POTATO MINITUBERS TECHNOLOGY – ITS DEVELOPMENT AND DIVERSITY: A REVIEW

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Abstract
The study consists of literature review on potato (*Solanum tuberosum* L.*)* initial seed material – minitubers production. This paper covers aspects of healthy potato microplants production techniques and subsequent greenhouse minitubers production methods. The diversity of conventional minituber growing techniques (on solid medium in greenhouses) is discussed. Review showed, that obtained minitubers number depends on growing methods and variety. Physical manipulation during *in vitro* phase could have positive effects on minitubers yielding capacities.
Key words: *Solanum tuberosum* L. *in vitro* plantlets, microplants, micropropagation, minitubers.

Introduction

Potato (*Solanum tuberosum* L.*)*, being vegetatively propagated crop, is prone to accumulation and further spread of several diseases affecting its yield and quality. Healthy planting material has essential role in potato production chain.

Clonal selection (a conventional seed production method), which had been used for decades, required intensive process control and seed programmes based on clonal selection could take 10 years and more.

Other propagation techniques have been developed and involved in seed production systems, thus decreasing time needed for seed multiplication These techniques include obtaining healthy stock material through virus eradication using meristem culture, rapid *in vitro* plants multiplication and increased number of individuals of the first year clones through minitubers production (Struik and Wiersema, 1999). Micropropagation techniques have widely been introduced in potato seed production systems (Jones, 1988; Struik and Wiersema, 1999) for more than three decades and development of *in vitro* multiplication system was a breaking point in the commercial production of high quality potato seed (Pruski, 2007).

Nowadays potato can be rapidly multiplied using nodal cuttings produced *in vitro* and involving following minitubers production. Methods, protocols and conditions to produce *in vitro* plantlets vary across laboratories, as well as methods for obtaining first generation potato seed tubers can be rather different, thus resulting in diverse outcomes.

The aim of this review is to cover aspects of the laboratory production of *in vitro* plantlets with an emphasis on the subsequent potato minitubers production. Aspects of microtubers (small tubers produced in *in vitro* conditions) production are skipped this time, as well as hydroponics and aeroponics techniques for minitubers production are noted without detailed study.

Materials and Methods

Monographic method has been used for this study. Available literature (journals, monographs, PhD thesis) have been studied with the aim to cover broad spectrum of methods developed for potato micropropagation and the following potato minitubers production. Additionally unpublished materials such as yearly reports since 1979 regarding potato minitubers production system establishment and development at State Priekuli Plant Breeding Institute have been studied in order to cover potato micropropagation and minitubers production techniques involvement in Latvia.

Results and Discussion

Microplant multiplication – obtaining of the stock material

Struik and Wiersema (1999) distinguish two major methods for obtaining a starting material in potato seed production: under semi *in vivo* conditions (greenhouses) using sprout, stem and leaf-bud cuttings and under artificial *in vitro* conditions.

In vitro produced plantlets are widely used as the base in potato seed programmes (Jones, 1988; Struik and Wiersema, 1999; Pruski, 2001; Tadesse, 2007) worldwide.

Jones (1991) claimed that large scale *in vitro* production of pathogen free plantlets was initiated in North America by New York State in 1978. In Latvia, Dr. Uldis Miglavs in 1974 was the first one who recommended including micropropagation methods in potato seed production programme (Миглавс, 1974). After that in 1979 newly developed laboratory at Priekuli Breeding and Experimental Station (now – State Priekuli Plant Breeding Institute) received its main objective of work – to develop potato seed production system in Latvia based on virus free plantlets (State Priekuli Plant Breeding Institute yearly reports 1979-1991).

Though liquid shaken cultures have been established (*in vitro* plantlets are cut into stem cuttings each with three to four nodes, and each stem piece is placed in liquid media, the flasks are shaken and after 2–3 weeks of rapid growth each flask contains 60 to 70 nodes (Dodds, 1988)), i*n vitro* single node cuttings are probably the most common multiplication method for mass propagation of *in vitro* plantlets (Roca et al., 1978; Ortiz-Montiel and Lozoya-Saldana, 1987; Ranalli et al., 1994; Grigoriadou and Leventakis, 1999; Struik and Wiersema, 1999; Pruski, 2001; Tadesse et al., 2001a; Gābere, 2004; Otroshy, 2006; Veeken and Lommen, 2009; Särekanno et al., 2010a; Asakaviciute, 2011; Ozturk and Yildirim, 2011; Milinkovic et al., 2012).

