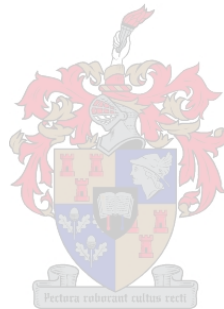


**Improved potato (*Solanum tuberosum* L.) seed production through
aeroponics**

By

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of Master of Science in Agriculture (Agronomy) at the Faculty of AgriSciences at
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Declaration

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Date: December 2013

Steve Ndongji Tshisola

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Abstract

The potato can be considered as one of the most important food crops in many African countries. The potential of this crop is reflected in the large increase in area of production where Africa showed the highest rate of growth within the developing world over the past twenty years. The multiplication rate of potatoes is very low compared to other crops. Therefore it is essential to investigate methods of increasing the number of minitubers produced from disease free in-vitro plantlets. There is a number of potato propagation procedures that are currently being used worldwide to multiply seed potatoes. As aeroponics is still a relatively new technique that has not been researched extensively for the production of minitubers, a study relating to the production practises including the nutritional requirements of potato minitubers produced in a aeroponic system were undertaken.

Potato plantlets, cv BP1, were grown aeroponically at two different densities (20 and 30 plants/m²) and four harvest intervals (7, 10, 14 and 18 days). The interaction between harvesting intervals and plant densities did not influence plant growth, minituber quality or yield. Best results were realised when harvesting every 7 days with a higher total tuber number over the growing period. Harvest interval also influenced the phosphorus and copper concentration in minitubers.

To study the effect of Calcium (Ca) application rate, potato plantlets of cultivars Up-to-date, Mnandi, Buffelspoort and BP1 were grown at four different Ca levels (8.40, 6.75, 5.10 and 3.45 meq/L). The interaction between Ca application levels and cultivars significantly influenced the percentage stolon branching. BP1 had more stolons at the lowest Ca application level and Buffelspoort had more stolons at the full Ca application levels. However, low Ca treatments produced the highest yield. The minituber number and weight harvested were three times more for Mnandi.

An aeroponic study on the irrigation frequency (20, 30, 40 and 50 minutes interval) was conducted on four potato cultivars (Up-to-date, Mnandi, Buffelsoort and BP1). Significant differences were noted in the interaction between irrigation frequencies and cultivars for the percentage tuberised plants and stolon and tuber dry mass. When irrigated every 40 minutes, 48% of the Buffelspoort plants produced tubers. Plant height was also significantly affected by the interaction between irrigation frequencies and potato cultivars, with Mnandi producing taller plants when irrigated

every 30 minutes. Total tuber number and tuber fresh and dry weight was higher at the irrigation frequency of 20 minutes. The interaction between irrigation frequencies and cultivars on the response to macro and trace elements was not significant for sodium and iron but was for phosphorus, potassium, calcium, zinc and aluminium.

A field study was conducted in a greenhouse where potato seed of BP1 obtained from the first trial were graded into different sizes (Small: >20, medium: 20–40 and large: > 40 mm of diameter) and stored at 3 different temperatures (3, 16 and 25°C) for 2 supplementary months before being planted. Sprouting capacity was mostly influenced by temperature regardless of other factors applied to potato seed minitubers such as harvest intervals and sizes. The higher storage temperature of 25°C resulted in tubers with a higher number of sprouts, longer sprouts and with a higher sprouting capacity.

Uittreksel

Aartappels is een van die belangrikste voedselgewasse in baie lande in Afrika. Die potensiaal van die gewas word gereflekteer in die groot toename in produksie areas, met Afrika wat die vinnigste van al die ontwikkelende lande gegroei het die laaste 20. In vergelyking met ander gewasse is die tempo van planvermeerdering by aartappels baie stadig. Dit is dus essensieel om metodes te ondersoek wat sal help om die aantal miniknolle wat per in vitro plantjie verkry kan word te verhoog. Daar is heel party plant vermeerderings prosedures wat tans wêreldwyd gebruik word om saad aartappels te vermeerder. Aangesien aeroponika nog steeds 'n relatiewe nuwe tegniek is wat nog nie ekstensief ondersoek is vir die verbouing van miniknolle nie, is 'n studie geloods om te kyk na die produksie praktyke, wat insluit die voedingsbehoefte van aartappel miniknolle in 'n aeroponika sisteem.

Aartappel plantjies, kultivar, BP1, is aeroponies verbou by twee plant digthede (20 en 30 plante/m²) en vier oesintervalle (7, 10, 14 en 18 dae). Die interaksie tussen oesintervalle en plantdigtheid het geen effek gehad op plant groei, miniknol kwaliteit of opbrengs nie. Die beste resultate is verkry waar die knolle elke 7 dae geoes is met 'n hoër totale aantal knolle oor die groeiseisoen. Die oesinterval het ook 'n effek gehad op die fosfaat en koper konsentrasie van die miniknolle.

Om die effek van die Kalsium (Ca) toedieningspeil te ondersoek is aartappel plantjies; kultivars Up-to-date, Mnandi, Buffelspoort en BP1 gekweek by vier verskillende Ca peile (8.40, 6.75, 5.10 en 3.45 meq/L). Die interaksie tussen Ca toedienings peile en kultivars het 'n beduidende effek gehad op die persentasie stolon vertakking. BP1 het meer stolons gehad by die laagste Ca toedieningspeil en Buffelspoort het meer stolons gehad by die volle Ca toedieningspeil. Die hoogste opbrengste is egter waargeneem by die laagste Ca toedieningspeil. Die aantal miniknolle en oes massa was drie keer meer vir Mnandi.

'n Aeroponiese studie op die besproeiingsfrekwensie (20, 30, 40 en 50 minuut intervalle) is gedoen met vier aartappel kultivars (Up-to-date, Mnandi, Buffelsoort en BP1). Beduidende verskille is opgemerk in die interaksie tussen besproeiings frekwensie en kultivars vir die persentasie plante met knolle en stolon en knol droë massa. Met besproeiings elke 40 minute het 48% van die Buffelspoort plante knolle produseer. Plant hoogte is ook beduidend beïnvloed deur die interaksie tussen besproeiingsfrekwensie en aartappel kultivar met Mnandi plante wat hoër was wanneer dit elke 30 minute besproei is. Die totale aantal knolle en knol vars- en droë massa was hoër wanneer daar elke 20 minute besproei is. Die interaksie tussen besproeiings frekwensie en kultivars op die makro- en mikro element inhoud van die knolle was nie beduidend vir natrium en yster nie, maar wel vir fosfaat, kalium, kalsium, sink en aluminium.

'n Potproef is gedoen in 'n kweekhuis waar aartappel saad van BP1 verkry vanaf die eerste proef nadat knolle verdeel is in verskillende grootte klasse (klein: < 20mm, medium: 20-40mm en groot: >40mm) en gestoor is by drie verskillende temperature (3, 16 en 25°C) vir 2 addisionele maande voor plant. Spruit ontwikkelings kapasiteit was meestal beïnvloed deur temperatuur ten spyte van ander behandelings soos oes intervalle en knol grootte. Die hoër bergings temperatuur 25°C het aanleiding gegee tot knolle met 'n hoër aantal spruite, langer spruite en 'n hoër spruit ontwikkelings kapasiteit.

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The referencing style in this thesis for all chapters was written according to the requirements of the South African Journal of Plant and Soil. This thesis represents a compilation of manuscripts where each chapter stands as an individual unit. Repetition between chapters that may occur was thus unavoidable.

Chapter 1

Introduction and literature review

Introduction

Potato (*Solanum tuberosum*) is a worldwide cultivated tuber-bearing plant which is the fourth main food crop in the world after rice (*Oryza sativa*), maize (*Zea mays*) and wheat (*Triticum aestivum*), in terms of both areas cultivated and total production (Douches et al. 1996). The potato does not require special growth conditions and is a major field crop in temperate regions and increasingly in warmer regions (Haverkort 1990). Potatoes are grown as a major crop in countries with very large populations, in different climatological zones including temperate regions, the sub-tropics and tropics, under very different agro-ecological conditions, lowlands as well as highlands, and in very different socio-economic environments (Struik and Wiersema 1999). The potato can be considered as one of the most important food crops in many African countries. The potential of this crop is reflected in the large increase in area of production where Africa showed the highest rate of growth within the developing world over the past twenty years (Hesen 1986). Potato production in the developing world has increased faster in recent years than that of any other major food crop (Hesen 1986). The increase in area planted with potato in the developing world has been facilitated by the development of new cultivars and the funding of seed production schemes utilizing modern multiplication techniques. Besides being the largest vegetatively propagated crop in the world, the potato has become an important staple food in parts of the world where there is a limited (but increasing) purchasing power, an increasing demand for food and an increasing pressure on scarce land (Struik and Wiersema 1999). In most African countries with low income farmers and erratic rainfall, there is a need to produce the highest tonnage of food in the shortest time. Potato is known to produce the highest protein per hectare per day. Nutritional studies also show how healthy potatoes can be in terms of vitamins, minerals, proteins, essential amino acids and carbohydrates (Buckenhüskes 2005, Struik et al. 2006).

Seed potato production is mostly vegetative and based on the use of *in vitro* plantlets or microtubers (Ranalli 1997). The multiplication rate of potatoes is very low compared to other crops, from between four to six times under optimal conditions. For this reason, a large portion of crop area is devoted to the production of seed tubers and it takes a considerable time to build up a sufficient amount of commercial tubers. With every field multiplication the build-up and transfer of pathogens can increase, leading to seed degeneration. Therefore it is essential to investigate methods of increasing the number of minitubers (G0) produced from disease free *in-vitro* plantlets.

Agronomists are faced with the high water requirements and low fertilizer use efficiencies of this crop (Struik et al. 2006) although many studies have shown the high use efficiency of other resources (e.g. land and energy) (Linnemann et al. 1999). New techniques must be promoted in seed production due to the increasing role the potato is playing as a major food crop and the potential which still exist for its exploitation.

Aeroponic techniques optimise root aeration which is the major factor leading to a yield increase of many crops compared to classical hydroponic systems (Soffer and Burger, 1988) and consequently has the potential to drastically reduce the number of field generations for the production of potato seed. Other advantages such as solution recirculation and good monitoring of nutrients and pH are also achieved (Farran & Mingo-Caste, 2006). Aeroponics can produce up to 10 times higher yields, more rapidly, and at a lower cost than conventional growing methods. Research done by the International Potato Center (CIP) indicate that by using this system yields of up to 80 tubers per plant can be obtained (Otazu 2007). Harvesting in aeroponics is also convenient, clean, and allows a greater size control by sequential harvesting (Ritter et al., 2001). Lommen and Struik (1992) found that the number and timing of non-destructive harvests were key factors in the optimization of minituber production. The removal of the dominant large tubers, allows initiation of new tubers as well as the development of the already existing tubers, which can be harvested afterwards. Investigating the use of aeroponics for the production of minitubers is warranted since little is known about applying this production system on a commercial scale in South Africa. From the literature it is also evident that the nutrient solutions used will have to be adjusted for cultivars and local growing conditions (Zebarth et al., 2006). One of the main problems minituber producers face is plants that become too vegetative, diverting resources away from the stolons and tubers. In an aeroponics system where the root zone conditions will be optimized for nutrient uptake the nutrient solution may have to be adjusted to reduce the vegetative growth leading to earlier tuberization. Planting density may influence vegetative growth and therefore time to tuber initiation and will differ between seasons as well as between cultivars. These factors will have to be verified in order for this system to be used commercially.

Other cultivation practices that have not been verified for this system include optimal planting densities, harvesting interval, irrigation frequency and optimal tuber size at harvesting for South African cultivars.

1. Potato plant developmental phases

Potato growth is classified into four distinct growth phases (Mikkelsen 2006) and is determined by the environmental and management factors between locations as well as the cultivars. Traditionally identification of potato cultivars depends on key morphological traits such as tuber type, leaf type, growth habit, flower colour and other characteristics (McMorran and Mosley 2003). The four growth phases are:

1.1. Sprouting and plant establishment

According to Smith (1977), morphologically the tuber is a fleshy stem, bearing buds or eyes in the axil of scale-like leaves which soon shed, leaving a ridge or leaf scar subtending the bud. Bud primordia are present even in young tubers but the sprout does not begin to form until the later stages of tuber development. Potato growers in the Andean region in Latin America have traditionally been using naturally occurring compounds to control sprout growth (Oosterhaven 1995). Sprouting strongly responds to the physiological age of the seed tuber, to inter- and intra-plant competition, nitrogen and water supply and air temperature (Struik and Wiersema 1999). Irrigation during stolon formation is crucial to the manipulation of stolon number per stem (Lugt et al. 1964). The size of seed tuber has an effect on number of eyes which is directly related to the physiological status of the tuber, smaller tubers are generally physiologically younger. The number of potential sprouts and stems per seed and the plant growth vigour are thus influenced (Struik and Wiersema 1999). According to Steyn et al. (1998), small tubers such as microtubers or minitubers already bear a surprisingly high number of potentially active eyes, especially at the apical end. There is significant unevenness in sprout emergence depending on whether the meristem is apical or lateral on the tuber, with the apical buds sprouting first and displaying an apical dominance over the lateral buds (Burton 1989). Fluctuating the humidity of the storage atmosphere, mechanical or chemical (carbon, disulphide or GA) damage, can encourage sprouting (Claassens and Vreugdenhill 2000). The number of leaf primordia formed on each stem is determined as well by storage conditions of the seed and conditions after planting (Struik and Wiersema 1999).

At the onset of sprouting, mobilisation of reserves, which consist mainly of starch and proteins, is of great importance. Potato tubers turn into a source organ before phytohormones can exert their growth control. During this process starch and protein will be degraded into soluble sugars and amino acids providing energy for the developing shoot. Starch breakdown can occur via phosphorolytic and hydrolytic reactions (Biemelt et al. 2000). High temperatures during growth also resulted in more sprouts per tuber after dormancy had ended for certain cultivars. According to Kempen (2012) for optimal tuber formation and high yields a productive canopy is required. If crop growth rate is assumed to be proportional to the rate of photosynthesis and thus net assimilation, then

maximum radiation interception is needed for as much of the growing season as possible. A positive correlation between leaf area and tuber number has been established (Kahn et al. 1983).

1.2. Tuber initiation

The potato crop is now also produced in areas where it is exposed to stress conditions that can lead to the failure of the crop or reduce its yield (Jefferies and Mackerron 1987). According to Kempen (2012), there are many factors that affect tuber formation, even the bacteria living in the root zone are reported to have an influence, however, nitrogen levels, temperature and light have the greatest effect. According to Celis-Gamboa et al. (2003), under Dutch conditions, stolon formation occurred by 29 DAP (days after planting) and stolon tip swelling started from 29 to 36 DAP for seven cultivars of different maturity. This growth stage is very short, lasting only 10-15 days in cases where tubers form at the end of stolon tips (Cowan 1986). Tubers with the highest weight are usually produced by the lowest stolons on the mother tubers (Clark 1921). The optimal temperature for initiation is 20°C (Borah and Milthorpe 1962). At temperatures lower than 15°C, tuberization is delayed by one week and at temperatures higher than 25°C, tuberization is delayed by three weeks (Levy and Veilleux 2007).

During production, tubers are exposed to an environment that has characteristic ratios of water, gas and solid compounds, different from those in the air, and are exposed to soil life that can affect their quality, usually in a negative way (Struik and Wiersema 1999). Vreugdenhil and Struik (1989) found that tuber initiation needs the cessation of stolon growth, which is linked to ethylene synthesis. Delays of tuberization have been observed when the stolon environment did not provide mechanical stress (Lugt et al. 1964). Absolute darkness is necessary for tuber formation, otherwise with a minimum of light, stolon tips develop small bleached leaves and no tuber formation will occur (Ritter et al. 2001).

Tubers can also form on other parts of the plant above ground, habitually from axillary nodes on the stem, and in specific circumstances they can even form from flowers (Ewing and Struik, 1992). Tuberization in potato plants is mostly controlled by environmental and nutritional factors which are known to affect the levels of endogenous growth substances (Melis et al. 1984, Wareing and Jennings 1979). Short days and cool night temperatures promote tuberization whereas long days, high night temperatures, and high nitrogen fertilisation inhibit or delay the process (Menzel 1980, Sattelmacher and Marschner 1978).

1.3. Tuber bulking

The plant itself has stopped growing at this stage and only the tubers grow larger (Tantowijoyo 2006). The duration and rate of this phase varies among cultivars and depends on environmental conditions. Bulking rate is greater under short days and moderate temperatures. These conditions favour dry matter partitioning to the tuber, promote tuber growth and restrict haulm growth (Levy and Veilleux 2007). It is important to note that depending on fertilizer, a large percentage of nitrogen available during tuber bulking is in the form of NH_4^+ when N content is larger than the recommended dose, which is known to replace calcium by cation exchange (Tisdale et al. 1993). As tuber initiation starts and through tuber bulking, the amount of dry matter being allocated to tubers at the expense of the shoot increases (Kooman et al. 1996). According to Steyn (1999), cells in the tuber can increase up to 18 times their normal size due to the accumulation of starch and water. However, cultivars seem to differ in their ability to retain initiated tubers produced earlier in the season because of tuber re-absorption (Walworth and Carling 2002).

Tuber cells expand with the accumulation of water, nutrients and carbohydrates. Tubers become the dominant site for deposition of carbohydrates and mobile inorganic nutrients (Cowan 1986). The majority of plant nutrients are taken up during this stage and nitrogen application at this period via irrigation is crucial (Cowan 1986).

1.4. Tuber maturation

During this phase photosynthesis will decrease, and the vines will eventually die back while the growth rate of the tubers also slow down. The skin will also thicken, protecting the tuber from infections and damage. As the tuber matures the buds become successively dormant, starting from the stolon end (Kempen 2012, Burton 1989). This stage is characterized by yellowing leaves and dropping of stems. According to Tantowijoyo (2006), tuber skins gradually harden due to their increased starch content and it is a cultivar dependent cycle.

During this phase, high temperatures may interfere with the onset of tuber dormancy, shorten the rest period, or even negate the inhibition of tuber buds, resulting in pre-harvest sprouting. The increase of the endogenous content of growth-promoting substances like gibberellins is associated with pre-harvest sprouting (Levy and Veilleux 2007).

2. Aspects influencing growth, tuber initiation and yield

2.1. Temperature

High temperatures are inhibitory for tuberization in both short and long photoperiods. It affects the partitioning of assimilates by decreasing the partitioning to the tubers and increasing the partitioning to other parts of the plant (Ewing and Struik, 1992). Short daylengths and low temperature, especially at night, enhance tuber initiation, increase the number of tubers formed, allow longer periods of photosynthesis, enhance efficient translocation of assimilates from haulm to tubers and lowers respiration rates during the cool nights (Levy and Veilleux 2007). Under controlled environmental conditions seed tuber sprout emergence requires a temperature of at least 6°C with optimum stem development at 18°C (Borah and Milthorpe 1962). But haulm growth needs higher temperatures at around 27°C (Bodlaender et al. 1964). The rate of photosynthesis and respiration is affected by temperature. The respiration rates of potato leaves in the dark roughly doubled for each 10°C increase in temperature (Winkler 1971).

Increases in either day or night temperatures above optimal levels drastically reduce tuber yields, with high night temperatures considered to be more damaging (Gregory 1956). However, response to temperatures is different from one cultivar to another (Went 1959, Levy and Veilleux 2007). Davis et al. (1971) found that certain *Solanum* species are well adapted to higher temperatures.

2.2. Photoperiod and light intensity

The potato originated in the Andes of South America (Palta 1996) where it was cultivated at an altitude of between 2000 and 4000 m above sea level in a region characterised by short daylengths, high light intensity, cool temperatures and relatively high humidity (Levy and Veilleux 2007). In order to achieve high tuber yields in parts of the world where there are no such climatic conditions, Levy and Veilleux (2007) advised for the selection against the obligatory demand of short photoperiod for tuber initiation. However, short days generally favour tuberization instead of stolon growth (Ewing and Struik 1992) and under long days, vegetative growth will be favoured but tubers will eventually be formed three to five weeks later than plants growing under short days (Chapman 1958). According to Lorenzen and Ewing (1992), short days favour the photosynthetic rate per unit leaf dry weight increase, more starch accumulates in the leaves during the day and assimilate export from leaves increases.

Solar radiation during crop growth influences nutrient availability and the plants ability to take up and utilise the nutrients, influencing the final yields (Arkin and Taylor 1981). Light intensity has a direct effect on photosynthesis which in turn influences a plant's demand for nutrients (Fageria 1992). Wheeler et al. (1990) found in their trial that shoot growth progressed rapidly under continuous light for the first 28 days but periodic inspections of the stolons showed that only a few tubers had initiated on either cultivars

during this time. Within two weeks after switching to a 12-h photoperiod at 28-days-age, tuber initiation was progressing rapidly for both cultivars. Levy and Veilleux (2007) discovered that at low light intensity in the winter season, maximum tuber weight was obtained between 12 and 14°C but with high light intensity in summer, maximum tuber weight was obtained at 18 to 20°C. Hayden et al. (2004) found that roots of plants growing on the west side of the glasshouse, which received more light, were significantly larger than those growing on the east side of the glasshouse, which was partially shaded.

3. Factors influencing nutrition

3.1. Nutrient uptake

Plants that are efficient in the absorption and utilisation of nutrients greatly enhance the use efficiency of applied fertilisers. Estimates of overall efficiency of applied fertiliser use have been reported to be in the near or lower than 50% for nitrogen, less than 10% for phosphate and about 40% for potassium (Baligar et al., 2001). Blair (1993) defined a plant to be nutrient efficient when it has the ability to acquire nutrients from a growth medium and /or to incorporate or utilise the nutrient in the production of shoot and root biomass or useable plant material (seed, grain, fruits, forage). Baligar et al. (2001) pointed out that the genetic, physiological and morphological components of plants have a tremendous effect on their ability to absorb and utilise nutrients under various environmental conditions.

Optimal nutrient use efficiency can be achieved by incorporating best external management practices. According to several researchers such as Baligar and Duncan (1990), plants interact with environmental factors such as rainfall, solar radiation and temperature and respond to diseases and insects and root microbes which have a great influence on the nutrient uptake capacity of plants. Gerloff and Gabelman (1983) report that genetic variability explains the differences in nutrient use efficiency and the parameter of nutrient uptake. Many studies with potatoes have shown that yields of twice that of conventionally produced potatoes can be obtained in controlled environments, provided the photosynthetically active radiation, CO₂, temperature, and nutrients are maintained near optimal levels (Wheeler and Tibbitts 1987).

Underground morphological factors such as length, thickness, surface area and volume of roots have important effects on the ability of the plant to obtain and absorb nutrients in the soil (Barber 1995). Plant uptake and efficient utilisation of nutrient elements are governed by different physiological mechanisms and their response to deficiency, tolerance, toxicity and climatic variables (Gerloff 1987). Clark and Duncan (1991) indicate that the identification and use of cultivars with greater tolerance to suboptimal nutrient levels offer considerable possibilities for increasing the potential crop

production. Genetic variation among potato species controls the calcium uptake and accumulation thereof (Bamberg et al. 1993).

As with any crop, optimising nutrient utilisation will not only improve the economic performance, but will also minimise any environmental impact. Several sources (Stefańska et al. 2003) acknowledge that macroelements perform important building functions, are an integral part of enzymes and play an important role as regulators of metabolic processes.

3.2. Essential macroelements

a) Nitrogen

Nitrogen (N) is the most difficult nutrient to recommend and control, and is the most critical for potato quality (Cowan 1986). Smith (1977) found that qualities of the potato most likely to be affected are the specific gravity or dry matter content which is related to the texture of the cooked or processed potato, maturity and sugar accumulation. N is necessary for protein synthesis and production (Whitney 1975) however, the period of highest N demand varies by potato variety and is related to cultivar characteristics such as root density and time to maturity (Mikkelsen 2006).

With strict environmental legislation being implemented, controlling N fertilisation is a precision science. Supplying the crop with the optimum amount of nutrients at the correct time and the method of application is critical for optimum crop growth with a minimal environmental effect. N is an essential component of chlorophyll, cell walls, amino acids and enzymes. Crops therefore respond more strongly to N-fertilisation in both quality and yield, in comparison to other nutrients (Skoglund and Smit 1994). Ammonium assimilation takes place mainly in the root and stem bases whereas nitrate assimilation takes place in shoot and roots, most actively in the shoot. Roots of plants grown in nitrate N have a higher N content than those that grew in ammonium N (Smith 1977). Marguerite et al. (2006) revealed that tuber yield per unit area is increased with increasing N fertiliser up to the recommended level of between 112.1 and 224.7 kg/ha of $\text{NO}_3\text{-N}$. With increasing N application the number of stolons and tubers increase as well leading to a better final yield (Zabihi-e-Mahmoodabad et al. 2010). This may be attributed to vegetative increase of the aerial parts and thus transferring photosynthetic compounds into tubers.

Over application of N may lead to excessive vegetative growth and heavy canopy development, delaying maturity and developing a microclimate conducive to aerial stem rot, sclerotinia and higher insect numbers (Cowan 1986). There is an inverse relationship between haulm growth and tuber growth, if haulm growth is encouraged by the application of early fertilisers, particularly by N, tuber initiation is delayed (Moorby 1978).

The form in which N is present and absorbed from the rhizosphere can have an effect on the pH and in this way affect the uptake and availability of other nutrient elements. According to Chang et al. (2011) and Marschner (1995), the presence of N in the root cells in the ammonium form indicates that potato plants are characterised by high cation/anion uptake ratio resulting in an efflux of H^+ from root, acidifying the rhizosphere. Wilkinson et al. (1999) reported that ammonium N can increase phosphorus uptake in plants by increasing root growth and the ability to absorb and then translocate the phosphorus by increasing the solubility of phosphorus through decreasing the pH in the media as a result of the absorption of NH_4^+ . Micronutrient interaction with N occurs due to change in pH in the rhizosphere, associated with the N forms used (Fageria 2001). When N is applied predominantly as NO_3^- the anion uptake will dominate resulting in the soil pH increasing which will limit the uptake of most micronutrients. Application of N as NH_4^+ will result in cation absorption dominating which may decrease the soil pH and increase the uptake of some micronutrients.

N is mobile in the growing plant, therefore deficiencies manifest in the older leaves first. Other interactions of N with micronutrients and macronutrients occur when N stimulated growth causes increased demand for a nutrient resulting in a possible deficiency if these nutrients cannot be taken up readily enough (Fageria 2001).

b) Calcium

Calcium (Ca) is an important plant nutrient with many functions, such as strengthening of cell walls, maintaining membrane stability and cell integrity (de Villiers 2007). Without adequate Ca supplies to the plant, most crops will produce lower yields, and the quality of storage organs or fruits may be of a poor quality and more susceptible to diseases and mechanical damage (Marschner 1986). When it reaches the root system, Ca is taken up and released into the xylem (Marschner 1995), then translocated to the rest of the plant along with water by a series of cation exchange reactions (Bell and Biddiph 1963). Ca is required in stolon tips for tuber initiation (Balamani et al. 1986). Previous studies show that Ca in potato tubers is taken up by functional roots present on the tuber and stolons (Kratzke and Palta 1985). Sousa et al. (1992) reported that downward movement of Ca in soil has resulted in increased rooting depth and higher uptake rates of N, Ca, Cu, P and Mn by maize (*Zea mays L*) grown in Cerrado acid Oxisol of Brazil. Studies have reported Ca's role as a secondary messenger controlling the functions of the enzymes and cellular metabolism (Palta 1996). In terms of Ca interactions with other nutrients, Ishizuka and Tanaka (1960) reported that Ca stimulated the absorption of phosphorus and potassium under certain concentration ranges of ions in nutrient solutions.

Considering all the nutrient elements, Ca seems to play the most important role in determining tubers' susceptibility to cell damage associated with internal brown spot and a clear relationship between Ca content of tubers and the prevalence of internal brown

spot is noticeable (Kempen 2012). According to Bangerth (1979), Collier et al. (1980), localised deficiencies in Ca have been associated with medullary and cortical cell death, or tissue necrosis as sometimes named brown centre or internal brown spot. Potato tubers are underground storage organs with low rates of transpiration, because water and Ca move together; they are susceptible to Ca deficiency (Palta 1996). Despite the importance of Ca in the plant, high concentration of this element has an inhibiting effect on the uptake of potassium and magnesium which may relate to the decrease in the permeability of cells (Fageria 1983).

Ca deficiency is characterised by a reduction in growth of meristematic tissues, this deficiency can first be observed in the growing tips and youngest leaves. These organs become deformed and chlorotic, at an advanced stage necrosis occurs at the leaf margins. The affected tissue becomes soft due to dissolution of the cell walls. Brown substances occur which accumulate in intracellular spaces and also in the vascular tissue where they can affect the transport mechanism (Mengel and Kirby 1987, De Villiers 2007). Some studies (Fageria 2001) have shown that magnesium significantly decreases the uptake of Ca when optimum application levels have been exceeded.

3.3. Water quality and irrigation management

In order to assess the suitability of water to be used in soilless systems it is necessary to not only look at the quality in terms of pathogen content but also the type and quantity of salts present. The total salts present will be analysed as the electrical conductivity (EC) which is measured in mS/cm and the feeding water will also be analysed for macro- and micronutrients, including calcium, magnesium, potassium, sulphate nitrate and phosphat, before a nutrient solution can be formulated (DeHayr et al. 1997). Nutrients are not adjusted individually, but they are added in fixed proportion because in general practice, a concentrated mixture of nutrients is added to an aeroponics solution to maintain a certain EC required (Chang et al. 2011).

Fertilisers are salts and when dissolved in water they conduct electricity. Excess salt affects nitrogen uptake by plants and also contributes to reduced permeability of roots, consequently decreasing water and nutrient uptake (Frota and Tucker 1978) resulting in a greater osmotic potential (Ayers and Westcot 1986). Bruns and Caesar (1990) found in their research that potato leaves are most sensitive to salt applied at the beginning of tuber formation. Uptake of chlorine and sodium by leaves may induce toxicity, exhibited as leaf burn along the margin. A change to the chloroplast structure likely affects photosynthesis, causing increased starch levels in leaves, suppression of nitrate reductase activity and reduced growth and dry matter production in tuber (Ghosh et al. 2001).

Baligar et al. (2001) reported that either with the downward translocation of photosynthates and hormones are probably the driving forces of overall nutrient uptake

and utilisation efficiency. Under heat stress, leaf transpiration increases further, thus reducing water transport to tubers (Björn and Palta 2006). Water management prior to emergence is critical for maximum production because excessive moisture may favour blackleg or promote verticillium (Cowan 1986). Water deficiency is a yield-limiting factor (Harris 1978), in areas subjected to harsh conditions such as semi-arid and arid regions, where potato is exposed to high day and night temperatures combined with comparatively dry atmosphere, irrigation is crucial for successful crop production (Levy and Veilleux 2007).

4. Tuberization enhancement

4.1. Tuber induction

According to Jackson (1999), under non-inductive conditions such as long days or high temperatures, stolons may grow upward and form new shoots above the ground. A change in environmental conditions and a decreased supply of nitrogen, slows the vegetative growth followed by an important tuber induction period of three weeks during which both the tuberisation percentage and the number of tubers per plant will increase (Farran and Mingo-Castel, 2006).

The optimum temperature for foliage growth and net photosynthesis is 20 and 25°C, respectively. Low mean temperatures (15-19°C) and short photoperiods (12h) are favourable for tuberization and early tuber growth (Vandam et al. 1996). Under such conditions a transmissible signal is activated that triggers cell division and elongation in the sub-apical region of the stolon to produce tuber initials (Xu et al. 1998). High temperatures inhibit tuberization in both short and long day conditions, especially under long photoperiods (Jackson 1999), simultaneously foliage growth is promoted, rate of photosynthesis declines rapidly, assimilate partitioning to tubers is reduced and dark respiration increases (Thornton et al. 1996). Plant growth rate is strongly related to net photosynthesis and dark respiration (Leach et al. 1982). Levy (1992) found that at above 29°C, tuber growth is completely inhibited because at this point the carbohydrates consumed by respiration exceeds those produced by photosynthesis.

Under short day conditions gibberellin biosynthesis is reduced (Amador et al., 2001). Potato plants grown under non-inductive conditions are characterised by high levels of endogenous gibberellins (Vreugdenhil and Sergeeva 1999) that promote shoot growth and delay or inhibit tuberisation (Vandam et al. 1996). In addition, the accumulation of gibberellin in tuber tissue can specifically impede starch accumulation (Vreugdenhil and Sergeeva 1999).

4.2. The tuberization signal

Growth of buds on the main underground stem into rhizomes or stolons rather than into leaf-bearing branches depends largely on certain environmental conditions, especially the exclusion of light (Smith 1977). If lower buds are exposed to light early, green shoots will generally develop instead of stolons. Transfer of the chemical signal is shown to occur through the xylem (Jackson et al. 1978).

