primary cultures	half strength MS medium enriched with IBA (3.0 mg/l) and 2.0 g/l of activated charcoal	A high frequency of rooting (66.7%) with development of healthy roots is observed from shoots cultured	Manel., *et. al.*, 2010
EMLA111	Axillary buds and shoot apices	MS medium supplemented with 6-Benzylaminopurine (BAP), Kinetin (Kn), Gibberellic acid (GA_3)	70% explant establishment	0.3 mg/l IBA and 1 mg/l NAA	IBA (81–82%) NAA (71%)	Modgil *et al.*, 2010

5. Rootstock Tissue Culture

5.1 Suitable species as rootstocks for apple

M series of apple rootstocks (Table 7) have been found suitable for high density plantation. Some of the most promising varieties are mentioned in Table 8.

Table 8: Promising varieties of M series of apple rootstocks

Category	Rootstock	Characteristics
Dwarf	M 9	Short juvenile phase, weak anchorage, suitable for high density planting in flat and irrigated areas only.
Semi-dwarf	M 4 and M 7	Suitable for high density planting and well-drained soils; resistant to wooly apple aphid but susceptible to collar rot.
Semi-vigorous	MM 111	Tree size is 70% of the standard, drought tolerant and resistant to wooly apple aphid.

Source: https://horticulture.jk.gov.in/FAQs.html

5.2 Constraints and possible solutions

The low productivity of apple continues to trouble the stake-holders. Increasing population and enhanced purchasing power of people have correspondingly increased the demand for apple fruits. In order to meet with the requirement, the productivity per hectare needs to be increased through innovative and scientific methods such as use of high-density plantation and adopting modern technologies for apple cultivation. Thus, high density plantation through tissue culture-raised rootstock of apple has great potential to address this issue to a large extent.

As mentioned earlier in Section 3; Table 6, there are large number of viruses and viroids which infect apple in India. This infection results into losses such as slow plant growth, failure in grafting, poor quality fruits, lower yielding orchards. Thus, there is an urgent need to use virus-free planting materials produced through tissue culture to grow healthy apple trees by the farmers.

5.3 Tissue culture-raised rootstocks and its benefits

Rootstocks are very important as it determines important traits of grafted trees, including growth vigour, yield and resistance or tolerance to biotic and abiotic stresses. Tissue culture-based propagation plays an important role in rapidly propagating promising rootstocks for commercial production.

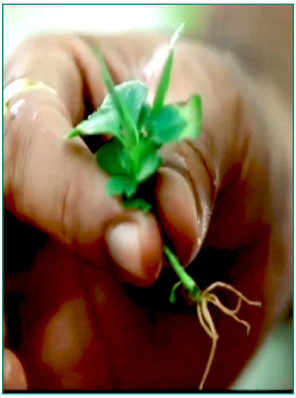
Key goal of apple tissue cultures is to multiply pathogen-free superior clones as per the market and field requirements. *In vitro* propagation of apple generally includes five stages, as in other plant species: (1) establishment of *in vitro* cultures from selected mother plants; (2) shoot regeneration and mass multiplication; (3) rooting of *in vitro* shoots; (4) acclimatization or hardening of *in vitro* plantlets; and (5) establishments of plant finally in natural environment (Fig. 8). The most frequently used explants at Stage 1 in apple tissue culture are shoot tips or axillary buds.

Plant tissue culture has become successful technique for producing rootstocks for fruit trees, namely, apple, pear, cherry, peach, almond, *etc.* In Europe and USA, maximum volume of peach and cherry rootstocks is propagated through plant tissue culture. Approximately 20 million peach rootstocks are propagated by tissue culture every year in Europe. Majority of pear, cherry, peach, almond, walnut, and pistachio rootstocks have been propagated by tissue culture in the USA (https://apples.extension.org/tissue-culture-propagation-of-apple-rootstocks/).

In India, demand for tissue culture-raised apple rootstocks for dwarf variety is increasing rapidly. Approximate 1 million plantlets/year of M series are being produced and sold in HP, J&K, Uttarakhand and North-East region of India.

Establishment of *in vitro* cultures

Shoot formation and mass multiplication



Root formation and mass multiplication

Acclimatization

Field plantation

Fig. 8: Stages of production of tissue culture apple rootstock

5.4 Commercialization

Commercial tissue culture was started in India in 1987 when AV Thomas and Company (AVT) established their first production unit in Cochin (Kochi), Kerala, for clonal propagation of superior genotypes of selected cardamom plants. This started with lab scale technology transferred to company by the National Chemical Laboratory (NCL), Pune, India. This technology was later refined and scaled up by the company to make the method more production-oriented so that the process is cost- and quality-effective and could guarantee an efficient delivery system. This pioneering effort in the application of tissue culture research by AVT was followed by several other entrepreneurs who entered into the field of plant tissue culture. In 1988, another company, namely, Indo-American Hybrid Seeds at Bangalore, Karnataka, who were in the nursery business in hybrid flowers and vegetables, established a tissue culture laboratory and green houses with a capacity to produce 10 million plants/annum. By 1988, the number of commercial tissue culture units increased to 4, and later showed a rapid rise till 1996 to around 50 commercial tissue culture labs in India (Mascarenhas, 1999). At present, there are around 200 commercial tissue culture companies in India with gross installed production capacity of about 500 million plantlets/annum. Banana, potato, sugarcane, apple, pineapple, strawberry, *Gerbera, Anthurium, Lillium,* orchids, bamboo, date palm, teak and pomegranate are some of the major plants which are produced through tissue culture in India.

The tissue culture industry witnessed a gross installed production capacity of about 500 million plantlets/annum with an actual production of approximately 350 million plants/annum. The plant tissue culture market in India is estimated valued INR 5000 million. Commercialization of tissue culture apple rootstocks has brought enhanced income not only for farmers but also for wholesalers and retailers throughout the seasons. Approximate 5 million plantlets of apple tissue culture rootstock have been produced and distributed in India in recent years.

5.5 Role of National Certification System for Tissue Culture Raised Plants (NCS-TCP) as comprehensive Quality Management System (QMS)

Quality management system has two main crucial components in commercial operation, namely, (i) freedom from viruses, and (ii) true-to-type plants produced through tissue culture. Without these two features, tissue culture-raised plants are not acceptable by the growers and it may also pose threat of undesirable spread of viruses and distribution of substandard quality of plants. To minimize the risk of inadvertent micropropagation of virus-infected plants, tissue culture-raised plants need to be thoroughly indexed for freedom from viruses and checked for quality. In view of this fact, Department of Biotechnology (DBT), Government of India, undertook responsibility as Tissue Culture Certification Agency (TCCA) under the Section 8 of the Seeds Act, 1966 (54 of 1966). Accordingly, DBT has established National Certification System for Tissue Culture Raised Plants (NCS-TCP) in India. NCS-TCP is a unique quality management system for tissue culture industry, which is first of its kind in the world. It is very comprehensive system involving many components for its effective implementation, namely, NCS-TCP Management Cell at Biotech Consortium India Limited (BCIL), two Referral Centres, and four Accredited Test Laboratories (ATLs). Recognized Tissue Culture Production Facilities are responsible for production and distribution of certified quality tissue culture plants through NCS-TCP, which has helped in capacity building in tissue culture sector. It is noteworthy fact that there has been no major virus outbreak since the inception of the NCS-TCP in the country. This outcome is due to increased number of testing of certification of plants (Shukla, 2017).

