

**The influence of different calcium levels, irrigation methods and storage temperatures on the yield, quality and growth potential of G0 mini-tubers**

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Thesis presented in partial fulfilment of the requirements for the degree of Master of Agricultural Sciences at the University of Stellenbosch.



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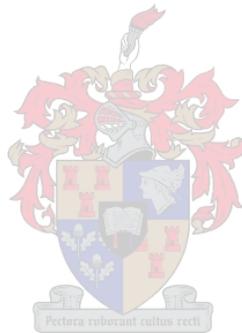
## Declaration

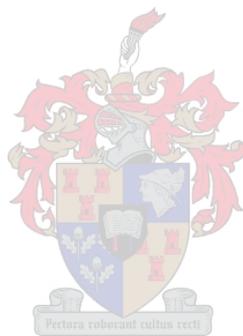
I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

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## Abstract

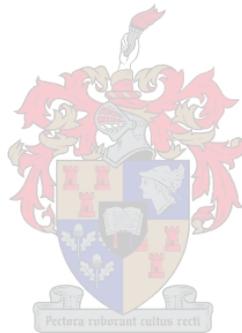
Calcium (Ca) is an important plant nutrient with many functions, such as strengthening of cell walls and maintaining membrane stability and cell integrity. A greenhouse experiment was conducted using an aeroponic production system, to evaluate the influence of different Ca: K & Mg ratios (consisting of a control [100% Ca], and three treatments in which the Ca levels were changed to 33%, 66% and 133% of the control, while the K and Mg levels were adjusted to compensate for the change in Ca) and two different irrigation methods (irrigation on roots only, and irrigation on roots and stolons) on tuber yield and mineral concentration. The treatment that received the highest Ca: K & Mg ratio had significantly more larger tubers than the lowest Ca treatment, although there was no significant difference in total tuber number between treatments. The high Ca treatment also had a significantly higher Ca concentration in the skin than the low Ca treatment. The site of irrigation did not have a significant effect on the total tuber number per plant, or on the Ca content of the tubers that were produced.

The tubers produced in the first experiment were divided into two weight classes, and stored at three different temperatures. The percentage weight loss during storage was determined by weighing the tubers before, and again after storage. The firmness of the tubers was also measured after storage. Tubers were then stored in a dark room at room temperature to allow sprouts to develop. The sprouts of each tuber were counted and weighed. Weight loss was the lowest for tubers stored at 3°C. Firmness of the tubers increased as the Ca: K & Mg ratio of the nutrient solution used during production was increased. Number of sprouts was the highest for tubers stored at 6°C. Sprout number was also significantly higher for the larger tubers compared to the smaller ones. Total sprout weight was the highest for the tubers stored at 6°C, and was also the highest for the larger tubers.

After sprouts started to develop, the tubers were planted again in the greenhouse, in sawdust and irrigated with a complete Steiner nutrient solution at 1.5 mS cm<sup>-1</sup>. After these plants were harvested, the leaf area and dry weight of the leaves were determined.

The first generation tubers were counted and weighed. The only factor that had a significant influence on the growth of the plants, was the size of the seed tubers that were used. The larger seed tubers produced plants that had significantly higher leaf areas, dry weight of leaves, as well as higher yields than that of the plants produced from the smaller seed tubers.

From the results of this study, it can be concluded that Ca has a definite positive effect on the quality of seed potatoes as well as the size of the tubers that are produced. This study also supported that seed tubers should be stored at low temperatures, around 3°C, to maintain the highest quality, while larger tubers proved to out-yield smaller ones.



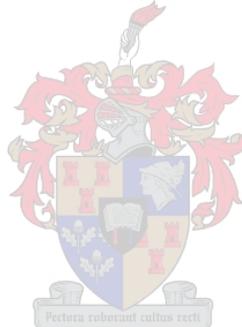
## Uittreksel

Kalsium (Ca) is 'n baie belangrike plant voedingstof met verskeie funksies, soos byvoorbeeld die versterking van selwande en die handhawing van membraan stabiliteit. 'n Kweekhuis eksperiment is gedoen om die invloed van verskillende Ca : K & Mg verhoudings (bestaande uit 'n kontrole [100 % Ca] en drie behandelings waar die Ca vlakke verander is na 33%, 66% en 133% van die kontrole, terwyl die K en Mg vlakke aangepas is om te kompenseer vir die verandering in Ca) en twee verskillende besproeiings metodes (besproeiing op slegs die wortels, en besproeiing op die wortels en die stolons) op die knolopbrengs en voedingstofinhoud van die knolle te toets. Daar was geen verskil in die totale hoeveelheid knolle per plant tussen behandelings nie, maar die behandeling wat die hoogste Ca : K & Mg verhouding ontvang het, het betekenisvol meer groter knolle produseer as die behandeling wat die laagste Ca ontvang het. Die knolle van die plante wat die hoogste Ca vlakke ontvang het, het ook 'n betekenisvolle hoër Ca konsentrasie bevat as die behandeling wat die laagste Ca vlak ontvang het. Die metode van besproeiing het nie 'n betekenisvolle invloed op die knolopbrengs of die Ca inhoud van die knolle gehad nie.

Die knolle wat in die eerste eksperiment geproduseer is, was verdeel in twee gewig klasse en is by drie verskillende temperature opgeberg. Die gewigsverlies persentasie van die knolle is bepaal deur die knolle te weeg voor opberging, en weer na opberging. Die fermheid van die knolle is ook gemeet nadat dit gestoor is. Die knolle is toe geplaas in 'n donker kamer by kamertemperatuur om die ontwikkeling van spruite te stimuleer. Die spruite van elke knol is getel en geweeg. Gewigsverlies was die laagste vir die knolle wat by 3°C gestoor is. Die fermheid van die knolle het toeneem soos die Ca : K & Mg verhouding van die voedingsoplossing wat gebruik is tydens produksie toeneem het. Die hoeveelheid spruite was die hoogste vir die knolle wat by 6°C gestoor is. Die hoeveelheid spruite was ook betekenisvol hoër vir die groter knolle as die kleiner knolle. Die gewig van die spruite was ook die hoogste vir die knolle wat by 6°C gestoor is. Dit was ook hoër vir die groter knolle in vergelyking met die kleiner knolle.

Nadat die knolle begin spruit het is dit weer in 'n kweekhuis geplant. Dit was geplant in saagsels, en is besproei met 'n standaard Steiner voedingsoplossing teen  $1.5 \text{ mS cm}^{-1}$ . Nadat die plante geoes is, is die blaaroppervlakte en die droë gewig van die blare gemeet. Die knolle wat geproduseer was, is getel en geweeg. Die enigste faktor wat 'n betekenisvolle invloed gehad het op die groei en opbrengs van die plante, was die grootte van die G0 knolle. Die groter knolle het plante produseer met groter blaaroppervlaktes, en hoër droë gewig. Die groter knolle het ook hoër opbrengste gelewer.

Uit die resultate van hierdie eksperimente kan daar gesien word dat Ca 'n betekenisvolle positiewe invloed op die kwaliteit van saadaartappels het, sowel as op die grootte van knolle wat geproduseer word. Daar kan ook afgelei word dat dit beter is om saadaartappels by lae temperatuur ( $\pm 3^\circ\text{C}$ ) op te berg om 'n goeie kwaliteit te behou, en dat groter knolle beter opbrengste lewer as kleiner knolle.



## Acknowledgements

I wish to express my sincere gratitude to the following persons:

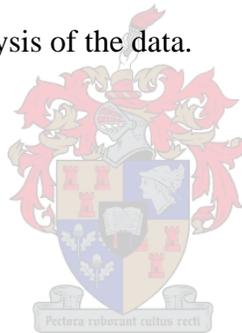
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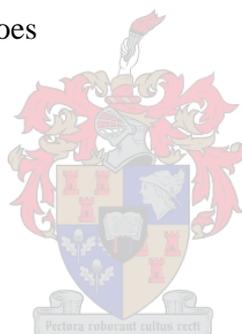


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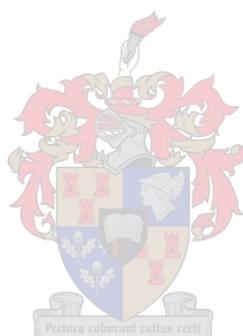
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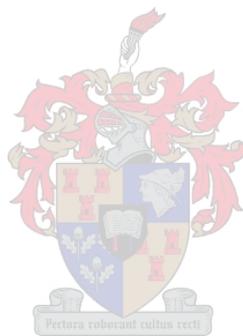
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**Appendix**

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Language and style used in this thesis are in accordance with the requirements of the *South African Journal of Plant and Soil*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some redundancy between chapters has, therefore, been unavoidable.



## Chapter 1

### Introduction and literature review

#### 1.1. Introduction

According to legend the first potatoes for planting purposes in South Africa came from Holland to provide food for mariners visiting the Cape. Since then the potato industry has grown to become one of the most important food providers in South Africa (National Department of Agriculture, 2003).

Within the South African context, the gross value of potato production accounts for about 43 % of major vegetables, 15 % of horticultural products and 4 % of total agricultural production (National Department of Agriculture, 2003). On average, approximately 55600 ha are planted with potatoes annually. Dryland production comprises approximately 27 % of the total area planted but contributes only 17 % towards total production (National Department of Agriculture, 2003). The average potato production is 157.8 million 10 kg pockets per year valued at R1.57 billion. Seed potatoes account for approximately 13 % of the total potato production, exports 7 %, while the rest is for consumption (National Department of Agriculture, 2003).

South Africa is not an important role-player in terms of world production. Major potato producing countries, namely China (15 %), Russian Federation (10 %), Poland (9 %), India (8 %) and the USA (8 %) jointly produce approximately half of the world total. South Africa is only ranked the 31st largest potato producer, supplying 0.5 % of the world's total production (National Department of Agriculture, 2003).

Potato cultivars available in South Africa can be divided into three groups according to the length of their growing periods. In the group for early cultivars (less than 100 days) Vanderplank is the most popular cultivar. The medium-growing season cultivars (100 to

120 days) form the bulk of potatoes grown in South Africa of which BP1 and Up-to-Date are the most popular cultivars at present. Together BP1 and Up-to-Date constitute almost 77 % of the potatoes grown in the country. The third group of cultivars is those with a longer growing season (longer than 120 days) such as Sackfiller, Late Harvest, Kimberley's Choice and Cedara (National Department of Agriculture, 2003).

According to Pieterse (1986), seed potatoes constitute about 30-40 % of the total production cost for potatoes in South Africa. However, seed potatoes account for only approximately 13 % of the total potato production. It is therefore much more expensive to produce seed potatoes than potatoes for consumption. Therefore it is important to produce seed tubers of good quality and attempt to increase yields.

*In vitro* material was first used in South Africa in the late 1980's (Kleingeld, 1997). The nuclear facility at Lydenburg was the first to use *in vitro* plantlets to produce mini-tubers in a greenhouse. The first certification of mini-tubers took place in 1993 (Kleingeld, 1997). Since then, four labs for the production of *in vitro* plantlets have been established.

The ratio of mini-tubers produced to the number of *in vitro* plants planted differs between varieties and greenhouses. The average ratio over two seasons for all the greenhouses were 2.85 mini-tubers for each *in vitro* plant planted (Kleingeld, 1997). This poor ratio can possibly be ascribed to the high mortality rate of *in vitro* plants in greenhouses.

Due to the high production costs for mini-tuber production, it is important to optimize the production systems. In this regard systems need to be implemented to maximize the tuber count per unit area, and per *in vitro* plant planted in the greenhouse.

According to Jones (1988), the advantages of using *in vitro* plantlets include:

1. The rapid multiplication of disease free planting material;
2. An interruption of the cycle of soil borne diseases;
3. Reduced labour;

4. Increased flexibility in respect of the scheduling of potato production and less dependence on the physiology of the mother tuber;
5. Production in a controlled environment; and
6. A reduction in the cost of transportation.

There are however disadvantages as well, which include:

1. Planting materials are expensive because specialized facilities and trained labour are needed for production;
2. *In vitro* plantlets produce small plants which require better management;
3. Contamination with pathogens can cause losses; and
4. Specific problems occur and the formation of roots can be a problem.

The mini-tubers that are produced in the greenhouses are usually planted in fields. Diseases multiply with each field multiplication and this leads to a decrease in quality (Kleingeld, 1997). The aim of the production and use of *in vitro* material is to improve the health status of existing seed stock by reducing the number of field multiplications (Kleingeld, 1997). Thus, if higher yields can be obtained, it is possible to decrease the number of field multiplications to prevent the reduction of quality.

There are many factors that can have an effect on the yield and quality of potatoes. The following topics were identified as the most important for the purpose of this study:

- Role of calcium in plant nutrition
- Role of calcium in potatoes
- Role of potassium and magnesium in potatoes
- Quality characteristics of seed potatoes
- Aeroponics in seed potato production
- Factors influencing tuberization
- Storage requirements for potatoes

The rest of this chapter will focus on the discussion of these topics and conclude with a detailed description of the study objectives.

## **1.2. Role of calcium in plant nutrition**

Calcium (Ca) is an important factor in determining quality and yield of most crops and plants. It has several functions in the plant, such as cell wall stabilization, cell extension, membrane stabilization and modulation of certain enzymes (Marschner, 1986). Without adequate Ca supplies to the plant, most crops will give lower yields, and the quality of storage organs and fruits may be poor and more susceptible to diseases and mechanical damage (Marschner, 1986). It is therefore essential that adequate amounts of Ca be available for uptake by the plant.

### **1.2.1. Ecological aspects of soil calcium**



Soils differ widely in their pH and Ca contents. This is particularly the case in uncultivated soils. During evolution, plant species have adapted to these varying pH and Ca conditions. For this reason remarkable differences in toleration occur between plant species and even varieties of a single species. In this respect plant species may be divided into *calcicoles* and *calcifuges* (Mengel & Kirkby, 1987). The calcicoles are typical of the flora observed on calcareous soils whereas the calcifuge species grow on acidic soils poor in Ca. Fairly clear differences occur in Ca metabolism of these two groups. Many of the calcicole species contain high levels of intracellular Ca and high concentrations of malate, whereas the calcifuges are normally low in soluble Ca. Species and even cultivars may differ considerably in their capability to precipitate Ca. It is supposed that this precipitation is mainly Ca oxalate (Mengel & Kirkby, 1987). Other Ca containing crystals, the true nature of which has yet to be established, may also be formed (Bangerth, 1979).

The level of Ca in the soil, however, is not the only factor of importance in the calcicole - calcifuge question, for calcareous and acid soils differ in other respects. Calcareous soils are higher in pH and carbonate content. They are richer in nutrients, the level of soluble heavy metals is usually lower and in addition the activity of the nitrifying and nitrogen fixing bacteria is higher. This, as well as the effects of Ca levels, has a bearing on the ecology of plants growing in these soils (Mengel & Kirkby, 1987).

### **1.2.2. Calcium uptake and translocation**

Higher plants often contain Ca in appreciable amounts. These high Ca contents, however, mainly result from the high Ca levels in the soil solution rather than from the efficiency of the Ca uptake mechanism of root cells (Mengel & Kirkby, 1987). Generally the Ca concentration of the soil solution is about 10 times higher than that of K. The uptake rate of Ca, however, is usually lower than that of K. This low Ca uptake potential occurs because Ca can be absorbed only by young root tips in which the cell walls of the endodermis are still unsubserved (Mengel & Kirkby, 1987). The uptake of Ca can also be competitively depressed by the presence of other cations such as K and  $\text{NH}_4$  which are rapidly taken up by roots (Mengel & Kirkby, 1987). The Ca content of plants is also to a large extent genetically controlled and is little affected by the Ca supply in the root medium, provided that the Ca availability is adequate for normal plant growth (Loneragan & Snowball, 1969).

In contrast to potassium and phosphate, transport of Ca is restricted to an area just behind the root tips (Clarkson & Hanson, 1980). This difference in behavior between nutrients has been explained by Clarkson & Hanson (1980) in terms of root structure and particularly the development of the casparian strip. As roots age the endodermis becomes subserved although continuity of the symplast is maintained through the endodermal walls by plasmodesmata. As the radial movement of Ca is prevented by the subserved endodermis, it is not transported effectively by the symplast. The movement of Ca from the cortex to the stele is therefore restricted to the apoplastic or free space

pathway which is only accessible in non suberized young roots (Mengel & Kirkby, 1987).

Calcium in the xylem sap is translocated in an upward direction with the transpiration stream. Thus the intensity of transpiration controls the upward translocation rate of Ca to a large extent (Mengel & Kirkby, 1987). In addition to transpiration, root pressure can also play a role. This is particularly true at night when transpiration more or less stops. The movement of Ca in the xylem vessels, however, can not be explained simply in terms of mass flow as Ca ions are absorbed by adjacent cells and also adsorbed to indiffusible anions in the xylem walls (Mengel & Kirkby, 1987).

The importance of exchange reactions in Ca movement is particularly clear from studies on individual plant organs where the correlation between intensity of transpiration and uptake of Ca is often much less close than for the plant as a whole. In leaves for example the influx of Ca sharply declines after leaf maturity even though a constant transpiration rate is maintained (Koontz & Foote, 1966). The same holds true for the influx of Ca into fruit. In growing plants there is evidence that Ca is translocated preferentially towards the shoot apex even though the transpiration rate here is much lower than in the older leaves. It now seems likely that this preferential movement is induced by the auxin indole acetic acid (IAA), which is synthesized in the shoot apex (Mengel & Kirkby, 1987). It is believed that during growth an IAA stimulated proton efflux pump in the elongation zones of the shoot apex increases the formation of new cation exchange sites so that the growing tip becomes a centre for Ca accumulation (Mengel & Kirkby, 1987).

The rate of downward translocation of Ca is very low due to the fact that Ca is transported in very small concentrations in the phloem (Mengel & Kirkby, 1987). The downward transport of Ca in cotton plants was inadequate to support root growth in the Ca deficient nutrient solution portion of a split root medium. Once Ca is deposited in older leaves it cannot be mobilized to the growing tips (Loneragan & Snowball, 1969). As a result of the low Ca concentration in the phloem, all plant organs which are largely provided with nutrients by the phloem sap are rather low in Ca. On the other hand the K

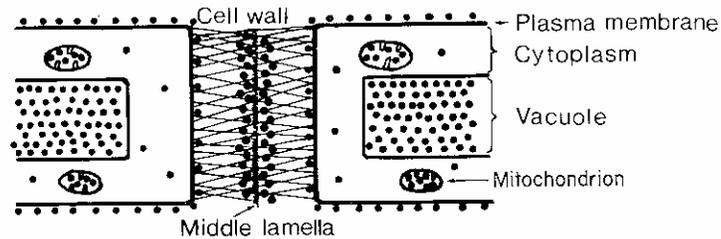
contents of these organs are relatively high, because K is present in the phloem sap in abundant quantities. This relationship is particularly evident when the Ca and K contents of leaves are compared with those of fruits and storage tissues. The poor supply of Ca to fruits and storage organs can result in Ca deficiency in these tissues (Mengel & Kirkby, 1987).

### **1.2.3. Functions of Calcium in Plants**

#### **1.2.3.1. Cell Wall Stabilization**

In contrast to the other macronutrients, a high proportion of the total Ca in plant tissue is located in the cell walls (apoplast). This unique distribution is the result of an abundance of binding sites for Ca in the cell walls as well as the restricted transport of Ca across the plasma membrane into the cytoplasm (Marschner, 1986).

The typical distribution of Ca in cells of fully expanded tissue is shown in Figure 1. There are two distinct areas in the cell wall with high Ca concentrations: the middle lamella and the exterior surface of the plasma membrane (Marschner, 1986). In both areas Ca has essential structural functions, namely the regulation of membrane permeability and related processes and the strengthening of the cell walls, respectively. The degradation of pectates is mediated by polygalacturonase, which is drastically inhibited by high Ca concentrations (Marschner, 1986). In agreement with this, in calcium-deficient tissue polygalacturonase activity is increased, and a typical symptom of calcium deficiency is the disintegration of cell walls and the collapse of the affected tissues, such as the petioles and upper parts of the stems (Marschner, 1986).



**Figure 1** Schematic representation of two adjacent cells with a typical distribution of  $\text{Ca}^{2+}$  (Marschner, 1986).

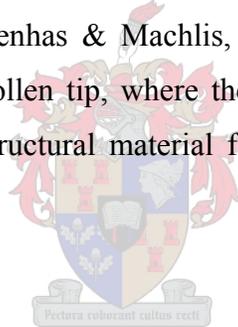
In the leaves of plants receiving high levels of Ca during growth or grown under conditions of high light intensity, a large proportion of the pectic material exists as calcium pectate. This makes the tissue highly resistant to degradation by polygalacturonase (Marschner, 1986). The proportion of calcium pectate in the cell walls is of importance for determining the susceptibility of the tissue to fungal infections and for the ripening of fruits. As shown by Rigney & Wills (1981) in experiments with tomato pericarp tissue during fruit development, the Ca content of the cell walls increases to the fully grown immature stage, but this is followed by a drop in the content just before the onset of ripening ("softening" of the tissue). Simultaneously a shift in the binding stage of Ca occurs in which water-soluble Ca is favored over wall-bound Ca. This shift is associated with a sharp increase in ethylene formation in the fruit tissue. Increasing the Ca content of fruits, for example, by spraying several times with calcium salts during fruit development or by post-harvest dipping in  $\text{CaCl}_2$  solutions, leads to an increase in the firmness of the fruit (Marschner, 1986) and delays or even prevents fruit ripening.

These results strongly support the view that solubilization of Ca binding sites in the cell walls and subsequent redistribution of Ca within the cells (probably via transport to the vacuoles) depresses polygalacturonase and also activates the ethylene-generating system, which is located in a cell wall plasma membrane complex at the membrane-cell wall interface. Intracellular redistribution of Ca is therefore the primary stimulus of, or acts as a secondary messenger in the onset of the ripening process of fleshy fruits (Rigney & Wills, 1981).

### 1.2.3.2. Cell Extension

In the absence of an exogenous Ca supply, root extension ceases within a few hours. This effect is more distinct in a Ca free nutrient solution than in distilled water, an observation consistent with the function of Ca in counterbalancing the harmful effects of high concentrations of other ions at the plasma membrane (Marschner, 1986). Although Ca is also involved in cell division (Marschner, 1986), the cessation of root growth in the absence of exogenous Ca is primarily the result of inhibited cell extension. The role of Ca in cell extension is not yet clear; there are indications, however, that it is required for the incorporation of material into the cell walls (Marschner, 1986).

Pollen tube growth is also dependent on the presence of Ca in the growth medium, and the direction of growth of the pollen tube is chemotropically controlled by the extracellular Ca gradient (Mascarenhas & Machlis, 1964; Marschner, 1986). The Ca level is highest in the growing pollen tip, where the plasma membrane fuses with the secretory vesicles, which carry structural material for cell wall formation (Marschner, 1986).



### 1.2.3.3. Membrane Stabilization and Enzyme Modulation

The fundamental role of Ca in membrane stability and cell integrity is reflected in various ways. It can be demonstrated most readily by the increased leakage of low-molecular-weight solutes from cells of calcium-deficient tissue (e.g., tomato fruits) (Goor, 1966) and, in severely deficient plants, by a general disintegration of membrane structures and a loss of cell compartmentation (Marschner, 1986).

In calcium-deficient tissues, increased respiration rates are related to enhanced leakage of respiratory substrates from vacuoles to the respiratory enzymes in the cytoplasm (Bangerth, Dilley & Dewey, 1972). Calcium treatment of deficient tissues therefore decreases respiration rates; it also enhances the net rate of protein synthesis. These

features of calcium deficiency are similar to those related to senescence. It is now well established that Ca, as well as phytohormones, is involved in the regulation of senescence (Marschner, 1986).

Calcium stabilizes cell membranes by bridging phosphate and carboxylate groups of phospholipids and proteins, preferentially at membrane surfaces (Marschner, 1986). There can be an exchange between Ca at these binding sites and other cations (e.g., K, Na or H), although the latter cannot replace Ca in its membrane stabilization role. To fulfill its functions at the plasma membrane, therefore, Ca must always be present in the external solution, where it regulates the selectivity of ion uptake and prevents solute leakage from the cytoplasm (Marschner, 1986). The membrane-protecting effect of Ca is most prominent under various stress conditions such as low temperature and anaerobiosis (Marschner, 1986).

In contrast to Mg, which is a strong activator of enzymes, Ca increases the activity of only a few enzymes. These include  $\alpha$ -amylase, phospholipases, and ATPases (Marschner, 1986). In general Ca stimulates membrane-bound enzymes, particularly ATPases at the plasma membrane of roots of certain plant species. Because the activities of many membrane-bound enzymes are modulated by membrane structure, Ca presumably enhances the activity of those enzymes even though it is bound to non-catalytic sites at the membranes (Marschner, 1986). On the other hand, the inhibitory effects of Ca on various enzymes located in the cytoplasm and in the chloroplasts have been well documented by Marschner (1986) amongst others. Hexodiphosphatase and PEP carboxylase are two examples of enzymes inhibited by Ca (Marschner, 1986).

Because PEP carboxylase is the key enzyme for  $\text{CO}_2$  fixation in  $\text{C}_4$  species, a very low level of free Ca in the chloroplasts is a prerequisite for a high photosynthetic rate. In  $\text{C}_3$  species as well,  $\text{CO}_2$  fixation ceases abruptly if the concentration of Ca in the stroma of the chloroplasts reaches the level of Mg (Marschner, 1986).

#### 1.2.4. Calcium Forms and Contents

Calcium occurs in plant tissues as free Ca, and as Ca adsorbed to indiffusible ions such as carboxylic, phosphorylic and phenolic hydroxyl groups. It is also present in Ca oxalates, carbonates and phosphates. These compounds often occur as deposits in cell vacuoles. In seeds, Ca is present predominantly as the salt of the inositol hexaphosphoric acid (phytic acid). As already indicated Ca in the cell wall is associated with the free carboxylic groups of the pectins and saturates most of these sites (Mengel & Kirkby, 1987).