Tuberization in cultivated potato is strongly influenced by photoperiod (Ewing and Struik 1992) because short days are required to initiate a series of adaptive and communicative events that result in tuber formation (Rodríguez-Falcón et al. 2006). The requirement for short days is so strong (long nights compared to days) that a 5-min interruption of the dark period with red light inhibits tuberization (Batutis and Ewing 1982), indicating the involvement of phytochrome (Jackson et al. 1996). The tuberization signal is graft-transmissible from the scion to the rootstock and not vice versa (Chapman 1958). Under noninducing long day conditions, GA₂₀ is not metabolized to GA₁ in the leaves but is transported to the stolon, where it is converted to GA₁ and inhibits tuber formation. Under short day conditions, conversion of GA₂₀ to GA₁ increases in the leaves and as result, less GA₂₀ is available for basipetal transport and conversion to active GA₁ in the stolon. The levels of inhibitory GA₁ in the stolon are thus reduced and tuber formation is initiated (Prat 2004).

4.3. Plant growth regulators

Plant growth regulators (PGRs) play important roles in plant growth and development. Changes in the presence, balance and distribution of PGRs communicate developmental, stress-related or environmental cues that alter growth. Short-distance communication involves changes in biosynthesis or metabolite conversion, whereas longer-distance communication may also require export and translocation of PGRs, their precursors or metabolites (Malladi and Jacqueline 2007). The shoot apex exerts a central coordinating influence on plant growth and development, the apical meristem contained within the shoot apex provides a source of basipetally moving auxin that inhibits the lateral bud outgrowth (Bangerth 1994). As long as the dominant apical meristem remains intact, auxin will be transported down the stem through xylem parenchyma (Leyser 2005). Studies of plant hormones in the tuberization process have been conducted to evaluate the nature of the tuberization stimulus, for example, tuberization of the stolons is promoted by cytokinins (Hussey and Stacey 1984, Mauk and Langille 1978, Palmer and Smith 1969) and inhibited by GA₃ (Hussey and Stacey 1984, Wareing and Jennings 1979). Ethylene inhibited tuberization (Mingo-Castel et al. 1976). Certain gibberellins (Ga's) have been shown to inhibit tuber formation and may mediate the photoperiod- and temperature- dependent tuberization responses (Jackson 1999).

5. Seed potato multiplication

5.1. Production systems

Potato plants can be grown for multiplication in the field, in green- or screenhouses and under artificial conditions in an *in vitro* or hydro- or aeroponics facility. According to Struik and Wiersema (1999), good quality seed tubers must be able to produce healthy, vigorous plants that produce a high yield of good quality within the time limits set by the growing season in which the seed is going to be used and given the socio-economic and agronomic environment in which the seed will be planted. Difficulties in obtaining healthy seed are a constraint on production throughout almost all countries (Jefferies and Mackerron 1987). Seed quality affects the number of plants and stems per unit area, the type of stems formed, the vigour of plants and stems, the length of the growth cycle, the balance between haulm and tuber growth and the number and growth rate of progeny tubers (Struik and Wiersema 1999, De Villiers 2007). Minitubers are usually defined as the 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 (laboratory) conditions on artificial media (Struik 2008). The minitubers offer a lot of advantages to storage, transport and mechanization due to their little size and reduced weight. They can be planted directly in the soil and they can be produced in any period of the year. They have similar morphology and biochemical features as traditional tubers (Nistor et al. 2010) and show a larger vigour than either microtubers or *in vitro* plantlets (Struik 2008).

5.2. Aeroponics in seed potato production

Aeroponic culture is an alternative method of soil-less culture in growth-controlled environments. In aeroponic culture the 'below-ground' plant parts are suspended in air and intermittently misted with nutrient solution. In hydroponic culture the transplants are grown in static nutrient solution whereas the nutrient flows along the lower roots in nutrient film culture (Bradshaw 2010). According to the International Potato Center (2010), aeroponics is a technology which involves growing the seed tubers in mid-air, with much higher multiplication rates than conventional methods. The advantages of container production systems over ground production systems are, greater water and nutrient efficiencies, less water consumption with an increased nutrient availability in recirculated systems and better cropping with higher salinity levels than soil grown plants (Mobini et al. 2009).

Aeroponics effectively exploits the vertical space of the greenhouse and air-humidity balance to optimize the development of roots, tubers, and foliage. Aeroponic systems allow the producer to precisely control root zone nutrient and water regimes and environmental conditions as well have a complete access to the roots throughout the life of the crop (Hayden et al. 2004).

According to the International Potato Centre (2010), most growers in developing countries do not use quality seed, because of high costs and lack of access. As a result, there is a high need for cost-effective methods to produce quality seed that can be accessed by smaller farmers at affordable cost. Aeroponic techniques optimise root aeration which is certainly the major factor leading to a yield increase compared to classical hydroponic systems (Soffer and Burger 1988) because of the ability to exclude toxic ions and to tolerate high osmotic pressure (Raviv et al. 2008). Cho et al. (1996) observed a growth and yield increase in cherry tomatoes aeroponics compared to classical hydroponic production. It shows other advantages such as solution recirculation, a limited amount of water used and good monitoring of nutrients and pH (Farran and Mingo-Caste 2006). This method can produce up to 10-times higher yields, more rapidly, and at a lower cost compared to conventional growing methods (International Potato Centre 2010). Studies have indicated that nutrient film techniques and aeroponics have an important role in commercial applications in the potato industry for certified seed production (Boersig and Wagner 1988). The danger of hydroponics is the appearance of gravitropism and hydrotropism which frequently leads to accumulation of root masses at the bottom, which may lead to oxygen deficiency due to the respiration of the dense root mass and existence of a water layer at the bottom of the container (Bhattarai et al. 2006). Mobini et al. (2009) found that by applying supplemental aeration increased the growth parameters such as leaf area index, root: shoot ratio, tuber yield, dry matter (up to 178%) and minituber production (up to 74%) significantly. When the oxygen in the root zone is in a favourable concentration, larger tubers will be produced, so potato plants will have more efficient and longer roots with a higher number of stolons and can produce more and larger tubers (Ritter et al. 2001).

Harvesting in aeroponics is convenient, clean, and allows a greater size control by sequential harvesting (Ritter et al. 2001). Lommen and Struik (1992) found that the number and timing of non-destructive harvests were key factors in the optimization of minituber production. The removal of the dominant large tubers allows initiation of new tuber formation as well as the development of the already existing ones, which can be harvested afterwards. The higher biomass yield of aerial parts from the aeroponic treatment indicate that this technique actually should not be limited to root crops, but should be considered for other types of crops as well (Hayden et al. 2004).

Power failure to pumps can produce irreversible damage and are thus the biggest risk to production. Despite this problem, this technique has been applied successfully for the production of different horticultural and ornamental species (Biddinger et al. 1998, He and Lee 1998, Molitor et al. 1999).

6. Potato seed quality

6.1. Size of seed

In general, total tuber yield is reduced by water stress at almost any stage during the growing season of a potato crop (Mould and Rutherford 1980, Steyn et al. 1998), but especially during the tuber bulking phase (Miller and Martin 1987, Ojala et al. 1990, Steyn et al. 1998). Apart from lower total tuber yield, water stress may also adversely affect the tuber-size distribution. The size of tubers affect the duration of the dormancy, the vigour of the seed tuber, the number of stems that can be successfully produced, the rate of emergence, the number of surviving plants and stems, the vigour of the individual stem and its yielding ability (Struik 2008).

According to Ritter et al. (2001) the influence of the cultivation method is highly significant for all parameters when compared to total production between hydroponic and aeroponic systems. The tuber yield per plant in the aeroponic system is 70% higher and tuber number is also 2.5 fold higher. The average tuber weight 33% less than in hydroponic system. The tuber size distribution must be manipulated in such a way that a maximum proportion of tubers are in the most desired size class, these elements are mutually interacting and dependent on seed handling, the prevailing ecological conditions and on the husbandry (Struik and Wiersema 1999). The selection of high-quality seed is essential for the production of a profitable potato crop (Bohl et al. 1992). Potato plants produced by meristem-tip culture or micro propagation are generally uniform in nature and rare variants are usually attributed to spontaneous mutations (McMorran and Mosley 2003). Larger tubers generally have higher rates of bruising as a result of the greater weight because more energy is absorbed at the point of impact as compared to smaller tubers which have a lower specific gravity (Brook 1996).

6.2. Dormancy and physiological age

According to Hemberg (1985), freshly harvested potato tubers are under a dormancy period, a physiological state which is characterised by the lack of visible growth. The duration of the dormancy period which varies between different cultivars is influenced by numerous factors such as temperature and photoperiod during growth and storage conditions. Dormancy and commencement of sprouting has been proposed to be under phytohormonal control by a gradual shift of the ratio between sprouting promoters and inhibitors. Burton (1989) suggested that potato tuber dormancy starts at tuber initiation. Seasonal stress usually manifests itself in storage by reducing the dormancy period, both heat and water stresses are seen symptomatically in storage as earlier sprout development (Kleinkopf et al. 2002). Similar symptoms in storage may be caused by frost or chilling injury and thus shortening the dormancy period (Kleinkopf et al. 2002).

Gibberellins and cytokinins are assumed to be involved in the termination of dormancy (Turnbull and Hanke 1985). Burton et al. (1992) demonstrated a direct decrease of

abscisic acid and an increase of gibberellin concentrations in tubers toward the end of the dormancy period with the start of sprout growth. Sprout growth is largely delayed under low temperature (Suttle 1995). Some research evidence suggest a prominent role of abscisic acid in controlling the length of the dormancy period of potato tubers, other reports do not agree with this view; Sorce et al. (1996) described an unexpected increase of abscisic acid in the eyes and sub-eye regions as sprouting approached. Moreover, after quantitative trait focus analysis, Simko et al. (1997) found an association between high abscisic acid content and long tuber dormancy. Potatoes cultivated under short-day conditions had a shorter dormancy period than those cultivated under long-day conditions and also the tuber weight had a significant impact in the duration of dormancy (Classsens and Vreugdenhill 2000).

Levy et al. (1993) found a correlation between the physiological age of seed with the response to salinity, plants developing from physiologically young or physiologically old seed tubers are more susceptible to salinity than those developing from seed tubers at the proper physiological age. According to Levy and Veilleux (2007), these differences could be due to healthy and more vigorous plant growth from seed tubers of the proper physiological age compared to very slow growth resulting from plants emerging from young seeds and fragile plants developing from old seed tubers.

6.3. Physiological disorders

Besides varietal differences, air and soil temperatures, soil type, soil moisture content and fertilisation are additionally associated with physiological disorders (Kempen 2012). Factors that influence the incidence and severity of physiological disorders include cold tuber temperatures and flaccidity during harvest or handling (Thornton and Timm 1989). The most important physiological disorders affecting potato tuber quality are hollow heart and growth cracks (Struik and Wiersema 1999). Tuber maturity at harvest may influence bruise rates with immature tubers presenting less susceptibility (Pavek et al. 1985). Short periods of high temperature stress were reported to cause tuber cracking (Lugt et al. 1964).

During blackspot bruising, the impact disrupts cells under the periderm allowing phenols to come into contact with polyphenoloxidase to ultimately produce the dark pigment melanin (Corsini et al. 1992). By increasing tuber calcium, Ozgen et al. (2006) in their experiment found a reduction in the internal defects such as internal brown spot and hollow heart. The presence of these disorders can lead to a reduction in the commercial value of the crop as growers are penalized depending on percentage of the tissue and tubers that are affected (Ozgen et al. 2006). Application of gypsum appears to increase calcium availability and improve the grade (Simmons et al. 1988). In another study by Simmons and Kelling (1987), a combination of calcium nitrate and gypsum was found to be more effective than gypsum alone.

High temperatures may cause various tuber disorders including irregular shape, chain tuberization or secondary tuber formation which is associated with excessive stolon elongation and branching as well as sprouted tubers (Bodlaender et al. 1964). High concentrations of specific ions may also cause physiological disorders in plant tissue due to salinity (Evers et al. 1998).

7. Factors influencing potato tuber quality multiplications

7.1. Storage conditions

Due to non-continuous crop production in developing countries coupled to a continuous demand, potato storage is necessary to fill the out-of-harvest periods. Most of the stored crop is used specifically in the seed industry although a small proportion is used for table consumption (Buckley et al. 2006).

The potato is a semi-perishable commodity (Rama and Narasimham 1991). According to Eltawil et al. (2006), the quality and storage life of this product is reduced by the loss of moisture, decay and physiological breakdown. These deteriorations are directly related to storage temperature, relative humidity, air circulation and gas composition. For these reasons, appropriate and effective post-harvest technology is critical to the entire production to consumption chain because of its bulkiness and perishability (Rama and Narasimham 1991). Workman and Holm (1984) discovered that machine harvest, handling, and storage of potato tubers can cause pressure due to impact injury resulting in tuber blackspot bruise and drastically reduce crop value by promoting premature aging. Elevated levels of respiration led to a loss in processing quality (Pisarczyk 1982), higher susceptibility to soft rot and led to decreased seed piece performance (Bartz and Kelman 1986).

The objective of storage is to maintain tubers in their most conservative condition and to supply a constant flow of tubers to the commercial potato growers throughout the year (Eltawil et al. 2006). The storability of potatoes as elucidated by Burton et al. (1992) is already determined before the beginning of storage, by factors such as cultivar, growing methods, weather conditions during the growth season, maturity of tubers at harvest and damages caused during lifting, transport and storage operations. Storage losses are occasioned by processes like respiration, water evaporation from tubers, sprouting, spread of diseases, changes in the chemical composition and physical properties of the tuber and damage caused by extreme temperatures (Eltawil et al. 2006). Björn et al. (2006) found that most blackspot bruising occurred in the cortical area, whereas deeper pressure blackspot bruising would more typically occur in the medullary tissue as a result of pressure exerted within potato storage piles.

In order to achieve optimum potato tuber storage, Mobini et al. (2009) advised to split it into different stages: (a) Equalisation or drying phase which consists of drying tuber

surface moisture with a ventilation fan running continuously to equalize average pile temperature within 2°C of average pulp temperature; (b) Wound Healing, pre-conditioning phase: 10 to 20°C at 85 to 95% relative humidity for 15 to 30 days. No water condensation and temperatures above 25°C are allowed during this phase; (c) Cooling phase where temperature is brought down to 7 to 10°C at about 0.5 to 1°C per day; (d) Holding phase: holding period requires low temperatures of 7°C, 4°C or 3°C for processing, fresh market or seed potatoes, respectively. Maintain 95 percent relative humidity. Intermittent ventilation only to control CO₂ built up and retains O₂ levels. Maintain potatoes at various locations within 1°C pulp temperature of one another; (e) Reconditioning phase: warming up of potato from holding temperature to preferably within 5°C of handling temperatures to avoid condensation and handling damage.

Objective

The objective of this work is to determine the effectiveness of aeroponics for the production of minitubers on a commercial scale and to investigate the effect of cultural practices on the yield and quality of tubers produced using this system. The generated data will facilitate the diffusion of aeroponics for quality seed potato production, increase access and lower production costs.

More specifically the effect of different calcium application levels will be evaluated to determine the optimal rate and timing of application for maximum yield and tuber quality. The results of these trials will also indicate if there are any yield differences between different planting densities as well as the optimal harvesting intervals and irrigation frequencies.

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CHAPTER 2

The effect of harvest interval and plant density on plant growth, tuber size distribution and yield of potatoes (*Solanum tuberosum* L.) in aeroponics

Abstract

One of the main constraints in the cultivation of the potato is the cost of producing seed tubers since this can account for between 30 to 50% of the total production expenses. There is a number of potato propagation procedures that are currently being used worldwide to multiply seed potatoes; conventional seed potato production, micro propagation, hydroponics and aeroponics. As aeroponics is still a relatively new technique in South Africa that has not been researched extensively for the production of minitubers, an experiment was done to assess the effects of different harvesting intervals and planting densities on the final yield and quality of potato minitubers. Potato plantlets, cv BP1, were grown aeroponically at two different densities (20 and 30 plants/m²) and four harvest intervals (7, 10, 14 and 18 days). Plants demonstrated a rapid vegetative development at the beginning when supplied with a nutrient solution at an EC of 1.5 mS.cm⁻¹. The interaction between harvesting intervals and plant densities did not influence plant morphological development, minituber quality or yield. Best yield in terms of tuber number were realised when harvesting every 7 days with a total tuber number of 299 per m² over the growing period; more than twice that harvested in other treatments. Harvesting interval did not have an effect on average individual tuber fresh weight. Plant density did not have any effect on average and total tuber number, neither on the average and total minituber fresh weight. At harvest, minitubers were graded into three classes: small (<20 g), medium (20-40 g) and large (>40 g) and stored for two months at 3°C, 16°C and 25°C. Large minitubers had longer sprouts with an average of 7.9 mm and a higher percentage of firmness. The higher storage temperature of 25°C resulted in tubers with a higher number of sprouts (5.08 sprouts per tuber), longer sprouts of about 11.03 mm and with a sprouting capacity of 61.16%. Harvest interval also influenced the phosphorus and copper concentration in minitubers, both being higher when harvested every 10 days. Size did not influence macro and trace content of the minitubers. Further research can be focused on further evaluating the efficacy of harvest intervals, plant densities and storage temperatures on more cultivars and postharvest potato seed quality.

Key words: potato, aeroponics, harvest interval, plant density, minitubers

Introduction

The potato is widely cultivated and consumed by most cultures across the world. The production in countries like South Africa has been growing due to increasing demand for food which has led to an increased area of production (FAO 2008). One of the main constraints in the cultivation of the potato is, however, the cost of producing seed tubers since this can account for between 30 to 50% of the total production expenses, depending on the country or region. A further limitation is the long asexual propagation cycle during which infection by viruses or bacteria can give rise to degenerative diseases (Corrêa et al. 2009).

There is a number of potato propagation procedures that are currently being used worldwide to multiply seed potatoes; conventional seed potato production, micro propagation, hydroponics and aeroponics. The uses of biotechnological techniques such as tissue culture and hydroponics have resulted in increased yields (Corrêa et al. 2009). Most farmers in developing countries, however, are not utilizing the more advanced methods of seed production due to a lack of resources (Chiipanthenga et al. 2012). Aeroponics is one of the most effective techniques because of its numerous advantages (Otazu, 2008) although it is still not used extensively. Over and above being safe and ecologically friendly, this system produces healthy plants and tubers (CIP, 2008) and the system also has the ability to conserve water and energy. The nutrient solution is recirculated in the aeroponics system, hence a limited amount of water and fertilisers is used (Farran et al. 2006). It provides the precise plant nutrient requirement for the crop and minimizes the risk of excessive fertiliser residues moving into the subterranean water table (Nichols 2005). The biggest challenge using an extensive system such as aeroponics is, however, the increased production cost. Management decisions become increasingly important due to the selection of the most profitable crops and cropping systems (Hagin and Tucker 1982). Research around the world is actively trying to improve the vigour and quality of seed potato tubers and consequently maximising production efficiency and increasing the crop yield (Corrêa et al. 2009).

Planting density is known to affect both crop morphology and yield. Several studies have shown that plant density is an important factor affecting the entire production process of a plant. The importance of the leaf area index size in translocation of assimilates has been acknowledged in different crops such as potato (Kempen 2007), tomato (Scholberg et al. 2000) and cucumber (Tanemura et al. 2008). Mojiri and Arzani, (2003) described that increasing plant population had an incremental effect on plant height and negatively affects stem girth and head diameter in sunflower. Maize differs in its responses to plant density (Luque et al. 2006) with stand density affecting plant architecture, altering growth and developmental patterns and influencing carbohydrate production. Many modern maize varieties at low densities do not tiller efficiently and quite often produce only one ear per plant whereas, the use of high plant populations increases interplant competition for light, water and nutrient which ultimately decreases the number of ears (Sangoi 2001). In soybean (Torigoe et al. 1982) narrow row sowing

and dense planting increases the risk of excessive growth which results in lodging due to the competition from the early stage. With a wide plant density (Cooper 1977), the competition decreases, resulting in rapid leaf area expansion, a higher crop growth rate and higher seed yields due to the development of branches, and an increase in the number of pods and nodes. Farran and Mingo-Castel (2006) observed similar results in the potato crops with a slightly lower number of nodes per plant grown in a higher plant density due to a more elongated type of growth as a consequence of lower light availability. At final harvest, low density plants produced higher yields than the higher densities. For a potato plant, row spacing and plant density vary considerably, depending on the cultivar, environmental conditions and production system and plant populations are often manipulated in order to ensure optimum yields and to reduce yield losses (Basha 2000, Allam et al. 2003). The purpose of this work was to assess the effects of different harvesting intervals and planting densities on the final yield of potato minitubers produced through an aeroponics system.

Materials and methods

Location and crop details

The research was carried out in a research glasshouse and tunnel at Welgevallen, Stellenbosch, the experimental farm of the University of Stellenbosch in the Western Cape of South Africa. Potato (*Solanum tuberosum* L.) *in vitro* plantlets from the Agricultural Research Council (ARC) were used for these studies (Plate B1 in the appendix). The cultivar BP1 was selected for this investigation since this cultivar is well adapted to the Western Cape climate with high minituber productivity. BP1 is a medium maturing cultivar which generally takes 90 to 110 days before maturing with a short dormancy period of about 50 to 70 days (Kempen 2007).

The *in vitro* plantlets were transplanted on Friday, the 21th of September 2012 into polystyrene seedling trays containing a mixture of vermiculite (50%) and perlite (50%) and placed in a temperature controlled glasshouse at 15/20°C night/day temperatures and a relative humidity (RH) of at least 60%. Seedling trays were kept covered with a transparent polyethylene sheet to maintain the high RH. Plantlets were irrigated twice daily with a nutrient solution at an electrical conductivity (EC) of 1.5 mS.cm⁻¹. Plants were transplanted to a naturally ventilated polyethylene covered tunnel on the 25th of October 2012.

Air mists were activated between 11:00 and 15:00 with the aim of reducing the air temperature and keeping the RH above 60%. After 25 days, the top part of the structure was covered up with a 40% aluminet shade net (Knittex Shade Net®) in order to reduce the light intensity and heat in the greenhouse as midday temperatures were approaching 40°C. The aeroponics system was made up of different mini chambers each consisting of a large compartment of 1 m deep, 1.25 m wide and 1.20 m in length, which had two

sided openings for controls and harvesting (Plate B2 in the appendix). The interior was made of black plastic to prevent light from entering the dark compartment and the exterior was covered with an aluminium sheet to prevent any heat build-up inside the chamber. Potato plantlets were fixed through small holes in the top surface. Between one-half and two thirds of the length of the stem was placed inside the dark chamber as suggested by Farran and Mingo-Castel (2006). In order to allow complete darkness, a silver stopper was rolled around the stems. Underground plant parts were periodically sprayed with the nutrient solution using six fog nozzles per chamber.

Plants were fertigated in the aeroponic system with a complete standard Steiner (1984) nutrient solution, differing in concentration at different growing stages (Table 2.1).

Table 2.1 Composition of the standard Steiner solution used for fertigation of potato plantlets in the aeroponic growing system. The different electrical conductivity (EC) of 1.5, 0.75 and 1mS.cm⁻¹ was used at different growing stages.

Macronutrients	Application (g 1000L ⁻¹)			Micronutrients	Application (g 1000L ⁻¹)
	EC of 1.5 mS.cm ⁻¹	EC 0.75 mS.cm ⁻¹	EC of 1 mS.cm ⁻¹		
KNO₃	227.25	113.63	505	Fe-EDTA	86.9
K₂SO₄	195.75	97.88		MnSO₄	18
KH₂PO₄	102	51	68	ZnSO₄	10
Ca(NO₃)₂·2H₂O	675	337.5	100	CuSO₄	1.2
MgSO₄·7H₂O	369	184.5	430.05	H₃BO₄	22
				MoCl₅	1.9

During the first week, fertigation was applied every 20 minutes for 2 minutes from 06:00 to 17:00 during the day and then reduced at night to simply three times; 22:00, 01:00 and 03:00. After 46 days the fertigation was reduced to every 30 minutes for 30 seconds, since by then plants showed a tendency of becoming further vegetative and more resistant to water stress. Night irrigation was also stopped at this time and air sprayers were shut down because plants are less active early morning or late in the afternoon. At 68 days after transplanting the EC was lowered from 1.5 to 0.75 mS.cm⁻¹ and the Ca content of the nutrient solution was reduced from 6.8 to 3.4 meq. This was done in an attempt to limit further vegetative growth and enhance tuber initiation. Barnard and Combrink (2004) reported that a Ca-deficiency can encourage tuber initiation probably by causing damage to the cell walls or membranes in stolon tips. Preventive disease management practices were followed (Table A1 appendix). The EC and pH in the four tanks utilised for this experiment were controlled manually each time when replenished. In order to sustain the large vegetative shoots, strings were attached at each plant from the base of stem and plants trellised.

Data collected

Temperature and relative humidity (measured with a thermohygrometer model HI 9161C, Hanna instruments) were collected inside growth chambers and light intensity (using a comptometer model LP-80 PAR/LAI Ceptometer, AccuPAR) collected in the tunnel throughout the entire growing season at two different times during the day; in the morning before 08:30 (cool period) and at 13:00 (warm period). During the cool period, the mean temperature was 28°C, RH 77.41% and light intensity of 173 $\mu\text{mol.m}^2.\text{s}^{-1}$ and at the warm period, the mean temperature was 34.26°C, RH 73.9% and light intensity of 173 $\mu\text{mol.m}^2.\text{s}^{-1}$ (Figure 2.1). Stolon branching was visually estimated according to the plant and root length.

Table 2.2 Minimum, maximum and average temperature and relative humidity in the greenhouse throughout the growing season

	Temperature (°C)	Relative humidity (RH)
Minimum Reading	7.9	21.9 %
Maximum Reading	42.8	100.0%
Average Reading	24.1	66.5 %
Mean Kinetic Temperature	27.0	

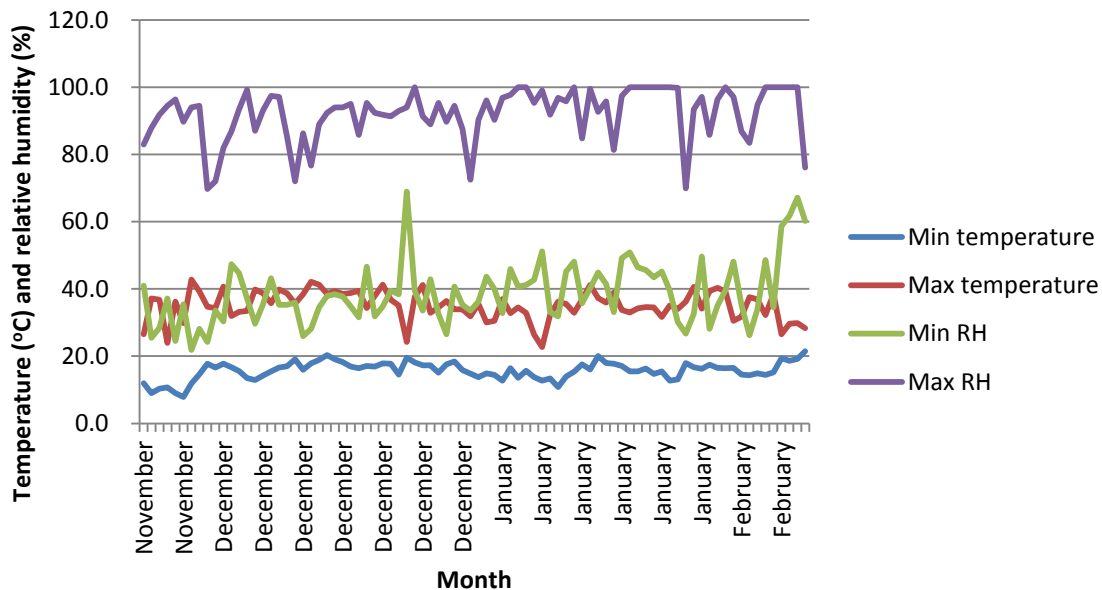


Figure 2.1 Temperature and relative humidity inside the polyethylene covered tunnel where the potato aeroponic system was tested.

At the transplanting of plantlets from the glasshouse into the tunnel, morphological parameters such as stem length, root length, stolon number, and leaf number were recorded.

A total of one hundred and eighteen days was allocated to this experiment. The study was based on two factors, harvest intervals and plant densities. Four harvest intervals of 7, 10, 14 and 18 were used with two plant densities of 20 plants/m² and 30 plants/m². At the end, 11 harvests were recorded when harvested minitubers every 7 days, 7 harvests when harvested every 10 days, 5 harvests when harvested every 14 days and finally only 5 harvests when the harvest interval was 18 days. Measurements were taken immediately after harvest, to prevent decline in accuracy of results that may occur due to water loss.

Minitubers larger than 20 mm in diameter were manually harvested according to each treatment at 7, 10, 14 and 18 days intervals and then grouped into three different size classes: <20 g, 20-40 g and >40 g. Total tuber number and fresh mass were determined per plant. The total fresh mass was calculated per plant as the sum of the shoot and tuber fresh mass. After the final harvest plants were measured (stem and root length) then separated into leaves and stem, tubers, stolons and roots. These plant parts were oven dried at 85°C for a week before determining their dry mass. Percentage tuberized and percentage stolon branching were also taken into consideration.

After a period of 4 months in a complete dark and cold (< 5°C) storage room, three tubers from each of the three size classes (<20 g, 20-40 g and >40g) were selected per treatment and placed at three different temperature regimes (3, 16 and 18°C) for two months to determine the sprouting capacity. Sprouting capacity was determined by the percentage of sprouts coverage on the tuber surface. Sprouts were manually counted. Each treatment had three replicates. A sprout of 2 mm indicated the end of dormancy. Firmness of the potato minitubers was measured with a densimeter. In order to determine mineral compositions, four tubers per treatment were washed and cut in pieces, dried at 80°C for 72 hours and then analyzed. For macro and trace element determinations, skin and medullary tissue samples were taken and processed following to the procedure described by Kratzke and Palta (1986) and Soltanpour et al. (1996). Samples were dried in an oven (49°C) for 5 days, grounded to pass a 40-mesh (0.635mm) screen, weighed, ached at 450°C for 8 hours, dissolved in 2 N HCl, and diluted with a lanthanum chloride (LaCl₃·xH₂O) solution and distilled, deionized water to obtain samples in 0.2 HCl and in Lanthanum (La) at 2000 µg/mL. Macro and trace element concentration were determined by atomic absorption spectrophotometry as described by Karlsson and Palta (2006).

Treatments and experimental design

The experiment used a factorial design, completely randomized in 24 growth chambers, where plant density was the first factor and harvesting interval the second. Plant densities of 20 and 30 plants.m⁻² were used and harvesting intervals of 7, 10, 14 and 18 days were evaluated. Data were analyzed using ANOVA, and means comparison ($P < 0.05$) using the general linear model of statistical software *statistica 11* (Statistica 2011) and *SAS Enterprise Guide 5.1*

Results and discussion

Effects of harvesting interval and plant density on vegetative plant growth.

The first flowers appeared thirty-three days after plants were transplanted into the aeroponic system. Flowering continued for the duration of the four month trial period for 50% of the plants. This could be explained by the extension of the growing season as plants were continually initiating new stems. In another greenhouse aeroponic study, the plants lasted even up to seven months before complete senescence though the cultivar was a medium-late maturing 'Nagore' (Ritter et al. 2001).



Plate 2.1 Potato plants at flowering in the aeroponic growing system.

There were no interactions between harvest intervals and plant densities on any of the measured parameters. In agreement with findings by Farran and Mingo-Castel (2006), there were no significant differences observed, between stem lengths root length, stolon branching and stolon number with different harvest intervals (Table 2.3). The harvesting interval also had no significant effect on the leaf, stem, stolon and root dry mass (Table 2.3). This might be due the fact that similar nutrient solutions and irrigation frequencies were supplied for all treatments and the fact that a single cultivar was used.

Table 2.3 Responses of different harvesting intervals on the growth of potato plants.

<i>Harvest interval</i>	Stem length (cm)	Root length (cm)	Stolon number	Percentage stolon branching (%)
1 (7 days)	174.11a	122.39a	52.66a	3.33a
2 (10 days)	169.70a	120.27a	42.86a	3.94a
3 (14 days)	175.00a	119.44a	40.15a	3.44a
4 (18 days)	184.48a	121.71a	41.95a	3.50a
	NS	NS	NS	NS
<i>Harvest interval</i>	Shoot dry mass (g)	Stolon dry mass (g)	Root dry mass (g)	
1 (7 days)	96.62a	17.80a	21.82a	
2 (10 days)	116.62a	20.81a	20.59a	
3 (14 days)	117.35a	23.69a	21.65a	
4 (18 days)	113.88a	20.61a	21.03a	
	NS	NS	NS	

*, **, NS: Significant F test at $P < 0.05$, $P < 0.01$ and not significant ($P > 0.05$), respectively. Means followed by the same letter within a column are not significantly different based on a 5% Least Significant Difference (LSD) Test.

Plant density had no significant effect on stem lengths, root lengths, percentage stolon branching or percentage tuberised plants (Table 2.4). Struik and Lommen (1990) using high plant densities of up to 800 plant/m² observed extreme differences between parameters measured such as plant stem length.

