

Effect of seed size on sprout growth of potato

by

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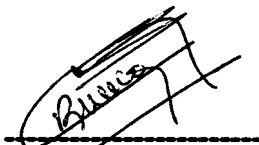
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LIST OF ABBREVIATIONS

ABA:	Absissic acid
ANOVA:	Analysis of variance
β :	Beta
cm:	Centimeter
C.V:	Coefficient of variance
d.f.:	Degree of freedom
DAP:	Days after planting
F:	Fischer
FAO:	Food and Agriculture Organisation
GA:	Gibberellic acid
GLM:	General linear Model
g:	gram
LSD:	Least significant difference
mmol/l:	millimole per litre
mm:	millimetre
mg:	milligram
mm ² :	millimetre square
ns:	Not significant
Pr:	Probabiity
Pacl:	Paclobutrasol
ppm:	Part per million
%:	Percentage
°C:	Degree Celsius
SAS:	Statistical Analysis System
t:	Ton
Temp:	Temperature
TPS:	True Potato Seed

DECLARATION

I, Charles BUCAGU, hereby declare that this dissertation for the degree M Sc (Agric.): Agronomy at the University of Pretoria is my own work and has never been submitted by myself at any other University. The research work reported is the result of my own investigation, except where acknowledged.



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ABSTRACT

The effects of seed size on sprout growth of potato seed pieces were investigated in controlled temperature conditions. The objectives were to quantify the effects of seed size, plant growth regulators (gibberellin and paclobutrasol) and calcium availability on sprout length, sprout and root dry mass, as well as the fraction of seed reserves utilised for sprout development. The interactive effect between seed size and temperature was also investigated.

Sprout length did not differ among seed pieces ranging from 1 to 8g in mass. For larger seed pieces sprout length, dry mass and dry mass per unit length, and the root dry mass increased with increasing seed size, reaching a maximum at a seed mass of 24g after 40 days. The smaller the initial seed piece the larger was the fraction of assimilates utilised for sprout growth.

Sprout length and dry mass as well as assimilate mobilisation for growth, were promoted by dipping seed pieces in gibberellin solutions, with the greatest effect obtained with 30 ppm GA, the highest concentration used in the experiment. Paclobutrasol showed inhibiting effects on all growth parameters except for sprout thickness. The root dry mass did not differ among treatments.

Sprout growth was enhanced by calcium availability, with the greatest effect obtained with exogenous supply of calcium at 10 mmol/l as CaSO_4 . A similar effect was noticed when complete nutrient solution was supplied. CaCO_3 was less effective than CaSO_4 . Calcium availability to sprout tips affected sprout growth only when the roots were deprived of nutrients.

Sprout length of the two seed pieces (3 and 5g in mass) was similarly affected by temperature over time, with the sprouts at the low temperature initially shorter, but reaching the same lengths as those at the high temperature after 30 and 40 days. Sprout dry mass and thickness of 3 and 5g seed pieces were similarly affected by temperature, with higher dry mass and greater thickening at 16°C than at 26°C. Exposure to low temperature (16°C) was associated with the transfer of a large fraction of seed reserves to the sprouts.

Keywords: seed piece, sprout growth, sprout length, sprout dry mass, sprout dry mass per unit length, root dry mass, gibberellin, paclobutrasol, calcium, temperature.

CHAPTER 1

GENERAL INTRODUCTION

Potato (*Solanum tuberosum* L.) belongs to the family *Solanaceae*, which also includes other commercial crops like tomato, pepper, eggplant and tobacco (Mc Collum, 1980). The potato is one of the most important crops worldwide and ranks fourth after rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.) and corn (*Zea mays* L.) in production (Taylor, 1988). According to recent figures released by CIP, developing countries annually produce 105.3 million tons of potatoes on 7.4 million hectares, compared to 189.8 million tons produced by industrialised countries on 10.9 million hectares (CIP, www.cipotato.org, 2002). Figure 1.1 illustrates the gradual declining trend in the production in developed countries and a substantial increase of production in developing countries since 1960. There is a growing interest in potato cultivation in developing countries where it has often provided famine relief during periods of crop failure. Over 98% of potatoes grown in developing countries are consumed domestically, rather than exported (Horton and Anderson, 1992).

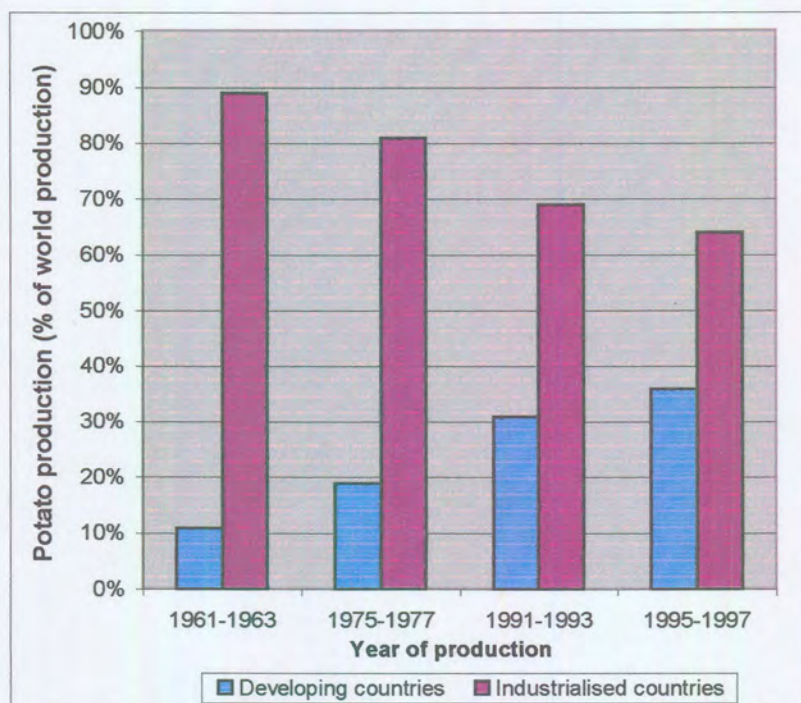


Figure 1.1 Global potato production 1961-1997

Source: FAO Database, www.fao.org, 2002

Despite the large global production, potato production costs remain higher than those of other staple foods due to expensive inputs (McCollum, 1980; Van der Zaag and Horton, 1983). In most developing countries imported certified potato seed is the most expensive input in potato production. Lack of adequate infrastructure and appropriate techniques often make local production of healthy seed impossible. The cost of imported seed constitutes a constraint to increasing potato production in developing countries. As an alternative to imported seed, farmers often retain small tubers from their own potato crops to be planted the next season. Unfortunately, this seed is of poor quality and prone to virus and micoplasm infection leading to rapid degeneration which negatively impacts on potato productivity (Goosen, 2001).

The acquisition of healthy seed is a prerequisite for any programme aimed at improving potato productivity. In developing countries appropriate strategies should be developed to efficiently utilise this expensive input. To optimise seed utilisation, it is necessary to establish the amount of mother tuber reserves required to produce a vigorous sprout. Such information is essential to develop strategies and practices to increase yield per unit of plant material.

From a practical point of view the success of such an approach requires a thorough understanding of the factors affecting sprout growth. Sprout growth between planting and emergence solely relies on seed reserves. The amount of available assimilates on one hand, and the levels of specific growth substances on the other hand, play critical roles in sprout growth. The external nutrient environment of the sprout after planting is a topic that has received little attention so far. The sprouting potato seed is constantly subjected to the effects of the ambient temperature that influences its physiological and biochemical reactions, which impact on sprout development.

It is evident that sprouting and subsequent stem development are determined by both physiological and environmental factors. A better understanding of these factors will enable growers to minimise seed costs without jeopardising yield and income. An approach that has received surprisingly little attention in research on sprout growth is to study sprout growth on

tuber seed pieces containing a single bud and producing a single stem. This approach eliminates all the complications of apical dominance, of differences in physiological age, of differences in the development of sprouts, and of competition between sprouts on the same tuber. In addition it allows easy manipulation of the seed piece for experimental purposes.

This investigation focuses on that specific phase of sprout growth after planting and before emergence, which has surprisingly received little research attention. Four different topics were covered. The effects of seed size, plant growth regulators, calcium application and the interaction between temperature and seed size on sprout growth after planting were investigated. In all the experiments the plants were kept in dark rooms, often for extended periods of up to 40 days in order to determine sprout growth characteristics. Apart from sprout length and mass, sprout thickness and root growth were determined.

The objectives of this study were to investigate factors affecting sprout growth after planting on single stem seed pieces by quantifying:

- (1) the effect of seed size on sprout length, dry mass of sprouts and roots, and utilisation of tuber reserves,
- (2) the effect of gibberellin and paclobutrasol on sprout length and dry mass of sprouts and roots,
- (3) the effect of calcium availability on sprout length and dry mass of sprouts and roots, and
- (4) the interactive effect between temperature and seed size on sprout growth.

CHAPTER 2

REVIEW OF LITERATURE

Although aspects in connection with sprout growth have been extensively reviewed in the past 40 years (Ivins and Milthorpe, 1963; Harris, 1992), there is still a lack of consensus on the definition of what is “a sprout”. Bates (1935) defined a sprout as “an eye showing viable sprouts”. Moorby (1967) called “sprout” the lateral shoots present on the tuber at planting. Wurr (1975) described a sprout as “a bud development on the seed tuber, which had leaf initials present and at least partly open”. This author ascribed the term “sproutlet” to a bud developing either directly from seed tuber or developing as a sprout branch. Although Wurr’s definition seems to give a more complete description of a sprout, a sprout taken in Wurr’s conception would lead to confusing interpretation of experimental observations on individual sprouts, especially when proliferation of lateral branching occurs on developing sprouts.

Sprout growth and development has mostly been documented during storage (Wurr and Allen, 1976; Wurr, 1979; McGee, Jarvis and Duncan, 1986; Hay and Hampson, 1991). Inversely the development of sprouts after planting seems to have received little research attention. This review focuses on developmental aspects of sprouts after planting and before emergence.

2.1 Sprout development after planting

Sprouts start developing after a dormant period during which the tubers do not sprout even when placed under conditions optimal for sprouting, then enters a phase of active sprout growth, environmental conditions permitting. In the last phase, sprout growth slows down with initiation of daughter tubers. Although the most part of sprouting process occurs in storage, there is usually a portion of sprout growth occurring in the field after planting (Toosey, 1964).

Growth of the sprout after planting is influenced by the degree of development the sprout has attained at planting time (Toosey, 1964). Morris (1967a) suggested that only sprouts that were actively growing at planting develop into stems and that sprout length can be used to identify the sprouts that can potentially develop into stems. Acceleration of sprout development shortens the interval between planting and emergence and allows quick emergence and plant establishment (Burghausen, 1962; Martin and Leonard, 1967).

Prior to planting seed pieces are often suberised so as to promote development of cork tissue on the cut surface (Martin and Leonard, 1967). The time of cutting is critical for the vigour of subsequent plants. If seed potatoes are sprouting at the time of cutting, apical pieces will produce a larger crop than do those from basal pieces. Inversely if the tubers are cut before sprouting has begun, no difference in the vigour of plants from apical and basal pieces is observed (Thompson and Kelly, 1959).

Root development depends on moisture conditions. Dry conditions restrict root growth while the root formation is favored in moist soils (Martin and Leonard, 1967). Root development is affected by a large number of factors. It is inhibited by gibberellic acid (Hartmans and Van Es, 1979), reduced by paclobutrasol (Wample and Culve, 1983; Wang, Byun and Steffens, 1985), reduced in low calcium soils (Marschner and Richter, 1974; Jones and Lunt, 1967) and depressed by high temperatures (Prange, McRae, Midmore and Deng, 1990).

2.2 Sprout growth measurements

During the process of sprout growth, both longitudinal and radial growths take place. Sprout growth can be assessed through parameters related to both types of growth. In some instances sprout dry mass is also determined to estimate sprout growth. Sprout elongation is measured by recording the length the sprout has attained at a specific time. Sprout length has been measured in different ways. Wurr and Allen (1976) determined total sprout length per tuber. Goodwin, Brown, Lennard and Milthorpe (1969) measured the length of the longest sprout and Shotton (1964) determined the mean apical sprout length. Measurement of sprout

length using the mean apical length can be complicated by difficulties in distinguishing between the apical bud and the axillary buds surrounding it (Wurr, 1978).

Radial growth is estimated by determining sprout thickness (sprout dry mass per unit length). Sprout thickness has rarely been accurately measured. It is often estimated by visual means or by using a rating system (Burton, 1966; Khedler and Ewing, 1985). However, the ratio sprout dry mass: sprout length was determined in some studies. Wurr (1978) found that the weight: length ratio for the sprouts was not a function of variety. Sadler (1961) found that the weight: length ratio of sprouts decreased with increase in size of sprouts but was independent of tuber size and temperature. Sprout diameter was reduced by application of gibberellic acid (Hartmans and Van Es, 1979) and temperature regime (Sadler, 1961, Burton, 1966).

In a number of studies, sprout dry mass was used to quantify sprout growth (Morris, 1967b; Hammes and Beyers, 1990; Engels, Bedewy and Sattermacher, 1993; Lommen, 1995). Although sprout dry mass is suggested as an accurate estimate of sprout development it presents a disadvantage in that its measurement involves destructive procedures (Wurr, 1978).

2.3 Mobilisation of seed reserves for growth

(a) Carbohydrates

Sprouts rely on carbohydrates stored in the seed tuber for their growth. The mother tuber reserves are made of 70 to 80% of polymeric carbohydrates (mostly starch) which are utilised by the sprout for its structural and functional requirements (Davies, 1990). The dependence of sprouts on mother tuber reserves can last long after emergence (Headford, 1962; Milthorpe, 1963).

Starch constitutes a source of carbon and energy for the developing sprouts. It is hydrolysed into sugars, which translocate to emerging sprouts in the form of sucrose and are partly hydrolysed into glucose and fructose (Burton, Van Es and Hartmans, 1992). Bailey, Philips

and Pitts (1978) showed an association between growth of sprouts and increase in reducing sugars in tubers. The mechanism of starch degradation in sprouting tubers was described by analogy to the hormonal mechanism involved in germination of cereal grains (Van Overbeek, 1968; Bailey *et al.*, 1978). Hormone involvement in the process of starch degradation was demonstrated by an increase in endogenous gibberellins during sprouting (Rappaport and Smith, 1962). Davies and Viola (1988) discovered that gibberellins do not operate by stimulating the development of starch mobilising enzymes but stimulate sprout growth by enhancing sink strength of sprouts for tuber assimilates. It was, therefore suggested that the pattern of starch mobilisation be studied in terms of sink-source relationship between sprouts and tubers (Coleman, 1987).

(b) Minerals

Along with other substances, minerals are translocated from tubers to sprouts. Nitrogen was cited as a tuber component which can limit sprout growth (Headford, 1961). In addition sprouts and root growth were reduced by omission of Ca, N, P and K from the growing medium (Davies and Ross, 1985). Of the effects of these mineral elements, that of calcium has been extensively documented. In potato calcium is actively translocated to sprouts, with the process being particularly rapid at 25°C (Dyson and Digby, 1975a). Calcium from the cortex and water-soluble calcium forms are preferably utilised during sprout growth but the quantity of calcium reaching the growing point is small (Davies and Millard, 1985). Although it is accepted that calcium requirements of the sprout can be met through root uptake for field-grown tubers, it is argued that most of the calcium is absorbed through the tuber surface, directly from the soil solution (Krauss and Marschner, 1971).

2.4 Factors affecting sprout growth in potato

2.4.1 Seed size

Potato is propagated using whole tubers or seed pieces. The factors determining whether seed tubers should be cut into several pieces or planted whole include the cost of labor for

cutting, the cost of seed and the relative efficiency of cut and whole seed. The comparative cost of seed and labor shows that it is more profitable to cut seed than to plant it whole (Wolfe and Kipps, 1959). Stem number is accepted as the most accurate unit of plant population (Bleasdale, 1965; Hammes, 1985). Sprout growth can be negatively affected by shortage of seed reserves in small seed pieces (Burton, 1966). It has been shown that shortage of seed assimilates slows down sprout growth, resulting in insufficient sprouting at planting and delay in the emergence (Emilsson, 1949; Ittersum and Struik, 1992).

Seed size affects all aspects of sprout growth. Sprout length and dry weight are altered by changes in seed size. Hammes and Beyers (1990) found little difference in sprout length of small seed pieces (1.5 to 10 g in mass) grown in the dark, but the sprout dry mass was correlated with the seed mass. Lommen (1995) observed that sprouts from heavier minitubers attained the first 6 mm length sooner than sprouts from smaller tubers. In single-sprout tubers planted in pots, early haulm growth increased with increasing tuber mass and emergence was delayed in plants from tubers less than 5 g in mass (Wiersema and Cabello, 1986).

Sprout morphology is also affected by seed size. More axillary branching occurs on plants developing from small seedlings (Moorby and Milthorpe, 1975; Engels *et al.*, 1993). This behaviour is described as a compensatory mechanism of small seed pieces for the limited number of sprouts (Allen, O'Brien and Firman, 1992 a). Sprouts from large seed pieces tend to be thicker than sprouts from small seed pieces (Hammes and Beyers, 1990; Lommen, 1995).

2.4.2 Plant growth regulators

The sprouting process is related to the levels of hormonal substances in tubers. Growth or lack of growth is described as visible expressions of hormonal activity, the most involved being gibberellins (promoters) and abscissic acid (inhibitor) (Rappaport and Wolf, 1969). Gibberellins increase sprout length due to stimulation of cell elongation (Brian, Hemming and Radley, 1955). When dipped into 1 ppm of gibberellin potatoes showed vigorous sprouts (Smith, 1977) with strong elongation of internodes (Lovell and Booth, 1967). The response

to gibberellin decreases with time, the effect being more pronounced at high temperature (25°C) (Timm, Rappaport, Primer and Smith, 1960; Smith, 1977). The rate of sprout elongation increases with increasing levels of GA up to 10^{-5} M (Hartmans and Van Es, 1979). In seed pieces, endogenous gibberellins increase in injured tissue. The effect is greater as seed size is reduced (Rappaport and Sachs, 1967).

Growth inhibiting substances play an opposite role to that of growth promoters. Abscissic acid (ABA) inhibits bud growth of potatoes (El-Antably, Wareing, and Hillman, 1967). The inhibition of nucleic acid synthesis by ABA constitutes probably its major contribution in preventing sprouting (Shik and Rappaport, 1970). Apart from ABA, other anti-gibberellins like paclobutrasol have been tested in a number of species. Paclobutrasol retarded growth in apples (Wang, Byun and Steffens, 1985), sunflowers (Wample and Culver, 1983) and citrus (Bauscher and Yelenosky, 1987; Hadlow and Allan, 1989). Paclobutrasol application resulted in internode shortening in strawberry (Stang and Weis, 1984) and reduced shoot and root weights of sunflowers and soybeans (Wample and Curve, 1983; Sankla, Davis, Jolley and Upadhyaya, 1986). Little has been published on potato response to paclobutrasol. However, Balamani and Poovaiah (1985) reported on the role of paclobutrasol on retardation of shoot growth and promotion of tuber growth of potato.

2.4.3 Calcium

(a) Calcium deficiency

Growth of plants can be restricted by a decrease in availability of calcium (Marschner, 1974). The problem resulting from calcium deficiency is mostly related to poor distribution of calcium within the plant (Loneragan and Snowball, 1969; Bangerth, 1979). In potato, the elongating regions of sprouts just below the apex can be deprived of calcium due to insolubility of calcium and low transport of calcium in the phloem (Tibbits and Palzkill, 1979). This condition leads to apical necrosis (Dyson and Digby, 1975a, 1975b) and a proliferation of branch sprouts (Davies and Ross, 1985).

Apart from its effect on sprout growth calcium availability can also influence root growth. Root development is restricted by low calcium levels (Marschner and Richter, 1974). Low levels of calcium in roots is partly due to the fact that calcium moves only in an upwards direction with the transpiration stream and not to the root tips (Bangerth, 1979).

(b) Interaction between calcium and other ions

The demand for calcium in plant systems depends on the level of other mineral elements. Generally, the need for calcium is decreased if heavy metal micronutrients are not in high supply (Wallace and Frolich, 1966). The reason for this phenomenon lies in an antagonism between calcium and some other elements. One of the functions of calcium in plant nutrition is to ameliorate toxic levels of some metals (Jones and Lunt, 1967). The cellular membrane is the site of interactive effects between calcium and other ions, but little is known about the biochemical reactions regulating Ca-membrane interactions (Bangerth, 1979).

2.4.4 Temperature

A number of environmental factors, namely temperature, moisture, soil fertility, light intensity and carbon dioxide concentration affect growth of potato plants, the most important among them is certainly temperature (Burton, 1966; Smith, 1977). In some areas such as tropical highlands, temperature is the single most important cause of reduction in the crop productivity (International Potato Center, 1985). In general sprout growth increases linearly with temperature over the range 4-20°C (Allen, O'Brien and Firman, 1992a). Exposure to high temperature results in loss of dry matter because of a high rate of respiration (Smith, 1977). Seed reserves are more rapidly exhausted in sprouting tubers exposed to high temperatures. For instance shortage of calcium has been detected in tubers grown at 25°C, this was associated with active growth under high temperature (Dyson and Digby, 1975a, 1975b). Temperature can affect biochemical reactions in sprouting buds. Menzel (1983) reported that high temperatures promoted synthesis of gibberellin in buds. Thomas and Wurr (1976) detected a high gibberellin activity in eye plugs treated initially with low temperature before being transferred to the sprouting temperature.

Temperature affects root growth. At low temperature, assimilates are diverted to roots, resulting in low shoot to root ratio (Smith, 1977). High temperatures detrimentally affect root growth. At high temperature root dry weight and length are considerably reduced and lateral roots are the most affected (Sattelmacher; Marscher and Kuhne, 1990). Inversely, high temperatures promote growth of the aerial part of plants (Khedler and Ewing, 1985; Bensalim, Nowak and Asiedu, 1998).

CHAPTER 3

EFFECT OF SEED SIZE ON SPROUT GROWTH OF SINGLE STEM SEED PIECES

3.1. ABSTRACT

Certified seed is the most expensive input in tropical potato production. The successful introduction of potatoes in farming systems requires better seed utilisation, which can be achieved by optimising stem production per unit seed mass. This suggests that a large fraction of seed reserves should be mobilised to sustain a greater number of vigorous sprouts. This aspect was investigated in two experiments using single “eye” seed pieces ranging from 1 to 32 g in mass. Seed pieces weighing 1 to 8g were used in the first experiment while seed pieces with mass of 8g or more were used in the second experiment.

Sprout length did not differ significantly among small seed pieces (Experiment 1) but positively correlated with seed mass in large seed pieces (Experiment 2). The longest sprouts were obtained with 24 g seed pieces 40 DAP. Sprout dry mass and sprout dry mass per unit length attained the maximum values with 24 g seed pieces. The limiting effect of seed mass at early stages of sprout development was evidenced by the thinner bottom segments of sprouts from small seed pieces compared to those from large seed pieces. Small seed pieces utilised a larger fraction of seed reserves for sprout growth compared to larger seed pieces.