5.6 Testing and batch certification of tissue culture-raised (TC) plants of apple

- ATLs entertain applications only from recognized Tissue Culture Production Facility (TCPF) for the purpose of issuance of Certificate of Quality under NCS-TCP.
- ATL would not accept the sample of TC plants for certification if the mother plant/stock culture has not been indexed for respective batch of TC plants.
- ATLs ensure receipt of complete information in the application for stock culture testing as well as certification of TC plants such as batch number and batch size (Shukla, 2016).

5.7 Tissue culture standards for apple

Tissue culture standards provide guidance for maintaining the TCPF as per the quality requirements and produce good quality TC plants. Standards for producing TC plants of apple is summarized on next page.

5.7.1 Standards for maintaining the Tissue Culture Production Facility

Requirements for maintaining TCPF are:

- TCPF should be recognized under the NCS-TCP. Recognition is based on compliance with the NCS-TCP guidelines which broadly includes infrastructure, package of practices of producing tissue cultured plants, effective technical supervision, record keeping and documentation.
- Entire facility should be free from pests or vectors of apple pathogen.
- Hygienic condition should be maintained throughout the operation and desired sterility class should be maintained at laboratory facility.
- Apple varieties should be tested for all the notified viruses at the time of initiation and it should be free from other endophytic or epiphytic bacteria and fungi.

5.7.2 Testing requirements as per quality norms

- TCPF must maintain passport data of variety covering unique code, date of initiation, origin, testing details *etc.*, that being propagated commercially.
- All the initiated material should be indexed by known viruses.
- *In vitro* cultures should not be multiplied beyond 12 passages to ensure uniformity or true-to-typeness.
- For batch of TC plants, a minimum of 0.1% of total number of plants should be tested for viruses and uniformity.

5.7.3 Requirements for greenhouse/polyhouse

- Effective procedure for insect and diseases monitoring should be in place.
- Field-grown apple plants should not be kept along with TC plants.
- Variety should be separated by physical barrier or proper tagging.

- Batch should be tested by ATLs and labelling process should be followed.

Source: https://dbtncstcp.nic.in

5.8 Importance of implementing Standard Operating Procedures (SOPs)

Standard Operating Procedures (SOPs) are set of written instructions which include step by step activities. Adoption of SOPs makes the system/operation process-dependent rather than making it person-centric. Package of Practices includes ensuring cleanliness particularly in washing area, proper procedure for discarding the used agar/media, labelling of media as well as culture, trays at each step, monitoring of air-borne microbes on monthly basis in addition to particle count at six monthly intervals and regular fumigation, storing the culture media for minimum 3-4 days prior to use in order to avoid wastage of precious culture and time due to problems such as media sterilization, maintenance of laminar air flow cabinet by cleaning of pre-filters, checking the air flow and efficiency of HEPA filter by exposing plates, maintaining uniform temperature in the growth room, grading of plantlets. Grading is done according to specific criteria for the plant species being micropropagated such as monitoring of health of *in vitro* cultures and plants in the hardening area, monitoring of light, temperature and humidity in the greenhouse, insect monitoring in the hardening areas, ensuring that good quality of water (potable) for watering of plants under hardening and avoiding excessive watering and undertaking regular weeding. Entire production process should be strictly monitored and supervised by in-charge and supervisors. Multiplication cycle should be restricted in order to avoid somaclonal variations (genetic instability). Maximum number of cycles can be determined by studying the uniformity at genetic level using molecular markers. Different species have different permissible limits of number of passages *e.g.* 12 cycle for apple. Facility must ensure that TC plants are fully hardened at the time of dispatch. In case of ex-agar plants, plantlets should be of appropriate size for survival during transport. Handouts to be given to the farmers while supplying plants covering the package of practices for cultivation (Shukla, 2015).

5.9 Virus indexing protocol

Reverse transcription–polymerase chain reaction (RT-PCR) is used to detect five Ribonucleic Acid (RNA) viruses and one viroid (ACLSV-Apple chlorotic leaf spot virus, AMV-Apple mosaic virus, PNRSV-Prunus necrotic ringspot virus, ASPV-Apple stem pitting virus, ASGV-Apple stem grooving virus and one viroid ASSVd - Apple scar skin viroid). For RNA virus, ELISA can also be used for primary screening being the economical option (except viroid). However, RT-PCR is the best available technique for confirmatory test. It includes following steps:

5.9.1 Isolation of total nucleic acid

Healthy samples (~100 mg) are cut into strips. Extract total nucleic acid are extracted using RNeasy kit. RNA is inactivated and used as template for RT-PCR.

5.9.2 cDNA Amplification

(i) RT-PCR reaction components are assembled on wet ice and prepare amplification mix by dispensing into ~ 200 μl microfuge tube.

(ii) RT-PCR reaction assembly are mixed by inversion and place the tubes in a thermal cycler and proceed with thermal cycling profile chosen for reaction.

(iii) The amplified product is analysed by electrophoresing 10 μl from the total reaction on 1 % agarose gel in Tris – acetate EDTA (TAE) containing ethidium bromide.

(iv) Temperature profile for RT-PCR:

Steps	Temperature	Time	Cycle
Initial denaturation	94°C	5 min	1
Denaturation	94°C	30 sec	–
Annealing	45-50°C	1 min	30
Primer extension	72°C	1 min	–
Final primer extension	72°C	10 min	1

5.9.3 Analysis of amplicons

Agarose (0.5g) is melted in 50 ml 1 x TAE running buffer and ethidium bromide (0.5 mg/ml) is added to the agarose after cooling to around 50°C. Melted agarose is poured into the casting tray for polymerization and the buffer tank is filled with running buffer (1 x TAE) and the comb is removed. RT-PCR product (10 μl) is loaded with 2 μl 6x loading dye and the gel is run at 60 volts for 2h along with marker. The gel is examined under ultraviolet transilluminator and photograph is taken (Dijkstra and de Jager, 1998; Pappu *et al.*, 1993).