Shoot dry mass was significantly higher at the lower plant density (130.88 g) compared to the higher density (91.36 g) (Table 2.4). Shoot growth tends to increase when a plant is not under competition. At a high planting density, a limitation in the availability of assimilates can result in stress which can together with the rapid growth of sink organs result in competition for nutrients, resulting eventually in rapid leaf senescence (Salisbury and Ross 1992). Stolon number and dry mass as well as root dry mass was also significantly different between different plant densities (Table 2.4), with more stolons and a higher stolon and tuber and root dry mass at the low planting density (20 plants/m²) compared to plants at the higher planting density (30 plants/m²). Total assimilate production and partitioning to both above and below ground plant parts were thus increased at the lower planting density due to less competition for space in these growth chambers. Stolon formation occurs under both tuber inducing and non-inducing conditions; the angle and amount of stolon growth has been interconnected with the strength of the inductive signal (Van den Berg et al. 1996). At the lower density the nutrient solution could also reach all roots, even at the mature stage. After three weeks in the aeroponics system, roots became longer and more fibrous, making it difficult to be sprayed by the six mists in growth chambers containing the higher plant populations of

30 plants/m². In contrast to our results, Resh (1995) reported that roots often account for a small portion of the biomass when plants are grown in soilless culture. In other studies it was also found that as plant density increase, there is a marked decrease in plant size and yield per plant (Masarirambi et al. 2012) due to a reduction in total biomass accumulation and also a decrease in tuber weight as biomass partitioning to tubers decrease (Gawronska and Dwell 1989).



Plate 2.2 Potato plants twenty-four days after being transplanted into the aeroponics system.

Table 2.4 Responses of potato plants in an aeroponic growth system at two planting densities on the plant growth

Plant density	Stem length (cm)	Root length (cm)	Stolon number	Percentage stolon branching (%)
1 (20 plants)	179.91a	130.66a	50.63a	3.71a
2 (30 plants)	171.73a	111.23a	38.12b	3.39a
	NS	NS	*	NS
Plant density	Percentage tuberised plants (%)	Shoot dry mass (g)	Stolon and tuber dry mass (g)	Root dry mass (g)
1 (20 plants)	26.78a	130.88a	25.53a	23.72a
2 (30 plants)	24.45a	91.36b	17.92b	18.83b
	NS	*	*	*

*, **, NS: Significant F test at P<0.05, P<0.01 and not significant (P>0.05), respectively. Means followed by the same letter within a column are not significantly different based on a 5% Least Significant Difference (LSD) Test.



Plate 2.3 Plantlets in the aeroponics system at final destructive harvest 118 days after transplanting.

Minituber quality and yield

Tuber formation started forty-seven days after transplant into the aeroponics system. Tuber initiation did increase after a reduction in the calcium content of the nutrient solution at thirty five days after transplanting. Other studies (Kang et al. 1996, Ritter et al. 2001, Farran and Mingo-Castel 2006, Adullateef et al. 2012) decreased the supply of nitrogen in order to stop the increase in vegetative growth and induce minituber initiation.

Some of the potato plantlets where transplanted from the glasshouse to the aeroponics system with tiny minitubers at the stolon ends. Tibbitts and Cao (1994) state that tuber initiation is enhanced under any stress condition. Due to the high nutrient solution concentration (from EC 0.5 to 1.5 mS.cm⁻¹) encountered in the new growing facility, secondary regrowth of minitubers was observed on the old ones which in corresponds with findings of Kang et al. (1996), Wheeler et al. (1990) and Farran and Mingo-Castel (2006). Repeated cycles of high nitrogen withdrawal can result in the formation of chain tubers (Krauss 1985). Hot weather can also cause this phenomenon in a process known as heat sprouting (Menzel 1985).

Harvesting plants at a 7 day interval gave the best result in terms of total number of tubers harvested throughout the season (Table 2.5). This indicates that frequent removal of the tubers enhanced initiation of new tubers. This result is similar to what Lommen and Struik (1992a) referred to by removing the dominant large tubers, initiation of new tubers and the development of the existing tubers would be influenced. Farran and Mingo-Castel (2006) reported that tuber production between consecutive harvests increased during development and decreased as the plant senesced. The best results in their study where achieved when harvesting every 7 days as in the current study.

Table 2.5 Effect of different harvest intervals on average tuber number per harvest and total tuber number of potato minitubers. Significant F test at P<0.05 (*) and P<0.01 (**) and non-significant (NS). Treatment means followed by different letters differ significantly (P<0.05).

<i>Harvest interval</i>	Average tuber number per harvest/m²	Total tuber number/m²
1 (7 days)	27.19a	299.17a
2 (10 days)	20.31a	142.17b
3 (14 days)	25.93a	129.67b
4 (18 days)	33.71a	134.83b
	NS	**

Harvest interval did not influence average or total tuber fresh weight (Table 2.6). For the first 4 harvests, minitubers harvested after every 18 days produced the highest average and total tuber fresh weight. This result was expected as the longer a tuber is attached to the mother plant, the longer the tuber bulking phase, resulting in bigger and physiologically more mature tubers. By harvesting more frequently the number of tubers was influenced but not the total weight of minitubers per plant (Table 2.6). This would indicate that tuber initiation is affected but not biomass accumulation which is more dependent on the availability of water, nutrients and optimum light conditions. The physiological processes inside tubers with frequent harvesting while using an aeroponics system need to be studied further for accurate explanations on tuber behaviour. Such a big difference between the values but without significantly differ could be due to a big variation in value from data recorded according to the intervals.

Table 2.6 Analyse of variance (ANOVA) on the effect of different harvest intervals on average tuber fresh weight (g), total fresh weight (g) and percentage firmness of potato minitubers (%). Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non-significant (NS). Treatments with different letter symbols differ significantly ($P < 0.05$).

<i>Harvest interval</i>	Average tuber weight (g)	Total tuber weight (g)	Percentage firmness (%)
1 (7 days)	252.17a	2773.8a	87.04a
2 (10 days)	223.30a	1563.1a	87.20a
3 (14 days)	258.39a	1291.9a	88.38a
4 (18 days)	483.17a	1932.7a	88.45a
	NS	NS	NS

Though there was a big difference between the two plant densities with a very strong trend toward the higher plant density having more minitubers, statistically no difference was found. Plant density did not have any significant effect on the average or total potato minituber number per m^2 or on the percentage firmness (Table 2.7). Probably the results could have been different if minitubers reached physiological maturity before being harvested as location and distribution of assimilate as well space would have been stressful to plants and affect final yield. The plant density used in this study was also lower compared to other research. For instance, Farran and Mingo-Castel (2006) used densities of 60 plants/ m^2 and 100 plants/ m^2 whereas Lommen and Struik (1992b) used a planting density of 350 plants/ m^2 in greenhouse. Both studies recorded higher yields with these high plant densities. The result of this current study is totally in contrast to findings by Georgakis et al. (1997) who reported that plant density strongly affected yield, both by number and by weight and that more tubers and a higher yield per square meter is expected at higher plant densities.

Table 2.7 Effect of different plant densities on average minituber number per harvest and total minituber number at the end of the experiment. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non-significant (NS). Treatment means followed by different letters differ significantly ($P < 0.05$).

<i>Plant density</i>	<i>Average minituber number/m²</i>	<i>Total tuber number</i>
1 (20 plants)	22.11a	148.83a
2 (30 plants)	31.46a	204.08a
	NS	NS

Though there was a tendency, the effect of plant density did not influence tuber fresh weight accumulation (Table 2.8). Although statistically there was no evidence, the low plant density of 20 plants/m² seemed to perform better during the two first and last harvests. But higher plant density of 30 plants/m² produced higher tuber fresh weights from the 3rd until the 10th harvest per m². Similar results had been achieved from field trials where both total weight per plot and total number of tubers increased as density increased (Midmore 1988, Muro et al. 1997). By increasing plant number, number of tubers will also be linearly influenced, unless plants become stressed or environmental resources such as water, light and space for both under and above ground development becomes limiting. In certain crops related to potato such as tomato, plants grown at a greater population density (4.2 plants/m²) had a reduced weight and lowered yield per plant (Wahle and Masiunas 2003). Moreover, Peres and Masiunas (1990) found that intraspecific competition resulted in smaller tomato plants. This negative circumstance is attainable in excessively overcrowded plantations. According to Beraga and Caesar (1990), increment of plant density increases tuber number because of high numbers of stems, but often decreases mean tuber size when interspecies competition occurs as observed in this study though there is no statistical evidence.

Table 2.8 Effect of different plant densities on average minituber fresh weight per harvest and total minituber fresh weight at completion of the trial. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non-significant (NS). Treatment means followed by different letters differ significantly ($P < 0.05$).

<i>Plant density</i>	<i>Average tuber fresh weight (g)/m²</i>	<i>Total fresh weight (g)/m²</i>
1 (20 plants)	252.09a	1586.5a
2 (30 plants)	356.42a	2194.3a
	NS	NS

The quality of the minitubers for use as seed tubers were also affected by the treatments although the interaction between tuber size, storage temperature, harvest interval and planting densities had no effect on the number of sprouts, sprout length or sprouting capacity. Temperature was the only determining factor regardless of the different correlations involved (Figure 2.2)

Harvesting interval had a significant effect on the sprout length with minitubers harvested every 10 days having longer sprouts (8.10 mm), compared to minitubers harvested every 18 days (7.00 mm), 14 days (5.91 mm) and 7 days (5.60 mm). Although it is primarily dependent on cultivar (Rolot and Seutin 1999), sprouting capacity was influenced by the different treatments. Harvesting every 7 days resulted in tubers with the highest sprouting capacity (46.47%) while tubers from plants harvested every 18 days had the lowest sprouting percentage (22.38%). This could be due to the size, smaller tubers tend to react rapidly to any kind of stress encountered by accelerating the sprouting phase due to not having sufficient nutrient reserves (Dam et al. 1996). This period may correspond to the optimum production interval when plants reach the recovery time and can readily transfer assimilate from the shoot to the underground parts, more specifically to stolons which enhance tuber initiation. Other studies need to be done to confirm this theory.

Though the number of sprouts was not influenced by the harvest interval alone (Table 2.9), the interaction between harvest interval and storage temperatures significantly influenced sprouting capacity (Figure 2.2). After spending two months in a completely dark storage chamber at three different temperatures of 3°C, 16°C and 25°C minitubers harvested every 7, 10 and 14 days and stored at 25°C had the highest rate of sprouting (80.6%, 69.2% and 59.8% respectively). This result could be explained by two hypotheses, the first one relating to the influence of high temperatures on the acceleration of the appearance and development of sprouts. Ranalli et al. (1994) found a linearly and inverse correlation between the length of dormancy duration and minitubers' storage temperatures. The second hypothesis concerns the size of the minitubers. Treatment 1 consisted of harvesting potato minitubers every 7 days when still immature and smaller with less reserves. This in combination with high temperatures could easily stress tubers and activate sprout emergence through growth regulators.

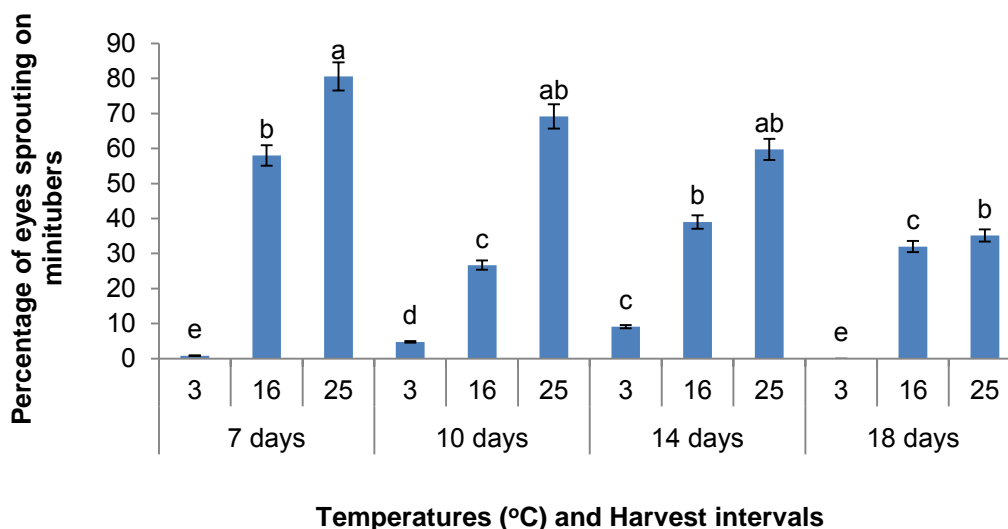


Figure 2.2 Effect of temperatures (°C) and harvest intervals on sprouting capacity of potato minitubers after two months of storage at different storage temperatures. Treatments with different letters differ significantly ($P < 0.05$)

Percentage firmness of the minitubers in this aeroponics experiment was influenced by tuber size (Table 2.9). Large minitubers (>40 g) showed a well-structured skin with an even and hard surface, with a firmness significantly higher than that of medium (20-40 g) and small minitubers (<20 g). This could indicate that the smaller tubers might have suffered from early stress because they had been harvested while still developing (Iritani and Sparks 1985). The description of deformations at cell level has largely been based on the early work of Nilsson et al. (1958) who describe the effect of turgor pressure on tissue elasticity. According to Hertog et al. (2004) firmness is mostly regulated by the water status, and changes as a function of time (Tijskens and Evelo 1994, Schouten et al. 1997) Tuber size is strongly related to the physiological age of the tuber, with larger tubers generally being well developed which furthermore will affect the number of stems per seed at sprouting. Smaller size minitubers, which are physiologically younger seem to be sensitive to both cell wall-related firmness and low water status which are related to tuber stiffness (Hertog et al. 2004). Wills et al (1998) reported that turgor decreases exponentially with moisture content decreasing.

With regards to the number of sprouts developing there was no interaction between storage temperature and tuber size. Also, the number of sprouts was not affected by the size of the minitubers but sprout length was (Table 2.9). However the larger minitubers (>40 g) had significantly longer sprouts (7.79 mm), compared to the small minitubers (5.38 mm). This could be explained by the fact that the small tubers were still in the growth process and not yet fully developed. Masarirambi et al. (2012) had similar results in their trial on the effect of plant density and tuber size and potato yield. A

uniform progression was noted where 85% of the small seed tubers sprouted, compared to 99% of the very large potato seed tubers. This could be explained by the fact that larger seed tubers have greater initial meristematic reserves compared to the smaller seed tubers (Kabir et al. 2004). Furthermore, smaller tubers had the lowest ground coverage; this could be explained by the fact that, the dormancy process in smaller tubers is hormonally prolonged and have an effect on the duration and sprout appearance rate. Though duration of dormancy is cultivar dependent (Struik and Wiersema 1999), the rate of sprouting is relatively slow at the beginning, but increases as the tuber gets older (Burton 1989). These results could have important implications for the use of these tubers as seed tubers as a delay in sprout development can have a negative effect on vegetative growth and thus crop establishment. It can therefore be suggested to only harvest the minitubers once they have reached at least 20g but preferably more than 40g.

As expected, storage temperature played a pivotal role on the number of sprouts, sprout length and sprouting capacity and profound differences were observed (Table 2.9). At 25°C, tubers showed a high number of developing sprouts (5.08); followed by 16°C (3.81) and the lowest temperature of 3°C had an average of 0.66 sprouts. Sprout length also was significantly influenced by different temperatures used during storage. Tuber stored at 25°C had the longest sprouts and tubers stored at the lowest temperature of 3°C had the shortest sprouts (Table 2.9). Tubers stored at 25°C had a sprouting capacity of 61.16%, compared to 38.91% at a storage temperature of 16°C and 3.66% at a storage temperature of 3°C (Table 2.9).

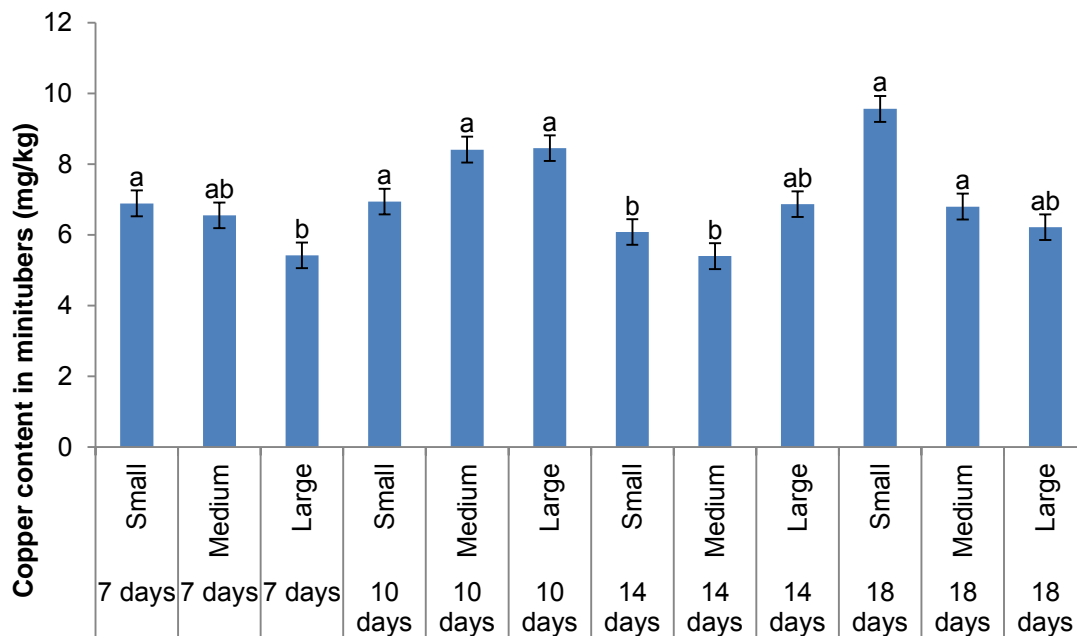
Whether the final product is to be marketed for fresh table stock, for the processing market, or as seed, it is suggested that the storage temperature have to be between 10 and 12.7°C for fresh use and 8.9°C for later use and that the temperature should not drop below 5.5°C (Iritani and Sparks 1985; Raghmi 2009). For long term storage, the recommended storehouse temperature is 2 to 4°C for seed potatoes (Holly 2005), which had been confirmed with this experiment as minitubers kept at 3°C showed almost 0% sprouting result after being stored for a period of 2 months.

Table 2.9 Responses of tuber sizes and storage temperatures ($^{\circ}\text{C}$) of potato minitubers. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non-significant (NS). Treatment means followed by different letters differ significantly ($P < 0.05$).

Sizes (mm)	Number of sprouts	Sprout length (mm)	Sprouting capacity (%)	Percentage firmness (%)
<i>Small (<20g)</i>	2.83a	5.38b	28.77a	86.91b
<i>Medium (20-40g)</i>	3.43a	6.75ab	37.70a	87.36b
<i>Large (>40g)</i>	3.29a	7.79a	37.25a	89.03a
	NS	*	NS	*
Temperatures ($^{\circ}\text{C}$)	Number of sprouts	Sprout length (mm)	Sprouting capacity (%)	
3	0.66c	0.18c	3.66c	
16	3.81b	8.71b	38.91b	
25	5.08a	11.03a	61.16a	
	**	**	**	

*, **, NS: Significant F test at $P < 0.05$, $P < 0.01$ and not significant ($P > 0.05$), respectively. Means followed by the same letter within a column are not significantly different based on a 5% Least Significant Difference (LSD) Test.

In terms of the elemental analysis, the interaction between harvest interval and tuber size significantly influenced only the Copper (Cu) concentration in the minitubers (Figure 2.3). The need for adequate Cu supply to potato has been clearly demonstrated. According to McCauley et al. (2009), Cu is needed by potato plant for chlorophyll production, respiration, and protein synthesis especially for tubers. But over application of nitrogenous fertilisers can increase the severity of Cu deficiency which can excessively delay maturity of plant with severe consequences on tuber production.



Harvest intervals and sizes (mm) of potato minitubers

Figure 2.3 Analyse of variance (ANOVA) on the interaction between harvest intervals and tuber sizes (mm) on the copper content of potato minitubers grown in an aeroponics system. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non-significant (NS). Treatments with different letter symbols differ significantly ($P < 0.05$). With small tubers (<20 g), medium tubers (20-40 g) and large tubers (>40 g).

There was no significant difference in the mineral composition of the tubers harvested at different time intervals for most of the elements tested with only the Potassium (K) and Cu content of the tubers differing significantly (Table 2.10). Harvesting minitubers at 10 day intervals resulted in the highest K content while tubers harvested most frequently, every 7 days had the lowest K content at frequent harvesting therefore tended to yield tubers with a lower K content. Unlike nitrogen and phosphorus, K does not form any vital organic compounds in the plant growth because it is an enzyme activator that promotes metabolism (Uchida 2000). The Cu content of tubers harvested every 10 days and 14 days was significantly higher than for tubers harvested every 7 days. Further investigations using the same harvesting intervals need to be done on Cu content in potato tubers to explain how at 7 and 14 days harvests the copper content was lower than at 10 and 18 day harvest interval. A better understanding of the physiology of the tuber might be necessary to understand this reaction from tubers.

Table 2.10 Effect of different harvesting intervals on the nutritional values of potato minitubers grown in an aeroponics system. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non-significant (NS). Treatment means followed by different letters differ significantly ($P < 0.05$).

<i>Treatments</i>	Phosphorus %	Potassium %	Calcium %	Magnesium %	Sodium mg/kg	Iron mg/kg
1 (7 days)	0.67 a	4.00 b	0.03 a	0.21 a	156.89 a	171.01 a
2 (10 days)	0.71 a	4.70 a	0.03 a	0.21 a	148.33 a	163.00 a
3 (14 days)	0.69 a	4.55 ab	0.02 a	0.21 a	139.00 a	151.21 a
4 (18 days)	0.71 a	4.44 ab	0.03 a	0.21 a	140.11 a	193.84 a
	NS	*	NS	NS	NS	NS
<i>Treatments</i>	Copper mg/kg	Zinc mg/kg	Manganese mg/kg	Boron mg/kg	Aluminium mg/kg	
1 (7 days)	6.29 b	38.20 a	12.56 a	13.89 a	7.55 a	
2 (10 days)	7.93 a	40.72 a	13.13 a	14.28 a	6.59 a	
3 (14 days)	6.11 b	37.00 a	12.5 a	13.80 a	7.52 a	
4 (18 days)	7.53 a	40.57 a	13.30 a	13.49 a	7.55 a	
	*	Ns	NS	NS	NS	

The concentration of macro and trace elements within tubers did not differ between the different size classes of tubers harvested except for sodium (Table 2.11). The sodium content of the smaller sized tubers was higher at 174.83 mg/kg than the medium and large sized tubers (129.5 mg/kg and 134.17 mg/kg respectively). It appears that the sodium concentration diminished with an increase in the growth rate of tubers. According to Romero-Aranda and Syverstsen (1996), toxic accumulation of Na^+ and Cl^- has also been correlated with reduction of total chlorophyll content but do not affect tubers in their quality as seed tubers. The fact that there were no significant differences in the elemental content of different sized tubers and those harvested at different maturity levels is something relevant that will not interfere in potato seed size selection. It means that nutritionally the small tubers harvested early and very often are just as good as the others.

The potato raw with skin contains minerals analysed by the Nutrient Data Laboratory (2012) suggested that for nutritional value per 100 g of a fully grown potato tuber, minerals repartition was: Ca: 1%, Magnesium: 6%, phosphorus: 8%, Potassium: 9%, Iron: 0.78 mg, Manganese: 0.153 mg, Sodium: 6 mg and zinc: 0.29 mg. Ca, Magnesium and phosphorus content in this current study were lower compared to the previous study, but other minerals were extremely higher. Maybe analyses of the skin, cortex, perimedulla and medulla should be done separately. Moreover, these results of ours could be compared to other aeroponics studies in the future when tubers would be used as potato seeds and used directly such as without previously being washed. Lommen and Struik (1992) reported the presence of a sodium layer around potato tubers when

using aeroponics at a higher EC level than the recommended dose depending on the temperature environmental conditions such as light intensity and humidity.

Table 2.11 Nutritional values of different sizes (mm) of potato minitubers grown in aeroponics system. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non-significant (NS). Treatment means followed by different letters differ significantly ($P < 0.05$).

<i>Sizes (mm)</i>	Phosphorus %	Potassium %	Calcium %	Magnesium %	Sodium mg/kg	Iron mg/kg
<i>Small</i>	0.69 a	4.45 a	0.03 a	0.21 a	174.83 a	208.27 a
<i>Medium</i>	0.70 a	4.34 a	0.03 a	0.21 a	129.5 b	145.13 a
<i>Large</i>	0.70 a	4.47 a	0.02 a	0.21 a	134.17 b	155.90 a
	NS	NS	NS	NS	**	NS
<i>Sizes (mm)</i>	Copper mg/kg	Zinc mg/kg	Manganese mg/kg	Boron mg/kg	Aluminium mg/kg	
<i>Small</i>	7.36 a	39.59 a	12.85 a	14.12 a	8.64 a	
<i>Medium</i>	6.79 a	39.12 a	13.24 a	14.12 a	6.98 a	
<i>Large</i>	6.74 a	38.65 a	12.58 a	13.48 a	6.28 a	
	NS	NS	NS	NS	NS	

Conclusions

At the beginning of the trial, plants grown at an EC of 1.5 mS.cm^{-1} became too vegetative, which delayed tuber initiation for all treatments. In order to activate tuber initiation and production, the nutrient solution EC was brought down to 1 mS.cm^{-1} and the Ca content of the nutrient solution was reduced. The reduction in Ca concentration was successful in inducing stolon tips to form tubers.

Harvest interval did not influence shoot, root or stolon growth and development but the total number of tubers and the quality of the tubers were affected. The highest yield, in terms of number of tubers was obtained from plants harvested every 7 days - 11 harvests over the 118 day growing period. The correlation between harvesting interval and storage temperatures suggested that tubers, harvested every 7, 10 and 14 days and stored at 25°C resulted in the optimal sprouting capacity. Concerning yield, harvesting minitubers at a 7 day intervals produced the best result with $299.17 \text{ minitubers/m}^2$ harvested during harvesting intervals did not play a major role in the distribution of macro and trace elements within minitubers except for K and Cu for tubers harvested at a 10 day interval.

The size of tubers at harvest significantly influenced sodium content with a higher sodium concentration in the smaller or physiologically younger potato tubers. Plant densities had a significant effect on stolon number, leaf and stem dry mass, stolon and tuber dry mass and root dry mass. The result observed in this study illustrated that

stolon initiation and elongation is related to main stem vigour and that later stolon initiation produces longer stolons. After transplantation of plantlets in the aeroponics system, tuber initiation was faster and more tubers initiated at the higher plant density. In the middle of the harvesting season, however, the plants at the lower planting density had longer and more vigorous stolon development and more tubers per plant initiated. This could be explained by the lack of space at high planting density which augmented competition when plants reached the adult phase. Regarding yield, plant density did not have a significant influence. Maybe more plants per treatment than used in this study need to be applied for further conclusions.

Temperature played a significant role on the post storage phase. Sprouting of minitubers was gradually influenced with the increase in temperature. Higher storage temperatures seemed to increase the capacity of sprout appearance regardless of tuber size. Conversely, firmness was influenced by size with the bigger (>40 g) tubers being significantly firmer and therefore less prone to moisture loss during storage.

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Chapter 3

The role of different calcium application rates on the growth, tuber size distribution and yield of potato (*Solanum tuberosum* L.) minitubers using aeroponics

Abstract

A healthy potato production system relies on the use of good quality seed potatoes and for this reason a large percentage of the total potato production worldwide is dedicated to the production of seed potatoes. The role of calcium in the maintenance of membrane integrity and cell wall strength is well established. An experiment was conducted to determine the effect of different calcium application levels on the growth and yield of four different potato cultivars in an aeroponic system and also to assess the quality of the tubers for use as seed potatoes. Potato plantlets, cultivars Up-to-date, Mnandi, Buffelspoort and BP1 were grown at four different calcium levels (8.40, 6.75, 5.10 and 3.45 meq/L) in a factorial, split plot design with five replications per treatment combination. The interaction between Ca application levels and cultivars significantly influenced the percentage stolon branching. BP1 had more stolons at the lowest Ca application level of 3.45 meq/L and Buffelspoort had more stolons at the full Ca application level of 8.4 meq/L. Mnandi was the least influenced by the different Ca treatments. Low Ca treatments with 3.45 and 5.1 meq.L⁻¹ respectively in the nutrient solution produced the highest yield (25.5 and 30.8 tubers respectively). There was also a strong correlation between the total tuber fresh weight and dry weight for the different Ca applications. However, the lowest Ca application levels of 3.45 meq/L resulted in the highest fresh and dry weight. The tuber number and weight of minitubers harvested was three times more for Mnandi (52.05 tubers/178.34 g) compared to Up-to-date (14.10 tubers/52.22 g). Dry weight in Up-to-date was the highest with an average total of 8.65 g. Potato plants supplied with lower Ca contents produced higher tubers yields than at higher Ca concentrations in the nutrient solution. These results indicated that Ca supply regulated crop productivity.

Key words: Potato, calcium, cultivars, seed, yield, size distribution, minitubers.

Introduction

Potato (*Solanum tuberosum* L.) cultivation is ideally suited to places where land is limited and labour is abundant (Lang 2001) and can be invaluable in creating food and jobs in many parts of the developing world. Potato tubers which consist of below-ground storage organs represent a reserve of carbohydrates, vitamins and mineral elements enabling subsequent plant growth (Subramanian et al. 2011). These organs also contribute considerably to the human diet, supplying many of the minerals required for well-being (White and Broadley 2005). It is ranked fourth after wheat, rice and maize in terms of business perspective (Ebadi and Iranbakhsh 2011) but presents the highest harvest index of 75-85 percent. That means that less than one-fourth of the plant material produced by sunlight, water, nutrients, labour and other inputs is wasted (Lang 2001). These aspects are attractive to ecologists, agriculturists and economists interested in developing sustainable food production systems (Hagenimana et al. 1999). A healthy potato production system relies on the use of good quality seed potatoes and a large percentage of the total potato production worldwide is dedicated to the production of seed potatoes. About 14% of the South African potato production comes from the Sandveld region (Knight et al. 2007). Understanding how the tubers develop is of great importance in assisting in improving the quality of tubers, especially seed potatoes.

The role of calcium (Ca) in the maintenance of membrane integrity and cell wall strength is well established (Palta 1996, Clarkson and Hanson 1980, Marschner 1995). Poor quality due to internal defects such as brown centre, internal brown spot (IBS), and hollow heart can substantially reduce the value of potato tubers (Clough 1994). IBS which is characterised by specks of fibrous or corky reddish-brown tissue interior to the vascular bundle (Wolcott and Ellis 1956) has been associated with localised Ca deficiencies in the tuber (Tzeng et al. 1986). In addition, there is some evidence indicating the involvement of Ca in tuberization (Balamani et al. 1986). It appears that during heat stress calcium is able to help sustain cell division and cell elongation in the apical meristem (Harris and Palta 1999). Several studies demonstrated how the tuberization signal is under complex biochemical control involving hormones, especially gibberellic acid (GA) (Ewing 1995; Xu et al. 1998; Jackson 1999). Cytosolic Ca is an important metabolic regulator in the plant transduction signal pathway (Poovaiah 1985)

and Bush et al. (1993) found a good correlation between GA and Ca/calmodulin, providing evidence for the interaction between GA and cytosolic Calcium action. In their study on the influence of supplemental Ca application on potato tuber number and size, Ozgen and Palta (2004) found that supplemental Ca application could alter tuberization in that an increased Ca concentration in the soil may suppress the tuberization signal. The fact that both calcium chloride and calcium nitrate were able to reduce tuber number further suggests that the observed decrease in tuber number is due to Ca and not the counter anion. According to Subramanian et al. (2011), the suberized periderm of mature tubers limits direct uptake of minerals, leading to their relative lower concentrations in the flesh compared to the potato tuber surface layer. However, in a developing tuber prior to the full development of the periderm and before the process of suberization, the direct uptake of minerals across the living epidermis would be possible. This is where aeroponics could play a major role compared to other techniques since it enhances the availability of nutrients throughout the tuber growing phase (Weathers and Zobel 1992; Farran and Mingo-Castel 2006). Aeroponics is a technique that uses a hydroponic growing system where underground plant parts, roots, stolons and tubers, are suspended in a dark enclosed chamber and sprayed with a nutrient solution as a fine mist (Christie and Nichols 2004). Many researchers reported that the benefits of the aeroponics system includes the ability to access roots with minimal disturbance, decreased plant water stress, enhanced plant growth rates and optimal aeration of the root zone (Kratch et al. 2004, Callaham et al. 1998). It is well known that plant roots function best at a temperature between 15°C and 25°C and at suboptimal root temperatures nutritional problems can ensue (Berry and Bjokman 1980). In this particular environment plants exhibit no signs of water stress and develop vigorous root systems and many root hairs (Kratch et al. 2004). Moreover, the nutrient solution can be easily adjusted, and the nutrients and pH value can be monitored accurately, improving the efficiency of seed-potato production (Soffer and Burger 1988). Plant material grown aeroponically is also considerably healthier because there is no medium to spread disease (Tello 1990). But water quality must be analysed often.