#### 1.2.5. Calcium Deficiency and Disorders

Ca deficiency is characterized by a reduction in growth of meristematic tissues. The deficiency can be first observed in the growing tips and youngest leaves. These become deformed and chlorotic and at a more 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 & Kirkby, 1987).

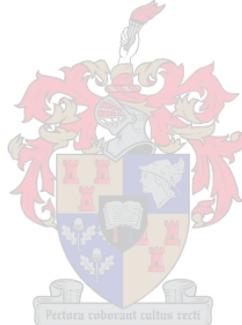
Absolute Ca deficiency as described above occurs relatively seldom as most mineral soils are rich in available Ca. Indirect Ca deficiency resulting from an undersupply of Ca to fruit and storage tissues, however, is an often observed disorder (Shear, 1975). In apple the disease is called bitter pit as the whole of the surface of the apple is pitted with small brown necrotic spots. In tomato the disease is known as blossom-end rot and is characterized by a cellular breakdown at the distal end of the fruit (Shear, 1975). All these tissues are mainly supplied with Ca by the transpiration stream which translocates Ca directly from the soil solution (Mengel & Kirkby, 1987). If the xylem sap is low in Ca or the rate of transpiration of the fruits is poor, as occurs under humid conditions, inadequate levels of Ca may be supplied to the fruits and deficiency symptoms may result. Calcium translocation in the xylem sap may be depressed by  $\text{NH}_4$  - nutrition, soil

water stress and high salt concentrations in the soil. These factors have therefore been found to favour the occurrence of blossom-end rot in tomatoes (Mengel & Kirkby, 1987).

Calcium appears only to be transported from the soil solution to the upper plant parts *via* root tips (Mengel & Kirkby, 1987). Any factor which prevents the growth of new roots (poor aeration, low temperatures etc.) may therefore be expected to prevent Ca uptake and thus induce deficiency. This may account for the observation that Ca related disorders often occur on soils inadequately supplied with Ca, and that the weather appears to be a controlling factor (Bangerth, 1979).

Fruits and storage tissues growing in the soil, such as peanuts, potatoes and celery bulbs are not supplied by the transpiration stream and for this reason Ca must be absorbed directly from the soil medium (Mengel & Kirkby, 1987).

### **1.3. Role of Calcium in potatoes**



#### **1.3.1. Crop Needs**

Calcium is a key component of cell walls, helping to build a strong structure and ensuring cell stability. Calcium enriched cell walls are more resistant to bacterial or fungal attacks. It is critical during cell division, and is therefore essential prior to, and during, the rapid growth phase of tubers (Harris, 1992).

Calcium also helps the plant adapt to stress by influencing the signal chain reaction when stress occurs, it has a key role in regulating the active transport of potassium for stomatal opening, and is particularly effective at helping reduce summer heat stress, minimizing wilting and leaf damage (Harris, 1992).

### 1.3.2. Calcium Movement

The vast majority of calcium taken up by the plant is through the main root system. It is then transported upwards to the leaves through the xylem, by water flow through the plant. Once in the leaf, calcium is immobile and unlike other nutrients, such as nitrogen, phosphorous and potassium, it doesn't move to other leaves or down to the tubers at a later stage (Westermann, 2005).

Calcium movement into the tubers is via the stolon and tuber root hairs, as well as through the tuber skin (Westermann, 2005). Readily available calcium supplied in the soil close to the stolons and tubers is the most effective way to increase tuber calcium levels (Karlsson & Palta, 2003). This can be best achieved by top-dressing with calcium nitrate at tuber initiation (Westermann, 1993). Where levels of calcium in the leaves are low, foliar applications will quickly increase supplies. This will improve the plant's tolerance to stresses such as heat and frost (Mengel & Kirkby, 1987).



### 1.3.3. Quality Effects

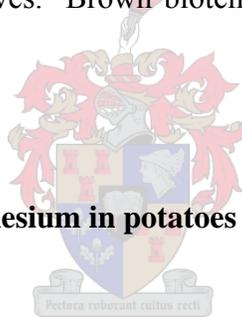
Calcium has an important effect on tuber cooking and storage quality. Internal disorders such as internal rust spot or internal brown spot can be reduced by a good calcium supply (Karlsson & Palta, 2003), in the form of calcium nitrate, at ridging or during tuber initiation. Ensuring there is a minimum of 0.15 % calcium in the peel, improves potato skin finish, boosts disease tolerance and minimizes internal rust spot (Ulrich, 1993). Therefore peel analysis is a good way of confirming whether a disease or skin finish problem is calcium related. Even small amounts of calcium in the tuber can make a big difference.

Strengthening of the tuber cell wall with calcium can help reduce the severity of *Erwinia* soft rot in storage (Harris, 1992). Calcium also reduces the level of tuber skin diseases including Black Scurf and Powdery Scab, leading to better skin finish. However, high

levels of calcium in the soil may increase the level of common scab (Mienie & Theron, 1999). High levels of calcium in the tuber also reduce bruising risks at harvest and subsequent transportation. The use of calcium in crops grown for seed boosts the following crop's performance. A mother tuber enriched with calcium is faster growing and in better condition, thereby boosting yield per plant (Harris, 1992).

### **1.3.4. Deficiency Symptoms**

In the field, moderate calcium deficiencies are unlikely to show leaf symptoms, but brown blotches may develop around the stolon end of the tuber. Severe deficiencies will show symptoms in the leaves. The youngest leaves are pale and small, and curl downwards at the ends of the leaves. Brown blotches may appear on the leaf margins (Mengel & Kirkby, 1987).



## **1.4. Role of potassium and magnesium in potatoes**

### **1.4.1. Potassium**

Starch development is increased by potassium, as well as some reactions in the photosynthesis process. Too much potassium can cause poor tuber quality (Steyn & Prinsloo, 1999). Potatoes need relatively high levels of available potassium. Mixing of the fertilizer in the seedbed will produce the best results, because potassium has low mobility in the soil (Mcdole & Westermann, 1998).

Potassium fertilization can increase the yield, especially where potassium levels in the soil is low. Increase in yield is mainly due to bigger tubers (Panique *et al.*, 1997). Too much potassium, however, can cause lower yields (Harris, 1992).

Potassium can reduce the specific gravity of tubers, which is undesirable for the processing industry, thus it is important not to use too much potassium (Panique *et al.*, 1997; Mcdole & Westermann, 1998). Mcdole & Westermann (1998) found that specific gravity was lower when potassium chloride (KCl) was used as compared to potassium sulphate ( $K_2SO_4$ ).

Potassium doesn't have a big effect in the early stages of growth, but it increases the LAI at the end of the growing season. It inhibits the abscission of the leaves, thus the plant will live longer and can photosynthesize longer to further increase yields (Harris, 1992).

### **1.4.2. Magnesium**

Magnesium is of great importance for the potato plant since it is indispensable to a number of enzymatic reactions occurring in the plant cells. One of the most important functions of magnesium is that it enables proper utilization of nitrogen by the plant and its transformation into organic compounds (Cieslik & Sikora, 1998).

The calcium/magnesium ratio is of significant importance in potato plants. A deficiency of both elements is equally as disadvantageous as an excess of magnesium in relation to calcium, since magnesium, due to its antagonistic action, causes a decrease in calcium absorption by plants (Cieslik & Sikora, 1998).

### **1.4.3. Deficiency symptoms**

#### **1.4.3.1. Potassium**

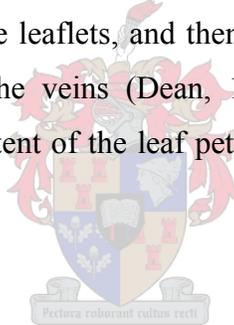
Potassium deficiency symptoms are first visible when the young leaves are spread open fully. The leaves are bluish-green in colour, and later the older leaves will turn yellow with brown margins and apices. The foliage may be crinkled, and the veins sunken

(Dean, 1994). In severe cases necrotic batches of leaves will appear. The internodes of the plant are also shortened (Mengel & Kirkby, 1987).

Deficiencies can usually be found on sandy loam soils with low cation exchange capacity. Deficiencies can also occur in clay-loam soils and soils with high levels of exchangeable magnesium. Deficiency symptoms will develop when the potassium content of the leaf petioles are less than 2 % and that of the leaf sheaths less than 1 % (Ulrich, 1993).

#### **1.4.3.2. Magnesium**

With magnesium deficiencies, the lower leaves are more lightly coloured than normal, first on the tips and margins of the leaflets, and then extending between the veins with, later, necrotic patches between the veins (Dean, 1994). Deficiency symptoms will develop when the magnesium content of the leaf petioles or sheaths are lower than 0.09 % (Ulrich, 1993).



#### **1.5. Quality characteristics of seed potatoes**

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 (Struik & Wiersema, 1999). Many of the factors determining yield are affected by seed quality. This occurs through the effects of seed quality on the number of plants and stems per unit area, the types 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 & Wiersema, 1999). Physiological seed quality (size and physiological age) is thus a very important determinant of yield, but through a complex of factors and their interactions.

### **1.5.1. Size of seed tuber**

The size of seed tubers is a main quality characteristic, because it affects the number of eyes per tuber. Larger tubers have more eyes than smaller tubers, because as tubers grow larger, new lateral buds are continuously initiated (Nielson, Iritani & Weller, 1989). Larger tubers can therefore produce more sprouts, and this can affect the number of main stems developing per seed tuber. Because larger tubers develop more main stems, ground cover increases at a faster rate than that of smaller tubers. Consequently, the amount of radiation accumulated by the crop will be increased, and therefore the yields of crops grown from larger seed tubers are higher (Struik & Wiersema, 1999).

### **1.5.2. Physiological age of seed tuber**

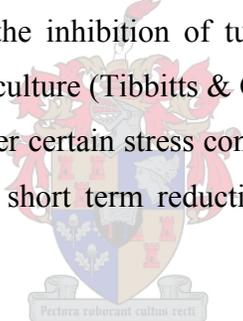
Physiological age may be defined as “the stage of development of a tuber, which is modified progressively by increasing chronological age (age from the time of tuber initiation), depending on growth history and storage conditions”. Physiological age is a crucial aspect of seed quality. It not only influences the behaviour of the seed, but also the behaviour of the crop grown from it. Physiological age affects the number of sprouts and the sprout behaviour, but also the growth pattern of the plant that originates from it, and thus sometimes the tuber yield of the crop produced from it (Struik & Wiersema, 1999).

## **1.6. Aeroponics in seed potato production**

Soil-less production techniques, such as the Nutrient Film Technique (NFT) and aeroponics have successfully been employed in tuber production, with good prospects for certified seed production (Farran & Mingo-Castel, 2006). However, Wheeler *et al.* (1990) described injury to periderm tissue by salt accumulation from the nutrient solution on the surface of the tuber using the NFT technique, and Tibbitts & Cao (1994) found

that tuber initiation in nutrient solution without solid media was poorer than in porous media.

Aeroponic culture is an alternative method of soil-less culture in growth-controlled environments. The underground organs are enclosed in a dark chamber and supplied with a nutrient solution by way of a misting device. Aeroponics optimizes root aeration, which is a major factor leading to yield increases as compared to classical hydroponics (Farran & Mingo-Castel, 2006). It shows other advantages such as solution recirculation, a limited amount of water used, and good monitoring of nutrients and pH. The worst inconvenience relies on the low volumes available to the root system, and any losses of power to pumps can produce irreversible damages (Farran & Mingo-Castel, 2006). Despite these problems, this technique has been applied successfully for the production of different horticultural and ornamental species (Farran & Mingo-Castel, 2006). Although some authors reported the inhibition of tuberization in immersed organs or roots subjected to continuous mist culture (Tibbitts & Cao 1994), tuberization under these procedures could be promoted under certain stress conditions such as nitrogen deficiency (Krauss & Marschner, 1982) and short term reductions in solution pH (Wan, Cao & Tibbitts, 1994).



Harvesting in aeroponics is convenient, clean, and allows a greater size control by sequential harvesting (Ritter *et al.*, 2001). The number and timing of non-destructive harvests are key factors in the optimization of mini-tuber production. To optimize the system, appropriate nutrient solutions, plant densities, number of harvests and harvesting intervals, as well as possible interactions between them should be considered (Farran & Mingo-Castel, 2006).

## 1.7. Factors influencing tuberization

Tuberization in potatoes is a complex process during which the stolon differentiates to form the tuber. This process is characterized by significant anatomic, hormonal and biochemical changes in the plant (Sharma, Kaur & Gupta, 1998). There are many factors that have an influence on tuberization. The most important however is photoperiod, temperature, irradiance and nitrogen levels (Jackson, 1999). Other factors that also have an influence are drought stress and soil saturation.

### 1.7.1. Photoperiod

Photoperiod has a significant influence on the time of tuberization, as well as on the number of tubers that develops. Induction to tuberize is promoted by short photoperiods (long nights) (Ewing & Struik, 1992), and the signal is perceived in the leaves (Gregory, 1956). In controlled environments, Gregory (1956) proved that tuberization was inhibited by high temperatures and long days. However, it is important to remember that different cultivars will not have the same sensitivity for changes in the photoperiod.



### 1.7.2. Temperature

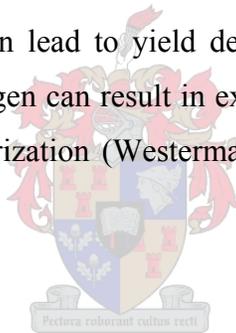
Like photoperiod, temperature exerts a major influence on tuberization, with cool temperatures favoring induction to tuberize (Ewing, 1981). The optimum air temperature for tuberization is in the range of 10-17 °C while it will be inhibited above an average daily temperature of 30 °C (O'Brien, Allen & Firman, 1998). According to Slater (1968) night temperature, as well as the temperature of the soil or growing medium, plays an important role in tuberization. An average temperature of 12-15 °C during the night is expected to be most favorable for tuberization. Tuberization will stop above an average night temperature of 24 °C (O'Brien *et al.*, 1998).

### 1.7.3. Irradiance

Like long photoperiods and high temperatures, low levels of irradiance during the day decrease the induction to tuberize (Demagante & Vander Zaag, 1988; Ewing & Struik, 1992). Menzel (1985) suggested that the effects of both low light intensity and high temperature are brought about by increased production of growth substances that inhibit tuber formation. Gibberellins are the most likely candidates for such a role. As might be expected, a combination of high temperatures and low irradiance is especially inhibitory to tuberization (Ewing & Struik, 1992).

### 1.7.4. Nitrogen

Although nitrogen deficiencies can lead to yield decreases, too much nitrogen is also disadvantageous. Too much nitrogen can result in excessive vegetative growth, and this will inhibit, or even prevent tuberization (Westermann *et al.*, 1994; Steyn & Prinsloo, 1999).



### 1.7.5. Drought stress

Drought stress has a significant effect on the induction to tuberize. The effects of drought stress in restricting potato yields may be associated with reductions in the canopy and consequent restrictions in the ability to produce assimilate rather than with direct effects on induction (Ewing & Struik, 1992). It is uncertain whether water around the stolon tip is needed for tuberization to occur. Struik & Van Voorst (1986) observed that the formation of tuber initials was greatly enhanced when the stolon medium was kept air dry during tuberization. This effect was more pronounced when the uptake of water by the roots was sufficient, but was also seen when drought occurred both in the root and the stolon medium. However, the large increase in the number of swollen stolon tips caused by the dry stolon medium did not result in more tubers.

### **1.7.6. Soil saturation**

Excessive soil water was reported to have deleterious effects on the number of tubers, either by restricting the number initiated, or by causing too many to be initiated (Harris, 1978; Ewing & Struik, 1992). The nature of these effects is not understood, but they presumably involve changes in soil gas exchange or soil compaction.

### **1.7.7. Implications for tuber yield**

In view of the effects of induction on overall plant development, it is obvious that the highest tuber yields are not necessarily associated with the highest levels of induction. Neither extreme (very strong or very weak induction) is desirable (Ewing & Struik, 1992). If there is no induction, there can be no yield of tubers, but very strong induction occurring early in the growth of the plant will severely limit tuber yields because of the stunted canopy and roots.

If there is a premium for early yields, it may be desirable to sacrifice potential yield by aiming for fairly strong induction early in the season. This would mean selection of a cultivar with a long critical photoperiod, choosing physiologically old seed tubers and using a moderate to low rate of nitrogen fertilizer (Ewing & Struik, 1992). Cultivar selection, seed handling, nitrogen fertilization and time of planting provide opportunities for controlling the level of induction, thereby influencing the harvest index, earliness of harvest, duration of canopy cover, and tuber yield (Ewing & Struik, 1992).

## 1.8. Storage requirements for potatoes

### 1.8.1. General requirements

Potatoes can freeze at around  $-1\text{ }^{\circ}\text{C}$ , and chilling injury in particularly susceptible cultivars can occur after prolonged storage at  $2\text{ }^{\circ}\text{C}$ , although others can withstand  $0\text{ }^{\circ}\text{C}$  (Struik & Wiersema, 1999). The minimum safe storage temperature for some cultivars for prolonged periods is thus  $>2\text{ }^{\circ}\text{C}$ , although brief periods down to  $0\text{ }^{\circ}\text{C}$  give no cause for concern. Breakdown of tubers attributable to high temperatures can occur above  $35\text{ }^{\circ}\text{C}$ , but it is better to regard the maximum safe temperature to avoid breakdown as  $30\text{ }^{\circ}\text{C}$ . Potato storage should thus be within the limits of  $2\text{-}30\text{ }^{\circ}\text{C}$ , although neither limit, certainly not the upper, would be advisable for prolonged storage (Burton, 1989). These are limits for survival of the tubers, not for fitness for use.

In this chapter it is assumed that disease control during the growing of the crop has been adequate, because there is no point in storing potatoes in which widespread infection by tuber-rotting organisms has already occurred. However good the disease control, it could always be expected that a few storage rots will develop (Burton, 1989). These, once established, are less serious the lower the temperature, but storage temperature also plays a part in whether or not some of them become established. Damage during the course of harvesting and handling is inevitable, and unless these wounds are healed, infection by wound pathogens is inevitable (Dean, 1994). Storage conditions can thus reduce the incidence of rotting by wound pathogens if they facilitate rapid wound healing (Burton, 1978). For wound healing, the higher the temperature and relative humidity (RH), the better, up to  $20\text{ }^{\circ}\text{C}$  and 98 % RH. However, a normally adequate rate of healing can be achieved at  $10\text{ }^{\circ}\text{C}$  and  $>75\text{ }^{\circ}\text{RH}$  (Burton, 1989). Two weeks under the latter conditions would be sufficient to allow suberization and periderm formation, compared with less than a week at  $20\text{ }^{\circ}\text{C}$  (Dean, 1994). Whatever may be the desired storage conditions for potatoes for any particular use, wound healing is nearly always an essential first step in successful storage, immediately after any operation which may have caused damage.

Apart from wound pathogens and any rots which may have been present at the commencement of storage, bacterial soft rots are the main cause of rotting (Burton, 1989). They normally only occur if the tubers are wet, either as a result of rain when the potatoes were being transported to the store, or of leakage of water onto the stored tubers or condensed water dripping on them, or of water condensing from warm humid air on the surface of cold tubers. It is essential for successful storage that the surface of the potatoes remains dry. This does not mean that the air in contact with the tubers should be at a low relative humidity. For several reasons this is often undesirable, but an actual film of water should be avoided. As in the case of other pathogens, the progress of the rots, once established, is less the lower the temperature (Burton, 1989).

Evaporative loss of water may quite often be the most serious cause of weight loss during storage, and in general is second only to rotting by pathogens as a source of loss. It is greater the greater the water vapour pressure deficit of the surrounding air - i.e. at any one temperature water loss is less the higher the relative humidity (Burton, 1978). It is much increased by harvesting- or handling wounds, and wound healing makes an important contribution to its limitation (Burton, 1989).

Respiratory loss of dry weight is the least important storage loss. The effect of storage temperature on respiration is in general at a minimum at about 5 °C, but the rate varies comparatively little over the range 4-10 °C (Burton, 1989). The reactions which occur during respiration, result in the production of CO<sub>2</sub> and the absorption of O<sub>2</sub>. Accumulation of CO<sub>2</sub> is undesirable at low levels and harmful at high levels. Considerable depletion of O<sub>2</sub> is also harmful (Burton, 1989). To maintain the CO<sub>2</sub> among the potatoes at an acceptably low level necessitates good ventilation (Burton, 1989). Under ordinary storage conditions a good rate of ventilation occurs by natural means and there is no need to make specific provision for it. If for any reason a gas-tight store were used, it would need to be ventilated.

It can be concluded that the best conditions to satisfy the general requirements for potato storage, that is to keep loss of weight and rotting at a minimum, and to allow for wound

healing, are storage for about two weeks immediately after harvest, transport, grading, or any other handling operation, at a temperature in the range of 10-20 °C, preferably at a high humidity (Dean, 1994). Thereafter the temperature should be held at 2-4 °C and the humidity as high as possible without the risk of condensation occurring on the tubers (Burton, 1989). These conditions may be contrary to the requirements for some specific purposes as described below, but marked deviation from these increases the risk, or the amount of loss. In many parts of the world, because of environmental conditions and lack of means of overcoming them, the storage conditions described represent a completely unattainable ideal, and considerable deviations from them, with associated losses, have to be accepted (Burton, 1989).

### **1.8.2. Requirements for seed potatoes**

The fundamental property expected from seed potatoes is that they should grow well when planted and that the crop from them should give a high economic yield. This means that by the time of planting the endodormant period should be over, but not to the stage beyond an optimal capacity to grow, i.e. the tubers should not be physiologically old (Struik & Wiersema, 1999). If the endodormant period is over, and environmental conditions permit, sprout growth will occur. With careful hand planting, sprouts 2-3 cm long or more may survive the planting procedures and grow. With machine planting or bulk handling, sprouts longer than a few millimeters are knocked off or damaged, but even so the potential of a previously well-sprouted tuber for immediate growth, from other buds, may be greater than that of a tuber on which the buds have just started to grow (Burton, 1989). This is true up to the stage beyond which sprouting capacity starts to decline. Sprouts several centimeters long which will be knocked off in planting need not be a disadvantage - they may indicate a potential for immediate vigorous growth. On the other hand, very long sprouts may be an indication that the physiological age of the tubers has reached the point of declining vigour. It must not be thought, however, that sprout growth is an infallible index of physiological age - unsprouted tubers can be physiologically too old after prolonged storage at a low temperature. It is quite possible

to hold seed tubers in superficially good condition, and unsprouted, for about 18 months or more at 1-2 °C, but would unlikely be capable of satisfactory growth (Burton, 1989).

Storage conditions, apart from their effect upon the sprouting potential of seed tubers, should also discourage disease and water loss and avoid the possibility of freezing, chilling, or high-temperature injury (Burton, 1989). Conditions for these requirements are outlined under 'General requirements'.

Seed tubers are very often stored by two people - the grower of the seed, and the ware grower who buys and plants it - and the importance they attach to the various requirements may be rather different. The former sells by weight and it is worth his while to avoid evaporative loss; the latter plants by number and evaporative loss is of no importance unless it impairs the capacity of the buds to grow and establish a plant (Burton, 1989). Both wish to avoid disease, and frost, chilling and high-temperature injury, but control of sprouting behaviour, as outlined below, is usually the concern more of the ware grower than of the seed grower. The seed grower is expected to supply healthy seed tubers which have not been stored or treated in any way which impairs their value, but getting the tubers to the right stage before planting, and the storage conditions necessary to achieve this are the responsibility of the ware grower, and he should arrange to take delivery sufficiently early to be able to exercise his options (Burton, 1989).

Cultivars differ greatly in their sprouting behaviour and a storage regime optimal for one cultivar could be different for another (Burton, 1989). Conclusions as to the storage requirements of a particular cultivar can only be reached on the basis of familiarity with its behaviour. The extent to which it is worth adapting storage practice to meet these particular requirements, needs to be assessed in relation to such variables as the method and speed of planting and the seriousness or otherwise of the consequences of not meeting them. Also it must be remembered that the state of the land at planting time may have an influence which overrides those resulting from refined storage techniques. Very often the practical conclusion, certainly if farming operations are largely mechanized, is to disregard the finer points of differences between cultivars, and adopt storage

conditions which give reasonably good results in general (Burton, 1989).

Temperature has an effect on the endodormancy of the tubers (Struik & Wiersema, 1999). The higher the temperature, up to perhaps 24 °C, the more rapidly endodormancy will end, although there are differences, not necessarily consistent, between cultivars, samples and seasons. Thus, if it is desired to bring the tubers to the state at which they will grow, usually the higher the storage temperature the better up to 24 °C (Burton, 1978). If it is desired to prolong the period of endodormancy, the lower the temperature the better, but not lower than 2 °C because of the risk of chilling injury (Burton, 1989).

Sprout growth subsequent to the break of dormancy is negligible or absent at 2 °C and in many cultivars at 4 °C (Burton, 1989). Above this temperature there is a rapid increase in the rate of growth up to a maximum at about 25 °C, although this rapid growth cannot be sustained and the eventual amount of sprout growth may be greater at 20 °C or even 15 °C. Light has a marked inhibiting effect on sprout elongation (Burton, 1989). If it is wished to encourage sprout growth, the storage temperature should be well above 4 °C and preferably in the range of 10-20 °C. To encourage sprout growth without excessive elongation, the high storage temperature should be combined with exposure to light. To prevent sprout growth, a temperature of 2-4 °C may be employed (Burton, 1989). If it is not possible to achieve such a low temperature, then the lowest temperature possible should be maintained (Struik & Wiersema, 1999).

#### **1.8.2.1. Storage by seed grower**

The seed grower should harvest under dry conditions or, if this is not possible, dry the crop by ventilation (Burton, 1978). Storage for about the first two weeks after harvest should be in the temperature range of 10-20 °C. Thereafter storage should be at the lowest temperature possible, down to 2 °C, and, if the health of the crop does not preclude this, at a high relative humidity. If, however, the seed grower intends exporting after a fairly brief storage period for immediate planting, the storage temperature

throughout should be as high as is considered safe, up to 20 °C (Burton, 1989).

