Canadian Micropropagation Technology for Nepali Fruit Crops

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Introduction

Over the past fifty years plant tissue culture has sprouted alongside the growth of fruit tree crop production in Nepal (Dodds, 1983). The pairing of the two will allow for the continuous expansion of micropropagation plant varieties, and increased ease of commercial fruit crop production in Nepal's agricultural sector; benefits spreading across seas and advancing academic research internationally through the use of information communication technology. Plant tissue culture in trees is the key to largescale propagation and rapid scale-up production for growers in Nepal. Canada holds the fastest and most economical means for achieving a commercial-level expansion of deciduous fruit tree production: tissue culture (AgriForest Bio-Technologies Inc., 2010).

1.0 Plant Tissue Culture: A Background

Micropropagation, or plant tissue culture, is the agricultural science of the regeneration of plantlets from the tissue cells of existing plants. This is achieved by culturing plant cells, tissues and organs on synthetic media under an aseptic environment, with controlled conditions of light, temperature and humidity (Dagla, 2012). Success using in vitro conditions is highly dependent on the quality of the nutrient media (Dagla, 2012). Optimal growth and morphogenesis of tissues will vary for different plants according to their nutritional requirements; macronutrients, micronutrients, vitamins, amino acids, carbon, organic supplements, growth regulators and solidifying agents provide the base-layer essentials for general plant tissue culture medium (Saad and Elshahed, 2012).

The purpose of in vitro propagation is to multiply plants of interest vegetatively. Cultures planted in the medium, in small sterile petri dishes, develop many axillary shoots without growing more than a few millimetres in length (Wilson, 2010). The masses of shoots are

periodically divided and subcultured, and are either further multiplied or transferred for rooting and potting (Wilson, 2010). Advantages of this technology are as follows: the growth of a large number of clones from one single seed; a shortened growing period and life cycle of seed development; by-passed seasonal restrictions for seed germination; efficient storage; freedom from disease; for species that have long generation time, low levels of seed production; and addressing seeds that do not readily germinate, rapid propagation is possible (Wilson, 2010).

The critical points for the effective in vitro culture establishment from woody fruits or nuts are associated with the efficacy of sterilization of collected explants (Dobranszki and Teixeira da Silva, 2010). Tree tissue culture requires sophisticated facilities and demands an appropriate skillset; Canada is the tool to eliminating these limitations, and has the necessary means for micropropagation (Wilson, 2010). For optimum performance, tree clippings will be taken from fruit trees currently growing in Nepal for further propagation of fruit trees with guaranteed suitable characteristics, initially limiting the start date of micropropagation. This will be the first step in the partnership between Canada and Nepal.

1.1 AgriForest Bio-Technologies Ltd.

In order to achieve custom micropropagation and supply Nepal with fruit trees best suited to their niche demands, customization alongside research and development interests are essential in Canadian company candidates for the task. AgriForest Bio-Technologies Ltd. (AFBT) undertakes custom propagation projects, and is already supplying small fruit tree crops through micropropagation (AgriForest Bio-Technologies Inc., 2010). Initially established in 1984, the company's mission was to provide tissue culture dwarf fruit tree rootstocks to local orchardists; now one of the largest suppliers of tissue culture plants in North America, AFBT Ltd. is

constantly invested in the development of new tissue culture varieties and research opportunities (AgriForest Bio-Technologies Inc., 2010).

Subject to a minimum order quantity, custom propagation projects are readily conducted by AFBT (AgriForest Bio-Technologies Inc., 2010). Most varieties are available all year round; however in requesting new varieties of propagated plantlets, a time allowance of six months is required (AgriForest Bio-Technologies Inc., 2010). As a clean nursery, certified by the Canadian Food Inspection Agency under the Canadian Fruit Tree Export Program, AgriForest products include a plant health and safety guarantee (AgriForest Bio-Technologies Inc., 2010). Offering free samples and high quality, this Canadian company best suits the needs of Nepal.

AgriForest Bio-Technologies Ltd. guarantees the following to complement their tissue culture fruit trees: disease-free trees, fibrous root systems free of root rot issues, dense branching habits, high survival rates, and vigorous growth (AgriForest Bio-Technologies Inc., 2010). The company's values stress their interest in research and development, and their philosophy incorporates constant improvement and innovation in growing the industry. Offering custom propagation and secure shipment methods, costs vary from $1.79 to $2.99 per unit in large orders (AgriForest Bio-Technologies Inc., 2010). For individual resale to Nepali farmers, this keeps the cost to a minimum, and for Canadians, employs many, from the plant specialists to the transportation industry.