As mentioned by Abdullateef et al. (2012), despite more than 20 years of research in aeroponically potato cultivation, little scientific knowledge is available about this fascinating technique. This study was therefore conducted to determine the effect of

different calcium application levels on the growth and yield of four different potato cultivars in the aeroponic system, and how the quality of the tubers to be used as seed potatoes will be affected.

Materials and methods

Plant material

The experiment was carried out at Welgevallen, the experimental farm of the University of Stellenbosch in the Western Cape, South Africa. Potato *in vitro* plantlets (*Solanum tuberosum* L.) from the Agricultural Research Council (ARC) were used for this study. Up-to-date, Mnandi, Buffelspoort and BP1 cultivars which are well adapted to the Western Cape climate with high minituber productivity were selected (Potatoes South Africa 1998/99, Franke et al. 2011). Buffelspoort has a strong, upright stem with a rapid development and good leaf coverage. It is a medium-short (80 to 100 days) growing cultivar with tuber dormancy period of 50 to 70 days (Denner and Venter 2011). Up-to-date also has a strong stem with good leaf coverage. The growing period of this cultivar lasts between 90 to 120 days with a dormancy length of 50 to 70 days. The dormancy of progeny tubers are often shortened with periods of heat and/or water stress during the growing season (Niederwieser 2003). Mnandi is a well-adapted easy growing variety that does well under various growing conditions. Plants are tall with erect stems which develop rapidly and give fair to dense foliage cover with a growing period ranging between 90 to 120 days. Tuber dormancy is short to long, 70 days at least (<http://www.potatoe.co.za> 2013). BP1 is a medium maturing cultivar which generally takes 90 to 110 days before maturing with a short dormancy period of about 50 to 70 days (Kempen 2007).

In vitro plantlets were planted in the glasshouse in a mixture of perlite (50%) and vermiculite (50%) and acclimatized for thirty-two days at 15/20°C night/day temperatures. An overhead transparent polyethylene sheet was used for the first week to shade the plantlets and reduce the stress of transplanting. Each plantlet was irrigated twice daily (before 09:00 in the morning and after 16:00 in the afternoon) with a nutrient solution with an electrical conductivity (EC) of 0.5 mS.cm⁻¹.

Aeroponic system

Acclimatized plants were transplanted to their permanent polyethylene covered tunnel with a natural cooling system on Tuesday the 16th of April 2013. The aeroponics system consisted of 20 growth chambers each 1 m deep, 1.25 m wide and 1.20 m long with two sided openings for access to the roots, stolons and tubers. The interior was made of black polyethylene sheets to prevent light from entering with perforations at the bottom for draining. The exterior was covered with an aluminium sheet to reflect light and prevent any heat to build up inside the chamber. A transverse cut was made to permit insertion and removal of the seedlings and allow for radial expansion of the plant stems. Plant density was 20 plants m². Between one-half and two thirds of the length of the stems was placed inside the dark chamber, similar to the procedure followed by Farran and Mingo-Castel (2006). Underground plant parts were periodically sprayed with a nutrient solution using six fog nozzles per compartment. Plants showing symptoms of chlorosis or growth defects, as well as dead plants, were removed 7 days after transplanting and replaced by healthy ones. In order to keep a disease free trial, Syngenta spraying program was followed (Table A1 appendix). A standard Steiner (1984) nutrient solution with four different calcium application levels (8.40, 6.75, 5.10 and 3.45 meq.L⁻¹) was used with an EC of 1.5 mS.cm⁻¹ (Table 3.1). Plants were fertigated intermittently every 30 minutes for 30 seconds throughout the entire trial and the nutrient solution was not recirculated.

Table 3.1 Composition of the standard Steiner nutrient solution with an electrical conductivity (EC) of $1.5 \text{ mS}\cdot\text{cm}^{-1}$ and four Ca levels (8.40, 6.75, 5.10 and $3.45 \text{ meq}\cdot\text{L}^{-1}$) used to fertigate potato plantlets in an aeroponic growth system.

	Application (g 1000L ⁻¹)			
	Ca 1 8.4 meq.L ⁻¹	Ca 2 6.75 meq.L ⁻¹	Ca 3 5.1 meq.L ⁻¹	Ca 4 3.45 meq.L ⁻¹
Macro-nutrients				
KNO ₃	60.60	227.25	393.90	560.55
K ₂ SO ₄	247.95	195.75	143.55	91.35
KH ₂ PO ₄	102.00	102.00	102.00	102.00
Ca(NO ₃) ₂ ·2H ₂ O	840.00	675.00	510.00	345.00
MgSO ₄ ·7H ₂ O	295.20	369.00	442.80	516.60
Micro-nutrients	Application (g 1000L ⁻¹)			
Fe-EDTA	86.9			
MnSO ₄	18.0			
ZnSO ₄	10.0			
CuSO ₄	1.2			
H ₃ BO ₄	22.0			
MoCl ₅	1.9			

Measurements and Analysis

Data collected

The pH and EC were controlled manually each time the four nutrient solutions were replenished. Temperature and relative humidity inside the growth chambers were measured with a thermohygrometer model HI 9161C (Hanna instruments). Light intensity was measured with a ceptometer (model LP-80 PAR/LAI Ceptometer, AccuPAR) throughout the entire growing season at two different times during the day, in the morning before 08:30, when still cool, and at 13:00. During the cool period, the average temperature was 14.26°C , RH 89.22% and light intensity of $34.15 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. However, during the warm period the average temperature was 20.23°C , RH 93.68% and light intensity $288.48 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Table 3.2 Minimum, maximum, average, kinetic temperature and relative humidity in the greenhouse throughout the growing season.

	Temperature (°C)	Relative humidity (RH)
Minimum Reading	3.1	14.7 %
Maximum Reading	42.1	100.0%
Average Reading	15.3	77.9 %

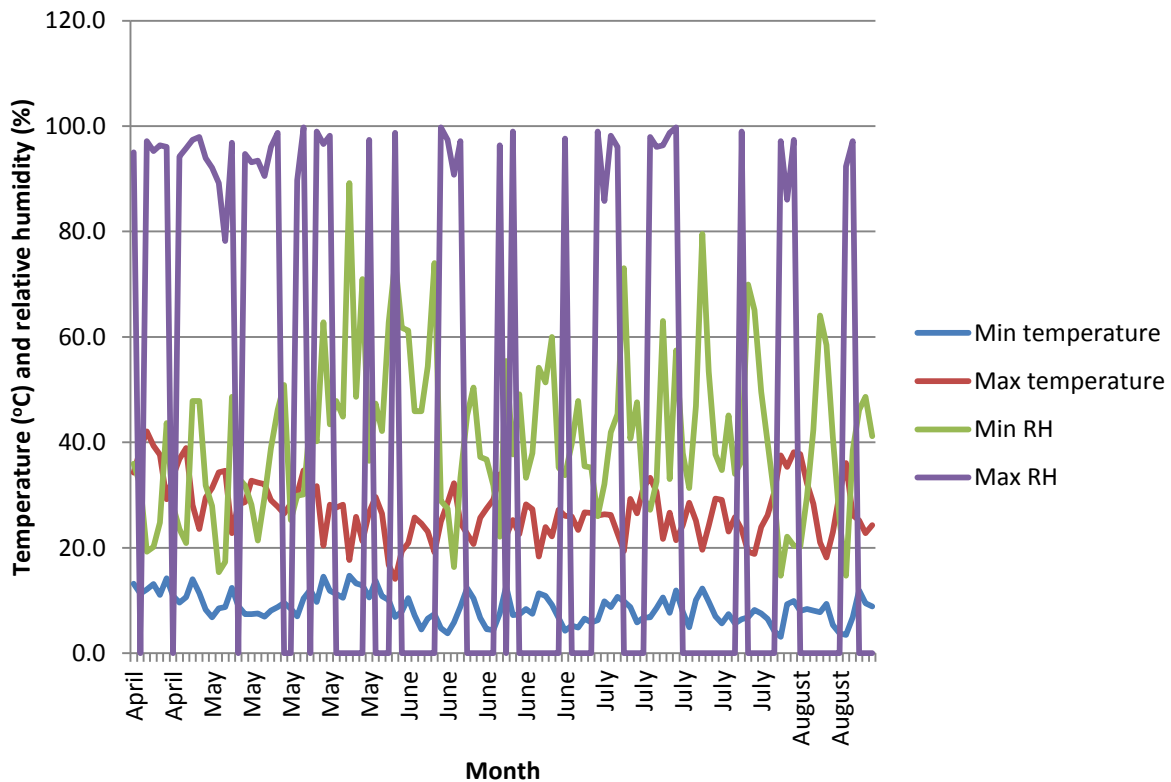


Figure 3.1 Temperature and relative humidity inside the polyethylene covered greenhouse where the potato plants were grown aeroponically.

At transplanting into the aeroponic system the following morphological parameters were taken: plant height, leaf number, shoot weight, root length and stolon number. All morphological measurements were recorded for three plants per Ca treatment from each cultivar. Plant heights were measured as the vertical length between the apical

growth point of the plant and the lowest base of the stem. At harvest, plant height was measured again before determining the dry mass of the shoots.

Potato minitubers larger than 20 mm in size were harvested at ten day intervals from the beginning of the initiations until the last day before plants could be completely removed and cut into parts for different analyses. The height of plants was recorded weekly and the number of minitubers and total tuber weight per plant were recorded at every harvest. The mean number of potato minitubers per harvest and total tuber number per plant were recorded for five plants per treatment combination. As recommended by Lommen (1995), a hand pulling down of the plants after each harvest was done to increase the formation of new stolons with tuber initiation sites and tubers. Percentage firmness of minitubers was performed at each harvest with a densimeter. At final, destructive harvest other parameters were also taken into consideration such as percentage tuberised plants, percentage stolon branching and stolon number. After counting and weighting the minitubers they were stored in brown paper bags in a dark room at 5°C.



Plate 3.1 Samples of minitubers after eight harvests from potato plants in an aeroponic growing system.

The fresh and dry weight – after being oven dried at 85°C for 48 hours of the minitubers were determined at each harvest. For macro and trace element determinations, skin and medullary tissue samples were taken and processed according to the procedure described by Kratzke and Palta (1986) and Soltanpour et al. (1996). Samples were washed and dried in an oven at 49°C for 5 days, grounded to pass a 40-mesh of 0.635mm of screen, weighed, burnt at 450°C for 8 hours, dissolved in 2 N HCl, and diluted with a lanthanum chloride (LaCl₃/H₂O) solution and distilled –deionized water to obtain samples in 0.2 HCl and in La at 2000 µg/mL. Samples were replicated 4 times by reading on the inductively coupled plasma-atomic emission spectrophotometry wavelength table. Macro and trace element concentrations were determined by atomic absorption spectrophotometry as described by Karlsson and Palta (2006).

Treatments and experimental design

The trial was a factorial, split plot design with five replications and five plants per replication with two factors. Four calcium levels was the main plot and the four potato cultivars (Up-to-date, Mnandi, Buffelsoort and BP1) constituted the second sub plot. Twenty completely separated mainplots were utilised, and each compartment received four different cultivars as sub plot. Data were analyzed using ANOVA, and means comparison ($P < 0.05$) using the general linear model of statistica software *statistica 11* (Statistica 2011).

Results and Discussion

Morphology and vegetative growth

The interaction between Ca application levels and cultivars significantly influenced the percentage stolon branching (Figure 3.2). For Up to Date and Buffelspoort the lowest Ca application rate (Ca4, 3.45 meq/L) resulted in fewer stolons while BP1 had more stolons at the lowest Ca application level. Buffelspoort showed the highest percentage stolon branching at the high Ca application rate (Ca1, 8.4 meq/L). Mnandi was the least influenced by the different Ca treatments. Unfortunately in this study we did not focus on stolon length itself. These results indicate that there may be an effect of Ca on stolon development but further studies using aeroponic systems on the study of stolon development could be more informative. According to Engels and Marschner (1986)

stolon size influences sink strength for photosynthates and therefore subsequent tuber growth. Although significant, these results look very variable with no clear trends.

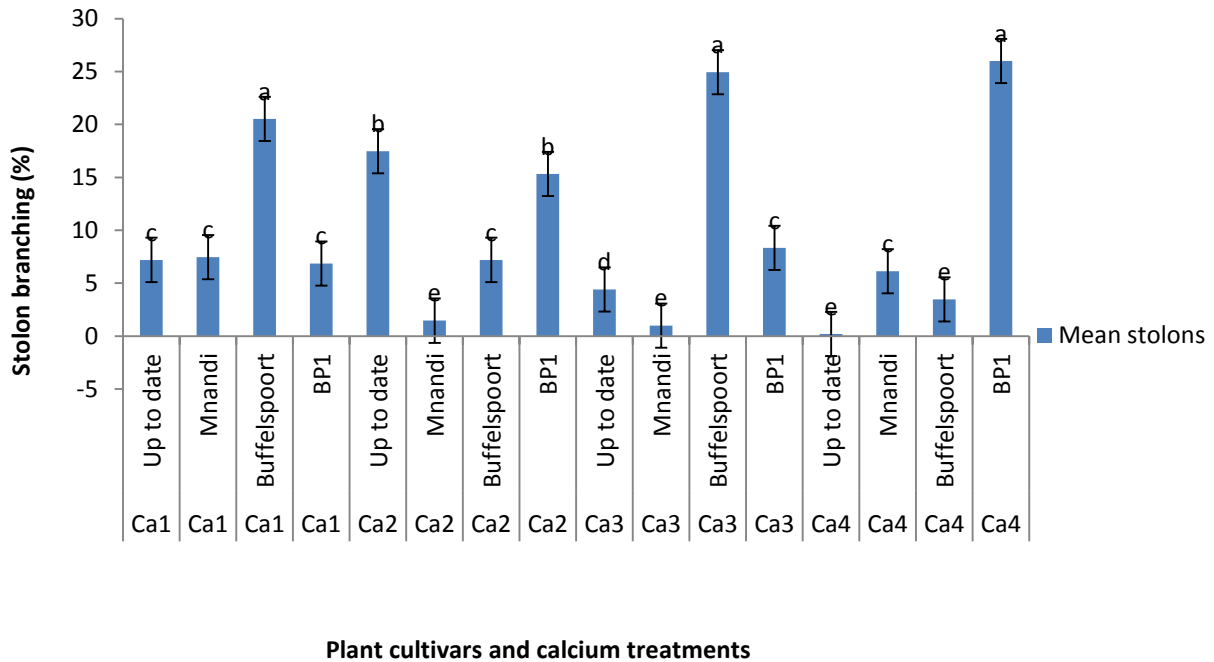


Figure 3.2 Interaction between Ca application level and cultivar on percentage stolon branching. With Ca1 supplied at 8.4, Ca2 at 6.75, Ca3 at 5.1 and Ca4 at 3.45 meq.L⁻¹. Significant F test at P<0.05 (*) and P<0.01 (**) and non-significant (NS). Treatments followed by different letters differ significantly (P<0.05).

No flower initiation was observed among the different cultivars. Vegetative plant growth, represented by stem number, stem length, root length, percentage tuberised plants, percentage stolon branching, stolon number, shoot, stolon and root dry weight (Table 3.3) was not statistically influenced by the different calcium application levels. There were, however, wide variations in stolon dry mass (2.68-10.95 g/treatment). As reported by Özgen et al. (2000) calcium does not affect plant development under good (ideal) growing conditions since calcium is expected to have a greater influence on plant parts under stress conditions like heat. As these experiments were done in the middle of winter, it could explain these insignificant differences as the temperature was mostly low and unfavourable for potato plant growth. With the lower temperatures water and fertilizer was also reduced but due to the slower growth rate at these temperatures no

nutrient deficiencies developed. The Ca application level did not have any influence on the growth of above ground parts visually or statistically. Higher application levels could probably have been more effective but this also indicates that at an application level of 3.45 meq/L Ca, this element will not result in deficiency in the vegetative plant growth. Further studies need to be done in aeroponics during a better growth environment because during summer it is easy to get Ca-deficient tubers.

Table 3.3 The means of morphological characteristics measured on different Ca application levels for potato plants grown in an aeroponic system. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non significant (NS). Treatments with different letter symbols differ significantly ($P < 0.05$)

Treatments (meq/L)	Stem Number	Stem Length (cm)	Root Length (cm)	% tuberised Plants	% Stolon Branching	Stolon number	Shoot dry weight (g)	Stolon dry weight (g)	Root dry weight (g)
Ca 1 (8.40)	0.90a	19.09a	36.85a	22.00a	10.83a	2.18a	5.50a	2.89a	4.79a
Ca 2 (6.75)	1.11a	20.09a	33.85a	21.83a	19.34a	2.03a	6.04a	4.04a	5.11a
Ca 3 (5.10)	0.95a	18.98a	35.37a	21.00a	12.75a	2.05a	6.27a	3.75a	5.07a
Ca 4 (3.45)	0.9a	20.21a	35.48a	17.33a	11.62a	1.80a	5.76a	3.79a	4.93a
	NS	NS	NS	NS	NS	NS	NS	NS	NS
Weekly heights (cm)									
Treatments (meq/L)	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	
Ca 1 (8.40)	16.99a	16.05 a	17.97a	17.93a	19.18a	17.03a	16.11a	16.08a	
Ca 2 (6.75)	16.74a	15.49 a	17.01a	17.02a	16.93a	19.87a	15.88a	16.01a	
Ca 3 (5.10)	17.00a	15.40a	17.03a	17.48a	17.10a	16.66a	16.43a	16.20a	
Ca 4 (3.45)	17.11a	14.90a	16.86a	17.54a	17.33a	16.90a	16.50a	16.05a	
	NS	NS	NS	NS	NS	NS	NS	NS	



Plate 3.2 Vegetative growth and tuber initiation three weeks after the potato plantlets were transplanted into the aeroponic system.

There were significant differences in vegetative plant development between the different cultivars (Table 3.2). Mnandi had the highest average stem number (1.38), stem length (22.51 cm), root length (average of 40.31 cm), shoot dry weight (7.01 g) and root dry weight (5.17 g). This is expected as Mnandi is an easy growing variety that can develop well under various growing conditions. From the weekly height measurements it can be seen that Mnandi is taller compared to other cultivars from the beginning until the end of this experiment (Table 3.4). Buffelspoort also gave good results with an average root length of 36.95 cm and together with BP1 it reached the highest percentage stolon branching (average of 16.72 and 18.71 % respectively). These results are similar to what Kratzke and Palta (1992) reported in their studies with eight different cultivars where all plants were subjected to similar environmental conditions, but cultivars displayed different results in terms of adaption to the growing media, indicating the inherent genetic variability in determining the adaptability of a crop.

Table 3.4 The means of morphological characteristics measured on different cultivars of potato plants grown in an aeroponics system. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non-significant (NS). Treatments followed by different letters differ significantly ($P < 0.05$)

Cultivars	Stem Number	Stem Length (cm)	Root Length (cm)	% tuberised Plants	% Stolon Branching	Stolon number/plant	Shoot dry weight (g)/plant	Stolon dry weight (g)/plant	Root dry weight (g)/plant
Up to date	0.73b	19.01ab	34.39ab	12.66a	15.11b	1.93a	5.28b	3.74a	4.95b
Mnandi	1.38a	22.51a	40.31a	24.50a	4.03c	1.36a	7.01a	3.67a	5.17a
Buffelspoort	0.96b	19.34ab	36.95ab	24.66a	16.72a	2.43a	5.75ab	3.49a	5.01ab
BP1	0.80b	17.49c	29.91b	20.33a	18.71a	2.33a	5.52abc	3.58a	4.97bc
	**	**	**	NS	*	NS	**	NS	*
Weekly height									
Cultivars	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	
Up to date	17.91ab	15.86ab	16.92b	16.80b	16.86a	19.55a	15.46cb	14.90b	
Mnandi	19.35a	17.24a	19.90a	20.01a	19.78a	19.16a	18.75a	19.34a	
Buffelspoort	16.39b	14.70b	16.98b	17.26b	17.15a	17.30a	16.60b	16.09b	
BP1	14.16c	14.05b	15.08b	15.90b	16.75a	14.47a	14.11c	14.01b	
	**	*	**	**	NS	NS	**	**	

Yield

Ca application level influenced the yield of potato minitubers significantly (Table 3.5). The total tuber number was the highest for plants grown at the two lowest Ca treatments with 25.50 and 30.85 tubers per plant at 3.45 and 5.1 meq.L⁻¹ applied Ca. This could be the result of Ca stress which caused stolons to initiate more tubers as root cell membranes were damaged (Abolitz and Zieslin 1996). According to Tibbits and Cao (1994), tuber initiation is enhanced under any stress condition. Higher Ca application rates (6.75 and 8.4 meq.L⁻¹) produced fewer minitubers (21.05 and 24.10 respectively). There was also a strong correlation between the total tuber fresh weight for the different Ca applications (Table 3.5). The lowest Ca application levels (3.45 and 5.1 meq/L) resulted in the highest tuber fresh weight (99.38 g/plant). The higher tuber fresh weight in conjunction with the increase in tuber number indicate that more tubers initiated but also that enough assimilates was available for tuber growth.



Plate 3.3 Plant growth at the final destructive harvest ten weeks after transplanting the potato plantlets into the aeroponic system. .

Table 3.5 The total tuber number per plant, total tuber fresh weight (g) per plant, and total tuber dry weight (g) per plant for eight different harvest intervals. Potato minitubers were grown in an aeroponic system using four different calcium treatments. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non significant (NS). Treatments followed by different letters differ significantly ($P < 0.05$)

Ca application rate (meq/L)	Total tuber number of 5 plants	Total tuber fresh weight (g)	Total tuber dry weight (g)
8.4	24.10b	89.18ab	8.37ab
6.75	21.65b	73.39b	7.67a
5.1	30.85a	92.17a	7.76a
3.45	25.50a	99.38a	8.56a
	*	*	*

There was no interaction between Ca application levels and cultivars for tuber yield. However, cultivars differed with regards to the number of tubers produced (Table 3.6). More tubers were harvested from Mnandi compared to the other cultivars from week one up to week eight. A total number of 52 tubers were recorded for Mnandi, followed by Buffelspoort with 18.7 tubers which was statistically the same tuber number as BP1 with 17.3 and Up to date with 14.1 tubers. As reported by Kratze and Palta (1992), there are occasionally conflicting results reported in studies looking at different cultivars used as they differ in their responses and final yield capacity and productivity duration as the patterns of tuber formation and the extent of tuber growth varied widely among cultivars (Wurr 1977). For instance, tubers of some cultivars such as “Russet Burbank” have been shown to have tiny roots growing directly out of the tubers (Struckmeyer and Palta 1986). Such supplementary roots are capable of supplying water and perhaps some nutrients to the tuber (Kratzke and Palta 1985) and this could explain why some cultivars performed better than others who do not have these functional roots. Further studies focusing on the physiological behaviour of cultivars used in the current study will be very beneficial to better understand these results. According to Steyn (1999), cultivars differ in terms of number of tubers initiated and retained, and also tuber bulking can continue for up to three months depending on the cultivar and environmental conditions. Ideally this trial should be repeated under different environmental conditions to determine if these differences could be linked to temperature and light intensity or day length.

Though Lommen (1995) noted in his study that repeated harvesting increased tuber number due to the removal of the dominant larger tubers which allowed initiation of new tubers; in this current study, the number of minitubers harvested increased only with the third harvest (Mnandi giving 2.8 tubers per plant) and then decreased at each successive harvest (Table 3.6). Probably due to poor leaf canopy development. A similar trend had been noticed with Up-to-date which had the lowest number of minitubers for all eight harvests with a total of 14.1 tubers. The total tuber number was however substantially higher than would be expected in a conventional minituber production system where plants are planted in a growing medium and tubers are harvested only once at the end of the growing season.

Analysis of variance showed that the total fresh weight per treatment per cultivar varied significantly among cultivars. Mnandi (52.1 tubers/178.3 g) had three times more than Up-to-date (14.1 tubers/52.2 g) in terms of number and weight of minitubers harvested (Table 3.6). These results are consistent with a previous study by Engels and Marschner (1986) which demonstrated that stolon length, tuber volume, and stolon diameter and tuber volume were interdependent for eight potato cultivars tested. Similarities have been noticed in this current study (Table 3.4) where the longest stem, root and stolon and total fresh weight (average of 178.34 g) were recorded with Mnandi. This could be explained by the increased carbohydrate sink strength provided by the long root as the plants could have better access to water and nutrients (Kunkel et al. 1973), and BP1 had the lowest average shoot height which was almost similar to the total fresh weight. If tubers are initiated when only a small leaf area has developed, branch and leaf production cease earlier and existing leaves senescence more quickly, giving smaller final yields (Burt 1961). In their studies Jelodar and Hassanpanah (2012), found a positive significant correlation between minituber number per square meter with plant height and negative significant correlation with minituber size and plant height with root length.

Dry weight partitioning within minitubers was significantly different between the cultivars used and Up to date had the highest dry weight accumulation (average of 8.65 g) (Table 3.6). There is some evidence that stolon characteristics may influence tuber growth as tuber bulking is primarily due to the transport of carbohydrates and water flow into the tuber via the phloem (Hanson 1984). Up-to-date had a higher total dry weight probably because this cultivar produced fewer minitubers and consequently more assimilates were partitioned to fewer minitubers resulting in improved quality. Mnandi had a lower total dry weight. Mnandi is known for its high yields (fresh) but low specific gravity/dry matter content (Potatoes South Africa 2013).

Table 3.6 The means of number of potato minitubers per harvest (H), total tuber number, total tuber fresh weight per plant (g) and total tuber weight per plant (g) for minitubers grown in an aeroponic system using four different cultivars. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non significant (NS). Treatment means followed by different letters differ significantly ($P < 0.05$)

Ca application rate (meq/L)	Number of tubers per plant								Total tuber number
	H1	H2	H3	H4	H5	H6	H7	H8	
Up to date	1.04b	1.38b	0.89b	1.04b	0.8b	0.62b	0.24b	0.24b	14.10b
Mnandi	1.17a	1.95a	2.83a	1.63a	2.00b	1.81a	1.24a	0.72a	52.05a
Buffelspoort	1.1ab	1.17b	0.83b	0.63b	1.00b	0.63b	0.63b	0.22b	18.65b
BP1	0.77b	1.39b	0.82b	0.66b	3.20a	0.45b	0.3b	0.20b	17.30b
	*	**	*	**	**	**	**	**	**

Ca application rate (meq/L)	Fresh weight of tubers per plant (g)								Total tuber fresh weight
	H1	H2	H3	H4	H5	H6	H7	H8	
Up to date	6.14a	5.84b	3.20b	3.27b	1.86b	1.84b	0.54b	0.51b	52.22b
Mnandi	5.79a	7.82a	7.19a	6.07a	6.79a	6.27a	3.57a	2.21a	178.34a
Buffelspoort	6.09a	4.45b	3.03b	2.03bc	2.56b	1.90b	1.83b	0.76b	67.96b
BP1	3.69b	5.1b	2.49b	1.79c	1.85b	1.15b	0.76b	0.54b	55.60b
	*	**	**	**	**	**	**	**	**

Ca application rate (meq/L)	Dry weight of tubers per plant (g)								Total tuber dry weight
	H1	H2	H3	H4	H5	H6	H7	H8	
Up to date	2.04a	1.95b	2.4a	1.09b	0.62b	0.61b	0.18b	0.17b	16.65a
Mnandi	1.93a	2.61a	2.39a	2.02a	2.26a	2.09a	1.19a	0.73a	14.86ab
Buffelspoort	2.03a	1.48b	1.01b	0.68b	0.85b	0.63b	0.61b	0.25b	16.16ab
BP1	1.23b	1.7b	0.83b	0.6b	0.61b	0.38b	0.25b	0.18b	14.70b
	*	**	**	**	**	**	**	**	*

Aeroponics is a convenient way to study the effects of nutrient use efficiency and deficiency on physiological, biochemical, and molecular processes in plant roots because of the misters that continuously provide plants with fresh nutrient solution (Peterson and Krueger 1988; Waisel 1996). The interaction between Ca application levels and potato cultivars used in the current trial influenced the concentration of macro

and trace elements within potato minitubers (Table 3.7). In general, Potassium (K) was equally distributed among treatments and cultivars as shown in table 3.5. This could be due to its very high mobility in plants as described by Bromley and County (2010). K content was however lowest for Mnandi at the highest Ca application level (8.4 meq/L) with 2.26%. This might be a characteristic of this cultivar as the potassium content tended to be lower at the other Ca application levels as well, although not significantly so.

The tuber Ca content was higher at the high Ca application level for Mnandi minitubers with 0.11% and lowest at the low Ca application level for Up to date with 0.05%. These results showed a marginal trend for all the cultivars tested towards a decrease in tuber Ca content as the Ca application level was decreased. This finding is consistent with previous studies where supplemental Ca application improved the Ca level of tubers (Kleinhenz et al. 1999, Ozgen et al. 2006). Harris and Palta (1999) reported that an application of Ca to the tuber and stolon, which is easier with aeroponics, resulted in a three-fold increase in the Ca concentration in the tuber peel and medullary tissue especially during the bulking stage. Furthermore, to maximize Ca uptake by potato tubers, Simmons et al. (1988) in their study observed that Ca had to be sprayed around the tubers and stolons. As Ca is not mobile and is not easily translocated in the plant there is also a huge difference between the Ca content of the stems and tubers. A deficiency of Ca in the tubers will therefore not necessarily be noticed through leaf analysis or observing deficiency symptoms on the young leaves and leaf tips (Uchida 2011). Ozgen et al. (2006) found that Ca tissue concentration was uniformly higher, irrespective of treatment as all soluble sources of Ca were equally effective in the study with “Russet Burbank” where supplemental Ca was applied. According to the same author, split nitrogen application improved tuber tissue Ca as compared to non-split nitrogen application. As no sign of deficiency in Ca was noticed among the cultivars used in this study, the four Ca application rates were thought to be appropriate for the development of plants.

Sodium (Na) content was higher in BP1 tubers at Ca application of 6.75 meq/L.

Tubers from Mnandi had a lower Copper (Cu) content compared to the other cultivars at the high Ca application level (Table 3.5). The tuber Cu content tended to decrease with

a decrease in Ca application level but not to the same degree for the different cultivars. For Buffelspoort the tuber Cu content was higher at the highest Ca application level while for BP1 the tuber Cu content was lower at the two lower Ca application levels and for Up to Date the tuber Cu content was lower only at the lowest Ca application level. Though cultivars responded differently to different Ca concentrations in the nutrient solution, it appeared that low Ca in the nutrient supplied to plants increased Zn content in tubers. The zinc (Zn) content was highest in Buffelspoort plants when fertigated with Ca at 3.45 meq/L having 35.40 mg/kg of zinc in tubers. Mengel and Kirby (2001) described manganese as particularly active in plant organelles where photosynthesis occurs namely chloroplasts. Buffelspoort displayed the lowest manganese concentration at the highest Ca application level (table 3.5). It therefore seems that there was an antagonistic relationship between Ca and Cu against Mg, Zn and Mn since generally the tuber magnesium (Mg), manganese (Mn) and Zn content was higher at the lowest Ca application level while the tuber Cu content was higher in the two higher Ca application levels. The boron content in Up to date tubers was high at higher Ca fertigation.

Table 3.7 Analyse of variance (ANOVA) on the interaction between Ca application levels and potato cultivars on the nutritional values of potato minitubers grown in an aeroponic system. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non-significant (NS). Treatments with different letter symbols differ significantly ($P < 0.05$).