Table 9: Primers used for detection of apple viruses

Primer	Primer Sequence	Genomic Region	Amplicon Size
ASPV F	ATGTCTGGAACCTCATGCTGCAA	ASPV-CP	370
ASPV R	TTGGGATCAACTTTACTAAAAAGCATA		
ASGV5641 F	ATGAGTTTGGAAGACGTGCTTC	ASGV-CP	750
ASGV 6396R	CTGCAAGACCGCGACCAAGTTT		
ApMV F	ATCCGAGTGAACAGTCTATCCTCTAA	ApMV-CP	262
ApMV R	GTAACTCACTCGTTATCACGTACAA		
ACLSV F	GATCAGAAGRMGRAGGAT	ACLSV-CP & UTR	800
ACLSV R	GTAGTAAAATATTTAAAAG		
PNRSV F	AACTGCAGATGGTTTGCCGAATTTGCAA	PNRSV-CP	675
PNRSV R	GCTCTAGACTAGATCTCAAGCAGGTC		
ASSVd F	CGGTGAGAAAGGAGCTGCCAG		330
ASSVd R	GCCTTCGTCGACGACGACAG		

Source: Rana *et al.*, 2011

6. Challenges

There are many challenges in adoption of tissue culture technologies for production of apple rootstocks. These challenges have been categorised as below:

6.1 Production of TC rootstock

Production of tissue culture rootstock requires setting up of sophisticated facility which includes commercial tissue culture lab, greenhouse and net house structures. These facilities require high-cost inputs (investment of approx. INR 25 – 30 million for the facility of producing 25 million plants/annum). Moreover, expertise is required in the tissue culture technology for establishing and operating the unit. Logistics, perishable/shelf life, contamination and mortality are other constraints for achieving effective production as per the market demand.

6.2 Managing quality

Managing quality for production of virus-free and true-to-type superior clones is very crucial. Effective quality management system in commercial operation depends on two major factors, namely, (i) freedom from viruses and (ii) true-to-type progeny plants. In the absence of any of these two characters, the TC plants are not acceptable to the farmers (Shukla, 2017). Quality procedure has to be adopted from the beginning *i.e.* selecting right mother plants, indexing for virus or viroid, minimizing contaminations, passages of sub-culturing under prescribed limits are essential.

6.3 Distribution

Distribution and logistics for TC rootstock are key challenges, being the live material. Moreover, it adds to the cost of plant. Cost of the TC plants/rootstocks can be decreased by transporting semi-hardened or ex-agar plants and further growing it at the regional hardening centres at the area of transportation and plantation.

6.4 Technology transfer

Technology/protocol of producing apple TC rootstock is available with research institutes. However, scalability remains an issue when it is applied for large scale propagation. Translation of research protocol into commercial production requires refining the technology with hand-holding support to entrepreneurs. Establishment of TCPF can be achieved as per the NCS-TCP guidelines. BCIL extends technical hand-holding to any entrepreneur for establishing new TCPF.

6.5 Adoption by farmers

Cost of TC rootstock remains a hurdle as it is 4-5x costly than conventional plants. Lack of awareness about the benefits of tissue culture quality material which compensate the initial high cost of plantation remain the hurdle. Due to this, farmers continue to maintain old orchards with low productivity.

6.6 Capacity building

Access to capacity building programme is one of the key challenges which hinders the growth of tissue culture sector and expansion of area adopting high tech plantation using tissue culture technology. This can be addressed at government level by organizing frequent training and skilling programmes at different modules for various categories of stakeholders.

7. Economics of Apple Cultivation using TC Rootstocks

7.1 Cost of TC rootstock plantlets

Cost of fully hardened TC plant is nearly 5x *i.e.* INR 100 per plant (equivalent to USD 1.37) as compared to INR 20 (equivalent to USD 0.27) of the plant raised as seedlings. The fully grafted plants through TC rootstock estimated about INR 300-400 per plant (equivalent to USD 4.11-5.49) (depending on variety of scion) whereas grafted plants through seedling are available at the rate of INR 200 per plants (equivalent to USD 2.74). Seedling plants will have undesirable variation in growth pattern as well as fruit production and field will have heterogeneous population. Plants raised through layering is also clonal propagation which is found superior over seedling-based plants, however, requirement of rootstocks in large quantity for a field can be met only through mass propagation by TC rootstocks where similar batch of virus-free plants can be provided for grafting and cultivation as compared to production of on an average 5 plants from one tree in a year through layering. High returns of tissue culture plants compensate the initial one-time investment of farmers towards procurement of superior plants.

7.2 Financial benefits to farmers using TC rootstocks over conventional planting of apple

Economic advantage of TC apple rootstock over the conventional seedling grafted plants is compared and summarized below:

7.2.1 High density plantation

TC rootstock plants has made it possible to adopt high density plantation. Standard apple plants raised on seedling rootstocks are planted at a spacing of 7.5 x 7.5 m with a planting density of 178 plants/ha. Some other varieties on seedling rootstocks are planted at a spacing of 5.0 x 5.0 m with a planting density of 400 plants/ha. In high-density planting, apple plants on clonal rootstocks approx. 5000 plants/ha can be planted which increase the production per unit area basis and improve the quality of the produce.

7.2.2 About 10-time higher yield

Due to a smaller number of plants in seedling-raised rootstock orchard, the average production of these orchards is approx. 6 to 8 MT/ha, which is much less than the production in high-density TC orchards (60-80 MT/ha). Dwarf varieties of TC rootstock has revolutionized the apple production. It is highly beneficial to meet the demand and need of apple growers when pressure on land is increasing in order to increase the production and their income.

7.2.3 Early maturity and fruit harvesting

Due to vigorous root system, TC rootstock-based cultivation starts yielding after two years whereas traditional seedling yield after 10 years. It saves not only time but resources of the growers that required to maintain the orchard for longer period awaiting fruiting.

7.2.4 Assured annual income

Tissue culture rootstock is clonal propagation which yield into homogenous population with uniform growth pattern. Orchardists get 10x better income. Importantly, these plants bear fruits every year whereas traditionally-grown plants result into low income and uncertain fruiting due to variation/heterozygous population of trees.

7.2.5 Orchard management

In TC rootstock-based high-density plantation, management of orchard becomes easy and economical. It requires less labor since cutting, pruning and spraying become easy to low canopy. Traditional plantation is comparatively high labor-intensive, therefore, less economical.

7.2.6 Better fruit quality

Fruit quality is uniform in TC rootstock plants due to which grading and packaging become more organized and less time-consuming resulting into better market acceptability, particularly for export. In traditional plants, after fruit setting, fruit were seen patching whereas in apple rootstock plant, trees were laden with flowers and fruits. The interesting thing was that orchardist had to do thinning of the fruit. As the size of the plant is small the root was able to transport nutrient quickly to the stem and branches. As a result, fruit obtains an ample nutrients and its size as well as quality improved and become better in contrast to that borne on old conventional trees.

In high density plantation through tissue culture, farmers get option for different densities ranging from 1500 trees/ha to 5000 trees/ha. Although, high density plantations are capital-intensive in the beginning due to inputs on a greater number of plants, but in long run the benefit-cost ratio is very high (Cahn and Goedegebure, 1992). The productivity of developed countries, namely, Italy, Netherlands, New Zealand is almost 70-75 tonnes/ha (Robinson *et al.,* 2004). These countries use TC rootstock-based high density plantation. In India, with increased awareness now adoption of TC rootstock is yet to attain its highest potential.