3.2 INTRODUCTION

The high cost of healthy seed is one of the major limiting factors in potato production in developing countries in general, and particularly in tropical areas. Most tropical countries do not produce certified seed and are forced to import seed from developed countries. Estimates for several developing countries show that potato seed is the most expensive input, accounting for 20 to 50% of total production costs (Van der Zaag and Horton, 1983). In the rare sub-tropical countries where seed multiplication programmes are locally operating, like Bangladesh (Midmore, 1992) and Rwanda (Goosen, 2001), the lack of support infrastructures such as cold storage facilities for seed and virology laboratories for post-harvest virus testing, can undermine the production of disease-free planting material.

The use of true potato seed (TPS) was suggested as a possible alternative to reduce seed costs. TPS have the merit of being of small mass and thus easy to transport and store, and most plant viruses are not transmitted via true seeds. However their value is limited by non-uniformity in crop growth, higher susceptibility of seedlings to environmental stresses and low yield (Howard, 1983; Taylor, 1988).

The use of small seed pieces rather than whole seed is another possibility. Given the fact that the number of mainstems is closely correlated with tuber yield irrespective of seed size (Bleasdale, 1965), the most economical approach may be to increase the number of developing stems per unit seed mass by using the minimum seed size. This approach has surprisingly received little research interest so far (Allen and Wurr, 1992).

Investigations on the effect of seed tuber mass on potato growth have extensively focused on aspects associated with the number of stems and tuber yield (Allen, 1979; Allen and O'Brien, 1987; Wiersema, 1989; Wurr, Fellows, Sutherland and Allen, 1990; Allen, O'Brien and Firman, 1992 b; O'Brien and Allen, 1992a, b). Little attention has been given to the study of individual sprout growth as a function of seed mass. Although Hammes and Beyers (1990), using single stem seed pieces, demonstrated that sprout elongation was not affected by seed size while sprout dry mass was proportional to the seed size both in conventional seed tubers and minitubers, no quantified data were provided to substantiate the above conclusions. Only seed pieces weighing up to 10 g of mass were used in their study.

The objectives of this experiment were:

1. to determine the effect of seed size on sprout length and thickness,
2. to determine the effect of seed size on dry mass of sprouts and roots and
3. to establish the fraction of seed tuber dry mass that is effectively utilised for sprout development.

3.3. MATERIALS AND METHODS

Two experiments were carried out at the Experimental Farm of the University of Pretoria. Single “eye” seed pieces on cylindrical cores of uniform size and shape were cut from certified seed tubers of the cultivar BP1. The cut surfaces were sterilised for 10 minutes in 3% (v/v) calcium hypochlorite and rinsed with distilled water. Before planting, the seed pieces were kept under cool conditions to allow formation of the cork layer, and 10 seed pieces were retained to determine the initial seed dry mass. To obtain seed pieces with comparable sprout lengths at planting, 100 seed pieces from each size category were planted in vermiculite. One week after planting those with uniform 2 cm long sprouts were selected and used for the experiments.

Seed pieces were planted in plastic crates in a dark room at 22°C/14°C and 80% relative humidity and grown for 40 days. Plants were watered as needed and no fertiliser was applied. Dry mass was determined after oven drying at 65°C for 24 hours. Data collected included sprout length, dry mass of the sprouts, roots and seed pieces, and partitioning of dry mass between plant components.

The results were subjected to analysis of variance using the General Linear Model (GLM) procedure of the Statistical Analysis system (SAS)¹ and Tukey’s t-test was used to test for differences between treatment means.

Experiment I

In the first experiment seed pieces of 10, 15 and 20 mm diameter, representing 1, 3 and 8 g of fresh mass respectively, were used. The experiment comprised two harvest procedures. Firstly sequential harvests with four replications (one plant per replicate) were carried out 10, 20, 30 and 40 days after planting (DAP). At each harvest roots were washed to remove the vermiculite. Sprout length and the dry mass of sprouts, roots and remaining seed pieces were

¹ SAS System is an integrated system of software providing control over data management analysis and presentation, and may be obtained from SAS Institute South Africa (Pty) Ltd, 93 Central Street, Houghton, P.O.Box 2837, Houghton, 2041, Republic of South Africa.

determined. The treatments comprised all combinations of three seed sizes and four harvest dates arranged as a randomised complete block design with four replicates per treatment.

Secondly four plants per treatment were sampled as soon as the sprouts had reached 10, 20, 30 and 40 cm respectively. Sprouts were dried and dry mass determined. The treatments consisted of all combinations of three seed sizes and four sprout lengths replicated four times. Data collected were subjected to analysis of variance to determine the effect of main factors and the interaction between them.

Experiment 2

In the second experiment seed pieces of 8, 16, 24 and 32 g fresh mass were obtained by cutting tubers longitudinally into halves. To obtain single “eye” seed pieces all but one eye were removed. Seed pieces were planted with twelve plants in the row. At 20 and 40 days after planting 20 plants were lifted from the substrate, roots washed to remove the vermiculite, and sprout length and dry mass of the plant components were determined. The experiment was arranged as a randomised complete block design with four seed sizes and two harvest dates with 20 replicates with one plant per replicate.

In a sub-experiment two separate harvests of four plants each were made in order to study the distribution of sprout dry mass per unit length within the sprout. Both harvests were made at the end of the experiment. At the first harvest segments of 5cm were cut from the top and bottom parts of the sprout. They were dried, weighed and the sprout dry mass per unit length recorded. At the second harvest sprouts were cut in a series of 10 cm segments, namely 0-10, 30-40, 50-60, 70-80 and 80-90 cm from the seed, respectively. Sprout dry mass per unit length was determined using the same procedure as in the first harvest. The data from these additional harvests were analysed separately. The effects of main factors as well as their interactions were determined using the GLM statistical procedure as described earlier.

3.4. RESULTS

EXPERIMENT 1

Sprout length

The results of sprout length over the growing period are presented in Figure 3.1. When harvested 10, 20 and 30 days after planting the sprouts from the large seed pieces (8g) tended to be shorter than those from the medium seed pieces (3g) which in turn were slightly shorter than sprouts from the small seed pieces. Though the differences were not significant (ANOVA Table A 3.1 of the Appendix) sprout elongation tended to be faster for small than for large seed pieces during the first 30 days. However, after 40 days sprouts from 8g seed pieces were the longest. The interaction between seed size and harvest date was significant. This apparently is due to a sharp increase in sprout length of the large seed pieces at the last harvest, and may be an indication that carbohydrate reserves in the small seed pieces were depleted at that stage.

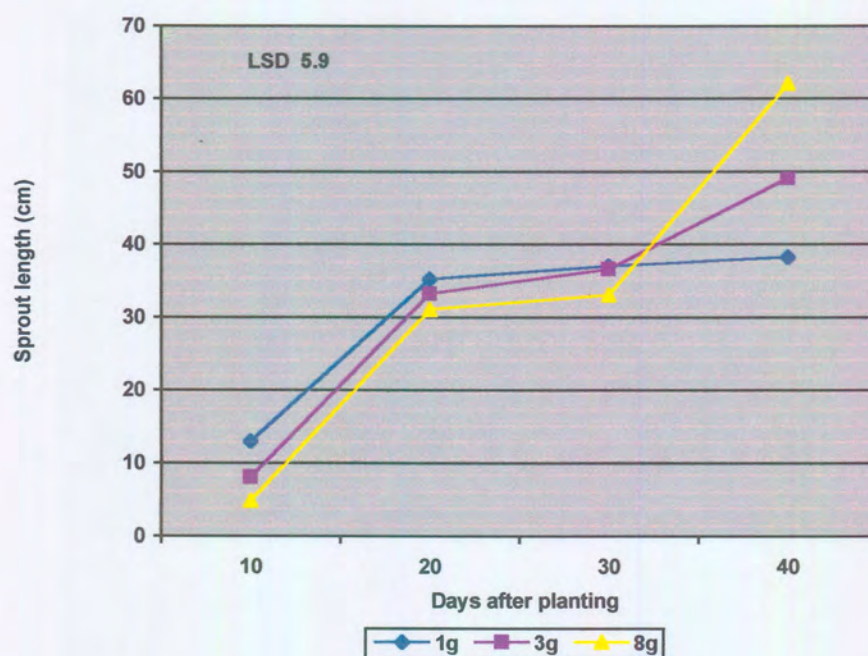


Figure 3.1 Effect of seed size and harvest date on sprout length (Appendix Table A 3.1)

Sprout dry mass

Data collected over the growing period show that the sprout dry mass increased sharply between 10 to 20 days after planting, then slowed down for the rest of the growing period in the case of the 1g and 3g seed sizes. For the 8g seed pieces the sprout dry mass increased almost linearly till the experiment was terminated after 40 days (Figure 3.2). The main effects of seed size and harvest date as well as their interaction were significant (ANOVA Table A 3.2 of the Appendix). The significant interaction between the main factors was the result of a slight increase in sprout mass of the small seed pieces compared to a large increase in sprout mass of 8g seed pieces from 10 to 40 days after planting. At 20 and 30 DAP sprout dry mass was two times greater in plants from 8g seed pieces than that of plants from 3g seed pieces which in turn was two times greater than that of plants from 1g seed pieces. After 40 days differences in sprout dry mass almost directly reflected the initial differences in the mass of the seed pieces.

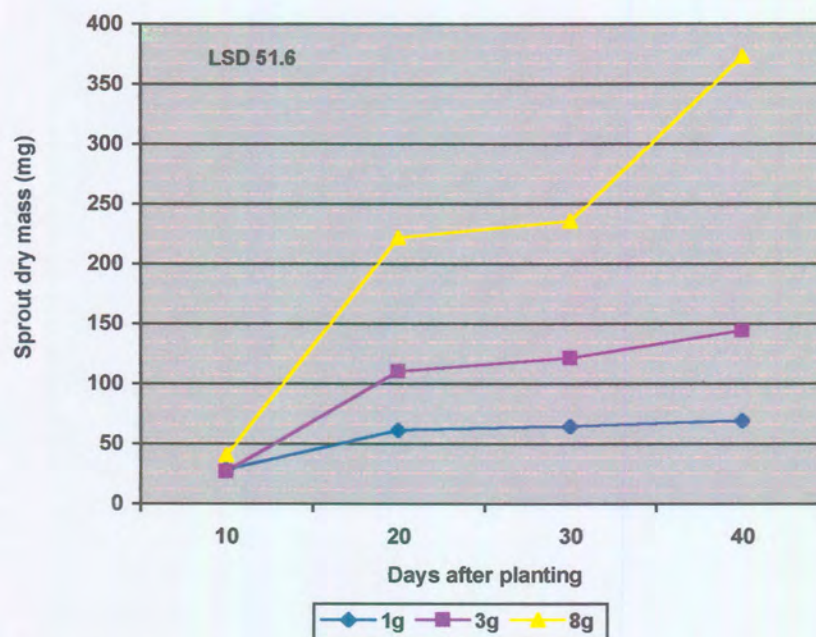


Figure 3.2 Effect of seed size and harvest date on sprout dry mass (Appendix Table A 3.2)

Sprout dry mass per unit length

The sprout dry mass per unit length was deduced from the results on total sprout dry mass and total sprout length. The effect of seed mass on the sprout dry mass per unit length is illustrated in Figure 3.3. From this figure it is evident that the sprout dry mass per unit length of 8g seed pieces was always the greatest despite a gradual decline over time. At all harvest dates the sprout dry mass per unit length of the 8 g seed pieces exceeded those of other seed pieces more than two times. The interaction between seed size and harvest date was significant as indicated in the ANOVA summary (Table 3.3 of the Appendix). This was due to a significant decline in the mass per unit length of the 8g seed pieces during the 40 days, while for the smaller seed pieces the mass per unit length, though much lower, remained fairly constant over time.

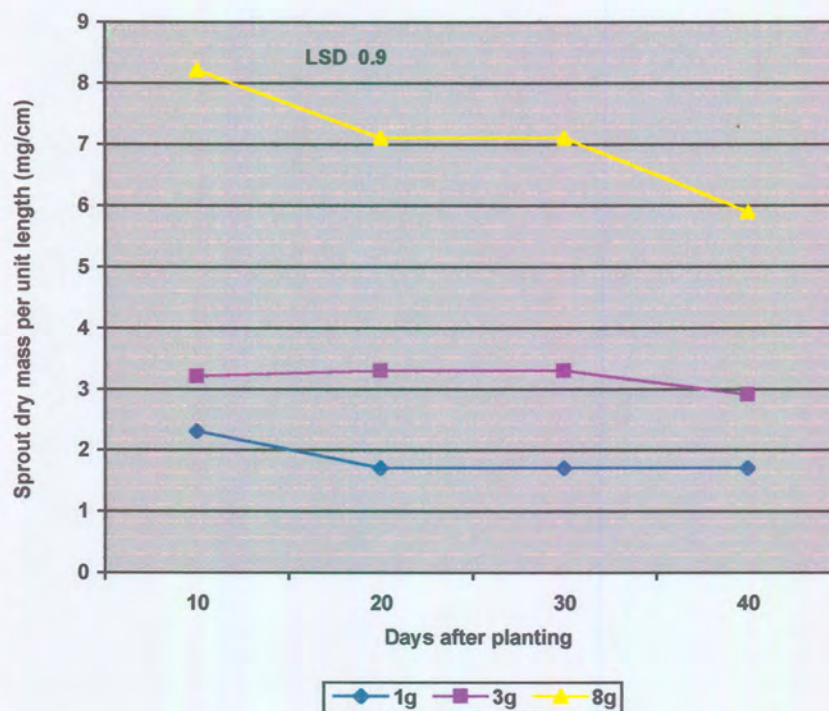


Figure 3.3 Effect of seed size and harvest date on sprout mass per unit length (Appendix Table A 3.3).

Sprout dry mass per unit length was determined regardless of sprout length. Sprout length is one of the components of sprout dry mass per unit length. Sprout dry mass per unit length

depends on the extent to which sprouts elongate. It is therefore important to establish the relationship between sprout length and sprout dry mass per unit length for different seed sizes when the sprouts are of the same length. In order to study this aspect sprouts were harvested when they had attained specific lengths (10, 20, 30 and 40 cm respectively) and the sprout dry mass per unit length was determined.

As expected the sprout dry mass per unit length significantly differed between seed sizes, differences becoming bigger as sprouts became longer (Figure 3.4). The results also indicate that the sprout dry mass per unit length decreased with increasing sprout length in the case of the 1 and 3g seed pieces. Inversely the sprout dry mass per unit length of the 8g seed pieces remained fairly constant for all sprout lengths. For example when sprouts were 20 cm long the ratio weight: length was 1.9, 4.9 and 7 mg/cm for plants from 1, 3 and 8g seed pieces respectively. When sprouts reached a length of 40 cm the sprout dry mass per unit length had decreased 47 % in 1g seed pieces, 26 % in 3g seed pieces and remained unchanged at 7mg/cm for the 8g seed pieces.

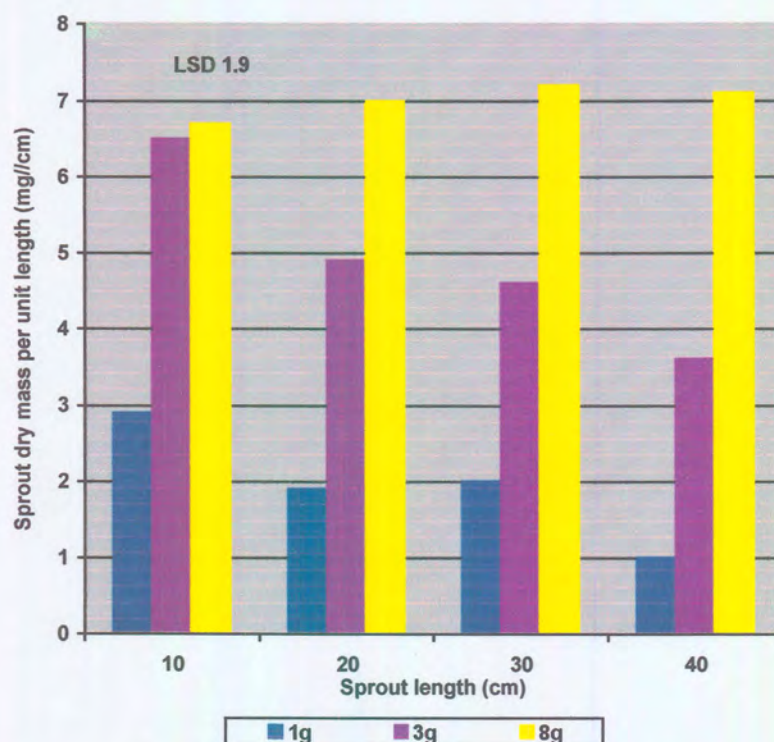


Figure 3.4 Influence of seed size on sprout dry mass per unit length at specific sprout lengths (Appendix Table A 3.4).

Root dry mass

At harvest some roots retained vermiculite, making accurate recovery of the roots impossible. The results showing the effect of seed mass on root dry mass are illustrated in Table 3.1. The root dry mass from 3 g seed pieces was almost two times higher than the root dry mass from 1g seed pieces at the first three harvests but the difference was smaller at 40 DAP. The root dry mass of plants from 8g seed pieces was 1.5 times larger than that of plants from 3g seed pieces at the first harvest. Differences became greater at the later harvests.

Table 3.1. Effect of seed size and harvest date on root dry mass

Seed size (g)	Root dry mass (mg)				
	Days after planting				
	10	20	30	40	Mean
1	19.2	22.7	18.7	24.5	21.2 c*
3	35.5	41.5	42.2	36.0	38.8 b
8	50.7	90.2	95.0	96.0	82.9 a
Means	35.1 b*	51.4 a	51.9 a	52.1 a	47.6
C.V.(%)	10.7				
LSD _{TUKEY}	4.4				
(0.05)	5.6				
	12.8				

* Values followed by the same letter in the column or row are not significantly different at P=0.05 according to Tukey (ANOVA in Appendix A 3.5)

Shoot plus root dry mass

Shoot plus root dry mass followed a similar trend to that of sprout dry mass (Figure 3.5), reflecting the fact that sprout dry mass contributed a large fraction of the total plant dry mass. Shoot plus root dry mass was significantly affected by seed mass as shown in Table A 3.6 of the Appendix. The greater shoot plus root dry mass associated with the 8g seed pieces was the result of higher sprout dry mass (Figure 3.2) and root dry mass (Table 3.2) which were

obtained from those seed pieces. Differences between seed sizes were smaller at the first harvest but increased at the later harvests.

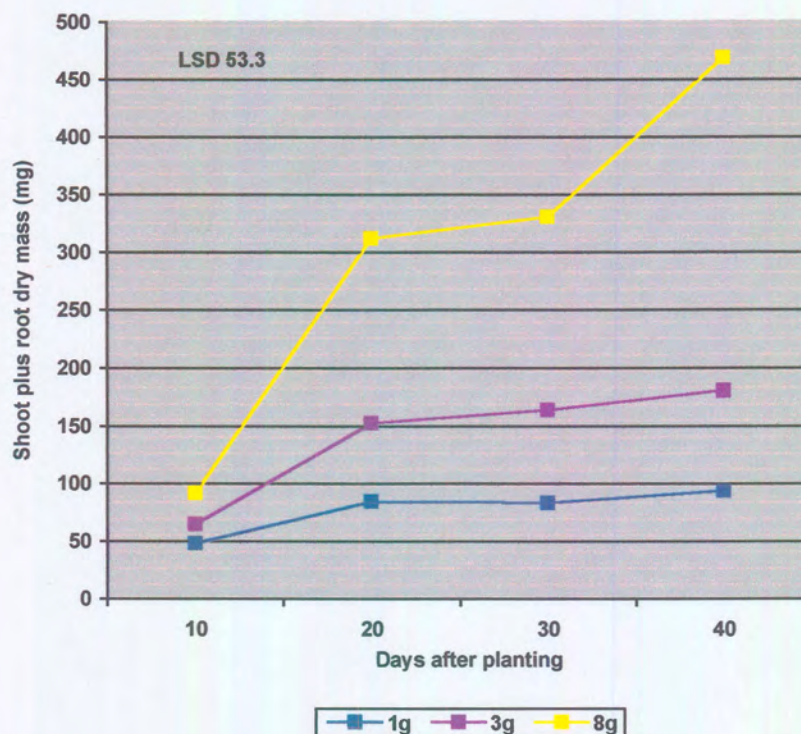


Figure 3.5 Effect of seed size and harvest date on shoot plus root dry mass (Appendix A 3.6)

Utilisation of seed reserves

During sprouting of seed pieces their reserves are mainly used for growth and respiration processes. The fractions of dry mass allocated to each process were determined and the results are presented in Figure 3.6. The percentage of seed material utilised for growth decreased with increasing seed mass. In 1g seed pieces more than 82 % of initial seed reserves were allocated to processes conducive to plant growth and only 18% remained in the seed. In contrast to this 8g seed pieces still retained 40% of their reserves at the end of the experiment.

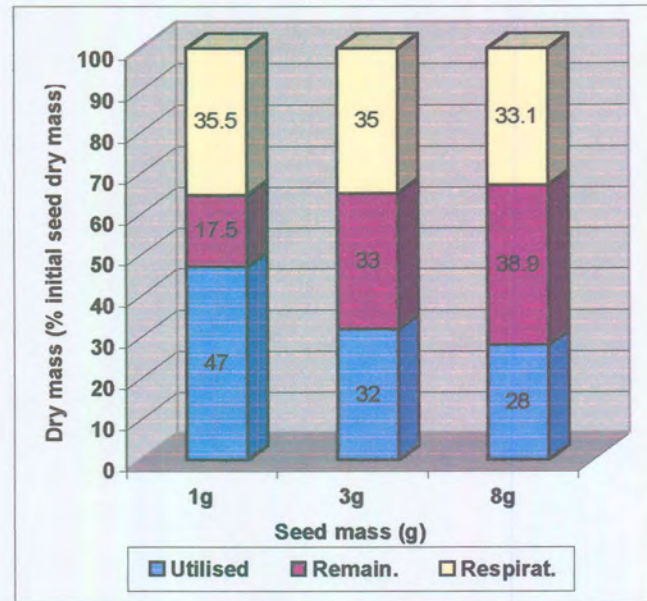


Figure 3.6 Effect of seed size on utilisation of seed reserves 40 days after planting

EXPERIMENT 2

Sprout length

Contrary to experiment 1, sprouts in this experiment showed lateral branching due probably to the effect of physiological ageing of the seed. This complicated sprout length measurements. All secondary sprouts were removed daily using a scalpel.

At 20 DAP the sprouts of 16g seed pieces were the longest at 53 cm of length, while at 40 DAP the sprouts of 24 g seed pieces were the longest at 103 cm of length (Figure 3.7). The interaction between seed size and harvest date was significant as indicated in Table A 3.7 of the Appendix. This was due to the fact that at 20 DAP sprout lengths did not differ significantly, while at 40 DAP the 8g seed pieces had shorter sprouts than those from larger seed pieces.