Treatments (meq/L)	Cultivars	Phosphorus %	Potassium %	Calcium %	Magnesium %	Sodium mg/kg	Iron mg/kg
Ca1 (8.40)	BP1	0.48a	2.65a	0.07ab	0.15a	110.25c	96.46a
Ca1 (8.40)	Mnandi	0.55a	2.26b	0.11a	0.15a	101.50c	113.00a
Ca1 (8.40)	Up to date	0.52a	2.75a	0.10a	0.19a	93.00ad	93.27a
Ca1 (8.40)	Buffelspoort	0.55a	2.79a	0.10a	0.20a	137.25b	114.44a
Ca2 (6.75)	BP1	0.51a	2.73a	0.09a	0.21a	156.50a	130.64a
Ca2 (6.75)	Mnandi	0.55a	2.37a	0.10a	0.17a	146.50a	123.83a
Ca2 (6.75)	Up to date	0.53a	2.68a	0.08a	0.20a	133.00b	81.53a
Ca2 (6.75)	Buffelspoort	0.50a	2.60a	0.07b	0.10a	93.50d	91.88a
Ca3 (5.10)	BP1	0.49a	2.67a	0.07ab	0.21a	133.00b	115.72a
Ca3 (5.10)	Mnandi	0.54a	2.36ab	0.08a	0.17a	123.00c	110.30a
Ca3 (5.10)	Up to date	0.51a	2.68a	0.06b	0.22a	119.00c	112.95a
Ca3 (5.10)	Buffelspoort	0.49a	2.57a	0.07ab	0.18a	138.25b	149.19a
Ca4 (3.45)	BP1	0.50a	2.73a	0.05b	0.22a	130.00bc	124.83a
Ca4 (3.45)	Mnandi	0.57a	2.58a	0.07ab	0.19a	128.50c	137.18a
Ca4 (3.45)	Up to date	0.51a	2.53a	0.05b	0.20a	107.00c	116.89a
Ca4 (3.45)	Buffelspoort	0.51a	2.76a	0.06b	0.22a	139.75ab	115.66a
		NS	*	*	NS	*	NS
Treatments (meq/L)	Cultivars	Copper mg/kg	Zinc mg/kg	Manganese mg/kg	Boron mg/kg	Aluminium mg/kg	
Ca1 (8.40)	BP1	2.71a	20.34b	14.07a	10.24b	12.12a	
Ca1 (8.40)	Mnandi	2.05b	23.50b	11.89a	9.25d	11.32a	
Ca1 (8.40)	Up to date	2.69a	20.34b	13.09a	11.04a	9.42a	
Ca1 (8.40)	Buffelspoort	3.28a	24.98ab	0.20c	10.38b	13.29a	
Ca2 (6.75)	BP1	3.30a	25.13ab	15.35a	10.96a	12.46a	
Ca2 (6.75)	Mnandi	2.04b	21.83b	12.94a	9.80cd	11.32a	
Ca2 (6.75)	Up to date	3.14a	21.90b	14.29a	11.13a	10.49a	
Ca2 (6.75)	Buffelspoort	2.11b	21.33b	6.61b	10.16bc	10.04a	
Ca3 (5.10)	BP1	2.19b	18.81b	13.22a	10.08c	12.25a	
Ca3 (5.10)	Mnandi	2.24b	20.61b	12.53a	9.67cd	12.42a	
Ca3 (5.10)	Up to date	2.60a	22.30b	14.77a	10.26b	14.20a	
Ca3 (5.10)	Buffelspoort	2.07b	19.58b	8.94a	9.46d	14.25a	
Ca4 (3.45)	BP1	2.25b	20.71b	16.53a	10.15b	12.27a	
Ca4 (3.45)	Mnandi	2.53ab	27.57ab	14.20a	10.60a	11.32a	
Ca4 (3.45)	Up to date	2.06b	20.12b	13.90a	9.84cd	13.39a	
Ca4 (3.45)	Buffelspoort	2.24b	35.40a	14.10a	10.17b	13.62a	
		**	*	**	*	NS	

The macro and trace elements content of the tubers were significantly different between the cultivars (Table 3.8). Mnandi had a higher Phosphorus (P) and Ca content but lower Mg and B content compared to the other cultivars. The tuber Cu content was higher in Mnandi compared to BP1 and Up-to-Date and the Zn content lower in Up-to-Date compared to Buffelspoort and Mnandi. It is possible that macro and trace element uptake and accumulation among cultivars is controlled by a specific gene as reported by Bamberg et al. (1993). It appeared that there was a tendency of interaction between Ca and B. For instance Mnandi plants produced tubers with a higher Ca content of 0.09% and lower B content of 9.83 mg/kg. According to Marschner (1995), tubers deficient in B can have ruptured surfaces and are smaller with brown specks and patches through the flesh reported. This finding was not observed in this current study, probably due to low difference in terms of B concentration between tubers from different cultivars (Table 3.6).

Table 3.8 Nutritional values of different potato cultivars grown in an aeroponics system. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non-significant (NS). Treatment means followed by different letters differ significantly ($P < 0.05$).

Cultivars	Phosphorus %	Potassium %	Calcium %	Magnesium %	Sodium mg/kg	Iron mg/kg
BP1	0.50b	2.69a	0.07b	0.20a	132.50a	116.91a
Mnandi	0.55a	2.39b	0.09a	0.17b	124.88a	121.08a
Up to date	0.51b	2.66a	0.07b	0.20a	113.00a	101.16a
Bufelspoort	0.51b	2.68a	0.08b	0.20a	127.19a	117.79a
	**	**	**	*	NS	NS
Cultivars	Copper mg/kg	Zinc mg/kg	Manganese mg/kg	Boron mg/kg	Aluminium mg/kg	
BP1	2.61a	21.24ab	14.79a	10.36a	12.27a	
Mnandi	2.21b	23.37a	12.89b	9.83b	11.60a	
Up to date	2.62a	21.16b	14.01a	10.57a	11.87a	
Bufelspoort	2.42ab	25.32a	7.46c	10.04a	12.80a	
	*	*	**	*	NS	

Conclusion

Calcium is an important plant nutrient with many functions, such as strengthening of cell walls, maintaining membrane stability and cell integrity. Without adequate Ca supplies to the plant, most crops will produce lower yields. Overall, the results of this study indicate that different calcium application levels did not influence the morphological development of plants. Yield of potato minitubers was not significantly influenced by different treatments of Ca applied for single harvest, but the total tuber number was affected. Tubers from plants where Ca was applied at 3.45 meq/L in the nutrient solution displayed higher fresh and dry weight content as expected. In terms of macro and trace element content, treatment with a Ca concentration of 8.4 meq/L had as expected the highest Ca and Cu content in minitubers, but had the lowest Mg and Mn concentration.

Mnandi plants performed better in terms of stem number, stem length, root length and shoot dry weight. BP1 plants produced a higher percentage of stolon branching. Cultivars played a significant role in yield distribution where Mnandi plants produced both a higher number of minitubers per harvest and a higher total fresh weight of potato minitubers. Up-to-date tubers had the highest dry weight. Nutritional results concluded that Mnandi minitubers had a higher concentration of P and Ca. BP1 minitubers had a higher concentration Mg, Cu and Mn. Up-to-date minitubers had a higher Mg, Cu and B content. Buffelspoort minitubers were rich in Mg and Zn.

For Ca nutrition, the use of aeroponics is more effective as it reaches all parts susceptible to increased Ca assimilation into tubers as the spray can attain underground parts such as roots and even stolons for better absorption.

Aeroponics appears to have a potential advantage to make potato production and multiplication more efficient and convenient by reducing generations of seed potato multiplication, allowing bulking up of large numbers of potato seeds. Thus, by reducing unnecessary costs of potato seed tubers, potato producers will eventually lower potato tubers price on the market of fresh and processing potatoes. In East Asia, Africa and Latin America there is need for increased potato production to meet increasing demands for food from human population growth during a period of environmental and climate change (Ducreux et al. 2005). With aeroponics, the use of chemicals such as pesticides, fertilisers and energy are easier to be monitored, especially when using a recirculating

system of nutrient solution and reduce the risk of residue moving to rivers and other subterranean water table. The system could also be associated to environment friendly energy by using solar panels or wind to sustain its functionability and avoid the use of fossil energy. The most important benefit aeroponics offer is that the harvesting of minitubers is very convenient, clean, and permits a greater size control through sequential harvesting (Ritter et al. 2001). These cultivars would need to be studied in the same system to confirm the actual trend.

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Chapter 4

The effect of irrigation frequency on the growth, tuber size distribution and final yield of potato (*Solanum tuberosum* L.) minitubers produced in aeroponics

Abstract

Water is the most important factor controlling plant growth. Improving the water and fertiliser use efficiency of potato production, including seed production should be a priority for the agriculture sector. Therefore, soilless production of potato seed tubers is suitable for areas having a limited water supply. The present study attempted to analyse the effect of fertigation frequency using aeroponics on the growth, final yield and nutritional content of four different potato plant cultivars. The trial was laid out as a factorial based split plots design with two factors and five replications per treatment combination. Irrigation frequency (20, 30, 40 and 50 minutes interval) was the main plots and the four potato cultivars (Up-to-date, Mnandi, Buffelsoort and BP1) constituted the subplots. Significant differences were noticed in the interaction between irrigation frequencies and cultivars for the percentage tuberised plants and stolon and tuber dry mass. When irrigated every 40 minutes, only 48% of the Buffelsoort plants produced tubers at final destructive harvest. Stem number, root length, stolon number, shoot, stolon and root dry mass was significantly lower at the low irrigation frequency. When irrigated every 20 minutes, BP1 plants produced 4.68g of stolon and tuber dry mass. Plant height was also significantly affected by the interaction between irrigation frequency and potato cultivars with Mnandi producing taller plants when irrigated every 30 minutes. BP1 plants produced the shortest stems with an average of 18.71 cm, while Mnandi plants initiated the longest roots with an average of 40.81cm and the longest shoot dry mass with an average of 6.24g. The interaction between irrigation frequencies with cultivars on the total fresh and dry weight was significant. Up to date produced the highest fresh weight when irrigated every 20 minutes while Mnandi had higher fresh weights when plants were irrigated every 30 minutes. Total tuber number and tuber fresh and dry weight was higher at the low irrigation frequency with a total of 39.95 tubers. Mnandi produced the highest total tuber number of 35.90 tubers at a fresh weight of 142.66 g. The interaction between irrigation frequencies and cultivars on the response to macro and trace elements was not significant for sodium and iron but was significant for phosphorus, potassium, calcium, magnesium, copper, zinc, manganese, boron and aluminium. Irrigation frequencies applied did not influence potassium and aluminium. Of all macro and trace elements analysed, only sodium and zinc were not influenced by cultivars.

Keywords: irrigation frequency, water, cultivar, potato.

Introduction

The potato (*Solanum tuberosum* L.), indigenous to the Peruvian Andes Mountains in South America (Steyn 1999), is globally the fourth most important staple food after wheat, rice and maize (Hawkes 1992). Existing projections for the future show that production is going to decrease moderately in Europe, increase marginally in the United States of America and more significantly in Africa, Latin America and Asia (Alonso 1996). It is estimated that potato consumption per capita annually ranges from 55 kg in developed countries to 11 kg in developing countries (FAO 1995). South Africa is among the five largest potato producers in Africa, annually producing approximately 42 tons per hectare (Potatoes South Africa 2013). One of the major potato production areas in South Africa is the semi-arid Sandveld region in the South-west coast, where 14% of the national potato production comes from (Franke et al. 2011). One of the main constraints in the culture of potato is the cost of producing seed tubers since this can account for between 30 and 50% of the total production expenses. Another limitation is the long asexual propagation cycle during which infection by viruses or bacteria can give rise to degenerative diseases (Correa et al. 2009).

Water is the most important factor controlling plant growth (Wesseling and Feddes 2006). Prediction of how water flows to plant roots has been a scientific challenge for centuries, and if we could be able to determine exactly when to irrigate to optimise plant growth, water could be saved (Geen et al. 2005). The impact of climate change on potato yields will vary per region, but in general the impacts of changing rainfall and temperature will be negative in sub-tropical regions (Haverkort and Verhagen 2008). In semi-arid climates like in South Africa where potatoes are cultivated primarily on irrigated land and shows a positive response both in quantity and quality aspects (Wright and Stark 1990, Shock et al. 1993, Lynch et al. 1995), indirect impacts of climate change such as saline intrusion could hamper potato production and irrigation potential (Hengsdijk and Verhagen 2013). Improving the water use efficiency of potato production, including seed production should be a priority for this sector of agriculture.

Water requirements for greenhouse crops are usually high. However, as high yields are obtained on relatively very small areas, the water use per unit of production can be very low. Therefore, greenhouse cultures are suitable for areas having a limited water supply (Hanan et al. 1978). Several scientific studies describe how adequate irrigation water supplied before and during tuber initiation can increase the number of tubers per plant and the average tuber size (Kleinkopf 1983, Shock et al. 1992; Hang and Miller 1986, Eldredge et al. 1996, Shock et al. 1998). Considering that irrigation water is expensive and limited in the semi-arid areas like Southern Africa, and that fertilisers above the threshold level often prove ineffective for production purposes while eventually damaging the environment (Irena et al. 2001) a balance should be found between expected profits, capital availability, quality and the amount of water available (Hagin and Tucker 1982). This would improve producers' income by saving water and reducing fertilisers costs and loss.

Development of sustainable irrigation practices will require a better understanding of the biophysical processes of root-water uptake, and transpiration from plant canopies (Green et al. 2005). Improvements in irrigation management are a way of increasing agricultural production and reducing the demand for water (Perry et al. 2009). Because of the easy access to the roots, aeroponics has been used as a research tool since the 1940s, with work done using vegetable crops first done in the early 1970s (Nir 1981). Aeroponics consists of culturing plants in a system in which the roots of the plants are suspended in a closed dark chamber and a balanced nutrient solution containing all of the essential components necessary for plant growth and development is sprayed directly onto the roots (Correa et al. 2009). There are several advantages to the use of aeroponics in the cultivation of seed potatoes over more conventional methods such as a high rate of tuber multiplication, absence of risk of tuber contamination by soil pathogens, lower incidence of physiological disorders, elimination of the need for soil sterilisation and easier system management (Ranalli 1997, Medeiros 2001, Correa et al. 2008, Correa et al. 2009).

The present study attempted to analyse the effect of fertigation frequency using aeroponics on the growth, yield and nutritional content of four different potato plant cultivars.

Materials and methods

Plant material

The experiment was carried out at Welgevallen, the experimental farm of the University of Stellenbosch in the Western Cape, in South Africa. Potato plantlets (*Solanum tuberosum* L.) from the Agricultural Research Council (ARC) were obtained by *in vitro* subculture procedures which were supplemented with 0.5 mg L⁻¹ thiamine, 100 mg L⁻¹ myo-inositol, 0.5 mg L⁻¹ pyridoxine, 0.5 mg L⁻¹ biotin, 30 g L⁻¹ sucrose and solidified with 7 g L⁻¹ agar with pH adjusted to 5.5±0.1 using 0.1 M NaOH (Murashige and Skoog 1962) were used for this study. Mnandi, Up to date, Buffelspoort and BP1 cultivars which are all commonly used cultivars in the Western Cape were selected (Potato South Africa 1998/99). Buffelspoort, a medium-short (80 to 100 days) growing cultivar with tuber dormancy period of 50 to 70 days has a strong, upright stem with a rapid development and good leaf coverage (Denner and Venter 2011). Up-to-date also has a strong and spreading stem, good leaf coverage with a growing period of 90 to 120 days and a dormancy period of 50 to 70 days. Progeny tuber is often shortened with periods of heat and/or water stress during the growing season (Niederwieser 2003). Mnandi plants are tall with erect stems which develop rapidly and give good to dense foliage cover. It is a well-adapted easy growing variety that does well under various growing condition with the growing period ranging between 90 to 120 days. Tuber dormancy generally takes 70 days from off (Potatoes South Africa 2013). BP1 is a medium

maturing cultivar which generally takes 90 to 110 days before maturing with a short dormancy period of about 50 to 70 days (Kempen 2007).

In vitro plantlets were removed from the culture medium and the roots washed to remove the agar growing medium. Plantlets were planted in a mixture of perlite (50%) and vermiculite (50%) and acclimatized for thirty-two days in a temperature controlled glasshouse at 15/20°C night/day, where they easily developed roots. An overhead transparent polyethylene sheet covered the plantlets for the first week to reduce the stress of transplanting. Each plantlet was irrigated twice daily (before 09:00 in the morning and after 16:00 in the afternoon) with a nutrient solution at an electrical conductivity (EC) of 0.5 cm⁻¹.

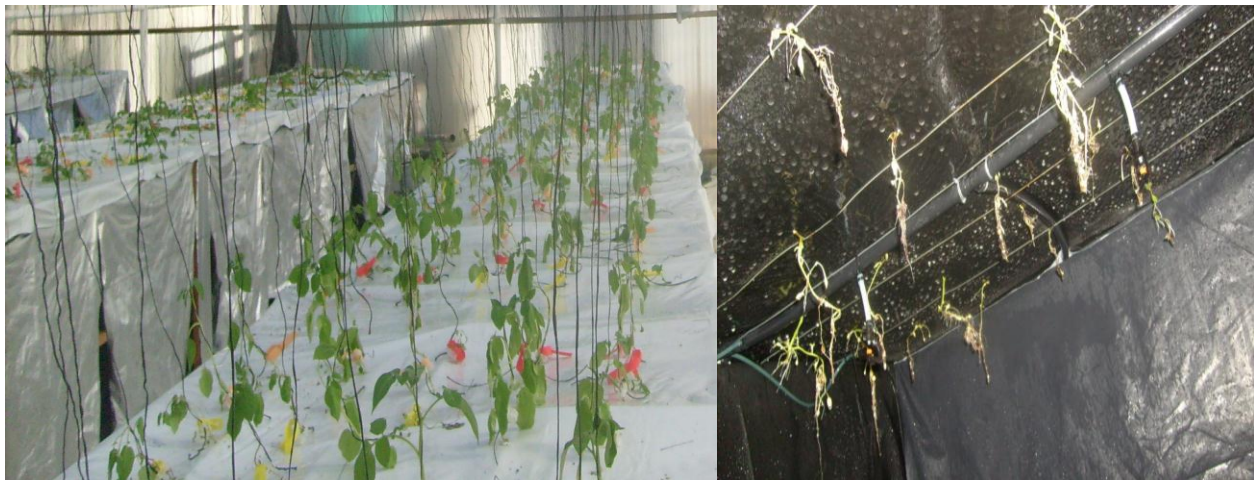
Aeroponics system

Acclimatized plants were transplanted to their permanent polyethylene covered tunnel on Tuesday the 16th of April 2013. The aeroponic system consisted of 20 different growth chambers, each 1 m deep, 1.25m wide and 1.20 m long, with two sided openings for controls and harvestings. The interior was made of black polyethylene sheet to prevent light from entering the dark compartment and the bottom liner sheet had perforations for draining. The exterior was covered with an aluminium sheet to prevent any heat to build inside the chamber. A transverse cut was made to permit insertion and removal of the seedling and allow radial expansion of the plant stem. Between one-half and two thirds of the length of the stems was placed inside the dark chamber (Farran and Mingo-Castel 2006). In order to allow complete darkness, silver tape was rolled around the stems. Underground plant parts were periodically sprayed using six fog nozzles (Farran and Mingo-Castel 2006). Plants showing symptoms of chlorosis or growth defects as well dead plants were removed 7 days after transplanting and replaced by healthy ones. In order to keep the trial disease free, a spraying program from Syngenta was applied (Table A1 appendix).

Four different irrigation frequencies were used; once every 20 minutes, once every 30 minutes, once every 40 minutes and once every 50 minutes. A complete Steiner (1984) nutrient solution with fixed nutrient ratios at an EC of 1.5 mS.cm⁻¹ was used (Table 4.1).

Table 4.1 Composition of the standard Steiner nutrient solution used for fertigation at an EC of 1.5 mS.cm⁻¹.

Macronutrients	Application (g 1000L ⁻¹)
KNO ₃	227.25
K ₂ SO ₄	195.75
KH ₂ PO ₄	102
Ca(NO ₃) ₂ .2H ₂ O	675
MgSO ₄ .7H ₂ O	369
Micro-nutrients	Application (g 1000L ⁻¹)
Fe-EDTA	86.9
MnSO ₄	18
ZnSO ₄	10
CuSO ₄	1.2
H ₃ BO ₄	22
MoCl ₅	1.9

**Plate 4.1** Potato plantlets just after transplantation into the aeroponic system.*Data collected*

The EC and pH were controlled manually each time when refilling the nutrient solution tank every two weeks. Temperature and relative humidity (RH) was measured with a thermohygrometer model HI 9161C (Hanna instruments) inside the growth chambers and light intensity measured using a ceptometer (model LP-80 PAR/LAI Ceptometer AccuPAR) throughout the entire growing season. Measurements were taken twice daily, at 08:30 and at 13:00. During the cool period, the mean temperature was 14.26°C, RH

89.22 and light intensity $34.15\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. During the warm period, the mean temperature was 20.23°C , RH 93.68% and light intensity $288.48\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Table 4.2 Minimum, maximum, average temperature and relative humidity in the greenhouse throughout the growing season.

	Temperature ($^{\circ}\text{C}$)	Relative humidity (RH)
Minimum Reading	3.1	14.7 %
Maximum Reading	42.1	100.0%
Average Reading	15.3	77.9 %

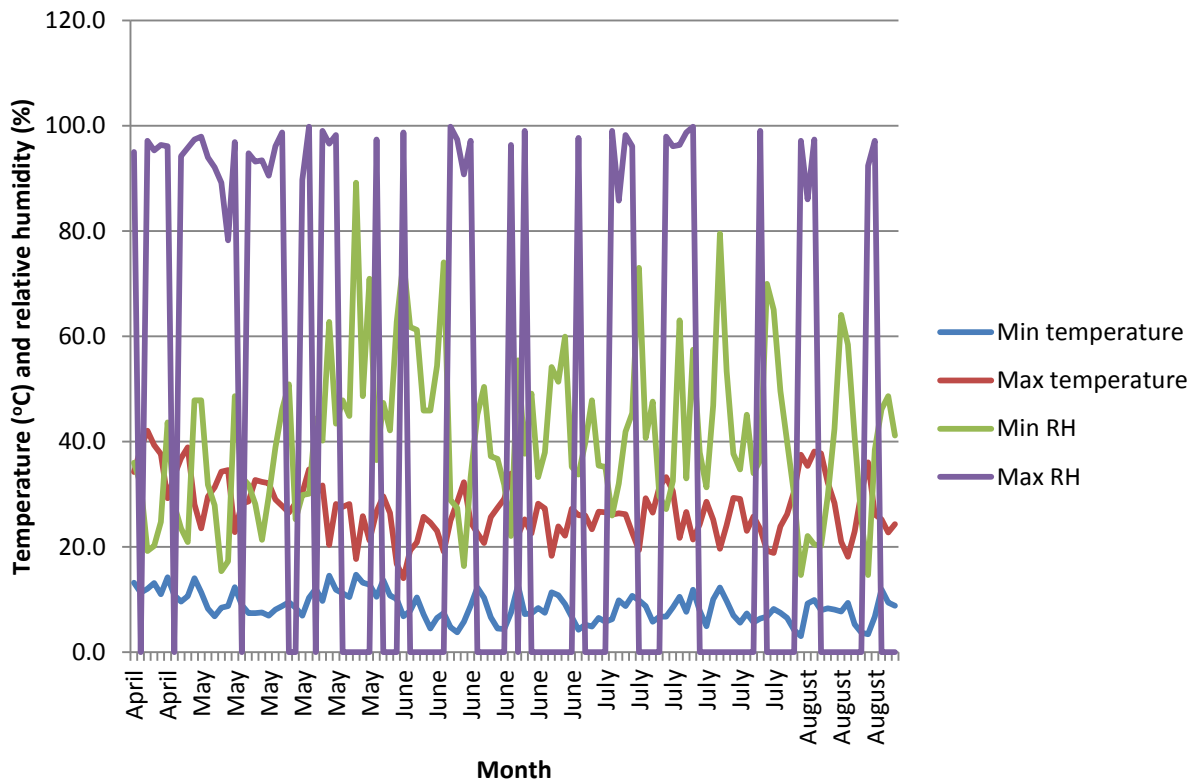


Figure 4.1 Five day interval measurement of temperature and relative humidity inside the polyethylene covered greenhouse.

Morphological parameters such as leaf number, root length and stolon number were noted upon transplanting plantlets into the aeroponic system. Plant height was recorded weekly and minitubers larger than 20 mm in size were removed every ten days from all plants in a total of eight harvest. The numbers of minitubers and total tuber weight as well as the percentage firmness were recorded at every harvest. At the final destructive harvest the percentage tuberised plants, percentage stolon branching and stolon number was also taken into consideration.



Plate 4.2 Samples of minitubers after final harvests.

After harvest tubers were cut into small pieces, washed and dried in an oven at 80°C for two days, and grounded. Tubers were processed with their skin. For each cultivar, five samples, each consisting of three tubers from one plant, were used for macro and trace elements content analyses. For these analyses, the procedure described by Andre et al. (2007) was followed. Samples, 300-500 mg dry weight, were subjected to acid digestion. Reagent (4.4 mL) (0.42 g of selenium, 14 g of Li₂SO₄, 350 mL of H₂O₂, and 420 mL of H₂SO₄) was added to the sample in a Kjeldahl flask. Acid digestion was performed by increasing temperature until the digest had cleared. At the end of the procedure, samples were diluted with H₂O up to 75 mL and kept at 4°C prior to analysis. Blank digestions were performed in the same way. Samples were analysed by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Varian, Palo, CA).

Treatments and experimental design

The trial was laid out as a factorial based split plot design with two factors and five replications per treatment combination. Irrigation frequency (20, 30, 40 and 50 minute interval) was the main plots and the four potato cultivars (Up to date, Mnandi, Buffelsoort and BP1) constituted the sub plots. Data was analyzed using ANOVA, and mean comparison ($P < 0.05$) using the general linear model of Statistica software *Statistica 11* (Statistica 2011). Data was also subjected to the variance analysis technique through the method of minimum significant differences (Box et al. 1989).

Results and discussion.

Morphology

Significant differences were noted in the interaction between irrigation frequencies and cultivars for the percentage tuberised plants and stolon and tuber dry mass (Figure 4.2). When irrigated every 40 minutes (F3), 48% of the Buffelspoort plants produced tubers at final destructive harvest, while 41% of the Up to date plants and 39% of the Mnandi plants produced tubers. The lowest rate of tuber production was observed with the Up to date plants with 2% when irrigated every 50 minutes (F4) and Mnandi plants with 5.33% when irrigated every 20 minutes (F1). Although not significantly so, it appears that tuberisation of plants was encouraged when crops were fertigated at a 40 minute interval regardless of the cultivar used. This could be due to the level of moisture content and stress level of plants encountered while using the 40 minute intervals. Plants may have suffered from stress at 20 and 30 minute irrigation intervals. The higher water content and humidity in the growth chamber where underground parts were enclosed could have resulted in the potato plants slowing down physiological processes such as tuberization and assimilate translocation. At 50 minute intervals, plants suffered from lack of water and nutrients necessary for the plant growth and development. Figure 4.2 shows that a low rate of tuberisation took place when potato plantlets were irrigated at 20 (F1) and 50 (F4) minute intervals, indicating that extreme conditions of moisture negatively influenced tuber development. In terms of the percentage tuberized plants the cultivars used also responded differently to the irrigation frequency. Mnandi seemed more sensitive to over-irrigation (F1) while Up-to-Date was more sensitive to long dry periods between irrigations (F4). The availability of different potato cultivars is important for optimum production since specific cultivars are adapted to specific environmental conditions and final uses (McGregor et al. 2000). From results obtained in Figure 4.2, it is relevant for the producer to know the duration of the growing season, irrigation availability and mostly the final aim of potato tubers before using the aeroponic system.

The stolon dry weight was significantly affected by the irrigation frequencies applied to the different cultivars (Figure 4.2). Irrigation frequencies did not strongly affect stolon growth of the different cultivars except that it was significantly lower for Up-to-Date at the low irrigation frequency (F4) compared to all the other treatments. Mnandi had a lower stolon dry weight at the high irrigation frequency (F1) compared to the other cultivars at this irrigation frequency and the highest stolon dry weight at the low irrigation frequency. Gawronska et al. (1990) reported the existence of varietal differences with respect to rate of net photosynthesis and dry matter production.

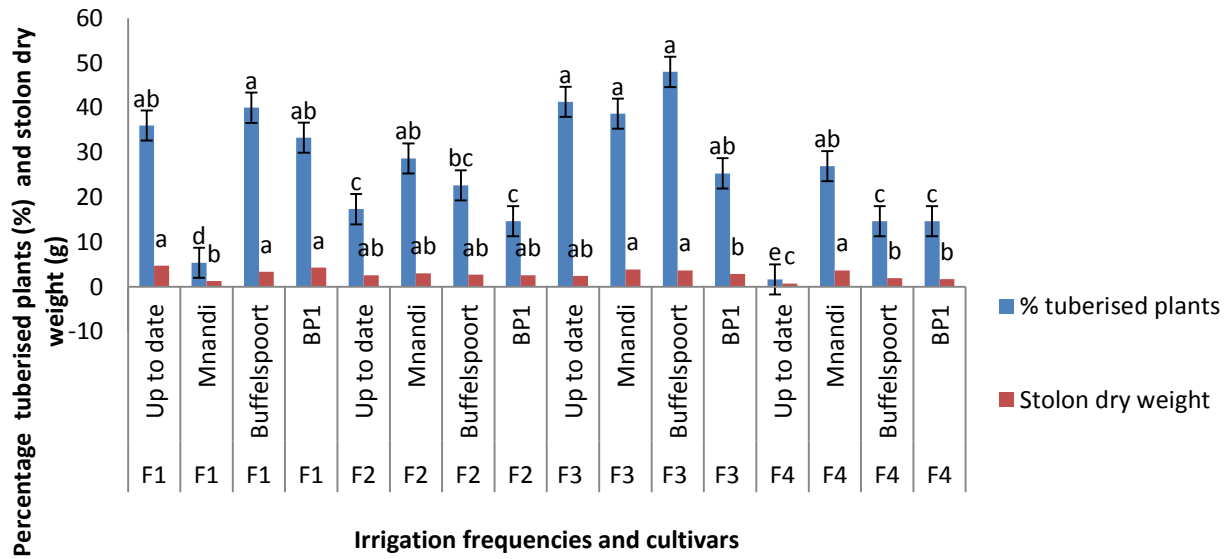


Figure 4.2 Interaction between irrigation frequencies and cultivars on percentage tuberized plants and stolon dry weight of potato plants grown in an aeroponics system. With F1 as fertigation every 20 minutes, F2 as fertigation every 30 minutes, F3 as fertigation every 40 minutes and F4 as fertigation every 50 minutes. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non-significant (NS). Treatment means followed by different letters differ significantly ($P < 0.05$).



Plate 4.3 Three weeks after plantlets were transplanted into the aeroponics system.

There was a significant interaction between the irrigation frequencies and potato cultivars on the plant height (Figure 4.3). At the highest irrigation frequency (F1, every 20 min) plant heights for BP1 and Buffelspoort were significantly lower than that of Mnandi and Up-to-date. Irrigating plants every 30 minutes resulted in a significant decrease in plant height for BP1 compared to all the other cultivars and irrigation frequencies. Comparing different cultivars and locations, Caesar et al. (1981) noted that the potato crop loses a higher quantity of water than of a dry matter. This could explain the presence of an important stem number in F1 were plants received water and nutrient solutions regularly than others besides genetic traits of cultivars that could influence height of crops as well. However, biomass production depends on leaf canopy size and duration over the growing period to intercept radiant energy which is related to growth regulators influenced by genes in the cultivar (Van der Zaag 1984).

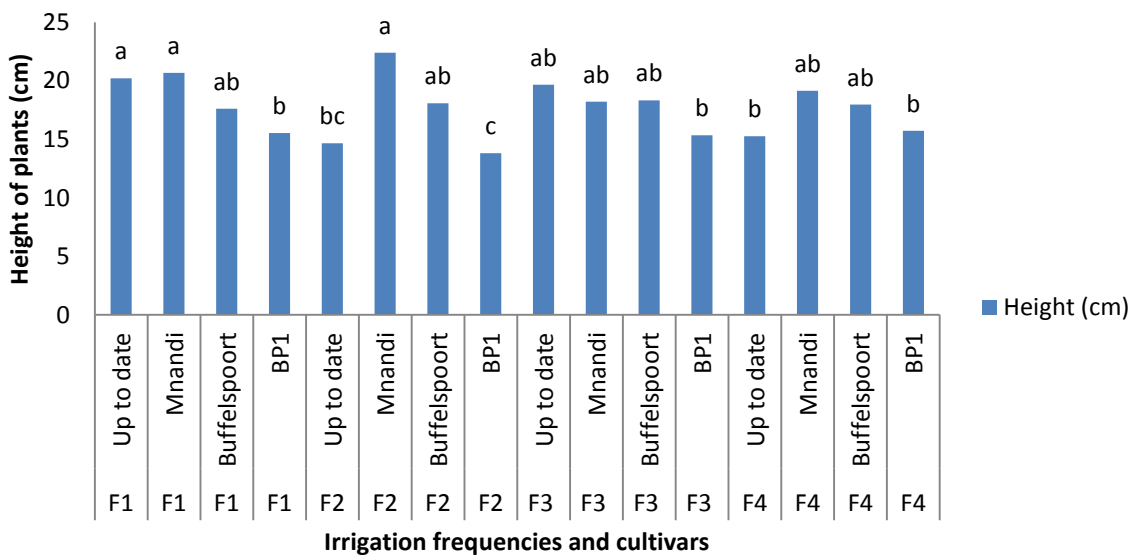


Figure 4.3 Interaction between irrigation frequencies and cultivars of potato plants grown in aeroponics system on height after (cm). With F1 as fertigation every 20 minutes, F2 as fertigation every 30 minutes, F3 as fertigation every 40 minutes and F5 as fertigation every 50 minutes. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non-significant (NS). Treatment means followed by different letters differ significantly ($P < 0.05$).