Detailed studies were carried out on performance of the high-density apple plantation scheme in J&K, India, which was launched in 2016 aimed at boosting the productivity and production of apples (Hassan *et al.,* 2020). This study demonstrated very interesting findings on high density plantation. Average yield of 11.43 tonnes/ha was reported in J&K which was above the national yield (9.73 tonnes/ha) however, it was recorded much below than the global production 55-63 tonnes/ha (National Horticulture Board, India, 2018-19). This emphasizes that there is a need for vigorous efforts for promotion of tissue culture and high-density plantation in apple. Field Survey of Hassan *et al.* (2020) reveals that high density plantation (4000 trees/ha) starts yielding 35 tonnes/ha which crosses 70 tonnes/ha after 7 years in J & K (eventually increased up to 100 tonnes/ha) which is comparable with global scenario of developed countries. It attains break even period in sixth year, however, with government support (subsidy), it becomes feasible to have early break even at 4/5 years. Indicative profile regarding investment and returns out of one ha orchard is given in Table 10.

Table 10: Investment and return per hectare (indicative project profile of 10 years)

Investment				Returns	
				Harvest per plant/Total yield (in kg)*	Returns (INR in million) (@ INR 100/kg
Year 1	Plant @ INR 400 per unit	Total 3000 plants x INR 600	INR 18,00,000	0.0	0.0
Upfront cost	Farm Yard Manure (FYM), Cost of Pit-digging and plant support @ INR 100/pit digging				
	Drip/Weed mat @ INR 100/plant				
Recurring cost of maintenance and harvesting (in INR)					
Year 2	100 per plant	3,00,000		0.0	0.0
Year 3	100 per plant + INR 500 per plant for Trellises	18,00,000		5/ 15,000	1.5
Year 4	125 per plant	3,75,000		8 /24,000	2.4
Year 5	125 per plant	3,75,000		10 / 30,000	3.0
Year 6	125 per plant	3,75,000		15 /45,000	4.5
Year 7	150 per plant	4,50,000		25 /75,000	7.5
Year 8	150 per plant	4,50,000		25 /75,000	7.5
Year 9	150 per plant	4,50,000		30 /90,000	9.0
Year 10	150 per plant	4,50,000		30 /90,000	9.0

● On conservative figure of yield. However, better yield (35 kg/plant reported by farmers used TC plants from 3rd/4th years) ● Total expenses: INR 6.83 million in 10 years Breakeven will be attained in 5th year ● Net return would be more than INR 7.5 Million from 7th year onwards which may further increase to INR 9.0 million from 9th year. Productive life will continue of orchards till 25 years.

Note: Cost of land /lease rent and interest on loan have not been taken into account. These factors may impact the net profit.

Disclaimer: Its generalized indicative profile for apple TC rootstock based high density plantation. The same should not be conceived as feasibility report as it will vary depending on various financial factors.

8. Scope of Agribusiness

Tissue culture technology has been successfully commercialized due to various advantages as depicted at Fig. 9.

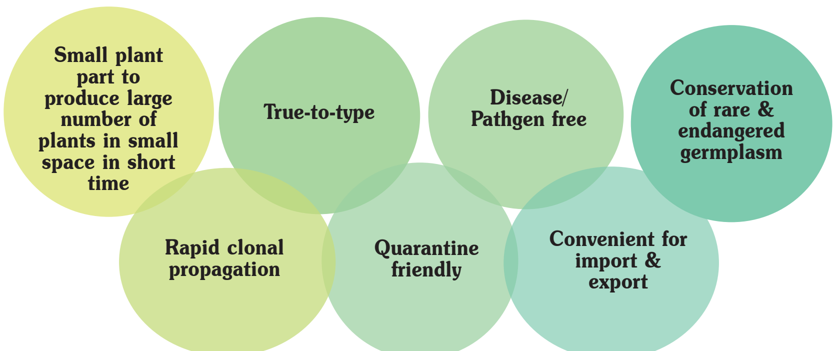


Fig. 9: Advantages of tissue culture technology

Entrepreneurship in plant tissue culture is the act of being an entrepreneur, who starts plant tissue culture production facility for being self-employed and creating job opportunities. Although, pursuing entrepreneurship is not the easy path. It has multiple challenges which need to be addressed with suitable strategies. Challenges and strategies to overcome the same are presented in Fig. 10.

Technical constraints can be effectively managed through adopting standards and guidelines. These national standards of NCS-TCP provide guidance to entrepreneurs for meeting the quality requirements in setting up of commercial unit in terms of infrastructure, package of practices and maintaining quality of tissue culture plants. Layout of the facility is the basic requirement for ensuring unidirectional personnel and material movement and

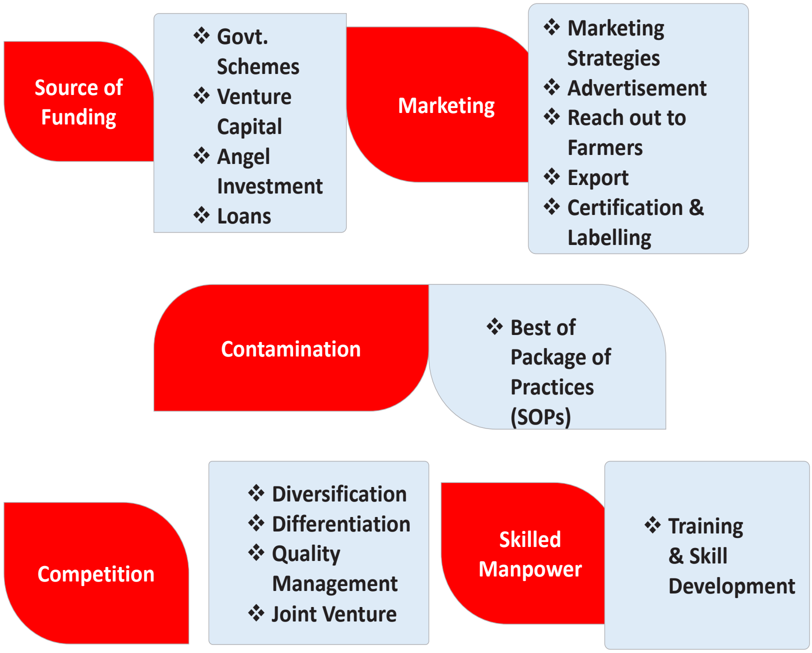


Fig. 10: Challenges and strategies

avoiding possibility of criss-cross movement between sterile and non-sterile zone. TCPF should have clearly demarcated area for entry, washing, media preparation, media storage, inoculation, growth room and plantlets washing/grading area. It is also necessary to ensure connectivity to various components of TCPF and availability of basic equipment particularly for maintenance of minimum sterility class 100,000 in media storage, inoculation, growth rooms and corridor connecting the sterile areas (Shukla, 2015).

8.1 Successful examples of established agribusiness unit

It is noteworthy to mention about the success story of Mr Vinod Soni as a successful entrepreneur based at Bilaspur, HP (Fig. 11) who established and operationalised his commercial tissue culture laboratory under the technical guidance and mentorship of BCIL, New Delhi.