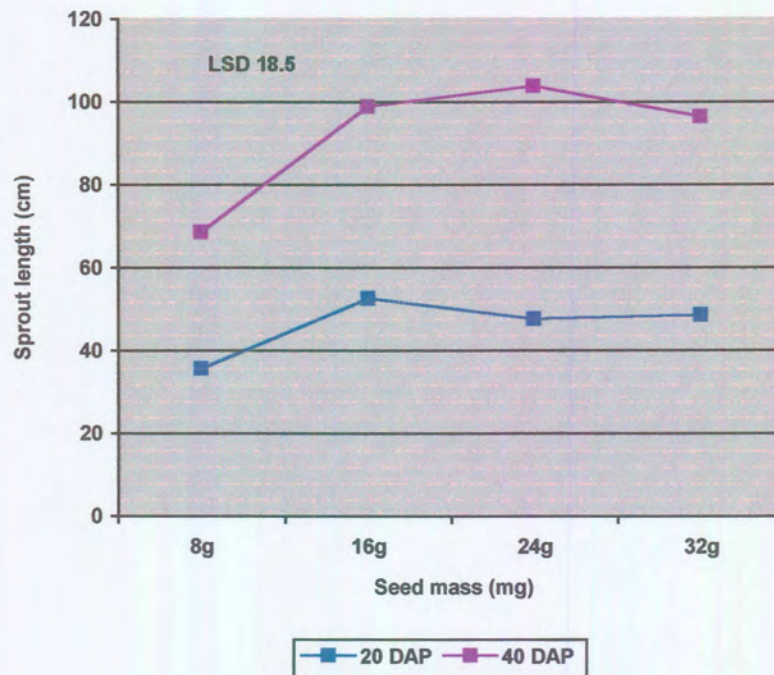


Figure 3.7 Effect of seed size and harvest date on sprout length (Appendix Table A 3. 7)

Sprout dry mass

Sprout dry mass increased with seed size and time of harvest (Figure 3.8). It can be seen that the sprout dry mass increased sharply when seed mass increased from 8 to 24g at both dates. The highest sprout dry mass was obtained with 24 g seed pieces. Increasing seed mass from 24 g to 32 g resulted in no change in the sprout dry mass. A significant interaction between seed piece size and harvest date was found as indicated in Table A 3.8 of the Appendix. This was attributed to the fact for the 8g seed pieces the sprout dry mass was similar at both 20 and 40 DAP while for the larger seed pieces the sprout dry mass at 20 DAP was significantly smaller than that at 40 DAP. The results indicate that although sprouts were almost two times longer at 40 DAP than at 20 DAP for all seed pieces (Figure 3.7) sprout dry mass increased much more between 20 and 40 DAP. The results indicate an exhaustion of seed reserves in the 8g seed pieces.

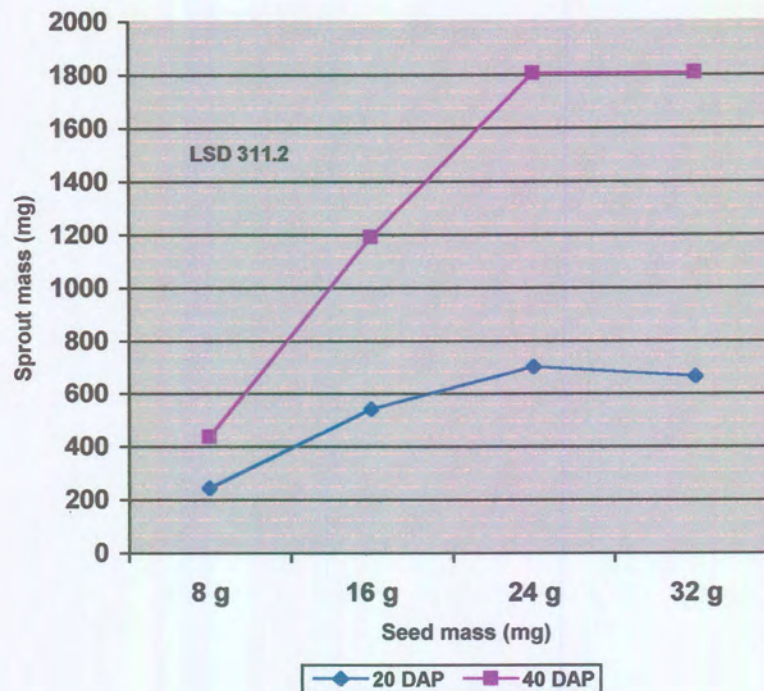


Figure 3.8 Effect of seed size and harvest date on sprout dry mass (Appendix Table A 3. 8)

Sprout dry mass per unit length

Contrary to the results of the first experiment where a decline (with 8g seed pieces) or no change (with 1 and 3g seed pieces) with time were noticed, sprout dry mass per unit length consistently increased with time for seed pieces larger than 8g. Inversely, the sprout dry mass per unit length from the 8g seed pieces remained constant over time. The sprout dry mass per unit length increased with seed size, the highest value of 17 mg/cm was obtained with the 24g seed pieces at 40 DAP. A significant interaction between seed size and harvest date was found as shown in Table A 3.9 of the Appendix. This was due to the fact that for seed pieces smaller than 24g in mass the sprout thickness was similar at both 20 and 40 DAP while for seed pieces of 24g and larger the sprout thickness was significantly larger at 40 DAP than at 20 DAP.

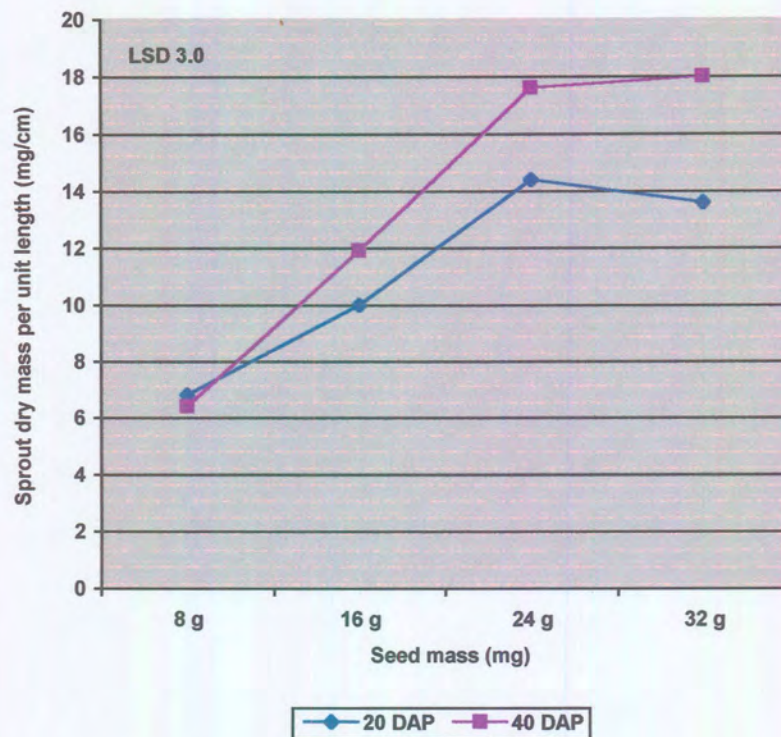


Figure 3.9 Effect of seed size and harvest date on sprout dry mass per unit length (Appendix Table A 3. 9).

In order to investigate whether sprout dry mass per unit length is uniform over the length of the sprout, sprout dry mass per unit length was examined for segments cut at different positions of the sprout. Information on the distribution of the sprout dry mass per unit length is important in that sprout vigour is associated with sprout thickness and, thus the degree to which dry mass is distributed in the sprout.

In the first part of this study the sprout dry mass per unit length of 5 cm segments cut at the top was compared to that of segments of similar length cut at the base of the sprout. The results are presented in Figure 3.10. Although the sprout dry mass per unit length increased with seed size up to 24g seed pieces both in the top and bottom segments the sprout, dry mass per unit length was always greater in the bottom segments. This indicates that assimilates were not uniformly distributed in the sprout and that the bottom segments of the sprouts accessed more assimilate. The sprout dry mass per unit length of the bottom

segments was little affected by harvest date. The maximum sprout dry mass per unit length of the top segments was obtained with 24 g seed pieces (Figure 3.10).

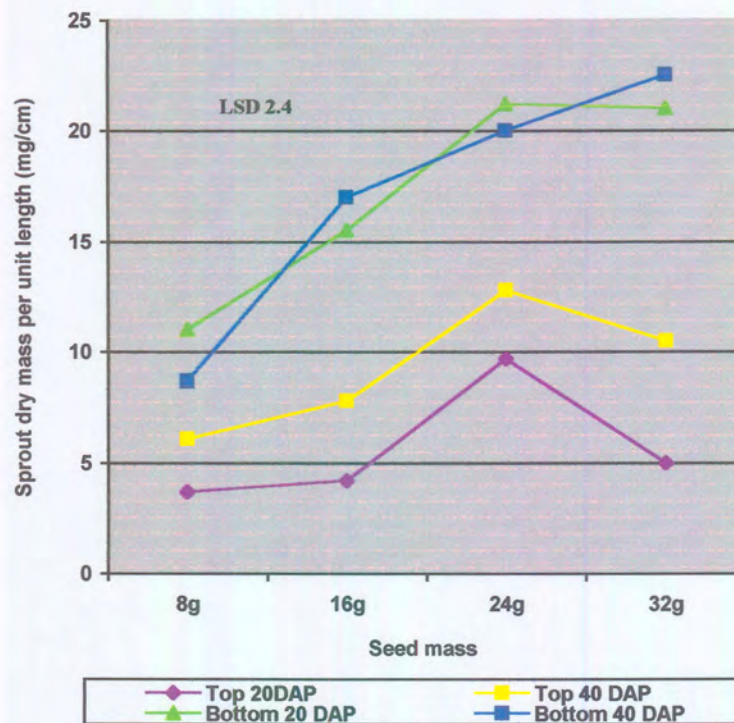


Figure 3.10 Effect of seed size and harvest date on sprout dry mass per unit length in the top and bottom segments of the sprouts (Appendix Table A 3. 10).

In addition a significant interaction between seed size and segment position was found as evidenced by the summary of the ANOVA in Table A 3.10 of the Appendix. This was due to the fact that for the bottom segments the sprout thickness increased consistently with seed mass while for the apical segments the sprout thickness increased with seed mass up to 24g before declining with seed pieces larger than 24g in mass. A significant interaction between segment position and harvest date was also found. This was ascribed to the fact that the sprout dry mass per unit length of the bottom segments was similar after 20 and 40 days while the sprout dry mass per unit length of the top segments was significantly smaller at 20 DAP than at 40 DAP. The results emphasise the fact that seed assimilates accumulate more rapidly in bottom segments of the sprouts.

In the second part of the study segments of 10 cm were cut from different positions on the sprout and the dry mass per unit length determined. The results are presented in Figure 3.11. The sprout dry mass per unit length declined sharply with increasing distance from the seed for all seed pieces. A significant interaction between seed size and segment position was noticed (Table A 3.11 of the Appendix). This was due to the fact that the sprout dry mass per unit length of the 8g seed pieces decreased less with distance from the seed than those of the larger seed pieces.

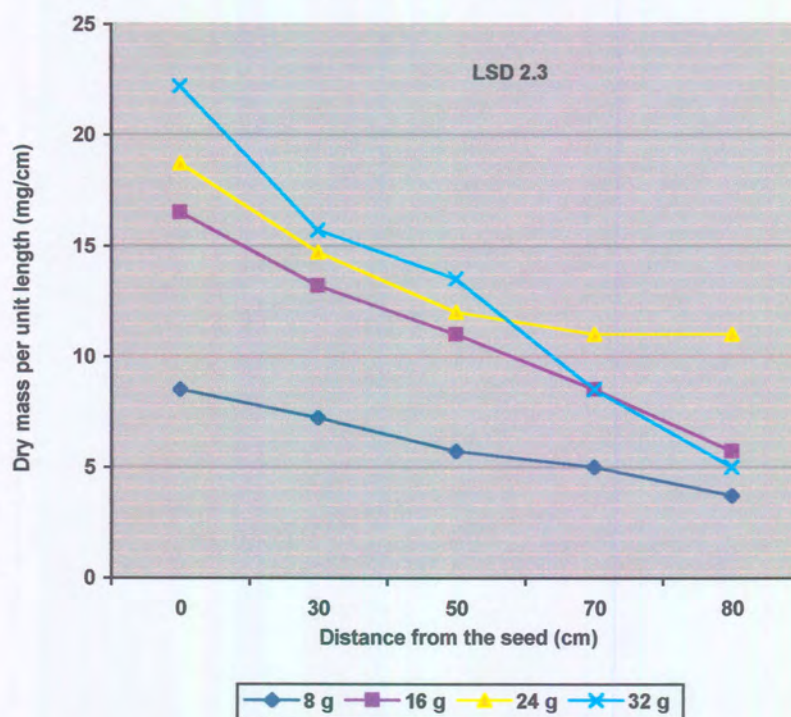


Figure 3.11 Effect of seed size on distribution of the sprout dry mass per unit length in the sprout (Appendix Table A 3.11).

Root dry mass

At harvest, it was impossible to recover all the roots due to the difficulties in separating vermiculite from certain parts of the roots. As in Experiment 1 the root dry mass increased with seed size at both harvest dates (Table 3.2). When the seed mass was doubled from 8 to 16g the root dry mass increased more than two times. But with further increase in seed mass,

the root dry mass did not increase proportionately. Significant root growth continued between 20 and 40 days after planting, especially in the case of the larger seed pieces.

Table 3.2 Effect of seed size and harvest date on root dry mass

Seed size (g)	Root dry mass (mg)		Mean
	<u>Days after planting</u>		
	20	40	
8	47.5	57.3	52.4 c*
16	103.5	123.3	113.4 b
24	159	182.6	170.8 a
32	148.9	201.3	175.1 a
Mean	114.7 b*	141.1a	127.9
C. V.(%)		34.1	
LSD _{TUKEY} Seed size		26.8	
(0.05%) Harvest date		14.5	
Seed size x Harvest date		48.5	

* Values followed by the same letter in the column or row are not significantly different at $P = 0.05$ according to Tukey (ANOVA in appendix A 3.12)

Shoot plus root dry mass

The shoot plus root dry mass followed a similar trend to that of the sprout dry mass (Figure 3.12) reflecting the importance of sprout dry mass in the total mass of the shoot plus root. It was significantly affected by seed size as shown in Table A 3.13 of the Appendix. The plants from 24g seed pieces attained a maximum of 2000 mg of total dry mass, which was three times higher than that attained by the 8g seed pieces at 40 DAP. The shoot plus root dry mass was also affected by harvest date. The value for this parameter was the greatest at 40 DAP for all seed sizes, exceeding by two times that attained at 20 DAP, except for the 8g seed pieces where differences between harvests were smaller (Figure 3.12). Similarly to sprout dry mass, the total dry mass of the shoot and roots was significantly affected by the interaction between seed size and harvest date. This was due to the fact that for the 8g seed

pieces the shoot plus root dry mass was similar at 20 and 40 DAP while for the larger seed pieces the shoot plus root dry mass was much larger at 40 DAP than at 20 DAP.

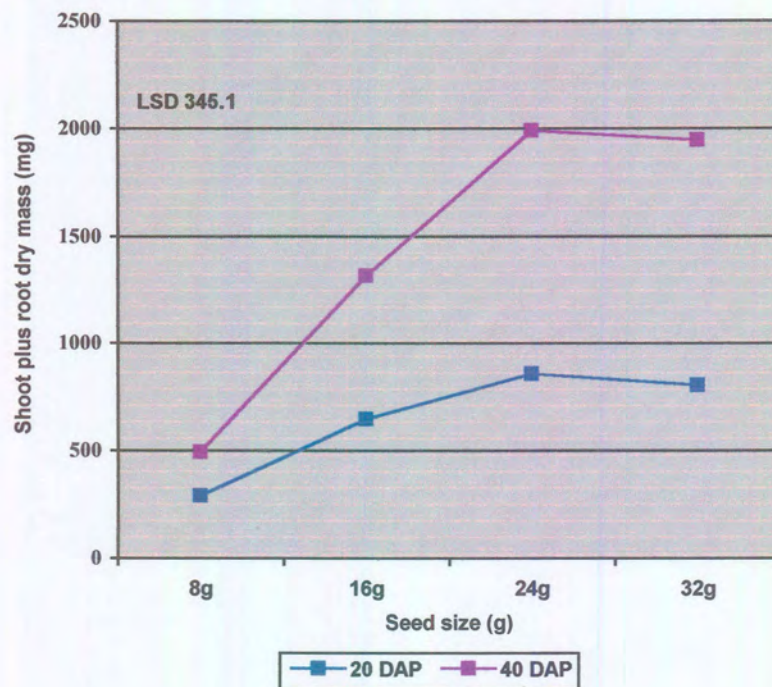


Figure 3.12 Effect of seed size and harvest date on shoot plus root dry mass (Appendix Table A 3.13).

Seed reserve utilisation

The percentage of seed reserves utilised for growth was determined on basis of shoot plus root mass expressed as percentage of the initial seed mass. Although the 8g seed pieces transferred the largest fraction of their seed reserves for plant growth, differences between the size categories were relatively small, indicating a similar trend in the utilisation of seed reserves.

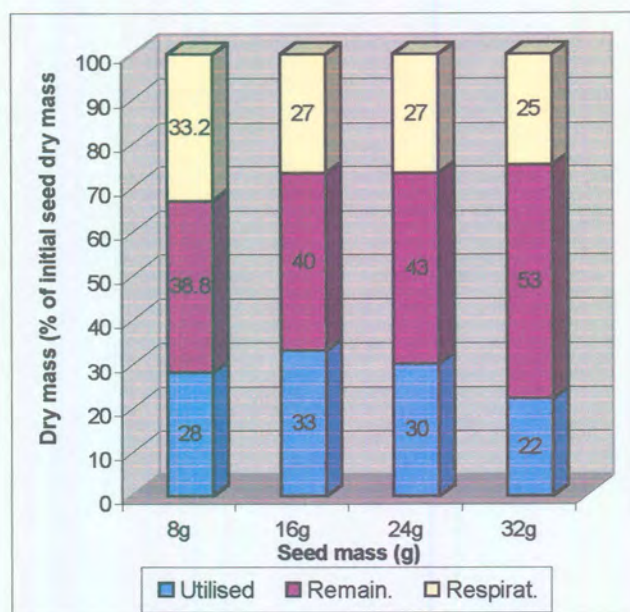


Figure 3.13 Effects of seed mass on utilisation of seed reserves 40 days after planting

3.5 DISCUSSION

Sprout length was not affected by seed size ranging from 1 to 8g in mass (Figure 3.1). These results corroborate with those of Hammes and Beyers (1990) who reported little difference in sprout lengths among seed pieces smaller than 10g. However, the results contrast with those found by other authors (Bean, 1981; Lommen, 1995) who stated that small seeds usually have shorter sprouts than large seeds. The discrepancy between their results and those of the present study may be due to the difference in the type of planting material used. They drew their conclusion from studies on whole seed tubers (minitubers in the case of Lommen, 1995) while seed pieces were used for this study. In seed pieces biochemical changes follow seed wounding. It is reported that endogenous gibberellins (GA) increase in response to seed cutting, and that the effect is greater in small than in large seed pieces (Rappaport and Sachs, 1967). Gibberellin is known to be active in inducing cell enlargement resulting in the predominance of longitudinal over radial growth for the tissue (Brian, Hemming and Radley, 1955; Jones, 1982). Longer sprouts on small seed pieces may be an indication of the effect of GA-like substances.

With larger seed pieces sprout length increased as seed mass increased from 8 to 16g, but seed pieces larger than 24g did not result in longer sprouts (Figure 3.7) despite a larger supply of seed reserves. These results support the argument that as long as appreciable material for growth is present in the tuber, no effect of seed size is likely to be encountered, but with a decreasing seed size, a point is reached where shortage of seed reserves restricts sprout growth (Burton, 1966).

Sprout dry mass increased in proportion to the initial seed mass for both experiments, this in agreement with the results from other studies (Wurr, 1978; Hammes and Beyers, 1990; Engels, Bedewy and Sattelmacher, 1993). Similarly the sprout dry mass per unit length correlated positively with seed mass in both experiments, confirming the results of Hammes and Beyers (1990) and Lommen (1995) who found that bigger seed pieces produced higher sprout dry mass per unit length. The low sprout mass and sprout dry mass per unit length associated with small seed pieces is clear evidence that the amount of available seed reserves is a factor limiting growth in the sprouts.

It is important to notice that small seed pieces compensated for this by producing thinner sprouts of almost the same length as those produced from large seed pieces. The exact scientific explanation for this phenomenon remains unclear, but the action of wound-induced gibberellins can be tentatively proposed as an explanation to this. An advantage of this reaction is that sprouts from small seed pieces grow at the same rate as those from large seed pieces, allowing early emergence and plant establishment despite limited reserves. A possible role of gibberellin in sprout growth of seed pieces will be part of the investigation in the following chapter.

The 24 g seed pieces produced the highest sprout dry mass. This means that under the experimental conditions, 24g of seed mass were adequate for the development of a vigorous sprout. This observation has practical significance. In Rwanda for instance potato stem density is generally estimated at 44.400 stems per hectare (MINAGRI, 2000). If 24g seed pieces were to be used, only 1.06 t of seed would be sufficient to plant one hectare, which is much lower than the 2 to 2.5 t commonly recommended by the Ministry of Agriculture and

Livestock (MINAGRI, 2000). This may imply that half of the current seed tonnage used by Rwandan farmers could be adequate to satisfy the seed requirements. A large percentage of the seed tuber mass planted per hectare is often not utilised for sprout growth and crop establishment. In terms of seed costs, farmers will be able to realise large savings if expensive seed resources can be used more efficiently.

The differences in the sprout dry mass per unit length between the bottom and the top segments is clear evidence that changes in the distribution of dry mass within sprouts does occur during sprout development. Appreciable seed reserves at the commencement of sprout development is responsible for greater sprout dry mass per unit length in bottom segments. As sprouts develop seed reserves are depleted and less assimilates are available for the growth of apical segments.

Translocation of seed reserves reflects the ability of the seed pieces to utilise assimilates for growth. In this respect 1g seed pieces utilised 82% of seed reserves for growth and respiration, 18% of seed reserves remained in seed residues. In contrast less than 70% were transferred from large seed pieces and 33 to 53% remained as seed residues after the extended periods of sprout growth in my experiments. This is an additional indication that significant amounts of the seed tuber reserves are normally not utilised during crop establishment. This is of importance to commercial and small scale potato growers, and warrants additional research attention. As a general guideline it can be stated that the objective should be to obtain at least one main stem per 20-25 g of seed material planted. This translates to 2 stems per 50 g tuber, or 4 stems per 100 g tuber.