One cycle of multiplication using nodal cuttings takes about four weeks (Ranalli et al., 1994; Struik and Wiersema, 1999; Pruski, 2001; Asakaviciute, 2011; Milinkovic et al., 2012) and on average 3–5 new cuttings can be obtained from one plantlet (Rannali, 1997).

Murashige and Skoog (1962) inorganic salts and vitamin medium (known as MS medium) with added sucrose 30 g l^{-1} and agar 6–8 g l^{-1} is common nutrient medium for potato micropropagation *in vitro* (Pruski, 2001; Gābere, 2004; Otroshy, 2006; Corrêa et al., 2008; Asakaviciute, 2011; Milinkovic et al., 2012). Application of half strength MS medium has been reported by Ahloowalia (1994) and Ranalli et al. (1994). Several authors outline usage of plant growth regulators (PGR) such as kinetins (Kotkas and Rosenberg, 1999), gibberellins (Roy et al., 1994; Kotkas and Rosenberg, 1999), auxins (Grigoriadou and Leventakis, 1999; Ozturk and Yildirim, 2011). Adding auxins to medium has been reported as a promoter of rooting (Ozturk and Yildirim, 2011), but Rannali (1997) has stated that separate rooting phase is not required for potato microplants, as cultured shoots of potato quickly develop roots. Application of PGR Alar (daminozide) during the last subculture has been mentioned as promoter of microplants survival after their transfer to greenhouse (Lommen, 1995; Grigoriadou and Leventakis, 1999; Tadesse et al., 2001a; Veeken and Lommen, 2009). Additional inorganic salts tetrahydrated calcium nitrate and diammonium phosphate can be added to medium (Muro et al., 1997), but high grade sucrose can be successfully replaced by ordinary sugar (Ahloowalia, 1999).

Vast diversity of culture containers are in use for potato microplants propagation. Jones (1988) explored that in North America glass test tubers and Magenta polycarbonate boxes are used most frequently, while in Europe glass test tubes dominate. Glass test tubes had been and still are popular in Europe as containers for the plant micropropagation (Миглавс, 1974; Roca et al., 1978; Jones, 1988; Roy et al., 1994; Tadesse et al., 2001a; Veeken and Lommen, 2009; Särekanno et al., 2010a; Ozturk and Yildirim, 2011). Some authors mention plastic containers with 100 mL of medium (Milinkovic et al., 2012), 300 mL flasks (Corrêa et al., 2008), suitable container with 16–25 single nodes (Dodds, 1988) not specifying entire information about material, volume of container, number of explants per container. More clear data are given by Wattimena et al. (1983) who have reported the use of 3 to 5 shoots per 120 mL culture vessel containing 30 mL medium and sealed with parafilm. Ahloowalia (1994) writes about usage of 120 mL clear plastic containers with 20–25 mL medium, 10–15 explants per container, Veeken and Lommen (2009) outline plastic jars (10 cm diameter and 5 cm height), containing 75 mL of medium and 25 single node cuttings grown in jars. The authors state that bigger containers are used only in the last phase of multiplication. Pruski (2001) mentions usage of GA7 Magenta vessels (produced by Magenta Corporation, USA and having certain volume), where 16 explants are placed. It was already explored by Jones (1988) that in North America Magenta polycarbonate boxes were used more frequently than in Europe.

The utilization of glass test tubes has both advantages and disadvantages over the usage of bigger containers for cultivation of microplants. Glass test tubes are very costly, but once they are purchased, they can be reused almost for unlimited time. One of the main disadvantages of the usage of glass test tubes is a lot of labor required for washing the tubes, especially when laboratory has to produce tens of thousands of potato microplants.