Fig. 11: TCPF at Nishant Biotech, Bilaspur, HP

Journey of Mr Vinod Soni has been remarkable as successful tissue culture entrepreneur of Himalayan region. He has been under constant mentorship of Dr Shiv Kant Shukla, BCIL (one of the authors), since year 2009. He continued his passion successfully following the trajectory as described below:

No formal background on agri-entrepreneurship: His father was a teacher in primary education and mother was working in medical department. His grandfather was a goldsmith and a shopkeeper of jewellery.

Tissue culture as business diversification: Mr Soni and his brother were also going with their family profession and established a jewellery shop in 1995 after completion graduation. Gradually, they settled in family business. In year 2005 to 2006, he wanted to diversify his business. In year 2004-2005, there was a revolution towards greenhouse construction and cultivation of flower plants in green house, and they thought about production of planting material through tissue culture. But they have no idea of tissue culture lab and this technology.

Orientation/training from horticulture department and research Institute: In year 2005, Mr Soni visited the horticulture department and he was advised to undergo training on tissue culture from Institute of Himalayan Bioresource Technology (IHBT), Palampur. After initial training, he applied for establishing tissue culture laboratory in 2005 at Department of Horticulture, Himachal Pradesh and got sanction from department and established a laboratory and started propagation of apple rootstock.

Hand-holding and mentoring by BCIL resulted into transforming tissue culture as successful venture: During conference at IHBT Palampur, he came to know about BCIL and NCS-TCP. He established a bigger tissue culture lab named as Nishant Biotech under scheme of MIDH and got 50% incentive from this scheme in year 2012. He applied for Recognition under NCS-TCP. This was the only laboratory in India producing TC apple plants which is recognized under NCS-TCP. He increased production every year and has staff of approxmately 80 persons in his unit.

Quality management system through NCS-TCP and market expansion: Market response was very good. Orchardist got good result from TC plants and demand of TC plants has been increasing every year. He supplies plants to J&K, HP, Uttarakhand and North-East region of India.

Awareness through social media revolution: His company organized awareness festivals in the villages and displayed TC plants with the aim of imparting first-hand information to the orchardists. Orchardists planted apple rootstock in their neighborhood villages. Awareness was created when survival rate of found good and it started fruiting in 2010-11. Many farmers started planting TC rootstock on small scale. With the passage of time, when 3G,4G network became stronger, videos of orchardists with fruiting were prepared and circulated with the help of social media. On seeing videos demand of TC rootstock increased. In 2012, scaled up unit in the name of Nishant Biotech with the production capacity of which is 1.5 million plants/annum was established to meet the increasing demand.

Easy availability and economical than imported plants: Small scale growers were unable to buy TC plants from abroad so Nishant Biotech became a good economically feasible option for them. Those growers who tried TC plants, got excellent results and progressed by leaps and bounds. The growers bought fresh rootstock from Nishant Biotech and grafted themselves at their orchards. The growers obtained virus-free rootstocks through tissue culture which proved to be good alternate for them. Nishant Biotech provides technical guidance and support also to growers.

Market expansion: Orchardist was able to get good quality of planting material in their own region. Besides providing plants to temperate region like J&K, HP and Uttarakhand, they also supplied plants to sub-tropical and tropical regions like Punjab, Maharashtra and plain areas of HP. From time-to-time seminars for orchardist were organized in the tissue culture labs as well as in the orchards. Many nurseries purchased primary plants from Nishant Biotech, grew in their nurseries, grafted themselves and eventually raising their incomes. As per the demand of growers, they are providing primary hardened, secondary hardened, fully-grown rootstocks and grafted plants on TC rootstocks.

Growth of the tissue culture laboratories through upliftment of growers: Orchardists are getting saturated from the conventional method of cultivation as the expenditure was more than income in many cases. Nishant Biotech provided them better option. The better varieties of apple increased income to 3x to 4x. Many vegetables farmers started planting apple rootstock for sale to other apple growers.

Future plan: Mr Soni has imported walnut, almond and apple new series almond (prunes) for large scale propagation. He has ambitious plan to export TC plants to neighbouring countries in near future.

9. Potential Benefits to Farmers and their Perception to Use Tissue Culture Rootstocks

Farmers are adopting TC rootstock due to its substantial benefits over the conventional planting materials with impressive results in the orchards. There is significant difference in traditional and TC rootstock farming. Seedling plants (traditional farming) includes heterozygous population with morphological variations and it generally does not yield effective fruiting up to 10 years. Moreover, size of plants becomes very large resulting into difficulty in operational management of orchards like spray, cutting, pruning and harvesting, *etc.* In one ha of orchard, only 500 to 600 plants can be cultivated due to their big size. The quality of fruits is also comparatively poor. Clonal planting rootstocks raised through layering has its own limitations of soil-borne infections and producing only a few progenies in a year. In contrast to traditional plants, rootstocks raised through tissue culture offers various benefits. It attains stage of effective fruiting from 3rd years onwards and in one ha and cultivation of about 2000 to 5000 plants is feasible (depending on type of varieties). Operational management like cutting, pruning and spray becomes very easy due to low height of plants. Quality of fruits is found very good that meeting the requirement of international export standards due to less chance of any viral infection.

The most important aspect of TC stock is that it has tremendous impact on farm yield. High density TC rootstock yields up to 50-100 MT whereas traditional plantation yields only up to 5-10 MT of apple fruits in a hectare which is about 10x more than traditional farming.

It is noteworthy that tissue-cultured rootstock plants are free from diseases particularly viruses which instilled high level of confidence among orchardists while adopting TC rootstocks. The quality of roots has been observed very good and, thus, overall growth of plants. Tissue culture plants grow faster than conventional plants. Due to a good rooting cluster, survival rate of plants in the field is about 95 to 98%. Economy of farmers is growing rapidly in Northern and Himalayan region in India spreading happiness among farming communities. This is evident from the case studies provided in subsequent section which includes experience sharing of apple growers through personal interaction. Authors made visits to facilities producing apple rootstocks and plantation to have in-depth understanding of impacts on the field of this technology (Fig. 12).

Fig. 12: Authors visiting tissue culture and field facilities

9.1 Experience sharing by Progressive Growers highlighting their success stories who adopted tissue cultured rootstocks for apple production

☞ ***Jiggi Chauhan and Jitu Chauhan***

Jiggi Chauhan

Jitu Chauhan

Address: Village Baggi P.O. Gawass Tehsil Chirgaon Distt. Shimla-171207 (HP)

Jiggi Chauhan and Jitu Chauhan came to know that tissue culture is modern technique of propagation of plants and realized that this is the only technique which can provide surety of virus-free rootstocks. They feel fortunate that tissue culture quality rootstocks are provided at affordable prices through NCS-TCP recognized TCPF at Bilaspur, HP. Above orchardists had planted total 6500 TC apple (varieties – Emla-9, M7 and MM106) plants from NCS-TCP recognized TCPF. These rootstocks were grafted with Redlum Gala, Superchief, Granysmith, Scarlet-2 and Pink Lady. Orchardists happily informed that TC plants made canopy in minimal time and reached maturity stage and started quality production in just 3 years. In Singh Apple Orchards, owned by them, an average of 35 kg of fruits from Emla-9 which was grafted with Redlum gala cultivar were harvested. Average production of HP is 8-9 million tonnes/ha. But they have succeeded to harvest 30-40 million tonnes/ha. With these encouraging results, now orchardists are spreading

this awareness amongst the mass apple growers in different parts of India.