It may not always be possible to achieve temperatures sufficiently low to prevent sprout growth, and control of sprouting by minimal applications of sprout suppressant chemicals is sometimes practiced (Struik & Wiersema, 1999). This saves weight loss for the seed grower but there is always a tendency for this method to result in delayed emergence, and sometimes in significantly reduced yields. In such a case, loss to the seed grower is avoided at the expense of the ware grower (Burton, 1989).

### **1.8.2.2. Storage by ware grower**

The ware grower should store for about two weeks, immediately after receipt of the potatoes, in the range of 10-20 °C (Burton, 1989). Thereafter the storage conditions should be managed in such a way that the tubers are in an optimum condition to grow after planting. Two extremes should be avoided. If the tubers are held back by a low storage temperature, growth initiation may be slow. This could happen even if the endodormant period is over, because the buds have not yet reached their maximum capacity to grow (Burton, 1989). Emergence is thus delayed and bulking of the crop occur later (Burton, 1978). A main-crop, if harvested late, may not suffer in final yield, but it is not always convenient to harvest late, because then it would be too late for the early market. At the other extreme, too high a storage temperature would result in excessive sprout growth (Struik & Wiersema, 1999). This would be removed before planting, or if not would be destroyed during planting, but the tubers could well be beyond their stage of maximum sprouting capacity. In this case replacement growth after planting would be slow and weak, and emergence could be much reduced. Yields could therefore suffer considerably.

It is suggested that seed potato storage by the user should be devoted to producing sprouts by planting time which are of the order of 10-20 mm long and starting to grow rapidly. Such sprouts will for the most part be destroyed or damaged during the handling

and planting of bulk-stored seed, but their presence indicates that the tuber is at a physiological state in which the vigour of growth from its buds is approaching at an optimal rate (Burton, 1989).

The yield penalty for exceeding this stage up to sprouts reaching 75 mm may not be serious, but much greater sprout growth gives increased loss of yield and in some cultivars the possibility of complete crop failure (Burton, 1989). Holding the seed tuber back by low-temperature storage, results in slower emergence and the possibility of a yield penalty in a short growing season, though not if the crop can be grown to maturity. Storage regimes which will give the desired degree of development depend very much on the cultivar and to some extent on the samples, but temperatures in the range of 5-10 °C may be envisaged. However, precise control of temperature, beyond that necessary to encourage some growth but prevent it becoming excessive, is unlikely to be economic (Burton, 1989).



### **1.9. Objectives of this study**

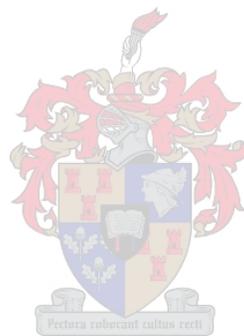
The main objective was to evaluate different Ca: K & Mg ratios and irrigation treatments for G0 mini-tuber production in South African greenhouses and to further evaluate the effect of different tuber sizes and post-harvest storage temperatures on the sprouting, growth, first-generation (G1) yield and -quality of the G0 mini-tubers in follow-up experiments.

To achieve this, the main objective was further divided into more specific objectives:

1. To determine the influence of different Ca: K & Mg ratios on the yield and quality of G0 mini-tubers, as well as to determine the mobility of calcium in the plant.
2. To determine the influence of different tuber sizes and post-harvest storage

temperatures on the water-loss, firmness and sprouting behavior of G0 mini-tubers produced from tissue cultured plantlets from the first objective.

3. To determine the influence of different tuber sizes and post-harvest storage temperatures on the growth, first-generation (G1) yield and -quality of G0 mini-tubers produced from tissue cultured plantlets from the first objective.



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## Chapter 2

### **Influence of different Ca : K and Mg ratios, and different irrigation methods on potato tuber number, size and nutrient content**

#### **Abstract**

Calcium (Ca) is an important plant nutrient with many functions, such as strengthening of cell walls and maintaining membrane stability and cell integrity. In this study the influence of different Ca levels on tuber yield and mineral concentration was tested. Plants were planted in 10 L buckets without a growing medium (aeroponic system) and irrigated with four different nutrient solutions with different Ca concentrations, namely a control (100 % Ca), and three treatments in which the Ca levels were changed to 33 %, 66 % and 133 % of the control, while the K and Mg levels were adjusted to compensate for the change in Ca. Plants were harvested 78 days after planting. Tubers were divided into different weight classes, and also analyzed for nutrient content. The treatment that received the highest Ca level had significantly more larger tubers than the lowest Ca treatment, although there was no significant difference in total tuber number between treatments. The high Ca treatment also had a significantly higher Ca concentration in the peel than the low Ca treatment.

#### **Introduction**

Calcium is an important plant nutrient. Calcium ion binding pectins in the middle lamella are known to be essential for strengthening the cell walls (Marschner, 1986). Calcium bound to the outer surface of the plasma membrane maintains membrane stability and cell integrity (Palta, 1996). In addition, calcium acts as a second messenger, coupling stimuli such as stress, light and plant hormones to a response (Bush, 1995).

The vast majority of calcium taken up by the plant is through the main root system. It is then transported upwards to the leaves through the xylem, by water flow through the plant. Once in the leaf, calcium is immobile and unlike other nutrients, such as nitrogen, phosphorous and potassium, it doesn't move to other leaves or down to the tubers at a later stage. Calcium movement into the tubers is mainly via the stolon, tuber root hairs, and through the tuber skin (Westermann, 2005). It is therefore important for the tuber to have a readily available supply in an area surrounding the tuber.

Tuberization in potato is a complex process that is known to be influenced by factors such as photoperiod, temperature and nitrogen nutrition (Jackson, 1999; Menzel, 1985). For example short days promote tuberization, whereas high temperatures and nitrogen inhibit tuberization. Although the exact mechanism of how these factors affect tuberization is not known, it is believed that plant hormones also play an important role (Ewing, 1995). The most convincing case for a critical role in tuberization has been made for gibberellin (Ewing, 1995). Tuberization was inhibited by high levels of gibberilic acid (GA), but it was promoted by reducing the GA level.

Evidence exists for calcium also having a role in tuberization (Balamani, Veluthambi & Poovaiah, 1986). Tuberization was inhibited in a single node leaf cutting by a Ca chelator EDTA and Ca ionophore A 23187. Tuberization was restored by including  $\text{CaCl}_2$  in the medium. Studies on the changes in barley aleurone during germination contain evidence for the modulation of GA by the Ca/calmodulin pathway (Gilroy & Jones, 1993). Evidence was provided for a powerful interaction between cytosolic Ca and GA action. Free cytosolic Ca is also a major metabolic regulator taking part in the signal transduction. Evidence also exists for the presence of Ca-dependent and calmodulin-independent protein kinase which is thought to modulate plant growth and development (Marschner, 1986). It is thus possible that calcium could have a role in regulating the tuberization process in potatoes.

The aim of this experiment is to determine whether calcium can be transported to the tubers via the roots, or if it needs to be supplied in an area close to the tubers for direct

uptake by the tubers. The influence of calcium on tuber number and size will also be determined.

## Material and Methods

On the 8<sup>th</sup> of September 2006, *in vitro* plants of the cultivar BP1 were planted in a glasshouse to harden off the plants. The plants were planted in a vermiculite medium and irrigated with a Steiner nutrient solution (Steiner, 1984) with an electrical conductivity (EC) of 0.75 mS cm<sup>-1</sup>. The temperature in the glasshouse was kept at 20 °C during the day and 15 °C during the night. After two weeks, on the 21<sup>st</sup> of September 2006, the plants were transplanted into a closed greenhouse with a pad-and-fan cooling system. An aeroponic growing system was used where the roots hang in the air and were frequently kept moist by sprinklers. Plants were planted through holes in the lids of 10 litre buckets, and held in position by sponges. The lids with the plants were then put on the buckets, with the roots hanging into the bucket without a growing medium. A virus net was placed halfway between the top and the bottom of the bucket which allowed the roots to grow through but not the stolons, therefore the tubers would stay on top of the net. Irrigation sprinklers were put in the buckets, one above the net and one below, allowing for irrigation to be done only on the roots in half of the buckets, and in the other half on both the stolons/tubers and the roots. Plants were irrigated with four different nutrient solutions which differed with regard to K, Mg and Ca levels. These nutrient solutions consisted of a standard Steiner solution as control (100 % Ca), and three treatments in which the Ca levels were changed to 33 %, 66 % and 133 % of the control, while K and Mg levels were adjusted to compensate for the change in Ca (Table 2.1.1-2.1.4). The EC for the solutions were kept at 1.5 mS cm<sup>-1</sup>. The sprinklers were switched on every 20 minutes for 15 seconds. Water drained from the buckets through holes in the bottom.

On the 8<sup>th</sup> and 23<sup>rd</sup> of November 2006, a foliar application of Cultar was applied. Cultar is a growth regulator that stems growth and helps with tuber initiation. Plants were harvested on the 8<sup>th</sup> of December 2006, 78 days after transplanting. Swelling of the

stolon to twice the normal size qualified as a tuber. The tubers were counted and weighed, and divided into six weight classes, namely smaller than 9 g, 9-17 g, 17-25 g, 25-50 g, bigger than 50 g and unmarketable (very small tubers). Tuber samples were also sent to a laboratory (Callab, Somerset West) to determine the nutrient content of the skin as well as the whole potato.

**Table 2.1.1** Ion concentrations (milli-equivalents per litre [meq L<sup>-1</sup>]) and application rates (mg L<sup>-1</sup>) for the fertilizers used to prepare Nutrient solution 1 (33% Ca) at an EC of 1.5 mS cm<sup>-1</sup>

Fertilizer	Application						
	(mg L <sup>-1</sup> )	K <sup>+</sup>	Ca <sup>++</sup>	Mg <sup>++</sup>	NO <sub>3</sub> <sup>-</sup>	H <sub>2</sub> PO <sub>4</sub> <sup>-</sup>	SO <sub>4</sub> <sup>-</sup>
KNO <sub>3</sub>	681.8	6.75			6.75		
K <sub>2</sub> SO <sub>4</sub>	52.2	0.6					0.6
KH <sub>2</sub> PO <sub>4</sub>	102	0.75				0.75	
Ca(NO <sub>3</sub> ) <sub>2</sub>	225		2.25		2.25		
MgSO <sub>4</sub> ·7H <sub>2</sub> O	571.9			4.65			4.65
<b>Total</b>		<b>8.1</b>	<b>2.25</b>	<b>4.65</b>	<b>9</b>	<b>0.75</b>	<b>5.25</b>

**Table 2.1.2** Ion concentrations (meq L<sup>-1</sup>) and application rates (mg L<sup>-1</sup>) for the fertilizers used to prepare Nutrient solution 2 (66% Ca) at an EC of 1.5 mS cm<sup>-1</sup>

Fertilizer	Application						
	(mg L <sup>-1</sup> )	K <sup>+</sup>	Ca <sup>++</sup>	Mg <sup>++</sup>	NO <sub>3</sub> <sup>-</sup>	H <sub>2</sub> PO <sub>4</sub> <sup>-</sup>	SO <sub>4</sub> <sup>-</sup>
KNO <sub>3</sub>	454.5	4.5			4.5		
K <sub>2</sub> SO <sub>4</sub>	126.2	1.45					1.45
KH <sub>2</sub> PO <sub>4</sub>	102	0.75				0.75	
Ca(NO <sub>3</sub> ) <sub>2</sub>	450		4.5		4.5		
MgSO <sub>4</sub> ·7H <sub>2</sub> O	467.4			3.8			3.8
<b>Total</b>		<b>6.7</b>	<b>4.5</b>	<b>3.8</b>	<b>9</b>	<b>0.75</b>	<b>5.25</b>

**Table 2.1.3** Ion concentrations (meq L<sup>-1</sup>) and application rates (mg L<sup>-1</sup>) for the fertilizers used to prepare Nutrient solution 3 (Control) at an EC of 1.5 mS cm<sup>-1</sup>

Fertilizer	Application						
	(mg L <sup>-1</sup> )	K <sup>+</sup>	Ca <sup>++</sup>	Mg <sup>++</sup>	NO <sub>3</sub> <sup>-</sup>	H <sub>2</sub> PO <sub>4</sub> <sup>-</sup>	SO <sub>4</sub> <sup>-</sup>
KNO <sub>3</sub>	227.24	2.25			2.25		
K <sub>2</sub> SO <sub>4</sub>	195.8	2.25					2.25
KH <sub>2</sub> PO <sub>4</sub>	102	0.75				0.75	
Ca(NO <sub>3</sub> ) <sub>2</sub>	675		6.75		6.75		
MgSO <sub>4</sub> ·7H <sub>2</sub> O	369			3			3
<b>Total</b>		<b>5.25</b>	<b>6.75</b>	<b>3</b>	<b>9</b>	<b>0.75</b>	<b>5.25</b>

**Table 2.1.4** Ion concentrations (meq L<sup>-1</sup>) and application rates (mg L<sup>-1</sup>) for the fertilizers used to prepare Nutrient solution 4 (133% Ca) at an EC of 1.5 mS cm<sup>-1</sup>

Fertilizer	Application						
	(mg L <sup>-1</sup> )	K <sup>+</sup>	Ca <sup>++</sup>	Mg <sup>++</sup>	NO <sub>3</sub> <sup>-</sup>	H <sub>2</sub> PO <sub>4</sub> <sup>-</sup>	SO <sub>4</sub> <sup>-</sup>
KNO <sub>3</sub>	0						
K <sub>2</sub> SO <sub>4</sub>	264.4	3.05					3.05
KH <sub>2</sub> PO <sub>4</sub>	102	0.75				0.75	
Ca(NO <sub>3</sub> ) <sub>2</sub>	900		9		9		
MgSO <sub>4</sub> ·7H <sub>2</sub> O	270.6			2.2			2.2
<b>Total</b>		<b>3.8</b>	<b>9</b>	<b>2.2</b>	<b>9</b>	<b>0.75</b>	<b>5.25</b>

### Experimental design and statistical analysis of data

A complete block design with a 4 (Ca-levels) x 2 (irrigation methods) factorial arrangement and 4 replications was used. An experimental unit consisted of one plant. The data were analyzed using STATISTICA version 7.1 (Statistica, 2004), and the Bonferoni test was used to compare treatment means at a probability level of 5%.

For the nutrient content data, the tubers of all four replications of each treatment were analyzed together and only an average value was determined for the four replications.

Therefore no interaction could be determined between Ca level and irrigation method when the data were analyzed (Table 2.4).

## Results

### *Tuber number*

Results of the Analysis of Variance (ANOVA) done on data with regard to tuber numbers are summarized in Table 2.2.

**Table 2.2** Significant levels ( $Pr > f$ ) of main effects namely Ca level, irrigation method as well as interactions with regard to tuber numbers in the different weight classes, and total tuber number

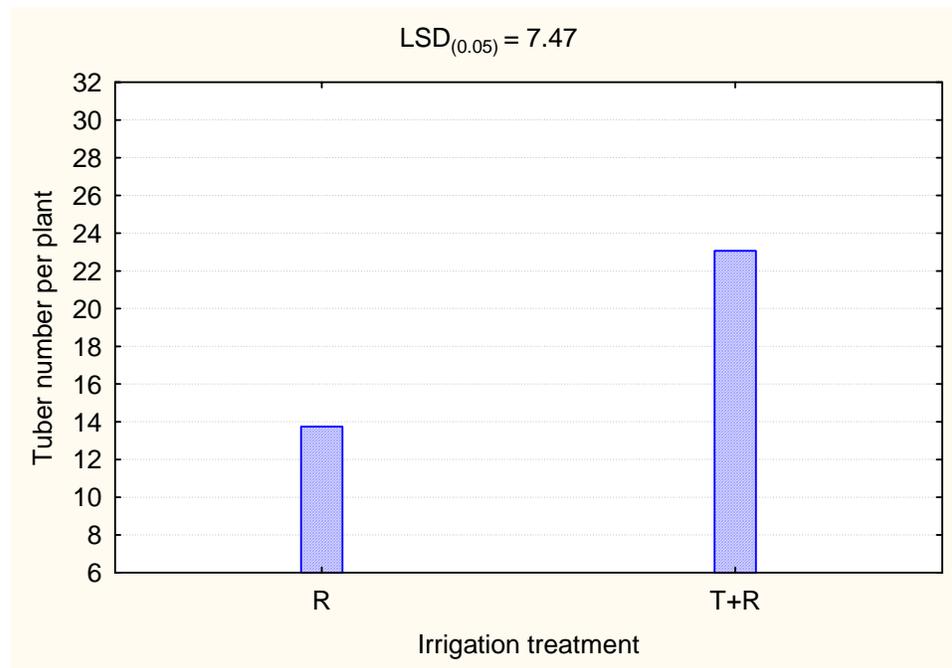
	<9 g	9-17 g	17-25 g	25-50 g	>50 g	Total tuber number
<b>Calcium</b>	0.2862	0.7966	0.2982	0.0324	0.3948	0.0774
<b>Irrigation</b>	0.0167	0.0060	0.3705	0.3268	0.1214	0.1367
<b>Calcium*Irrigation</b>	0.4702	0.5569	0.7916	0.7943	0.9001	0.6825

Ca levels had a significant effect on the number of tubers in the 25-50 g weight class, while irrigation method affected the number of tubers in both the <9 g and 9-17 g weight classes (Table 2.2). There was no interaction found between Ca level and irrigation method for any of the weight classes.

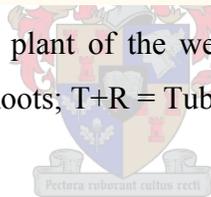
### **Weight class <9 g**

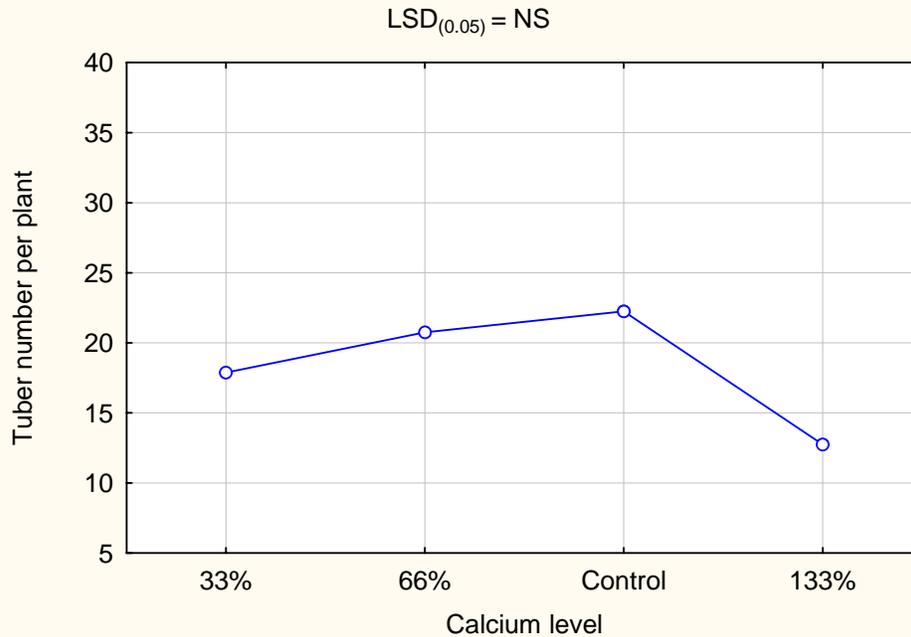
Number of tubers in this weight class was significantly increased where both the stolons and roots were irrigated (23 tubers) compared to irrigation on the roots alone (14 tubers) (Figure 2.1). Although Ca level did not have a significant effect on the number of tubers produced in this weight class, tuber number tended to decrease when the Ca-

concentration was either increased beyond the concentration in the control, or decreased below this concentration (Figure 2.2).



**Figure 2.1** Number of tubers per plant of the weight class <9 g as influenced by the different irrigation methods (R = Roots; T+R = Tubers + Roots).

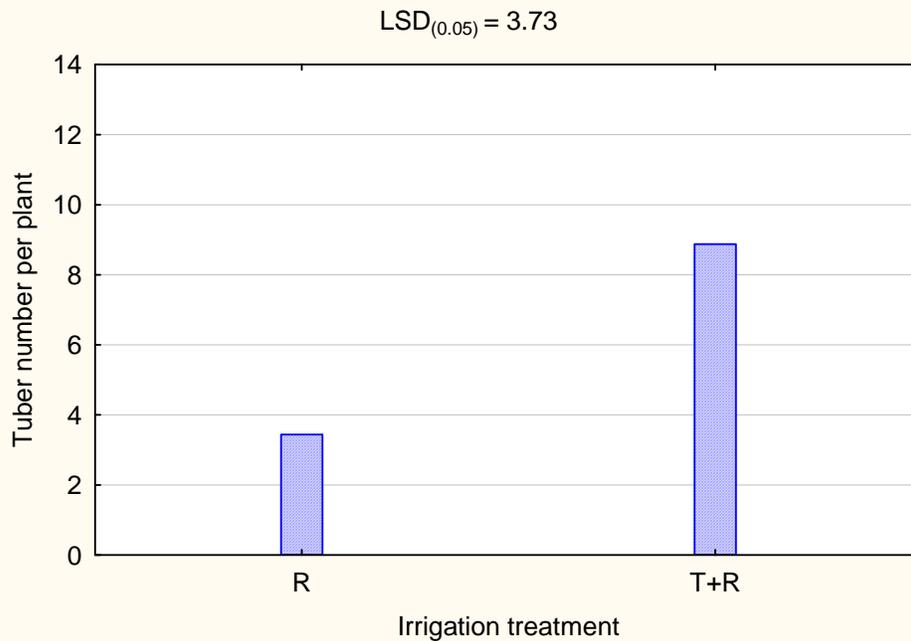




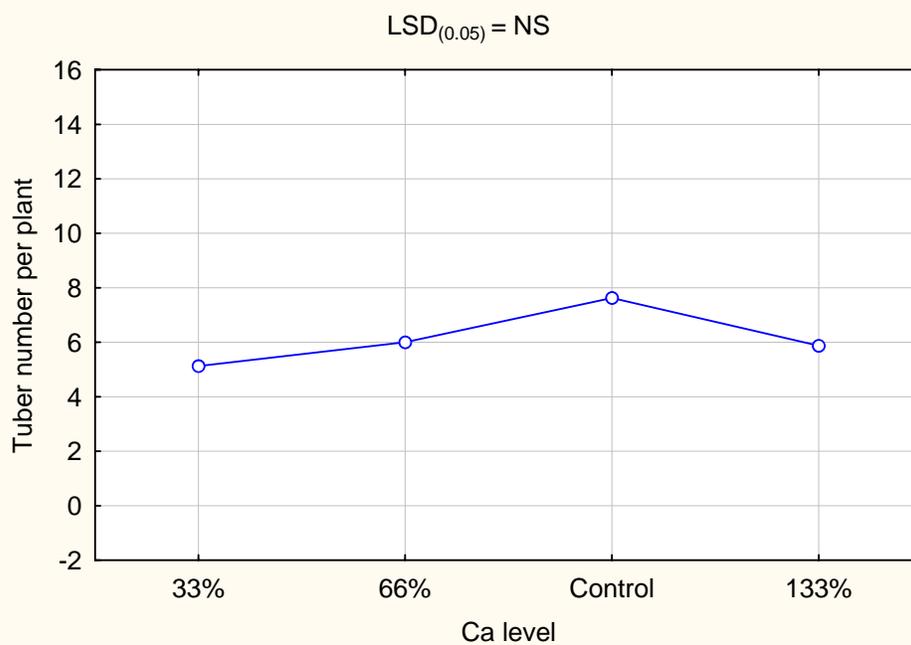
**Figure 2.2** Number of tubers per plant of the weight class <9 g as influenced by the different Ca levels (NS = not significant).

#### Weight class 9-17 g

Number of tubers in this weight class was significantly increased when both the stolons and the roots were irrigated (9 tubers) compared to irrigation on the roots alone (3.5 tubers) (Figure 2.3). There was no significant difference in the number of tubers due to the different Ca levels, but tuber number tended to decrease when Ca-concentration was either increased beyond the concentration in the control, or decreased below this concentration (Figure 2.4).



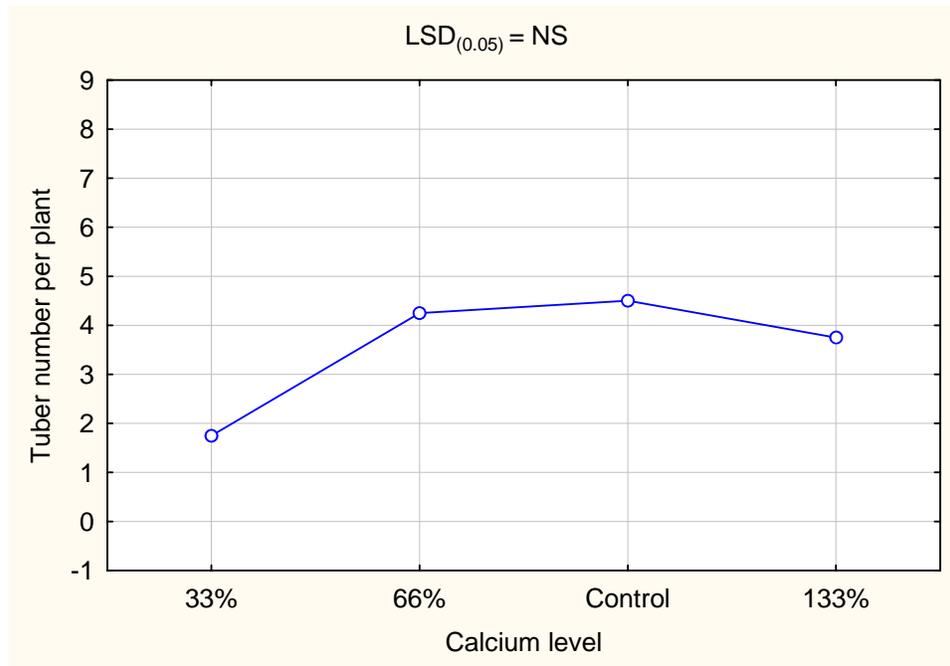
**Figure 2.3** Number of tubers per plant of the weight class 9-17 g as influenced by the different irrigation methods (R = Roots; T+R = Tubers + Roots).