Bigger containers can be both disposable and reusable. Reusable containers for microplants cultivations are usually costly, but the usage of disposable containers raises the question of sustainable development because a lot of waste is produced as a result of plants masspropagation. When contamination occurs, all plants grown in one bigger container are damaged unlike test tubes where only a single plant is lost per container.

The effect of culture container on potato microplants growth is rarely studied. Fal et al. (2002) claims that there are some facts often overlooked in tissue culture. Research on effects of culture vessel and its closure type on *in vitro* propagation of carnations (*Dianthus caryophyllus* L.) outlined the response of *in vitro* growth and morphogenesis of several *Dianthus caryophyllus* L. cultivars. It was dependent on the environmental differences inside various types of culture vessels. These findings were mainly related with the specific sensitivity of each cultivar to the gas exchange and medium desiccation determined

by the vessel type. It was stated that light inside the culture vessel depended on its type and was different from the light coming from the culture shelf, as well as various closures provided various gas exchange inside of culture vessels (Fal et al., 2002). The study on carnations could be possibly applied to potato micropropagation because both species are propagated through nodal cuttings. The study on influence of culture container volume, medium volume and culture density on the growth yields of lettuce (*Lactuca sativa* L.) and spearmint (*Mentha spicata* L.) shoots were conducted. In this study, culture vessel capacity greatly influenced the growth responses from lettuce and spearmint shoots (Tisserat and Silman, 2000). Concerning potato micropropagation it has been found that net photosynthetic rates per microplant and per leaf area were reduced at lower relative humidity rates in the culture container (Tanaka et al., 1992).

Further investigations on effects of tissue culture containers and their closures on potato microplants growth and quality as well as possible after effects of these treatments on minitubers formation in greenhouse might be necessary. New findings might contribute to improvement of efficiency of initial potato seed production.

A discussion about genetic stability of micropropagated plants has always been in the scope of researchers.

In the early years when *in vitro* multiplication of healthy stock material was introduced in potato seed production system, Roca et al. (1978) stated that no detectible changes due to *in vitro* procedures could be found using morphological and biochemical criteria. Plantlets derived from meristems could be more genetically stable that plantlets derived through other *in vitro* procedure - leaf discs etc. (Slack, 1980). On the contrary, it has been reported that mutations can arise when plants are derived from small explants such as meristems (Wright, 1983).

Ahloowalia (2000) has looked into phenotypic stability of microplants and minitubers by conducting the greenhouse experiment with thousands of plants. His findings showed only variegated leaves of one single branch of one single plant. Few off-type minitubers were observed, but they became true to type in the subsequent propagation (Ahloowalia, 2000).

Minitubers derived from microplants have been tested for genetic variation under field conditions (Rosenberg et al., 2007). No genetic variation has been reported; however, the authors stated that meristem clones differed in the intensity of flowering, height of stems and in the uniformity of plants.

There is still a different point of view whether the initial material for micropropagation should be renewed every year (by meristems, shoot tips cuttings and other methods) or it can be obtained from repositories where stock plants are maintained for a longer time.

Acclimatization (hardening) of microplants

Various authors have different views on the necessity of the acclimatization phase of *in vitro* plants before they are planted into production containers in greenhouses.

Potato microplants can be planted in small containers (e.g. paper pots (Muro et al., 1997), plastic rolls (Miglavs, 1987; Rosenberg et al., 2007; Särekanno et al., 2010a), in transplanting trays with small cells (Tadesse et al., 2001a)) filled with certain growing medium (e.g. in fertilized peat (Muro et al., 1997)). Plants can be kept under reduced light (Muro et al., 1997; Corrêa et al., 2008). After acclimatization plants are usually disturbed and replanted to other growing trays and growing medium.

On the other hand, it has been reported that microplants can be planted directly to the greenhouse without passing acclimatization phase (Ahloowalia, 1994; Grigoriadou and Leventakis, 1999).

Minitubers growing methods

Two major traits by which minitubers can be distinguished from microtubers and conventional seed tubers can be derived from literature.