1.1.1 Shipment

Located in Kelowna, British Columbia, transportation of tree tissue culture plants propagating in petri dishes to Nepal will average around two weeks, and incur high costs (AgriForest Bio-Technologies Inc., 2010). These costs can be mitigated through Canadian research grants available from AFBT's close partner, the National Research

Council of Canada (National Research Council of Canada, 2015). AFBT will use common carriers with protective services to ship plants on a custom built pallet with wooden racks (AgriForest Bio-Technologies Inc., 2010). Shipment materials can be reused for a variety of purposes once in Nepal; infrastructure and crop storage being among many examples, and deliveries can be left unplanted for up to three months.

1.1.2 Costs

Apple, pear, pomegranate, fig, plum, cherry and apricot trees are examples of common fruit trees in Nepal with the potential to be micropropagated through tissue culture (Yokoyama and Gauchan, 1999). While it is most economical to ship a large order of a variety of these tree tissue culture plants, each individual plant will have a slightly varying cost and will be sold to individual Nepali farmers for around two dollars Canadian, or about one hundred and sixty Nepali Rupees, per plant (Money Converter, 2015). The following is a sample price list taken for 2015 cherry tree tissue culture seedlings (AgriForest Bio-Technologies Inc., 2010):

Species	Variety	Price/Unit*		
		10"-12" LINERS		4" Pot
		100+	1000+	
*Prunus cerasus***	CARMINE JEWEL CHERRY**	$2.99	$2.79	$3.99
*Prunus cerasus***	CRIMSON PASSION CHERRY**	$2.99	$2.79	$3.99
*Prunus cerasus***	CUPID CHERRY**	$2.99	$2.79	$3.99
*Prunus cerasus***	EVANS SOUR CHERRY**	$2.99	$2.79	$3.99
*Prunus cerasus***	JULIET CHERRY**	$2.99	$2.79	$3.99
*Prunus cerasus***	ROMEO CHERRY**	$2.99	$2.79	$3.99
*Prunus cerasus***	VALENTINE CHERRY**	$2.99	$2.79	$3.99

** Plus applicable royalty fees*
*** CFIA Certified Virus Indexed Plants*

Fig. 1 Average plantlet price list (AgriForest Bio-Technologies Inc., 2010)

'Liners' provide the best value at $2.79 per unit, and do not require greenhouse facilities (AgriForest Bio-Technologies Inc., 2010). The minimum order size for tissue culture liners is 64 plants, or 2 trays, per variety, for a total of $500 (AgriForest Bio-Technologies Inc., 2010). The plantlets range between six and 24 inches when shipped, providing a high value seedling ready to be transplanted directly into Nepali soil (AgriForest Bio-Technologies Inc., 2010).

1.2 Benefits to Canada

In exporting tree tissue culture crops to Nepal, Canada gains ample opportunities for further research, more jobs and more revenue in the hands of entrepreneurial businesses such as AFBT. By outsourcing Nepal's research and development to Canada, Canada, and specifically AFBT increases its sales potential, reduces risks the company may be exposed to from fluctuations in local business cycles, helps to put idle production capacity to work, and provides

economies of scale to Canada, AFBT Ltd, and other relatable companies on a global scale (Almodovar, 2014).

Partnering with Nepal will create a framework for similar programs with other countries. Investing in the development of tree tissue culture plantlets suitable for growth in Nepal will allow for divestment in other countries possessing similar climate conditions (Woods and Welsh, 1965). Canada and AFBT will gain a competitive advantage over other countries with the success of this strategic investment, while mitigating general investment liquidity constraints through geographic sales diversification (Shaver, 2011). Relaxation of investment liquidity constraints allows systematically profitable long-term investments, and aids Nepal's agricultural sector and the environment on a global scale (Shaver, 2011).

Micropropagation creates a high volume of plants at a low cost, and in a condensed area. With approximately 80% of Nepal's population engaged in agriculture, and a population of almost 30 billion inhabitants, there is a large market opportunity for this product, and a great research opportunity for Canadian scientists in the AFBT program and for the National Research Council of Canada (Chadha, 1998).

2.0 Nepal

Nepal, as a predominantly agricultural country, is composed of about 3.3 million families engaged in subsistence agriculture (Chadha, 1998). Commercial fruit growing is not traditional in Nepal, and has been until recently limited largely to homesteaded gardens harvested for domestic consumption (FAO United Nation, 2015). From this is sourced an indication of the potential for growing temperate fruits in Nepal.

With three major ecological zones: the high mountains accounting for 34% of the total area, the hills composing 44% and the plains (terai) region, 21%, agricultural practices are variable (Devkota and Upadhyay, 2013). However, with deep roots and hardy temperaments, fruit trees are able to sustain well in each region, see *Fig. 2* (Yokoyama and Gauchan, 1999). The incorporation of fruit tree crop production into farming systems in the hill region of Nepal has shown decreases in female labor by 20%, while the use of hired male labor increased by 10%, encouraging equality, employment, food security and a new income source (Yokoyama and Gauchan, 1999).

