Utilization of Tissue Culture Techniques in a Seed Potato Tuber Production Scheme

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This work is dedicated to those who are trying for human and humanity

ABSTRACT

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Recently, rapid multiplication systems have been developed in potato seed tuber production to provide large quantities of plantlets, microtubers and minitubers of high quality. Microtubers, plantlets and minitubers are high quality starting materials that can be produced year round in in vitro conditions (microtubers, plantlets) or ex vivo conditions (minitubers) at a high density.

In Iran, the genetic quality, the sanitary quality, and the physiological quality of seed potatoes need to be improved. The objectives of this PhD programme were to contribute to the improvement of seed tuber quality by (i) identifying the role of different types of propagules in the system, (ii) investigating the effects of environmental conditions and growth regulators on the performance of these different propagules and (especially) their progeny, and (iii) exploring the options to manipulate quality of propagules at the end of the production scheme (minitubers or normal seed tubers) through treatments during storage (either storage temperature or chemical treatment).

Effects of temperature during two phases per period of 24 hours (Phase 1 and Phase 2) on microtuber production were studied. Number and size of microtubers and also status of bud were affected to some extent by temperatures during both phases. More microtubers with larger size were obtained at lower temperatures than at higher temperatures. This was observed for temperatures during both phases. There was no effect of temperature during any of the two phases on the weight of the microtubers. Effects of diurnal fluctuations in temperature during the in vitro phase on microtuber production were surprisingly small.

In vitro plantlet characteristics such as plantlet height, number of branches, leaf area, number of leaves, root number and length of roots can be affected by growth regulator application in vitro. Fewer branches were related to plantlets treated with higher doses of chlormequat; the highest dose of CCC also resulted in short plantlets with only a few leaves. The highest dose of CCC and the highest dose of gibberellic acid both reduced leaf area compared to the control. Shortest root system was found for plantlets grown on the medium with the highest doses of CCC but root numbers were increased by gibberellic acid.

Growth regulator application significantly influenced number and size of microtubers: nodal cuttings grown on the media with gibberellic acid produced lowest numbers of microtubers with smallest size. Yet, there was no significant direct effect of growth regulator on the weight of microtubers. The three cultivars tested gave similar values for number, size and weight of microtubers. Growth regulator application during in vitro plantlet production had a highly significant after-effect on

the number, size and weight of microtubers. Nodal cuttings taken from plantlets grown on the medium with gibberellic acid produced highest numbers of microtubers with largest sizes and highest weights. Highest and lowest dose of gibberellic acid showed similar after-effects on the number, size and weight of microtubers. However, also the untreated control performed well. After-effects of three doses of CCC resulted in lowest values for number, size and weight of microtubers. Temperature during the in vitro phase significantly affected above ground dry matter, size and weight of minitubers, and weight of sprouts of minitubers produced in the greenhouse, but no effects of temperature during the in vitro phase on the plant height, number of minitubers, the number of sprouts or the length of the longest sprout were found. Highly significant effects of photoperiod on the above ground dry matter, the height of the plant, and the number, size, individual weight and yield of minitubers were observed but there was no effect of photoperiod during the in vitro phase on the sprouting behaviour of the minitubers as reflected by the number, length and weight of sprouts.

When in vitro plantlets were produced at different combinations of temperature and photoperiod, above ground dry matter of the transplants grown in the greenhouse was higher with the lowest temperature than with the higher temperatures while longer photoperiods resulted in higher above ground dry matter than the shortest photoperiod. Temperature during the in vitro phase did not significantly affect height of potato plants but increasing photoperiod resulted in taller potato plants; shortest photoperiod resulted in the shortest potato plants. Number of minitubers produced was not affected by temperature during the in vitro phase but longer photoperiods resulted in more minitubers than the shortest photoperiod. Lowest in vitro temperature and longer photoperiods gave larger minitubers with higher average weight. Although temperature and photoperiod during the in vitro plantlet production could affect minituber production in the greenhouse, no effect was observed on the growth vigour and performance of minitubers produced from these plantlets in the greenhouse.

Storage temperature of 8 ^{o}C resulted in higher minituber weight losses (both relative weight loss and absolute weight loss) than other storage temperatures tested. During the storage larger minitubers showed higher weight losses than smaller minitubers while relative weight loss was higher with smaller minitubers than with larger minitubers. The higher ratio between surface area and tuber volume in smaller minitubers caused higher evaporation and subsequently higher weight loss in smaller minitubers.

Growth regulator application during storage influenced the effect of storage temperature on minituber sprouting. Before growth regulator application more sprouts with larger size and higher weight were found for the storage temperature of 12 ^{o}C while after growth regulator application highest values for number, length and weight of sprouts were observed at a storage temperature of 8 ^{o}C. Larger minitubers produced more sprouts with larger size and highest weight. The effect of growth regulators on the tuber sprouting was highly significant, a concentration of 5 mg/l gibberellic acid gave highest number of sprouts. Gibberellic acid enhanced length and weight of sprouts, but the sprout

weight of the minitubers was also increased by a low dose of CCC. The results were very complicated. Two-way and three-way interactions of storage temperature, tuber size, growth regulators and cultivars on the tuber sprouting were statistically significant. Some parameters of tuber growth vigour assessed by planting the minitubers in the greenhouse were also affected by storage temperature, minituber size and growth regulator application during the storage phase.

At the end of the project, we tested effects of growth regulators applied to normal seed tubers. Three tuber sizes (35 – 45 mm, 45 – 55 mm and 55 – 65 mm) of three potato cultivars (Frieslander, Marfona and Santé) were treated with GA (2.5 mg/l and 5 mg/l) and CCC (10 mg/l and 100 mg/l); also two controls, tubers cut and tubers uncut, were used. The sprouting behaviour showed some interesting results. GA had different effects with different cultivars. Frieslander produced more sprouts, but not longer sprouts, while Marfona produced longer sprouts, but not more sprouts in response to GA. The growth vigour was also affected by the growth regulators, but also in a different way with different cultivars. GA increased the days of growth with Marfona and Santé, but not with Frieslander. Consistent with its effects on number and length of sprouts of the different cultivars, GA let Frieslander produce more stems, but not longer stems, while it let Marfona produce longer stems, but not more stems. Only Marfona produced more foliage with GA. Tuber yield also showed a difference among the cultivars when treated with GA. Frieslander produced more tubers and tuber yield, while Marfona and Santé produced fewer tubers and lower tuber yield after GA application.

CCC did not have a large influence on the growth vigour. Only Santé got faster and better emergence and a few more days of growth in response to CCC.

This thesis has illustrated that it is important to test after-effects of treatments applied during in vitro production of plantlets on the performance of the propagules produced and their progenies. It is also important to realize that the response of in vitro propagules to environmental conditions is not always similar to what can be found for plants from normal seed tubers grown under field conditions. The thesis has also illustrated that there are significant and complicate interactions between storage temperature, growth regulator application and cultivar, both for minitubers and normal seed tubers. Such interactions provide opportunities for cultivar-specific manipulation of performance of propagules, but also complicate the design of general strategies to increase the performance of a seed production scheme.

Key words: Potato, *Solanum tuberosum* L., in vitro plantlet, nodal cutting, microtuber, minituber, in vitro tuberization, seed tuber, photoperiod, day temperature, night temperature, temperature amplitude, storage temperature, after-effect, growth regulator, gibberellic acid, CCC, sprout behaviour, vigour.

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Mahmoud Otroshy
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Chapter 1

General introduction

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The potato crop

Potato (*Solanum tuberosum* L.) or Irish potato or white potato is an annual crop from the genus *Solanum* (family Solanaceae) (Khurana et al., 2003), originated in the Andes near the border of Peru and Bolivia in South America (Ahmad, 1977; Pushkarnath, 1978; Horton, 1987; Rowe, 1993). It is introduced from South America into Europe at the end of the 16th century, first to Spain and then into England. After the introduction into Europe it travelled from Europe to Asia and other countries throughout the world (Harris, 1992). Over one billion people in different countries consume potatoes (Khurana et al., 2003). Potato consumption is increasing and it has doubled every 10 to 15 years (CIP, 1984).