The first trait is the way of obtaining minitubers. A majority of authors agree that minitubers are produced from *in vitro* derived potato plantlets under greenhouse conditions (Lommen and Struik, 1992a; Ahloowalia, 1994; Rannali, 1997; Struik, 2007) either on a soil or in soil-less systems such as hydroponics and aeroponics or under field conditions (Jones, 1991; Särekanno et al., 2010a). The way of production distinguishes minitubers from microtubers, which are produced under *in vitro* conditions. Some authors mention that minitubers can also be produced from microtubers (Ahloowalia, 1994).

The second trait is the size of minitubers and this trait is less unambiguous. Struik and Wiersema (1999) summarize that the size of minitubers may be in the range from 5–25 mm although in many potato seed production systems larger minitubers are also common.

Some seed production systems involve growing of minitubers directly under field conditions (Wattimena et al., 1983; Särekanno et al., 2010a; 2010b; 2012) regardless the delicate planting material (Wiersema et al., 1987) and careful handling that is required (Lommen and Struik, 1992b).

Most seed programs already more than two decades ago involved greenhouse minituber production (Miglavs, 1987; Dodds, 1988; Jones, 1991). Regardless large-scale development of soil-

less systems worldwide, growing of potato minitubers in greenhouses in normal potting substrates (soil, peat etc.) is still considered a simple and cheap way of production; therefore, it can be called a conventional minitubers production system.

The main purpose of initial potato seed production is obtaining as many medium sized minitubers with good health status per one *in vitro* plantlet or per area unit of greenhouse as possible.

Lommen and Struik (1992a) have stated five main parameters which can be manipulated in minitubers production phase: '(1) the number of minitubers per *in vitro* plantlet, (2) the number of minitubers per unit area, (3) the average weight per minituber, (4) the minituber yield per plantlet, and (5) the minituber yield per unit area'.

Many crop husbandry techniques have been utilized in order to manipulate minituber yield parameters. These techniques include planting density, growing medium, fertilizing, growing container used and others. In many cases all these treatments can interact; therefore, when one of them is changed, other yield parameters can be obtained.

A wide diversity of planting densities is described in literature covering densities from 24–25 plants per m^{-2} (Wiersema et al., 1987; Roy et al., 1994; Veeken and Lommen, 2009) to even 800 plants per m^{-2} (Lommen and Struik, 1992a). Authors provide information about planting densities of 40–48 plants per m^{-2} (Wiersema et al., 1987; Gābere, 2004; Dimante, 2013), 100 plants m^{-2} (Dodds, 1988; Roy et al., 1994), 200 plants m^{-2} (Grigoriadou and Leventakis, 1999). In the study of Veeken and Lommen (2009), three planting densities were compared – 25 plants m^{-2}, 62.5 plants m^{-2}, 145.8 plants m^{-2}. Similarly, Roy et al. (1994) compared minituber yield at three planting densities 25 plants m^{-2}, 49 plants m^{-2}, 100 plants m^{-2}. The density of 280 plants per m^{-2} was authorized by the Netherlands General Inspection Service of Agricultural Seeds and Seed Potatoes (Struik and Wiersema, 1999). On average 200–400 plantlets per m^{-2} can be planted, when a repeated harvesting method is used (Lommen and Struik, 1992a).

The most popular growing medium mentioned in literature is peat (Miglavs, 1987; Kotkas and Rosenberg, 1999; Gābere, 2004), various mixtures containing peat such as 1:1 mixture of sand and peat (Wiersema et al., 1987), 2:1 peat sand mixture (Muro et al., 1997), 2:1:1 soil, vermiculite and sand substrate (Ranalli et al., 1994), 5:1 peat – perlite mixture (Roy et al., 1994). 1:1 peat – perlite (Grigoriadou and Leventakis, 1999), peat-clay mixture 1:1 (Veeken and Lommen, 2009). Commercial ready-made substrates can be used for minitubers production as well, e.g. mixture of perlite and potting soil 1:1 is mentioned by Loomen and Struik (1992a), ready-made potting compost obtained from commercial company (Ahloowalia, 1994), nutrient rich potting soil without any specification is described by Ostroshy (2006).