Impact of success on larger farming community: Since 2013 when Chauhans started cultivation of apple with organic practices and high-density planting, they started organizing a Farm Fest every year. Thousands of farmers join them from different States *e.g.* J&K, Uttarakhand and Arunachal Pradesh who were trained for apple cultivation and shared practices and experience of adoption of TC apple rootstock. Apart from Farm Fest, they maintain Facebook page (https://www.facebook.com/Himganic/) and through this page experiences in orchards are shared regularly for the benefits of other orchardists.

During 2019, Chauhans organized Farm Fest in association with Department of Horticulture, HP, with active participation from large number of farmers along with officials from state agriculture and horticulture departments. During adverse period of COVID-forced lock down, Fest was organized at virtual platform connecting hundreds of farmers from different states. They have also registered a Farmer Producer Organization (FPO) under the name Himganic Farmers Producer Company LTD., to provide advantage to fellow orchardists. There are approximate 200 farmer members from different regions of HP in addition to some active members from other states.

☞ ***Thakur Surender Singh***

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Village Revi P.O. Shilaroo
Tehsil Theog, Distt Shimla-171213 (HP)

Professional Background
Retd. Chief Electrical Engineering

Mr Singh retired as an Electrical Engineer in the year 2012. Just after retirement, he visited tissue culture facility for exploring to become an apple grower. He wanted good quality rootstock and opted for M9 as per advice of experts. Initially, he planted 200 plants

in January 2013 and got it grafted in 2014 with Super Chief as scion wood. Subsequently, additional 100 TC plants were planted and grafted after one year with Super Chief only. All the 300 plants survived successfully. During 2018, Mr Singh got full grown orchard with excellent quality of fruits in terms of size and colour. On an average, he marketed around 300 boxes of 10 kg each. He is experiencing success in apple farming in spite of non-agriculture background and now planning to scale up the apple production. He gives credit to the supply of quality TC rootstock plants.

☞ ***Dr Nishar Ahmad Latoo***

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Drabgam, Tehsil Rajpora,
Distt. Phulwama-192302 (J&K)

Dr Latoo is medical doctor by profession but now also into high-density apple rootstock farming and got good success.

While visiting commercial tissue culture laboratory, Dr Latoo was impressed with tissue culture technology and understood its field application. Later, he also visited apple orchards and witnessed the success of farmers who had adopted TC rootstock. Dr Latoo planted around 400 plants of different TC rootstocks in year 2016. He was very much impressed by the shape, colour and size of the apples, as all apples followed a uniform pattern of shape, size and colour in contrast to that of the traditional ones.

He experienced that farming based on TC apple rootstock is cost effective due to requirement of less labour. Currently, he has expanded cultivation in phased manner and possess varieties of Jeromine (300 plants), Superchief (800 plants), Shalit Super (Washington; 300 plants), SS2 (200 plants) and R. Gala (300 plants). Yield was recorded up to 30-40 kg/plant. Maturity of plants were attained within 3-5 years of plantation.

☞ ***Mr Kushal Singh***

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Village Rahidhar P.O. Bagsiad Tehsil Thunag Distt. Mandi-175035 (HP)

Mr Singh had a long experience of traditional apple farming. He faced many problems like incidence of diseases, low quality fruit, *etc.* Expenses for pruning and maintenance cost of plants were higher than the earnings. Later, he came to know about the latest technology and methods by which the production of apples can be increased exponentially. He bought 3500 plants of apple rootstock from renowned commercial tissue culture unit of HP. These plants had 100% survival rate and reached at maturity within 3 years. He got average yield of 30 kg fruits per tree with uniform quality.

☞ ***Mr Atul Kanwar***

Address
Village Kutiage P.O.
Piplage Tehsil Bhuntar, Distt.
Kullu -175125 (HP)

Mr Kanwar was growing vegetable in which cost of labour and inputs were higher than outputs. In year 2014, he planted 1500 apple plants using TC rootstocks and got fruiting in 3 years. Now in 2020, he has harvested about 40 kg fruits/plant. In this area, apple was not cultivated before 2014. When he established apple orchards, it created revolution in this region and now there is a group of 100 farmers who are moving towards apple farming based on TC rootstocks.

☞ ***Mr Vishwa Mahant***

Address
Village Kinjanoli, P.O. Kuid Distt.
Kullu-175125 (HP)

Mr Mahant lives in Kullu. He was growing pomegranate and vegetables till 8 years from now. He was familiar with TC plants and he planted TC plants of pomegranate. He came to know about TC apple plants and started plantation of TC apple rootstock. He got very encouraging results and could earn much better income than vegetable and pomegranate. As per his feedback, people from valley are shifting from vegetable to apple rapidly. He also found that the cost of locally available TC apple plants was 50% less than the imported plants of apple.

☞ ***Mr Mohammad Sohail***

Address
Old exchange Road
Distt. Anantnag-192101
(J&K)

In year 2013, Mr Sohail took 200 plants from Rajat Biotech - A TCPF recognized under NCSTCP and got very encouraging results. As the performance and outcomes were found excellent, he decided to grow a big orchard with TC apple plants in year 2020 and planted 3000 plants of M9, M7, MM106 and MM111. He highlighted with great enthusiasm that TC apple rootstocks provide great opportunity to Kashmiri apple growers.

☞ ***Mr Mohammad Shafi***

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H.D. Apple Orchard,
Matipora, Anantnag-192101
(J&K)

Mr Shafi found that his TC rootstock-based orchards completed its vegetative phase within 3 years. He, along with more than 200 growers, got planting materials from the NCS-TCP recognized tissue culture lab, feel happy to adopt progressive farming technology. He realized that TC rootstocks are single parent progeny, so have little chances of variation as compared to seedlings.

Experiences of the above farmers clearly indicate that TC apple rootstock has great impact in increasing yield and improving quality of fruit. High density plantation (HDP) is very important to enhance the productivity. HDP coupled with TC plants is the key for success, however, field survey of 2019-20 indicates that only about 19% farmers have adopted 3300 plants/ha whereas only about 8% farmers have adopted high density plantation of 4400 plants/ha in J&K (Hassan *et al.*, 2020). Very limited number of farmers adopted HDP in spite of its benefits due to high cost associated with it, lack of awareness, old orchards still owned by parents who have affinity or emotional bonding with the plantation and sometimes, it is difficult to have break in recurrent source of income during the shifting from low density old orchard (which is still yielding) to HDP. However, there is a great potential of expansion of cultivation of TC apple rootstock by adopting HDP method with increased awareness and new generation of progressive farmers ready for adopting new technology.