Features	Terai Region	Hill Region	Mountain Region
Land Area (Million ha)	3.1 (44%)	4.4 (29.5%)	2 (19.7%)
Geology	Quaternary alluvium	Phyllite, quartzite limestone and islands of granites	Gneiss quartzite and mica shists
Elevation	100-300 m	800-2400 m	1000-4000 m
Climate	Tropical	Subtropical, warm temperate	Warm to cool temperate, alpine
Moisture Regime	Subhumid	Humid, perhumid above 2000 m	Subhumid to perhumid
Rainfall Intensity	High	Medium	Low
Horticultural Crops	Mango, lychee, pineapple, jack-fruit, potato, tomato	Mango, papaya, banana, orange, lime, lemon, peach, plum, nectarine, persimmon, Asian pear, potato, cauliflower	Chestnut, walnut, apple, peach, plum, apricot, cherry, almonds, potato

Fig. 2 Characteristics of Physiographic Regions of Nepal (Devkota, 2013)

Agrobiodiversity, the incorporation of a variety of annual and perennial plants in agricultural systems, diversifies species composition leading to the enhancement of stability and productivity on farmland in Nepal (Baul et al, 2013). By supplying Nepal with a large quantity – achievable through plant tissue culture techniques – of fruit tree plantlets, the emphasis is taken away from current monoculture crop production. Rather than encourage the traditional monocropping tendencies in Nepal by supplying fertilizers, diversification can be promoted through the establishment of fruit trees.

2.1 Target Market

As a country composed of inhabitants reliant upon subsistence agriculture, plant tissue culture is applicable to a broad audience, as opposed to a niche market. Farmers seeking an additional monetary source, increased yields, or fertilizers can turn to Canada's AFBT for the solution (AgriForest Bio-Technologies Inc., 2010). Crop biodiversity and the addition of trees underpins the range of dietary needs and services consumers and subsistence farmers in Nepal demand (Smale, 2005). An incorporation of individual smallholder farmers, villages and government will comprise the target market.

2.2 Storage

Receiving shipment crates from Kelowna, British Columbia in Kathmandu, Nepal incorporates further employment and procedural ease. Plantlets in sealed petri dishes can be left in transportation crates possessing appropriate lighting and sanitation requirements for up to three months; available for distribution for around two months after the shipment is received (AgriForest Bio-Technologies Inc., 2010).

In the transfer of rooted shoots to the natural environment, acclimatization incorporates various aspects atypical to current practices in Nepal. Micropropagated plants are mixotrophic; they are not fully dependent on photosynthesis due to the low light intensity in vitro and the carbohydrate content of the medium (Dobranszki and Teixeira da Silva, 2010). Additionally, there is often high humidity in culture vessels, resulting in morphological and physiological modifications (Dobranszki and Teixeira da Silva, 2010). Such modifications can cause rapid water loss when moved to external conditions, and it is necessary that acclimatization incorporate a gradual decrease of high humidity and an accompanying increase in light intensity (Dobranszki and Teixeira da Silva, 2010). With Nepal's climate and

seasonality, rainfall will work advantageously to diminish this consideration (Ghimire et al, 2012).

2.3 Benefits to Nepal

Developing deciduous fruit cultivation is the next step for Nepali farmers. Branching from current monoculture crop fields, and establishing orchards and applying permaculture techniques with the use of micropropagation, will allow both farmers and consumers in Nepal to experience numerous direct food security advancements and health gains (Paudyal and Haq, 2008). Expanding fruit crop production to established farms will increase plant fertility by encouraging variance from monocrop culture, and reduce topsoil loss due to the growing season of trees (Pandey, 2015). Plant tissue culture in the multiplication of fruit trees allows this to be achieved at economies of scale and at an escalated speed (AgriForest Bio-Technologies Inc., 2010).

With 10.2% of households in Nepal suffering from chronic food insecurity, a dependable source of annual produce is necessary (Maharjan and Joshi, 2011). AFBT Ltd. will provide fruit tree plants with the benefit of frequent bountiful produce (AgriForest Bio-Technologies Inc., 2010). While fruit trees will not bear fruit every year, but rather on a cyclical basis, a balance of multiple trees, neighbouring trees, or in future applications, communal trees, at two dollars each will supply sources of nutrients in times of food scarcity (Wilkie et al, 2008).

