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Rapid Multiplication Techniques (RMTs): A Tool for the Production of Quality Seed Potato (*Solanum tuberosum* L.) in Ethiopia

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ABSTRACT

Productivity of potato is constrained primarily by use of low quality seeds in Ethiopia. Many field multiplication generations of vegetatively propagated basic seed result in build-up of seed-borne diseases and subsequent dissemination to new fields. Use of soil-less media is an alternative to reduce soil borne disease infections in production of vegetatively propagated planting materials. The main bottleneck in potato seed tubers is slow multiplication rate on the field and contamination of the seed with viral and bacterial diseases. This has therefore, called for use of Rapid Multiplication Techniques (RMTs) such as *in vitro* propagation of potato to eliminate viral and bacterial infections. The production of potato seed under conventional system has not been effective in avoiding or reducing the build-up of pathogens and has consequently led to reduced quality seed and low crop yields. Holetta Agricultural Research Center (HARC) aims to improve production and productivity of potato in Ethiopia through promotion of the use of quality seed tuber. RMTs have been used at the research center to improve the multiplication ratio of disease free potato seed in short period of time. Using RMTs, large quantities and quality minitubers have been produced at HARC starting from Tissue Culture (TC) *in vitro* plantlets in the laboratory followed by screen house pot multiplication. Recently, aeroponics unit were introduced and under use to produce high quality minitubers for released potato varieties and clones as one of the RMTs tools. Thus, the existing potato seed multiplication using conventional methods alone cannot cope up with the current national seed demand for improved quality seed tubers. Therefore, the use of TC mass propagation of *in vitro* plantlets and further multiplication using RMTs such as screen houses and aeroponics techniques are an efficient way of assisting the process of multiplying large quantity clean seed tuber production. It also aims to contribute to the growing national seed demand as well as to produce disease free planting materials that will be helpful to eradicate the dissemination of potato viruses and bacterial wilt. Thus, this review paper outlines the progress and achievements in RMTs for production of high quality seed potato at HARC. The challenges and the need to develop certification standards to increase capacity for potato minitubers production by RMTs in Ethiopia were also explicitly discussed.

Key words: Aeroponics, quality seed production, potato, minitubers, tissue culture, RMTs

INTRODUCTION

In Ethiopia, potato (*Solanum tuberosum* L.) can significantly contribute to food security improvement by increasing food availability and cash income of smallholder farmers (Tufa, 2013).

Hirpa *et al.* (2010) also described that, potato is regarded a high potential food security crop because of its ability to provide a high yield of high quality product per unit input with a shorter crop cycle (mostly <120 days) than major cereal crops like maize. The national average yield is approximately 10.5 t ha^{-1}, which is very lower than the world's average yield of 17 t ha^{-1} (Muthoni *et al.*, 2011). Moreover, Hirpa *et al.* (2010) stated that, the potential of potato crop has not been adequately exploited due to poor quality seed tubers and unavailability of seed tubers of improved varieties. The crop is mainly grown at high altitudes of 1500-3000 masl by small scale farmers, who account for over 90% of national potato production. Currently, potato production is expanding from highland areas to mid and lowland parts of the country due to its potential for production in a short period of time, high yield per unit area, source of food and cash crops to large number of food-insecure smallholder farmers and pastoralists in the country. Nowadays the main production season for ware potato represents only 22% (34,000 ha), while the off-season production is around 128,000 ha in northern and central Ethiopia (Haverkort *et al.*, 2012). The reason for a gradual shift from Meher (main growing season) to Belg (off season) is the fact that the late blight pressure is increasing and farmers experience less risk with cultivation during the 'small' rains combined with irrigation.