10. Conclusions

Plant tissue culture has revolutionized agriculture, horticulture and forestry. It has immense potential for uplifting the farmers' income and agro-economy leading towards sustainable agriculture. It has been widely applied in horticultural crops like banana, pomegranate, apple, *etc*. Producing disease-free quality rootstock of apple has proven a great success for HDP by increasing production and productivity. When there is immense pressure for providing quality food and nutrition to increasing population with limited cultivable land, such technology has provided a solid base for sustainability. When tissue culture technology is coupled with quality management through effective indexing of viruses, it instils high level of confidence among apple growers. However, there is need to promote entrepreneurship for plant tissue culture and build the capacity of farmers through training and awareness for large scale adoption of apple tissue culture rootstocks in order to realize the full potential of tissue culture technology. This will not only increase apple production but substantially increase income of apple growers also. This proven technology can be adopted by apple orchardists in other apple growing countries of Asia-Pacific region.

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12. Important APAARI Publications[1]

Expert Consultations/Meetings/Training Programmes

1. Regional Workshop on Underutilized Fish and Marine Genetic Resources and their Amelioration – Proceedings and Recommendations (2020)
2. Satellite Symposium on Dryland Agrobiodiversity for Adaption to Climate Change: Proceedings and Recommendations (2019)
3. Regional Expert Consultation on Gene Editing and its Regulation- Proceedings and Recommendations (2019)
4. Satellite Symposium on Dryland Agrobiodiversity for Adaptation to Climate Change: Proceedings and Recommendations (2019)
5. *In Vitro* and Cryopreservation Approaches for Conservation of Plant Genetic Resources: Training Manual (2019)
6. International Hands-on Training on Genome Editing Technology: Training Manual (2019)
7. Regional Workshop on Underutilized Animal Genetic Resources and their Amelioration: Proceedings and Recommendations (2019)
8. Training on Transformation of Agricultural Education through Knowledge Management and Capacity Development for More Effective Agricultural Innovation System (AIS) (2019) (softcopy)
9. Regional Conference on Role of Soil and Plant Health Towards Achieving Sustainable Development Goals (SDG) in Asia-Pacific 21-23 November 2018, Bangkok, Thailand - Proceedings and Recommendations (2021)
10. Regional Workshop on Knowledge Management for More Effective Agricultural Innovation Systems (AIS) (2018) (softcopy)
11. Regional Expert Consultation on Agricultural Biotechnology-Scoping Partnerships to Improve Livelihoods of Farmers in Asia and the Pacific: Strategic Papers and Country Status Reports (2018)
12. Regional Expert Consultation on Agricultural Biotechnology – Scoping Partnerships to Improve Livelihoods of Farmers in Asia and the Pacific: Proceedings and Recommendations (2018)
13. Regional Expert Consultation on Underutilized Crops for Food and Nutritional Security in Asia and the Pacific: Proceedings and Recommendations (2018)

[1] Copies of all the publications are available at: www.apaari.org

14. Investment in Agricultural Research for Sustainable Development in Asia and the Pacific: Country Status Reports (2017)
15. Expert Consultation on Best Practices in Agri-food Innovations in Asia and the Pacific: Proceedings and Recommendations (2017)
16. 14th General Assembly Meeting (GAM): Proceedings (2017)
17. High Level Policy Dialogue on Investment in Agricultural Research for Sustainable Development in Asia and the Pacific: Proceedings (2016)
18. High Level Policy Dialogue on Investment in Agricultural Research for Sustainable Development in Asia and the Pacific: Papers Presented (2016)
19. Development of Communication Strategies for Adoption of Agri-Biotechnology in the Asia-Pacific Region: Proceedings and Recommendations (2015)
20. Capacity Development Workshop on Planning, Monitoring and Evaluation towards Measuring Outcomes and Impacts: Proceedings (2015)
21. Expert Consultation on Assuring Food Safety in Asia-Pacific: Proceedings and Recommendations (2015)
22. 13th General assembly Meeting (GAM): Proceedings (2014)
23. National Workshop on Outscaling Farm Innovation: Proceedings and Recommendations (2014)
24. 12th Asian Maize Conference and Expert Consultation on "Maize for Food, Feed, Nutrition and Environmental Security": Recommendations (2014)
25. NARS-CGIAR Interactive Session for Strengthening Partnership in South Asia: Proceedings and Recommendations (2014)
26. Proceedings: APAARI-AVRDC-COA - Asia-Pacific Symposium on Molecular Breeding (2014)
27. Expert Consultation on Strengthening Linkages between Research and Extension to Promote Food and Nutrition Security (2014)
28. Expert Consultation on Promotion of Medicinal and Aromatic Plants in the Asia-Pacific Region: Proceedings (2014)
29. Asia-Pacific Symposium on Molecular Breeding: Proceedings (2014)
30. International Conference on Innovative Approaches for Agricultural Knowledge Management: Global Extension Experiences: Proceedings (2013)
31. Training Workshop on Open Access Publishing Using Open Journal Systems: Proceedings (2013)
32. Foresight and Future Pathways of Agricultural Research Through Youth: Proceedings and Recommendations (2013)
33. Expert Consultation on Managing Trans-boundary Diseases of Agricultural Importance in the Asia-Pacific: Proceedings and Recommendations (2013)
34. Stakeholders' Dialogue on Biosafety Regulations in the Asia-Pacific Region Proceedings and Recommendations (2013)

35. Regional Consultation on Agricultural Research for Development: Proceeding and Recommendations (2013)
36. Regional Consultation on Collective Actions for Opening Access to Agricultural Information and Knowledge in the Asia-Pacific Region: Proceedings (2012)
37. Prioritization of Demand-driven Agricultural Research for Development in South-Asia (2012)
38. Regional Consultation on Improving Wheat Productivity in Asia: Proceedings and Recommendations (2012)
39. Workshop on Climate-Smart Agriculture in Asia: Research and Development Priorities: Proceedings and Recommendations (2012)
40. First Global Conference on Women in Agriculture (GCWA): Proceedings (2012)
41. Regional Workshop on Implementation of Suwon Agrobiodiversity Framework: Proceedings (2012)
42. Regional Dialogue on Conservation Agriculture in South Asia: Proceedings and Recommendations (2012)
43. Expert Consultation on Agricultural Biotechnology, Biosafety and Biosecurity: Proceedings and Recommendations (2012)
44. Workshop on Moving Beyond Strategy to Improve Information and Knowledge Management for Agricultural Development in the Pacific Islands Countries and Territories: Proceedings (2012)
45. Stakeholders' Interface on GM Food Crops: Proceedings and Recommendations (2011)
46. Expert Consultation Meeting on Postharvest and Value Addition of Horticultural Produce – Strengthening Technologies for Linking Farmers to Market: Proceedings and Recommendations (2011)
47. International Symposium on Sustainable Agricultural Development and Use of Agrobiodiversity in the Asia-Pacific Region (2010)
48. APAARI-ADB Asia-Pacific Consultation on Agricultural Research for Development (AR4D) in Asia and the Pacific-The Way Ahead (2009)
49. Expert Consultation on Biopesticides and Biofertilizers for Sustainable Agriculture (2009)
50. Symposium on Global Climate Change: Imperatives for Agricultural Research in Asia-Pacific (2008)
51. Expert Consultation on Agricultural Biotechnology for Promoting Food Security in Developing Countries (2008)
52. Workshop on Development and Management of ARD Information Resources (2008)
53. Asia-Pacific Regional Workshop on Agricultural Research for Development (2008) (for establishment of NGO Consortium-NAARAP)