Bauhin in 1596 gave the Latin name *Solanum tuberosum* to the species introduced into Europe and Linnaeus in 1753 confirmed that (Ahmad, 1977; Tadesse, 2000). Potato is also called batata and patata in Latin America, papa in the Queehua language, Aaloo in Hindi, Alu or Bilati Alu in Bengali, Alooguti in Assamese and Bilati Alu in Oriya (Ahmad, 1977) and Sibe-Zamini (meaning earth apple) in Persian.

Different species (including *Solanum tuberosum* L., *S. ajanhuiri, S. curtilobum, S. caucha, S. goniocalyx, S. phureja, S. juzepczukii,* and *S. stenotomum*) are recognized as potato species (Struik & Wiersema, 1999) and over 230 wild potato species are known (Harris, 1992).

Potato is a short-day plant (Went, 1957), a cool season crop (Ewing, 1981) and a C3 plant with a low light saturation point (Demagante & Vander Zaag, 1988). It is known as a herbaceous, succulent, dicotyledonous plant with alternate stolons underground and alternate leaves on the stem above ground; stems are about 30 – 100 cm tall. A potato plant can have three kinds of stems including sprouts (leafy stems), stolons and tubers. Tubers are underground, fleshy stems with eyes and they are suitable for ware, food processing, seed, animal feed and non-industrial use (Beukema & Van der Zaag, 1979; Struik & Wiersema, 1999).

A potato crop, grown from seed tubers goes through five different stages: sprout development stage, vegetative growth stage, tuber initiation stage, tuber bulking stage and finally the maturation stage (Rowe, 1993).

Trends in potato production

Potato is one of the most important food crops, cultivated in many countries in different climatic zones, including temperate, tropical and sub-tropical regions. At present potato is the fourth most important food crop in the world (Li, 1985), after wheat, rice and maize, with – in 2003 – a harvested area of 18,897,000 ha and a production of 311 million tons (CIP website at http:// www.cipotato.org; FAO, 2003). In the period 1995 – 1997, the total potato area was 18,381,000 ha and China with 3,489,000 ha, Russia with 3,389,000 ha and Poland with 1,390000 ha had the largest potato areas (Struik & Wiersema, 1999). In year 2002 and 2003, the harvested area in China had even increased to 4,402,000 and 4,502,000 ha, respectively (FAO, 2002, 2003).

In the first half of the last century 90% of world potato was produced by Europe and USSR (Horton, 1987). In the world during the period 1995 – 1997 average potato yield was about 16 Mg/ha fresh tubers, and the Netherlands with 38 t/ha and Malawi with 7 t/ha produced the highest and lowest potato yields per hectare, respectively (Struik & Wiersema, 1999). In the period 1997 – 2003 average potato productivity was 15.88 Mg/ha and the highest yields (42.75 Mg/ha) were obtained in New Zealand (in Oceania) and the lowest yields (2000 kg/ha) were found in Swaziland in Africa (FAO, 2002; Khurana et al., 2003).

World annual consumption of potato per capita in the period of 1994 – 1996 was 28 kg and it was highest in Poland (136 kg per year) and lowest (10 kg per year) in Bangladesh (Struik & Wiersema, 1999). Potato consumption in Asia in 1991 – 1992 was 12 kg/capita. In developing countries the consumption in the period 1961 – 1963 was 9 kg/capita but it increased to 14 kg/capita in developing countries in 1994 – 1996 (CIP website at http//www.cipotato.org/potato/potato.htm).

Recently the demand for processed potato products in the international markets has increased. The main reasons for this increase are increasing income, more working women, urbanization, and rapidly expanding tourism (Khurana et al., 2003). According to a CIP report (see

http://www.cipotato.org/market/potatofacts/growprod.htm) annual growth rate for potato production for the period 1993 – 2020 will be about 2.71% and potato will be an important food crop in the food basket of developing countries in the future. During this period an increase by 40% in worldwide demand for potato is expected.

Potato as a food source and a raw material

Potato can be used as human food or animal feed, but also for seed tuber production and industrial use; the food processing industry uses potatoes to produce crisps, French fries, flakes, and canned potato, whereas the non-food industry uses potatoes to produce starch, alcohol, etc. (Struik & Wiersema, 1999; Khurana et al., 2003). Before World War II, in western European countries about 50% of the potato was used as animal feed but this proportion has decreased to less than 20% in the mid-1980s (Horton, 1987) and to well below that in recent years. In 2000 59.8 % of potato was used as food, 14.5% as feed, 11% as seed, 4.3% for processing in the food industry, 8% as waste, and 2.2% for other industrial uses (Khurana et al., 2003). Potato is also used for starch production especially in the Netherlands, Japan and Eastern Europe (Beukema & Van der Zaag, 1990) and it can also be used for alcohol production. Potato starch is the basis for many special products: starch can be used for thousands of products that people use daily around the world. For example, starch is one of the most important inputs in the paper and board industry (Avebe website at www.avebe.com).

According to Talburt & Smith (1967) white potato tubers contain 77.5% water and 22.5% solids such as: protein 2.0%, carbohydrates (with 0.6% crude fibre) 19.4%, fat 0.1% and ash 1.0%. Potato contains important mineral elements such as: iron 0.01%, sulphur 0.15%, magnesium about 0.1%, calcium 0.05%, potassium, boron, copper, silicon, manganese, iodine and fluorine which are needed for health (Salaman, 1985). Three forms of sugar (sucrose, fructose and glucose) and also different nitrogeneous compounds (including free amino acids, storage proteins and nitrate) are present in the potato tuber (Ahmad, 1977).

Potato contains very low fat, about 5 percent of the fat content of wheat, and one-fourth of the calories of bread, while boiled potato has more protein than maize, and nearly twice the amount of

calcium. A single potato tuber (of medium size) has about half the daily adult requirement of vitamin C whereas some other staples like rice and wheat have none (CIP website at http:// www.cipotato.org).

The potato is a wholesome food with all the extremely important and necessary dietary constituents, which are needed for health and growth (Pushkarnath, 1978; Li, 1985; Burton, 1989). Compared to other roots and tubers and also many cereals, potato tubers have a high ratio protein to carbohydrates with a high nutritional value of the protein (Shekhawat et al., 1994).

Potato propagation

Potato can be propagated sexually (by botanical seed, also called true potato seed) and asexually (vegetatively) by means of tubers (Beukema & Van der Zaag, 1990). Tubers are shortened and thickened underground stems with auxiliary buds. The physiological status and health of seed tubers are among the most important factors influencing potato yield (Wiersema, 1984). Potato needs about 15% of its area to produce the required seed tubers (Lommen, 1995; Struik & Wiersema, 1999) while for cereals only one thirtieth of their area is necessary for seed production.

In conventional systems generally seed potato tubers are utilised for multiplication and production (Struik & Wiersema, 1999). This method has a number of disadvantages. Some of these are: low rate of multiplication, it is inefficient, it has a high risk of catching various diseases (fungal, viral, and bacterial diseases) and different pests, and it requires intensive control and a high number of field multiplications (Beukema & Van der Zaag, 1990; Struik & Wiersema, 1999). Many pests and diseases can be transferred via potato tubers and these cause degeneration of the seed tubers. Moreover, high quality storage structures are necessary to store seed tubers to acquire a suitable physiological condition at planting time. These demands result in high costs for seed potato tubers.

Ease of planting, uniformity of tubers and vigorous plant growth are advantages of utilizing seed tubers for potato multiplication (Gopal, 2004). Generally 2.5 – 3.0 tons of potato tubers per hectare are used for multiplication and potato production (Khurana et al., 2003). This amount is in great contrast with the only 50 – 250 g TPS needed per hectare (Struik & Wiersema, 1999).

Compared to grain seed, seed tubers are less storable and soil-borne diseases can be transferred with seed tuber. The potato is prone to seed degeneration because it can be propagated vegetatively and continued propagation decreases the quality of the seed tubers, mostly caused by an increase in virus frequency and concentration which decreases health status. Tubers naturally are prone to accumulate and transmit pathogens (bacterial, fungal and viral diseases) to the next generation and this can weaken the plant production potential (Rolot & Seutin, 1999). According to Struik & Wiersema (1999) two methods can control the degeneration: 1) reducing the number of field multiplications can shorten duration of degeneration; 2) rate of degeneration can be reduced by taking all protective measures possible such as protecting the crop against re-infection.