3.6. CONCLUSIONS

Sprouts from seed pieces smaller than 8g did not differ in length, suggesting that not only assimilate were involved but also other growth factors could have played a role in sprout elongation of small seed pieces. High levels of gibberellin in small seed pieces may have influenced elongation of the sprouts.

It is clear that under the experimental conditions, 24 g of seed mass was adequate for the development of a vigorous sprout. A better seed management based on use of seed pieces of 20 to 25g can offer possibility of realising large savings in areas where expensive quality seed is a limiting factor in potato productivity.

The distribution of sprout dry mass per unit length was not uniform in the sprout length. For a specific seed size the bottom segments of the sprouts was thicker than the top segments of the sprouts, suggesting that seed assimilate were limiting as sprouts were developing. The bottom segments of the sprouts from the large seed pieces were thicker than the bottom segments of the sprouts from the small seed pieces, demonstrating that sprout thickness was affected by seed mass even before assimilate became limiting.

There was a significant interactive effect between seed size and harvest date on all sprout growth characteristics studied, suggesting that the time at which measurements are made should be considered in interpreting results from studies related to sprout growth of seed pieces.

The ability of small seed pieces to transfer stored material in the seed to the sprouts was evidenced by a large fraction of seed material allocated to sprouts and roots growth. The large fraction of assimilate released by small seed pieces, despite limited seed reserves is a further indication that growth factors other than assimilate are involved in sprout growth of small seed pieces.

CHAPTER 4

EFFECT OF GIBBERELLIN AND PACLOBUTRASOL ON SPROUT GROWTH

4.1 ABSTRACT

A greenhouse trial was undertaken to assess the effects of gibberellin and paclobutrasol on sprout growth. Three concentrations of gibberellic acid (5, 10 and 30 ppm) and paclobutrasol (0.05, 0.1 and 0.2 %) were used. Seed pieces of uniform size were dipped in the hormonal solutions and grown in vermiculite in a dark room and at 25°C/14°C for three weeks. GA treatments significantly increased sprout elongation by 23% at 5 ppm and 10 ppm concentrations and by 31% at the 30 ppm concentration. Paclobutrasol markedly inhibited sprout growth. GA increased sprout dry mass but decreased sprout dry mass per unit sprout length. Root development was not affected. A higher shoot/root ratio was noticed with gibberellin as compared to paclobutrasol treatments. GA stimulated mobilisation of assimilates for growth, with 37% of initial seed dry mass utilised at the 30 ppm concentration. In paclobutrasol-treated seed pieces only 10.7% of the initial dry mass was utilised at 0.2% concentration.

4.2 INTRODUCTION

The use of small seed tubers is often proposed as an alternative to reduce the cost of seed in potato production (Allen and O'Brien, 1987; O'Brien and Allen, 1992a). However, delayed emergence associated with small size is a major concern in potato growing when small seeds are used (Allen, 1979; Lommen, 1995). This is attributed to a long dormant period and insufficient sprout growth in the case of minitubers (Lommen, 1966). The challenge is to promote rapid and adequate sprout growth to make better use of small seeds. Rapid development of sprouts allows quick emergence, and early plant development is essential for improving productivity in potato (O'Brien, Allen, Bean, Griffith, Jones and Jones, 1983; Midmore, 1992).

A number of chemicals of widely diverse nature have been identified and recommended to enhance sprouting. Of all growth-promoting substances gibberellic acid was found to be the

most effective (Claassens and Vreugdenhil, 2000). The positive effect of GA on percentage of sprouting, sprout length and number of sprouts has been clearly demonstrated (Timm, Rappaport, Bishop and Hoyle, 1962; Lovell and Booth, 1967; Morris, 1967b). In addition, GA influences endogenous carbohydrate levels in sprouting tubers. Clegg and Rappaport (1970) reported that GA stimulates formation of reducing sugars but does not affect enzyme activities involved in the process. Bailey *et al.* (1978) demonstrated that an increase in reducing sugars also occurs in disbudded tubers but at a lower rate compared to that in budded tubers. This led to the suggestion that buds exert a direct control over mobilisation of reserves, possibly by secreting hormones which may influence enzyme activity (Morris, 1967b). It was not clear whether the increase in the level of gibberellin precedes, or is a consequence of, sprouting. Seed cutting has been shown to increase endogenous gibberellin (Rappaport and Sachs, 1967) but evidences of gibberellin involvement in sprout growth of seed pieces have not been presented yet.

Application of GA to excised buds or intact seed tubers resulted in an increased number of long and thin stems (Timm *et al.*, 1962; Hartmans and Van Es, 1979). This, together with the results of Chapter 3 where sprouts from small seed pieces were shown to be as long as sprouts from large seed pieces though thinner, support the hypothesis that morphological changes on sprouts developing from small seed pieces could be associated with an increase in endogenous GA. The higher wound surface area to volume ratio of small seeds pieces excised from larger tubers may result in a higher concentration of endogenous gibberellin, favoring sprout elongation.

In order to gain more insight in the extent to which endogenous GA influences sprout growth on potato seed pieces the level of GA was modified by the application of exogenous GA or paclobutrasol. Effects were assessed in conditions favorable to sprouting. The objective of the study was to quantify the effects of gibberellic acid and paclobutrasol on sprout length, dry mass and thickness, and rate of sprout growth on seed pieces.

4.3 MATERIALS AND METHODS

The experiment was carried out using certified seed tubers of the cultivar BP1. Cylindrical cores of 1.5 cm diameter and 2g fresh mass containing a bud (eye) were excised from the tubers. Seed pieces were planted in vermiculite in a dark room where the temperature was maintained at 25°C/20°C and the relative humidity between 70 and 80%. As soon as the sprouts reached a length of 2cm they were treated with gibberellic acid (GA) or paclobutrasol. Seed pieces were removed from the growing substrate and soaked for 10 minutes in 50 ml of gibberellin or paclobutrasol solutions. Three concentrations of gibberellin (5 ppm, 10 ppm and 30 ppm) and paclobutrasol (0.05 %, 0.1 % and 0.2 %) were respectively used. The treated seed pieces were left in a cool area to dry before being replanted. Seed pieces dipped in distilled water were used as a control.

The treated seed pieces were re-planted 1 cm deep in the vermiculite. The experiment comprised seven treatments and was analysed as a randomised complete block design with five replications, one single plant representing one replicate. Sprout length was measured every three days starting from the date of hormonal treatment. After three weeks sprout length was measured before plants were divided into shoot and root fractions for dry mass determination. The root growth was measured by scanning the roots and the resulting images analysed with the “GSRoot” Computer Program to determine root length and root surface. Roots were grouped in three classes, namely with diameters less than 1mm, between 1 and 2 mm and larger than 2 mm. Data on sprout and root growth was subjected to analysis of variance using the SAS programme and means separated using appropriate test as described in Chapter 3.

4.4 RESULTS AND DISCUSSION

Sprout length

Sprout length plotted over time describes typical S-shaped curves where three distinct phases can be noticed for the GA treatments and the control (Figure 4.1a). The first phase corresponds to a period of exponential growth during which sprout elongation was moderate.

The linear phase follows for a relatively short time during which sprout length increased considerably. During the last phase sprout growth slowed down.

The rate of sprout growth is illustrated in Figure 4.1b. Shortly after GA treatment sprout length increased at moderate growth rate. The second phase corresponds to a sharp increase in sprout length during which higher growth rates are noticeable. The last phase corresponds to that of declining growth rate. The stimulation of sprout elongation by GA is due to its effect on cell extension and cell division (Brian, Hemming and Radley, 1955; Jones, 1982), but the activity of gibberellin decreases progressively with time as demonstrated by Timm, Rappaport, Primer and Smith (1960). With the small seed pieces (2g fresh mass) in this trial the relatively early decline in sprout growth may be due to exhaustion of tuber reserves.

The results of the analysis of variance for the final sprout length are illustrated in Table 4.1 with the ANOVA summarised in Table A 4.1 of the Appendix. There were significant differences between treatments with regards to the sprout length. Gibberellin stimulated sprout growth and the sprout lengths of the 10 and 30 ppm gibberellin treated seed pieces were significantly longer than sprouts of untreated seed pieces three weeks after treatments. Applied at 5 ppm GA had no significant effect on sprout length. Increase in sprout length following GA application has previously been reported (Timm *et al.*, 1962; Hartmans and Van Es, 1979). Morris (1967b) reported an increase in sprout length from 2 to 4 cm five days after tubers were treated with 10 ppm GA in the presence of light.

Paclobutrasol application severely restricted sprout growth (Figure 4.1 and Table 4.1). There were no significant differences between the different paclobutrasol concentrations. Sprouts of paclobutrasol treated seed pieces were significantly shorter than the sprouts of gibberellin treated seed pieces and those of untreated seed pieces. This is a typical action of triazoles (of which paclobutrasol is a member) as plant growth retardants (Davis, Steffens and Sankla, 1988). Paclobutrasol operates as an anti-gibberellin. It reduces internode length as a result of inhibition of cell enlargement (Wample and Culver, 1983; Stang and Weis, 1984).

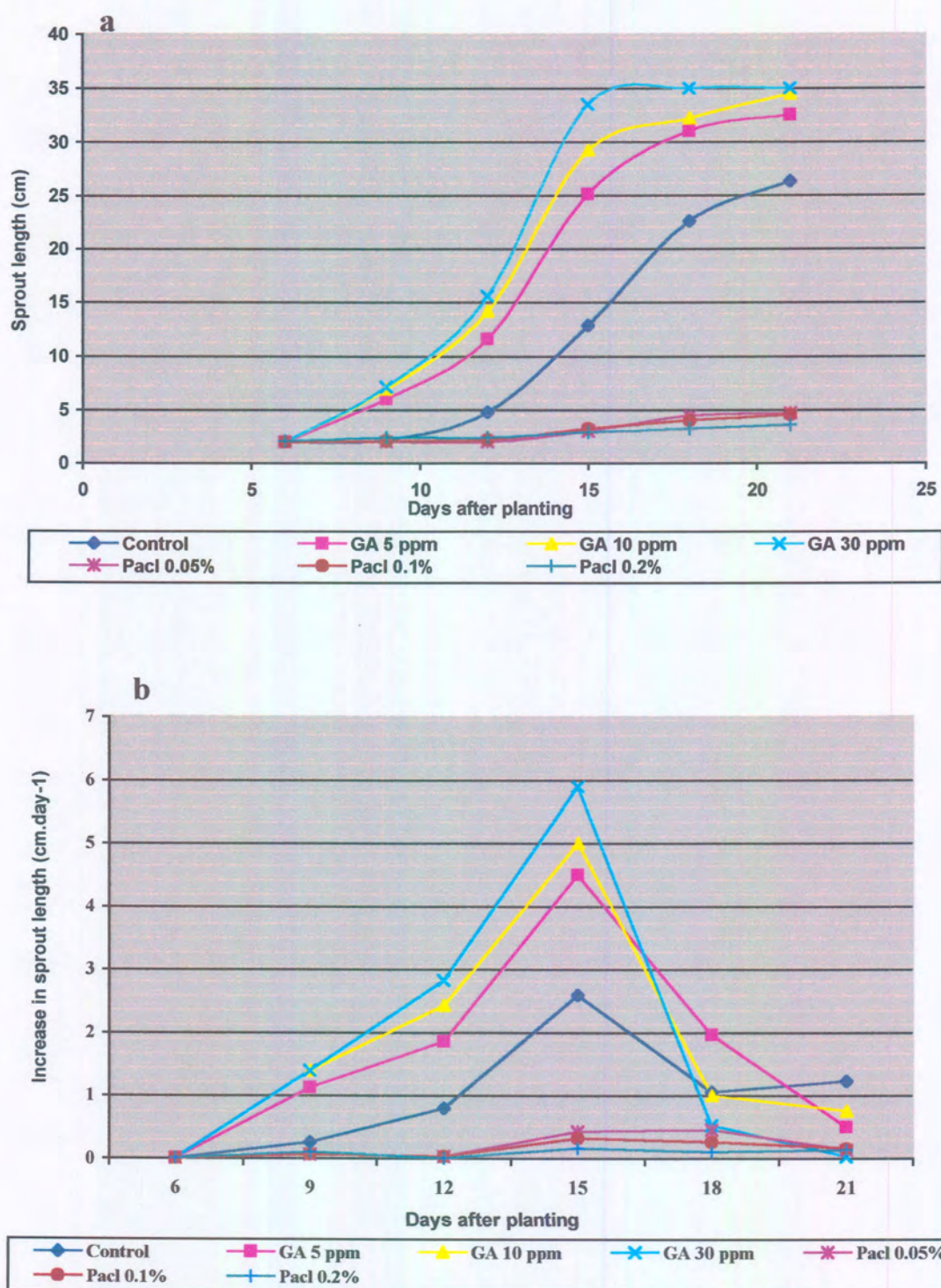


Figure 4. 1 Effect of gibberellin and paclobutrasol on (a) sprout length and on (b) rate of increase in sprout length.

Table 4. 1. Effect of gibberellic acid and paclobutrasol on sprout length, root length, root surface area and diameter

Treatment	Sprout length	Root length (mm)	Root area (mm ²)	Root diameter	
				D<1mm	D>1mm
Control	26.3 b *	1784	4570	51.2	48.8
GA 5 ppm	32.6 a b	1691	5225	44	56
GA 10 ppm	34.6 a	1059	3270	48.1	51.9
GA 30 ppm	35.0 a	701	6014	37.5	62.5
Pacl. 0.05 %	4.7 c	1455	4321	47.6	52.4
Pacl. 0.1 %	4.5 c	759	2697	48.9	51.1
Pacl. 0.2 %	3.6 c	824	2758	31.8	68.2
Means	20.2	1305.2	4121.4		
CV (%)	16.7	49.6	46.9		
LSD _{TUKEY(0.05)}	6.8	ns	ns		

*Values followed by the same letter in the column are not significantly different at P=0.05 according to Tukey (Appendix Tables A 4. 1, A 4. 2 and A 4. 3)

Root length, surface area and diameter

The coefficients of variations for the root parameters were very high resulting in none of the differences between the treatments being statistically different. Root development can be expressed in terms of root length, thickness as well as root area. The effects of gibberellic acid and paclobutrasol on root development are illustrated in Table 4.1 and also in the ANOVA summaries (Tables A 4.2 and A 4.3 of the Appendix). There were no significant effects on root length and root area for any of the treatments. GA had no effect on root elongation, contrary to the view that GA inhibits root length (Stowe and Yamaki, 1957).

Paclobutrasol tended to decrease root length particularly at the 0.1 and 0.2% concentrations. Similar observations were made by Sankla *et al.*, (1986) who reported short roots on soybean plants treated with paclobutrasol. Inversely the roots of a paclobutrasol treated sunflower did not exhibit any visual change in root morphology (Wample and Curve, 1983). This may be an indication that plant species differ in their sensitivity to paclobutrasol. The pattern of root thickness distribution did not differ among treatments (results of ANOVA not shown). This contrasts with previous reports that paclobutrasol treated plants exhibit a greater number of

short roots with an enlarged diameter (Steffens, Wang, Steffens and Brennam, 1983; Narendra, Davis, Upadhyaya, Sankla, Wasler and Smith, 1985; Sankla *et al.*, 1986).

Sprout dry mass

The sprout dry mass significantly differed among treatments as shown in Table 4.2 and in Table A 4.4 of the Appendix. Despite absence of significant differences between GA treatments and the control, the sprout dry mass of GA treated seed pieces tended to be greater than that of untreated seed pieces. The effect of gibberellin as a promoter of sprout dry mass was also reported by Morris (1967b) and Lovell and Booth (1967).

Contrary to the effects of GA, paclobutrasol treatments significantly reduced the sprout dry mass up to 50 % at 0.05, 58% at 0.1% and 65% at 0.2% concentrations, compared to that of untreated seed pieces (Table 4.2). The inhibition of shoot growth is a characteristic effect of paclobutrasol in most plant species (Wample and Culver, 1983; Narendra *et al.*, 1985).

Sprout dry mass per unit length

The results on sprout dry mass per unit length for different treatments are illustrated in Table 4.2. GA application decreased the sprout dry mass per unit length at all concentrations. At 30 ppm GA concentration, the radial growth was reduced by 13% compared with the control. This observation was also made by Rappaport, Blumenthal-Goldschmidt, Hartmans and Van Es (1979) who observed that plants treated with GA had thin stems. Inversely paclobutrasol treatment considerably increased the sprout dry mass per unit length due to sprout shortening (Figure 4.2). The sprout dry mass per unit length attained the highest value of 14 mg/cm with paclobutrasol applied at 0.2 % of concentration.

Shoot to root ratio

The values of the shoot to root ratio for the different treatments are presented in Table 4.2. GA treatments resulted in higher shoot to root ratio. The shoot to root ratio for GA

treatments ranged from 2.7 to 3.6, suggesting that the sprout dry mass was two to three times higher than the root dry mass for plants from GA treated seed pieces. Paclobutrasol, due to a marked reduction in sprout dry mass, resulted in a lower shoot to root ratio (Table 4.2). The ratio shoot to root was approximately 1 for all paclobutrasol treatments, indicating that the sprout dry mass was almost equal to the root dry mass for plants from paclobutrasol treated seed pieces. The results indicate that the plant growth regulators influenced the partitioning of assimilates in the plant components. GA tended to promote allocation of seed reserves to the shoots whereas paclobutrasol tended to inhibit that effect.

Root dry mass

The root dry mass did not differ among treatments, as indicated in Table 4.2 and also in the ANOVA summary (Table A 4.5 of the Appendix). As in the previous chapter, the roots could not be recovered reliably at harvest and this may have influenced the results on root dry mass.

Table 4. 2 Effect of gibberellin and paclobutrasol on sprout dry mass, dry mass per unit length, root dry mass and root: shoot ratio

Treatment	Mean sprout dry mass (mg)	Sprout dry mass per unit length (mg/cm)	Root dry mass (mg)	Shoot/root ratio
C	113 a*	4.3	44 a	2.5
GA 5 ppm	110 a	3.4	30 a	3.6
GA 10 ppm	114 a	3.2	26 a	4.3
GA 30 ppm	110 a	3.1	40 a	2.7
PP333 0.05 %	57 b	12	40 a	1.4
PP333 0.1 %	48 b	12	30 a	1.6
PP333 0.2 %	40 b	14	36 a	1.1
Means	84.7		5.1	
CV (%)	22.3		29.9	
LSD _{TUKEY} (5%)	38.4		ns	

*Values followed by the same letter in the column are not significantly different at P=0.05 according to Tukey (Appendix Tables A 4. 4 and A 4. 5)

Utilisation of seed reserves

The pattern of mobilisation of seed reserves is illustrated in Figure 4.3. In GA treated seeds more than 30% of tuber reserves were utilised for growth, while only 10 to 22 % were used in paclobutrasol treated seeds. On daily basis 6.7 mg of dry mass was mobilised at 5 ppm GA, 7.4 at 10 ppm and 7.9 mg of dry mass at 30 ppm GA. In paclobutrasol treated seed pieces these values were 4.6 mg at 0.05 % Pacl., 4.2 at 0.1 % and 3.6 mg of dry mass at 0.2 % Pacl. The increases in sprout length and sprout dry mass associated with GA were a result of stimulating effect on the mobilisation of materials from the seed. During sprout growth carbohydrates are degraded into reducing sugars and proteins are broken down into amino acids. These soluble compounds are translocated to growing sprouts (Moorby and Milthorpe, 1975). These results conform to those of Morris (1967b) who found that high doses of GA resulted in a greater translocation of mobilised materials.

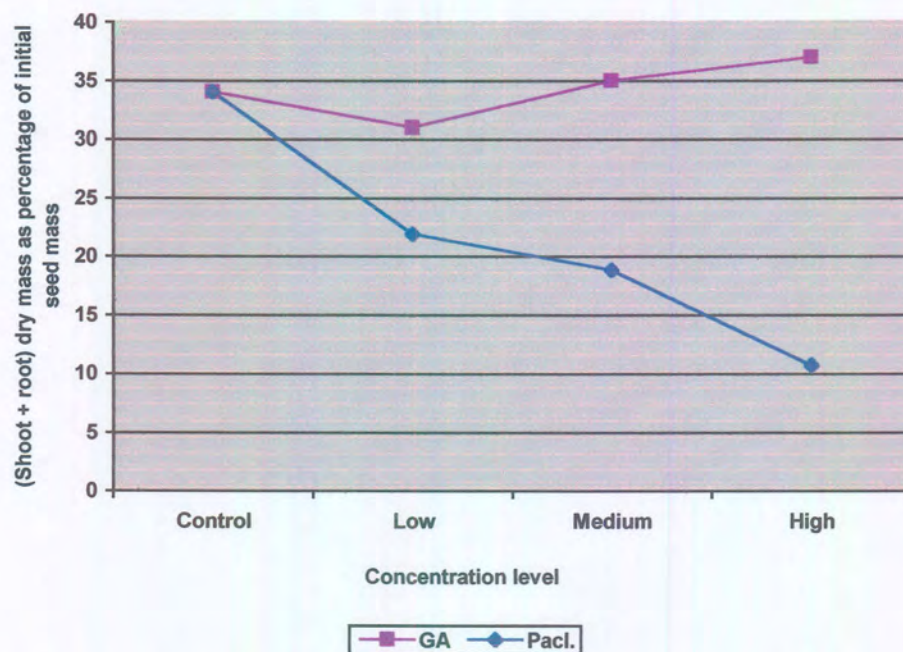


Figure 4.2 Effect of gibberellin and paclobutrasol on shoot plus root dry mass expressed as percentage of initial seed mass

Control: distilled water Low: 5 ppm GA, 0.05% Pacl. Medium: 10ppm GA, 0.1% Pacl. High: 30 ppm GA, 0.2% Pacl.

4.5 CONCLUSIONS

The application of gibberellin increased sprout length, the rate of increase in sprout length, sprout dry mass and mobilisation of seed reserves, but reduced sprout dry mass per unit length. Inversely, paclobutrasol, acting as an antigibberellin, operates by inhibiting the effects of gibberellin. It can be concluded that levels of gibberellins-like substances play a central role in sprout growth of seed pieces. The optimum effect for GA was obtained at a concentration of 30 ppm.

The pattern of sprout development following application of exogenous gibberellin in this experiment is similar to sprout development on small seed pieces (Chapter 3), and provides some circumstantial evidence that the long, thin sprouts produced by small seed pieces may be the result of increased gibberellin activity in smaller seed pieces.

Gibberellin-like substances can be successfully used in commercial potato production to enhance the rate of sprout growth in order to break dormancy and improve emergence and plant establishment in case of physiologically young seedlots.