54. Expert Consultation to Review Progress of Agricultural Research Networks and Consortia in Asia-Pacific (2007)
55. ICT/ICM Sensitization and Awareness Building Workshop for NARS Leaders and Senior Managers (2007)

Success Stories

56. Sheep and Goats in Fiji and Papua New Guinea – A Success Story (2021), Alan Quartermain and Vinesh Kumar
57. Success Story on Induced Systemic Resistance – A New Hope for Malaysian Papaya Industry (2021) Ganisan Krishnen and Mohamad Roff Mohd Noor
58. GM Maize in the Philippines – A Success Story (2019), Carlo G. Custodio Jr., Virma Rea G, Lee and Maria Monina Cecilia Q. Arcelo-Villena
59. Banana Tissue Culture in India – A Success Story (2019), VK Baranwal, Reetika Kapoor and Shiv Kant Shukla
60. Linking Farmers to the Global Market, Tea Production and Export Development in Nepal – Success Story (2018), Murari Prasad Gautam Upadhyay
61. Success Story on Bt Brinjal in Bangladesh (2018), Md. Rafiqul Islam Mondal and Nasrin Akter
62. Climate Smart Farmers' Field School: The BICOL, Philippines Experience (2018)
63. Durian in Thailand: A Success Story (2018)
64. Success Stories on Information and Communication Technologies for Agriculture and Rural Development (2015)
65. ITC *e*-Choupal: Innovation for Large Scale Rural Transformation - A Success Story (2014)
66. Wax Apple Industry in Taiwan: A Success Story (2014)
67. Agricultural Information and Knowledge for All: Success Stories on ICT/ICM in AR4D in Asia and the Pacific Region (2013)
68. Linking Farmers to Market: A Success Story of Lettuce Export from Chinese Taipei (2012), Min-Chi Hsu *et al.*
69. Biofuel Growers Market Network (2012), K. Narayan Gowda
70. Success Stories on ICT/ICM in AR4D in Asia and the Pacific Region, Malcolm Hazelman and S. Attaluri
71. Short Duration Mungbean: A New Success in South Asia (2010), M.L. Chadha
72. Taro Improvement and Development in Papua New Guinea (2009), Abner Yalu *et al.*

73. Cotton-Wheat Production Systems in South Asia: A Success Story (2008), C.D. Mayee *et al.*
74. Linking Farmers to Market: Some Success Stories from Asia-Pacific Region (2008), Rosendo S. Rapusas *et al.*
75. Rainbow Trout (Oncorhynchus mykiss) Culture in the Himalayan Kingdom of Nepal (2005/1), A.K. Rai *et al.*
76. Sustaining the Green Revolution in India (2004/3), S. Nagarajan
77. Lentil Improvement in Bangladesh (2004/1), Ashutosh Sarker *et al.*
78. Success Story on the Control of Newcastle Disease in Village Chickens (2003/1), Robyn Alders
79. Hybrid Rice in China – A Success Story (1994), Lou Xizhi and C.X. Mao
80. Tilapia Farming in the Philippines – A Success Story (1994), Rafael D. Gurrero III
81. Dairying in India – A Success Story (1994), R.P. Aneja

Status Reports

82. Regional Workshop on Underutilized Fish and Marine Genetic Resources and their Amelioration – Country Status Reports (2020)
83. Regional Workshop on Underutilized Animal Genetic Resources and their Amelioration: Regional Status Reports and Strategic Papers (2019)
84. Regional Expert Consultation on Underutilized Crops for Food and Nutritional Security in Asia and the Pacific: Thematic, Strategic Papers and Country Status Reports (2018)
85. High Level Policy Dialogue on Investment in Agricultural Research for Sustainable Development in Asia and the Pacific: Scoping Paper (2015)
86. Jackfruit Improvement in the Asia-Pacific Region: A Status Report (2012)
87. Information and Communication Technologies/Management in Agricultural Research for Development in the Asia-Pacific Region: A Status Report (2011)
88. Strengthening of Plant Genetic Resources for Food and Agriculture: Conservation and Utilization in the Pacific (2011)
89. Bt-Cotton in India – A Status Report (2nd Edition) (2009)
90. Production and cultivation of Virus-Free Citrus Saplings for Citrus Rehabilitation in Taiwan (2008)
91. Biosafety Regulation of Asia-Pacific Countries (2008)
92. Micropropagation for Quality Seed Production in Sugarcane in Asia and the Pacific (2008)

93. Commercialization of Bt-Corn in the Philippines (2005)
94. Information and Communication Technologies in Agricultural Research for Development in the Asia-Pacific-Region (2004)

Other Publications

95. Agricultural Research in Southeast Asia: A Cross-Country Analysis of Resource Allocation, Performance, and Impact on Productivity (2020)
96. Vietnam (online) ASTI Country Brief | February 2020, Thailand (online)
97. ASTI Country Brief | April 2020, Malaysia (online)
98. ASTI Country Brief | December 2019: Papua New Guinea (online)
99. ASTI Country Brief | December 2019: LAOS (online)
100. ASTI Country Brief | November 2019 Myanmar (online)
101. APAARI Resource Mobilization Strategy (2020)
102. APAARI Membership Strategy: Strategic Considerations and Guiding Principles for Management and Mobilization of APAARI Membership (2020)
103. APAARI Knowledge Management and Communication Strategy 2017-2022: Making Agri-food Systems More Knowledge Intensive (2020)
104. APAARI Constitutions (2019)
105. APAARI Capability Statement (2018)
106. Abridged Version: APAARI Strategic Plan 2017-2022: Pathways to Strengthened Agri-food Research and Innovation Systems in Asia and the Pacific (2017)
107. APAARI Strategic Plan 2017-2022: Pathways to Strengthened Agri-food Research and Innovation Systems in Asia and the Pacific (2016)
108. APAARI Vision 2030: Strengthened Research and Innovations for Sustainable Agricultural Development (2016)
109. High Level Policy Dialogue on Investment in Agricultural Research for Sustainable Development in Asia and the Pacific: Abstracts of Presentations (2015)
110. Twenty-Two Years of APAARI – A Retrospective (2014)
111. Benchmarking Agricultural Research Indicators Across Asia-Pacific: ASTI Regional Synthesis Report
112. Training Workshop on Open Access Publishing Using Open Journal Systems

113. APAARI on CD
114. Priorities for Agricultural Research for Development in South Asia
115. Improving Wheat Productivity in Asia
116. Fifteen Years of APAARI – A Retrospective
117. APAARI Vision – 2025
118. APAARI Newsletter (half yearly)

ISBN 978-85-99249-50-5

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