Storage conditions can affect quality of seed tuber, storage losses, vigour of tuber and degeneration as well. According to CIP (International Potato Center, 1984) problems during seed potato storage include: reduction of tuber vigour, high level of storage losses, and excessive sprouting.

In some countries true potato seed (TPS) is used as planting material in a seed potato production scheme. Size of each true seed is 1.2 – 1.8 mm and the weight of 100 seeds is only 50 – 100 mg (Struik & Wiersema, 1999). In China, botanical seeds have been used for many years (Accatino & Horton, 1980). TPS can solve some of the problems associated with conventional systems. True potato seed has advantages such as: ease to transport and store (it can be stored at room temperature and dark conditions for many years), lower cost**,** less transmission of diseases and pests (Wiersema, 1984; Harris, 1992; Khurana et al., 2003). When TPS is used a large amount of potato tubers which would have been used for potato production and multiplication can now be used for other purposes, such as food (Wattimena et al., 1983; Gopal, 2004). However, TPS also has some disadvantages, including high labour requirement, less uniformity in plant type and maturity for crops grown from TPS, and more variation in colour and shape and dry matter content of tubers produced from TPS. Moreover, potato seedlings are weak and can be damaged easily by environmental stress and pests (Khurana et al., 2003; Wiersema, 1984).

Why rapid multiplication?

Recently some new multiplication techniques have been developed and they are now used all over the world. One of these new techniques is rapid multiplication (or micropropagation or tissue culture technique). This technique is now widely used in many different countries (Murashige, 1974; Hussey & Stacey, 1981). Rapid multiplication is very flexible and gives a high rate of multiplication. It also provides seed potato tubers free from seed borne diseases (Roca et al., 1978; Wang & Hu, 1982; Jones, 1988; Beukema & Van der Zaag, 1990). Rapid multiplication can solve some of the problems associated with the conventional multiplication system (Beukema & Van der Zaag, 1990; Lommen, 1995; Struik & Wiersema, 1999). Tissue culture revolutionized seed potato production in some countries such as Vietnam and doubled potato yield and potato area (Uyen & Vander Zaag, 1983, 1985). A large number of virus-free potato microplants can be obtained by the micropropagation technique (Khurana et al., 2003).

Higher rate of multiplication, disease-free material (including plantlets, transplants, microtubers and minitubers), more convenient storage and transport, and a small demand for space during multiplication are known as advantages of rapid multiplication. Moreover, multiplication can be done whole year round (Wang & Hu, 1982; Marinus, 1983; Struik & Wiersema, 1999).

A very high rate of multiplication (8 to 84 fold over a period of 40 days) is reported by Marinus (1983). Goodwin & Brown (1980) reported that in a period of 4 months ten tubers gave 2500 tips (as Goodwin and Brown called it), thus limiting the number of propagation steps and greatly increasing the number of available tubers. According to Wang & Hu (1982) through rapid multiplication techniques 36,300 minitubers were produced from 1,210 culture flasks on a bench area of 10 m^2 in a short period (4 months) and 1800 kg seed potato tubers were obtained after three plantings in the soil. Micropropagation techniques can be a feasible alternative for potato multiplication (Wattimena et al., 1983). Theoretically a number of 14.3 million (3^{15}) microplantlets can be obtained from a microplant within one year with an interval of cutting of 25 days (Khurana et al., 2003). A more practical estimate of the number of plantlets from a single bud has been provided by Beukema & Van der Zaag (1990). They estimated that 3^6 (= 729) to 10^6 (= 1,000,000) plantlets can be obtained from a bud in a period of 6 months.

Different methods of rapid multiplication are used in various laboratories and different countries (Jones, 1988). In all techniques a very small part of the plant (called the explant) is cultured on a standard nutrient medium (usually a Murashige and Skoog medium) in a test glass tube or glass jar under aseptic conditions for a short period of around 3 – 5 weeks (Murashige & Skoog, 1962; Wang & Hu, 1982; Tadesse, 2000; Struik & Wiersema, 1999). There is little difference in composition of nutrient media for plantlet production and in vitro tuber production. Generally in case of in vitro plantlet production the cutting will be cultured on a nutrient medium containing 4.4 g/l Murashige & Skoog medium with vitamins, 30 g/l sucrose, 8 g/l agar, pH will be adjusted at 5.8 before autoclaving, sealed containers with cutting will be placed at 24 ^{o}C and a photophase of 16 h supplied by fluorescent tube lamps with a light density of around 30 μmol m^{-2} s^{-1}. For in vitro tuber production (microtuber production) different protocols are used to induce microtubers, but generally the same nutrient media with 80 g/l sucrose, 6 g/l agar will be used, 500 ppm of CCC will be added to the medium after autoclaving (cytokinins and some other substances can also be used) to induce microtuber production, pH will be adjusted at 5.7 after autoclaving. In some laboratories other substances, such as jasmonic acid, NAA, coumarine, acetic acid, abscisic acid, and triazole cytokinines (especially BA) are added to nutrient media to induce microtuber production (Khurana et al., 2003).

Through tissue culture, different types of disease free materials can be obtained (see also below) such as: 1. plantlets or transplants, 2. microtubers, and 3. minitubers in large quantities (Hussey & Stacey, 1984; Jones, 1988; Gopal et al., 1998; Struik & Wiersema, 1999). The relations among these different types of propagules are illustrated later in this General Introduction. The quality of the in vitro plantlets and of the microtubers can be affected by a combination of growth regulators, environmental conditions during culture and in vitro medium (e.g. Otroshy & Struik, 2004).

Plantlets are very small plants produced under completely sterile conditions (called in vitro conditions) but transplants can be produced by planting in vitro plantlets under non-sterile condition (called in vivo conditions) or conditions between in vitro and in vivo (called ex vivo conditions or semi in vitro conditions or semi in vivo conditions (Struik & Wiersema, 1999)).

Microtubers are very small tubers produced in in vitro conditions. Generally each plantlet or explant can produce one microtuber with a weight of 0.2 – 0.7 g and 3 – 10 mm diameter (Struik & Lommen, 1990). Recently some larger microtubers with higher weight are produced in some laboratories (10 g or more). These large microtubers are still called microtubers as the term microtubers is determined by the method of production rather than based on size. Microtubers can be produced in in vitro conditions under dark condition or light conditions both with a temperature of 24 ^{o}C but microtuber production could be better in light (a short day of 8 h) than in dark (Ranalli, 1997). According to Pruski (2001) better results for microtuber production (higher number of uniform microtubers with larger size and higher weight) can be obtained under light exposure (8 h) than under conditions without light, and also on solid medium than on liquid medium. Pruski also reported that such microtubers perform better in the greenhouse or field than when the in vitro tuberization has taken place in the dark. Generally, microtubers have a long period of dormancy.

Minitubers are small tubers of 5 – 25 mm in diameter and a range in weight between 0.1 – 10 g and sometime higher. Minitubers can be obtained on in vitro plantlets under ex vivo conditions or under in vivo conditions after planting them in a soil medium. Generally they are produced under ex vivo conditions. The number of minitubers can be 2 – 10 per plantlet and sometimes it can be more, depending on the mother plant management. Minitubers can be planted directly in the field. According to Struik & Wiersema (1999) also some soil-less production systems have been developed for minituber production: minitubers can also be produced through hydroponic systems (soil-less systems). Less risk of infection by different pathogens, high rate of multiplication and higher number of minitubers per unit area are reported as advantages of the soil-less system (hydroponic system) for minituber production compared to minituber production in the soil such as peat (Rolot & Seutin, 1999). Also Muro et al. (1997) reported that total production and number of tubers were higher in hydroponic systems compared to traditional culture, meaning culture in a peat/sand mixture. Nutrient film technique is another soil-less technique that can be used for tuber production (Wheeler et al., 1990).