**Figure 2.4** Number of tubers per plant of the weight class 9-17 g as influenced by the different Ca levels.

**Weight class 17-25 g**

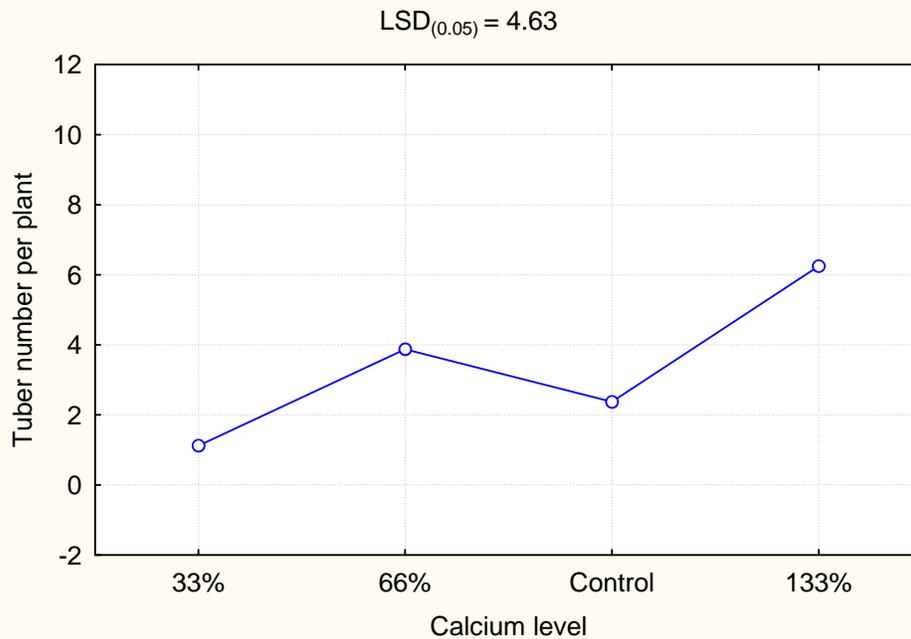
There was no significant difference in the number of tubers due to the different Ca levels or the different irrigation methods, although the tuber number of the 33 % Ca level were lower ( $\pm 1.8$ ) than the three higher levels ( $\pm 4.2$ , 4.4 & 3.8 for 66 %, 100 % and 133 % respectively) (Figure 2.5).



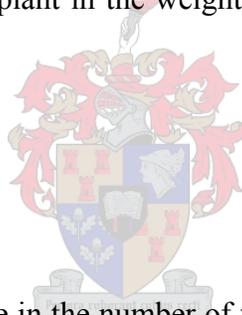
**Figure 2.5** Number of tubers per plant of the weight class 17-25 g as influenced by the different Ca levels.

**Weight class 25-50 g**

There was a significant difference in tuber number between the lowest and the highest Ca levels used during production (Figure 2.6). Significantly more tubers (6) were produced when irrigated with the 133 % Ca nutrient solution, compared to irrigation with the 33 % Ca nutrient solution (1 tuber). There was no significant difference due to the different irrigation methods.

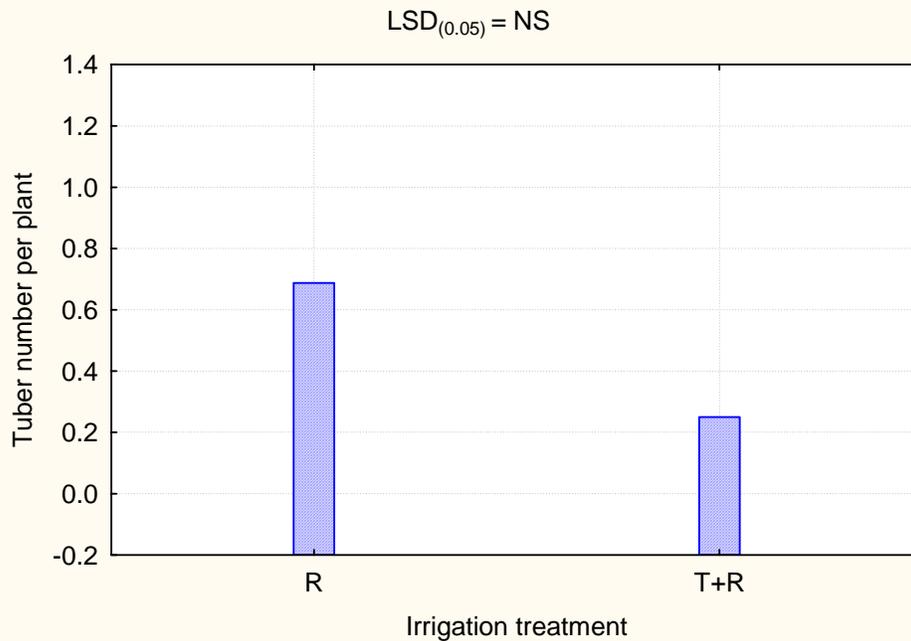


**Figure 2.6** Number of tubers per plant in the weight class 25-50 g as influenced by the different Ca levels.



**Weight class >50 g**

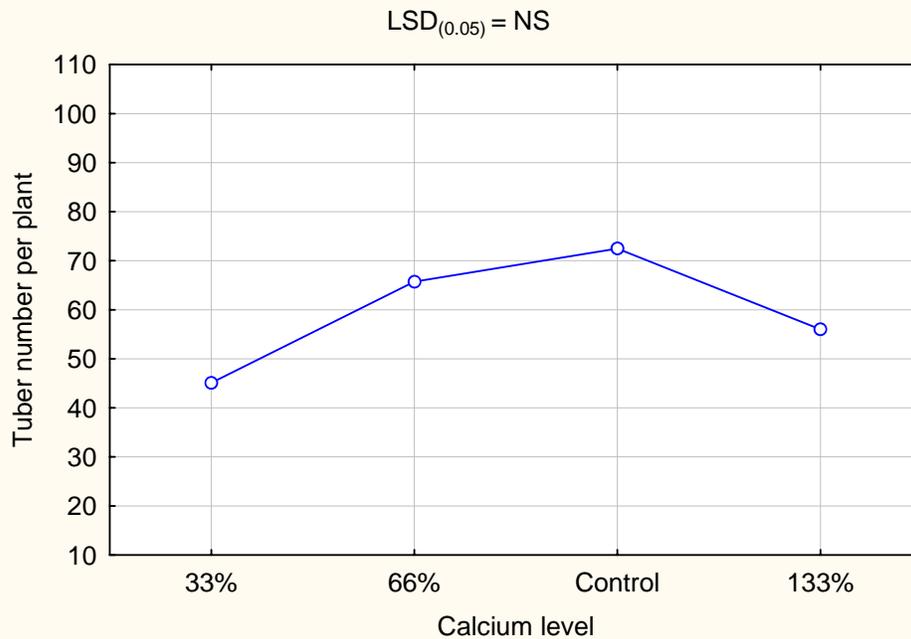
There was no significant difference in the number of tubers due to the different Ca levels or the different types of irrigation methods, although irrigation on the roots alone yielded slightly more tubers in this weight class than irrigation on the roots and the stolons (Figure 2.7).



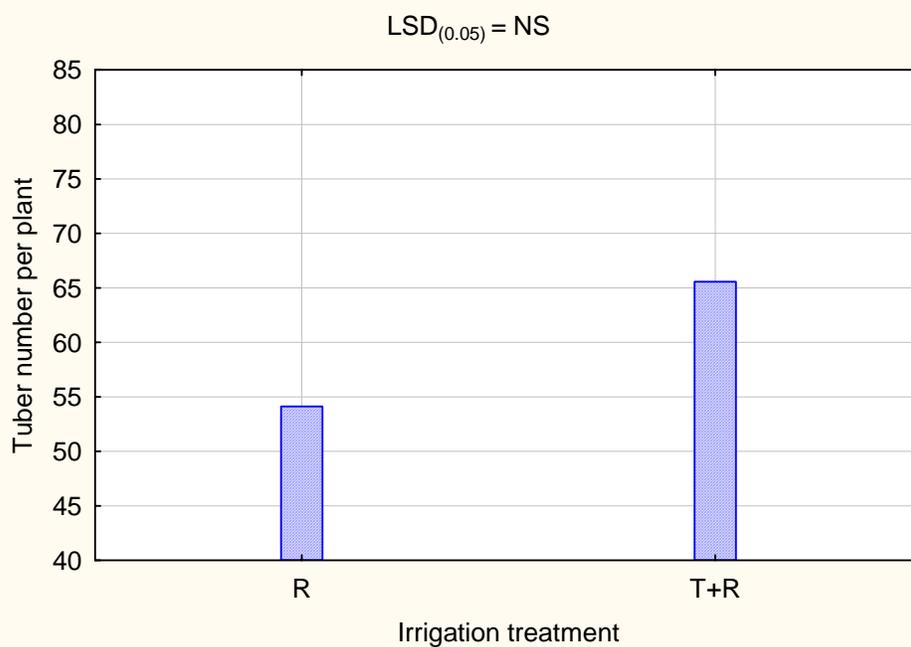
**Figure 2.7** Number of tubers per plant of the weight class >50 g as influenced by the different irrigation methods (R = Roots; T+R = Tubers + Roots).

#### Total tuber number per plant

There was no significant difference in the total number of tubers due to the different Ca levels or the different irrigation methods. However, tuber number tended to decrease when the Ca-concentration was either increased beyond the concentration of the control, or decreased below this concentration (Figure 2.8). The tuber number was also higher where both the stolons and roots were irrigated, compared to irrigation on the roots only (Figure 2.9).



**Figure 2.8** Total number of tubers per plant as influenced by the different Ca levels.



**Figure 2.9** Total number of tubers per plant as influenced by the different types of irrigation. (R = Roots; T+R = Tubers + Roots)

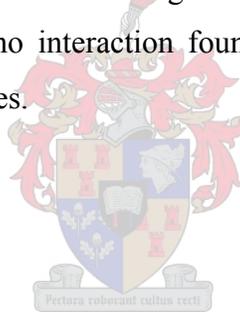
### *Tuber Weight*

Results of the Analysis of Variance (ANOVA) done on data with regard to tuber weights are summarized in Table 2.3.

**Table 2.3** Significant levels ( $P > f$ ) of main effects namely Ca level, irrigation method as well as interactions with regard to total tuber weight in the different weight classes

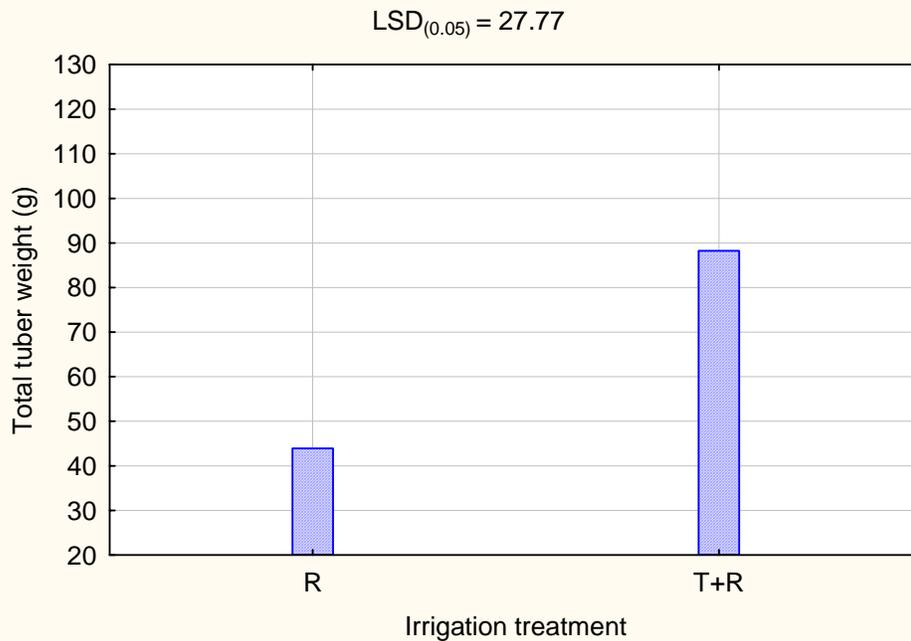
	<b>&lt;9g</b>	<b>9-17g</b>	<b>17-25g</b>	<b>25-50g</b>	<b>&gt;50g</b>
<b>Calcium</b>	0.3915	0.7878	0.2757	<b>0.0271</b>	0.4014
<b>Irrigation</b>	<b>0.0031</b>	<b>0.0070</b>	0.3859	0.1950	0.0800
<b>Calcium*Irrigation</b>	0.5245	0.5414	0.7753	0.7101	0.8491

Ca level had a significant effect on the total tuber weight in the 25-50 g weight class, while irrigation method affected the tuber weight in both the <9 g and 9-17 g weight classes (Table 2.3). There was no interaction found between Ca level and irrigation method for any of the weight classes.



#### **Weight class <9 g**

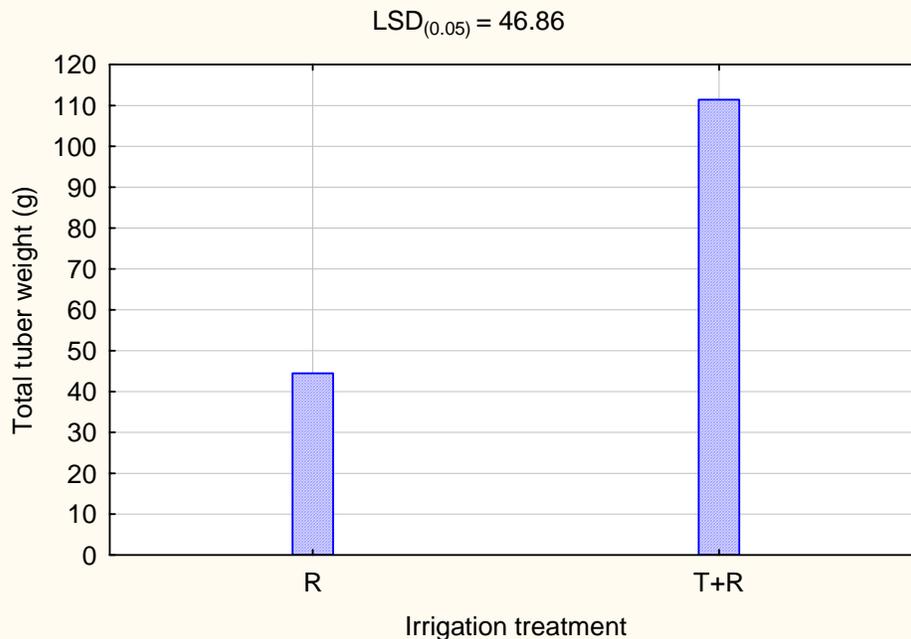
Total tuber weight for this weight class was significantly increased where both the stolons and roots were irrigated (89 g) compared to irrigation on the roots alone (44 g) (Figure 2.10). No significant difference occurred as a result of the different Ca levels.



**Figure 2.10** Total tuber weight of the weight class <9 g as influenced by the different irrigation methods (R = Roots; T+R = Tubers + Roots).

#### Weight class 9-17 g

Total tuber weight for this weight class was significantly increased when both the stolons and the roots were irrigated (111 g) compared to irrigation on the roots alone (45 g) (Figure 2.11). There was no significant difference due to the different Ca levels.



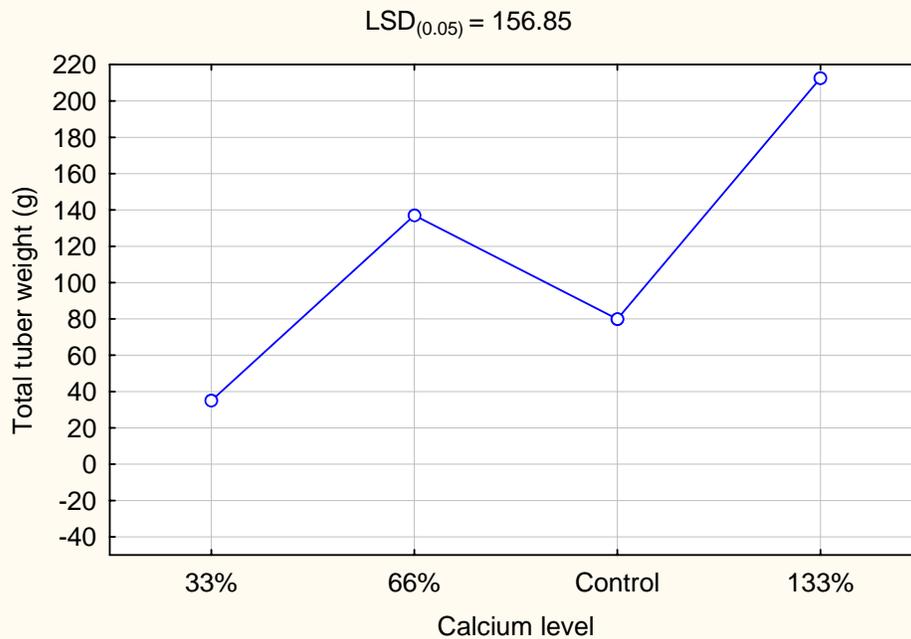
**Figure 2.11** Total tuber weight of the weight class 9-17 g as influenced by the different irrigation methods (R = Roots; T+R = Tubers + Roots).

#### Weight class 17-25 g

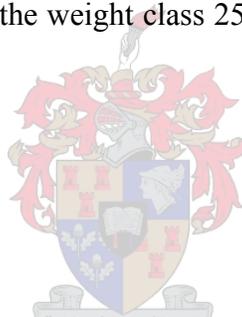
There was no significant difference in the total tuber weight due to the different Ca levels or the different irrigation methods.

#### Weight class 25-50 g

There was a significant difference in total tuber weight between the lowest and the highest Ca levels (Figure 2.12). Total tuber weight was significantly higher when irrigated with the nutrient solution containing 133 % Ca (215 g), compared to irrigation with the 33 % Ca nutrient solution (35 g). There was no significant difference due to the different irrigation methods.



**Figure 2.12** Total tuber weight in the weight class 25-50 g as influenced by the different Ca levels.



#### Weight class >50 g

There was no significant difference in the total tuber weight in this weight class due to the different Ca levels or the different irrigation methods.

#### *Nutrient Content*

Results of the Analysis of Variance (ANOVA) done on data with regard to nutrient content of the skin and the whole tuber are summarized in Table 2.4.

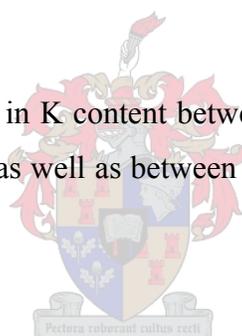
**Table 2.4** Significant levels ( $P > f$ ) of main effects namely Ca level and irrigation method with regard to nutrient content of the skin and the whole tuber

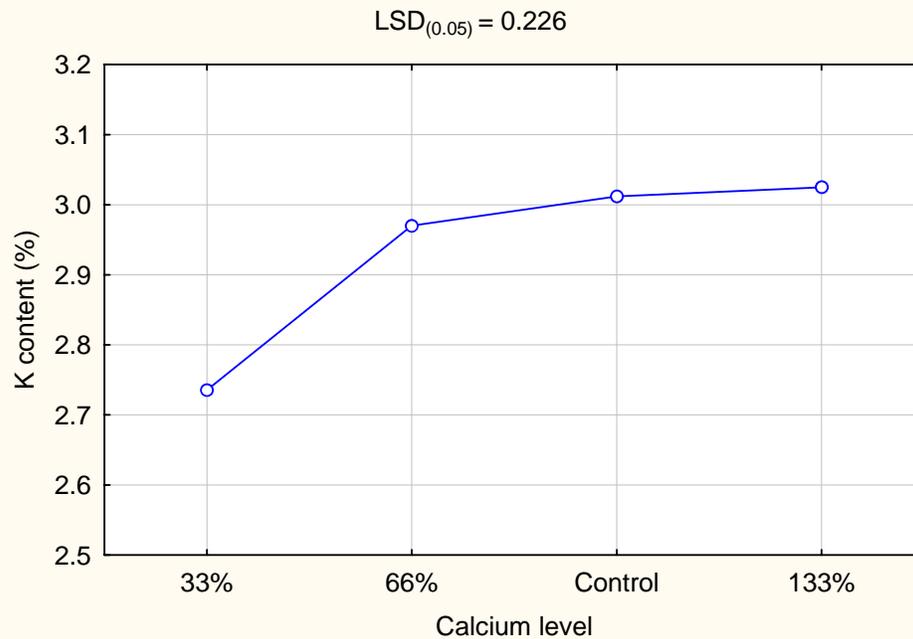
	Skin			Whole Tuber		
	Ca	K	Mg	Ca	K	Mg
<b>Calcium</b>	0.0250	0.0396	0.0019	0.2547	0.0504	0.0009
<b>Irrigation</b>	0.9443	0.5134	0.0133	0.2293	0.3840	0.0018

The different Ca levels had a significant effect on the Ca, K and Mg contents of the skin, as well as on the Mg content of the whole tuber. The different irrigation methods only had a significant effect on the Mg content of both the skin and the whole tuber (Table 2.4).

#### Skin

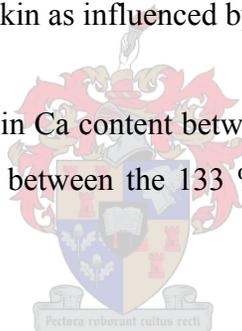
There was a significant difference in K content between the 133 % Ca level (3.02 % K) and the 33 % Ca level (2.73% K) as well as between the control (3.01% K) and the 33% Ca level (Figure 2.13).

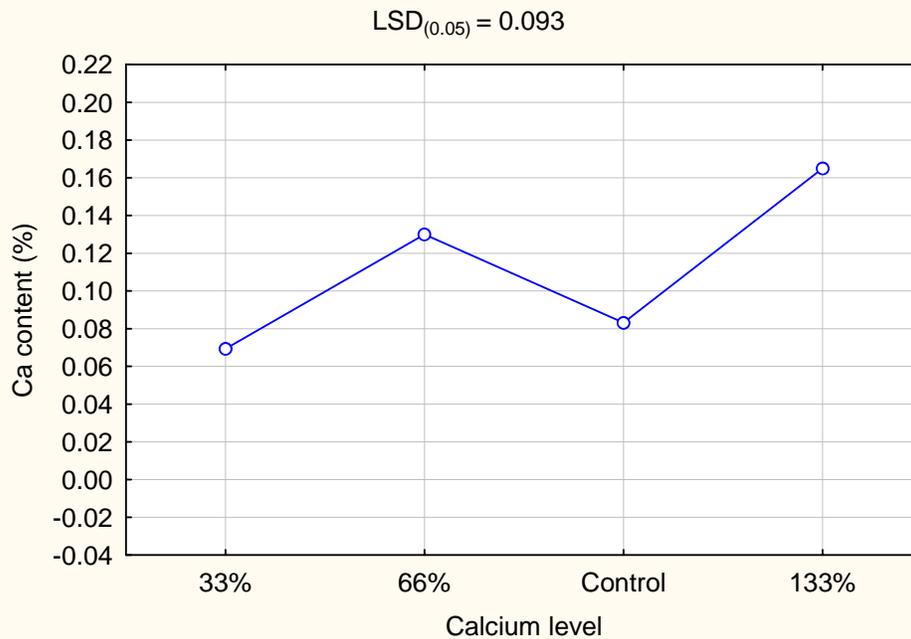




**Figure 2.13** The K content of the skin as influenced by the different Ca levels.

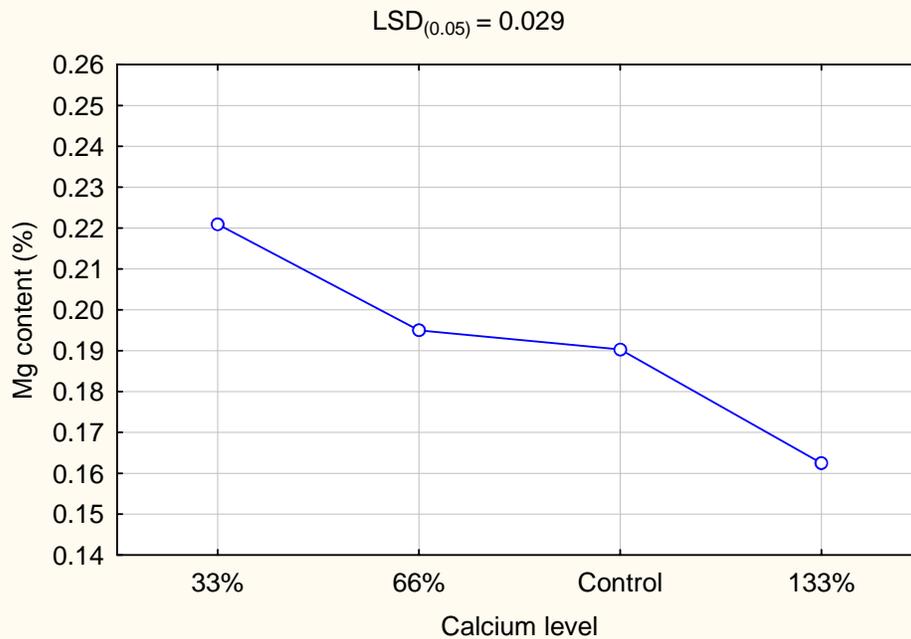
There was a significant difference in Ca content between the 133 % Ca level (0.16 % Ca) and the control (0.08 % Ca), and between the 133 % Ca level and the 33 % Ca level (0.07 % Ca) (Figure 2.14).