CHAPTER 5

CALCIUM AVAILABILITY AS A FACTOR IN SPROUT GROWTH

5.1 ABSTRACT

The availability of external calcium may affect sprout growth after planting. This aspect was investigated in two experiments conducted in dark conditions at 22°C. In the first experiment four concentrations of calcium (0, 0.1, 1 and 10 mmol/l) were supplied as CaSO₄ or CaCO₃ to sprout tips of 3g seed pieces. A treatment with a full nutrient solution was included. In the second experiment the effect of calcium was studied on growth of nutrient deprived or nutrient supplied sprouts. Sprout length, dry mass and sprout dry mass per unit length were greater with the high concentration of calcium (10 mmol/l) as CaSO₄ and with the full nutrient solution treatment. These treatments completely prevented apical necrosis. The root dry mass was not affected by calcium. CaSO₄ was more effective than CaCO₃. In nutrient deprived conditions, sprout length, sprout dry mass and sprout dry mass per unit length were significantly greater on plants treated with calcium compared to the control. Inversely, when plants were supplied with the full nutrient solution sprout length, sprout dry mass and sprout thickness remained unchanged irrespective of whether calcium was added to sprout tips or not. It can be concluded that the response of sprout growth to calcium supply depends on calcium level and form, and on whether other nutrient elements are present in the growing substrate or not. The calcium requirements of the sprout apex can be supplied either by the roots or by absorption of calcium by the sprout tips.

5.2 INTRODUCTION

Several investigations reported that sprouts depend on mother tuber reserves for growth (Borah, Burt, Headford and Milthorpe, 1960; Davies and Ross, 1985; Davies, 1990). This dependence could last even long after emergence (Milthorpe, 1963). It follows that sprout growth will ultimately be affected by shortage of seed reserves. On basis of the results from the previous experiments (Chapters 3 and 4) it clearly appears that sprout growth is limited by shortage of tuber reserves in small seed pieces, suggesting that either stored assimilates per se, or specific growth substances, were exhausted during sprout development. The limiting effect of some of the growth factors is not yet fully established. The question of whether a lack of calcium in small seed pieces affects sprout development deserves attention.

Calcium deficiency is very common in potato production and is responsible for deleterious effects on plant growth and subsequent tuber yield and quality. Potato plants developed poorly in acid soils due to calcium deficiency (Wallace and Hewitt, 1948). Calcium deficiency was characterised by apical necrosis followed by proliferation of lateral sprout development (Toosey, 1963; Coleman, 2000). Similar symptoms were also observed in micropropagated plants (Abousalim and Mentell, 1994).

Calcium deficiency is not only attributable to a shortage of soluble calcium but the presence of excessive levels of some ions can also negatively impact on calcium absorption. For instance K^+ , Mg^{++} and NH_4^+ have been shown to depress calcium uptake (Bangerth, 1979; Wallace and Mueller, 1980). Furthermore Na^+ and many trace elements may be phytotoxic when calcium supply is low (Wallace and Mueller, 1980). Application of calcium with the aim to correct effects of calcium deficiency must therefore take into account the level of other ions.

Even though the effectiveness of calcium in preventing apical necrosis is largely recognised, its role in sprout growth is not yet clearly established. There are conflicting opinions as to whether calcium is a factor in sprout growth. Davies (1984) found that sprouts developing in compost were longer and had higher calcium concentrations than those placed in chitting trays. Inversely, Davies and Ross (1985) observed that although calcium was effective in preventing apical necrosis, no evidence of its role as a factor influencing the degree of inter-sprout competition was provided. Tzeng, Kelman, Simmons and Kelling (1986) found no significant differences between tubers with high internal calcium content and tubers with low internal calcium content with regards to the number of stems and stem vigour.

It is hypothesised that a lack of calcium in small seed pieces is partly responsible for the development of long, thin sprouts and that the utilisation of stored assimilates for sprout growth can be improved by supplying calcium to sprouts. The objective of this study was to determine the effect of calcium on the sprout length, sprout thickness and dry mass of sprouts and roots.

5.3 MATERIALS AND METHODS

The effect of calcium on sprout growth was evaluated in two experiments conducted at the Experimental Farm of the University of Pretoria. Single “eye” tuber pieces were cut with a 1.5 cm cork borer from the middle portion of tubers. The inner tissue distal from the eye end was cut away to obtain sets of 3 g of uniform size and shape.

Seed pieces were planted in crates containing vermiculite with the buds at the surface of substrate. The crates were placed in the dark at a 22°C constant temperature and 80 to 90% relative humidity. These conditions were maintained throughout the experiment. Any secondary sprouts were removed daily with a scalpel.

Measurements were taken on sprout length. Dry mass of sprouts and roots were determined after oven drying samples at 65°C for 24 hours. The analysis of variance was performed using the GLM statistical procedure and means were compared using Tukey’s least significant differences computed at the 5% significance level as described in Chapter 3.

Experiment 1

In this experiment calcium or distilled water was applied daily to the sprouts. Three concentrations of CaSO_4 and CaCO_3 corresponding to three levels of calcium (0.1, 1 and 10 mmol/l) were applied. Sprouts treated with distilled water were used as a control. An additional treatment with a full nutrient solution was included to assess the effect of other minerals. Calcium and the other treatments were applied on the sprout tips only using a small brush. The average daily application was approximately 0.005 ml. The experiment comprised eight treatments with 6 plants per treatment. Due to variability in sprout growth, more seed pieces were initially planted, and the six best growing sprouts per treatment were retained for data collection. Measurement of the sprout length was made 3 days after the date of the first application and was repeated every three days on the six plants for each treatment. After 26 days the plants were carefully removed from the vermiculite and the final sprout length, sprouts and roots dry mass were determined.

Experiment 2

Seed pieces were grown in two separate groups of crates filled with nutrient free vermiculite or vermiculite watered with a full nutrient solution. The complete nutrient solution used was a commercial nutrient solution (Hydro-gro)² supplied twice a week at a rate of 250 ml per crate. Calcium sulphate (10 mmol/l) was applied to the tips of half of the sprouts growing in each medium using a small brush. The average daily application was about 0.005 ml. The other sprouts were similarly treated with distilled water only. Four treatments were compared:

- 1- Control, nutrient free medium + untreated sprout tips
- 2- Nutrient free medium + calcium applied to sprout tips
- 3- Nutrient supplied medium + untreated sprout tips
- 4- Nutrient supplied medium + calcium applied to sprout tips

The experiment consisted of four treatments with 16 replicates. After 30 days the final sprout length was recorded and dry mass determined.

5.4 RESULTS

EXPERIMENT 1

Sprout length

The results on sprout length are presented in Figure 5.1 and Table 5.1. Three days after the treatments commenced differences in sprout growth could be observed, with sprouts receiving the high concentration of CaSO₄ (T3) and the full nutrient solution (T7) longer

² Hydro-gro may be obtained from Hortichem, Division of Ocean Agriculture (Pty) Ltd. P.O. Box 741, Muldersdrift 1747, Republic of South Africa.

than those of the other treatments. This trend continued during the 18 days the experiment lasted. At the final harvest the sprouts of these two treatments were significantly longer than those of all the treatments except the low CaSO_4 (T1) and the high CaCO_3 (T6). Sprouts from the untreated control (T8) and the low levels of CaCO_3 (T4 and T5) were the shortest while the low concentrations of CaSO_4 resulted in intermediate sprout lengths.

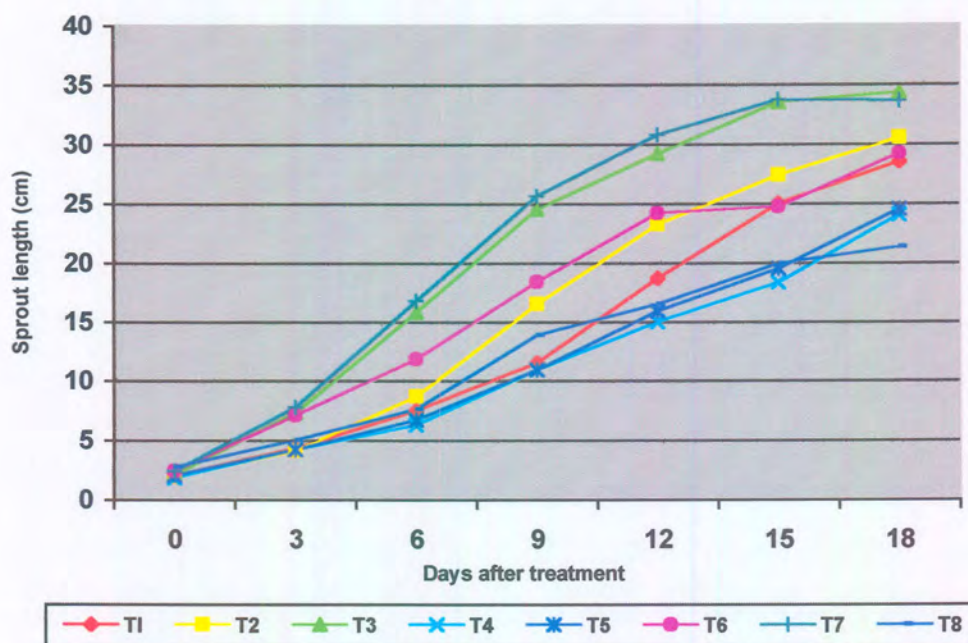


Figure 5.1 Effect of CaSO_4 , CaCO_3 and full nutrient solution on sprout length during the 18 days experimental period (Appendix Table A 5.1)

Table 5. 1 Effect of calcium on sprout growth characteristics 18 days after planting

Treatment	Sprout length (cm)	Sprout dry mass (mg)	Sprout dry mass per unit length (mg/cm)
T1	28.5 a b c*	101.3 c d	3.5 b
T2	30.5 b	108.3 c	3.6 b
T3	34.3 a	200 a	5.8 a
T4	24.0 c d	97.3 c d	4.0 b
T5	23.5 b c d	137.5 b	5.8 a
T6	29.5 a b c	136.5 b	4.6 b
T7	33.7 a	197.5 a	5.8 a
T8	21.3 d	78.1 d	3.6 b
Mean	28.5	132.0	4.6
C.V (%)	11.6	10.8	13.8
LSD _{Tukey}	6.1	26.8	1.1

* Values followed by the same letter in the column are not significantly different at $P=0.05$ according to Tukey (Appendix Tables A 5.1, 5.2 and 5.3) T1: CaSO_4 0.1 mmol/l T2: CaSO_4 1 mmol/l T3: CaSO_4 10 mmol/l T4: CaCO_3 0.1 mmol/l T5: CaCO_3 1mmol/l T6: CaCO_3 10 mmol/l T7: full nutrient solution T8: distilled water (control)

Sprout dry mass

The results of sprout dry mass as presented in Table 5.1 were similar to the results on sprout length with the largest dry mass (in the order of 200 mg per sprout) obtained from the high CaSO_4 treatment (T3) and the full nutrient solution treatment (T7). The control (T8) and the low CaCO_3 and CaSO_4 treatments (T1, T2 and T4) resulted in much lower sprout dry mass (in the order of 100 mg per sprout). It is clear that the external supply of calcium enabled the growing sprouts to utilise twice the amount of assimilates from the mother tuber than the control treatment.

Incidence of apical necrosis

The degree of apical necrosis was assessed in the different treatments (Figure 5.2). In the untreated control 33% of the sprouts exhibited signs of apical necrosis. At low calcium supply, CaSO_4 (T1) was more effective in controlling apical necrosis than CaCO_3 (T4). When supplied at 1 mmol/l, CaSO_4 had a comparable effect to that of CaCO_3 supplied at high concentration (T6). The high concentration of CaSO_4 (T3) and the full nutrient solution (T7) completely prevented apical necrosis.

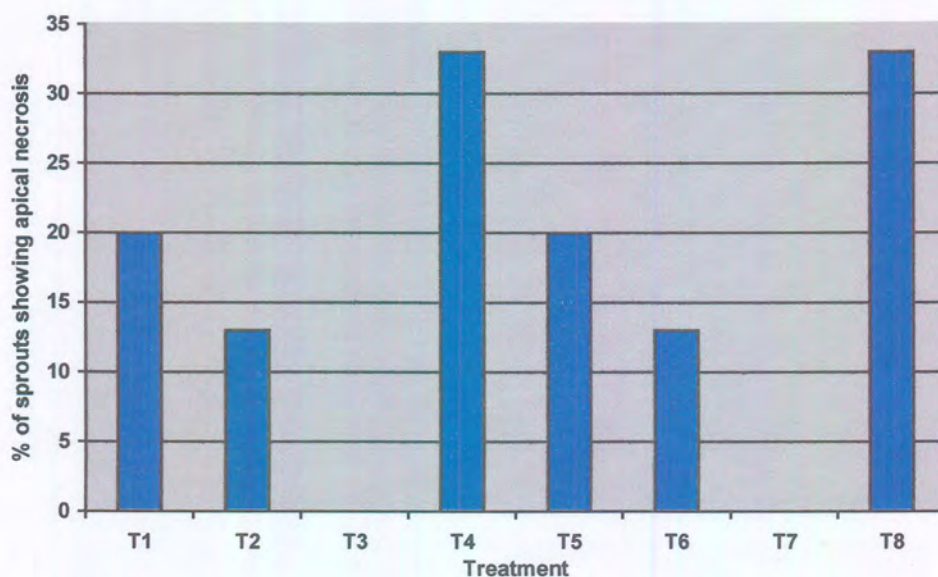


Figure 5.2 Number of sprouts with apical necrosis (as % of total sprouts)

T1: CaSO_4 0.1 mmol/l T2: CaSO_4 1 mmol/l T3: CaSO_4 10 mmol/l T4: CaCO_3 0.1 mmol/l T5: CaCO_3 1mmol/l T6: CaCO_3 10 mmol/l T7: full nutrient solution T8: distilled water (control)

Sprout dry mass per unit length

The results illustrating sprout dry mass per unit length for the different treatments are reported in Table 5.1, with the ANOVA summarised in Table A 5.3 of the Appendix. There were significant differences between treatments. The treatments resulting in the best sprout growth (T3, T5 and T7) also had sprouts with greater dry mass per unit length compared to the other treatments. For these treatments sprout dry mass per unit length was 5.8 mg/cm. The sprout thickness from other treatments was comparable to the sprout thickness from the control and ranged from 3.5 to 4.6 mg/cm.

Root dry mass

Data on root dry mass as illustrated in Table 5.2 and Table A 5.4 of the Appendix show that the root dry mass did not differ among treatments. Despite absence of significant differences it clearly appears that low concentrations of calcium (T1 and T4) had lower root dry mass (in the order of 25 mg per plant) compared to that of other treatments (more than 30 mg per plant). It was impossible to reliably recover all the roots at harvest and a possible influence of this on the reliability of the data cannot be excluded.

Shoot plus root dry mass

The results on shoot plus root dry mass as illustrated in Table 5.2 were similar to those on sprout dry mass, with the largest dry mass (in the order of 230 mg per plant) obtained from the high CaSO_4 treatment (T3) or the nutrient solution treatment (T7). The total shoot and root dry mass from treatments with moderate and high CaCO_3 (T5 and T6) did not differ from the dry mass obtained from the treatment with moderate CaSO_4 (T2). The control (T8) and the low calcium treatments (T1 and T4) resulted in low shoot plus root dry mass. It is clear that supply of calcium or nutrient solution promoted accumulation of dry mass in the developing plants.

Table 5.2 Effect of calcium on root dry mass and shoot plus root dry mass 18 days after Planting

Treatment	Root dry mass (mg)	Shoot plus root dry mass (mg)
T1	27.8 a	129.1 c
T2	31.3 a	139.6 b c
T3	31.5 a	235.1 a
T4	25.7 a	123.1 c
T5	29.8 a	169.3 b
T6	32.3 a	168.8 b
T7	36.3 a	233.8 a
T8	34.2 a	112.2 c
Mean	31.1	163.8
C.V (%)	25.0	10.2
LSD _{Tukey}	ns	31.3

*Values followed by the same letter in the column are not significantly different at $P=0.05$ according to Tukey (Appendix Tables A 5. 4 and A 5. 5) T1: CaSO_4 0.1 mmol/l T2: CaSO_4 1 mmol/l T3: CaSO_4 10 mmol/l T4: CaCO_3 0.1 mmol/l T5: CaCO_3 1mmol/l T6: CaCO_3 10 mmol/l T7: full nutrient solution T8: distilled water (control)

EXPERIMENT 2

Sprout length

The results on sprout length for the different treatments are presented in Figure 5.3 and also in the ANOVA summarised in Table A 5.6 of the Appendix. Where the vermiculite substrate was deprived of nutrients, calcium treated sprouts (T2) were 40% longer than the untreated sprouts. However for the sprouts grown in a nutrient supplied substrate, untreated sprouts (T3) were as long as the calcium treated sprouts (T4). It was also noticed that calcium treated sprouts did not differ in length irrespective of whether sprouts were deprived of nutrients (T2) or not (T4). These results indicate that by supplying the roots with a full nutrient solution (including calcium) adequate calcium was transported to the apical meristem of the sprouts, and no additional advantage was obtained by supplying calcium directly to the sprout tips.

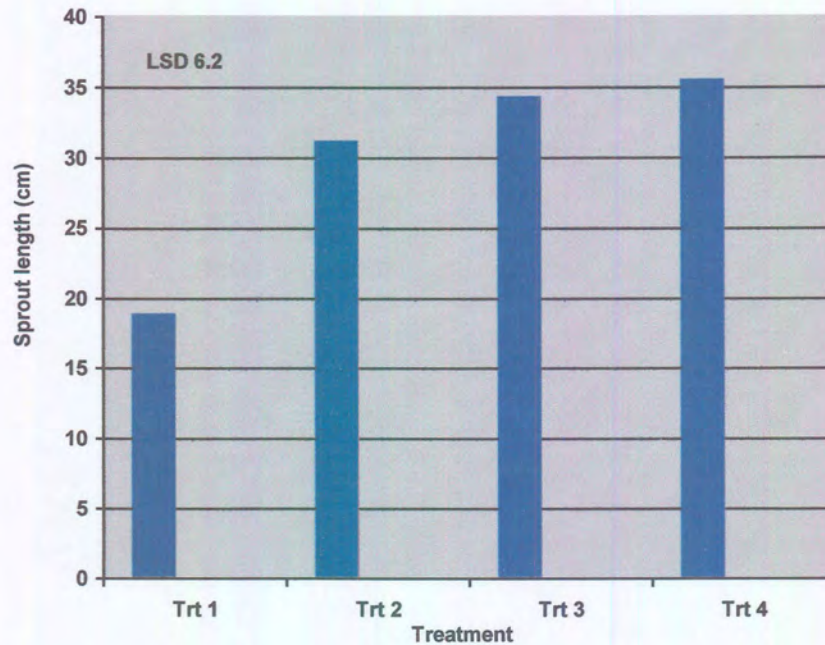


Figure 5.3 Effect of calcium and nutrient supply to the sprout tips on sprout length
Trt 1: nutrient free medium + untreated sprout tips Trt 2: nutrient free medium + calcium applied to sprout tips Trt 3: nutrient supplied medium + untreated sprout tips Trt 4: nutrient supplied medium + calcium applied to sprout tips (Appendix Table A 5. 6)

Sprout dry mass

The results on sprout dry mass are illustrated in Figure 5.4. The sprout dry mass was significantly smaller in the control (T1). The sprouts deprived of nutrients but treated with calcium (T2) had twice the dry mass of the control (T1). For sprouts growing in a substrate supplied with nutrients, calcium treated sprouts (T4) did not differ from the not supplied with additional calcium (T3). As indicated in Table A 5.7 of the Appendix sprout dry mass did not differ between calcium treated sprouts irrespective of whether sprouts were deprived of nutrients (T2) or supplied with nutrients (T4). The results clearly show that by supplying complete nutrient solution to the roots large amount of seed material was transferred to the sprouts and no additional effect was obtained by supplying calcium directly to the sprout tips.

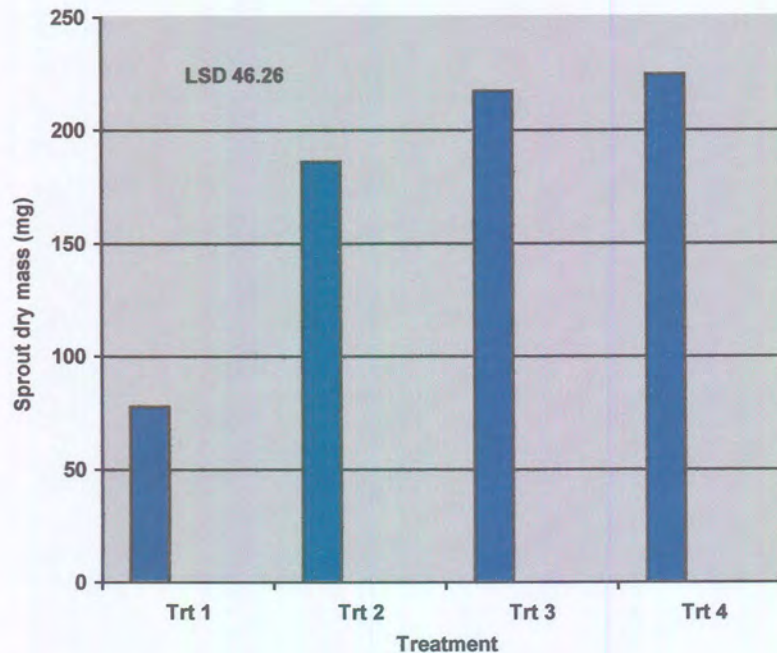


Figure 5.4 Effect of calcium and nutrient supply to the sprout tips on sprout dry mass
 Trt 1: nutrient free medium + untreated sprout tips Trt 2: nutrient free medium + calcium applied to sprout tips Trt 3: nutrient supplied medium + untreated sprout tips Trt 4: nutrient supplied medium + calcium applied to sprout tips (Appendix Table A 5.7)

Sprout dry mass per unit length

Data on sprout dry mass per unit length as illustrated in Figure 5.5 reflect the results on sprout dry mass. For the sprouts deprived of nutrient the application of calcium to sprout tips (T2) increased significantly the sprout thickness. No difference in sprout thickness was found among the sprouts growing in a nutrient supplied substrate irrespective of whether calcium was applied to sprout tips (T4) or not (T3). The sprout dry mass per unit length of these treatments (T3 and T4) was 40% higher than that from the control (T1). In addition, the results indicate that there was no difference in sprout thickness between the calcium treated sprouts (T2 and T4) irrespective of whether the sprouts were growing in a nutrient supplied substrate or not. The results indicate that by supplying a full nutrient solution to the roots, large amount of dry matter of seed pieces was transported to the sprouts, resulting in high sprout dry mass per unit length. But no additional effect was obtained when calcium was applied directly to the sprout tips.