There are a number of production problems. The major ones are unavailability and high cost of seed tubers; lack of well adapted cultivars to the major agro-ecological zones; suboptimal agronomic practices; the prevalence of diseases and insect pests and inadequate storage, transportation and marketing facilities. To address these problems, the Ethiopian Agricultural Research Institute (EIAR) then the Institute of Agricultural Research (IAR) in collaboration with International Potato Centre (CIP) and national higher learning institutions like Haramaya University initiated potato improvement program in Ethiopia. The research had as its main objectives to develop adaptable and high yielding potato cultivars with good resistance to the biotic and abiotic stresses; identify the best agronomic practices and storage systems; adopt the use of botanical seed as an alternative propagation method; develop seed production system in the country and train farmers and other stakeholders.

A number of high yielding, wider adaptation and late blight disease resistance/tolerant potato varieties have been released and under production by large number of farmers in Ethiopia. However, one of the key production bottlenecks that contribute to low yield of potato in Ethiopia is the lack of healthy and quality seed tubers in the required quantity and quality (Lemaga and Gebremedhin, 1994). There is no formal seed certification system operating for clean/healthy potato seed multiplication and distribution in Ethiopia. Hirpa *et al.* (2010) reported that potato seed production in Ethiopia is basically informal, which in most cases farmers are recycles planting materials from previous crop harvest which results in viral and bacterial diseases accumulation. According to the authors, 98.7% of the current national seed potato requirements supplied through the informal seed system such as seed access from neighboring farmers, friends, relatives, merchants, local market, part of ware potato and commercial markets where potato is sold for consumption. Moreover, it is a common practice to save the smaller size and inferior tubers that are not sold for consumption for seed purposes; consequently, this practice allows disease and viruses to build up and yield may gradually decline, as seed gets degenerated.

The conventional method of bulking potato seed tubers is by repeatedly multiplying a set of tubers that has been proved to be disease-free in a process known as clonal multiplication (Bryan, 1981). However, this method has low multiplication rates of 6-8 tubers $plant^{-1}$. Therefore, it is expensive and time-consuming to produce enough seed tubers. Consequently, the seed tubers are expensive; it is estimated that the cost of seed tubers may account for 20-70% of the total production costs of commercial potatoes (Accatino and Malagamba, 1982). In addition, multiplication takes place in the field thereby exposing the seeds to soil-borne diseases. The alternative to field seed multiplication of potato is use of rapid multiplication techniques (Chandra and Birhman, 1994). Over the last three decades rapid multiplication systems became an important technique to provide disease-free propagules. These techniques yield *in vitro* plantlets, transplants, microtubers and minitubers, which are used in the initial phases of a seed tuber production scheme (Murashige, 1974; Roca *et al*., 1978; Hussey and Stacey, 1981; Wang and Hu, 1982; Jones, 1988).

The production of clean seed is very crucial to sustain the production and productivity of potato in the country. Currently, the common method for propagation of commercially important potato cultivars is through tubers. However, this propagation method has encouraged accumulation of tissue-borne viruses, fungi and bacteria in subsequent seasons. This has lead to significant losses in yield and tuber quality over seasons (Tsoka *et al*., 2012). The low multiplication rate of conventional potato seed production and bulk nature of the community are other challenges that not attract many private and public seed companies not to engage in potato seed business.

This study seeks to review and assess the various options available for clean seed tuber production or multiplication and suggest the way forward, with particular emphasis on the applicability of Rapid Multiplication Techniques (RMTs) such as Tissue Culture (TC), aeroponics and sand hydroponics technologies for minituber production in Ethiopia.

CONVENTIONAL TECHNIQUES

Conventional techniques of seed potato production involve the use of potatoes that are propagated by harvesting and replanting the tubers in the field. The tubers used for planting are known as "seed potatoes", as opposed to "potato seeds". Seed potato growers select better quality tubers for seed and discard those of poor quality. The diseased tubers are separated from healthy tubers and discarded while the healthy tubers are used for the next season's production. However, this method of seed production has proved to be laborious (labour intensive), prone to pest and disease infestation and time consuming. Considering that vegetatively propagated crops especially potatoes are prone to both viral and bacterial diseases, the conventional production of seed potatoes favors disease build-up, which drastically reduces crop yield (Badoni and Chauhan, 2010; El-Komy *et al*., 2010). If the mother potato plant becomes infected with a disease during the growing season, each of the new daughter tubers is likely to be infected as well.