Potato in Iran

Iran is located in the Middle East on the Asian continent. Some of the general characteristics of Iran are:

Latitude: 25^{o} 03′ – 39^{o} 47 ′N

Longitude: 44^{o} 05′– 63^{o} 18′E

Area: 1,648,195 km²

Total population in 1994: 59.6 million

Climatic conditions:

Average annual rainfall is about 240 mm with a wide range from 50 mm (in central parts) to more than 1500 mm in northern parts. There is a wide variation of climatic conditions in the country. In the south it is warm and semi arid or arid; in the north there is a Mediterranean climate, with relatively moderate temperatures and moist conditions. In the west, northwest and some central parts there is a cold semi-Mediterranean climate. In the central region there are also regions with an arid to semi arid climate. In the east the climate is arid.

Given this wide diversity of climatic conditions, many different arable crops are grown in Iran. Some of them are: wheat, potato, rice, barley, sugar beet, sugarcane, vegetables, and cotton. Horticultural crops include apple, citrus, grape, peach, pistachio and cherry.

Potato is one of the most important food crops in Iran and it ranks third after rice and wheat. Sir John Malcolm (one of the English Advisors to the Iranian government) imported potato to Iran in the period of Fathe-Ali shah (one of the Qajar Kings). It is known as Melcom's Plum and in Persian it is called Sibe-Zamini (which means earth apple). For the first time this crop was cultivated around Tehran (in a region which is called Pashand) and in the Fereydan area (a region in the Esfahan Province).

Different (local) potato cultivars are cultivated in Iran including Pashandy, Istanbouli, Damavandi and Mahalati, which are local varieties. Pashandy and Islambouli are popular for their culinary and cooking qualities. Other (imported) varieties include Agria, Ajax, Alpha, Aola, Baraka, Brina, Concord, Cosima, Cosmos, Désirée, Diamont, Draga, Marfona, Moren, Picasso, Perima,

Sandra, Spartaan, and Wital. Cv. Cosima is imported from the Netherlands and it is valued for its taste, high yield, and its suitability for storage; it has been one of the most preferred cultivars for many potato producers in Iran and has been multiplied and cultivated for many years in different potato areas.

Almost in all provinces of Iran potatoes are grown (Fig. 1), but the main regions for potato cultivation are

* the Alborz Mountains, which run from west to east. The provinces Azarbaijan, Ardebil and Gorgan are the most important potato areas in this part of Iran.

* Zagros Mountains including the provinces Esfahan, Hamadan, Markazi, and Chahar Mahal Va Bakhtiyari.

In some provinces, such as Hormozgan, Boushehr, Khouzestan, Fars, Sistan and Baluchestan, potato is scarcely cultivated. Potato is cultivated in other parts of Iran as well.

Figure 2 shows the trend over time of the land area cropped to potato. During the last years the area under potato has increased substantially compared to the situation in the early 1980s. In the meantime, a decrease in potato area in the rest of the world was observed.

From a study of the geographical distribution of potato land areas in 1992 – 1993 it was found that 29,994 hectare of potato land (20%) belonged to the Ardabil province. More than one third of the potato area is located in the western part (Azarbayejan, Ardebil, Kermanshah, Kordestan and Hamadan) because of the favourable climatic conditions in these areas.

The potato production in the period of 1982 – 2003 is presented in Fig. 3. A considerable increase in potato production after 1988 was realized (Fig. 3). The total potato production in 2002 and 2003 was 3,500,000 and 3,550,000 tons respectively (FAO, 2002, 2003).

A study of the geographical distribution of potato production in Iran shows that in 1992 –1993 Ardebil in the northwest of Iran produced 633,807 tons potato (19.75% of total production) and was the province with the highest production in the country.

Average of potato yield in Iran during the period 1992 – 1993 was 21.5 Mg/ha, which is an increase by 37.3% compared to 1981 – 1982. The Fars province showed the highest potato yield. The

province of Hamadan with 29.5 Mg/ha ranked second and Tehran with 28.3 Mg/ha was on the third place. During this period, Kermanshah had the lowest yields (5.5 Mg/ha). In recent years the average of potato yield per hectare in Iran has increased (with some exceptions): in the Yazd province it increased from 9.4 to 28.2 Mg/ha, in Sistan and Baluchestan from 10 to 28 Mg/ha and in the Fars province from 14 to 34 Mg/ha. According to FAO (2002) average potato yields in Iran in the years 2000, 2001, 2002, 2003 have been 20.8, 20.0, 20.0, and 19.7 Mg/ha, respectively (Fig. 4).

Annual consumption of potato per capita in Iran was 26.8 kg in 1980 and 38.0 kg in 1990.

Potato production constraints in Iran

The main factors limiting potato production in Iran are: shortage of certified seed tubers, inadequate methods of multiplication and production, and storage problems.

Generally most of the farmers who grow potato, are trying to select their seed tubers from the last year's yields or buy uncertificated tubers from local potato producers or local markets. This method causes significant problems including:

1. incidence and spread of fungal, bacterial and viral diseases and some pests such as potato tuber moth;
2. mixing of different varieties.

The lack of proper storage facilities causes problems with seed quality, especially in certain seasons, but also limits the availability of ware potatoes to a few months per year. Moreover, there is no well functioning potato processing industry.

This means that there are serious problems with potato seed tuber quality with respect to the genetic quality, the sanitary quality, and the physiological quality.

In recent years, solutions for these problems were sought by stimulating:

1. Import of basic seed tubers and seed multiplication under well controlled conditions;
2. Use of rapid multiplication methods and training experts to apply these methods;
3. Regulation of seed potato production.

With these measures, the genetic quality and sanitary quality of the seed potatoes could be improved.