Regrettably few authors specify composition of fertilizers used for enrichment of growing medium, as well as there is a lack of clear information about additional feeding during growing season promoting good tubers set. Moreover, fertilizer composition is a production secret for private enterprises producing potato minitubers.

Very common situation is when only description of either fertilizing of growing substrate or additional feeding can be found.

Application of Nitrofoska® at the rate of 3.3 kg m^{-3} of peat is mentioned in literature (Miglavs, 1987). Wiersema et al. (1987) specify usage of NPK 1:1:1 40 g m^{-2} three times per season. Roy et al. (1994) describe application of NPK 14:14:14 100 mg l^{-1} N until four weeks before harvest. Rannali et al. (1994) indicate a weekly application of solution containing Nitrofoska® fertilizer N:P:K:Mg (12:5:14:1.5). Gābere (2004) describes composition of peat mixture indicating the usage of N 0.14 kg, P 0.07 kg and K 0.07 kg per m^{-3} of peat, as well as the usage of $CaNO_3$ solution at rate 2 g l^{-1} as foliar applications two times per season. In addition, Lommen and Struik (1992a, 1992b) specify that 131.4 mg l^{-1} of N is added to the mixture of perlite and potting soil. The authors describe a complete composition of nutrient solution including macro and micro salts. The solution is used at a low concentration (which is not specified) twice a week with respect of 100 to 200 ml per six plants. Struik and Wiersema (1999) introduce the procedure followed by the Netherlands General Inspection Service for Agricultural Seeds and Seed Potatoes, which include the usage of potting soil with nutrients for the first 2 months. When tubers are initiated, two types of fertilizers are used – NPK 17:17:17 applied by hand at the amount of 1g per m^{-2}. NPK 18:18:18 including trace elements is dissolved in water and applied in quantity 1 g of fertilizer per m^{-2} every 2 weeks until irrigation is stopped.

Various growing containers have been in the use for minitubers production. Pots made from paper, plastics and other materials with various diameters (e.g. 25 cm (Miglavs, 1987), 13 and 19 cm (Vanaei et al. 2008), 20 cm (Milinkovic et al., 2012) etc.) are very common. Bigger nursery beds could be considered as very suitable containers for masspropagation of minitubers. In the study of Wiersema et al. (1987) 1 m wide nursery beds are mentioned. Roy et al. (1994) describe wooden beds, which are 1 meter wide and 50 cm high. In addition, other dimensions of planting beds are described in literature – 25 m long and 1.25 m wide benches (Muro et al., 1997), plastic beds 50×360×20 cm (Gābere, 2004). The usage of plastic

boxes is reported as well (Grigoriadou and Leventakis, 1999; Gābere, 2004; Veeken and Lommen, 2009).

According to Otroshy (2006), 100 days is a normal production cycle for minitubers. Time for destructive harvest can be from 70 days after planting (Ahloowalia, 1994) to even 121 days after planting (Roy et al., 1994).

Alternative method in conventional minitubers production system – repeated harvesting

Lommen and Struik (1992a; 1992b) have developed a distinct approach to minitubers growth. This technology involves non-destructive harvesting of minitubers, and thus is called – repeated harvesting.

The authors describe procedure clearly 'Plants were lifted carefully from the soil mixture, tubers > 0.3 g were removed and plants were replanted into the soil mixture. Whether the weight of the removed tubers was > 0.3 g had to be estimated, using a diameter of approximately 8 mm as a criterion. Plants were always replanted deeper than before. Replanting depth was not recorded but depended on the harvest date, and increased as the length of the stem part without leaves increased. Care was taken not to damage stems and stolons. Damage of roots, however, could not be avoided. Removing tubers > 0.3 g in a non-destructive harvest, many new tubers were initiated on existing stolons, newly formed stolons and directly on the below-ground part of the main stem' (Lommen and Struik, 1992b). Such harvesting procedure was used at the first two harvests; the third harvest was destructive (Lommen and Struik, 1992a). Later Veeken and Lommen (2009) summarize that repetitive harvesting results in relatively small tubers, and in addition has a high labor demand. Therefore, repetitive harvesting method may be less interesting and effective for commercial production.