The conventional method of propagation system is one of the slowest of seed multiplication rates of 1:10 ration compared with other seed propagation techniques like TC and aeroponics in the course of a year (Otazu, 2010). This method has also shown to be time specific particularly in tropical and sub-tropical regions where potato is a winter crop (Burton, 1989). The main disadvantages of a conventional seed potato program are the low multiplication rate of field-grown potato plants, resulting in a slow and inflexible system and the rising risk of catching viral, fungal or bacterial diseases with an increasing number of field multiplication. A reduction in the number of multiplication year requires a propagule that can be produced in large numbers in protected environments in a short period (Lommen, 1995). However, pathogen-free planting material of selected potato varieties and clones have been multiplied at Holetta and Jeldu research fields through a conventional method and distributes to producers as a source of planting materials. To avoid diseases like bacterial wilt and viruses a new system has been established for producing healthy seed based on systemic virus testing and *in vitro* rapid multiplication of virus free planting materials. Thus, different RMT's have been used for bulking up minitubers of released potato varieties and promising clones for distribution to growers.

Table 1: No. of minitubers produced under aeroponics at HARC from 2011-2013

	Year of production			
Variety	2011	2012	2013	Total
Belete	38,390	67,078	32,426	137,829
Awash	4,659	-	-	4,659
Gudenie	864	1,278	2,684	4,826
Jalene	233	2,362	-	2,595
Total	44,146	70,718	35,110	149,974

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RAPID MULTIPLICATION TECHNIQUES (RMTs)

RMTs are extensive methods used to increase the amounts of nuclear seed stocks for further seed multiplication. RMTs provide a better multiplication method than the conventional method of vegetative multiplication of potato (Endale *et al.*, 2008). The conventional method gives a lower multiplication ratio ranging from 1:3 to 1:15 and more likely rapid virus infection. However, RMTs provide higher multiplication ratios (1:40 to 1: several thousand per year) and lower rate of contamination, particularly from soil and seed-borne pathogens. Approximately 15% of the total area under potato cultivation around the world is used for the production of seed tubers. With the conventional methods, potatoes are often prone to pathogens such as fungi, bacteria and viruses, thereby resulting in poor quality and yield (FAO, 2008), whereas using healthy and quality seed is essential for growing an optimal potato crop (Parrot, 2010).

Different RMTs such as TC produced plantlets, screen house and aeroponics have been used to bulk up selected potato varieties for multiplication and distribution to growers. Selected improved potato varieties of Tolcha and Menagesha, have been multiplied using stem cuttings before the establishment of the new system and varieties, Jalene, Gudenie, Belete and Awash have been multiplied using aeroponics (Table 1). For the stem-cutting activities, healthy and clean *in vitro* plantlets received from TC and minitubers imported form CIP were planted in pots in the screen houses where they get intensive care and management. Although, these plants reach 20-30 cm high, the growth point of each stem was removed to stimulate growth of lateral shoots from the auxiliary buds. Cuttings, developed from auxiliary buds at each leaf, were taken to root in moist, coarse sand at a distance of 5×5 cm between individual cuttings for minituber production. Recently, several techniques have been evaluated and proved to be suitable for minituber production, including aeroponics culture (Kang *et al.*, 1996; Kim *et al.*, 1999; Nugaliyadde *et al.*, 2005). In Ethiopia, techniques for minituber production that have been used include stem cuttings and TC produced *in vitro* plantlets are then planted either in pots in screen house or under aeroponics facility. Since the establishment of aeroponics at HARC in 2011, the multiplication of quality planting materials was improved in terms quality and quantity. As a result, recently released potato varieties and promising clones have been multiplied using rapid multiplication techniques for further research and dissemination.