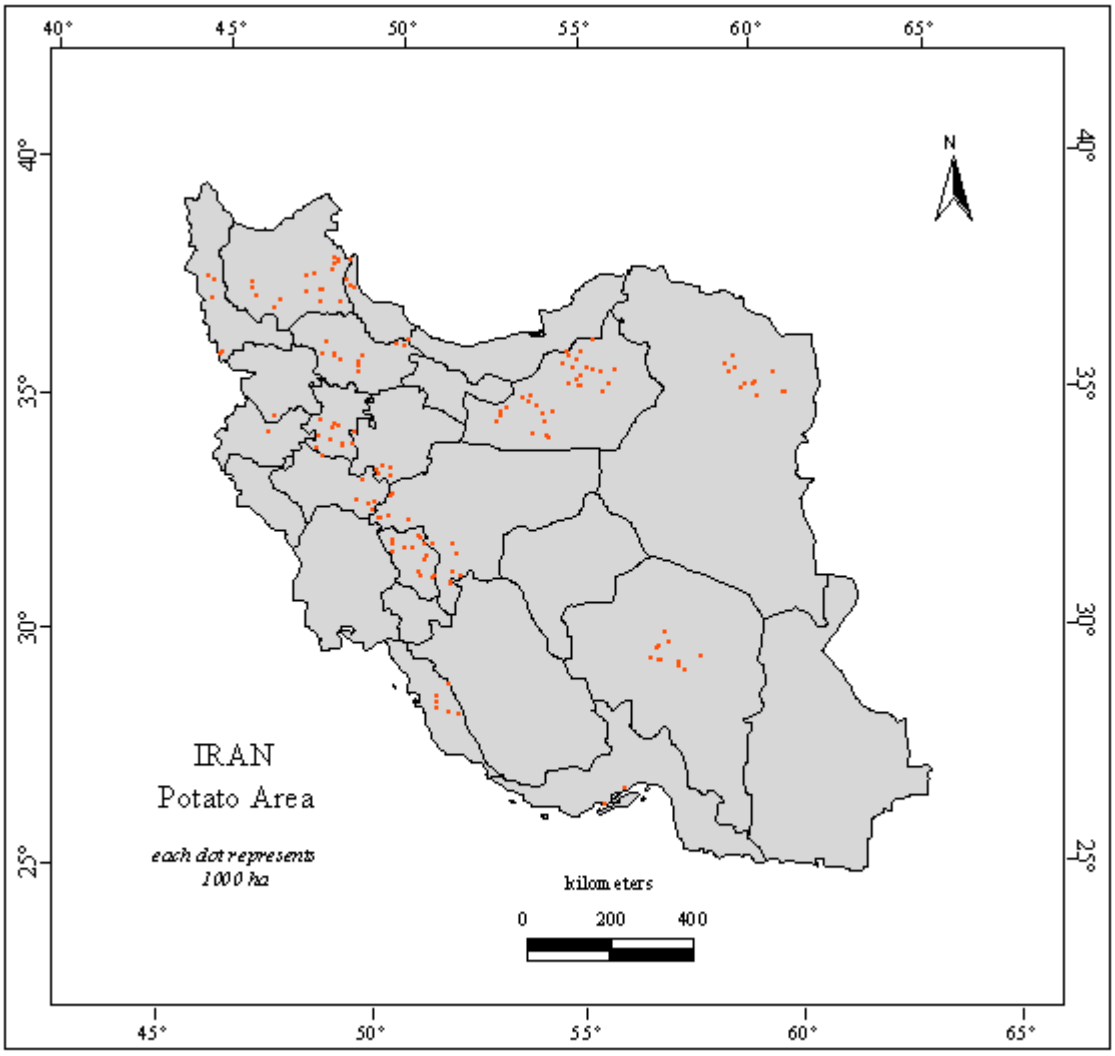

Fig. 1. Potato in Iran (from CIP website: http://www.cipotato.org).

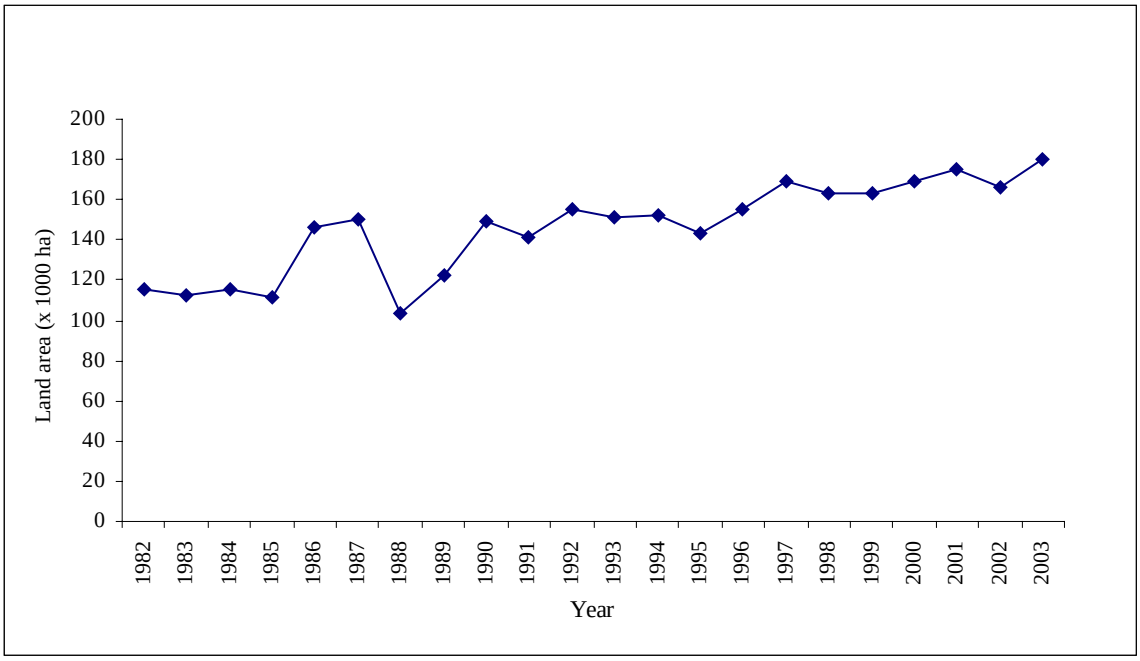

Fig. 2. Land area cropped to potato in Iran (1982-2003).

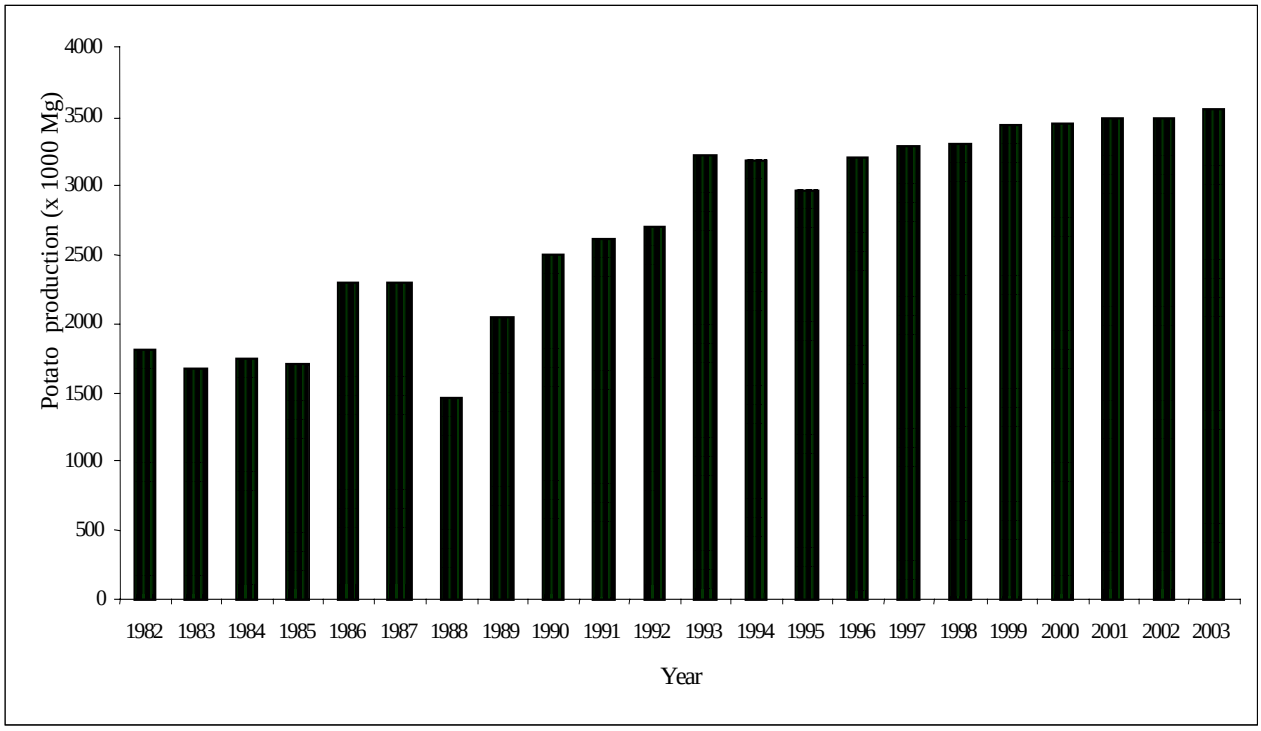

Fig. 3. Potato production in Iran (1982 – 2003).