Beside stem length, irrigation frequencies also significantly influenced the plants on other morphological parameters measured (Table 4.3). Increasing water and nutrient availability increases the plants development throughout the crop cycle. Although the differences were not statistically significant for all measured factors, the crop growth tended to be negatively affected at the lowest irrigation frequency (F4; every 50min) while remaining the same at all the other irrigation frequencies (Table 4.3). As expected,

treatments receiving more water developed well established crops with larger above and underground parts plant. At the high irrigation frequency biomass accumulation was higher, represented by an increase in root and shoot development. While the potato crop is characterised by a small and fine structured root system with a maximal root depth of 60 cm (Brouwer et al. 1976), these results showed that when potato plants are irrigated as often as every 20 minutes, abundant roots will develop (Table 4.3). Stem number, root length, shoot, stolon and root dry mass was significantly lower at the low irrigation frequency when plants were irrigated every 50 minutes compared to the highest irrigation frequency when plants received fertigation every 20 minutes (Table 4.3). Under the experimental conditions described here, there is a clear pattern showing crop vegetative performances correlated to the amount of fertigation allocated. The increase in irrigation frequency therefore increased the availability of both water and nutrients for plant uptake. According to De Wit (1967), the maximum productivity of a crop is reached when it has a closed leaf canopy and is well watered, with transpiration meeting the evaporative demand. Carbohydrates derived directly from current photosynthesis account for 90% of the dry weight in a crop (Guo et al. 2012).

Table 4.3 The means of morphological characteristics measured for potato plant grown in an aeroponics system using four different irrigation frequencies. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non significant (NS). Treatment means followed by different letters differ significantly ($P < 0.05$)

Irrigation frequencies	Stem Number	Stem Length (cm)	Root Length (cm)	% tuberised Plants	% Stolon Branching	Stolon number	Shoot dry weight (g)	Stolon dry weight (g)	Root dry weight (g)
F 1 (20 min)	1.31a	23.19a	47.13a	28.66ab	15.86ab	2.85a	7.03a	3.42a	4.78a
F 2 (30 min)	1.10ab	20.93a	34.98b	22.33b	9.64ab	1.96a	5.56b	2.91ab	4.84a
F 3 (40 min)	1.21ab	21.24a	31.97b	38.3a	21.56a	2.76a	4.80b	3.34ab	4.62b
F 4 (50 min)	0.93b	22.41a	30.69b	17.65b	8.69b	1.55a	5.23b	2.48b	4.77ab
	*	NS	**	**	**	NS	**	NS	*

Stem number, percentage tuberised plants, percentage stolon branching, stolon number, stolon dry weight and root dry weight were not significantly different between the cultivars used (Table 4.4). Although not statistically significant, Mnandi tended to develop more roots and shoots while BP1 had shorter stems (Table 4.4). BP1 plants produced shortest stems with an average of 18.71 cm, while Mnandi plants initiated longest roots with an average of 40.81 cm and highest shoot dry mass with an average of 6.24 g. Kolbe and Stephan-Beckmann (1997) found that the development of the root and stolon dry matter happens during the vegetative period on a 1:10 scale in relation to the tuber dry matter comprising about 2% roots and 1% stolons.

Table 4.4 The means of morphological characteristics measured for potato plants grown in an aeroponics system using four different cultivars. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non significant (NS). Treatment means followed by different letters differ significantly ($P < 0.05$)

Cultivars	Stem Number	Stem Length (cm)	Root Length (cm)	% tuberised Plants	% Stolon Branching	Stolon number/plant	Shoot dry weight (g)/plant	Stolon dry weight (g)/plant	Root dry weight (g)/plant
Up to date	1.16a	22.16ab	37.13ab	24.91a	12.53a	2.39a	5.76ab	2.78a	4.68a
Mnandi	1.21a	24.12a	42.36a	25.56a	12.58a	2.04a	6.43a	3.06a	4.84a
Buffelspoort	1.13a	21.80ab	33.87b	34.33a	16.79a	2.51a	5.27b	3.39a	4.79a
BP1	1.05a	19.49b	31.40b	22.66a	13.85a	2.19a	5.17b	2.93a	4.70a
	NS	*	**	NS	NS	NS	*	NS	NS



Plate 4.4 Plant growth at the final destructive harvest ten weeks after transplanting into the aeroponic system.

Yield

The interaction between irrigation frequencies and cultivars on tuber fresh and dry weight was significant (Figure 4.4). Up-to-Date plants irrigated every 20 minutes produced the highest tuber fresh and dry weight (40.93 g and 10.92 respectively). None of the other cultivars showed a significant difference in tuber fresh or dry weight as the irrigation frequency was adjusted. Buffelspoort had a lower tuber fresh weight at the low irrigation frequency (F4) compared to Mnandi and Up to Date at the higher irrigation frequency (F2). Mnandi and Up to Date therefore seems to benefit more at the higher irrigation frequencies while Buffelspoort is more sensitive to the lower irrigation frequency. Up to Date had a lower tuber dry weight at the low irrigation frequency (F4) compared to the high irrigation frequency (F1). Overall the tuber fresh and dry weight was not greatly affected by the irrigation frequency and we can assume that this is more a function of the capacity of the plant to produce assimilates. According to Baumann (1957), the potential osmotic values increase by about 25% during the growth of tubers under optimum environmental conditions. Moreover, very high respiration rates are shown in young tubers followed by a clear decrease until the period during which rates of starch accumulation decline (Kolbe and Stephan-Beckmann 1997). For all potato cultivars, Hunnius (1974) found that the maximum starch yield accumulation is reached when more than half of the leaves are dead and the stems begins to die. With this current experiment, plants were harvested before natural senescence in order to do the analysis mentioned in Tables 4.1 and 4.2. Though tuber dry matter content is influenced by tuber size, environmental conditions and cultural practices, tuber dry matter content appears to be genetically controlled (Dean 1994). Onder et al. (2005) suggested that potato seed producers and farmers in general should not be advised to grow potato under water deficiency of more than 33% of the irrigation water requirement.

Irrigation frequency significantly influenced the number, fresh and dry weight of minitubers harvested during the 8 harvests (Table 4.3). Each irrigation frequency displayed a particular trend in tuber initiation over time. When plants were fertigated every 20 minutes for 30 seconds, the higher water and fertiliser availability to plants resulted in a higher productivity capacity for tuber growth and the number of tubers as well as the fresh and dry weight was higher compared to the low irrigation frequency. Total tuber number was higher when irrigated every 20 minutes, with a total of 39.95 tubers/plant (Table 4.3). Clearly water and fertiliser regulate development and yield of potato crop. Probably the low frequency irrigation of every 50 minutes produced a small architecture and small sized-minitubers and a low number of tubers because of the limitation in water necessary for the synthesis and translocation of assimilates. Our estimates are not significantly different from conclusions of water use efficiency reported by Steduto and Albrizio (2005), for instance, Kirda (1982) reported a yield of 79% with relative water use efficiency of 1.06 was obtained when 25% deficit of evapotranspiration was prevailed for the whole season of potato.

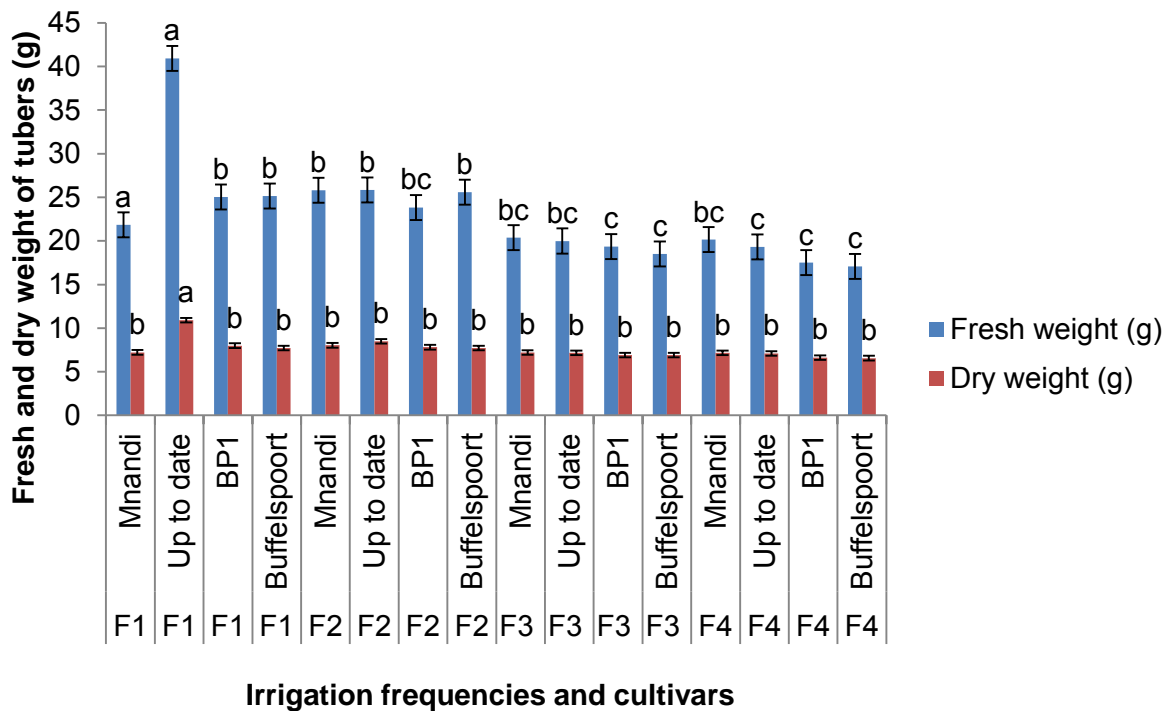


Figure 4.4 Interactions between irrigation frequencies and cultivars on fresh and dry weight of potato minitubers grown aeroponically. F1 = fertigation every 20 minutes, F2 = every 30 minutes, F3 = every 40 minutes and F4 = every 50 minutes. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non-significant (NS). Treatment means followed by different letters differ significantly ($P < 0.05$).

Total plant number alive was also significantly affected by irrigation duration (data not shown). Statistically, irrigation is acceptable up to 40 minutes intervals and above that threshold, mortality dramatically increased among crops. Plants irrigated each 30 minutes had the best plant survival. Low canopy coverage during the early growing season might increase evaporative water losses and lead to severe consequences. Death of plants can occur if sufficient measures such as proper irrigation are not taken in time (Yuan et al. 2013). This result could explain the high rate of mortality localised among plants irrigated only between 40 and 50 minutes. The trial also started later than the optimum growing season of potato plant, crop could not get enough light and heat for optimum growth.

The tuber fresh weight of the eight different harvest intervals was significantly influenced by irrigation frequencies used, and in most cases linear to the mean number of minitubers (Table 4.5). According to Perry et al. (2007) a higher yield can be achieved by maintaining relatively high water content conducive to plants. The high irrigation frequency facilitated better water and nutrient availability to the crop root zone (Segal et al. 2000). The good performances recorded with irrigation at an interval of 20 minutes

could also be due to more resources that were available and less stress to initiate more minitubers. According to Kolbe and Stephan-Beckmann (1997), after tuber initiation very high respiration rates are localised in young tubers followed by a clear decrease until the period during which rates of starch accumulation decline. The higher moisture content as well as the lower temperature when irrigating plants every 20 minutes could be very beneficial for tuber respiration and allow better tuberization and dry matter accumulation. According to several authors (Van Loon 1981, Schapendonk et al 1989), in summer the potato has a low tolerance for water stress because of a shallow root zone system. Therefore, timing and duration of water stress during the different growth stages influences the crop yield (Patel and Rajput 2007). King et al. (2003) reported that the total potato yield to be reduced mainly when deficit irrigation was applied during mid and mid late bulking regardless of water stress intensity. Results of differential irrigation experiment by Patel and Rajput (2007) showed that potato yield decreased with decreasing amount of irrigation water while using 5 different treatments with drip irrigation in sandy soil. This result is not in accordance with ours, irrigation of plants every 40 minutes had higher yield than irrigation of plants every 30 minutes (Table 4.5). Steyn et al. (1998) reported significant tuber yield and size reductions with the reduction of applied water, but they also noticed significant differences among genotypes in response to water stress. So, our result is not in agreement with previous studies (Meiros et al. 2001, Hegney and Irena et al. 2011) which found that potato yield responds linearly to applied water. Maybe the answer is in the agricultural system applied as neither of them used aeroponics but traditional field with a unique destructive harvest.

Total production tuber fresh and dry weights were significantly influenced by different irrigation frequencies used, and linearly closely related as illustrated in table 4.5. Plants irrigated every 20 minutes had the highest tuber fresh and dry weights, (161.02 g and 20.13 g respectively). Increasing irrigation frequency increased the canopy size, which in return increased the accumulation of photosynthetic radiation throughout the increase of leaf area index and leaf area duration (Tei et al. 2002). After tuberization, a distinct increase in the rate of photosynthesis can be seen which is often associated with an exponential tuber growth (Kolbe and Stephan-Beckmann 1997). In general for potato tuber, the course of starch accumulation is similar to that of dry matter and reaches maximal values between 90-110 days after planting, thereafter, the starch content decreases slightly until complete maturity (Putz 1978). Desborough (1985) found that young and small tubers in comparison to larger and older ones have relatively high protein and very high nitrate contents and only low values of starch. This result is similar to dry weight shown in Table 4.5 where plants irrigated every 50 minutes produced the smallest biomasses, with reduced tuber sizes and a higher rate of resorptions.

Table 4.5 The number of tubers per harvest (H), total tuber number, fresh weight of tubers (g), total tuber fresh weight, dry weight of tubers (g) and total tuber dry weight (g) of eight different harvest intervals of potato minitubers (in g) grown in an aeroponics system using four different irrigation frequencies. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non significant (NS). Treatment means followed by different letters differ significantly ($P < 0.05$)

Frequencies	Number of tubers per plant								Total tuber number
	H1	H2	H3	H4	H5	H6	H7	H8	
F1 (20 min)	0.75a	1.27a	1.27a	1.26a	1.42a	1.34a	1.16a	0.60a	39.95a
F2 (30 min)	0.47ab	0.39c	0.52b	0.66ab	0.63b	0.32b	0.15b	0.09b	15.20b
F3 (40 min)	0.18b	1.05ab	0.8ab	0.74ab	1.00ab	0.73ab	0.32b	0.32ab	22.35ab
F4 (50 min)	0.29b	0.75bc	0.65b	0.51b	0.31b	0.44b	0.44b	0.44ab	14.45b
	**	**	*	*	**	**	**	*	**
Frequencies	Fresh weight of tubers								Total tuber fresh weight
	H1	H2	H3	H4	H5	H6	H7	H8	
F1 (20 min)	4.54a	5.41a	5.40a	4.94a	4.63a	4.90a	3.57a	1.95a	161.02a
F2 (30 min)	2.46ab	1.49c	1.67b	1.87b	1.34b	0.82c	0.34b	0.19b	47.86c
F3 (40 min)	1.17b	4.30b	2.35ab	2.35ab	2.02b	2.15b	0.97b	1.00b	92.34b
F4 (50 min)	1.15b	2.90bc	2.33b	2.34b	0.75b	1.2bc	0.75b	0.98b	45.29c
	**	**	**	*	**	**	**	*	**
Frequencies	Dry weight of tubers								Total tuber dry weight
	H1	H2	H3	H4	H5	H6	H7	H8	
F1 (20 min)	1.51a	1.80a	1.8a	1.64a	1.54a	1.63a	1.19a	0.65a	20.13a
F2 (30 min)	0.82ab	0.49c	0.56b	0.62b	0.45b	0.27c	0.11b	0.06b	5.98c
F3 (40 min)	0.39b	1.43b	0.78ab	0.78ab	6.06b	0.71b	0.32b	0.33b	11.54b
F4 50 min)	0.38b	0.96bc	0.77b	0.78b	0.25b	0.4c	0.15b	0.33b	5.66c
	**	**	**	*	**	**	**	*	**

The number of tubers, fresh and dry weight of tubers differed significantly between the different cultivars (Table 4.6). A strait linear correlation between total numbers of minitubers per cultivar and fresh weight of tubers for the eight different harvest intervals was noticed. Mnandi plants produced the highest yield with a total tuber number of 35.90 tubers and a fresh weight of 142.66 g. Tuber growth had a depressing effect on tuber initiation, which may partly due to competition for assimilate as at certain point, the number of minitubers harvested started to decrease for all cultivars. The only explanation to this result could be genetic because all cultivars received the same treatments, but ended up with different yields, shape and colour of tubers (Plate 4.2). Using aeroponics is advantageous in terms of tuber number and size as there is a possibility of harvesting several times during the same growing season compared to traditional ways of producing potato seed where only one destructive harvest is allowed; consequently less space is used for producing higher yields of potato. According to literature where potato seed is produced using the traditional way, they found that the tuber number can drop markedly until harvest, in case from 18 to 14 tubers per plant due to resorptions (Struik et al. 1988). Also, the resorptions of already initiated tubers take place especially during the second part of the vegetative period (Krijthe 1955, Patzold and Stricker 1964, Moll 1992). According to Tekalign and Hammes (2005), the fact that cultivars exhibited differences regard to tuber yielding could be attributed to variation in days to tuber initiation, rate of photosynthesis, efficiency of assimilate partitioning to the tubers and maturity period length. According to certain studies (Lehmann 1926), there is a high positive correlation between the weight of the tubers and the diameter of cells which is cultivar specific, and is established early in the season. A higher rate of assimilate storage can be observed (Kolbe and Stephan-Beckman 1997) in those particular large tubers.

Table 4.6 The means of number of potato minitubers per harvest (H), total tuber Number, fresh weight of tubers, total tuber fresh weight (g), dry weight of tubers and total tuber weight (g) of minitubers grown in aeroponics system using four different cultivars. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non significant (NS). Treatment means followed by different letters differ significantly ($P < 0.05$)

Cultivars	Number of tubers per plant								Total tuber number
	H1	H2	H3	H4	H5	H6	H7	H8	
Up-to-date	0.51a	0.94a	0.79a	0.58b	0.51b	0.52b	0.38b	0.26ab	19.05b
Mnandi	0.48a	1.1a	1.10a	1.27a	1.28a	1.24a	1.04a	0.63a	35.90a
Buffelspoort	0.36a	0.72a	0.71a	0.90a	0.77ab	0.59ab	0.46b	0.41ab	20.40b
BP1	0.34a	0.71	0.64a	0.52b	0.65ab	0.5b	0.11b	0.14b	16.60b
	NS	NS	NS	*	*	*	**	*	**

Cultivars	Fresh weight of tubers								Total tuber fresh weight
	H1	H2	H3	H4	H5	H6	H7	H8	
Up-to-date	3.55a	4.65a	3.02a	2.23ab	1.48a	1.99b	1.10b	0.9ab	80.46b
Mnandi	2.38a	4.58a	4.62a	4.40a	3.76a	3.76a	3.02a	1.87a	142.66a
Buffelspoort	1.78a	3.00a	2.63a	2.77ab	2.01a	1.75b	1.19ab	0.96ab	66.81b
BP1	1.86a	2.99a	2.26a	1.54b	1.58a	1.38b	0.33b	0.35b	56.59b
	NS	NS	NS	*	NS	*	*	*	**

Cultivars	Dry weight of tubers								Total tuber dry weight
	H1	H2	H3	H4	H5	H6	H7	H8	
Up-to-date	1.18a	1.55a	1.00a	0.74ab	0.49a	0.66b	0.36b	0.3b	8.42a
Mnandi	0.79	1.52a	1.54a	1.46a	1.25a	1.25a	1.00a	0.62a	7.40ab
Buffelspoort	0.59a	0.90a	0.87a	0.92ab	0.67a	0.58b	0.39b	0.32ab	7.34ab
BP1	0.62a	0.99a	0.75a	0.51b	0.52a	0.46b	0.11b	0.11b	7.22b
	NS	NS	NS	*	NS	*	*	*	*

There was a significant interaction between irrigation frequencies and cultivars on the response to macro and trace elements content in potato minitubers grown under aeroponics system, except for that of sodium and iron (Table 4.7). Concentration of Calcium (Ca), Sodium (Na), Iron (Fe), Boron (B) and Aluminium (Al) is very different throughout the development of tubers. In this current study, all minitubers were harvested at the same physiological age of 10 days interval with a total of 8 harvests. The Phosphorus (P) concentration tended to be lower for Up-to-Date and higher for Mnandi and Buffelspoort. The Potassium (K) content was higher for BP1 plants and for

tubers from Buffelspoort and Up to date at the lower irrigation frequencies. The tuber Ca concentration was higher when Mnandi and Buffelspoort plants were irrigated less frequently and lower overall for tubers from BP1 and Up to date. Sufficient Ca content in tubers may also be beneficial because it increases the tuber quality and storability (Olsen et al. 1996). The Magnesium (Mg) content was generally higher in tubers of BP1 plants and increased in tubers from Mnandi, Up-to-Date and Buffelspoort as the irrigation frequency was reduced. The Copper (Cu) content of tubers tended to increase as the irrigation frequency was decreased. A higher Zinc (Zn) content was noticed when Mnandi plants were irrigated every 30 minutes while a lower Zn content was observed when Up to date plants were irrigated every 20 minutes. Zn plays an important role in protecting cellular components from oxidation (Ho 2004). The tuber Manganese (Mn) content did not vary much between treatments but tended to be higher for BP1 plants and reduced in tubers of Up-to-date at the high irrigation frequency. The tuber Boron (B) content was higher when BP1 and Up to date plants that were irrigated every 30 minutes. The tuber Aluminium (Al) content tended to be higher for BP1 plants and lower for tubers from Mnandi. The Buffelspoort tubers had a high Al content at the two higher irrigation frequencies but this was reduced as the irrigation frequency was reduced. Andre et al. (2007) in the study of 74 Andean genotypes found a low level of relationship between genetic diversity and nutritional diversity in potato tubers by using molecular markers between the genetic distances, on the other hand, and distinct or combined nutritional parameters. Where in this current study, the interaction between different irrigation frequencies and cultivars were in some cases strongly influenced by both as shown in Table 4.7. It appears that micro or trace-elements content in particular were significantly correlated to cultivars more than to irrigation frequencies. It is important to underline that besides the genetics, other factors are known to affect the levels of macro and trace element like the environment and technique used to produce potato seed tubers. In most cases, macronutrient concentrations were influenced by moisture content of tubers related to the irrigation frequency in growth chambers.

Irrigation frequencies applied did not have an influence on P, Zn and Al distribution in potato minitubers. However, significant differences had been observed in the partitioning of other macro and trace elements such as illustrated in Table 4.6. Potato is naturally recognised as a source of high quality proteins, carbohydrates, vitamin C, vitamin B₃, vitamin B₆, and certain minerals such as P, K and Mg (Subar et al. 1998). Irrigation supplied at a 20 minute interval yielded tubers with the lowest K concentration of 2.46%, Ca concentration of 0.09% and Fe concentration of 76.01 mg/kg. Potato minituber being a slow transpiring organ could not attract and conserve less mobile minerals such as Ca, Mg, Fe, Cu, Mn and B (Jackson and Carter 1976) similar to what we observed in this study at the higher irrigation frequency of every 20 minutes (Table 4.5). According to Habib and Donnelly (2002), Ca is directly taken up by tubers, and the roots attached to tubers and stolons along with the water uptake although its removal by tubers is not large at optimum temperature for tuberization. It has been noticed in this

experimentation that higher irrigation and moisture levels reduced the concentration of elements in tubers, although there is an increase in total fresh weight and size of tubers. Furthermore, Salisbury and Ross (1992) reported that growing plants in greenhouses where there is a reduced transpiration due to high humidity may cause Ca deficiencies in particular in certain tissues and too rapid transpiration can lead to a toxic build-up of certain elements. In the current study, it has been noticed that the concentration in macro and trace elements at the high irrigation was reduced probably due to the higher moisture content that hinder tubers transpiration in growth chambers.

Table 4.7 Analyse of variance (ANOVA) on the interaction between treatments and potato cultivars on the nutritional values of potato minitubers grown in aeroponics system. Significant F test at $P<0.05$ (*) and $P<0.01$ (**) and non-significant (NS). Treatment means followed by different letters differ significantly ($P<0.05$).

Treatments	Cultivars	Phosphorus %	Potassium %	Calcium %	Magnesium %	Sodium mg/kg	Iron mg/kg
F1 (20 min)	Up to date	0.48b	2.47c	0.08c	0.16c	83.25a	70.67a
F1 (20 min)	BP1	0.53ab	2.65cb	0.08c	0.19ab	95.25a	84.30a
F1 (20 min)	Buffelspoort	0.53ab	2.36c	0.10ab	0.17bc	124.25a	72.35a
F1 (20 min)	Mnandi	0.55a	2.37c	0.09bc	0.16c	105.75a	76.73a
F2 (30 min)	Up to date	0.49b	2.68b	0.09bc	0.20ab	87.50a	80.46a
F2 (30 min)	BP1	0.55ab	2.80a	0.09bc	0.21a	112.25a	82.50a
F2 (30 min)	Buffelspoort	0.55a	2.48c	0.09bc	0.18b	102.75a	79.57a
F2 (30 min)	Mnandi	0.53ab	2.38c	0.13a	0.17bc	91.50a	68.19a
F3 (40 min)	Up to date	0.49b	2.60cb	0.08c	0.19ab	132.00a	93.25a
F3 (40 min)	BP1	0.53ab	2.79a	0.08c	0.21a	130.25a	105.25a
F3 (40 min)	Buffelspoort	0.54ab	2.57c	0.13a	0.18b	130.00a	76.02a
F3 (40 min)	Mnandi	0.57a	2.63b	0.13a	0.19ab	153.00a	92.46a
F4 (50 min)	Up to date	0.52b	2.76a	0.08c	0.20a	141.25a	73.44a
F4 (50 min)	BP1	0.51b	2.72ab	0.08c	0.21a	138.50a	108.5a
F4 (50 min)	Buffelspoort	0.56a	2.81a	0.13a	0.21a	150.25a	85.47a
F4 (50 min)	Mnandi	0.56a	2.59cb	0.14a	0.19ab	114.00a	71.39a
		*	*	*	**	NS	NS
Treatments	Cultivars	Copper mg/kg	Zinc mg/kg	Manganese mg/kg	Boron mg/kg	Aluminium mg/kg	
F1 (20 min)	Up to date	1.85c	23.64d	12.19b	10.64b	10.12c	
F1 (20 min)	BP1	2.59ab	29.87bc	13.78ab	10.39bc	19.23b	
F1 (20 min)	Buffelspoort	1.97bc	29.11c	13.28ab	10.22bc	23.75a	
F1 (20 min)	Mnandi	1.79b	36.33b	13.10ab	9.51c	11.72c	
F2 (30 min)	Up to date	2.23b	27.10cd	14.02ab	11.24a	11.99c	
F2 (30 min)	BP1	2.66ab	29.13cd	15.14a	11.48a	22.06a	
F2 (30 min)	Buffelspoort	2.40ab	32.67c	13.50ab	10.05bc	18.54b	
F2 (30 min)	Mnandi	2.14b	46.65a	13.77ab	10.39bc	10.84c	
F3 (40 min)	Up to date	2.61ab	35.26b	13.36ab	10.14bc	12.91b	
F3 (40 min)	BP1	3.33a	28.52c	15.82a	10.96b	28.57a	
F3 (40 min)	Buffelspoort	2.41ab	33.52bc	13.83ab	10.21bc	11.77c	
F3 (40 min)	Mnandi	2.96a	34.47b	13.97ab	10.55b	12.30c	
F4 (50 min)	Up to date	2.94a	30.99bc	13.26ab	10.57b	12.57bc	
F4 (50 min)	BP1	3.03a	34.26bc	14.18ab	10.36bc	31.90a	
F4 (50 min)	Buffelspoort	3.38a	47.50a	15.00a	10.38b	12.37c	
F4 (50 min)	Mnandi	2.26ab	31.55bc	13.08ab	10.11bc	9.94c	
		*	*	*	*	**	

Irrigation supplied every 30 minutes had an average Ca content of 0.10% in tubers, Mn with 14.10 mg/kg and B with 10.79 mg/kg among cultivars. It appeared that Ca, Mn and B availability increased with augmentation of plant transpiration and are correlated in the distribution within the tuber (Table 4.8). According to Mondy and Munshi (1993), B appears to regulate not only the flux of substrate into the pentose-phosphate cycle, but also lignin biosynthesis via formation of stable phenolic acid-borate complexes, particularly with caffeic acid. These two compounds are the chief phenols of potatoes (Perkins and Aronoff 1956). Whereas Owns (1961) found that B application significantly increased the polyphenol oxidase activities of potatoes which positively influence firmness of tubers.

Irrigation supplied to potato plants every 40 minutes resulted in a higher tuber concentration of Na with an average of 136.31 mg/kg, Fe with an average of 91.74 mg/kg, Cu with an average of 2.82 mg/kg. Some authors found that application of Na-selenite to potatoes increases both total protein and amino acid contents (Munshi et al. 1990) while decreasing nitrate content and the total glycoalkaloid (Munshi and Mondy 1992). A determination coefficient of 0.13 indicates that tuber size may explain 13% of the variability in Fe content, and also Fe composition of fruits and vegetables is a reflection of the environment in which the plant grew in (Anderson et al. 1999). Our potato was cultivated in aeroponics system with a specific environment, which is known to increase the availability of Fe around the underground part plants with a direct nutrient solutions spray (Sarkar and Jones 1982).

Irrigating plants every 50 minutes for 30 seconds with nutrient solution produced the minituber with a high concentration of K averaging at of 2.72% magnesium (Mg) and Ca were also relatively high. Higher concentrations of elements when irrigating plants every 50 minutes displayed a possible link between the stress level of potato minitubers and a higher content of macro and trace elements within the minituber. According to Andre et al. (2007), the ratio of skin to flesh may vary between the samples, according to the tuber size and shape, but may be attributed to a genotypic characteristic and extreme growing environment also need to be considered. For instance, Cieslik and Sikora 1997 found that potassium and calcium affected assimilation and translocation of magnesium. Mg is of important use in future seed potatoes. It enables proper utilisation of nitrogen by the plant and its transformation into organic compounds (Cieslik and Sikora 1997). K has various functions in physiological process in plant such as antiion in transport of ion NO_3 from the roots to overground portions of plants (Kzobus 1990). Literature data indicate that the calcium/magnesium ratio is of significant importance in plants, and a deficiency of both elements is equally as disadvantageous as an excess of Mg in relation to Ca (Buczek et al. 1980). The higher tuber Na concentration when irrigating plants after every 40 and 50 minutes could be explained by a lack of physiological dilution due to the young age and small size of tubers (Kolbe and Stephan-Beckmann

1997) at harvest. A higher content of macro and trace-elements when irrigating plants every 50 minutes could be explained by the effect of encouragement of plants and tubers to transpire more. This association strengthens the hypothesis that an increased rate of transpiration enhances the rate of mineral uptake (Tekalign and Hammes 2005). According to Yuan et al.(2013), under water limited conditions such as in rainfed agriculture in arid or semi-arid regions, the crop yield might be much more sensitive to water availability.

Table 4.8 Effect of different irrigation frequencies on the nutritional value of potato minitubers grown in an aeroponics system. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non-significant (NS). Treatment means followed by different letter symbols differ significantly ($P < 0.05$).

Irrigation frequencies	Phosphorus %	Potassium %	Calcium %	Magnesium %	Sodium mg/kg	Iron mg/kg
F1 (20 min)	0.52a	2.46c	0.09b	0.17c	102.12b	76.02c
F2 (30 min)	0.53a	2.58b	0.10a	0.19ab	98.50b	77.68b
F3 (40 min)	0.53a	2.65ab	0.10a	0.19ab	136.31a	91.74a
F4 (50 min)	0.54a	2.72a	0.11a	0.20a	136.00a	84.70ab
	NS	**	*	*	*	*
Irrigation frequencies	Copper mg/kg	Zinc mg/kg	Manganese mg/kg	Boron mg/kg	Aluminium mg/kg	
F1 (20 min)	2.05b	29.74a	13.09b	10.19b	16.20a	
F2 (30 min)	2.35b	33.89a	14.10a	10.79a	15.85a	
F3 (40 min)	2.82a	32.94a	14.24a	10.47ab	16.39a	
F4 (50 min)	2.90 a	36.07 a	14.03 a	10.35 ab	16.70 a	
	**	NS	*	*	NS	

Of all macro and trace elements analysed in Table 4.9, only sodium and zinc were not influenced by cultivars. Like in the study of Andre et al. (2007), statistically a difference between cultivar concentration of elements could be observed. P concentration was higher in Mnandi plants with an average of 0.55% but low in Up-to-date plants with an average of 0.49%. These results were different from Tekalign and Hammes (2005) where none of the cultivars used had a P content of more than 0.40%. These tubers were however physiologically mature tubers.

The K concentration in tubers depends on the kind and rate of fertilisation, weather and cultivar (Ciecko et al. 1994). In this study K was higher in BP1 plants with an average of 2.74% and lower in Buffelspoort plants with an average of 2.55% and in Mnandi plants with an average of 2.49%. Similar results were noticed by Mazurczyk (1988) where cultivar could be affected by tuber genetic trait rather than external influences. Heisler et al. (1962) established a correlation between low K content and a tendency for potato

tubers to blacken since potassium content is directly proportional to citrate content. No similar description was found among minitubers in the current study, probably due to very weak differences found in potassium content in minitubers.