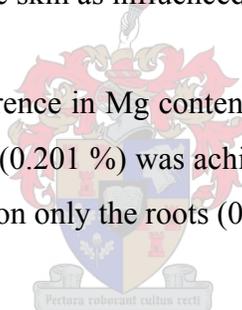
**Figure 2.14** The Ca content of the skin as influenced by the different Ca levels.

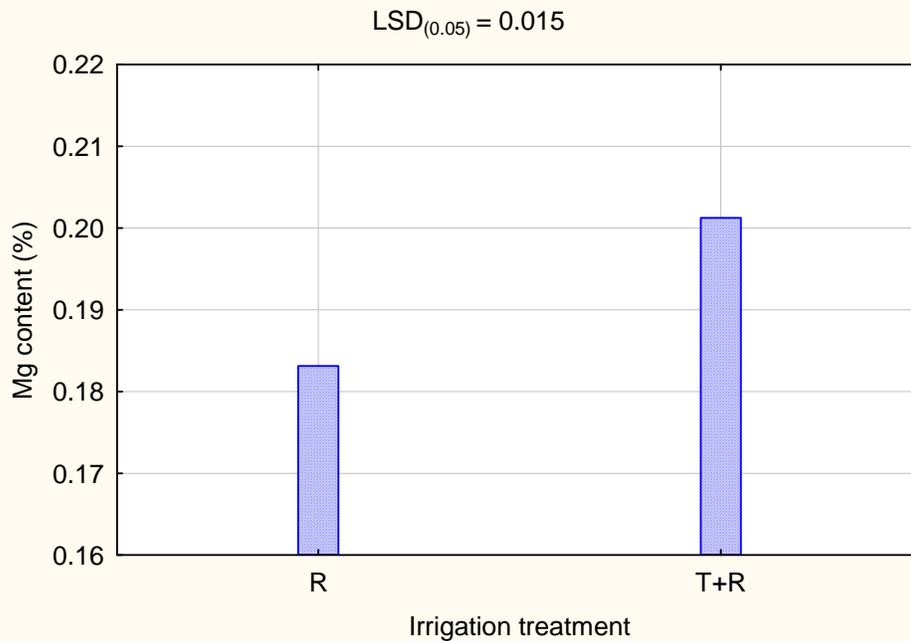
There was a significant difference in Mg content due to the different Ca levels. Tubers produced with the 33 % Ca level (0.22 % Mg) was significantly higher in Mg than all of the other levels and both the 66% Ca level (0.195 % Mg) and the control (0.19 % Mg) was significantly higher than the 133 % Ca level (0.16 % Mg) (Figure 2.15).



**Figure 2.15** The Mg content of the skin as influenced by the different Ca levels.

There was also a significant difference in Mg content due to the two irrigation methods. A significantly higher Mg content (0.201 %) was achieved by irrigation on both the roots and tubers, compared to irrigation on only the roots (0.182 %) (Figure 2.16).

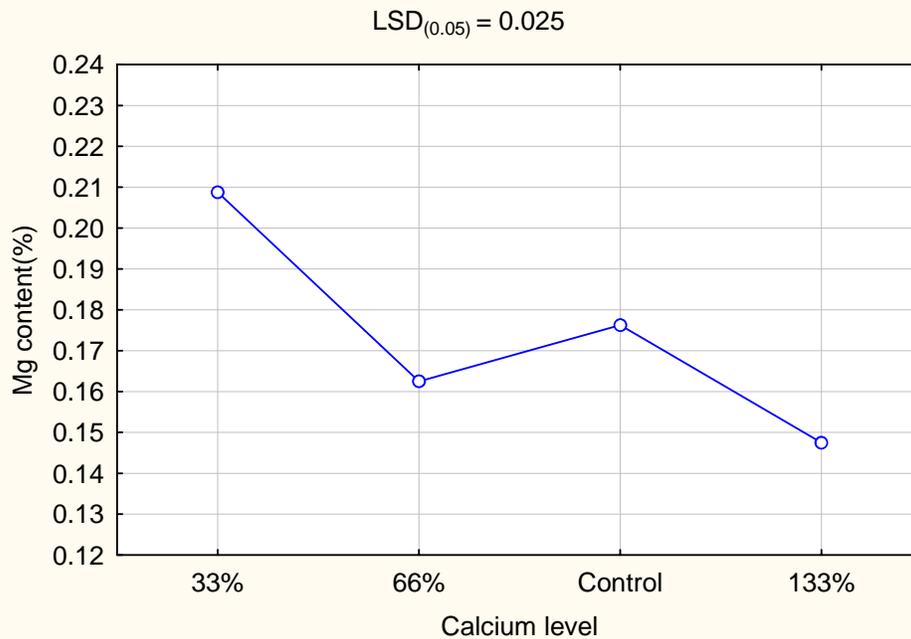




**Figure 2.16** The Mg content of the skin as influenced by the different irrigation methods. (R = Roots; T+R = Tubers + Roots)

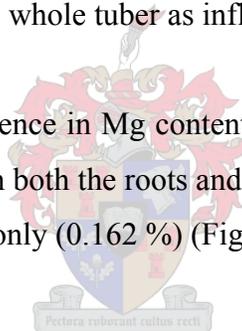
#### Whole tuber

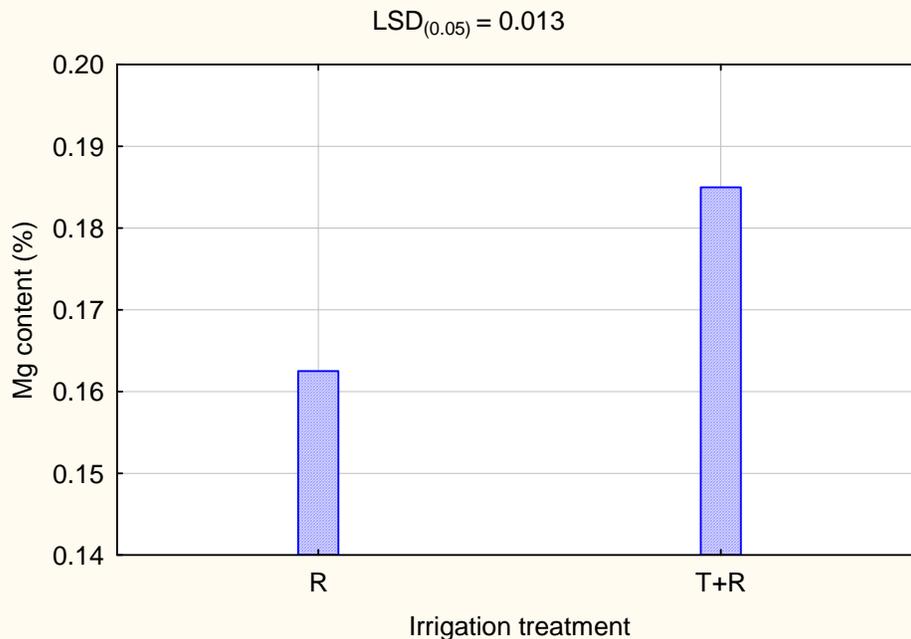
The only significant difference was found for the Mg content. The 33 % Ca level (0.21 % Mg) was significantly higher than all of the other levels. The control (0.176 % Mg) was also significantly higher than the 133 % Ca level (0.158% Mg) (Figure 2.17).



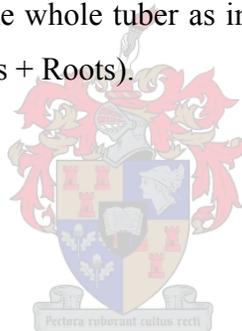
**Figure 2.17** The Mg content of the whole tuber as influenced by the different Ca levels.

There was also a significant difference in Mg content due to the two irrigation methods. The Mg content of the irrigation on both the roots and stolons (0.185 %) was significantly higher than irrigation on the roots only (0.162 %) (Figure 2.18).





**Figure 2.18** The Mg content of the whole tuber as influenced by the different irrigation methods (R = Roots; T+R = Tubers + Roots).



## Discussion

### *Tuber number and weight*

No significant difference was found in the total number of tubers per plant between the different Ca levels. This was in contrast to the results of previous studies done by Ozgen & Palta (2004) who suggested that an increased Ca-concentration in the soil during the tuberization period can reduce the tuber number per plant. They also found that increased calcium during bulking increased tuber weight. In this study it was also found that the bigger tubers (25-50 g) were significantly more with the highest Ca level than with the lowest level. This agrees with research done by Simmons & Kelling (1987). Thus it is possible that higher Ca applications might increase tuber weight. Balamani *et al.* (1986) did an experiment where freshly excised leaf cuttings were grown in H<sub>2</sub>O, CaCl<sub>2</sub> and CaCl<sub>2</sub> + MgCl<sub>2</sub> respectively. They found that all the cuttings produced tubers, but the tubers that developed in CaCl<sub>2</sub> or CaCl<sub>2</sub> + MgCl<sub>2</sub> were generally larger than the

tubers that developed in the H<sub>2</sub>O control. Thus, they also found that Ca could increase tuber size. In the weight classes smaller than 9 g and 9-17 g, the tuber number was significantly higher where both the roots and tubers were irrigated. This might suggest that it is better for the tubers to have minerals available for direct uptake.

### ***Nutrient Content***

In this experiment it was found that the K concentration in the peel of the 133 % Ca level was significantly higher than that of the 33% Ca level (Figure 2.13). Ca may therefore have an influence on K uptake, because the 33 % Ca treatment received more K in the nutrient solution than that of the 133 %. Studies done by Clough (1994) also suggested an increase in tuber K concentrations with increased Ca supply.

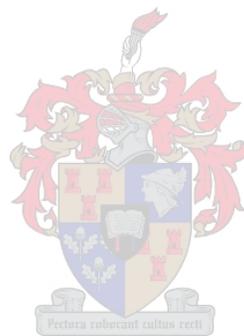
In the peel, the Ca content of the 133 % Ca level was also significantly higher than that of the 33 % Ca level. This agrees with studies done by Clough (1994), which also found an increase in tuber Ca with increased fertilization. In this study there was no significant difference in Ca content due to the two different irrigation methods. The results therefore suggest that it is possible for Ca to be transported from the roots to the tubers via the xylem. This is in contrast to results suggesting that most of the tuber Ca was taken up by the tuber itself (Westermann, 2005). The significant decreases in tuber Mg content as Ca increases might be due to decreased Mg in the nutrient solution as Ca increases.

### **Conclusion**

The 133 % Ca level produced more large tubers (25-50 g & >50 g) than any of the other Ca levels. There was, however, no difference between the total number of tubers per plant between the different Ca levels. Since bigger tubers are favoured more by local producers, it might prove advantageous to increase the Ca : K and Mg ratio in the nutrient solution.

Increasing the Ca : K and Mg ratio in the nutrient solution increased the K and Ca content in the peel, while decreasing the Mg content in the peel and the whole tuber. This ratio should therefore not be increased too much, as it could induce Mg deficiencies in the tubers. The Mg content of the peel and the whole tuber also varied significantly between the two different irrigation methods, where Mg content was higher when irrigation occurred on both the stolons and roots as compared to roots only.

There was, however, no significant difference in the Ca content of the peel and the whole tuber between the different irrigation methods. As this is in contrast to the results of previous studies, further research can be done to determine the mobility of Ca in the plant.



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### Chapter 3

#### **Influence of different Ca : K and Mg ratios, different irrigation methods as well as different storage temperatures on seed potato tuber quality**

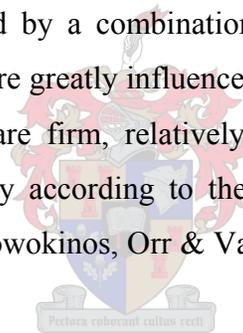
##### **Abstract**

Storage of potatoes is very important to ensure a good quality, whether it is for use as seed potatoes or for the consumer market. Storage temperature plays a major role in the quality of potatoes. Tubers harvested from plants that were treated with four different nutrient solutions (namely a control [100 % Ca], and three treatments in which the Ca levels were changed to 33 %, 66 % and 133 % of the control, while the K and Mg levels were adjusted to compensate for the change in Ca) and two different irrigation methods (irrigation on roots only, and irrigation on roots and stolons) were divided into two weight classes (<14 g & >14 g) before storing at three different temperatures (3, 6 & 10 °C) for five weeks. The percentage weight loss during storage was determined by weighing the tubers before, and again after storage. The firmness of the tubers was also measured after storage. Tubers were then stored in a dark room at room temperature to allow sprouts to develop. The sprouts of each tuber were counted and weighed. Weight loss was the lowest for tubers stored at 3 °C. Firmness of the tubers increased as the Ca level of the nutrient solution was increased. Firmness was also significantly influenced by an interaction between tuber weight class and storage temperature, although no clear trend could be recognized. Number of sprouts was the highest for tubers stored at 6 °C. Sprout number was also significantly influenced by an interaction between tuber weight class and the different Ca levels and irrigation methods used during tuber production. Total sprout weight was also the highest at 6 °C.

## Introduction

Potato supplies are needed for several reasons including domestic consumption, food-processing industries, starch production, as well as seed potatoes (Sukumaran & Verma, 1993). Therefore good storage is needed to increase potato availability and avoid large quality deterioration. Tubers should be maintained in their marketable conditions by preventing large moisture losses, spoilage by pathogens and quality deterioration. While cold storage may provide the necessary environment to prevent loss of weight and spoilage, the quality of potatoes continues to change as a result of physiological activity. Tubers can become soft and unusable due to freezing injuries caused by low temperatures. On the other hand, higher storage temperatures will result in greater quality loss and increased respiratory activity.

Quality of potatoes is determined by a combination of various physico-chemical and nutritional characteristics, which are greatly influenced by storage conditions (Guenther, 1995). Good-quality potatoes are firm, relatively smooth and without any defects. These factors may, however, vary according to the degree of maturity, harvest time, variety, and storage conditions (Sowokinos, Orr & Varns, 1987; Laza, Scanlon & Mazza, 2001).

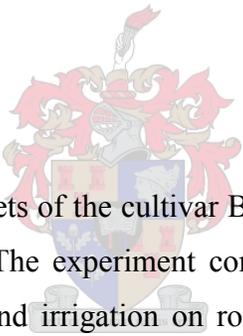


Extensive changes in chemical composition of tubers, which are also considered as quality attributes, can be promoted by storage conditions (Rodriguez-Saona & Wrolstad, 1997). Sugar and starch are the primary components affected by post-harvest metabolism in potato tubers. The rate of sugar accumulation depends largely on the variety and temperature of storage (Spychella & Desborough, 1990) and occurs most rapidly in cold temperatures. Compositional changes in turn influence the textural and appearance quality of the potatoes. Chemical and biochemical changes are important not only in determining potato quality, but also in the quality of the finished product (Hertog, Tuskens, & Hak, 1997; Peshin, 2000).

The number of sprouts per tuber is a very important factor in potato cultivation, as it has a strong correlation with the number of main stems that will develop in the field (Struik & Wiersema, 1999). More sprouts may result in more stems per seed tuber. However, although a certain proportionality exists, not all sprouts at planting develop into main stems. The proportion of sprouts developing into main stems decreases with increasing tuber size. The relationship between proportion of sprouts developing into main stems and seed tuber size may also depend on the season and year.

The objective of this experiment is to determine the influence of different Ca levels and irrigation methods used during the production of the seed tubers, as well as different storage temperatures on the quality of the seed tubers, and on the sprouting behaviour of the tubers.

## Material and methods



Hardened-off *in vitro* potato plantlets of the cultivar BP1 were cultivated in an aeroponic system in a closed greenhouse. The experiment comprised of two irrigation methods (irrigation on stolons and roots, and irrigation on roots only) as well as four different nutrient treatments with different Ca: K and Mg ratios. The nutrient solutions differed with regard to K, Mg and Ca levels. These nutrient solutions consisted of a Steiner (1984) solution as control (100 % Ca), and three treatments in which the Ca levels were changed to 33 %, 66 % and 133 % of the control, while K and Mg levels were adjusted to compensate for the change in Ca and to keep the electrical conductivity (EC) at 1,5 mS cm<sup>-1</sup> (Chapter 2).

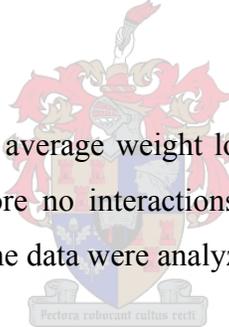
Harvested tubers of each treatment combination (irrigation method x nutrient solution) were divided into two different weight classes (<14 g and >14 g). They were then stored at three different temperatures (3 °C, 6 °C and 10 °C) for 5 weeks. The tubers were weighed before storage and again after storage to determine the percentage weight loss. The firmness of the tubers was also measured after storage, using a densi-meter which

indicates the relative resistance of the potato skin to applied pressure (kPa). The tubers were then stored in a dark room at room temperature for 3 months to allow the sprouts to develop. The sprouts of each tuber were counted and weighed to determine the growth vigour of the tubers.

### **Experimental design and statistical analysis**

A 4 (Ca levels) x 2 (irrigation methods) x 2 (weight classes) x 3 (storage temperatures) factorial arrangement with four replications was used for this experiment. An experimental unit consisted of one tuber. A repeated measures analyses of variance (ANOVA) was done on the data, and the Tukey HSD test was done to compare treatment means at a probability level of 5 %. The data were analyzed using STATISTICA version 7.1 (Statistica, 2004).

For the weight loss data, only the average weight loss for the four replications of each treatment was determined, therefore no interactions could be determined between Ca level and irrigation method when the data were analyzed (Table 3.1).



## **Results**

### ***Weight loss***

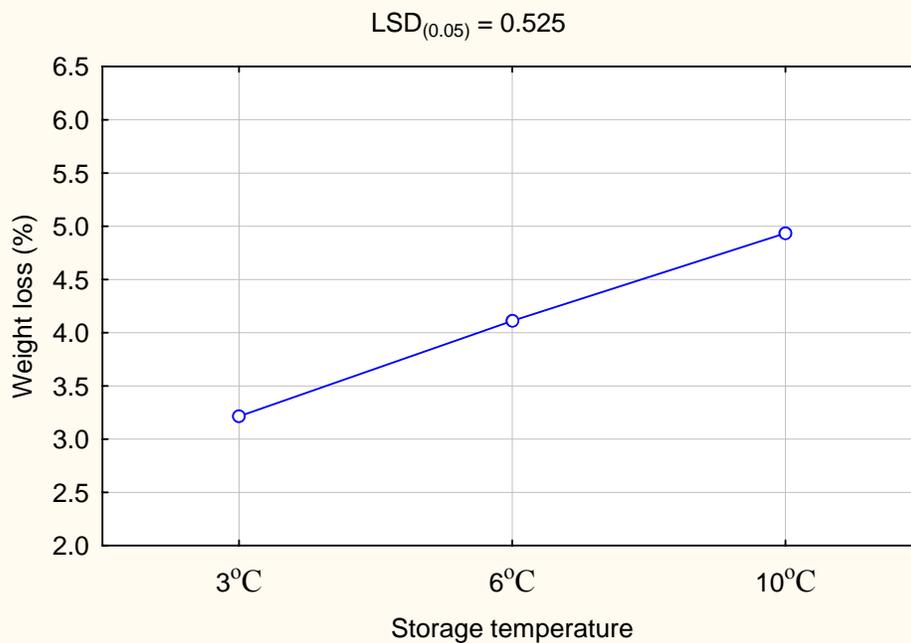
Results of the Analysis of Variance (ANOVA) done on data with regard to weight loss are summarized in Table 3.1.

**Table 3.1** Significant levels ( $Pr > f$ ) of main effects namely Ca level, irrigation method, weight class and storage temperature, as well as interactions with regard to weight loss of tubers

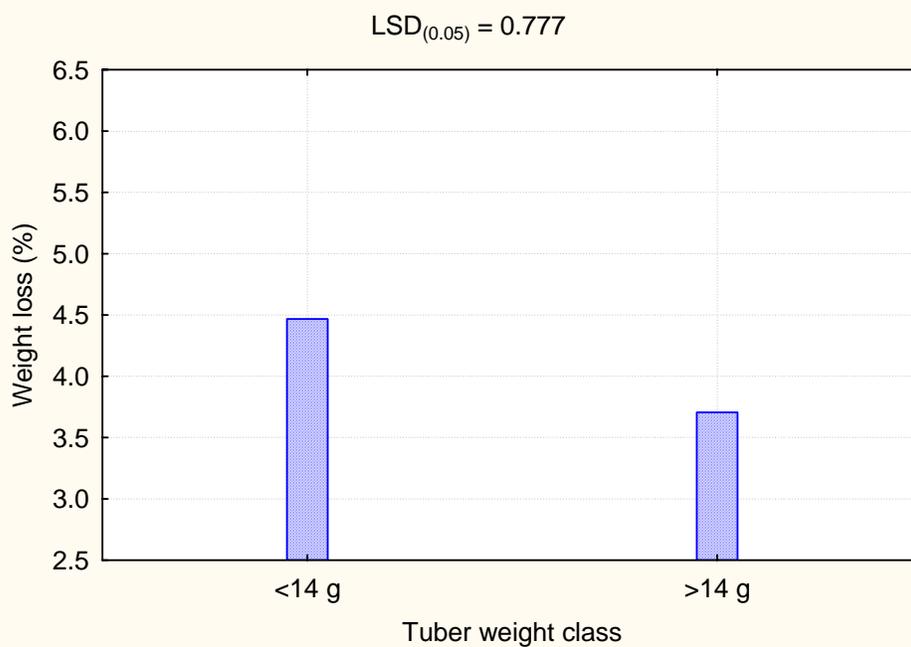
	<b>Weight loss</b>
<b>Ca</b>	0.1162
<b>Irrigation</b>	0.4838
<b>Weight class</b>	0.0522
<b>Weight class *Ca</b>	0.2806
<b>Weight class *Irrigation</b>	0.3773
<b>Temperature (Temp)</b>	<b>0.0002</b>
<b>Temp*Ca</b>	0.2369
<b>Temp*Irrigation</b>	0.7835
<b>Weight class *Temp</b>	0.3175
<b>Weight class *Temp*Ca</b>	0.9609
<b>Weight class *Temp*Irrigation</b>	0.4672

The different storage temperatures had a significant effect on the weight loss of the tubers during storage ( $Pr > f = 0.0002$ ) (Table 3.1). No significant difference was found for the other main effects and no interactions were found either.

Weight loss of the tubers increased significantly as the storage temperatures was increased from 3 °C to 10 °C (Figure 3.1). Weight loss was significantly higher when stored at 6 °C (4.1 %) compared to storage at 3 °C (3.2 %). Weight loss was also significantly higher at 10 °C (4.95 %) compared to storage at both 3 °C and 6 °C. Although weight class didn't have a significant effect on weight loss, it was slightly higher for the small tubers (4.5 %) than the larger tubers (3.7 %) (Figure 3.2).



**Figure 3.1** Percentage weight loss of the tubers as influenced by the different storage temperatures.



**Figure 3.2** Percentage weight loss of the tubers as influenced by the different weight classes.

### *Firmness*

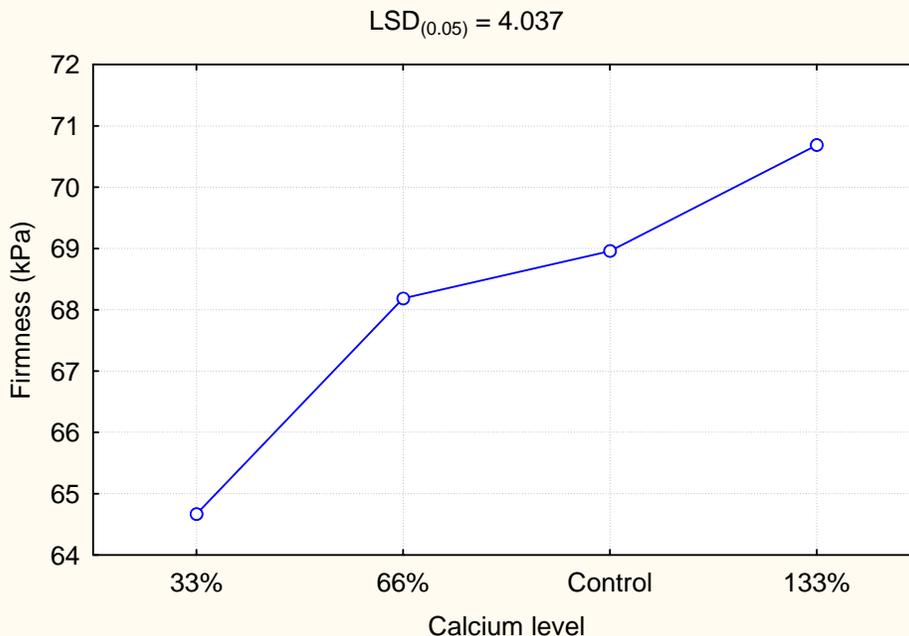
Results of the Analysis of Variance (ANOVA) done on data with regard to tuber firmness are summarized in Table 3.2.