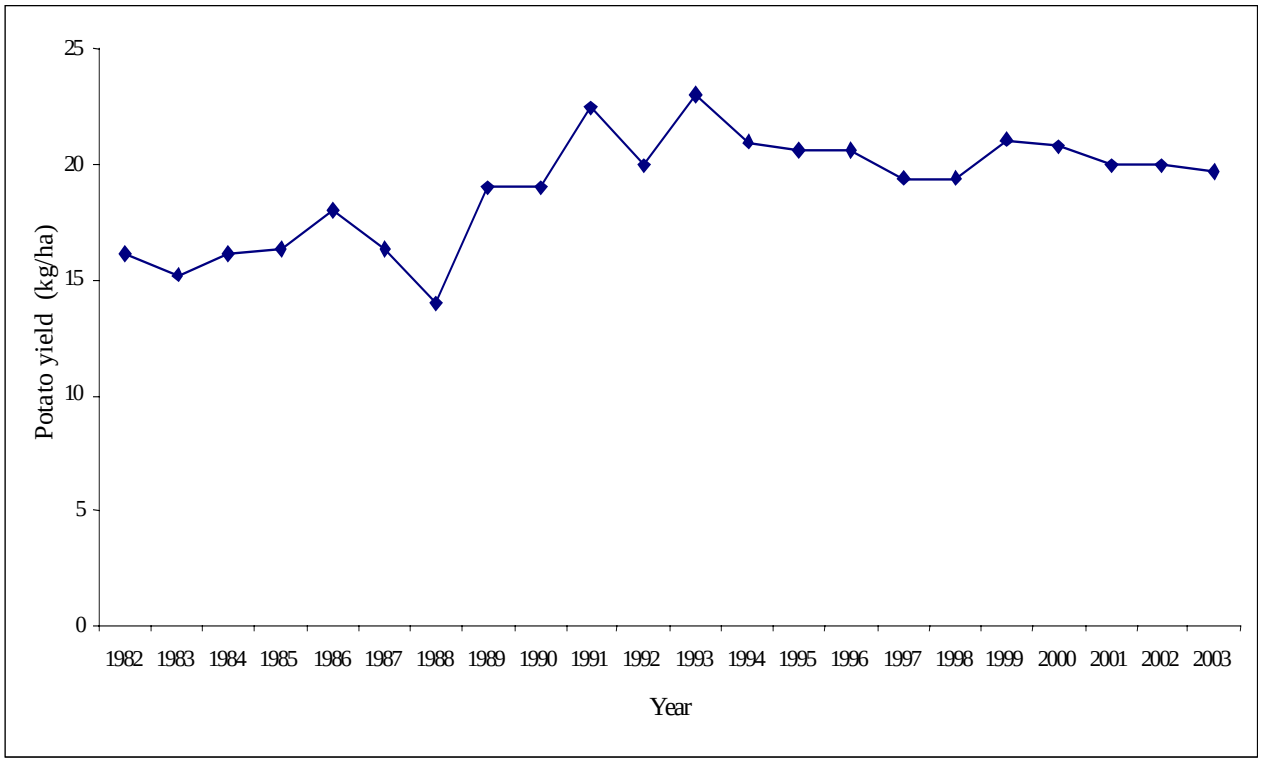

Fig. 4. Average potato yield per hectare of irrigated potato in Iran (1982 – 2003).

Moreover, the Ministry of Agriculture of Iran established several modern potato stores in some areas to improve the physiological quality. The Ministry of Agriculture also trained manpower, built

laboratory facilities and made agricultural machinery available, in order to improve the technology level of potato cultivation in Iran.

Objectives of the research project

The objectives of the PhD programme were to contribute to the efforts of the Ministry of Agriculture of Iran to improve potato cultivation in the country by investigating approaches to solve the current constraints and by creating expertise in rapid multiplication techniques and storage issues. Within this framework, the PhD project aimed at:

- obtaining experience with the production of in vitro plantlets, microtubers and minitubers; identifying possible specific roles of different types of propagules (in vitro plantlets, microtubers, minitubers) during different multiplication phases of a seed production scheme;
- understanding the influences of temperature and photoperiod on the production of in vitro plantlets, microtubers and minitubers and the possible after-effects of these environmental factors on the subsequent progeny;
- understanding the influence of some growth regulators on the in vitro plantlets, microtubers and minitubers and especially on the quality of their subsequent progeny;
- understanding the behaviour of minitubers and normal tubers during storage and exploring the possibilities to manipulate seed tuber quality by combinations of storage temperature and chemical treatments.
- to determine what are the best environmental conditions to obtain maximum seed tuber yields with highest quality.

The focus was on the effects of factors studied on the performance of the progeny. The treatments in some of the experiments were selected in such a way that they would provide relevant insight and experience rather than provide practical tools.

Nevertheless, the practical aim of the project was to come up with suggestions for changes in protocols during different stages of the seed potato production scheme allowing high yields, high rates

of multiplication and adequate quality of the progeny (including proper length of dormancy and optimal growth vigour).

Structure of the thesis

A view of the structure of the thesis is presented in Fig. 5.

The first chapter of this study provides some information about the potato crop, reproduction methods of potato and new techniques of potato multiplication.

Chapter 2 deals with microtuber production under different temperature regimes to identify the effect of different thermal phases during the in vitro phase on the microtuber production.

Some information regarding plantlet and microtuber production as affected by different growth regulators during the in vitro phase is presented in Chapter 3. In this chapter some information about the direct effects and the possible after-effects of growth regulators on the number, size and weight of microtubers, and on some characteristics of potato plantlets such as plantlet height, number of branches, leaves, roots and also length of longest root are presented.

Minituber production is studied in Chapter 4 to determine the effect of three different temperatures and three different photoperiods applied during the in vitro phase on quality of minitubers including their subsequent vigour.

The effect of storage conditions and minituber size and growth regulators application on the sprout behaviour and vigour of minitubers are discussed in Chapter 5. Losses occurring during the storage, sprout characteristics, vigour and characteristics of plants grown from these minitubers are presented.

Chapter 6 explains how tuber size and growth regulators during storage affect sprouting behaviour of normal tubers, their date of emergence, characteristics of plants grown from these tubers, date of senescence and available growing days and some other parameters in various potato cultivars.

Finally, the general discussion, Chapter 7, reflects on the initial objectives and the results.

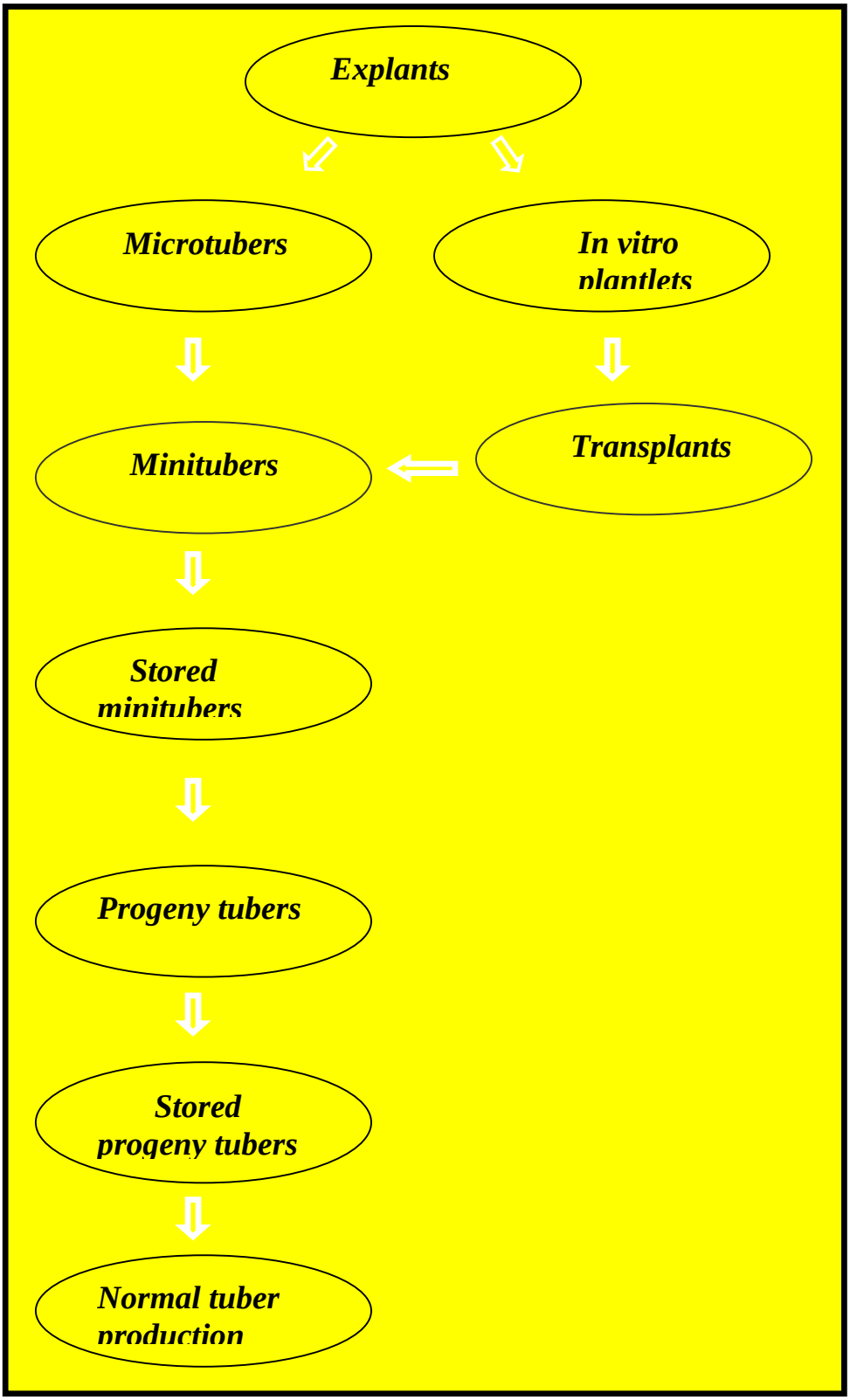

Fig. 5. A scheme of the research project showing the relations between the different types of propagules as well as the phases of the seed production scheme studied.