Ca was higher in Mnandi minitubers with 0.12% and Buffelsoort with 0.11% but lower in Up to date and BP1 minitubers with both having 0.08%. Calcium is an essential component of the plant cell walls, giving mechanical strength (Budde and Chollet 1988), and as a divalent cation, it has structural roles in the cell membranes as a counter cation for anions in the vacuoles (White and Broadley 2003). Calcium deficiency provokes disorders in horticultural crops such as blossom end rot, internal brown spot, hollow heart and soft rot in potatoes (Nookaraju et al. 2012). No minituber showed any internal disease or disorder after dissection and visual analysis of tubers in the current study.

Mg was particularly higher only in BP1 minitubers with 0.20% and lower in other cultivars. Mg is required for the activity of several calcium-regulated enzymes such as protein kinases (Salimath and Marme 1983). In tuber materials a close positive correlation can be found between the contents of K and the contents of Mg as well as of Mn (Kolbe 1995).

Fe as well was only higher in BP1 minitubers with 95.14 mg/kg. Levitt and Todd (1952) reported that as much as one-third of the total Fe is associated with the protein and chemically bound to the protein. However, excess in Fe induces blackening of tubers (Heisler et al. 1963). Our result is completely different from Andre et al. (2007) where a weak but significant correlation was found between Zn and Fe content; but is in accordance to no significant correlation between Zn and Ca contents. The contents in Fe are in the same range as those published by Anderson et al. (1999) for unpeeled potatoes.

Cu was higher in BP1 minitubers with 9.90 mg/kg and lower in Up-to-date minitubers with 2.41mg/kg. Cu serves as an activator for several oxidating enzymes in plant metabolism and is a constituent of an enzyme important in the utilisation of protein and chlorophyll formation (Sprague 1964). Mn was also higher in BP1 with 14.73 mg/kg and lower in Up-to-date 13.21 mg/kg. In general, Cu and Mn become most available at pH 6 or lower, though usually adequate amounts are soluble at pH 7 or less (Buckman and Brady 1969).

B was higher in BP1 minitubers with 10.80 mg/kg and lower in Mnandi with 10.14 mg/kg. B is an essential micronutrient element required for growth and development of vascular plant (Loomis and Durst 1992). Several physiological impairments as a result of B deficiency such as sugar transport, cell wall synthesis, lignification, carbohydrate metabolism, respiration, phenol metabolism and membrane integrity were reported by Parr and Loughman (1983). Al as well was extremely higher in BP1 minitubers with 25.44 mg/kg. Higher aluminium concentration in mature potato tubers grown in clay soil with 51.8 mg/kg was observed by Scancar et al. (2003). This difference could be

explained by the maturity physiologic and the particularity of higher aluminium content in clay soils (Muller et al. 1998, Oniawa et al. 1997).

Minitubers from BP1 were rich in K, Mg, Fe, Cu, Mn, B and Al, pointing out a certain genetic predisposition for this group to accumulate these elements. Mnandi tubers were rich in P and Ca (Table 4.9). As also noticed by Andre et al. (2007), published data on the extent of variation with regard to mineral contents within the potato cultivars used in this paper is scarce.

Table 4.9 Nutritional values of different potato cultivars in an aeroponics system. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non-significant (NS). Treatment means followed by different letters differ significantly ($P < 0.05$).

Cultivars	Phosphorus %	Potassium %	Calcium %	Magnesium %	Sodium mg/kg	Iron mg/kg
Up-to-date	0.49b	2.63a	0.08b	0.18bc	111.00a	79.46b
BP1	0.53a	2.74a	0.08b	0.20a	119.06a	95.14a
Buffelspoort	0.54a	2.55c	0.11a	0.19b	126.81a	78.35b
Mnandi	0.55a	2.49d	0.12a	0.17c	116.06a	77.19b
	**	**	**	**	NS	**
Cultivars	Copper mg/kg	Zinc mg/kg	Manganese mg/kg	Boron mg/kg	Aluminium mg/kg	
Up-to-date	2.41b	29.24b	13.21c	10.65b	11.90c	
BP1	2.90a	30.44a	14.73a	10.80a	25.44a	
Buffelspoort	2.54b	35.70a	13.90ab	10.21bc	16.61c	
Mnandi	2.29b	37.25a	13.63bc	10.14c	11.20c	
	**	*	**	**	**	

Conclusions

Significant differences were noticed in the interaction between irrigation frequencies and cultivars for the percentage tuberized plants and stolon mass. Irrigation frequency, transpiration, biomass and yield varied substantially and significantly between treatments and cultivars. Potato plants that received water regularly at 20 minutes interval presented an impressive architecture. Mnandi plants registered the longest stems and roots, highest dry weight and grew taller with an average of 20 cm. According to Hagin and Tucker (1982), irrigation change is an important component of climate-water relations, and fertilisers change an important function of growing media-fertility relationships. Local potato producers will have to adapt their current management practices to speculations predicting a decline in ground water resources in South Africa (Hengsdijk and Verhagen 2013). Knowledge about water-use by plants is still required for the design of sustainable irrigation practices. As Green et al. (2005) suggested, science can be implemented in planning policies and irrigation practices for better yield.

The interaction between irrigation frequencies with cultivars on fresh and dry weight was significant. Concerning yield efficiency, plants irrigated every 20 minutes produced more minitubers per harvest, a higher total tuber number, tuber fresh and dry weight. Mnandi plants produced more minitubers per harvest with a higher fresh weight and total tuber number but Up-to-date plants produced the highest dry weight among all four cultivars.

The interaction between irrigation frequencies and cultivars on the response to macro and trace element content in potato minitubers grown under aeroponics system was not significant for sodium and iron. Other elements were significantly affected according to different associations used. Minitubers from plants irrigated less frequently - every 50 minutes - had the highest concentration of most macro and trace elements, compared to tubers from plants that were irrigated every 20 minutes that had the lowest concentration. Results of the current experiment demonstrated that cultivars exhibited differences with respect to their mineral concentration. In general, BP1 plants mostly had higher macro and trace element contents, except for phosphorus and calcium in which Mnandi plants performed better.

In conclusion, higher irrigation frequency increased potato tuber yield. Total fresh tuber yields and marketable tuber yields increased with increasing amount of irrigation frequency. A good balance between tuber yield and quality is when plants were irrigated after every 40 minutes. Variations within potato cultivars offer potato producers and consumers the option of choosing a high-ranking cultivars in terms of mineral content (Andre et al. 2007), depending on the final use.

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Chapter 5

Post-harvest quality of potato minitubers (*Solanum tuberosum* L.) produced aeroponically

Abstract

Diseases become increasingly common, especially those transmitted through seed tubers and therefore the trend in seed potato production systems is to minimise the number of field multiplications. The production and multiplication of a large volume of potato seed in protected environments using hydroponics or aeroponics is a need and can reduce the number of years needed for seed propagation. Seed potatoes of BP1 (*Solanum tuberosum* L.) produced through aeroponics at two different plant densities, 20 plants/m² (low density) and 30 plants/m² (high density), and four different harvest intervals, every 7, 10, 14 and 18 days, were used. Tubers were graded into different sizes: small tubers, <20 mm, medium, 20–40 mm and large > 40 mm in diameter. Minitubers were stored in a dark and cold room at 5°C for almost 4 months and then at 3 different temperatures (3, 16 and 25°C) for 2 supplementary months before being planted. The interaction between harvest intervals and storage temperatures strongly affected tuber yield, with fresh weight generally higher for potato plants from minitubers stored at 25°C and lower for plants from seeds stored at 3°C. Minitubers harvested every 7 days produced plants with the highest average dry shoot weight of 13.15 g. Mother tuber harvest interval did not influence stem number, leaf area index, tuber size distribution, total tuber number and total tuber weight at harvest of potato plants grown in the sand. Temperatures of potato seed storage strongly influenced different morphological parameters. Seed tubers kept at 25°C produced the highest total tuber weight with an average of 148.88 g, followed by seed stored at 16°C with an average of 99 g and seed stored at 3°C produced an average of 43.65 g.

Keywords: Potato seed, aeroponic, storage temperature

Introduction

The potato (*Solanum tuberosum* L.) is one of the most important food crops in the world, and timely availability of high quality planting material of suitable potato varieties is considered to be one of the most limiting constraints of this crop (Crissman et al. 1993). In many regions, the potato is grown in different seasons per year, making it difficult to obtain seed in the right condition (Struik and Wiersema 1999).

Diseases become increasingly common and especially those transmitted through seed tubers and knowing that conventional seed methods take more than 10 years to overcome an infected area (Ranalli 1997), the trend in seed potato production systems is to minimise the number of field multiplications (Lung'aho et al. 2010). The production and multiplication of a large volume of potato seed in a protected environment such as hydroponics or aeroponics is a needed and can reduce the number of field generations traditionally needed for seed propagation in the field. With the repeated harvesting when using aeroponics about 3500 minitubers larger than 5 mm of diameter per m² were harvested from *in vitro* propagated plantlets that were planted in a glasshouse under tuber-inducing conditions (Lommen and Struik 1992), where. However, final potato seed size will vary depending on the climatic conditions where they need to be planted; smaller tubers in temperate zones and larger tubers in warmer zones. Larger potato seeds are more resistant to harsh conditions as they contain more reserves than smaller sized tubers. According to Struik and Lommen (1990) plantlets from small minitubers are also less tolerant to early heat and drought stress due to less reserves.

For potato minituber production, storability should be equally important as disease resistance or quality as it has a major effect on the future of the seed depending on whether the end-use is for fresh consumption, processing, or planting as seed. For potato seed, keeping quality, dormancy period, sprouting behaviour and weight loss are major criteria (Pavlista 2004). The investigations by Ranalli (1997) showed that minitubers are dormant and must be stored before use. According to Nowak and Skwiercz (1975), low temperature limits tuber sprouting by slowing metabolic processes, especially synthetic process and at 2-3°C induces an increase in hydrolytic activity in tubers (Nowak 1977). Induced rapid sprouting and increased protein level was observed above 20°C (Pinto et al. 1993). Farran and Mingo-Castel (2006) in their study on the field performance of aeroponic minitubers found no difference in tuber yield and number

of tubers between seed from both aeroponics and hydroponics, and their physiological behaviour under field conditions were similar. However, the aeroponics system produced more minitubers than the hydroponics. According to Weathers and Zobel (1992), one of the driving forces behind the development of aeroponics for commercial application has been the need for making maximum efficient use of available space.

The present research evaluated the post -harvest storage conditions that could suit potato seeds produced through aeroponics. The performance of four different harvesting intervals and three sizes of potato minitubers were evaluated.

Materials and methods

Plant material

The experiment was carried out at Welgevallen, the experimental farm of the University of Stellenbosch in the Western Cape, South Africa. Potato seed of BP1 (*Solanum tuberosum* L.) obtained from a previous study where minitubers were produced in an aeroponic system at different planting densities (20 and 30 plants/m²) and harvested at different intervals (every 7, 10, 14 and 18 days). Tubers were graded into different sizes: Small tubers with a diameter of <20 mm, medium tubers comprised of tubers between 20 – 40 mm and large tubers above 40 mm in diameter. Minitubers were stored in a dark and cold room at 5°C for almost 4 months. Thereafter, samples of each treatment and size constituted of 4 tubers each were moved and stored at 3 different temperatures (3, 16 and 25°C) for 2 supplementary months before being planted. Minitubers were cultured in a evaporatively cooled greenhouse from Thursday, the 11th of July 2013 and harvested on Monday, the 23rd of September 2013.

Tubers were planted in 5L bags filled with silica sand and fertigated with a standard Steiner (1984) nutrient solution at an EC of 1.5 mS.cm⁻¹ (Table 5.1). The pH and EC were manually controlled during the whole trial.

Table 5.1 Composition of the standard Steiner nutrient solution used for fertigation of the potato plants in an aeroponic system at EC of 1.5 mS.cm⁻¹.

Macronutrients	Application (g 1000L⁻¹)	Micro- nutrients	Application (g 1000L⁻¹)
KNO₃	568.13	Fe-EDTA	86.9
K₂SO₄	489.40	MnSO₄	18.0
KH₂PO₄	255.00	ZnSO₄	10.0
Ca(NO₃)₂·2H₂O	1687.50	CuSO₄	1.2
MgSO₄·7H₂O	1033.20	H₃BO₄	22.0
		MoCl₅	1.9

One hundred and forty-four (144) minitubers were used for this experiment and each 5 L black plastic bag received one potato seed. For three weeks, fertigation was given twice per day, at 10:00 and 15:00 for 30 seconds in order to avoid water logging that could hinder sprout development. The irrigation frequency was increased to three times per day (8:00, 12:00 and 16:00) when 50% of bags had plantlets until the end of the trial.

Data collected

Rate of sprouting was determined throughout the growing season by visual counting. At harvest the following parameters were recorded: stem number, leaf area index (LAI), plant heights (cm) - measured as the vertical length between the apical growth point of the plant and the lowest base of the stem, number of tubers according to their sizes (mm), total tuber number, total tuber weight (g), shoot dry weight (g), tuber dry weight (g) and percentage of tuber firmness (%) - performed at harvest with a densimeter.

The temperature in the greenhouse was kept cool as encountered at the centre of its origin, with mean daily temperatures between 15 and 18°C (Haverkort 1990) for sprout elongation. It was kept at 25°C which is the optimum for haulm growth (Struik and Wiersema 1999) until tuber harvest.

Treatments and experimental design

The experiment used a three level factorial design, where the four harvest intervals during the preceding aeroponic production phase, the three tuber sizes and three storage temperatures constituted the main factors. Each treatment combination was replicated four times. Data was analyzed using ANOVA, and means of comparison ($P < 0.05$) using the general linear model of statistica software *statistica 11* (Statistica 2011).

Results and discussion

The interaction between harvest interval during growth in the aeroponic system and post-harvest storage temperatures strongly affected fresh and dry weight of potato tubers with the general tendency of increased yield for tubers from all harvest intervals as the storage temperature increased (Figure 5.1). The tuber fresh weight from tubers harvested every 18 days was highest for mother tubers stored at 25°C. For mother tubers harvested either every 10 or 14 days the resulting tuber fresh weight was only significantly lower for tubers stored at 3°C. The mother tubers harvested every 10 and 14 days before storage at 16°C, produced yields statistically similar to the tubers stored at 25°C. For the mother tubers harvested every 7 days the only significant difference in tuber fresh weight was between tubers stored at 3°C and tubers stored at 25°C. Minitubers harvested every 7, 10 and 14 days and stored at 3°C produced the lowest tuber dry weight.

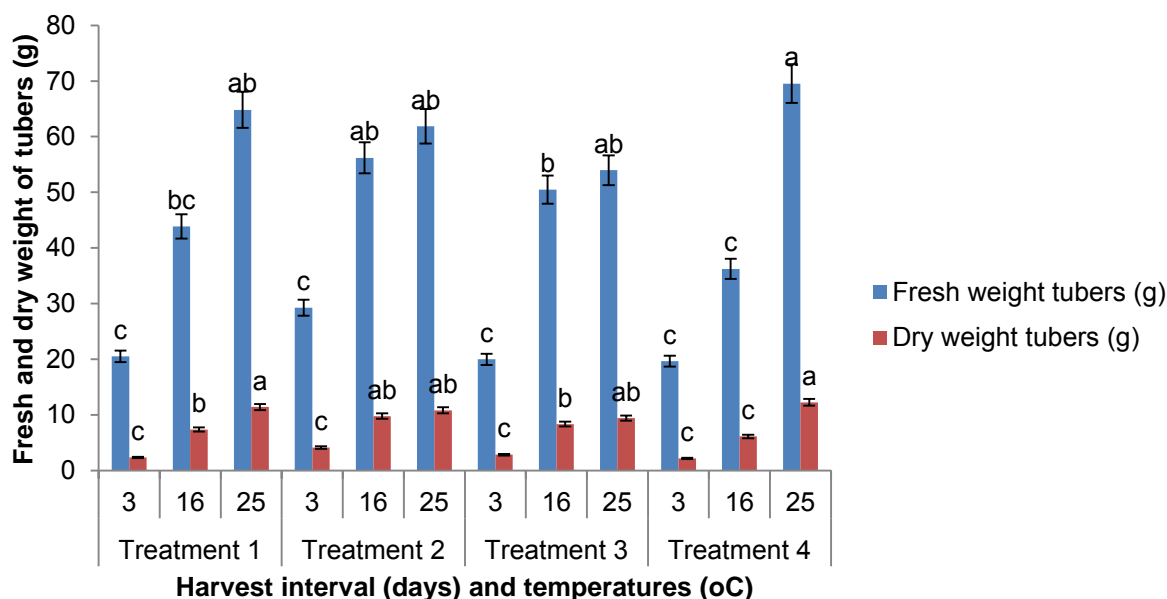


Figure 5.1 Interaction between harvest interval during aeroponic production and post-harvest storage temperatures ($^{\circ}\text{C}$) on the fresh and dry weight of tubers. With Treatment 1 = minitubers harvested every 7 days, Treatment 2 = minitubers harvested every 10 days, Treatment 3 = minitubers harvested every 14 days and Treatment 4 = minitubers harvested every 18 days. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non significant (NS). Treatment means followed by different letters differ significantly ($P < 0.05$). The data represented an average of the experiment.

The interaction between mother tuber size and post-harvest storage temperatures strongly influenced the fresh and dry weight of harvested tubers (Figure 5.2). The fresh and dry weight tended to be higher in tubers from all sizes stored at 25°C although it was only the small and large tubers stored at 3°C that had lower tuber fresh and dry weights. From these results, it is clear that seed storage temperature was the most important factor regardless of the minituber size at harvest. Higher fresh and dry weight of minitubers recorded from seeds stored at 25°C was probably influenced by the physiological status at planting. Weight loss in unsprouted tubers occurs through the periderm and for a minimum portion through the lenticels, hence, tubers that are physiologically more mature with a thicker periderm and fewer lenticels on the tuber surface loses less weight and should have more reserves to sustain sprout development (Pande et al. 2007).

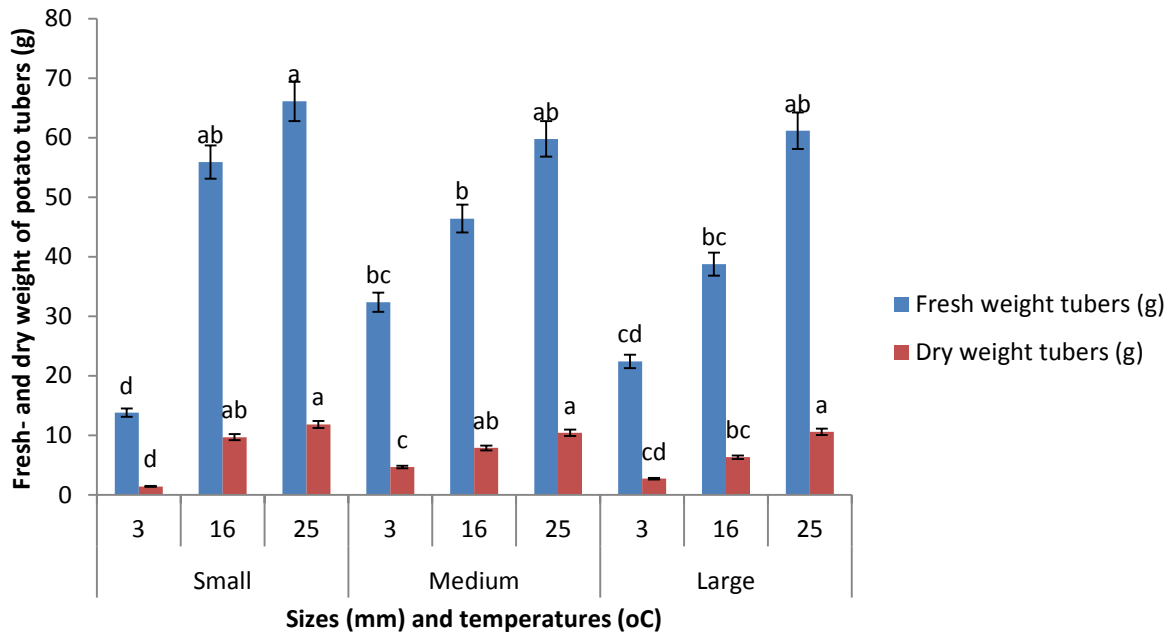


Figure 5.2 Interaction between tuber sizes (mm) and post-harvest storage temperatures ($^{\circ}\text{C}$) on the fresh and dry mass of tubers. With small = minitubers <20 g, medium = minitubers: 20-40 g and large = >40 g. Significant F test at $P<0.05$ (*) and $P<0.01$ (**) and non significant (NS). Treatment means followed by different letters differ significantly ($P<0.05$). The data represented an average of the experiment.

The interaction between harvest intervals and different storage temperatures applied to potato seeds influenced the percentage firmness (Figure 5.3). All interactions produced tubers with an average firmness between 84.33% and 90.77% except for minitubers harvested every 14 days and stored at 3°C which produced the lowest percentage firmness of 58.22%. According to De Belie et al. (1999) fruit or tubers stored at a high relative humidity showed a greater firmness than fruit stored at a low relative humidity. Besides, water status, temperature also plays a reversible physical effect on firmness (Jeffery and Banks 1994). An increased cell tension due to increased turgor or temperature will increase the firmness and the elastic modulus of the tissue (Chen 1993). Minitubers harvested every 14 days and stored at 3°C possibly faced a similar situation as described by these authors and also by Bajema et al. (1998) who state that

cell walls facing temperature fluctuations might become more brittle resulting in an increased firmness but reduction in the cell wall strength. The potato seed kept at lower storage temperatures could be stressed by higher temperatures above 20°C found in the greenhouse after being transplanted. Also, we should keep in mind that tubers harvested did not reach physiological maturity in order to give an accurate conclusion. At the cellular level, firmness depends on cell size, cell wall thickness and strength, turgor pressure and the manner in which cells bind together (DeEll et al. 2001). According to Struik and Wiersema (1999) tubers on the same stem show a tremendous variation in size, dry matter content, dry matter composition and physiological condition. Therefore, seed tubers differ in their behaviour, not only between seed lots but also within seed lots.

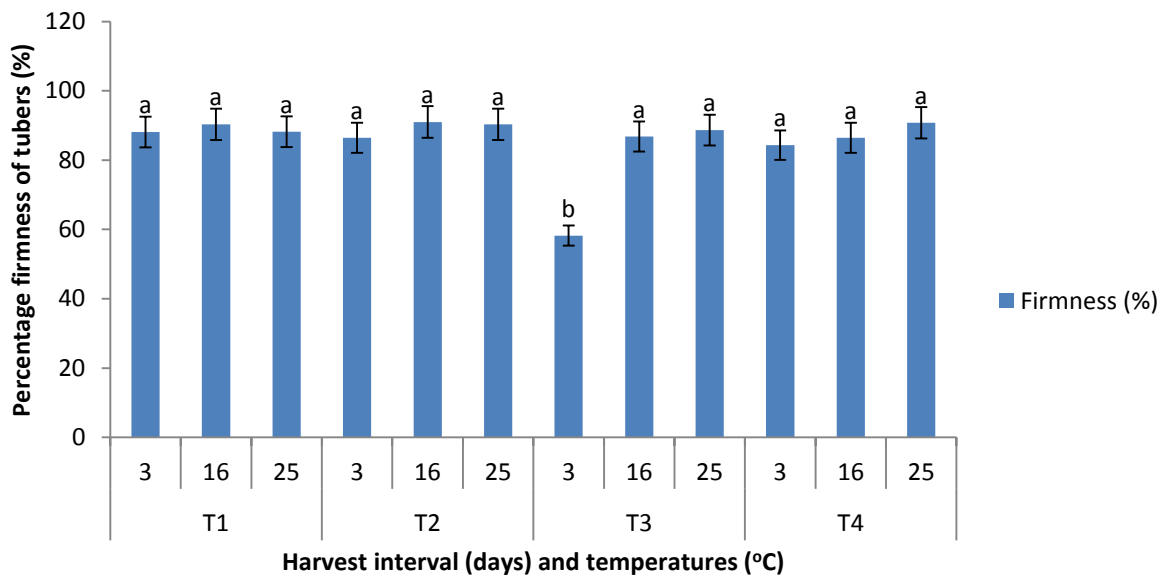


Figure 5.3 Interaction between harvest interval and storage temperatures (°C) on the firmness of tubers. With Treatment 1 = minitubers harvested every 7 days, Treatment 2 = minitubers harvested every 10 days, Treatment 3 = minitubers harvested every 14 days and Treatment 4 = minitubers harvested every 18 days. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non significant (NS). Treatments with different letters differ significantly ($P < 0.05$). The data represented an average of the experiment.

A larger percentage of the minitubers harvested every 10 and 14 days initiated sprouts after 4 months of storage at 5°C (Table 5.2). The same trend was seen for sprout length for minitubers harvested every 10 days. However, sprouting appearance on tubers was higher in minitubers harvested every 7 days with an average of 46.47%. Number of sprouts per tuber was not significantly influenced by the different harvest interval treatments (Table 5.2).

Table 5.2 Interaction between harvest interval and storage temperature of potato minitubers grown in an aeroponic system on sprout development. Results are the average of treatments combined. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non significant (NS). Treatments with different letter symbols differ significantly ($P < 0.05$). The data represented an average of the experiment.

<i>Harvest interval</i>	Tubers with sprouts (%)	Sprouts length (mm)	Sprouting appearance (%)	Number of sprouts
1 (7 days)	17.53b	5.55b	46.47a	3.00a
2 (10 days)	32.3a	8.10a	33.52bc	3.80a
3 (14 days)	31.59a	5.91b	35.94ab	3.36a
4 (18 days)	21.04b	7.0ab	22.38c	2.58a
	*	*	**	NS



Plate 5.1 Left hand side, sample of mother tubers after 74 days spent in the sand. At the right hand side, a sample of potato daughter tubers after harvest. The growing season could have been extended as these mother tubers were still producing sprouts.

No interaction between the size of potato seed tubers and storage temperature was found. However, the interaction between harvest intervals and storage temperatures strongly influenced sprouting capacity (Table 5.3). After two weeks, minitubers harvested every 7, 10, 14 and 18 days and stored at the higher temperatures of 25°C sprouted faster, followed by minitubers stored at 16°C and finally minitubers stored at 3°C had the lowest rate of sprouting, but continued sprouting even after 9 weeks. At week 7 as observed in Table 5.3, seed tubers harvested every 7 days had the highest sprouting rate.

Increased storage temperature of potato minitubers before planting improved sprouting capacity as observed in this current study. High storage temperatures possibly positively influenced ethylene production in tubers due to onset of the sprouting (Rapport et al. 1957) at optimal sprouting conditions, i.e., darkness, 15°C to 20°C, relative humidity about 90% (Wiersema 1985). According to Pratt and Goeschl (1969), ethylene is well known to be a plant hormone initiating fruit ripening and regulating many aspects of plant growth in several plants. In the case of potato tubers, the ethylene evolution is also

stimulated with gibberellic acid which is one of the most effective agents for breaking their dormancy (Poapst et al. 1968).

Though it is not shown, size also influenced tuberisation. Larger minitubers sprouted faster and initiated more stems at the beginning than medium and smaller minitubers. Investigations by Lommen and Struik (1994) reported as well that lighter tubers took longer to produce sprouts of 2 mm than heavier tubers, and when tubers with sprouts of the same length were planted in pots, sprouts from lighter tubers took longer to emerge. However, 74 days after planting, these differences could not be noticed as all plants had almost similar sizes in this current study because storage temperature had a greater influence than tuber size.

Table 5.3 Interaction between harvest interval and storage temperature of potato minitubers grown in an aeroponic system on sprout appearance. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non-significant (NS). Treatments with different letters differ significantly ($P < 0.05$).

Harvest interval (days)	Temperature (°C)	Week 2 (%)	Week 6 (%)	Week 7 (%)
1 (7 days)	3	0.11c	0.00b	0.55a
1 (7 days)	16	0.77ab	0.00b	0.00c
1 (7 days)	25	0.88a	0.00b	0.00c
2 (10 days)	3	0.00c	0.00b	0.44a
2 (10 days)	16	0.44b	0.00b	0.00c
2 (10 days)	25	1.00a	0.00b	0.00c
3 (14 days)	3	0.11c	0.00b	0.00c
3 (14 days)	16	0.44b	0.00b	0.00c
3 (14 days)	25	0.66ab	0.00b	0.00c
4 (18 days)	3	0.00c	0.11a	0.22ab
4 (18 days)	16	0.66ab	0.00b	0.11bc
4 (18 days)	25	1.00a	0.33a	0.00c
		**	**	**

Results shown in table 5.4 displayed sprouting capacity of minitubers of different sizes stored at different temperatures for 2 months. As noticed, sprouting was regulated by storage temperature. After 2 weeks, small, medium and large minitubers stored at 25°C sprouted more than at other storage temperatures applied especially during the second week after planting. But after 6 weeks, minitubers stored at 3°C showed an increase in sprouting capacity. Though it is not shown, medium minitubers sprouted more at 16°C and at 3°C the rate of sprouting was lower. Sprouting capacity was very retarded at 3°C as observed in plate 5.2.

At 25°C all treatments without distinction initiated more sprouts during the first and second week, and then gradually decreased for this particular storage temperature but marginally increased at 16°C (Table 5.4). After 7 weeks, it increased among minitubers stored at 3°C. As illustrated in table 5.3 and 5.4, after 9 weeks more plants were observed from minitubers stored at 25°C as these tubers sprouted faster. Besides genes, dormancy periods depends on soil and weather conditions during growth, tuber maturity at harvest, storage conditions, and whether the tuber is injured or not (Ezequiel and Singh 2003).

Table 5.4 Number of weeks to sprouting associated to storage temperature of potato seed. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non significant (NS). Treatments with different letter symbols differ significantly ($P < 0.05$). The data represented an average of the experiment.

Number of weeks to sprouting									
Temperature									
(°C)	1	2	3	4	5	6	7	8	9
3	0.00b	0.05c	0.00b	0.11a	0.02a	0.02a	0.30a	0.08a	0.02a
16	0.02b	0.58b	0.19a	0.08a	0.16a	0.00a	0.02b	0.00a	0.00a
25	0.30a	0.88a	0.05a	0.05a	0.02a	0.08a	0.00b	0.00a	0.00a
	*	**	*	NS	NS	NS	**	NS	NS



Plate 5.2 Potato plant growth 49 days after planting potato minitubers in the greenhouse.

When using potato seeds obtained from aeroponics, the harvest interval did not influence stem number, leaf area index, tuber number or total tuber weight during subsequent growth of plants in the sand (Table 5.6). However, dry shoot weight and the height of crops were significantly affected by the harvesting intervals applied. Therefore, plants from tubers that were harvested every 7 days produced the highest average dry shoot weight and plants from tubers that were harvested every 10 days produced the lowest shoot dry weights. Plant height was only lower for plants from tubers harvested every 14 days (Table 5.6). This study did not allow the potato plant to complete its growth cycle as the objective was the seed sprouting and early development of plantlets. The whole experiment took place in 74 days, while BP1 cultivar used in this study requires generally 90 to 110 days before maturing (Kempen 2007). Obviously height influenced dry weight partitioning within treatments and in both cases plants from minitubers harvested every 7 days had the highest scores. The taller the plants were, the higher the dry weight of the shoots was as leaves and stems of these plants were also bigger. Further studies need to be done to elucidate the difference in dry matter partitioning and other physiological aspects between plants from tubers harvested at

different intervals as the number of days do not differ that much. The same trend appeared for leaf area of the plants in the aeroponic system, although we do not have statistical evidence to support it. Okazawa (1973) reported that dehydration of senile tubers lead to a marked decline in the fresh weight and consequently increases ethylene concentration which is a plant growth regulator. This could be the reason why vegetative growth was enhanced for plants harvested at a 7 day interval. When harvested minitubers at an 18 day interval, minitubers might have had a significant amount of ethylene as it tended to be physiological more mature compared to tubers harvested at other intervals. However, the growth regulator gibberellic acid exerts a promoting effect on elongative growth of the sprout (Okazawa 1973). However, for potato tuber, dormancy and sprouting are controlled by the interactions of major plant growth regulators, predominantly gibberellin and abscisic acid (Femie and Willmitzer 2001), auxin and indole acetic acid (Pavista 2004).

Large minitubers were mostly found when harvesting minitubers at 18 day intervals. At sprouting, larger minitubers initiated more sprouts compared to medium and small minitubers. As Burton (1971) reported, since the sprouts depend on the tuber for the material for growth, if there are several sprouts on the tuber, an inter-sprout competition for growth factors will be imposed by the size of the tuber. However, the small size minitubers eventually produced more sprouts and at harvest, no difference could be noticed in terms of stem number. This result is very important for potato seed production as it is not only the tuber size that influences the number of sprouts as observed in this study. Moreover, harvest interval did not influence the number of small-, medium-, large-, total number of tubers or total tuber weight.