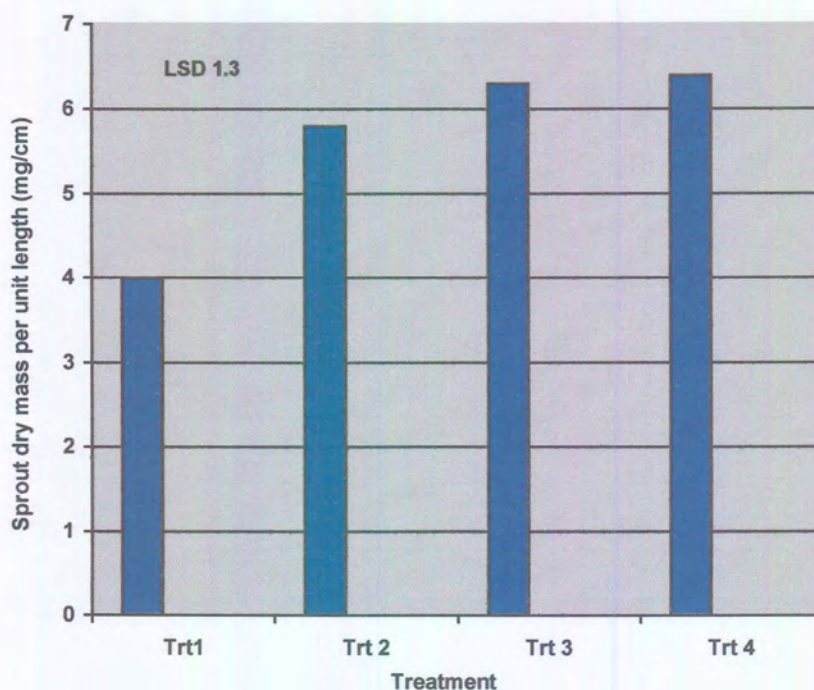


Figure 5.5 Effect of calcium and nutrient supply to the sprout tips on sprout dry mass per unit length Trt 1: nutrient free medium + untreated sprout tips Trt 2: nutrient free medium + calcium applied to sprout tips Trt 3: nutrient supplied medium + untreated sprout tips Trt 4: nutrient supplied medium + calcium applied to sprout tips (Appendix Table A 5. 8)

Root dry mass

Contrary to the results on sprout growth where growth was significantly increased by supplying calcium to the plants deprived of nutrients, the results collected on root dry mass showed no effect of calcium applied to the tips of the sprouts growing in a nutrient deprived substrate (Figure 5.6). No difference in the root dry mass was found among the sprouts growing in a nutrient supplied substrate irrespective of whether calcium was applied to sprout tips (T4) or not (T3). The significant difference between treatments (Table A 5.9 of the Appendix) was due to greater root dry mass from the sprouts of the treatment T4 compared to the control (T1).

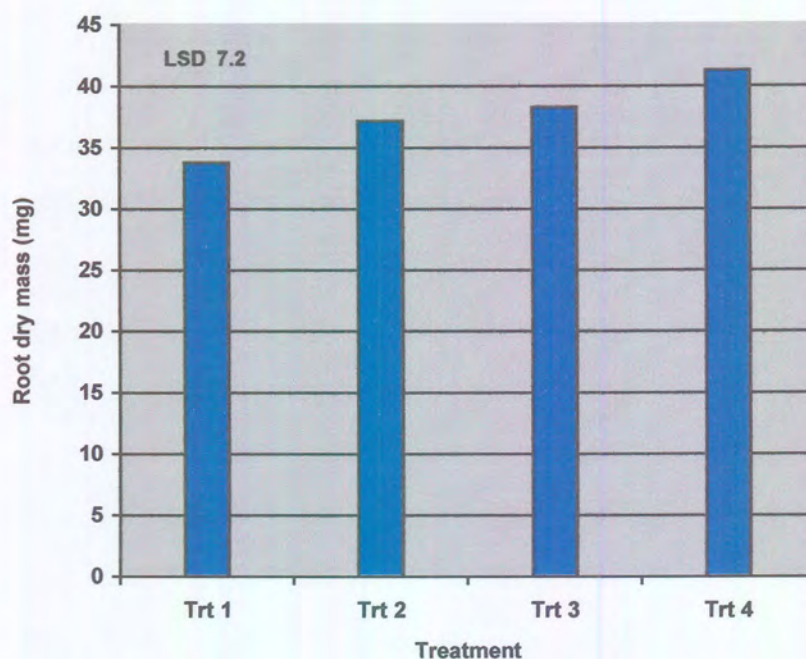


Figure 5.6 Effect of calcium and nutrient supply to the sprout tips on root dry mass

Trt 1: nutrient free medium + untreated sprout tips Trt 2: nutrient free medium + calcium applied to sprout tips Trt 3: nutrient supplied medium + untreated sprout tips Trt 4: nutrient supplied medium + calcium applied to sprout tips (Appendix Table A 5.9)

Shoot plus root dry mass

The shoot plus root mass followed closely the same trend as the sprout dry mass. The shoot plus root dry mass significantly differed among treatments as shown in Table A 5.10 of the Appendix. When the sprouts were developing in a substrate deprived of nutrient, calcium application to the sprout tips significantly increased the shoot and root of the plants compared to the untreated plants. In contrary, when the sprouts were developing in a substrate supplied with nutrient, calcium application to sprout tips resulted in no additional effect in terms of shoot plus root dry mass.

5.5 DISCUSSION

Sprout growth was influenced by calcium availability and the degree to which the sprouts were affected depended on the calcium source, the calcium level and on whether nutrients other than calcium were present in the root medium or not. The data indicate that CaSO_4

supplied to sprout tips at a concentration of 10 mmol/l or application of a complete nutrient solution to the root zone, had same effect on sprout length, with sprout lengths significantly longer than those of the control. At the high concentration CaSO_4 and CaCO_3 had the same effect on sprout length at the early stage of sprout development but the effect of CaCO_3 was smaller at a later stage. The results also indicate that calcium application had a significant effect on sprout length only when plants were growing in a substrate deprived of nutrient elements. A positive effect on sprout length was also observed on sprouts developing on high calcium tubers as compared to those developing on low calcium tubers (Tzeng *et al.*, 1986). The absence of a significant effect of calcium on length of the sprouts growing in a nutrient supplied substrate emphasises the fact that when sprouts are initially supplied with a complete nutrient solution including calcium, the functional requirements for calcium are decreased (Wallace and Mueller, 1980).

It is difficult to compare the results of the effect of calcium on sprout length with those obtained by Burton (1958) using lettuce hypocotyl sections. In lettuce, calcium accumulation in cell wall inhibited growth-promoting effect of gibberellin, resulting in reduced extensibility of the cell wall, suggesting that the application of high calcium might result in shorter stems due to inhibition of gibberellin. The relationship between calcium level and activity of gibberellin in sprouting seed pieces deserves further research attention.

The daily application of 0.005 ml of calcium solution at a concentration of 10mmol/l enabled greater sprout growth. This means that under the experimental conditions, a daily supply of 0.002 mg of calcium was adequate for the production of a vigorous sprout. The supply of the same volume of calcium solution at a concentration of ten-fold lower (1mmol/l) corresponding to a daily application of 0.0002 mg of calcium resulted in reduced sprout growth, demonstrating that calcium availability is a limiting factor of sprout growth.

Apical necrosis was completely prevented by high CaSO_4 concentrations. CaCO_3 was less effective in controlling apical necrosis. The results show that apical necrosis is a consequence of calcium deficiency in the apical region. The fact that apical necrosis could be prevented by supplying adequate levels of calcium shows that calcium is necessary for

normal growth of the apex. The efficiency of calcium in preventing apical necrosis was also reported in earlier studies (Dyson and Digby, 1975b; Davies and Ross, 1985). Dyson and Digby (1975a) demonstrated that calcium salts including calcium nitrate, calcium chloride and calcium sulphate were equally effective in preventing apical necrosis, but calcium nitrate increased sprout growth rate more than calcium sulphate and chloride.

The sprouts for which tips were treated with high CaSO_4 solution were thicker than the control, demonstrating that calcium plays a critical role in sprout thickness. The results of the present study resemble those of Tzeng *et al.* (1986) who observed that diameters of sprouts from the low calcium tubers were significantly smaller than those of the high calcium tubers.

Calcium applied to sprout tips enabled sprouts to utilise more assimilate, resulting in more growth. This argument is supported by the earlier observation that sprout growth was accompanied by a decrease in the concentration of calcium of the tuber (Davies and Millard (1985). Shortage of calcium in small seed pieces may partly be responsible for less dry mass in the sprouts. These results emphasise the importance of calcium in sprout growth as reported previously by Dyson and Digby (1975a). It also indicates that sprout growth can be improved by supplying adequate amount of calcium to sprout tips.

The root dry mass was not affected by the calcium application to sprout tips. These results are contrary to the observation made earlier that root growth was depleted by calcium deficiency (True, 1914). Edwards (1936) pointed out that omission of calcium from a complete nutrient solution exposed the roots to a highly toxic environment of potassium, magnesium and other micronutrients. The failure in recovering reliably the roots at harvest may have influenced the data on root dry mass.

5.6 CONCLUSIONS

The results indicate that calcium is essential for growth of the meristem. Low calcium results in reduced sprout dry mass and sprout dry mass per unit length. It can be concluded that low dry mass in the sprouts developing on small seed pieces (Chapter 3) may be partly due to

shortage of calcium in the small seed pieces. The results indicate that shortage of calcium reduces sprout length, demonstrating that low calcium levels in small seed pieces could not be associated with the development of long sprouts.

Sprout growth can be improved by supplying calcium to sprout tips. Calcium enables assimilate to move from the seed pieces to the developing sprouts, resulting in more growth. Calcium availability is a factor of sprout growth in seed pieces in potato.

CHAPTER 6

EFFECTS OF TEMPERATURE AND SEED SIZE ON SPROUT GROWTH

6.1. ABSTRACT

Sprout growth is affected by environmental factors and temperature is the most important among them. The purpose of this study was to determine how temperature (16°C and 26°C) affects sprout and root growth as well as utilisation of seed reserves of two seed pieces of different sizes (3 and 5g). Sprout length was similarly affected by temperature over time, with the sprouts at low temperature initially longer, but reaching the same lengths as those at the high temperature after 30 and 40 days. Sprout dry mass, sprout dry mass per unit length and root dry mass were generally greater at low than at high temperature and with large than with small seed pieces. The sprout dry mass per unit length was less affected by seed size and temperature in the top segments of the sprouts. The bottom segments of the sprouts from 5g seed pieces were thicker than the bottom segments of the sprouts from 3g seed pieces. For a specific seed size, the bottom segments were thicker than the apical segments. The root dry mass was adversely affected by high temperature, though not significantly. This resulted in a high shoot to root ratio for the sprouts grown at 26°C. For most variables tested, interaction between temperature and harvest date was noticed.

6.2 INTRODUCTION

Sprout growth is affected by a wide range of environmental factors with temperature the most important one (Toosey, 1964; Burton, 1966; Smith, 1977). The effect of temperature has been mostly investigated on sprouting of stored seed tubers (Goodwin, 1967; Wurr, 1979; McGee, Jarvis and Duncan, 1986). The response to temperature depends on the range of temperatures to which the growing sprout is exposed. It has generally been observed that sprout growth is severely inhibited at temperatures ranging from 0 to 5°C (Burton, 1966; Short and Shotton, 1970) but above this range, increases in temperature result in marked growth (Sadler, 1961; Short and Shotton, 1970). At temperatures higher than 25°C sprout growth declined (Dyson and Digby, 1975a, 1975 b) exhibiting an outgrowth of lateral sprouts and swelling of the base of the sprout (Davidson, 1958). Alternating low and high

temperatures rather than keeping tubers under a constant favorable sprouting temperature was shown to promote elongation (Wurr and Allen, 1976; Allen, Bean and Griffith, 1978) and thickening of the sprouts (Burton, Van Es and Hartmans, 1992).

Although temperature effect on sprouting of intact potato tubers is well documented, less is known about the response of seed pieces to temperature. The way in which temperature regulates sprouting of seed pieces may be of particular significance. Activation of gibberellins occurs following seed injury (Rappaport and Sachs, 1967) and levels of growth promoting substances play a central role in sprout growth as evidenced by the results of Chapter 4. This, together with reports on the influence of temperature on activity of plant growth regulators (Thomas and Wurr, 1976; Menzel, 1983) suggests possible interactive effects of temperature and seed size on sprout growth. This experiment was designed to investigate the sprout growth response of seed pieces of different sizes to two temperature regimes.

The objectives of this study were to determine:

1. the effects of temperature and seed size on sprout length and on dry mass of sprouts and roots, and
2. the effects of temperature and seed size on the fraction of dry matter that is utilised for sprout development

6.3 MATERIALS AND METHODS

An experiment was conducted in the controlled environment research facilities on the Experimental Farm of the University of Pretoria. Single “eye” seed pieces (1.5 and 2 cm in diameter) with sprouts of 2 cm long were cut with cork borers from tubers. Seed pieces were sterilised and left in cool conditions to allow formation of a cork layer before planting. They were planted in two temperature-controlled rooms at constant temperatures of 16 and 26°C respectively. The experiment was laid out as a randomised complete block design with all combinations of two temperatures, two seed sizes and four harvest dates replicated five

times. Plants were watered as needed and harvested 15, 20, 30 and 40 days after planting. At each harvest sprout length was measured before plants were divided into roots and shoots. Dry mass was determined after drying at 65°C for 24h. Sprout dry mass per unit length was examined in two different ways. Firstly the mean sprout dry mass per unit length was determined by obtaining the weight: length ratio for the sprouts. In addition, segments of 5 cm were cut both from the top and bottom ends of the sprout, dried and weighed in order to establish whether dry mass per unit length varies in a particular sprout.

The analysis of variance (ANOVA) was performed using the GLM statistical procedure in SAS to determine the effects of main factors and the interactions between them, and the means were compared using the Tukey's t-test computed at the 5% significant level as described in Chapter 3.

6.4 RESULTS AND DISCUSSION

Sprout length

Sprout length was significantly affected by seed size, temperature and harvest time as indicated in Table A 6.1 of the Appendix. The results presented in Figure 6.1 show that at the first two sampling dates (after 15 and 20 days respectively) there were no significant differences between sprout lengths of 3 and 5g seed pieces exposed to the same temperature, although the sprouts on the larger seed pieces tended to be a bit longer. Sampled after 30 and 40 days the sprouts of the 5g seed pieces were, however, significantly longer. Temperature had the same effect on large as on small seed pieces over time, with the sprouts at the low temperature initially shorter, but reaching similar lengths as those at the high temperature after 30 and 40 days.

The significant interaction found between temperature and harvest date (Table A 6.1 of the Appendix) was due to the fact that sprout lengths were initially greater at high than at low temperature and this situation was reversed at later harvest dates. Sprout length was affected by the interactive effect between temperature and seed size but this interaction was not

strong ($P = 0.0357$). This interaction was due to the fact that for 3g seed pieces sprouts were significantly longer at 26°C than at 16°C while for the 5g seed pieces sprout lengths were similar at both 16°C and 26°C (Table 6.1).

Table 6.1. Interactive effect of temperature and seed size on sprout length

Temperature (°C)	Sprout length (cm)		Means
	Seed size (g)		
	3	5	
16	23	38.3	30.6 b*
26	36.6	45.1	40.8 a
Means	29.8 b*	41.7 a	35.7
C.V.(%)	16.5		
LSD _{Tukey}			
(0.05)	Seed size	2.6	
	Temperature	2.6	
	Seed size x temperature	6.9	

* Values followed by the same letter in the column or row are not significantly different at $P = 0.05$ according to Tukey (ANOVA in Appendix A 6.1)

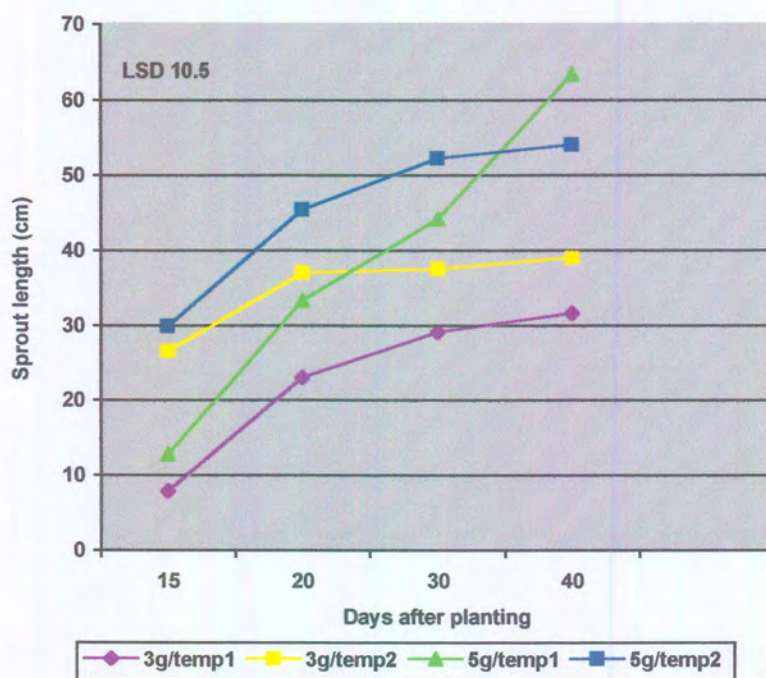


Figure 6.1 Effect of temperature, seed size and harvest date on sprout length

temp 1 = 16°C

temp 2 = 26°C (Appendix Table A 6.1)

The rapid growth of the sprouts in response to high temperature is in accordance with findings from previous studies (Dyson and Digby, 1975a; McGee *et al.*, 1986; Khanbari and Thomson, 1996). Dyson and Digby (1975a) found an increase in sprout length with increasing temperature, growth being the slowest at 16°C for the first 10 days after which sprouts held at that temperature grew rapidly. In contrast, growth was the most rapid at 25°C for the first two days but declined thereafter due to occurrence of necrosis of the sprout apex.

Sprout dry mass

It is evident from the results presented in Figure 6.2 that the sprout dry mass reflected the sprout length data. The effect of temperature on sprout dry mass followed the same trend for both seed pieces. For the small seed pieces (3g) the sprout dry mass was greater at the higher temperature but remained fairly constant over time, while the sprout dry mass at the lower temperature was initially lower, increased with time and was similar to that at the higher temperature after 40 days. For the large seed pieces (5g) the sprout dry mass at the lower temperature was initially low but exceeded that at the higher temperature after 40 days.

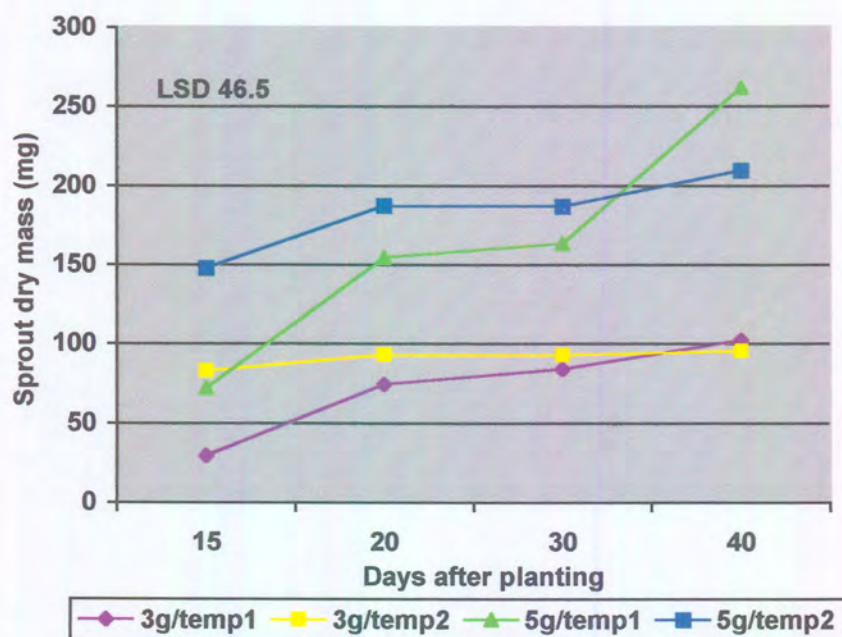


Figure 6.2 Effect of temperature, seed size and harvest date on sprout dry mass

temp 1 = 16°C

temp 2 = 26°C (Appendix Table A 6.2)

A significant interaction was found between temperature and harvest date as indicated in Table A 6.2 of the Appendix. This was due to the fact that from day 15 to day 30 the sprout dry mass at 26°C was greater than the sprout dry mass at 16°C while at last sampling date (day 40) the sprout dry mass was the highest at 16°C.

The initial increase in the sprout dry mass at the high temperature later slowed down, possibly due to exhaustion of carbohydrates reserves in the seed pieces towards the end of the experiment. The continued increase in sprout dry mass at the low temperature is at least partly due to the initial slower utilisation of seed reserves, resulting in reserves still being available for sprout growth 30 to 40 days after planting. These results corroborate with the findings of Burton (1958) who observed that sprout growth was greater at 25°C than at lower temperatures for a number of weeks before declining. The decline was attributed to the effect of limiting factors including assimilates and other growth factors such as calcium that was rapidly exhausted by the early rapid growth (Burton, 1966).

Sprout dry mass per unit length

The main effects of seed size, temperature and harvest date were highly significant but there were no significant interactions between them as indicated in Table A 6.3 of the Appendix. The results of sprout dry mass per unit length for the different treatments are presented in Figure 6.3. The sprout dry mass per unit length was greater for 5g seed pieces than for 3g seed pieces, and greater at 16°C than at 26°C, and decreased gradually over the growing period for all treatments. The sprout dry mass per unit length apparently remained constant during the last 10 days, except for 3g seed pieces grown at 26°C where the decline persisted, resulting in lower sprout dry mass per unit length (2.1 mg/cm) at the final harvest. For a specific seed piece (3 or 5g seed pieces) temperature did not affect the sprout dry mass per unit length (sprout thickness), although the sprout dry mass per unit length at 16°C tended to be somewhat higher than at 26°C.

It is difficult to compare these results with the results of other studies because sprout thickness has rarely been accurately measured. In most cases information reported on sprout

diameter was based on a simple observation or a rating system (Burton, 1966; Khedler and Ewing, 1985). Nevertheless the present results corroborate with those found by Sadler (1961) who pointed out the importance of radial over axial expansion of sprouts exposed to low temperatures.

The mechanisms by which temperature regulates the sprout growth may be explained on basis of physiological changes that occur in seed pieces during sprout development. The level of gibberellin is believed to increase in wounded seed pieces (Rappaport and Sachs, 1967). This, coupled with a report on increased activity of gibberellin under high temperature conditions (Menzel, 1983) may lend support to the hypothesis that the sprout dry mass per unit length at high temperatures results from increased activity of gibberellin, resulting in enhanced sprout elongation. The reduction in stem diameter, as indicated by low sprout dry mass per unit length, is known as a typical manifestation of the effect of high levels of gibberellins on stem growth (Timm *et al.* 1962; Hartmans and Van Es, 1979).