Agroforestry, as the integration of trees to produce food, fodder, fuel-wood and timber incorporates additional advantages such as carbon sequestration, enhancing water quality, protecting soil and conserving biodiversity (Dhakal et al, 2012). The following is an example of proportional distribution of fruit trees and crops for maximum positive impact of microporpagated seedlings in Nepal:

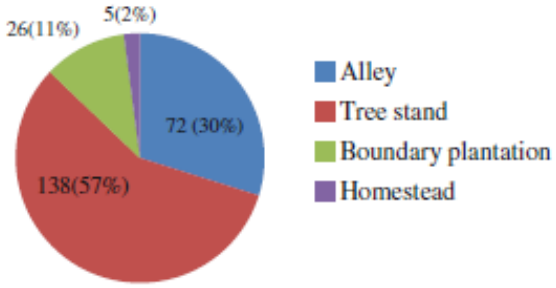

Fig. 3 Distribution of trees in agroforestry (Dhkal et al, 2012)

The combination of trees with ground crops has significant potential to be widespread in Nepal's successful and sustainable farming practices (Cleveland and Soleri, 2002). Utilizing the attributes of micropropagation allows Nepali farmers to benefit from access to plantlets progressed from germination and ready to be transplanted directly into the soil; reducing labour and increasing returns. Replacing chemical fertilizers with the nutrient substitution of monocrops with trees can increase soil fertility and yields for long-term growth and rewards. While posed constraints such as farm size, agricultural labour force and farmers' capacity to utilize farm inputs indicate potential difficulties; institutional support from Canada will be an essential support mechanism (Dhakal et al, 2012). Though Nepali people often travel abroad for jobs, biodiversity management plans support the employment of youth, the main agricultural force, in Nepal (Pandey, 2012). The overall socio-economic gains and cultural benefits of tree tissue cultured plantlets provide a healthy base upon which any limitations can be developed into attributes and opportunities for growth.

3.0 Between Uneasy Giants

Nepal, as a *yam between two stones,* has in recent history found itself intently surrounded by the uneasy relationship between China and India (Dixit, 2013). China is now India's largest trading partner, surpassing the United States in this context. This concentration of both countries on investment-led growth is showing increasing potential for collision due to the disparities between China's one-party command economy and India's democracy (Dixit, 2013). While China and India both hold the technology and resources to conduct similar research and export tree tissue culture plants, rather than emerge into relations with these conflict-prone neighbours, Canada provides a suitably stable alternative for Nepal.

3.1 Further Applications of Micropropagation in Nepal

In applying future uses of plant tissue culture, issues such as deforestation and food scarcity can be addressed. Many rural households in Nepal require access to public forest resources to complement their private resources for food and livestock production (Dhakal et al, 2010). Historically, less productive land in Nepal is managed as public or communal property for producing, for example, firewood, fodder, pasture and timber (Dhakal et al, 2010). As a result, the majority of Nepali farmers have no private pastureland (Dhakal et al, 2010). Instead, livestock graze in forests, and more than 60% of farming households have a food deficit from their own land (Dhakal et al, 2010). The attributes of plant tissue culture are again observed; promoting plantlets to the Nepali government and encouraging policy establishment of fruit tree growth in communal forests will add nutrients to the soil, and act as a food source for livestock and humans in times of drought and food scarcity.

3.2 Long-Term Applications of Canadian-Nepali Partnership

Permaculture and agro-environmental lessons stimulating sustainable agriculture are promoted by the introduction of large quantities of fruit tree plants (Fullen et al, 2011). Demand for increased food production and higher soil quality, coupled with rapid industrialization in Nepal place pressure on land use and fertility (Fullen et al, 2011). Soil erosion, decreased soil productivity and environmental quality link socio-economic and food scarcity issues and emphasize the demand fruit tree crops as a solution. Increasing the productivity and sustainability of cropping systems is achievable with Canada's research inputs and subsequent sharing of land literacy amongst Nepali people (Fullen et al, 2011). Short duration projects often fail to fully disseminate outcomes; studies show that alley cropping with hedgerows of fruit trees is the best cultural practise for sustainable sustenance agriculture on sloping highlands (Fullen et al, 2011). The outline for tree tissue culture is a long-term plan with continual progress and lasting effects within the country of Nepal.

Conclusion

Plant tissue culture is the key to widespread fruit production and consumption for growers in Nepal. The introduction of plant tissue culture seedlings to Nepal eliminates stresses stemming from poor germination rates, and acts as a greenhouse for Nepal's farming practices. Canada gains knowledge and dependable opportunities for future applications in the research of fruit tree crop micropropagation and in the exportation of resources (National Research Council of Canada and Prairie Regional Laboratory, 1982). Soil and human health gains in Nepal complementing job expansion and economic growth in Canada, allows for a strategically favourable partnership while creating prospective embryonic opportunities. Canada holds the fasted and most economical means to achieving a commercial-level expansion of deciduous fruit

tree production and Nepal holds the potential to thrive from more fruit trees and their bountiful produce (AgriForest Bio-Technologies Inc., 2010).

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