Table 5.6 The means of plant morphology and tuber yields of potato plants from tubers harvested at different intervals in the aeroponic system. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non significant (NS). Treatments with different letters differ significantly ($P < 0.05$). The data represented an average of the experiment.

<i>Treatments</i>	Stem		Shoot dry		
	number	LA (cm ²)	weight (g)	Height of plants (cm)	
1 (7 days)	1.36a	530.02a	13.15a	10.08a	
2 (10 days)	1.27a	458.86a	11.20b	8.914a	
3 (14 days)	1.14a	395.57a	9.15c	6.89b	
4 (18 days)	1.47a	455.40a	11.91b	9.15a	
	NS	NS	*	*	
Number of tubers per plant					
<i>Treatments</i>	Small	Medium	Large	Total	Total tuber weight (g)
1 (7 days)	3.22a	0.63a	1.11a	5.00a	97.29a
2 (10 days)	2.83a	0.67a	1.40a	4.91a	107.47a
3 (14 days)	2.20a	0.42a	1.37a	4.00a	93.92a
4 (18 days)	2.83a	0.72a	1.00a	4.55a	90.24a
	NS	NS	NS	NS	NS

Temperature of potato seed storage strongly influenced different morphological parameters found in Table 5.6, except for stem number and the number of small and medium sized seed produced. Seed stored at 25°C produced the highest leaf area index, while the lowest LA (leaf area) was for plants from tubers stored at 3°C before planting. Shoot dry weight was higher for tubers stored at 25°C and 16°C, compared to that of tubers stored at 3°C. Plant height was higher for plants from tubers stored at 25°C compared to plant height of plants from tubers stored at the lower temperatures. The fresh- and dry weight of tubers was also found to be higher from plants where the mother tuber was stored at 25°C before planting, followed by minitubers stored at 16°C (Table 5.7). The number of large tubers (>40 g) was strongly influenced by temperatures. Potato seed stored at 25°C in an incubator for 2 months before being

planted produced the most large tubers with an average of 2.04 tubers per plant, followed by 1.33 tubers per plant when mother tubers were stored at 16°C and only 0.29 tubers when mother tubers were kept at 3°C. The total number of tubers and tuber dry weight was also influenced by storage temperatures with seed stored at 25°C yielding the highest and seed stored 3°C yielding the lowest per plant. Using 25°C as storage temperature in the dark room for potato tuber seeds, Okazawa (1973) reported an increase in level of ethylene of tubers, which encouraged sprouting capacity and sprout growth as noticed in this current study. By having an advance from the beginning with an important level of ethylene, higher yield observed at the end of the experiment was not a surprise. Prat and Goeschl (1969) anticipated that production of ethylene in or near meristematic tissues suggested a possible role in cell division. Other authors (Morgan and Hall 1962, Burg and Burg 1966) reported that ethylene production of plants is high in tissues containing auxins. Okazawa (1967) suggesting that the presence of gibberellin and auxin may be a prerequisite for the stimulation of ethylene evolution in the potato tubers. Carli et al. (2010) reported that high temperatures, low soil moisture and high fertility during tuber growth accelerate physiological development and reduce the dormant period. In this current study, temperature was kept under 25°C in the greenhouse which is slightly above the optimum temperature (15-20°C) for sprouting; therefore temperature of storage played the most important role. It appears that lower storage temperature (3°C) has longer period of dormancy than those stored at higher temperatures.

Statistically, tubers harvested from seed which were harvested after every 10, 7 and 18 days produced tubers with a higher percentage firmness, respectively 89.25, 88.88 and 87.18%, and tubers from plants harvested every 14 days had the lowest percentage firmness of 77.88%. The physiological age of the tuber has a great effect, but the basis is genetic (Carli et al. 2010). As the same cultivar was used for this study, other factors mostly the physiological age of minitubers at 14 days day should be further investigated for percentage firmness.

Table 5.7 The means of plant morphological and yields characteristics of potato plants from three different storage temperatures. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non significant (NS). Treatments with different letter symbols differ significantly ($P < 0.05$). The data represented an average of the experiment.

Temperatures (°C)	Stem number	LA (cm ²)	Shoot dry weight (g)	Height plant at harvest	Fresh mass tubers (g)	Dry mass tubers (g)
3	1.10a	351.76c	9.46b	7.50b	22.37c	2.86c
16	1.35a	453.88b	11.25a	8.12b	46.80b	7.91b
25	1.47a	575.56a	13.39a	10.7a	62.31a	10.93a
	NS	**	*	**	**	**

Number of tubers					
Temperatures (°C)	Small	Medium	Large	Total	Total tuber weight (g)
3	2.31a	0.50a	0.29c	3.10b	43.65c
16	2.77a	0.75a	1.33b	4.85a	99.44b
25	3.25a	0.60a	2.04a	5.91a	148.88a
	NS	NS	**	*	**

Concerning weight, medium (88.58%) and large tubers (87.77%) had a higher percentage firmness compared to small tubers with an average of 81.05% (Table 5.8). This result could possibly be influenced by the fact that minitubers harvested at an immature stage have a longer post-harvest dormancy than tubers harvested at physiological maturity as mentioned earlier. Carli et al. (2010) described the same behaviour in their study. Investigations by Ranalli et al. (1994) showed that the length of dormancy is inversely correlated with tuber size, and that smaller tubers suffered dehydration when stored for a long time, and reduced growth vigour when planted directly in the field. In another experiment (Leclerc et al. 1995), smaller tubers of <250 mg had longer dormant periods than did those >250 mg. Temperatures of 16°C (88.63%) and 25°C (89.50%) produced tubers with higher firmness than 3°C (79.27%). According to Karanja et al. (2013), low temperature promotes conversion of starch to

sugar which can negatively affect quality. As potato minitubers were removed directly from different storage temperatures (3°C, 16°C and 25°C), this could have also influence on tubers produced by plants from seeds stored at 3°C. Another hypothesis is that tubers from seeds stored at 3°C were physiologically younger than seed from 16°C and 25°C as sprouted and initiating tubers later because dormancy was particularly prolonged. That is why processing potatoes are stored at 7°C to 10°C, while table potatoes are stored at 6°C to 7°C to avoid browning (Liu et al. 1990).

Table 5.8 The means of potato tubers firmness according to treatments, sizes (mm) and temperatures (°C). Significant F test at P<0.05 (*) and P<0.01 (**) and non significant (NS). Treatments with different letter symbols differ significantly (P<0.05). The data represented an average of the experiment.

Percentage firmness of tubers (%)					
Treatments		Weight (g)	Temperatures		
				(°C)	
1 (7 days)	88.88a	Small	81.05b	3	79.27b
2 (10 days)	89.25a	Medium	88.58a	16	88.63a
3 (14 days)	77.88b	Large	87.77a	25	89.50a
4 (18 days)	87.18a		**		**
	**				



Plate 5.3 Potato tubers harvested after 74 days

Conclusions

Potato minitubers from an aeroponics system used as seed would appear to have a positive future in the potato production industry. With its easy application, aeroponic technique can eliminate one generation of seed potato multiplication in the field thus lowering production costs and raising the quality of the first field production generation (Lung'aho et al. 2010, Otazu 2010). It has been noticed in this current study that sprouting capacity was mostly linearly influenced by storage temperature regardless other factors applied to potato seed minitubers such as harvest intervals and tuber sizes. Minitubers stored at 25°C (after an initial 4 months storage at 3°C) had the earliest and highest rate of sprout appearance. This factor could be a benefit to growers in planning of the growing season associated to the cultivars growth habit, or breaking the dormancy period for cultivars that have naturally long dormancy period by using temperature rather than growth regulators. Harvesting intervals of potato seed did not influence stem number, leaf area, dry shoot, height of plants, size repartitions of tubers, total tuber number- and total tuber weight. This observation is particularly important since sequential harvesting that aeroponic system offers enables the growers to harvest

frequently – at an interval of 7 days - and have more potato seeds without compromising on quality. The advantage of early sprouting from minitubers stored at 25°C encouraged plants to produce a large leaf area which increased the absorption of solar radiation, crucial to plant production and distribution of assimilates within the crop. As observed, seed stored at 25°C with the highest leaf area produced the highest total tuber number and total tuber weight. Distribution of small and medium tubers was not influenced by temperature but temperature significantly influenced large tuber distribution among plants with the highest number when seed was stored at 25°C.

Medium and large daughter tubers had a higher percentage firmness than small tubers. Statistically, daughter tubers from seed stored at 25°C and 16°C had a higher percentage firmness.

All interactions concluded that storage to be the most important factor in determining the potential of the following crop. The costs of producing high quality planting material are high, but seed of adequate quality is a necessary investment both in low-input and high-input agriculture (Struik and Wiersema 1999).

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Chapter 6

Summary and General Conclusions

The most limiting factor for potato seed production is the number of field multiplications needed, which takes long and adds a higher risk of disease contamination. Fewer field generations will not only guarantee a higher tuber health but will also be more environmentally friendly, since fewer pesticides need to be used. With the development of propagation technology, different ways of producing potato tubers from disease free *in-vitro* plantlets or the use of meristem culture already exist but multiplication of tubers lacks. Previous research had been applied in different countries and satisfactory results were achieved using techniques for potato seed multiplication like hydroponics and aeroponics, enabling year-around production to produce enough seeds during optimum seasons for potato production. According to literature, aeroponics particularly had shown higher productivity and easier management of underground plant parts responsible of tuber initiation. Aeroponic techniques optimise root aeration which is the major factor leading to a yield increase compared to classical hydroponic systems. Aeroponics can produce up to 10 times higher yields, more rapidly, and at a lower cost compared to conventional growing methods. Harvesting in aeroponics is also convenient, clean, and allows a greater size control through sequential harvesting. With conventional techniques, at higher planting density, plants suffer from competition of water and minerals necessary for plant growth and tuber development. With aeroponics, when there are enough sprayers in growing chambers, plants do not show any nutritional deficiency. The only damageable possibility would be the lack of electrical supply to functioning pumps.

The aim of this study was therefore to elucidate the cultivation aspects of producing quality potato seed tubers aeroponically under local conditions. A series of experiments were performed at Walgevallen experimental farm at Stellenbosch University from 2012-2013 to investigate the use of aeroponics for the production of minitubers since little is known about applying this production system on a commercial scale in South Africa, although it is being applied commercially on limited scale. Postharvest field performance of aeroponically produced minitubers was also studied in the greenhouse.

Harvest interval and plant density

Electrical conductivity was found to affect vegetative growth of potato plants grown aeroponically. In the experiment, harvest intervals of every 7, 10, 14 and 18 days were compared at two plant densities of 20 and 30 plants/m² of BP1 cultivar. At two months before sowing, minitubers were stored at 3 different temperatures (3, 16 and 25°C) for sprouting capacity determination. It appeared that knowledge of fertiliser application proportional to different plant growth stages is important in order to produce high quality and yield of potato seeds through an aeroponic system. This study indicated that lowering Ca concentration was successful to induce stolon tips to form tubers. From the results it became clear that harvest interval did not influence stem length, root length, stolon number, percentage stolon branching, leaves and stem dry weight, stolon dry weight and percentage firmness. The correlation between treatments and temperatures suggested that the smaller tubers that were harvested every 7 days and stored at 25°C resulted in the optimal sprouting capacity. Harvesting minitubers at 7 day intervals produced higher yields of minitubers compared to the other intervals for a growing period of 118 days allocated to the experiment. Harvesting intervals did not play a major role in the distribution of macro and trace elements within minitubers except for K and Cu harvested after every 10 days interval. The Cu content could positively be affected by harvesting large and medium tubers at 10 day intervals. The size of tubers at harvest significantly influenced Na content with a higher Na concentration in the smaller tubers.

Plant densities had a significant effect on stolon number, leaf and stem dry weight, stolon dry weight and root dry weight. After transplantation of plantlets in the aeroponics system, tuber initiation was faster and more tubers were initiated at the higher plant density. In the middle of the harvesting season, however, the plants at the lower planting density had longer and more vigorous stolon development and more tubers per plant initiated. This could be explained by the lack of space at high planting density which augmented competition when plants reached the adult phase. Regarding yield, plant density did not have a significant influence though a strong trend showed higher yield at higher plant density. Maybe more plants per treatment than used in this study needs to be applied for further studies.

Temperature played a significant role on the post storage phase. Sprouting of minitubers was gradually influenced with the increase in temperature. Higher temperatures seemed

to increase the capacity of sprout appearance regardless of tuber size. However, conversely, firmness was influenced by size with the bigger (>40 g) tubers being significantly firmer and therefore less prone to moisture loss during storage.

Future research should evaluate the role and timing of Ca application to enhance tuberization further and also look at using a higher plant density. At optimum minituber production, harvesting even more frequently than every 7 should also be explored. Other South African potato cultivars should also be tested using this system to determine if there is a variation in response.

Calcium rates

Seed tuber quality is a product of different factors during potato plant growth and the tuber initiation process. Producers are looking for good quality seed tubers but poor quality due to internal defects such as brown centre, internal brown spot, and hollow heart can substantially reduce the value of potato tubers. The role of calcium (Ca) in the maintenance of membrane integrity and cell wall strength and thus reducing the incidence of many physiological disorders and damage due to stress and handling is well established. Therefore, potato plantlets, cultivars Up-to-date, Mnandi, Buffelspoort and BP1 were grown aeroponically at four different calcium levels (8.40, 6.75, 5.10 and 3.45 meq/L). Results of this study indicated that different calcium levels used did not influence the morphological development of plants. Probably to the relative high Ca-levels used as treatments. Yield of potato minitubers was also not influenced by different treatments of Ca applied for single harvest, but the total tuber number was influenced by Ca in the nutrient solution. Ca at 3.45 meq/L displayed a higher fresh and dry weight content. In terms of macro and trace element content, at higher Ca concentration of 8.4 meq/L tubers had the highest Ca and Cu content, but had the lowest Mg and Mn concentration. The tuber Ca content was higher regardless of cultivar used.

Mnandi plants had taller plants from the first height measurements taken until the last day of destructive harvest and the plants performed better in terms of stem number, stem length, root length and shoot dry weight. BP1 plants produced a higher percentage of stolon branching. Cultivars played a significant role in yield distribution where Mnandi plants produced both a higher number of minitubers per harvest and higher total fresh

weight of potato minitubers. Up-to-date plants had the highest dry weight. Nutritional results concluded that Mnandi minitubers had higher concentration of P and Ca. BP1 minitubers had higher concentrations of P, Mg, Cu and Mn. Up-to-date minitubers had higher Mg, Cu and B content. Buffelspoort minitubers were rich in Mg and Zn. For Ca and other mineral absorption, the use of aeroponics is more effective as it reaches all parts susceptible to increased Ca and other elements assimilation into tubers as the spray can reach underground parts such as roots and even stolons for faster and better absorption. The most important benefit aeroponics offer is that the harvesting of minitubers is very convenient, clean, and permits a greater size control through sequential harvesting. Future research should be focused on evaluating on the use of Ca by Mnandi cultivar, maybe increasing the concentration even more in the nutrient solution, especially since this cultivar produced a higher yield and seemed to be well adapted for the aeroponic system.

Irrigation frequency

Water is the most important factor controlling plant growth. The present paper attempted to analyse the effect of fertigation frequency using aeroponics on the growth, final yield and nutritional content of four different potato plant cultivars (Up-to-date, Mnandi, Buffelsoort and BP1) using four different irrigation frequencies (20, 30, 40 and 50 minutes interval). Significant differences were noticed in the interaction between irrigation frequencies and cultivars for the percentage tuberized plants and stolon dry weight. An increase in the stolon dry weight indicates a higher starch accumulation rate in stolons. Irrigation frequency, transpiration, biomass and yield varied significantly between treatments and cultivars. Potato plants that received water most regularly, every 20 minutes, presented an impressive architecture. Mnandi plants registered the longest stem and root length, highest dry weight and grew taller. Knowledge about water-use by plants is still required for the design of sustainable irrigation practices.

The interaction between irrigation frequencies with cultivars on fresh and dry weight was significant. Concerning yield efficiency, plants irrigated every 20 minutes produced more minitubers per harvest, a higher total tuber number, fresh and dry tuber weight. Mnandi

plants produced more minitubers per harvest with a higher fresh weight and total tuber number but Up-to-date plants produced the highest dry weight among all four cultivars.

The interaction between irrigation frequencies and cultivars on the response to macro and trace element contents in potato minitubers grown under aeroponics system was not significant for Na and Fe. Other elements were significantly affected according to different associations used. For minitubers nutritional content, irrigating potato plants every 50 minutes for 30 minutes had the highest concentration of most macro and trace elements, and irrigating plants every 20 minutes resulted in the lowest concentration. In general, BP1 plants mostly had a higher macro and trace element content except for P and Ca in which Mnandi plants performed better. In conclusion, higher irrigation frequencies increased potato tuber yield, but decreased tuber quality. Total fresh tuber yields and marketable tuber yields increased with increasing amount of irrigation frequency. Further research in this section is needed regarding cultivars and nutritional content. However, higher irrigation increased yield and Mnandi produced the highest yield. Irrigating plants after every 40 minutes would be the recommendation. Maybe further knowledge should be oriented in the increase of nutritional content in Mnandi tubers.

Postharvest in the field

The production and multiplication of large volumes of potato seed in a protected environments such as hydroponics or aeroponics is a need. It requires a few years less than the traditional seed propagation in the field. Potato seed of BP1 obtained from a previous study which consisted of the production of minitubers through an aeroponics system at two different plant densities (20 and 30 plants/m²), and four harvest intervals (after 7, 10, 14 and 18 days) were used. Tubers were graded into different sizes (Small: >20 mm of diameter, medium: 20–40 mm and large: > 40 mm). Minitubers were stored in a dark and cold room at 5°C for almost 4 months. Thereafter it was stored at 3 different temperatures (3, 16 and 25°C) for 2 supplementary months before being planted. Sprouting capacity was mostly linearly influenced by temperature regardless other factors such as harvest intervals and tuber sizes. Minitubers stored at 25°C had the earliest and highest rate of sprouting. Harvesting intervals of potato seed did not

influence stem number, leaf area, dry shoot weight, height of plants, size distribution of tubers, total tuber number and total tuber weight. This observation is particularly important in the measure that, with the possibility of sequential harvesting that aeroponics system has, growers could for instance choose the interval of 7 days and have more potato seeds compared to harvesting every 18 days for the same length of the growing season.

All interactions between treatments and temperatures on fresh and dry weights, sizes and temperatures on fresh and dry weights, treatments and sizes on firmness, treatments and temperatures on firmness, sizes and temperatures on firmness concluded temperatures of storage to be the most affecting factor. The advantage of early sprouting from minitubers stored at 25°C encouraged plants to produce large leaf area. This increased the absorption of solar radiation, crucial to plant production and distribution of assimilates within the crop, especially toward the storage organ; the tuber. As observed, seed stored at 25°C with the highest leaf area produced the highest total tuber number and total tuber weight. Stem number, distribution of small and medium tubers were not influenced by temperature. However, temperature significantly influenced larger tuber distribution among plants with the highest number when seed was stored at 25°C. Statistically, tubers from seed stored at 25°C and 16°C had higher percentage firmness than seed stored at 3°C. Further research should be focused on testing on the efficacy of small tubers produced aeroponically using more local cultivars adapted to the climate.

Results from these studies indicate that aeroponics is an easy way of producing potato seed tubers. This system can monitor sequential harvesting interval of tubers at chosen size and increase life span of the plant, higher yield achievability with clean final material, fertigation is easy handled which reduces plant competition for water and minerals. Temperature is a key factor in potato seed sprouting. Higher temperature of 25°C improved sprouting that produced better plants for production. And low temperature of 3°C prevented sprouting and was successful, good for later planting dates.

This research is a call to commercial growers that are not well informed of the benefits of aeroponic systems this technique could help improve their production on a large scale in South Africa and other emerging countries.

Appendix

Table A1 Syngenta spraying program

Week	Date	Chemical	Dosis
3	15.11.2012	Karate	7.5 ml/10 L 20 ml/10 L
4	22.11.2012	Aphox Karate Bravo	5 g 7.5 ml 20 ml
5	27.11.2012	Selecron Bravo	15 ml 20 ml
6	29.11.2012	Aphox Score	5 g 15 ml
7	4.12.2012	Score Parton	15 ml 20 g
8	11.12.2012	Selecron Patron	15 ml 20 g
9	14.12.2012	Aphox Bravo	5 g 20 ml
10	18.12.2012	Selecron Patron	15 ml 20 g
11	25.12.2012	Bravo Score	20 ml 15 ml
12	27.12.2012	Patron Aphox	20 g 5 g
13	8.01.2013	Patron Aphox	20 g 5g
14	11.01.2013	Biomectin	6 ml



Plate A1 From left to right, 1. *In vitro* plantlets, 2. First day in seedling trays, 3. Fourteen days in seedling trays

Table A2 Interaction between harvest intervals and sizes (mm) on the nutritional values of potato minitubers grown in aeroponics system. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non-significant (NS). Treatment means followed by different letters differ significantly ($P < 0.05$).

<i>Treatments</i>	<i>Sizes (mm)</i>	Phosphorus %	Potassium %	Calcium %	Magnesium %	Sodium mg/kg	Iron mg/kg
<i>1 (7 days)</i>	<i>Small</i>	0.69 a	3.96 a	0.03 a	0.22 a	187.33 a	212.84 a
<i>1 (7 days)</i>	<i>Medium</i>	0.67 a	4.02 a	0.03 a	0.21 a	133.33 a	126.20 a
<i>1 (7 days)</i>	<i>Large</i>	0.68 a	4.02 a	0.02 a	0.21 a	150.00 a	174.00 a
<i>2 (10 days)</i>	<i>Small</i>	0.68 a	4.63 a	0.03 a	0.20 a	161.67 a	198.00 a
<i>2 (10 days)</i>	<i>Medium</i>	0.74 a	4.57 a	0.03 a	0.23 a	150.00 a	145.33 a
<i>2 (10 days)</i>	<i>Large</i>	0.71 a	4.89 a	0.03 a	0.22 a	133.33 a	145.67 a
<i>3 (14 days)</i>	<i>Small</i>	0.70 a	4.85 a	0.02 a	0.21 a	163.33 a	155.67 a
<i>3 (14 days)</i>	<i>Medium</i>	0.68 a	4.37 a	0.02 a	0.21 a	117.00 a	135.63 a
<i>3 (14 days)</i>	<i>Large</i>	0.70 a	4.44 a	0.03 a	0.20 a	136.67 a	162.33 a
<i>4 (18 days)</i>	<i>Small</i>	0.72 a	4.36 a	0.03 a	0.23 a	187.00 a	266.58 a
<i>4 (18 days)</i>	<i>Medium</i>	0.70 a	4.42 a	0.03 a	0.21 a	116.67 a	173.33 a
<i>4 (18 days)</i>	<i>Large</i>	0.71 a	4.56 a	0.02 a	0.21 a	116.67 a	141.60 a
		NS	NS	NS	NS	NS	NS
<i>Treatments</i>	<i>Sizes (mm)</i>	Copper mg/kg	Zinc mg/kg	Manganese mg/kg	Boron mg/kg	Aluminium mg/kg	
<i>1 (7 days)</i>	<i>Small</i>	6.89 a	38.32 a	12.23 a	13.02	8.31 a	
<i>1 (7 days)</i>	<i>Medium</i>	6.55 a	38.66 a	12.90 a	15.06 a	8.17 a	
<i>1 (7 days)</i>	<i>Large</i>	5.42 b	37.63 a	12.56 a	13.60 a	6.18 a	
<i>2 (10 days)</i>	<i>Small</i>	6.94 a	38.43 a	11.99 a	13.62 a	7.27 a	
<i>2 (10 days)</i>	<i>Medium</i>	8.41 a	42.43 a	14.76 a	14.60 a	6.58 a	
<i>2 (10 days)</i>	<i>Large</i>	8.45 a	41.30 a	12.63 a	14.63 a	5.92 a	
<i>3 (14 days)</i>	<i>Small</i>	6.08 b	38.03 a	12.66 a	14.50 a	8.30 a	
<i>3 (14 days)</i>	<i>Medium</i>	5.40 b	35.70 a	12.53 a	13.60 a	6.85 a	
<i>3 (14 days)</i>	<i>Large</i>	6.87 a	37.26 a	12.30 a	13.30 a	7.41 a	
<i>4 (18 days)</i>	<i>Small</i>	9.56 a	43.58 a	14.51 a	14.86 a	10.68 a	
<i>4 (18 days)</i>	<i>Medium</i>	6.80 a	39.70 a	12.96 a	13.23 a	6.33 a	
<i>4 (18 days)</i>	<i>Large</i>	6.22 ab	38.43 a	12.83 a	12.40 a	5.63 a	
		*	NS	NS	NS	NS	

Table B1 The means of number of potato minitubers per harvest (H) for minitubers grown in an aeroponic system using four different cultivars. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non significant (NS). Treatments with different letters differ significantly ($P < 0.05$)

Ca application rate (meq/L)	Number of tubers per plant							
	H1	H2	H3	H4	H5	H6	H7	H8
3.45	1.30a	1.59a	1.44a	1.23a	1.14a	1.08a	0.84a	0.29a
5.1	0.97a	1.47a	2.1a	0.90a	1.30a	0.98a	0.54a	0.29a
6.75	0.74a	1.23a	0.99a	1.07a	0.85a	0.73a	0.46a	0.29a
8.4	1.17a	1.64a	1.27a	0.92a	1.52a	1.05a	0.94a	0.71a
	NS	NS	NS	NS	NS	NS	NS	NS
Ca application rate (meq/L)	Fresh weight of tubers (g)							
	H1	H2	H3	H4	H5	H6	H7	H8
3.45	6.61a	5.99a	5.16a	4.33a	3.84a	3.46a	2.51a	1.00a
5.1	4.94a	5.86a	3.95a	3.36a	3.82a	3.3a	1.53a	0.83a
6.75	4.08a	4.78a	3.20a	3.22a	2.25a	2.02a	1.29a	0.72a
8.4	6.38a	7.54a	4.99a	3.01a	4.74a	3.73a	2.39a	2.18a
	NS	NS	NS	NS	NS	NS	NS	NS
Ca application rate (meq/L)	Dry weight of tubers (g)							
	H1	H2	H3	H4	H5	H6	H7	H8
3.45	2.05a	1.86a	1.60a	1.34a	1.19a	1.15a	0.78a	0.33a
5.1	1.98a	2.34a	1.58a	1.33a	1.53a	1.32a	0.61a	0.33a
6.75	1.54a	1.80a	1.21a	1.21a	0.85a	0.76a	0.49a	0.27a
8.4	1.81a	2.14a	1.40a	0.85a	1.34a	1.05a	0.68a	0.61a
	NS	NS	NS	NS	NS	NS	NS	NS



Plate B1 In vitro plantlets first week in the glasshouse for acclimatisation purpose.



Plate B2 Just after transplantation of potato plantlets of four different cultivars into the aeroponics system.

Table B2. Responses of different calcium levels on the nutritional values of potato minitubers grown in an aeroponic system. Significant F test at $P < 0.05$ (*) and $P < 0.01$ (**) and non-significant (NS). Treatment means followed by different letters differ significantly ($P < 0.05$).

Treatments	Phosphorus	Potassium	Calcium	Magnesium	Sodium	Iron
(meq/L)	%	%	%	%	mg/kg	mg/kg
Ca1 (8.40)	0.52a	2.61a	0.10a	0.17c	110.50a	104.29a
Ca2 (6.75)	0.52a	2.59a	0.08b	0.19b	132.38a	106.98a
Ca3 (5.10)	0.51a	2.57a	0.07c	0.19b	128.38a	122.04a
Ca4 (3.45)	0.52a	2.65a	0.06c	0.20a	126.31a	123.64a
	NS	NS	**	*	NS	NS

Treatments	Copper	Zinc	Manganese	Boron	Aluminium
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Ca1 (8.40)	2.68a	22.29b	9.81c	10.23a	11.54a
Ca2 (6.75)	2.64a	22.55b	12.30bc	10.51a	11.08a
Ca3 (5.10)	2.27b	20.32b	12.36bc	9.87a	13.28a
Ca4 (3.45)	2.27b	25.95a	14.68a	10.19a	12.65a
	*	*	*	NS	NS



Plate C1 Small minitubers stored at 3°C



Plate C2 Medium tubers stored at 3°C

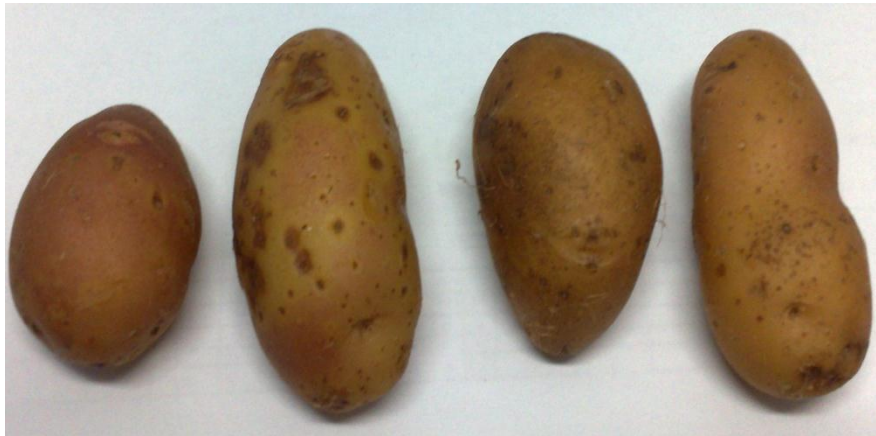


Plate C3 Large minitubers stored at 3°C

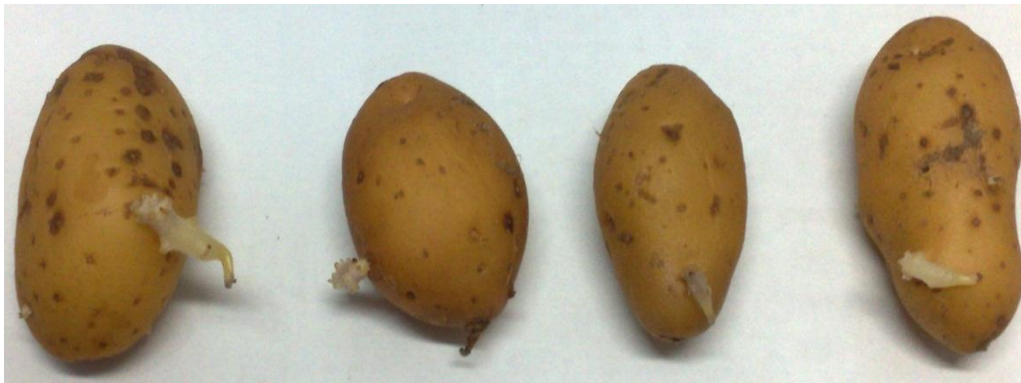


Plate C4 Small minitubers stored at 16°C



Plate C5 Medium minitubers stored at 16°C



Plate C6 Large minitubers stored at 16°C



Plate C7 Small minitubers stored at 25°C



Plate C8 Medium minitubers stored at 25°C

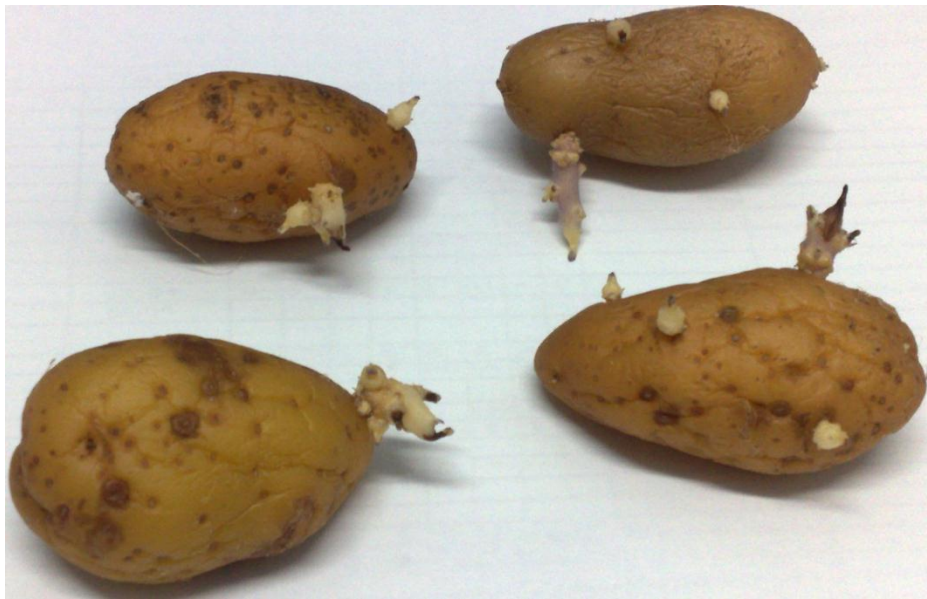


Plate C9 Large minitubers stored at 25°C



Plate C10 Potato plantlets at day 49 in the greenhouse.