TISSUE CULTURE TECHNIQUES (TC)

Plant TC is the science of growing plant cells, tissues or organs isolated from the mother plant, on artificial media. This is facilitated using liquid, semi-solid, or solid growth media in sterilized tubes or containers. The TC technique is one of the important new methods of plant propagation available to growers and its use in seed production has allowed mass production of potato plants in a very short time. The system is characterized by very flexible rapid multiplication giving a high rate of multiplication (Beukema and Van der Zaag, 1990). Meristem culture is one of the important plant tissue culture applications for elimination of viruses from planting materials (Naik and Karihaloo, 2007; Badoni and Chauhan, 2010). It is a procedure in which apical/axillary growing tip (0.1-0.3 mm) are dissected and allowed to grow into plantlets on artificial nutrient media under controlled conditions. This technique for virus elimination is based on the principle that, many viruses are unable to infect the apical/axillary meristems of a growing plant and that a virus free plant can be produced if a small piece of meristematic is propagated (Wang and Hu, 1982; Kassanis, 1950). Apical meristem has a number of unique characteristics that has made elimination of virus possible and some of the features include (1) Vascular system through which viruses are spread is not developed in the meristematic region (2) Chromosome multiplication during mitosis and high auxin content in the meristem may inhibit virus multiplication through interference with viral nucleic acid metabolism and (3) Existence of virus inactivating system with greater activity in the apical region than elsewhere (Naik and Karihaloo, 2007).

The TC techniques employed in the micro-propagation of potatoes consists of the aseptic cultivation of cells or fragments of plant tissues and organs in an artificial medium under controlled temperature and light conditions. Vigorous and disease free potato plantlets can be obtained in the laboratory using these methods and then transferred to screen house in pots and aeroponics conditions for the production of minitubers. Moreover, the seed materials should be free of disease causing pathogens. Clean stocks are first obtained by meristem culture and then these plantlets are transferred to seed beds, screen house in pots and aeroponics to produce minitubers. Minitubers are commonly used in seed potato production in order to increase seed tubers (Ozturk and Yildirim, 2010). One of the advantages of this method is the maintenance of genotype identity since meristem cell preserve their genetic stability more uniformly (Grout, 1990). When materials have been cleaned of the pathogens, they can be mass multiplied for use as planting materials.

MINITUBERS PRODUCTION USING AEROPONICS

Plantlet culture in aeroponics system is recently used as an efficient method to produce and propagate minituber, which are healthy seeds without any contamination to pathogens. Eradication of soil born plant diseases, prevention from inoculation of pathogens by sterilized root medium, increasing in growth rate, propagation and vigor of minitubers, multi harvesting, omission of terminal dominance, uniformity in size and higher number of minitubers are results of applying this method (Lommen and Struik, 1992; Lommen, 2007). The science of minituber production in soilless systems shows an improvement in seed potato production program. Aeroponics is the process of growing plants in an air or mist environment without the use of soil or an aggregate media. Aeroponics system refers to the method of growing crop with their roots suspended in a misted nutrient medium. This is an alternative method of soilless culture in growth controlled environments. Minitubers are those progeny tubers produced on *in vitro* derived plantlets. The term refers to their size, as they are smaller than conventional seed tubers but larger than *in vitro* tubers (or microtubers) produced under aseptic conditions on artificial media.

The size of minitubers may range from 5-25 mm in diameter, although in current systems larger minitubers have also become common (Hassanpanah *et al*., 2009). Minitubers can be produced throughout the year and are principally used for the production of clean seed by direct field planting (Ritter *et al*., 2001). The use of minitubers in a seed program reduces the number of field multiplications. This may increase the flexibility of seed production, improve the health status of the ultimate seed and reduce the time for adequate volumes of seed from new cultivars to become available for growers (Lommen and Struik, 1992). Though the technique is in its early stage, attempts have been to improve production healthy or high quality planting materials.