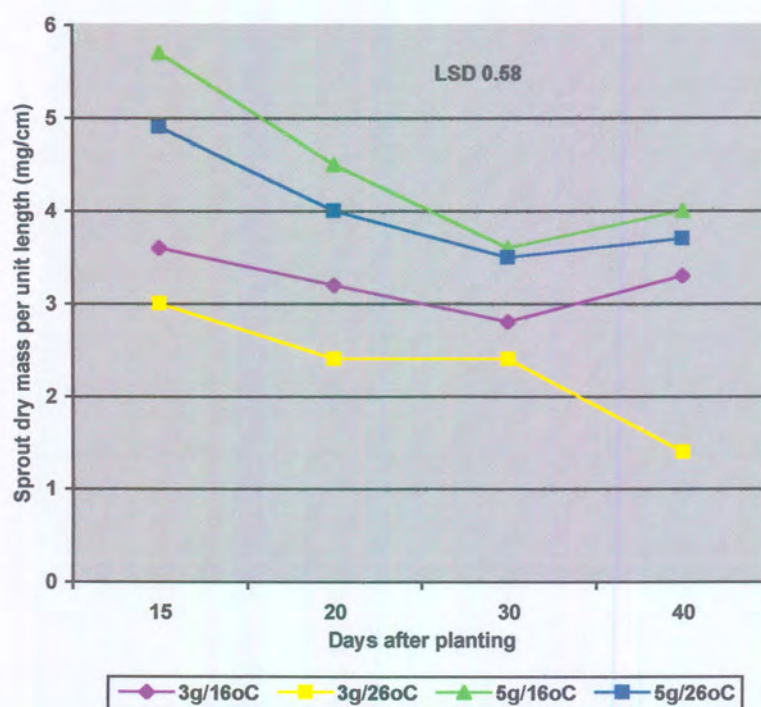


Figure 6.3 Effect of temperature, seed size and harvest date on sprout dry mass per unit length

temp 1 = 16°C

temp 2 = 26°C (Appendix Table A 6.3)

The low dry mass per unit length associated with high temperature can also be attributed to high losses of assimilates through respiration. Burton (1966) states that greater sprout development is accompanied by an increase in respiration.

In order to ascertain whether the sprout dry mass per unit length was uniform over the length of the sprout, or changed during sprout growth, this parameter was determined for the top and bottom segments of the sprouts. The effects of harvest date, seed size, temperature and segment position were highly significant as indicated in Table A 6.4 of the Appendix. For all four treatment combinations the sprout thickness (sprout dry mass per unit length) both at the top and at the bottom of the sprouts remained fairly constant with no clear trends over the observation period from 20 to 40 days after planting (Figure 6.4).

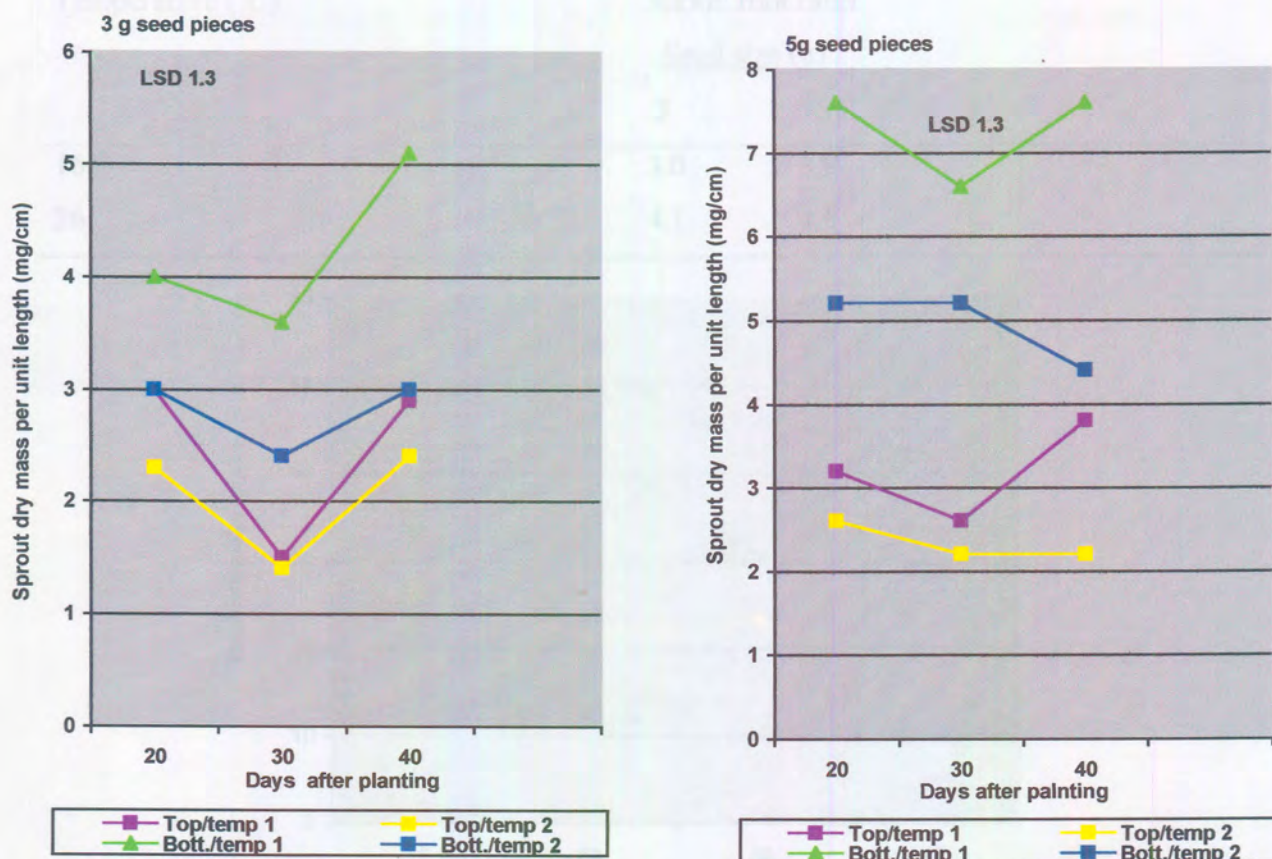


Figure 6.4 Effect of temperature, seed size and harvest date on the sprout dry mass per unit length of the top and bottom (Bott.) segments of the sprouts

temp1 = 16°C

temp 2 = 26°C (Appendix Table A 6. 4)

For the bottom sections it indicates that once a particular section of a sprout has formed little or no secondary growth occurred, neither did a net retranslocation of carbohydrates occur. The bottom segments from 5g seed pieces were significantly thicker than those from 3g seed pieces at both temperatures, indicating that even during early sprout growth when assimilates from the seed was probably not limiting, the seed size affected sprout thickness.

Temperature affected the thickness of the basal segments of the sprouts, with a significantly higher sprout dry mass per unit length at the low temperature. Thus the basal segments from 3g seed pieces grown at 16°C were similar to those of 5g seed pieces grown at 26°C. The significant interaction between temperature and segment position was due to the fact that differences in the dry mass per unit length between upper and bottom segments were higher at 16°C than at 26°C. The results indicate that during early sprout development the dry mass per unit length is affected by the amount of available assimilates, and also by temperature affecting the rate of sprout elongation. The dry mass per unit length of the apical segments of the sprouts was less affected by temperature, probably due to limited assimilates after more than 20 days of active sprout growth. The apical parts were significantly thinner than the basal segments.

Root dry mass

It was impossible to recover the roots reliably from the vermiculite substrate, and variation in root data was high. The root dry mass was affected by the seed size, but temperature and harvest date had no effect as shown in Table 6.5 of the Appendix. The root dry mass was markedly greater in plants from 5g seed pieces than in those from 3g seed pieces at all harvest samplings (Figure 6.5). The significant interaction between temperature and harvest date was due to the fact that from day 15 to day 20 the root dry mass was greater at 26°C than at 16°C while from the day 20 up to end of experiment the situation was reversed. Although sprouts were still growing (Figure 6.2) practically no root growth occurred after 15 days. This reflects investment in sprout growth to ensure emergence and plant establishment at the cost of root growth.

Deleterious effects of high temperature on development of lateral roots have been reported (Struik, Geerstema and Custers, 1989; Sattelmacher, Marschner and Kuhne, 1990). As lateral roots constitute the majority of the total root growth it may be reasonable to suggest that the reduction of the root dry mass at 26°C may be partly attributed to the inhibiting effect on lateral roots. The reduced root dry mass at high temperature resulted in high shoot: root ratios (Table 6.1). Promotion of shoot and reduction of root development for plants exposed to high temperature were also noticed in earlier studies (Sattelmacher *et al.*, 1990; Sarquis, Gonzalez and Bernal-Lugo, 1996).

Table 6.2. Effect of temperature and seed size on shoot: root ratio of plants from seed pieces of different sizes

Temperature (°C)	Shoot: root ratio	
	Seed size (g)	
	3	5
16	3.0	3.9
26	4.1	4.8

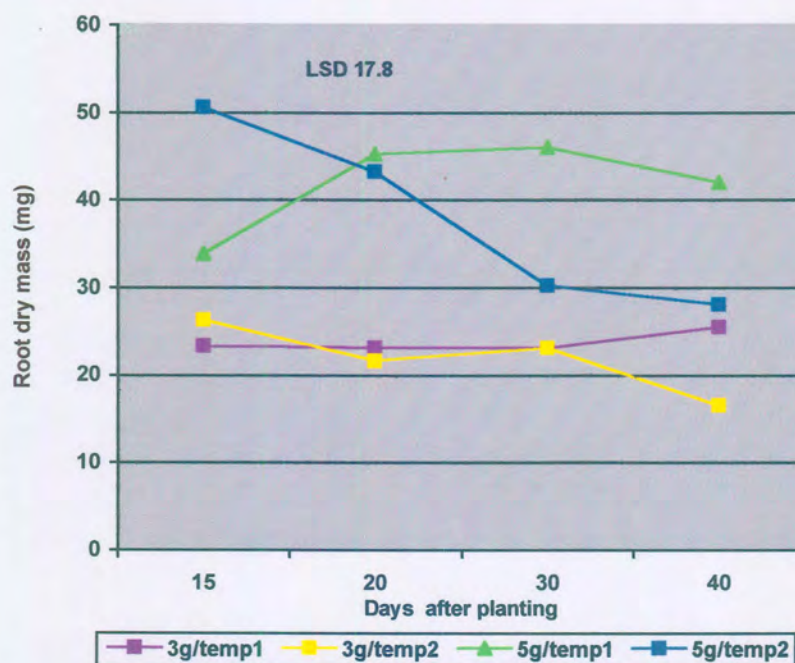


Figure 6.5 Effect of temperature, seed size and harvest date on root dry mass

temp 1 = 16°C

temp 2 = 26°C (Appendix Table A 6. 5)

Shoot plus root dry mass

The pattern of shoot plus root dry mass as illustrated by Figure 6.6 was similar to that of the sprout dry mass. The shoot plus root dry mass was initially higher in plants growing at 26°C than in those growing at 16°C. But plants kept at the low temperature recovered at later harvests, resulting in higher shoot plus root dry mass after 40 days. For the small seed pieces (3g) plants held at the low temperature had the same shoot plus root dry mass as that of plants held at the high temperature at the final harvest, while for the large seed pieces the shoot plus root dry mass was greater at low temperature after 40 days. The significant interaction between temperature and harvest date can be attributed to the fact that from day 15 to day 30 the shoot plus root dry mass was greater at 26°C than at 16°C while at the final harvest (day 40) the shoot plus root dry mass was the highest at the low temperature (16°C). A significant interaction between seed size and harvest date was also observed (Table A.6.6 of the Appendix).

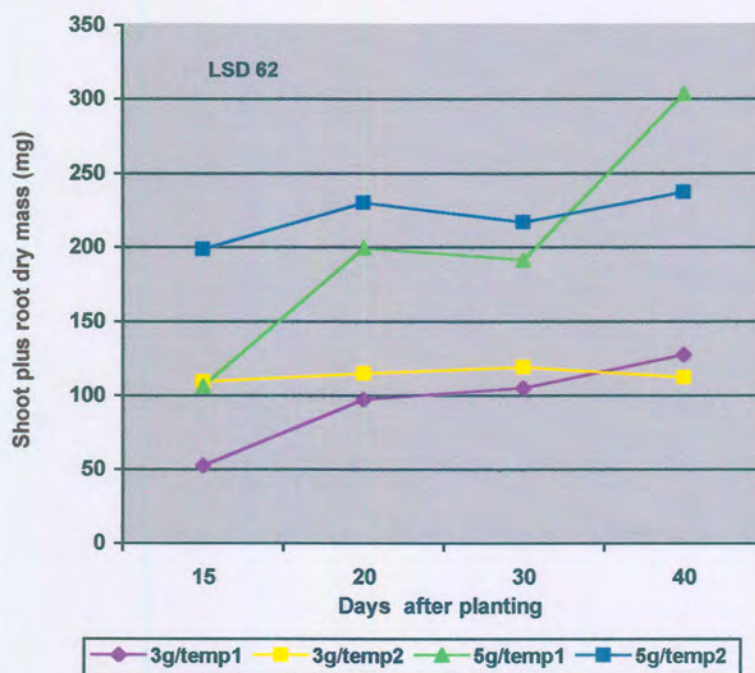


Figure 6.6 Effect of temperature, seed size and harvest date on shoot plus root dry mass

temp1 = 16°C

temp 2 = 26°C (Appendix Table A 6.6)

Utilisation of seed reserves

Some rotting occurred at the high temperature. Similar tuber decay was also observed by Khanbari and Thompson (1996) following exposure to high temperatures and was attributed to the increased levels of CO₂ and reduced levels of O₂. Due to this situation the seed residue at harvesting as well as the dry mass lost through the respiration process could not be quantified. However, the fraction of seed reserves utilised for growth was estimated by the shoot plus root dry mass expressed as percentage of initial dry mass of the seed pieces. The results are presented in Table 6.2. The fraction of reserves mobilised from the seed pieces in the low temperature treatment was the greatest for both seed sizes. At both temperatures the largest percentage of seed reserves was transferred from the large seed pieces (5g seed pieces).

Table 6.3 Sprout dry mass as percentage of original seed pieces dry mass illustrating the percentage of reserves mobilised as affected by temperature and seed size

Temperature (°C)	% of seed reserves mobilised	
	<u>Seed size (g)</u>	
	3	5
16°C	24.9	28.9
26°C	16	22.6

6.5 CONCLUSIONS

The results indicate that the increase in sprout length and sprout dry mass were slower but persisted longer at the low temperature, while the increase was more rapid but with rates declining with time at the high temperature. This suggests that low temperatures are more effective in prolonging sprout growth. The sprout thickness was also increased by low temperature. Temperature affected similarly sprout dry mass and sprout dry mass per unit length of 3 and 5g seed pieces. A small interactive effect between temperature and seed size was found on sprout length.

All characteristics of sprout growth except sprout dry mass per unit length (sprout thickness) were affected by interactive effect between temperature and harvest date, suggesting that temperature effect on sprout length and sprout dry mass should be assessed taking into consideration the time at which measurements are made.

Even though the mobilisation of seed reserves was generally greater at low than at high temperature for both seed sizes, the seed rotting that occurred towards the end of the experiment may have affected the rate at which the assimilates were mobilised.

CHAPTER 7

GENERAL DISCUSSION

The costs of inputs in potato production are very high and producers are constantly seeking ways to reduce expenditure in producing the crop. In developing countries, lack of infrastructure and absence of healthy seed programmes make local production of certified seed impossible, forcing those countries to depend on developed countries for supply of quality seed. Seed could be utilised more efficiently by using the minimum seed mass to produce acceptable stem growth. In this perspective, factors affecting sprout growth after planting and before emergence were investigated.

In this research project seed pieces of less than 10g were generally used because little information is available on the pattern of sprout development on seed pieces smaller than 10g in mass. However, larger seed pieces were included in one experiment. The experiments were conducted in temperature controlled dark rooms to simulate sprout growth before emergence. The experimental period was confined to 40 days after planting due to the fact that under field conditions the period of sprout growth between planting and emergence or the early stages of crop establishment often varies between 10 and 40 days.

In **Chapter 3** it was shown that sprouts from seed pieces of 1 and 3g in mass were of the same length as the sprouts from 8g seed pieces. For larger seed pieces the sprout length increased in proportion to seed mass up to 24g of mass, which was the optimum seed mass for sprout length 40 DAP. The sprout dry mass and sprout dry mass per unit length increased in proportion to seed mass, with the maximum values obtained with 24 g seed pieces. The bottom segments of the sprouts from large seed pieces were thicker than the bottom segments of the sprouts from small seed pieces. Small seed pieces transferred up to 82% of their seed reserves to the sprouts.

Strong elongation of the sprouts growing on small seed pieces suggests that not only assimilate were involved, but also other factors could have played a role in sprout growth. The ability of small seed pieces in transferring large fraction of assimilates for growth,

despite limited seed reserves also supports this view. Growth promoting substances are naturally present in potato tubers and regulate sprout growth (Smith, 1977; Rappaport and Wolf, 1969). It is also established that GA increase the rate of transfer of dry material from tubers to sprouts (Morris, 1967). In this investigation, gibberellin applied on seed pieces increased sprout length and sprout dry mass but decreased sprout dry mass per unit length (**Chapter 4**). GA enables sprouts to get more assimilate out of the mother tuber. The pattern of sprout growth on GA treated seed pieces resemble that of sprout development on small seed pieces, suggesting that long and thin sprouts on small seed pieces were associated with induction of wound-induced GA in small seed pieces (Rappaport and Sachs, 1967). The potential of small seed pieces to mobilise seed reserves for growth could also be associated with the stimulating effect of GA on assimilate transfer from the tuber to developing sprouts.

Calcium is essential for normal plant growth (Demarty, Morvan and Thellier, 1984) and maintenance of the structural integrity of the cell (Burstrom, 1968; Loneragan, Snowball and Simmons, 1968). In potato, calcium is requisite for growth of meristem (Dyson and Digby, 1975a). In the present study sprouts treated with high calcium concentrations were longer and had higher sprout dry mass (**Chapter 5**). Calcium applied to sprout tips enabled plants to utilise more assimilate, resulting in more sprout growth. These results may lend support to the argument that shortage of calcium in small seed pieces may be responsible for low sprout dry mass and thin sprouts. The effect of calcium as a factor of sprout growth was demonstrated.

Temperature is an important environmental factor affecting growth of potato plants (Smith, 1977). The influence of temperature on physiological processes causes noticeable effects on plant development (Poincelot, 1980). It is possible that temperature that was prevailing during experimental period affected sprout growth of seed pieces by acting on the physiological reactions involved in growth process. In previous studies, it was observed that occurrence of calcium deficiency was a function of temperature (Dyson and Digby, 1975a, 1975b). Changes in the activity of gibberellin in potato buds were associated with temperature to which tubers were subjected (Thomas and Wurr, 1976; Menzel, 1983).

The experiments clearly showed that sprout growth is a complex process with the length and thickness of the sprouts not only determined by the amount of carbohydrates available in the seed piece. Temperature affects the rate of mobilisation of reserves and also the growth rate of the sprout. Adequate evidence is presented to emphasise the role of endogenous gibberellins in sprout growth, with the long, thin sprouts typical of small seed pieces probably due to higher gibberellin activity. The possible role of calcium as a limiting factor in sprout growth was investigated because of the established importance of this element in developing sprouts (Dyson and Digby, 1975 a).

Other constituents of the seed piece (seed tuber) like amino acids, enzymes, macro and micro nutrients are obviously also involved in sprout growth, and the relative scarcity or availability of these constituents may also affect sprout length and thickness. The contribution of these factors was not investigated.

In the course of this research programme valuable information regarding the reaction of potato seed pieces to growth factors was gained. The information should contribute to expand existing knowledge on sprout growth and contribute towards better seed utilisation, especially in areas where scarcity of healthy seed is a limiting factor in potato productivity.

In the light of these findings future research should focus on field experiments aiming at:

- Establishing the best size of seed pieces to ensure optimum utilisation of stored seed material. The potential of small seed pieces in producing adequate stem growth may be considerable because 5g of tuber tissue has been shown to be sufficient for the growth of a mainstem (Hammes, 1985).
- Investigating stimulation of sprout development by means of gibberellin treatments.
- Investigating use of calcium application to sprout tips as an inexpensive mean to improve sprout growth and development.

SUMMARY

Potato (*Solanum tuberosum* L.) is one of the most important food crops worldwide and also in developing countries. Despite the growing interest in potato production, the high cost of imported certified seed remains a major constraint to potato production in developing countries (Van der Zaag and Horton, 1983). There is a need to set up strategies that can allow efficient utilisation of this expensive resource. Therefore, it is important to understand how sprout growth is affected by specific factors. In this study the emphasis was on sprout growth after planting. The objectives of this study were to:

- (1) investigate the effect of seed size on sprout growth and on the fraction of seed reserves used for sprout growth,
- (2) quantify the effect of gibberellic acid and paclobutrasol on sprout growth,
- (3) determine the effect of calcium on sprout growth and
- (4) determine the interactive effect between temperature and seed size on sprout growth.

The sprout length did not differ among seed pieces ranging from 1 to 8g in mass, while it significantly increased with seed mass for the larger seed pieces. The longest sprouts were obtained with 24g seed pieces. The sprout mass, sprout dry mass per unit length and root dry mass consistently increased with seed mass, with the maximum values for these parameters obtained with 24 g of seed pieces. For a specific seed size the sprouts were thicker at the bottom than at the apical end parts. The bottom segments of the sprouts from the large seed pieces were thicker than the bottom segments of the sprouts from the small seed pieces, indicating that even before seed reserves become limiting (at the commencement of sprout development) sprout thickness is affected by seed size. The explanation for this phenomenon remains unclear.

The fact that small seed pieces produced long and thin sprouts, and transferred a large fraction of assimilate to sprouts, despite limited seed reserves suggests that factors other than assimilate were involved in the growth process. The effect of plant growth regulators on

sprout growth was investigated by determining the effect of gibberellin and paclobutrasol on sprout growth of 3g seed pieces. Sprout growth was affected by the concentration of applied gibberellin (GA). High GA levels enhanced the rate of increase in sprout length, the absolute sprout length and the sprout dry mass, but reduced the sprout dry mass per unit length (sprout thickness). Paclobutrasol (an anti-gibberellin) inhibited sprout growth considerably. GA treated seed pieces transferred a higher percentage of seed reserves to developing sprouts compared to those treated with paclobutrasol.

The difference in sprout growth between small and large seed pieces suggests that availability of seed reserves other than carbohydrate could be a factor in sprout growth. This was examined by determining the effect of calcium availability on sprout growth. Sprout length, sprout dry mass and sprout dry mass per unit length were significantly improved by application of calcium to sprout tips at a rate of 10mmol/l as CaSO_4 . The application of a full nutrient solution produced a similar effect. The importance of calcium as a factor of sprout growth was demonstrated. However when roots were initially supplied with a complete nutrient solution, application of calcium to the sprout tips resulted in no effect in terms of sprout growth.

The interaction between temperature and seed size on sprout growth was investigated by determining the response of seed pieces of different sizes (3 and 5g) to two temperature regimes (16°C and 26°C). The pattern of sprout length as affected by temperature was similar for both 3 and 5g seed pieces, with the sprouts at the low temperature initially shorter, but reaching similar lengths as those at the high temperature after 30 and 40 days. Sprout dry mass and thickness responded similarly to temperature, with greater dry mass and thickening at 16°C than at 26°C.