Minitubers yield (numbers)

Ahloowalia (1994) has stated that obtained tuber number is the most important parameter in the production of minituber for seed.

Struik (2007) summarizes that the number of minitubers is usually in the range from 2–5 tubers per planted plant. Average data obtained by many authors across varieties fall in the frame marked by Struik (2007). The varietal differences in terms of tuber number per plant have been confirmed by authors (Hagman, 1990; Ahloowalia, 1994; Gābere, 2004; Otroshy, 2006; Struik, 2007; Dimante, 2013).

Very small average tuber number per plant of only 0.26–3.07 tubers was reported by Aahlowalia (1994), 1.85–2.52 tubers per plant reported by Grigoriadou and Leventakis (1999), but Corrêa et al. (2008) reported average yields of 7.00–8.31 minitubers per plant

Authors investigating the influence of planting density on minitubers yield have relevant finding that increasing of planting density decreases minituber number per plant and average minituber size, but increases tuber number per area unit (Roy et al., 1994; Veeken and Lommen, 2009). The study of Roy et al. (1994) and Veeken and Lommen (2009) overlaps in the smallest planting density, which is 25 plants per m^{-2}. Nevertheless, the authors have got significantly different results. Roy et al. reported the average yield of 11.1 minitubers per planted plant, but Veeken and Lommen mentioned the yield of 5.4 minitubers per plant as average 10 weeks after planting. These differences could be explained by several factors: thus, confirming the potential significance of various treatments. Different varieties, different planting containers were used (plastic boxes by Veeken and Lommen and relatively large propagation beds by Roy et al.). The layer of planting substratum was respectively 10 cm and 18 cm for Veeken and Lommen and Roy et al., as well as substratum mixture and fertilizing was different. Roy et al. performed additional treatments, providing supplementary irradiation and performing plant hilling during the season.

Effects of in vitro phase on subsequent minitubers production

Several efforts have been attempted in order to understand possible manipulation with plant status during *in vitro* phase and acclimatization and its effect on subsequent minituber yield parameters.

It has been reported that minitubers yield in the greenhouse could be improved by modifying *in vitro* growing conditions (Seabrook et al., 1995; Tadesse, 2000; Pruski, 2001; Otroshy, 2006; Milinkovic et al., 2012).

Ahloowalia (1994) reported that a longer duration of *in vitro* phase has a negative effect on minituber production. Milinkovic et al. (2012) reported contrary results – significantly positive effect of extended *in vitro* growing period on subsequent minitubers yield resulting in up to 97% higher number of minitubers in comparison with control.

Decreasing of photoperiod during the last subcultures of *in vitro* plant multiplication could have positive effect on subsequent minitubers yield in greenhouses (Seabrook et al., 1995). Milinkovic et al. (2012) did not confirm this finding, reporting that minitubers number per plant did not change significantly when a shorter photoperiod than standard 16 hours daylight during *in vitro* phase was used.

According to the study of Otroshy (2006), lower *in vitro* temperature subsequently resulted in larger minitubers, but it did not affect minitubers number. These results are opposite to Tadesse's et al. (2001b)

findings that lower temperature during *in vitro* phase did not affect minitubers average weight significantly.

Further studies are necessary to understand the influence of various physical treatments during *in vitro* phase on subsequent microplants yielding capacities in a greenhouse, especially on stable production of large enough number of minitubers.

Conclusions

1. Methods, protocols and conditions to produce *in vitro* plantlets vary across laboratories, as well as methods for obtaining first generation potato seed tubers can be rather different.
2. No common opinion about a necessity of hardening phase of potato microplants can be found.
3. Minitubers are obtained from *in vitro* grown microplants. The way in which minitubers are obtained is the main trait, which distinguishes them from microtubers and conventional potato seed tubers.
4. A wide diversity of planting densities, fertilizing protocols and other growing techniques are reported in literature. The influence of a variety on minitubers number has been approved.
5. Physical manipulation during *in vitro* phase could have positive effects on subsequent minitubers yielding capacities, but further investigations are required.
6. Not all of the minitubers growing methods described in literature can be suitable for commercial production. Seed producers have to adapt techniques, which are the most effective for their capacity.

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