Importance of aeroponics: Aeroponics can be used to produce higher yields, up to 10 times higher than the conventional method as well as reduce the rate of soil-based disease infections (Otazu, 2010). In aeroponics, plant roots grow in the air, tuber contact with soil-borne pathogens is avoided and production plant^{-1} increases considerably (Otazu, 2010). This system will help shift from six generations of multiplication in open fields to only three generations. However, potato cultivars respond differently to aeroponics and proper plant populations need to be determined for each cultivar (Otazu, 2010).

This method of propagation is one of the most rapid methods of seed multiplication. An individual potato plant can produce over 100 minitubers in a single row (Otazu, 2010). This contrasts with conventional methods that create only about 8-10 daughter tubers in a year and only 5-6 tubers plants^{-1} are produced using soil in the greenhouse in 90 days (Hussey and Stacey, 1981; Otazu, 2010). Another advantage of aeroponics is that nutrient and pH are easy to monitor. The system provides precise plant nutrient requirements for the crop, thereby reducing fertilizer requirement and minimizing risk of excessive fertilizer residues moving into the subterranean water table (Nichol, 2007).

Farran and Mingo-Castel (2006) reported that, soilless production techniques, such as aeroponics, have successfully been employed in tuber production, with good prospects for certification in seed production systems. However, the worst drawbacks are the low volumes available to the root system and any losses of power to pumps that can produce irreversible damages. Traditionally, minituber production in Sub-saharan African countries is done on soil-base substrates that are steam sterilized to avoid soil born diseases and pests. However, rising prices of fossil fuels and scarcity of firewood used for the steam boilers render this practice almost prohibitive. The best alternative is to use nutrient solutions instead of soil substrates which is a common practice of aeroponics already established for minituber production in industrialized Asian countries. CIP has developed an economic module that is being promoted for developing countries.

In aeroponics, plantlets are grown in specially designed boxes where shoots grow on top and roots grow suspended in the air within the box and in darkness. Roots are fed with pressurized nutrient solution mist at short intervals; as plants develop tubers are formed from stolons near the roots. There are several advantages of this technology as compared to the soil-based substrates for minituber production; the most important ones are: Healthier tubers because of no soil borne diseases, higher number of tuber set per plant and reduced costs per minituber. In this system, minituber production is 10 times that of soil substrate.

The Ethiopian Institute of Agricultural Research (EIAR), with support from the Common Fund for Commodities (CFC) and USAID, established two aeroponics units at Holetta Agricultural Research Center (HARC) in 2010 for the production of minitubers of both popular and newly released varieties. The technique is suitable for early stages of seed multiplication in which the production operations are handled by the best technical support system. The technique is effective in giving high number of minitubers upto 50 plant^{-1} but adoption rates will be determined by availability of stable and low-cost power supply and expansion in the seed market. Minitubers produced in the aeroponics unit multiplied in the aphid-proof screen house/net house in HARC to produce seed. The minitubers that are produced in the screen house and aeroponics are usually either planted in open field or in pots in the screen house based on the size of the tubers. The aim is to improve the health status of existing seed stock by reducing the number of field multiplications (Kleingeld, 1997). Harvesting in aeroponics is convenient, clean and allows a greater size control by sequential harvesting (Ritter *et al.*, 2001). The number and timing of non-destructive harvests are key factors in the optimization of minituber production. To optimize the system, appropriate nutrient solutions, plant densities, number of harvest and harvesting intervals, as well as possible interaction between them should be considered (Farran and Mingo-Castel, 2006).

Sand hydroponics: This new technology was established at HARC on 2013 for potato multiplication. Sand hydroponics is one of the RMTs used to produce clean planting materials as minitubers. It is adopted from CIP and all the nutrients required for plant growth and development was prepared in a container and supplied in the form of drip irrigation. Nonetheless, sterilized sand is used as a media for root development, to cover the new tuber and also to support the plants. In sand hydroponics system potato is planted at a spacing of 5×5 cm to produce minitubers. As a result and a remarkable yield was obtained from individual plants as compared to conventional potato multiplication on the field. For sand hydroponics, either the *in vitro* plantlets from TC lab or the minitubers from the aeroponics was used as a planting material. Thus, the resulting generation is free from viral and bacterial diseases. Therefore, about 3,159 minitubers of variety Belete was multiplied using sand hydroponics techniques that will be multiplied under a field conditions.