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C. BUCAGU

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APPENDIX

***, **, = Significantly different from zero at $P \leq 0.05$, $P \leq 0.01$ respectively**

ns = Not significant

d.f.: Degree of freedom

Pr : Probability

Table A 3. 1 ANOVA Table for the effect of seed size and harvest date on sprout length (Experiment 1)

Source	d.f	Sum of squares	Mean square	F value	Pr > F
Model	14	11925.360	851.811	89.2**	0.0001
Error	33	315.026	9.546		
Total	47	12240.386			
	R ²	C.V.	Root MSE		Mean
	0.974	9.725	3.089		31.766
Source	d.f	Type III SS			
Size	2	28.065	14.032	1.47ns	0.2446
Dates	3	10545.416	3515.138	368.22**	0.0001
Size*Date	6	1323.754	220.625	23.11**	0.0001
Rep	3	28.123	9.374	0.98	0.4131

TABLE A 3. 2 ANOVA Table for the effect of seed size and harvest date on sprout dry mass (Experiment 1)

Source	d.f	Sum of squares	Mean square	F value	Pr > F
Model	14	483753.333	34553.809	47.47**	0.0001
Error	33	24020.666	724.899		
Total	47	507774.000			
	R ²	C.V.	Root MSE		Mean
	0.952	21.6	26.979		124.500
Source	d.f.	Type III SS			
Size	2	223218.375	111609.187	153.33**	0.0001
Date	3	167121.166	55707.055	76.53**	0.0001
Size*Date	6	91912.958	15318.826	21.05**	0.0001
Rep	3	1500.833	500.277	0.69	0.5662

TABLE A 3. 3 ANOVA Table for the effect of seed size and harvest date on sprout dry mass per unit length (Experiment 1)

Source	d.f	Sum of squares	Mean square	F value	Pr > F
Model	14	255.834	18.273	74.09**	0.0001
Error	33	8.139	0.246		
Total	47	263.974			
	R ²	C.V.	Root MSE		Mean
	0.969	12.12	0.496		4.095
Source	d.f.	Type III SS			
Size	2	241.139	120.569	488.81 **	0.0001
Date	3	7.466	2.488	10.09 **	0.0001
Size*Date	6	5.985	0.997	4.04 **	0.0038
Rep	3	1.244	0.414	1.68	0.1899

Table A 3. 4 ANOVA Table for the effects of seed size on sprout dry mass per unit length at specific sprout lengths (Experiment 1)

Source	d.f	Sum of squares	Mean square	F value	Pr > F
Model	14	223.208	15.943	14.91**	0.0001
Error	33	35.290	1.069		
Total	47	258.499			
	R ²	C.V.	Root MSE		Mean
	0.863	22.832	1.034		4.529
Source	d.f.	Type III SS			
Size	2	200.001	100.000	93.51**	0.0001
Length	3	6.934	2.311	2.16ns	0.1113
Size*Length	6	6.263	1.043	0.98ns	0.4567
Rep	3	10.009	3.336	3.12	0.0391

TABLE A 3. 5 ANOVA Table for the effect of seed size and harvest date on root dry mass (Experiment 1)

Source	d.f	Sum of squares	Mean square	F value	Pr > F
Model	14	38563.833	2754.559	104.511**	0.0001
Error	33	868.083	26.305		
Total	47	39431.916			
	R ²	C.V.	Root MSE		Mean
	0.977	10.750	5.128		47.708
Source	d.f.	Type III SS			
Size	2	32342.041	16171.020	614.74**	0.0001
Date	3	2519.583	839.861	31.93**	0.0001
Size*Date	6	3346.791	557.798	21.20**	0.0001
Rep	3	355.416	118.472	4.50	0.0094

TABLE A 3. 6 ANOVA Table for the effect of seed size and harvest date on shoot plus root dry mass (Experiment 1)

Source	d.f	Sum of squares	Mean square	F value	Pr > F
Model	14	757318.125	54094.151	70.05**	0.0001
Error	33	25481.687	772.172		
Total	47	782799.812			
	R ²	C.V.	Root MSE		Mean
	0.967	16.114	27.787		172.137
Source	d.f.	Type III SS			
Size	2	424647.125	212323.562	274.97**	0.0001
Date	3	205320.729	68440.243	88.63**	0.0001
Size*Date	6	124296.208	20716.034	26.83**	0.0001
Rep	3	3054.062	1018.020	1.32	0.2849

TABLE A 3. 7 ANOVA Table for the effect of seed size and harvest date on sprout length (Experiment 2)

Source	d.f	Sum of squares	Mean square	F value	Pr > F
Model	26	90702.281	3488.549	24.8**	0.0001
Error	113	15863.063	140.381		
Total	139	106565.345			
	R ²	C.V.	Root MSE		Mean
	0.821	18.046	11.848		65.655
Source	d.f	Type III SS			
Size	3	14126.030	2224.969	33.54**	0.0001
Dates	1	63926.984	63926.984	455.38**	0.0001
Size*Date	3	2130.642	710.214	5.06**	0.0025
Rep	19	3427.690	180.404	1.29	0.2072

TABLE A 3. 8 ANOVA Table for the effect of seed size and harvest date on sprout dry mass (Experiment 2)

Source	d.f	Sum of squares	Mean square	F value	Pr > F
Model	26	42971907.102	1652765.657	21.63**	0.0001
Error	113	8636288.863	76427.335		
Total	139	51608195.965			
	R ²	C.V.	Root MSE		Mean
	0.832	31.858	276.454		867.755
Source	d.f	Type III SS			
Size	3	18872504.189	6290834.729	82.31**	0.0001
Date	1	18351663.681	18351663.681	240.12**	0.0001
Size*Date	3	5133106.235	1711035.411	22.39**	0.0001
Rep	19	829890.911	43678.469	0.57	0.9194



TABLE A 3. 9 ANOVA Table for the effect of seed size and harvest date on sprout dry mass per unit length (Experiment 2)

Source	d.f	Sum of squares	Mean square	F value	Pr > F
Model	26	2526.855	97.186	10.25**	0.0001
Error	113	1071.037	9.478		
Total	139	3597.882			
	R ²	C.V.	Root MSE		Mean
	0.702	24.996	3.078		12.316
Source	d.f.	Type III SS			
Size	3	2173.204	724.401	76.43**	0.0001
Date	1	211.391	211.391	22.30**	0.0001
Size*Date	3	148.224	49.408	5.21**	0.0021
Rep	19	101.073	5.319	0.56	0.9258

Table A 3. 10 ANOVA Table for the effect of seed size and harvest date on the sprout dry mass per unit length in top and bottom segments of the sprout (Experiment 2)

Source	d.f	Sum of squares	Mean square	F value	Pr > F
Model	18	2565.377	142.520	105.55**	0.0001
Error	45	60.760	1.350		
Total	63	2626.137			
	R ²	C.V.	Root MSE		Mean
	0.976	9.500	1.161		12.231
Source	d.f.	Type III SS			
Size	3	756.567	252.189	186.78**	0.0001
Position	1	1524.902	1524.902	1129.37**	0.0001
Date	1	49.702	49.702	36.50**	0.0001
Size*Position	3	147.847	49.282	36.50**	0.0001
Position*Date	1	42.902	42.902	31.77**	0.0001
Size* Date	3	2.887	0.962	0.71	0.5495
Rep	3	4.040	1.346	1.00	0.4028

Table A 3. 11 ANOVA Table for the effect of seed size on distribution of sprout dry mass per unit length in the sprout (Experiment 2)

Source	d.f	Sum of squares	Mean square	F value	Pr > F
Model	22	1943.700	88.350	55.65**	0.0001
Error	57	90.500	1.587		
Total	79	2034.200			
	R ²	C.V.	Root MSE		Mean
	0.955	11.613	1.260		10.850
Source	d.f.	Type III SS			
Size	3	678.700	226.233	142.49**	0.0001
Position	4	1014.575	253.643	159.75**	0.0001
Size*Position	12	220.425	18.368	11.57**	0.0001
Rep	3	30.000	10.000	6.30	0.0009

TABLE A 3. 12 ANOVA Table for the effect of seed size and harvest date on root dry mass (Experiment 2)

Source	d.f	Sum of squares	Mean square	F value	Pr > F
Model	26	320837.333	12339.897	6.20**	0.0001
Error	113	224818.066	1989.540		
Total	139	545655.400			
	R ²	C.V.	Root MSE		Mean
	0.587	34.127	44.604		130.700
Source	d.f.	Type III SS			
Size	3	264806.600	88268.866	44.37**	0.0001
Date	1	15916.033	15916.033	8.00**	0.0001
Size*Date	3	24638.600	8212.866	4.13**	0.0081
Rep	19	35090.066	1846.845	0.93	0.5500

TABLE A 3. 13 ANOVA Table for the effect of seed size and harvest date on shoot plus root dry mass (Experiment 2)

Source	d.f	Sum of squares	Mean square	F value	Pr > F
Model	26	47379134.732	1822274.412	20.21**	0.0001
Error	113	10187816.174	90157.665		
Total	139	57566950.907			
	R ²	C.V.	Root MSE		Mean
	0.823	30.337	300.262		989.745
Source	d.f.	Type III SS			
Size	3	22703136.473	7567712.157	83.94**	0.0001
Date	1	18745917.008	18745917.008	207.92**	0.0001
Size*Date	3	5440889.210	1813629.736	20.12**	0.0001
Rep	19	1321139.816	62405.994	0.69	0.8199

TABLE A 4. 1 ANOVA Table for the effect of gibberellin and paclobutrasol on sprout length

Source	d.f	Sum of squares	Mean square	F value	Pr > F
Model	10	7038.870	703.887	61.34 **	0.0001
Error	24	275.399	11.474		
Total	34	7314.269			
	R ²	C.V.	Root MSE		Mean
	0.962	16.767	3.387		20.202
Source		Type III SS			
Treatment	6	6897.277	1149.546	100.18**	0.0001
Rep	4	141.592	35.398	3.08	0.0349

TABLE A 4. 2 ANOVA Table for the effect of gibberellin and paclobutrasol on root length

Source	d.f	Sum of squares	Mean square	F value	Pr > F
Model	10	8882998.385	888299.838	2.12 ns	0.064
Error	24	10064812.722	419367.196		
Total	34	18947811.107			
	R ²	C.V.	Root MSE		Mean
	0.468	49.612	647.585		1305.291
Source		Type III SS			
Treatment	6	6493492.063	1082248.677	2.58 ns	0.045
Rep	4	2389506.321	597376.580	1.42	0.256

TABLE A 4. 3 ANOVA Table for the effect of gibberellin and paclobutrasol on root surface area

Source	d.f	Sum of squares	Mean square	F value	Pr > F
Model	10	678777107.142	67877710.714	1.81ns	0.1131
Error	24	89958339.599	3748264.150		
Total	34	157835446.742			
	R ²	C.V.	Root MSE		Mean
	0.430	46.974	1936.043		4121.485
Source		Type III SS			
Treatment	6	48315487.542	8052581.257	2.15ns	0.0846
Rep	4	19561619.599	4890404.900	1.30	0.2965

TABLE A 4. 4 ANOVA Table for the effect of gibberellin and paclobutrasol on sprout dry mass

Source	d.f	Sum of squares	Mean square	F value	Pr > F
Model	10	37837.828	3783.782	10.58**	0.0001
Error	24	8585.314	357.721		
Total	34	46423.142			
	R ²	C.V.	Root MSE		Mean
	0.815	22.326	18.913		84.714
Source		Type III SS			
Treatment	6	35521.542	5920.257	16.55**	0.0001
Rep	4	2316.285	579.071	1.62	0.2020

TABLE A 4. 5 ANOVA Table for the effect of gibberellin and paclobutrasol on root dry mass

Source	d.f	Sum of squares	Mean square	F value	Pr > F
Model	10	1617.142	161.714	1.46ns	0.214
Error	24	2657	110.714		
Total	34	4274.285			
	R ²	C.V.	Root MSE		Mean
	0.378	29.940	10.522		35.142
Source		Type III SS			
Treatment	6	1314.285	219.047	1.98ns	0.1087
Rep	4	302.857	75.714	0.68	0.6100

Table A 5. 1 ANOVA Table for the effect of calcium as CaSO₄, CaCO₃ and full nutrient solution on sprout length

Source	df	Sum of Square	Mean Square	F value	Pr > F
Model	12	1020.885	85.073	7.84**	0.0001
Error	35	379.844	10.852		
Corrected Total	47	1400.730			
R-Square		C.V.	Root MSE		V mean
0.728		11.651	3.294		28.5
Source	DF	Type III SS			
Treatment	7	911.353	130.193	12.0**	0.0001
Rep	5	109.532	21.906	2.02	0.1001

Table A 5. 2 ANOVA Table for the effect of calcium as CaSO₄, CaCO₃ and full nutrient solution on sprout dry mass

Source	DF	Sum of Square	Mean Square	F value	Pr > F
Model	12	91120.481	7593.373	36.53**	0.0001
Error	35	7274.553	207.844		
Corrected Total	47	98395.034			
R-Square		C.V.	Root MSE		V mean
0.926		10.877	14.416		132.732
Source	DF	Type III SS			
Treatment	7	88927.913	12703.973	61.12**	0.0001
Rep	5	2192.668	438.533	2.11	0.0870

Table A 5. 3 ANOVA Table for the effect of calcium as CaSO₄, CaCO₃ and full nutrient solution on sprout dry mass per unit length

Source	DF	Sum of Square	Mean Square	F value	Pr > F
Model	12	52.373	4.364	10.58**	0.0001
Error	35	14.444	0.412		
Corrected Total	47	66.818			
R-Square		C.V.	Root MSE		V mean
0.783		13.834	0. 642		4.643
Source	DF	Type III SS			
Treatment	7	47.446	6.778	16.42**	0.0001
Rep	5	4.926	0.985	2.39	0.0570

Table A 5. 4 ANOVA Table for the effect of calcium as CaSO₄, CaCO₃ and full nutrient solution on root dry mass

Source	DF	Sum of Square	Mean Square	F value	Pr > F
Model	12	751.625	62.635	1.03ns	0.442
Error	35	2123.804	60.680		
Corrected Total	47	2875.429			
R-Square		C.V.	Root MSE		V mean
0.261		25.018	7.789		31.135
Source	DF	Type III SS			
Treatment	7	481.901	68.843	1.13ns	0.364
Rep	5	269.723	53.944	0.89	0.498

Table A 5. 5 ANOVA Table for the effect of calcium as CaSO₄, CaCO₃ and full nutrient solution on shoot plus root dry mass

Source	DF	Sum of Square	Mean Square	F value	Pr > F
Model	12	97011.455	8084.287	28.50**	0.0001
Error	35	9926.768	283.621		
Corrected Total	47	106938.224			
R-Square		C.V.	Root MSE		V mean
0.907		10.264	16.841		163.843
Source	DF	Type III SS			
Treatment	7	94328.629	13475.518	47.51**	0.0001
Rep	5	2682.826	536.565	1.89	0.1200

Table A 5. 6 ANOVA Table for the effect of calcium and growing medium on sprout length.

Source	DF	Sum of Square	Mean Square	F value	Pr > F
Model	18	3345.961	185.886	4.26**	0.0001
Error	45	1962.156	43.603		
Corrected Total	63	5308.117			
R-Square		C.V.	Root MSE		V mean
0.630		22.04	6.603		29.956
Source	DF	Type III SS			
Treatment	3	2796.423	932.141	21.38**	0.0001
Rep	15	549.537	36.635	0.84	0.6300

Table A 5. 7 ANOVA Table for the effect of calcium and growing medium on sprout dry mass

Source	DF	Sum of Square	Mean Square	F value	Pr > F
Model	18	273094.906	15171.939	6.31**	0.0001
Error	45	108261.203	2405.804		
Corrected Total	63	381356.109			
R-Square		C.V.	Root MSE		V mean
0.716		27.762	49.049		176.671
Source	DF	Type III SS			
Treatment	3	221408.046	73802.682	30.68**	0.0001
Rep	15	51686.859	3445.790	1.43	0.1739

Table A 5. 8 ANOVA Table for the effect of calcium and growing medium on sprout dry mass per unit length.

Source	DF	Sum of Square	Mean Square	F value	Pr > F
Model	18	101.622	5.645	2.70**	0.0036
Error	45	94.126	2.091		
Corrected Total	63	195.749			
R-Square		C.V.	Root MSE		V mean
0.519		25.499	1.446		5.671
Source	DF	Type III SS			
Treatment	3	60.273	20.091	9.61**	0.0001
Rep	15	41.349	2.756	1.32	0.2316



Table A 5. 9 ANOVA Table for the effect of calcium and growing medium on root dry mass.

Source	DF	Sum of Square	Mean Square	F value	Pr > F
Model	18	994.875	55.270	0.94ns	0.5402
Error	45	2648.875	58.863		
Corrected Total	63	3643.750			
R-Square		C.V.	Root MSE		V mean
		20.357	7.672		37.687
Source	DF	Type III SS			
Treatment	3	461.125	153.708	2.61ns	0.0629
Rep	15	533.750	35.583	0.60	0.8555

Table A 5. 10 ANOVA Table for the effect of calcium and growing medium on shoot plus root dry mass.

Source	DF	Sum of Square	Mean Square	F value	Pr > F
Model	18	300442.250	16691.236	7.00**	0.0001
Error	45	106905.687	2375.681		
Corrected Total	63	394922.152			
R-Square		C.V.	Root MSE		V mean
		22.726	48.740		214.468
Source	DF	Type III SS			
Treatment	3	240597.312	80199.104	33.76**	0.0001
Rep	15	59844.937	3989.662	1.68	0.0905

Table A 6. 1 ANOVA Table for the effect of temperature, seed size and harvest date on sprout length

Source	DF	Sum of square	Mean square	F value	Pr>F
Model	19	16699.171	878.903	25.03**	0.0001
Error	60	2106.468	35.107		
Corrected total	79	18805.639			
R-Square		C.V.	Root MSE		V Mean
0.887		16.556	5.925		35.788
Source	Type III SS				
Temp	1	2079.780	2079.780	59.24**	0.0001
Size	1	3136.260	3136.260	89.33**	0.0001
Date	3	8892.740	2964.246	84.43**	0.0001
Temp*Size	1	162.165	162.165	4.62*	0.0357
Temp*Date	3	967.440	322.480	9.19**	0.0001
Size*Date	3	1172.920	390.973	11.14**	0.0001
Rep	7	167.599	23.942	0.681	0.4370

Table A 6. 2 ANOVA Table for the effect of temperature, seed size and harvest date on sprout dry mass

Source	DF	Sum of square	Mean square	F value	Pr>F
Model	19	291484.240	15341.275	22.38**	0.0001
Error	60	41125.556	685.425		
Corrected total	79	346685.421			
R-Square		C.V.	Root MSE		V Mean
0.876		20.582	26.180		127.156
Source	Type III SS				
Temp	1	7459.453	7459.453	10.88**	0.0016
Size	1	166303.203	166303.203	242.6**	0.0001
Date	3	71606.059	23868.686	34.82**	0.0001
Temp*Size	1	9.453	9.453	0.01ns	0.9069
Temp*Date	3	22392.759	7464.253	10.89**	0.0001
Size*Date	3	17436.909	5812.303	8.48**	0.0001
Rep	7	2533.343	633.335	0.52	0.4561

Table A 6. 3 ANOVA Table for the effect of temperature, seed size and harvest date on sprout dry mass per unit length

Source	DF	Sum of square	Mean square	F value	Pr>F
Model	19	67.694	3.562	8.06**	0.0001
Error	60	26.529	0.442		
Corrected total	79	94.223			
R-Square		C.V.	Root MSE		V Mean
0.718		18.515	0.664		3.591
Source	Type III SS				
Temp	1	7.021	9.522	15.88**	0.0002
Size	1	37.950	43.512	85.83**	0.0001
Date	3	16.516	5.505	12.45**	0.0001
Temp*Size	1	0.465	0.465	1.05ns	0.3092
Temp*Date	3	0.885	0.295	0.67ns	0.5753
Size*Date	3	2.844	0.948	2.14ns	0.1040
Rep	7	1.378	0.196	0.44	0.7243

Table A 6. 4 ANOVA Table for the effect of temperature, seed size and harvest date on sprout dry mass per unit length of the top and bottom segments of the sprouts

Source	DF	Sum of square	Mean square	F value	Pr>F
Model	16	357.376	22.336	30.72**	0.0001
Error	103	74.883	0.727		
Corrected total	119	432.259			
R-Square		C.V.	Root MSE		V Mean
0.826		22.742	0.852		3.749
Source	Type III SS				
Temp	1	44.286	44.286	60.92**	0.0001
Size	1	80.524	80.524	110.7**	0.0001
Date	2	14.652	7.326	10.08**	0.0001
Position	1	154.552	154.552	216.7**	0.0001
Temp*Size	1	1.850	1.850	2.54ns	0.1137
Temp*Date	2	6.195	3.097	4.26*	0.0167
Temp*Position	1	20.254	20.254	27.86**	0.0001
Size*Date	2	1.896	0.948	1.30ns	0.2759
Size*position	1	25.668	25.668	5.31**	0.0001
Rep	7	4.497	1.124	1.55	0.1944

Table A 6. 5 ANOVA Table for the effect of temperature, seed size and harvest date on root dry mass

Source	DF	Sum of square	Mean square	F value	Pr>F
Model	19	9077.603	477.768	4.61**	0.0001
Error	60	6219.268	103.654		
Corrected total	79	15296.871			
R-Square		C.V.	Root MSE		V Mean
0.593		32.456	10.181		31.368
Source	Type III SS				
Temp	1	89.253	89.253	0.86ns	0.3572
Size	1	5788.503	5788.503	55.84**	0.0001
Date	3	394.234	131.411	1.27ns	0.2936
Temp*Size	1	53.628	53.628	0.52ns	0.4748
Temp*Date	3	1205.959	401.986	3.88*	0.0133
Size*Date	3	193.609	64.536	0.62ns	0.6031
Rep	7	573.231	81.890	0.79	0.2508

Table A 6. 6 ANOVA Table for the effect of temperature, seed size and harvest date on shoot plus root dry mass

Source	DF	Sum of square	Mean square	F value	Pr>F
Model	19	353725.250	18617.118	15.32**	0.0001
Error	60	72897.500	1214.958		
Corrected total	79	426622.750			
R-Square		C.V.	Root MSE		V Mean
0.829		22.148	34.856		157.375
Source	Type III SS				
Temp	1	7605.000	7605.000	6.2*	0.0151
Size	1	224720.000	224720.000	184.9**	0.0001
Date	3	62371.750	20190.583	17.1**	0.0001
Temp*Size	1	31.250	31.250	0.03ns	0.8731
Temp*Date	3	33472.200	11157.400	9.18**	0.0001
Size*Date	3	16928.000	5642.666	4.64**	0.0055
Rep	7	3396.500	485.214	0.39	0.6957