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Chapter 2

Effects of temperature regime on microtuber production in different cultivars of potato

Otroshy, M., & P.C. Struik, 2006

Effects of temperature regime on microtuber production in different cultivars of potato

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Abstract

Microtuber production in potato is strongly affected by temperature, but the minimum and optimum temperatures for efficient microtuber formation and the influence of different temperature regimes with diurnal fluctuations at similar average temperature have not been studied in great detail. Two experiments including potato cultivars Gloria (very early), Marfona (mid-early) and Agria (late), were carried out to assess the effects of different temperature regimes during (in vitro) microtuber production of potato. In both experiments, nodal cuttings from 5-weeks old plantlets were cultured in sterilized petri dishes on 30 ml standard medium containing Murashige & Skoog salts with normal vitamins, 8% sucrose, 6 g/l agar and 500 mg/l CCC. The sealed petri dishes were randomly placed on a germination table in the dark. This germination table had 100 cells and the temperature in each cell could be precisely controlled and programmed separately. Per petri dish, four (Experiment 1) or five (Experiment 2) explants of each of the three cultivars were placed.

In Experiment 1, different temperatures were set during two thermal phases per period of 24 hours (Phase 1 from 0:00 h (midnight) till 12:00 h (midday) and Phase 2 from 12:00 h (midday) till 24:00 h (midnight)). All 25 possible combinations with diurnal temperature fluctuation of five temperatures during Phase 1 (12, 14, 16, 18 and 20 ^{o}C) and five temperatures during Phase 2 (17, 19, 21, 23, and 25 ^{o}C) were included in quadruplicate. These 25 combinations resulted in different average temperatures over the 24 hours period (ranging from 14.5 to 22.5 ^{o}C) and in different diurnal temperature fluctuations between the two phases (ranging from 1 to 13 ^{o}C).

In Experiment 2, temperature trends imposed over a 24 h period were either sinusoidal, linear or with a sudden switch. For each of these trends, there were six combinations of maximum and minimum temperatures: 30/10, 28/12, 26/14, 24/16, 22/18 and 20/20 ºC. Average temperature was 20 ºC for all treatments, but temperature differences between the two phases ranged from 0 to 20 ºC. Per temperature treatment there were five replicates.

In Experiment 1, a highly significant effect of temperature during both phases on the percentage of cuttings that produced microtubers was observed. The highest temperatures in either phase resulted in the lowest percentage of cuttings that produced microtubers. More microtubers per cutting and larger tuber sizes were associated with lower temperatures during either phase. There was no clear minimum temperature for microtuber formation within the range of temperatures studied. The effects of temperature on the microtuber weight were not statistically significant. Increasing the temperature during different thermophases increased length and weight of sprouts formed on the microtubers. The highest temperatures gave the lowest levels of tuberization and the highest sprout growth. There was a significant curvilinear relationship between temperature differential and the number of microtubers per explant, but this relationship was intertwined with the effect of average temperature on microtuber formation. For treatment combinations with the same average temperature but with different differentials no significant difference could be assessed. Yet, for some parameters significant interactions between the temperatures during the two phases were observed. The three cultivars showed different responses in the percentage of explants that produced microtubers. The percentage of explants that produced microtubers, the number of microtubers and the length of the sprouts increased after 45 days of incubation, but the status of the microtuber (sprouted or not-sprouted) did not change and the microtuber size did not increase beyond 35 days of incubation. Explants exposed to higher temperatures produced structures with a higher weight over the entire range of temperature regimes.

In Experiment 2, the gradual temperature regime gave on average more and heavier microtubers than the other two regimes. This might have been associated with a better temperature control caused by the gradual regime. Despite the wide range, there was surprisingly little effect of the

diurnal fluctuation of temperature. In this experiment this effect of diurnal fluctuation could be assessed independently of average temperature.

In general, the effects of different temperature regimes are small compared to the effects of average temperature. This finding is in agreement with the literature.

Key words: Potato, *Solanum tuberosum* L., in vitro, nodal cutting, microtuber, temperature, temperature amplitude, thermophase, temperature differential, seed tuber.

Introduction

Potato (*Solanum tuberosum* L.) is one of the most important crops world wide. The crop is grown under different agro-ecological zones. The tubers produced are used as cheap food, industrial raw material, animal feed and seed tubers. Nowadays scientists and policymakers are aware that the potato crop is important (Horton, 1987; Struik & Wiersema, 1999). It is grown in over 120 countries (Burton, 1989) and potato consumption is increasing and doubling every 10 to 15 years (CIP, 1984). According to FAOSTAT (cited in the website www.cipotato.org.) consumption in developing countries increased from 9 kg/capita in 1961 – 1963 to 14 kg/capita in 1995 – 1997.

Potato can be propagated sexually (by true potato seed) and asexually (Beukema & Van der Zaag, 1990) but generally in conventional systems the potato crop is propagated asexually and potato tubers are used for multiplication and propagation. In conventional potato seed tuber production systems tubers are prone to infection by different pathogens and the rate of multiplication is very low. Poor seed quality results in poor yields and quality of progeny crops (Haverkort et al., 1991; Beukema & Van der Zaag, 1990). The sanitary quality of seed potato tubers is (next to their physiological status) known as one of the most important factors influencing potato yield (Wiersema, 1984).

Nowadays much attention is paid to micropropagation (tissue culture) techniques to supply a generation of high quality and disease-free seed potato tubers, which then can be used for further propagation. Including tissue culture techniques in a seed potato system allows a higher flexibility of scheduling, less testing for health status and a faster rate of multiplication (Roca et al., 1978; Wang &

Hu, 1982; Jones, 1988; Struik & Wiersema, 1999). According to Struik & Wiersema (1999), for the rapid multiplication of potato in vitro, four types of potato propagules can be used: meristems, apical cuttings, nodal cuttings and microtubers. These different types of starting material then produce either cuttings, whole plants (called plantlets) or small tubers (called microtubers).

In vitro produced tubers are called microtubers, because of their (very) small size. Microtubers often play a special role in seed potato production schemes (e.g. Wang & Hu, 1982; Wattimena et al., 1983). The use of microtubers has several advantages compared to plantlets. These include (Struik & Wiersema, 1999):

- they can be produced any time and not necessarily have to be produced just before use;
- they are convenient and easier to transport and store for several months.

In vitro microtubers also have some disadvantages. These include (Struik & Lommen, 1990; Lommen & Struik, 1995; Struik & Wiersema, 1999):

- they are small and not uniform;
- they have a long dormancy period,
- the production step from cutting to microtuber usually does not contribute to the multiplication as one can only produce few microtubers per plantlet or cutting (mostly on average only one or less than one microtuber per plantlet).