MULTIPLICATION AND DISTRIBUTION SCHEME

In this program, high quality planting materials are either imported from CIP's regional office in Nairobi or multiplied at Holetta and Jeldu research fields as well as TC laboratory. The materials are distributed to growers and different research centers for seed production or research purposes. A total of 1,669.5 tons of seed potatoes of 14 released potato varieties have been produced on station at Holetta and Jeldu from 2008 to 2011. The distribution of the materials to farmers was practiced using farmer groups organized in to Farmer Field Schools (FFS) or Farmer Research Groups (FRG). This had led the foundation of reaching more farmers to use clean seed for their seed production.

The healthy planting materials/stocks multiplied using RMTs are of the selected varieties, which are maintained under strict hygienic conditions in an insect-proof screen house. Subsequent propagation is carried out using rooted cuttings in the screen house to obtain enough planting material before they are planted in open field. A total of 227, 333 MT of several clones and released varieties were multiplied in screen houses which include Awash (1,710 MT), Gudenie (39,518 MT), Jelene (13,179 MT) and Belete (145,870 MT). Source materials for this MT production were either *in vitro* plantlets or smaller MT produced from the previous season in the aeroponics units. These materials have been introduced in to the center's seed production program and have played a paramount role in regenerating the stock materials (Table 2).

Way forward: Lack of good quality seed is mostly a consequent of the prevailing seeds system; in most developing countries. Majority of farmers recycle their own seeds potato or get them from informal sources. This leads to seed degeneration and build-up of tuber-borne diseases and hence low yields. Therefore, the use of RMTs like aeroponics technology produced far more minituber than the conventional methods. Aeroponics system complemented by plant TC promises a great potential

Table 2: Minitubers produced under screen houses planted from *in vitro* plantlets and small sized minitubers of aeroponics

	Years of production					
Variety/clones	2008	2010	2011	2012	2013	Total
Different clones	15,041	1,692	6,043	-	25,825	48,601
Jalene	3,760	7,440	1,979	-	318	13,497
Guassa	1,200	1,080	-	-	-	2,280
Gudenie	1,800	1,352	9,231	27,135	5,412	44,930
Belete	-	-	42,871	102,999	-	145,870
Awash	228	228	1,254	-	-	1,710
Gorebela	1,264	-	-	-	-	1,264
Zengena	516	-	-	-	-	516
Tolcha	220	-	-	-	-	220
Total	24,029	11,792	61,378	130,134	31,555	258,888

CFC project annual report, 2013

to transform seed potato production in developing countries. Considering the potential benefits of the system such as rapid production of seed, spacious, good nutrient monitoring system, improvement of growth and survival rate of plantlets, constant air circulation and ecologically friendly, this system has a potential of revolutionizing potato seed production industry in developing countries. However, the system needs further and complete evaluation in terms of productivity, profitability and sustainability.

Aeroponics system itself can be inadequate in viral disease-free seed potato production if not complemented with tissue culture's meristem culture application. The meristem culture has to be applied in elimination of any viral pathogen from the desired clone of the potato before multiplying the plantlets to be used in the aeroponics system to produce pathogen-free seed tubers. A stock of viral-free planting materials can be maintained under both tissue culture conditions and in an insect-free screen house where re-infection cannot occur. The plant stocks maintained in a tissue culture laboratory have to undergo the hardening off stage each time before getting into the aeroponics system. In addition, a comparative cost analysis is required between aeroponics and conventional methods in terms of unit cost per tuber. The system has a potential of significantly increasing income and reduce time and cost of production of quality seed potatoes to make them more accessible to growers in developing countries.

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