**Table 3.2** Significant levels ( $Pr > f$ ) of main effects namely Ca level, irrigation method, weight class and storage temperature, as well as interactions with regard to firmness of tubers

	<b>Firmness</b>
<b>Ca</b>	<b>0.0034</b>
<b>Irrigation</b>	0.7805
<b>Ca*Irrigation</b>	0.1706
<b>Weight class</b>	0.1247
<b>Weight class*Ca</b>	0.9320
<b>Weight class*Irrigation</b>	0.7900
<b>Weight class*Ca*Irrigation</b>	0.1583
<b>Temperature (Temp)</b>	0.7422
<b>Temp*Ca</b>	0.6816
<b>Temp*Irrigation</b>	0.9461
<b>Temp*Ca*Irrigation</b>	0.0509
<b>Weight class*Temp</b>	<b>0.0085</b>
<b>Weight class*Temp*Ca</b>	0.8092
<b>Weight class*Temp*Irrigation</b>	0.1573
<b>Weight class*Temp*Ca*Irrigation</b>	0.4209

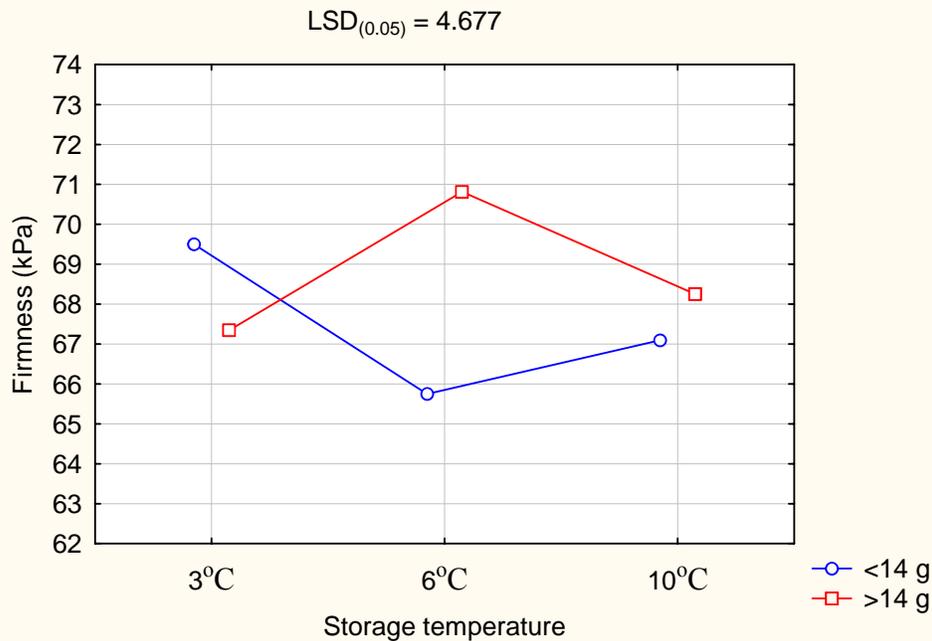
The different Ca levels had a significant effect on the firmness of the tubers after storage. A significant difference in firmness was also found due to an interaction between weight class and storage temperature (Table 3.2).

After a storage period of 5 weeks, the tubers produced at high Ca levels showed a higher firmness compared to tubers produced at low Ca levels (Figure 3.3). Firmness of the 133 % Ca level (70.7 kPa) and the control (69.0 kPa) were significantly higher than that of the 33 % Ca level (64.7 kPa).



**Figure 3.3** Firmness (kPa) of the tubers as influenced by the different Ca levels.

There was a significant interaction found between weight class and storage temperature (Figure 3.4). Firmness of the smaller tubers was decreased as storage temperature was increased from 3 °C to 6 °C, but increased again as the storage temperature was further increased to 10 °C. On the other hand, for the larger tubers the firmness increased as the storage temperature was increased from 3 °C to 6 °C, but decreased again at 10 °C. The only significant difference however, was between the small tubers and the larger tubers stored at 6 °C. At this temperature the firmness of the larger tubers (70.8 kPa) was significantly higher than that of the smaller tubers (65.8 kPa).



**Figure 3.4** Firmness (kPa) of the tubers as influenced by an interaction between weight class (<14 g & >14 g) and storage temperature.

### *Sprouts*

Results of the Analysis of Variance (ANOVA) done on data with regard to sprout number, total sprout weight and average weight per sprout are summarized in Table 3.3.

**Table 3.3** Significant levels ( $P > f$ ) of main effects namely Ca level, irrigation method, weight class and storage temperature, as well as interactions with regard to sprout number, total sprout weight and average weight per sprout

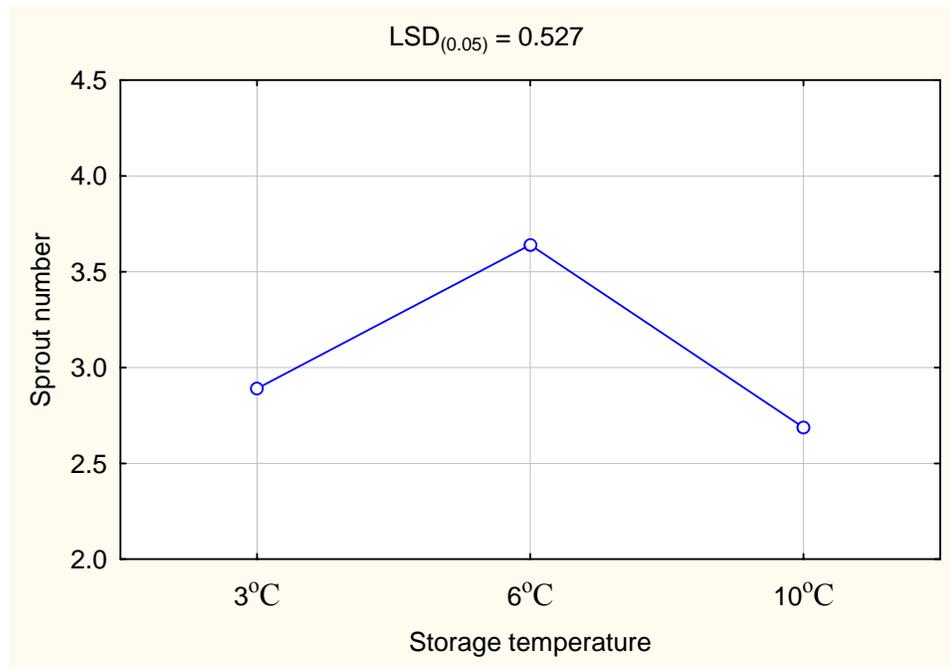
	<b>Sprout number</b>	<b>Total sprout weight</b>	<b>Average weight per sprout</b>
<b>Ca</b>	0.1743	0.0000	0.0000
<b>Irrigation</b>	0.3059	0.6113	0.0164
<b>Ca*Irrigation</b>	0.6779	0.1047	0.0056
<b>Weight class</b>	0.0000	0.0000	0.0000
<b>Weight class*Ca</b>	0.7229	0.0005	0.0024
<b>Weight class*Irrigation</b>	0.0030	0.0942	0.9307
<b>Weight class*Ca*Irrigation</b>	0.0029	0.7008	0.0376
<b>Temperature (Temp)</b>	0.0002	0.0246	0.3342
<b>Temp*Ca</b>	0.6837	0.7370	0.4740
<b>Temp*Irrigation</b>	0.4347	0.9332	0.3370
<b>Temp*Ca*Irrigation</b>	0.8220	0.6490	0.7180
<b>Weight class*Temp</b>	0.1600	0.1382	0.7615
<b>Weight class*Temp*Ca</b>	0.3715	0.9698	0.1121
<b>Weight class*Temp*Irrigation</b>	0.4075	0.9923	0.3318
<b>Weight class*Temp*Ca*Irrigation</b>	0.1951	0.6290	0.4275

An interaction between weight class and Ca level of the nutrient solution had a significant effect on the total sprout weight. An interaction between weight class and the Ca level and irrigation method used for the production of tubers, had a significant effect on both sprout number and average weight per sprout produced during the storage period. The different storage temperatures had a significant effect on both sprout number and total sprout weight (Table 3.3).

#### **Sprout number**

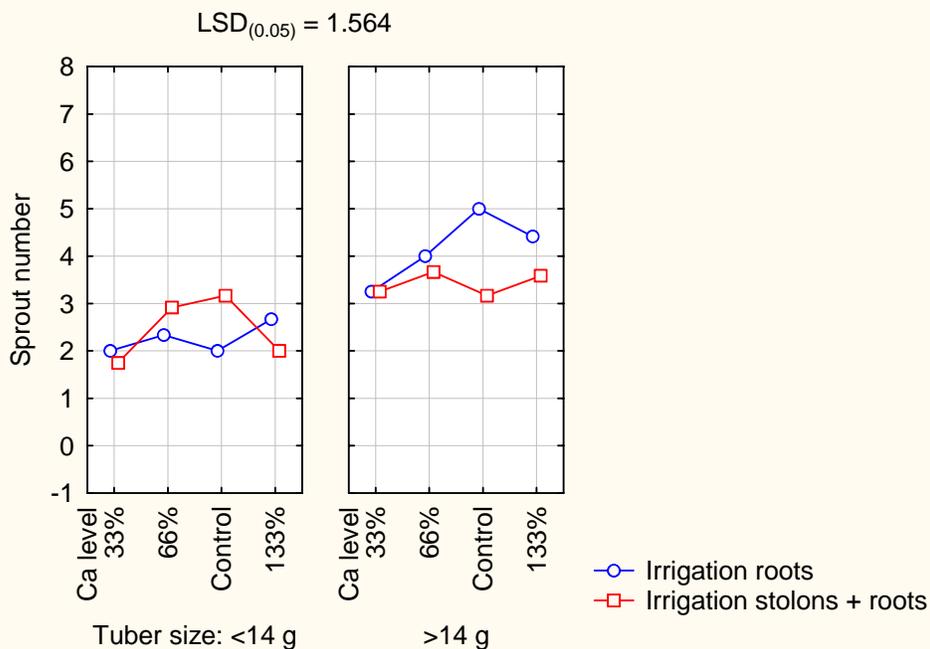
Temperature had a significant effect on the sprout number per tuber. Sprout number increased as the storage temperature increased from 3 °C to 6 °C, although it decreased again as the temperature was further increased to 10 °C (Figure 3.5). Sprout number for

storage at 6 °C (3.64) was significantly higher than those found at storage at both 3 °C (2.9) and 10 °C (2.7). There was no significant difference between storage at 3 °C and at 10 °C.



**Figure 3.5** Sprout number as influenced by the different storage temperatures.

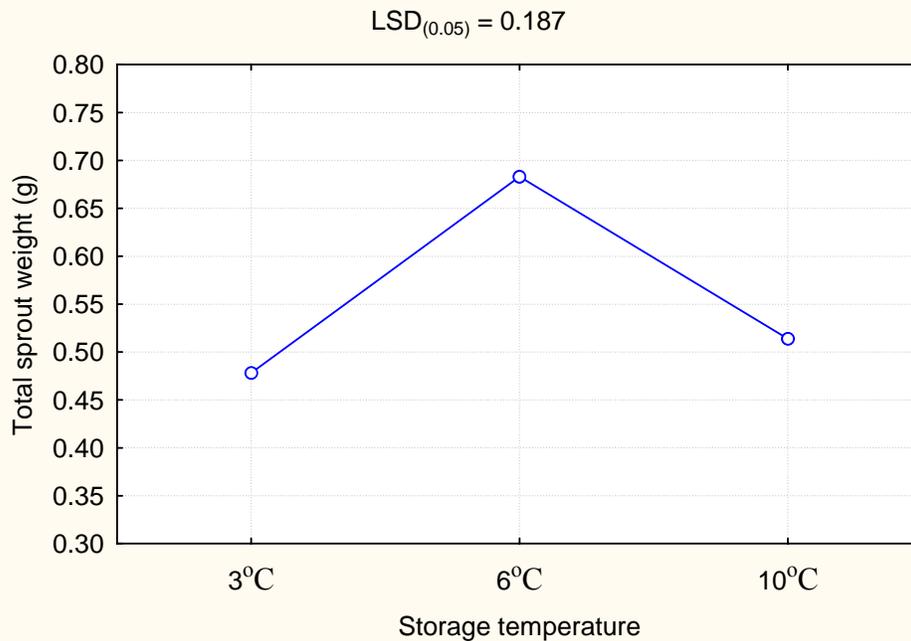
There was a significant interaction between tuber weight class and the different Ca levels and irrigation methods used during tuber production (Figure 3.6). For both irrigation methods, the larger tubers (>14 g) had more sprouts than the smaller tubers (<14 g) at all the Ca levels, except for the control, which gave the same number of sprouts, irrespective of the tuber size. However, only the two highest Ca levels of the larger tubers that was irrigated on the roots only, produced significantly more sprouts than tubers that were exposed to the nutrient solution during fertigation. Using <14 g tubers, Ca level and irrigation method had no significant effect. Using >14 g tubers, that were produced at increasing Ca levels, the number of sprouts on tubers that were exposed to fertigation did not change, but sprout numbers increased significantly from the 33 % and 66 % Ca levels to 100 % Ca, only where developing tubers were not fertigated.



**Figure 3.6** Sprout number per tuber as influenced by an interaction between weight class, irrigation method and Ca level.

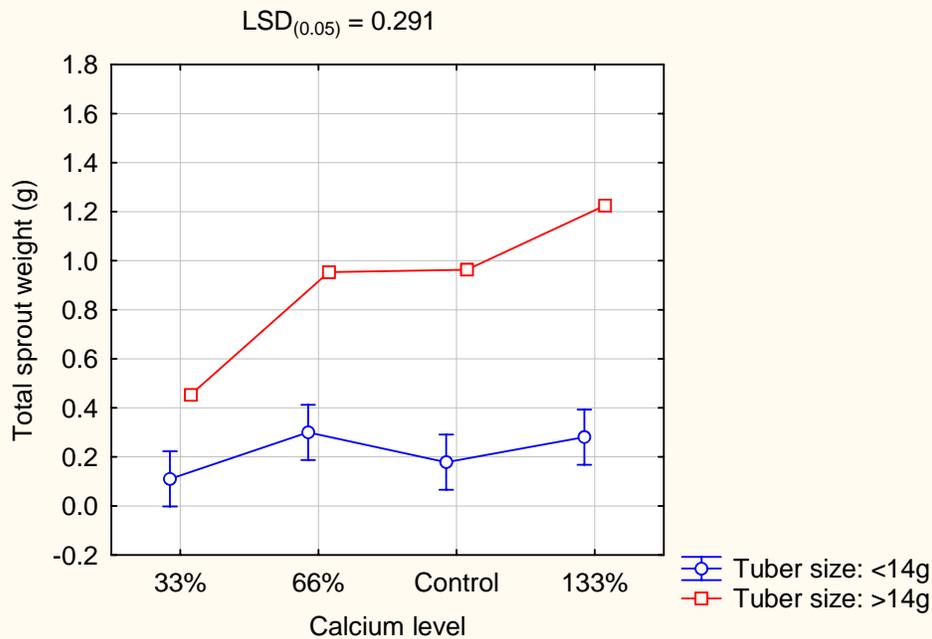
#### Total sprout weight

Storage temperatures had a significant effect on total sprout weight (TSW). TSW increased as the storage temperature increased from 3 °C to 6 °C, although it decreased again as the temperature was further increased to 10 °C. TSW for tubers stored at 6 °C (0.68 g) was significantly higher than that of tubers stored at 3 °C (0.48 g), but not significantly higher than those stored at 10 °C (0.52 g). There was also no significant difference between 3 °C and 10 °C (Figure 3.7).

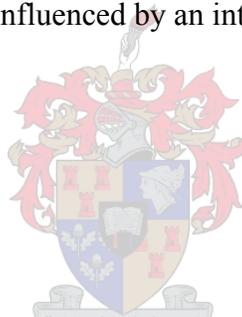


**Figure 3.7** Total sprout weight as influenced by different storage temperatures.

There was also a significant difference in TSW due to an interaction between weight class and the Ca level supplied during tuber production (Figure 3.8). TSW of the larger tubers was significantly higher than that of the smaller tubers at all the Ca levels, except for the 33 % Ca level. For the larger tubers TSW increased as Ca level was increased from 33 % to 133 %. TSW of the 33 % Ca level of the larger tubers was significantly lower than all of the three higher levels. There was no significant difference in TSW on the small tubers that were produced at different Ca levels.

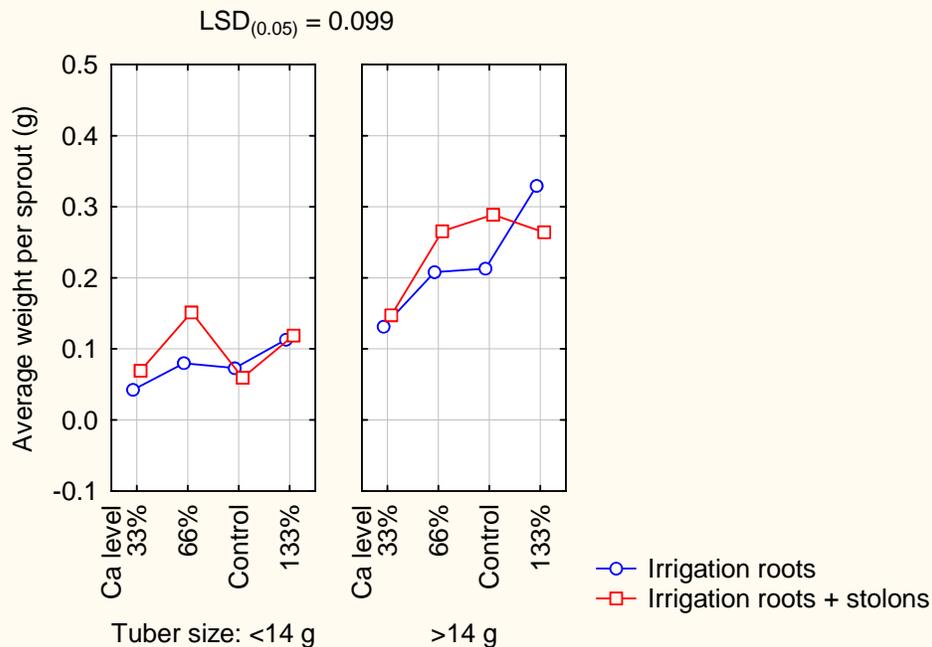


**Figure 3.8** Total sprout weight as influenced by an interaction between tuber weight class and Ca level.



#### Average weight per sprout

Average weight per sprout (AWS) was significantly influenced by an interaction between weight class and the different Ca levels and irrigation methods used during tuber production (Figure 3.9). For both irrigation methods, AWS of the larger tubers was significantly more than that of the smaller tubers at all the Ca levels, except for the 33 % Ca level. For irrigation on the roots only, there was an increase in AWS as the Ca level was increased, for both tuber weight classes. For the smaller tubers, irrigation on both the roots and stolons had a higher AWS than that of irrigation on the roots only, at the two lowest Ca levels, but at the control and the 133 % Ca level both irrigation methods had more or less the same AWS. For the larger tubers, irrigation on both the roots and stolons had a higher AWS than irrigation on the roots only, at the 33 % Ca level, 66 % Ca level and the control, but for the 133 % Ca level irrigation on the roots only had a higher AWS than that of irrigation on both the roots and stolons.



**Figure 3.9** Average weight per sprout as influenced by an interaction between tuber weight class, irrigation method and Ca level.



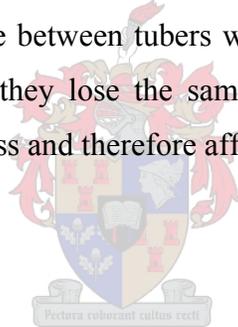
## Discussion

### *Weight loss*

According to Edgar (1968), the main reason for weight loss of the tubers is water loss. When tubers are stored, conditions must be favourable to minimize weight loss, because water loss will lead to lower quality. The only factor that had a significant influence on weight loss of the tubers was the temperature at which the tubers were stored. Weight loss increased as the temperatures at which the tubers were stored was increased, regardless of the Ca level or irrigation method that was used during production of the tubers. Other research workers also found that water loss increased at higher temperatures (Edgar, 1968; Dean, 1994). Veerman & Wustman (2005) also suggest that seed potatoes should be stored at 3-4 °C.

### ***Firmness***

The Ca level applied during production had a significant effect on the firmness of the tubers, even though weight loss was not significantly affected by Ca level. Firmness of the tubers was increased as the Ca supply was increased. Thus, higher levels of Ca applied during production are favourable for the tubers, because firmer potatoes will have better quality and shelf-life and has greater resistance to diseases and mechanical injuries (Harris, 1992). According to Marschner (1986) Ca plays an important role in the strengthening of cell walls. This is probably the reason why the firmness of the tubers was increased as the supply of Ca was increased. Firmness was also influenced by an interaction between tuber weight class and storage temperatures, although no clear trend could be recognized with regard to the effect of storage temperature on firmness. The highest firmness was found for the larger tubers at 6 °C and 10 °C. This can possibly be explained by the weight difference between tubers where the smaller tubers will have a higher percentage weight loss if they lose the same amount of water than the larger tubers, thereby leading to turgor loss and therefore affecting the firmness of the tubers.



### ***Sprouts***

For the tubers to sprout, starch within the tubers needs to be changed to sugars. Tubers with more sugars will sprout more easily and faster than tubers with less sugars (Smith, 1968). According to Smith (1968), tubers stored at lower temperatures will accumulate more sugars, so it can be expected that they will sprout more easily. In this experiment, tubers stored at 6 °C produced the most sprouts (Figure 3.5), and had the highest TSW (Figure 3.7).

Tuber weight class also has an effect on the number of sprouts that will develop. In this study, the larger tubers produced more sprouts and higher TSW than the smaller tubers. This agrees with results reported by Struik & Wiersema (1999). According to Struik & Wiersema (1999) the reason why larger tubers will produce more sprouts is mainly due to

the development of an increasing number of eyes as the tubers grow larger, since new lateral buds are continuously initiated.

The sprout number per tuber was significantly influenced by an interaction between weight class and the different Ca levels and irrigation methods used during tuber production (Figure 3.6). For the larger tubers which were irrigated on the roots only, there was an increase in sprout number with an increase in the Ca level. According to Combrink (1971), a higher supply of Ca could lead to an increase in starch of the tuber. Thus, it is possible that the higher Ca levels had more starch that was transformed to sugars than the lower Ca levels. The larger tubers irrigated on both the roots and stolons had less sprouts than those irrigated on the roots only at all the Ca levels. This could be due to the tubers having a longer shelf-life (time to sprouting) than those irrigated on the roots only.

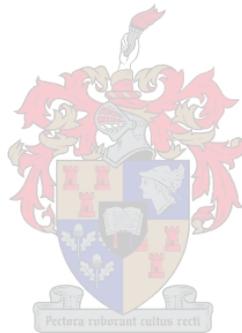
The TSW was significantly influenced by an interaction between tuber weight class and the Ca level used for the production of tubers. The larger tubers had an increase in TSW as the Ca level was increased. This could be due to the fact that the tubers treated with the higher Ca levels had more starch which could be transformed to sugars (Combrink, 1971). The smaller tubers had more or less the same TSW at all the Ca levels. This could be because they were not physiologically mature; therefore the influence of Ca was not yet established.

## **Conclusion**

Weight loss of the tubers increased as the storage temperatures was increased from 3 °C to 10 °C. Weight loss lead to reduced firmness and therefore quality of the tubers, therefore it is important to limit weight loss during storage. Thus it is better to store tubers at lower temperatures to limit weight loss.

Firmness of the tubers is also very important. Tubers with better firmness have better quality and have better resistance to diseases. In this experiment it was found that tubers which had a higher supply of Ca were firmer than those which had reduced Ca levels in their nutrient solution during production. Thus Ca plays an important role in the quality of harvested tubers.

For seed potatoes, tubers are should produce a higher number of sprouts. Several factors had an influence on sprouting. Larger tubers produced more sprouts than the smaller tubers. Best sprouting also occurred in tubers which were stored at 6 °C. Overall, the higher Ca level in the nutrient solution also produced tubers which had a higher TSW and also AWS. Thus, Ca applied during production plays an important role in sprouting of seed potatoes, and should therefore not be less than the Ca level used in the control nutrient solution.



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## CHAPTER 4

### **The effect of production techniques and post harvest storage temperatures of G0 seed tubers on the plant growth, yield and quality of first generation (G1) seed potatoes**

#### **Abstract**

Tubers harvested from plants treated with four different nutrient solutions (control with 100 % Ca, and a 33 %, 66 % and 133 % Ca solution) and two different irrigation methods (irrigation on roots only, and irrigation on roots and stolons) were divided into two weight classes (<14 g & >14 g) and stored at three different temperatures (3, 6 & 10 °C) for five weeks. Tubers were stored in a dark room at room temperature to allow sprouts to develop. Thereafter tubers were planted in a greenhouse and were irrigated with a standard Steiner nutrient solution (1.5 mS cm<sup>-1</sup>). The plants were harvested after two months. Leaves of all the plants were collected, the leaf area measured, before it was oven dried for a week and weighed to determine the dry weight. The tubers of each plant were counted and weighed to determine the yield. Tuber samples were sent to a lab to be analyzed for their nutrient content. The tuber production techniques, as well as the storage temperatures of the seed potatoes had no significant effect on the plant parameters measured. The larger seed tubers produced plants with significantly higher leaf areas, dry weight of leaves, as well as higher yields than the plants produced from the smaller seed tubers. Differences in nutrient content between treatments were inconsistent and difficult to explain.

## Introduction

Tuber size is very important in determining the quality of seed potatoes, because it has an effect on the number of eyes per tuber, and on the vigour of each sprout and stem developing from it (Struik & Wiersema, 1999).

Larger tubers have more eyes than smaller tubers, because as tubers grow larger, new lateral buds are continuously initiated (Nielson, Iritanie & Weller, 1989). The degree of tuber development, as already partly reflected in its size (larger tubers are usually further advanced in growth and development), also has an effect on the number of eyes per tuber, although very small tubers, such as microtubers or minitubers may already bear a surprisingly high number of potentially active eyes (Struik & Wiersema, 1999).

Seed tuber size has an effect on the plant vigour, as it affects the number of sprouts per tuber, and therefore also the amount of stems that will develop per tuber. Wiersema, Cabello & Booth (1987) tested the ground cover and tuber yield development for different seed tuber sizes planted at similar plant densities. They found that ground cover development took place at a faster rate for the larger seed tubers than that of the smaller ones, because larger seed tubers developed more main stems. Consequently, the amount of radiation accumulated by the crop increases, and therefore yields of crops grown from larger seed tubers are higher than those from smaller seed tubers (Wiersema, 1989).

Different reasons exist for the inferior performance of the stems of very small seed tubers. Firstly, plant emergence is lower for smaller seed tubers than for larger ones. Moreover, plants from small tubers are more sensitive to adverse growing conditions (Struik & Wiersema, 1999). Stems from small tubers also have a relatively low initial growth rate and are thinner, and therefore they are more susceptible to stem canker and other pests and diseases (Lommen, 1994). Small tubers use a larger proportion of their much smaller reserves to produce emerging stems, consequently the developing sprouts need to become self-supportive earlier than sprouts from larger tubers. In addition, the root production is poor, to the detriment of water and nutrient supply. Secondly, small

seed tubers have a longer dormancy period and therefore will sprout later than larger tubers, if stored in the same environment (Morris, 1966; Burton, 1989a).

Low levels of Ca used during the production of G0 mini-tubers can lead to lower levels of Ca in the peels of these tubers (Chapter 2). Ca is important for strengthening of cell walls (Marschner, 1986); therefore these lower levels of Ca in the peel might lead to mechanical injuries or infections by pathogens. Lower levels of Ca can also lead to smaller G0 tubers (Chapter 2). Both these factors may lead to fewer eyes emerging with retarded and poor growth from the seed, which in turn may lead to lower yields of the plants that are produced from them (Struik & Wiersema, 1999).

The storage temperature of seed tubers may have a marked effect upon subsequent growth and yield, apart from its effect upon sprout numbers (Burton, 1989b). The optimum pre-sprouting storage temperature can depend on the cultivar, but also on the sample, year and growing conditions. This optimum temperature can vary from 1 to 7 °C (Burton, 1989b). The effect of storage temperature on the subsequent behaviour of seed tubers, and the yield of the crop grown from them, can largely be attributed to one or more of a number of characteristics which can be influenced by storage temperature (Burton, 1989b). These characteristics may include the following:

1. A different time of emergence of the plants;
2. A different time-scale in achieving ground-cover;
3. A different size and longevity of the foliage; and
4. A different time of tuber initiation.

The objective of this experiment was to determine the effect of production techniques and post-harvest storage temperatures of G0 seed tubers on the plant growth and yield of first generation (G1) seed potatoes.

## Material and methods

Experiments were conducted in a greenhouse with evaporative cooling at Welgevallen, the experimental farm of the University of Stellenbosch, in the Western Cape of South Africa.

G0 minitubers used in this experiment incorporates the following seed sources:

1. *In-vitro* derived potato plantlets of the cultivar BP1 were cultivated in an aeroponic system in a greenhouse. The experiment comprised of two irrigation methods (irrigation on stolons and roots, and irrigation on roots only) as well as four different nutrient treatments with different Ca: K and Mg ratios. The nutrient solutions differed with regard to K, Mg and Ca levels. These nutrient solutions consisted of a Steiner (1984) solution as control (100 % Ca), and three treatments in which the Ca levels were changed to 33 %, 66 % and 133 % of the control, while K and Mg levels were adjusted to compensate for the change in Ca and to keep the electrical conductivity (EC) at  $1,5 \text{ mS cm}^{-1}$  (Chapter 2).
2. Harvested tubers of each treatment combination (irrigation method x nutrient solution, here-after referred to as tuber production technique [TPT]) were divided into two weight classes (<14 g and >14 g) and stored at three different temperatures (3, 6 & 10 °C) for five weeks. Tubers were stored in a dark room at room temperature to allow sprouts to develop (Chapter 3).

G0 mini-tubers obtained from the above-mentioned sources were planted individually in 10 L bags containing a pine sawdust and shavings substrate. Bags were irrigated with a standard Steiner nutrient solution (Steiner, 1984) at an EC of  $1.5 \text{ mS cm}^{-1}$ , using drip irrigation. Plants were irrigated four times a day for three minutes. This irrigation frequency was kept the same during the whole production phase of the plants. The plants were harvested after two months. Leaves of all the plants were collected, and their area measured. Leaves were oven dried at a temperature of 80 °C for one week, after which

they were weighed to determine the dry weight. The tubers of each plant were counted and weighed to determine the yield. Tuber samples were sent to a laboratory (Callab) to determine the nutrient content of the skin as well as the whole potato.

### Experimental design and statistical analysis

An 8 (4 Ca levels x 2 irrigation methods [TPT]) x 2 (weight classes) x 3 (storage temperatures) factorial arrangement with four replications was used for this experiment. A repeated measures analyses of variance (ANOVA) was done on the data, and the Tukey HSD test was done to compare treatment means at a probability level of 5 %. The data were analyzed using STATISTICA version 7.1 (Statistica, 2004).

### Results

Results of the Analysis of Variance (ANOVA) done on data with regard to leaf area (LA), dry weight of the leaves (DWL), tuber number (TN), total tuber weight (TTW) and average weight per tuber (AWT) are summarized in Table 4.1.

**Table 4.1** Significant levels ( $Pr > f$ ) of main effects namely Ca level combined with irrigation method (TPT), tuber weight class (size) and temperature (Temp), as well as interactions with regard to LA, DWL, TN, TTW and AWT

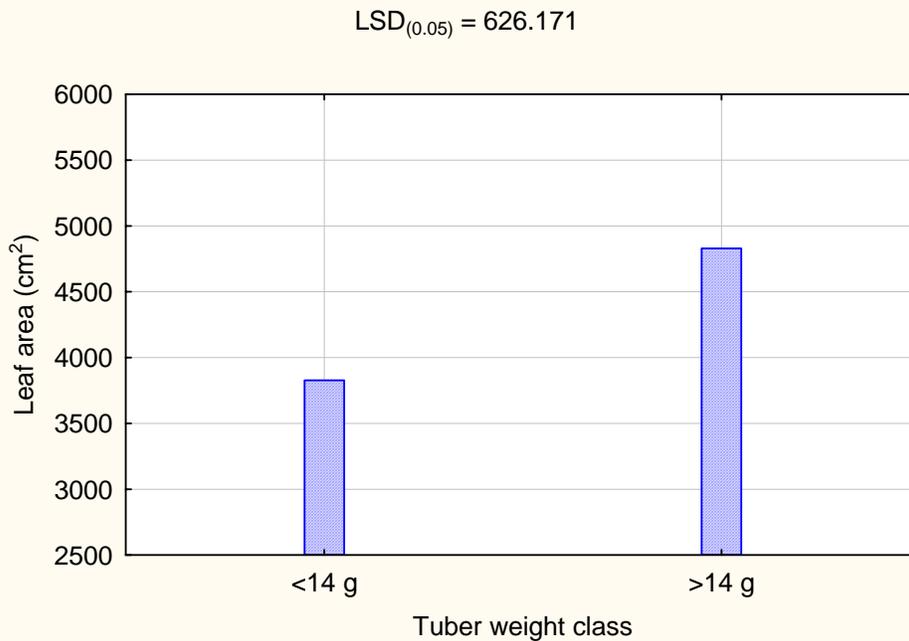
	LA	DWL	TN	TTW	AWT
TPT	0.372616	0.413739	0.016557	0.120846	0.055564
Size	0.002995	0.000007	0.000019	0.000002	0.003856
Size*TPT	0.496015	0.137055	0.178261	0.552801	0.165690
Temp	0.116456	0.557947	0.339905	0.196631	0.440220
Temp*TPT	0.054017	0.317267	0.613910	0.347874	0.071111
Size*Temp	0.470609	0.318441	0.405148	0.079317	0.067073
Size*Temp*TPT	0.009812	0.003477	0.188495	0.047833	0.416759

The different tuber production techniques (TPT) had a significant effect only on the TN per plant. Tuber weight class (size) had a significant effect on all the plant parameters measured, namely LA, DWL, TN, TTW and AWT. Storage temperature (temp) as a main effect did not have a significant effect on any of the measured plant parameters (Table 4.1).

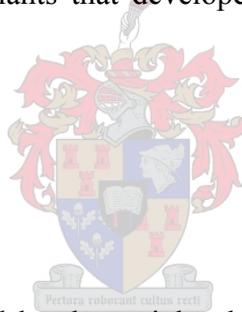
Although there was a significant interaction between tuber weight class, storage temperature and TPT for LA, DWL and TTW, only tuber weight class as a main effect had a significant effect on all the measured parameters, but was involved in interactions. Because there was no clear trend (Appendix A) for these interactions between tuber weight class, storage temperature and TPT, it is regarded as experimental errors and will not be discussed.

### ***Leaf area (LA)***

LA was significantly influenced by the weight class of the seed tubers (Figure 4.1). LA of the plants produced from the larger seed tubers ( $\pm 4825 \text{ cm}^2$ ) was significantly higher than that of the plants produced from the smaller seed tubers ( $\pm 3825 \text{ cm}^2$ ).

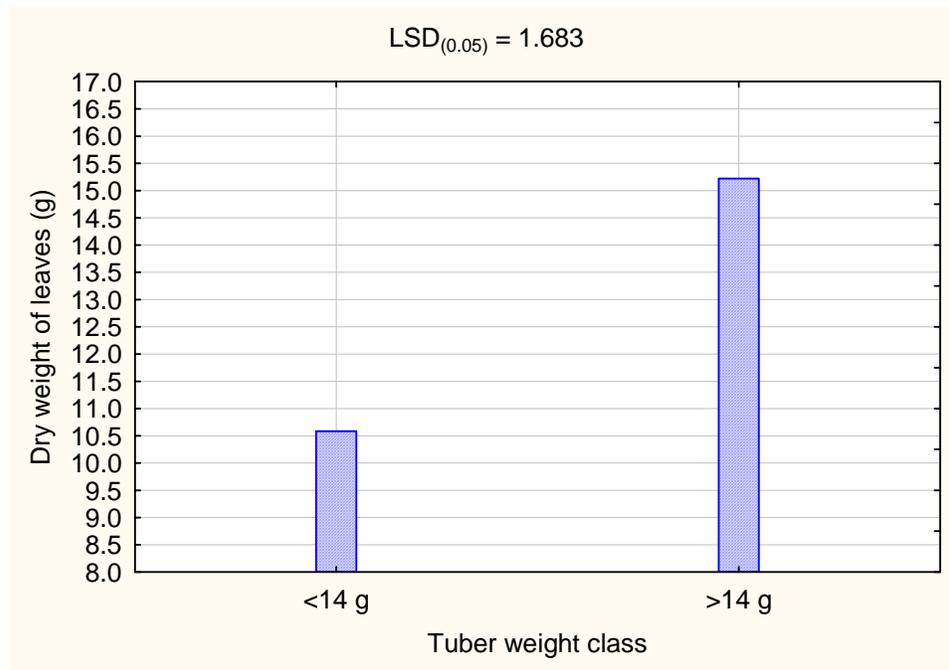


**Figure 4.1** Leaf area of potato plants that developed from seed tubers of two weight classes.

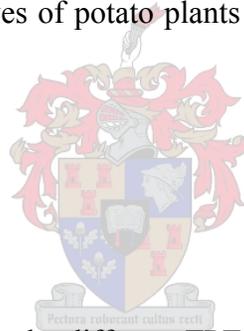


#### ***Dry weight of leaves (DWL)***

DWL was significantly influenced by the weight class of the seed tubers (Figure 4.2). DWL of the plants produced from the larger seed tubers (15.2 g) was significantly higher compared to that of the plants produced from the smaller seed tubers (10.6 g).

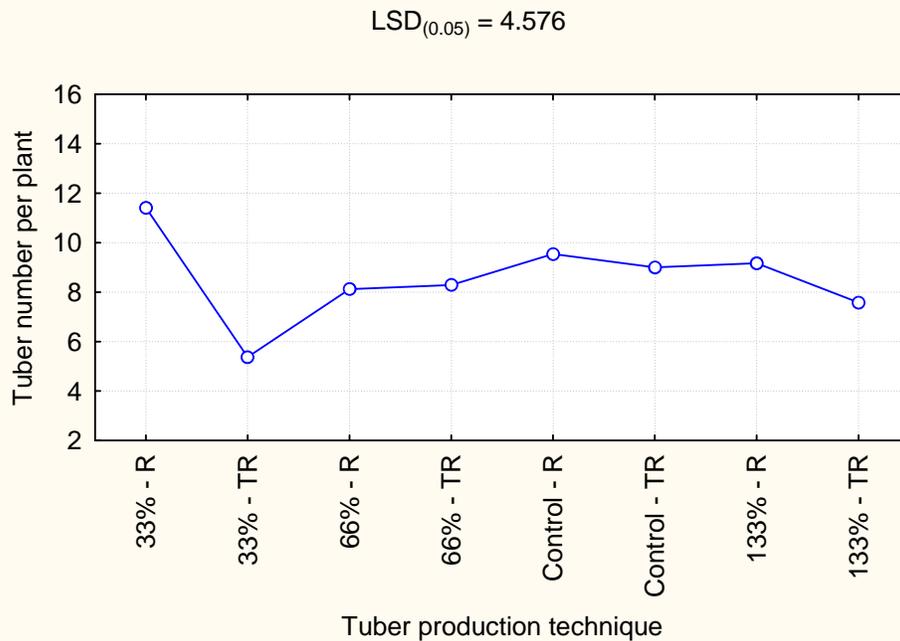


**Figure 4.2** Dry weight of the leaves of potato plants that developed from seed tubers of two weight classes.



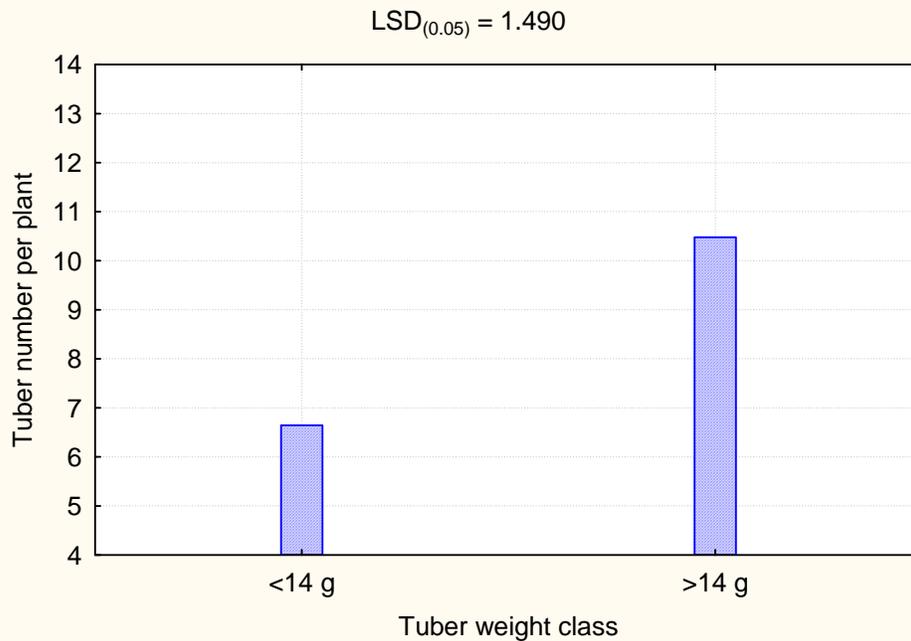
### ***Tuber number (TN)***

TN was significantly influenced by the different TPT's used during the production of the seed tubers (Figure 4.3). However, the only significant difference was between the 33 % Ca level that was irrigated on the roots only ( $\pm 11.5$  tubers) and the 33 % Ca level that was irrigated on both the stolons and the roots ( $\pm 5.5$  tubers). No other significant difference due to the TPT's used to produce the seed tubers was found and no general trend was shown if the first mentioned treatments were ignored. The above mentioned significant difference is difficult to explain and will be regarded as an experimental error.

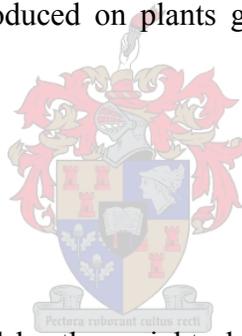


**Figure 4.3** Tuber number per plant as influenced by the different tuber production techniques (R = irrigation on roots only; TR = irrigation on stolons and roots).

TN was also significantly influenced by the weight class of the seed tubers (Figure 4.4). TN of the plants produced from the larger seed tubers ( $\pm 10.5$  tubers) was significantly higher than that of the plants produced from the smaller seed tubers ( $\pm 6.6$  tubers).

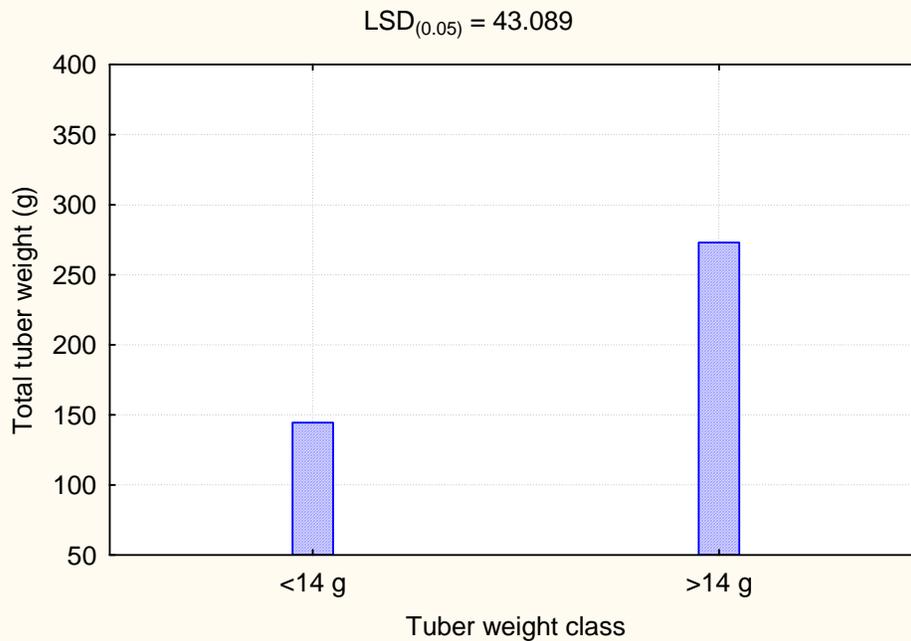


**Figure 4.4** Number of tubers produced on plants grown from small (<14 g) and big (>14 g) seed tubers.

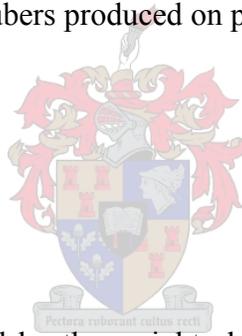


### ***Total tuber weight (TTW)***

TTW was significantly influenced by the weight class of the seed tubers (Figure 4.5). TTW of the tubers produced from the larger seed tubers ( $\pm 273$  g) was significantly higher than that of the tubers produced from the smaller seed tubers ( $\pm 145$  g).

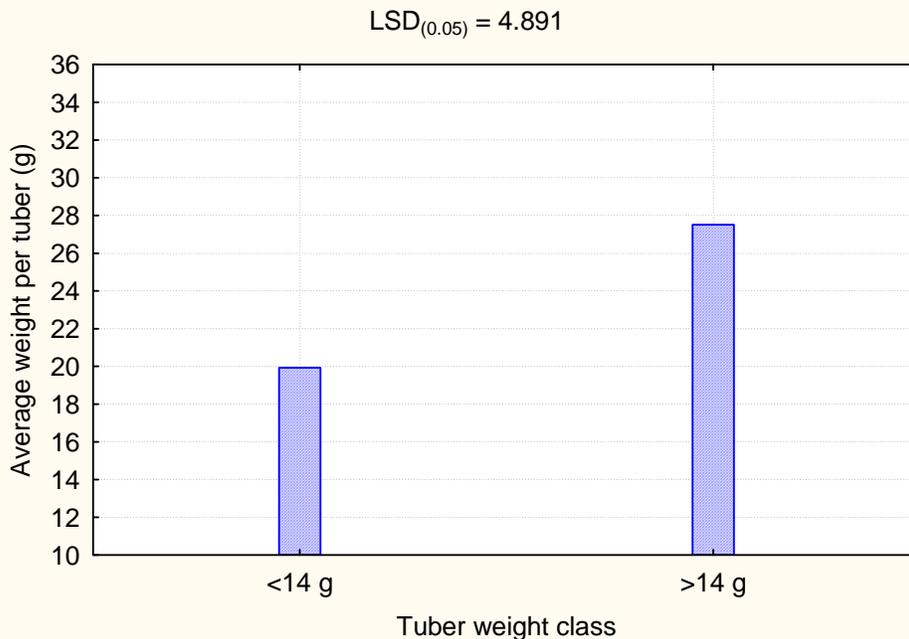


**Figure 4.5** Total tuber weight of tubers produced on plants grown from small (<14 g) and big (>14 g) seed tubers.

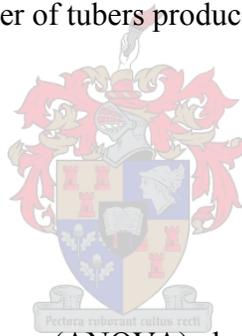


***Average weight per tuber (AWT)***

AWT was significantly influenced by the weight class of the seed tubers (Figure 4.6). AWT of the tubers produced from the larger seed tubers ( $\pm 27.8$  g) was significantly higher than that of the tubers produced from the smaller seed tubers ( $\pm 20$  g).



**Figure 4.6** Average weight per tuber of tubers produced on plants grown from small (<14 g) and big (>14 g) seed tubers.



### *Nutrient Content*

Results of the Analysis of Variance (ANOVA) done on data with regard to nutrient content of the skin and the whole tuber are summarized in Table 4.2.

**Table 4.2** Significant levels ( $Pr > f$ ) of main effects namely Ca-level combined with irrigation method (TPT), tuber weight class (size) and temperature (Temp), as well as interactions with regard to nutrient content of the skin and the whole tuber.

	Skin				Whole tuber			
	Ca	Mg	K	H <sub>2</sub> O	Ca	Mg	K	H <sub>2</sub> O
<b>TPT</b>	0.369537	0.000018	0.894580	0.111407	0.666488	0.000012	0.010628	0.026936
<b>Size</b>	1.000000	0.000792	0.223749	0.033474	0.336358	0.229237	0.005592	0.003767
<b>Temp</b>	0.169019	0.557199	0.841466	0.976040	0.584831	0.555692	0.100036	0.831657
<b>TPT*Size</b>	0.109373	0.271383	0.525318	0.332183	0.950072	0.164995	0.295420	0.074934
<b>TPT*Temp</b>	0.042476	0.097720	0.206199	0.573402	0.237115	0.044342	0.213062	0.073956
<b>Size*Temp</b>	0.502037	0.428684	0.910287	0.877816	0.183236	0.800722	0.352276	0.262687

The TPT's had a significant effect on the Mg content of the peel and the whole tuber, as well as the K and the H<sub>2</sub>O content of the whole tuber. Tuber weight class had a significant effect on the Mg and H<sub>2</sub>O content of the peel, as well as the K and H<sub>2</sub>O content of the whole tuber. There was also a significant interaction between TPT and storage temperature that had an effect on the Ca content of the peel, as well as the Mg content of the whole tuber (Table 4.2).

Although the above mentioned differences occurred, differences were inconsistent and no clear trend could be found, making the interpretation of the data not feasible. Since all the plants received a complete and balanced Steiner (1984) nutrient solution during the whole production phase, it is believed that nutrient deficiencies in the source seed potatoes did not have an effect on the nutrient content of the next generation tubers. These differences are therefore regarded as experimental errors, and will not be discussed (data are summarized in Appendix A).

## Discussion

### *Leaf area and dry weight of leaves (DWL)*

Both the leaf area and DWL were significantly influenced by the weight class of the seed tubers. Leaf area and DWL of the plants produced of the larger seed tubers were significantly higher than from the plants produced of the smaller tubers. This could be expected, because the larger tubers had more sprouts than the smaller tubers (Chapter 3) and therefore could develop more main stems. Also, the larger tubers had more reserves to support faster growth of the plants. Wiersema *et al.* (1987) also found that larger tubers planted at the same densities as smaller tubers developed ground cover much quicker than smaller tubers, because larger tubers produced more main stems. Nielson *et al.* (1989) also found that larger tubers had more eyes than smaller tubers, and therefore could produce more sprouts and thus more main stems.

In chapter 2 it was found that higher Ca: K & Mg ratios can produce larger tubers. Thus, the TPT's may have had an indirect effect on the growth vigour of the plants because of the effect that they had on the size of the seed tubers that were used to produce these plants.

### ***Tuber yield***

Tuber number, total tuber weight and average weight per tuber was significantly higher for the plants produced from the larger seed tubers than that of the plants produced from the smaller ones. This suggests that larger seed tubers produce higher yields than smaller seed tubers. According to Wiersema (1989) one of the reasons for this higher yield is that the larger tubers produce more ground cover than the smaller ones, and therefore the amount of radiation accumulated by the crop will be increased. Burton (1989a) also found that larger seed tubers yield better than smaller ones.

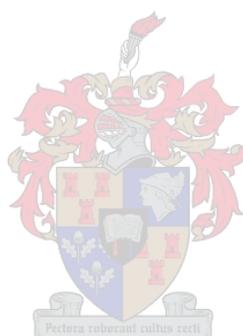
Although storage temperature of the seed tubers did not have a significant influence on the yield in this experiment, Wurr (1978) found that the highest yields were obtained from tubers stored at low temperatures. No significant differences were found between the different TPT's, and therefore this will not be discussed here.

### **Conclusion**

The larger seed tubers produced plants with higher leaf area and leaf dry weight, as well as plants that produced higher yields. This suggests that larger seed tubers are better to use than smaller ones.

Although the TPT's of the seed tubers, and the different tuber seed storage temperatures had no significant influence on the plants that were produced from them, it was shown in

chapters 2 and 3 that the TPT's significantly affected the size and quality of the seed tubers.



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## Chapter 5

### General conclusions

Potatoes are one of the most important food crops in South Africa. Good quality seed potatoes are therefore needed to provide an adequate amount of potatoes for consumption. However, the production of good quality, disease free seed potatoes is expensive. It is therefore important to find ways to increase the yield per plant to make the production of seed potatoes more cost efficient.

The objective of this study was to determine the influence of different Ca, K and Mg ratios and irrigation treatments, as well as storage temperature on the yield and quality of two generations of seed potatoes. The experiment was done in a greenhouse with an evaporative cooling system, using an aeroponic production system. The mobility of Ca in the plant was also tested by irrigating some of the plants on the roots only, and the other plants on both the roots and stolons.

The greenhouse studies for the production of G0 minitubers indicated that there was no difference in the total tuber yield per plant due to different Ca: K & Mg ratios, but the plants that received more Ca produced larger tubers than the plants that received less Ca.

A chemical analysis was done on the skin and flesh of the tubers produced in the first greenhouse experiment. The K concentration in the skin of the highest Ca treatment was significantly higher than that of the lowest Ca treatment. This suggests that Ca has an influence on the uptake of K, because the plants that received the highest Ca received less K than the plants that received the lowest Ca. It was also found that the Ca content of the skin increased as the Ca: K & Mg ratios in the nutrient solution used during production increased. Therefore it is better to increase the Ca supply to the plant, because Ca is an important factor determining the quality of tubers. The Mg content of both the skin and the whole tuber was decreased as the Ca level of the nutrient solution was increased.

This was probably due to lower Mg levels in the nutrient solution as the Ca level increased.

The tubers produced from the first experiment were divided into two weight classes, and stored at three different temperatures (3 °C, 6 °C and 10 °C). The tubers were weighed before, and again after storage to determine weight loss during storage. There was an increase in weight loss as the storage temperature was increased from 3 °C to 10 °C. This suggests that it is better to store the tubers at lower temperatures, because weight loss is mainly due to water loss, reducing seed tuber quality. The smaller tubers also had a higher percentage weight loss than the larger ones. This may be because the peels of the smaller tubers were not fully developed, and therefore more permeable.

The firmness of the tubers was measured after storage. It was found that the firmness increased as the Ca level used during production was increased. Firmer tubers are of better quality, because they will be more resistant to diseases and injury. Thus, the results suggested that an increase in Ca supply to the plant may increase the quality of the tubers.

After the tubers were stored at the different temperatures, they were placed in a dark room to allow sprouting. Sprouts of each tuber were counted and weighed. The tubers that were stored at 6 °C produced the most sprouts with the highest total sprout weight. It was also found that the larger tubers produced more sprouts than the smaller ones. Total sprout weight was also increased as the Ca level used during production was increased. As the sprouting behavior of a tuber is an indication of its growth vigour, tubers that produce more sprouts are favorable, because they will produce plants with more main stems, and thus more ground cover. Thus bigger tubers (high Ca: K & Mg ratios during production) have a better sprouting ability than smaller ones. The results also suggested that higher Ca: K & Mg ratios during production also enhance sprouting of the seed tubers.

The tubers that were allowed to sprout were re-planted in a greenhouse to determine the influence of Ca: K & Mg ratios and site of irrigation during production, as well as post-harvest storage of the seed tubers at different temperatures on the growth vigour and yield of the first generation plants. The leaf area and dry weight of the leaves were measured after the plants were harvested. The tubers were counted and weighed. The only factor that had a clear effect was the weight class of the seed tubers. It was found that the larger tubers produced bigger plants with higher leaf areas and dry mass of leaves than the smaller tubers. They also produced higher tuber yields (more tubers per plant, as well as larger tubers). This again suggested that larger seed tubers enhance growth and yield.

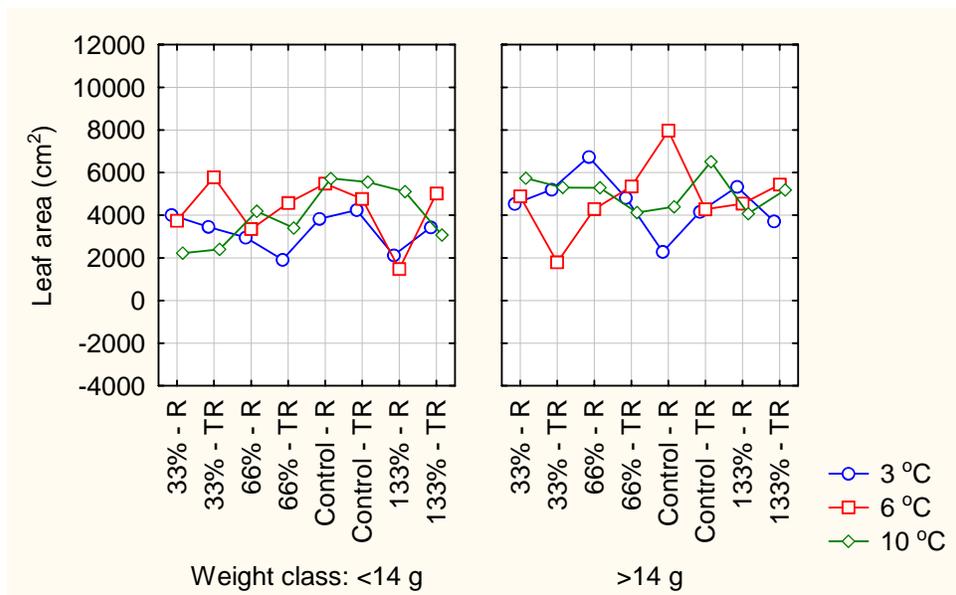
A chemical analysis was done on the harvested tubers from the first generation plants. Although there were significant differences between treatments, none of them showed clear trends and was difficult to explain. No conclusion could thus be made on the effect of the previous treatments (Ca: K & Mg ratios, site of irrigation and post-harvest storage temperatures) on the nutrient content of the tubers (skin and flesh).

It can thus be concluded that Ca has a positive effect on the size distribution and quality of seed potatoes. In this study, no difference in yield or Ca content occurred between tubers produced from the plants that were irrigated on the roots only, and plants that were irrigated on both the roots and stolons. Since this result is contrasting to other literature, more research is needed in this regard.

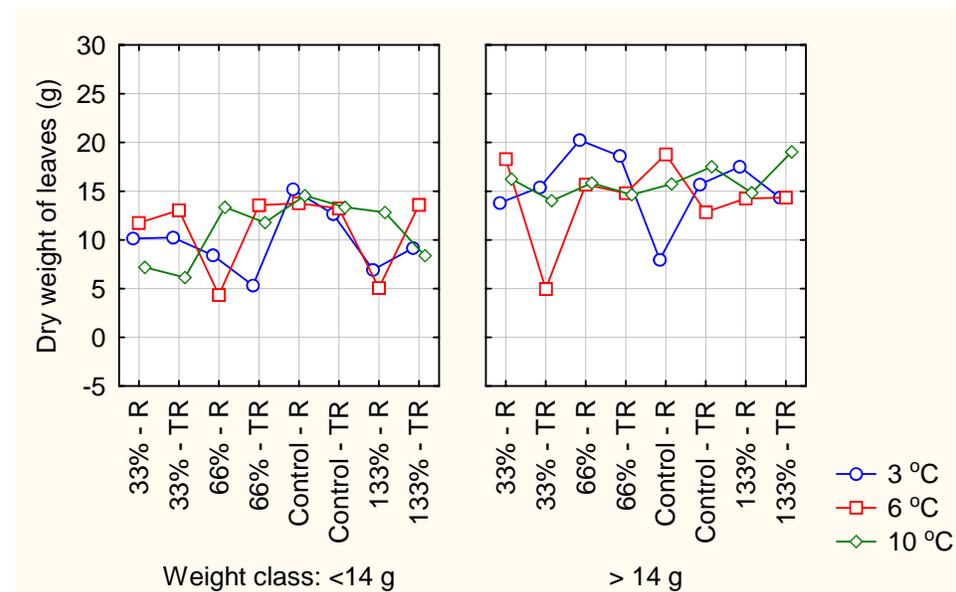
This study shows that seed tubers should be stored at low temperatures, around 3 °C, to maintain the highest quality, while larger tubers proved to out-yield smaller ones. It is possible to obtain larger G0 tubers if the Ca ratios are increased during production. This can lead to higher yields in the next generations. Thus it could be possible to reduce the number of generations needed to produce enough seed tubers for use of commercial potato production. This will lead to higher quality seed potatoes, and can also reduce the input costs for the production of seed potatoes.

## Appendix A

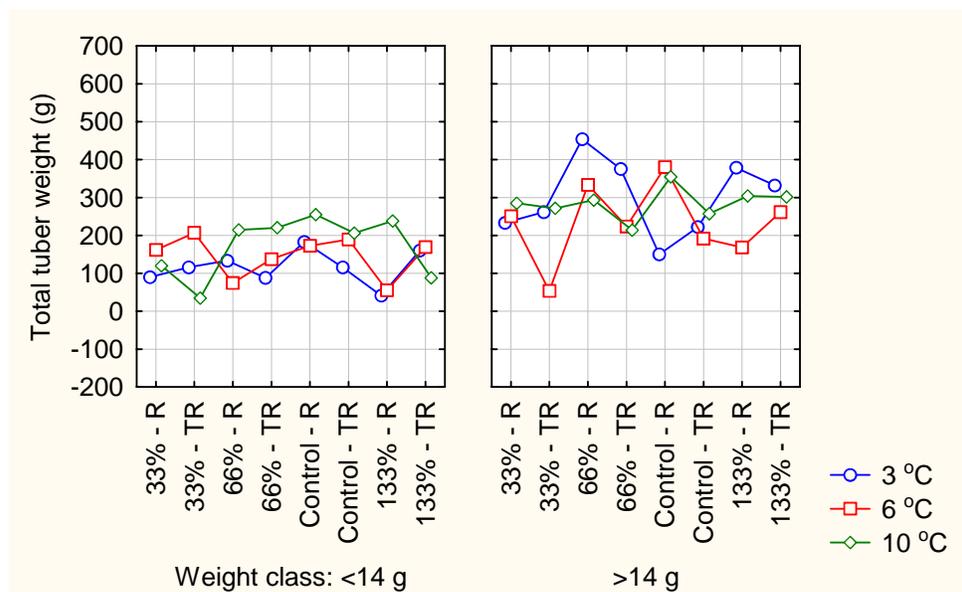
**4.1** Leaf area of the plants as influenced by an interaction between tuber weight class, storage temperature and tuber production technique (TPT) (R = irrigation on the roots only; TR = irrigation on the roots and the stolons)



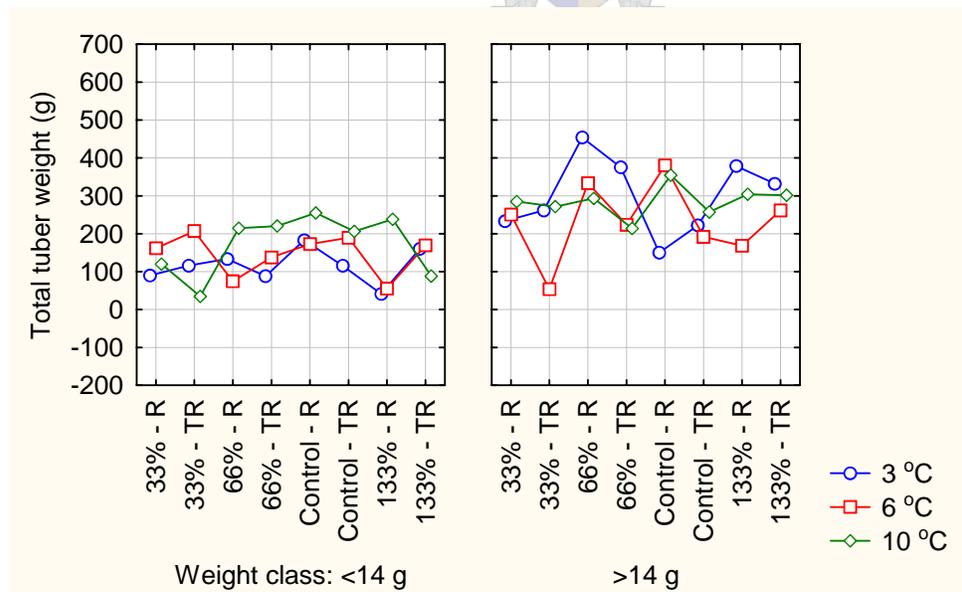
**4.2** Dry weight of the leaves as influenced by an interaction between tuber weight class, storage temperature and TPT (R = irrigation on the roots only; TR = irrigation on the roots and the stolons)



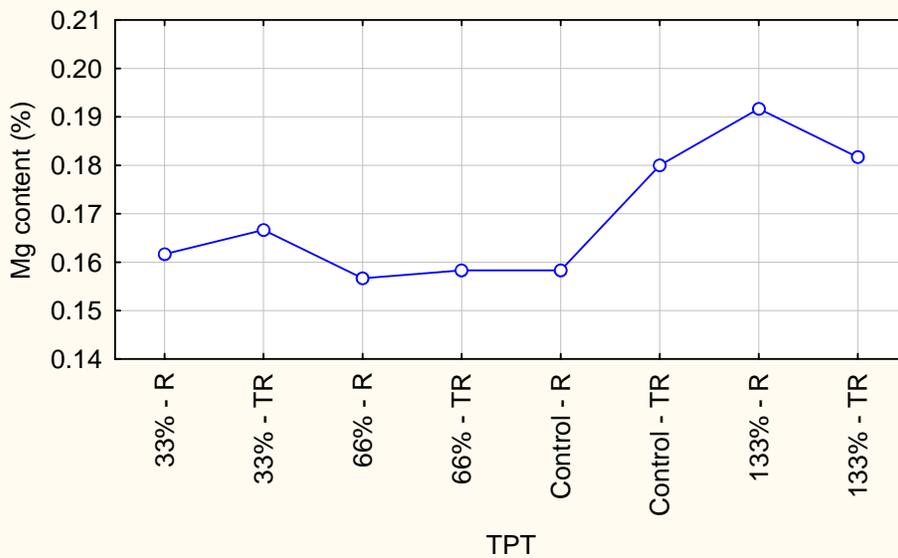
**4.3** Total tuber weight as influenced by an interaction between tuber weight class, storage temperature and TPT (R = irrigation on the roots only; TR = irrigation on the roots and the stolons)



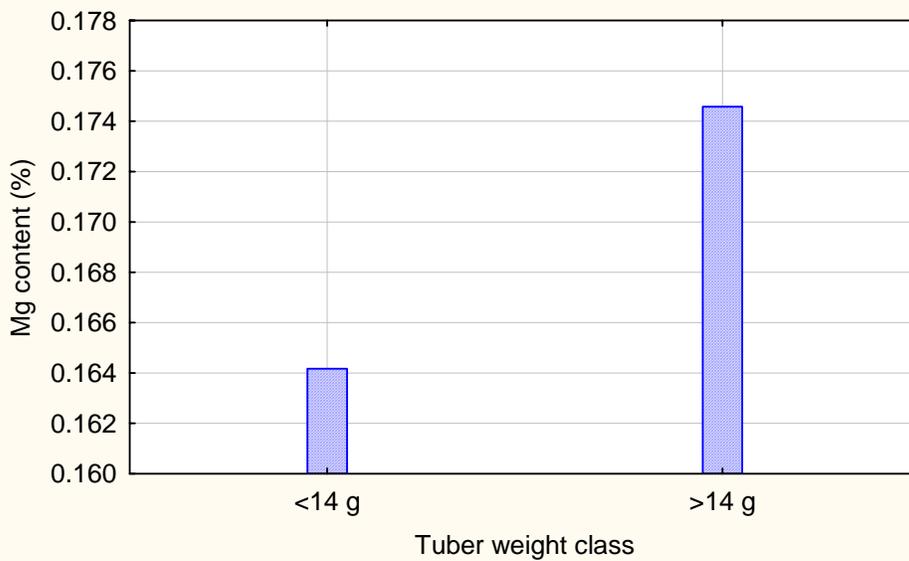
**4.4** Ca content of the peels of the tubers as influenced by an interaction between TPT and storage temperature (R = irrigation on the roots only; TR = irrigation on the roots and the stolons)



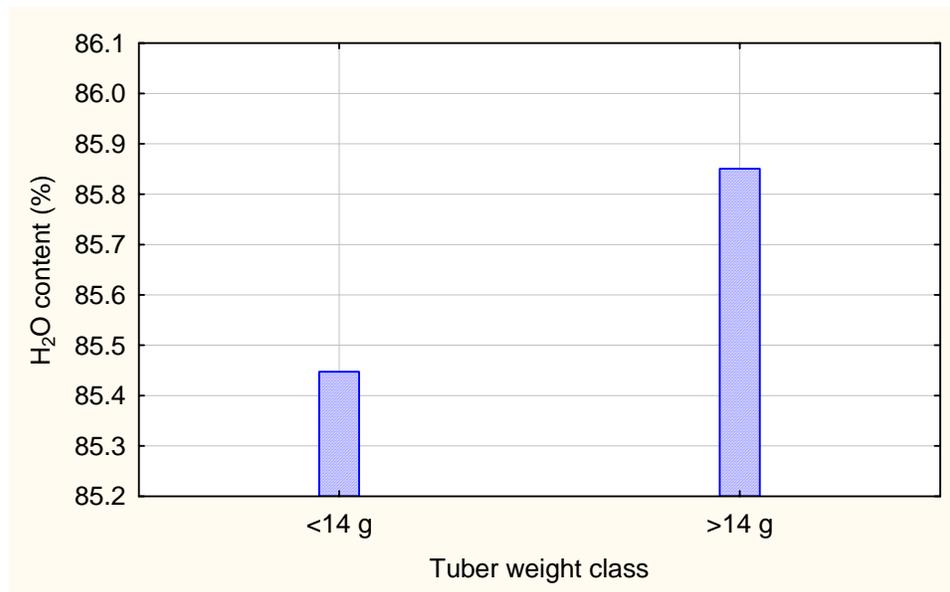
**4.5** Mg content of the peels of the tubers as influenced by the different TPT's (R = irrigation on the roots only; TR = irrigation on the roots and the stolons)



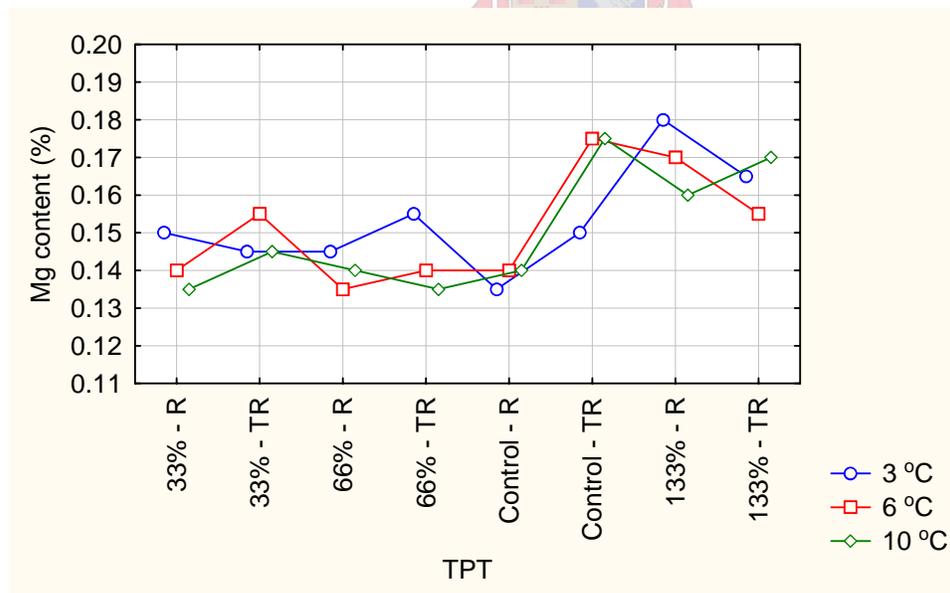
**4.6** Mg content of the peels of the tubers as influenced by tuber weight class



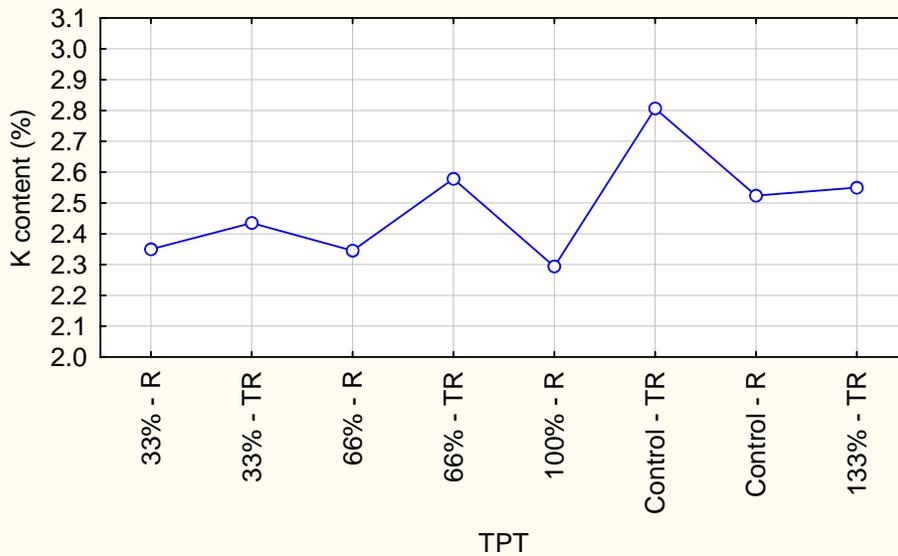
#### 4.7 H<sub>2</sub>O content of the peels of the tubers as influenced by tuber weight class



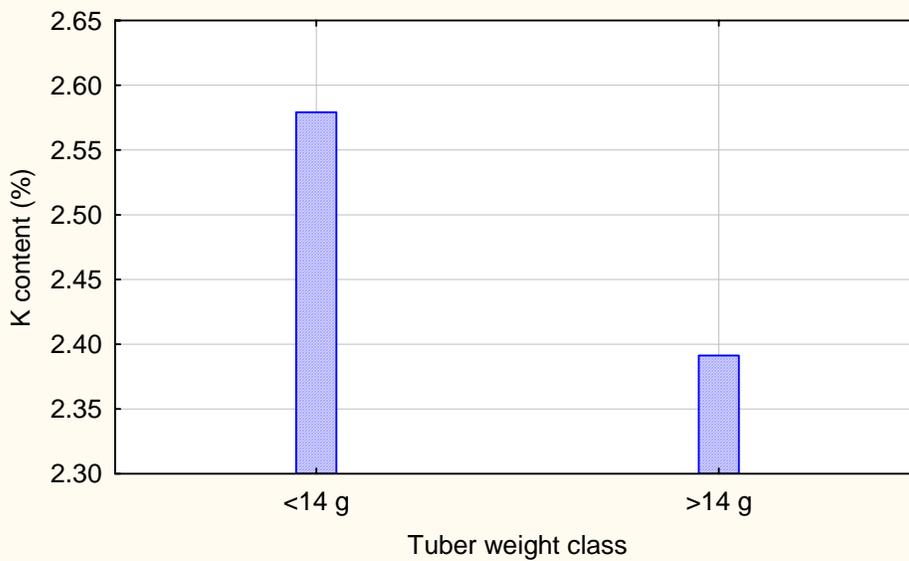
#### 4.8 Mg content of the whole tuber as influenced by an interaction between TPT and storage temperature (R = irrigation on the roots only; TR = irrigation on the roots and the stolons)



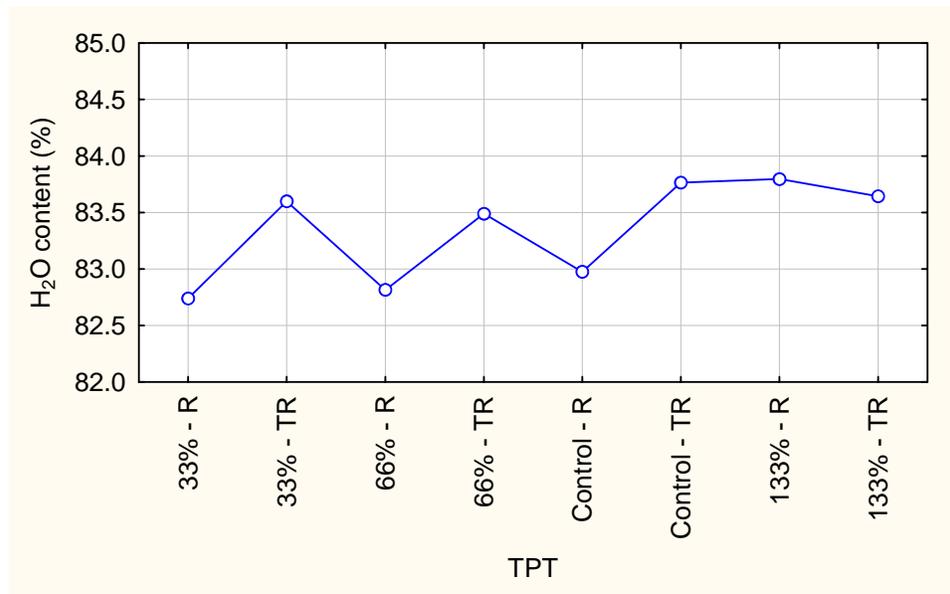
**4.9** K content of the whole tuber as influenced by the different TPT's ((R = irrigation on the roots only; TR = irrigation on the roots and the stolons))



**4.10** K content of the whole tuber as influenced by tuber weight class



**4.11** H<sub>2</sub>O content of the whole tuber as influenced by the different TPT's ((R = irrigation on the roots only; TR = irrigation on the roots and the stolons)



**4.12** H<sub>2</sub>O content of the whole tuber as influenced by tuber weight class