When one wants to study the effects of temperature regimes on microtuber production revisiting the literature on the effects of (fluctuations in) environmental factors in the field is very important. Potato plants are highly flexible in their response to environmental factors. Potato tuber formation is enhanced under short days (Went, 1957; Gregory, 1956; Slater, 1963). Ewing (1981) called potato a cool season crop, indicating that the crop performs best under temperate conditions. Gregory (1956) reported that *tuberization* could be promoted with low temperatures and short days; short days (8 hours of light) and low night temperatures promote yields of tubers while no tuber is formed under long days (16 hours or longer) and high night temperatures. Menzel (1983) indicated that in cuttings as well as in intact plants tuber production was completely inhibited at high temperatures (35/30 ^{o}C). According to Went (1957), at low night temperature a tuber inducing

hormone can be produced in the leaves which is then translocated to the stolon tip. At lower temperatures tubers are initiated more rapidly than under higher temperatures; at low temperatures, the concentration of soluble carbohydrates at the stolon tip is higher and tuber initiation is accelerated (Borah & Milthorpe, 1962), but at higher temperatures, the process of starch assimilation at the stolon tip is retarded. Under long photoperiods the negative effects of high temperatures on tuberization and tuber formation are much more pronounced (Wheeler & Tibbitts, 1986; Snyder & Ewing 1989) than under short days. Beukema & Van der Zaag (1990) indicated that tuber initiation can be stimulated by short days and lower temperatures but that low night temperature is more effective than low day temperature in enhancing tuber formation. Struik et al. (1989) indicated that tuber initiation can be delayed or even inhibited by high temperatures, whereas also the distribution of dry matter between tubers and haulm can be affected by temperature, particularly night temperature. High temperatures not only delay stolon and tuber initiation, but ongoing tuber formation can even be arrested temporarily by a short period of high temperatures (Struik & Ewing, 1995).

Effects of temperature on *tuber yields* are even more complicated than the effects on tuberization. Day and night temperatures can affect plant height, total dry weight of leaves, dry weight of stem, flowering, senescence of plant, root length, root diameter, and leaf starch accumulation (Ewing & Struik, 1992). Day and night temperature can influence haulm growth, dormancy of tubers and tuber yield; dormancy of tubers was significantly shorter at day/night temperatures of 32/12 ^{o}C than at 18/12 ^{o}C (Van Ittersum & Scholte, 1992). Yield of large tubers is significantly affected by short changes in environmental conditions (Struik, 1987). According to Struik et al. (1989) root and shoot temperature can affect haulm longevity, number of branches and total dry matter yield; combination of high shoot temperature and low root temperature increased haulm longevity while it was decreased by combination of high shoot temperature and high root temperature. According to Borah & Milthorpe (1962) at intermediate temperatures highest yields are obtained.

Light and temperature are also environmental factors that can influence microtuber induction and growth, modifying the effects of components of the culture medium (sucrose, growth regulators, nitrogen and other nutrients). Physiological responses of in vitro plantlets or explants, however, can

greatly differ from the responses commonly reported for normal field plants (e.g. Wang & Hu, 1982). Moreover, there are large discrepancies in the literatures on optimal values for different environmental factors influencing in vitro tuberization. These discrepancies may be caused by variation in cultivars used, but may also be due to differences in the basic medium, the type and concentration of growth regulators added to the medium, the type and size of the explant used, the other environmental factors imposed, etc.

According to Pruski (2001) fewer microtubers per plantlet with lower average weight were obtained in the dark than under light conditions (8 h photoperiod). Wang & Hu (1982) reported that more microtubers were produced under shorter photoperiod (8 h photoperiod) than under longer days (16 h photoperiod). Decreasing daylight from long to short days increased the size of microtubers (Seabrook et al., 1993) and resulted in earlier microtuber formation (Garner & Blake, 1989).

In vitro temperature can affect microtuber production (Hussey & Stacey, 1981; Wang & Hu, 1982; Akita & Takayama 1994). Although the growth of micropropagated plantlets is optimal at temperatures of 20 to 25 ^{o}C, microtuber formation is often best at (slightly) lower temperatures. Hussey & Stacey (1981) found an optimum temperature range of 20 – 25 ^{o}C for in vitro tuberization, with tuberization being slightly faster at 20 ^{o}C. According to Wang & Hu (1982) the average number of microtubers was lower at higher in vitro temperature (28 – 28 ^{o}C day/night temperature) than at lower temperature (20 – 20 ^{o}C day/night temperature). Wang & Hu (1982) claimed that the optimum temperature for in vitro tuberization is 20 ^{o}C. Akita & Takayama (1994), however, found optimal in vitro tuberization for temperatures from 15 to 18 ^{o}C. Wattimena (1984; cited by Wang & Hu, 1985) even found an optimum temperature of 15 ^{o}C for tuberization, with only a slight reduction in tuberization at 10 ^{o}C compared to the optimum at 15 ^{o}C. In contrast, Okazawa (1967) observed that temperatures below 12 ^{o}C strongly inhibited tuber formation. According to Wang & Hu (1985) these differences in temperature optima can be related to the light intensity (high light intensity increasing the temperature inside the vessels compared to the temperature environment) and to the use of growth regulators (in the absence of cytokinin, the low temperature may be needed to trigger tuberization). Wang & Hu (1982) also suggested that with equal average temperatures, constant temperatures were

giving higher levels of microtuber formation than alternating day-night temperatures. This negative effect of alternating temperatures is in strong contrast with findings of Steward et al. (1981) and Bennett et al. (1991) who found that diurnal temperature fluctuations were generally beneficial in whole plants (Ewing & Struik, 1992).

In vitro tuberization can also be affected by the interaction between temperature and the sucrose concentration in the culture medium (Koda & Okazawa, 1983). Koda & Okazawa (1983) reported that microtuber induction at 25 ^{o}C was enhanced as concentration of sucrose in the medium increased from 2% to 8%. The same sucrose effect could not be found at lower or higher temperatures investigated.

Sometimes the effects of environmental factors last beyond the period during which the explants or plantlets are exposed to these conditions. Otroshy & Struik (2002) reported a positive effect of lower temperature during the in vitro phase on the tuber yield, size and weight of minitubers produced in the greenhouse. Lower temperature (16 ^{o}C) during the in vitro phase resulted in heavier minitubers and therefore significantly increased the yield of potato plantlets compared to higher in vitro temperatures (20 and 24 ^{o}C). They also found that the lower in vitro temperature shifted the tuber size distribution toward larger tubers.

Although much work has been done on the effect of temperature (both day and night temperatures) on the developmental physiology and growth of the potato crop, relatively little is known concerning the effects of temperature on microtuber production. There seems to be a strong controversy in the literature on the minimum temperature for efficient microtuber production and on the optimum temperature for microtuber production. Moreover, the effects of alternating temperatures or diurnal temperature fluctuations are not well established. The few reports on this issue indicate that there are negative effects of diurnal fluctuations on microtuber production. These negative effects are in strong contrast with the beneficial effects commonly found in whole plants. The purpose of this paper is to contribute to our knowledge on optimal temperatures for in vitro microtuber production in the dark and on the effects of diurnal temperature fluctuations, thus helping to increase the efficiency of microtuber production systems.

Materials and methods

Plant material

Diseases-free tissue cultured plantlets of potato were obtained from the potato germplasm bank of Plant Research International of the Netherlands and cultured at the tissue culture laboratory of the Laboratory of Plant Breeding of Wageningen University. Three cultivars of potato (CVs) were used: Gloria (very early cultivar), Marfona (mid-early cultivar) and Agria (late cultivar).

Nodal cuttings with one leaf node were taken from 5 weeks old in vitro plantlets and cultured on a standard medium containing 4.4 g/l MS medium (Murashige & Skoog 1962), with standard vitamins, 8% sucrose, 6 g l^{-1} agar and 500 mg l^{-1} CCC (chlormequat chloride). The pH of the medium was adjusted at 5.7 before using agar and before autoclaving, CCC was added to the media after autoclaving and before solidification. Thirty ml of media was dispensed to 96 × 16 mm sterilised glass petri dishes. Each petri dish was divided into three parts and each part was allocated to a cultivar. Four (Experiment 1) or five (Experiment 2) viable nodes (discarding tops) of each cultivar were cultured in one part of the petri dish. The petri dish was closed with glass caps and sealed with household plastic foil and were randomly placed in the allocated cells mounted on the seed germination table (see below) for 45 days (Experiment 1) or 60 (Experiment 2) days.

The experimental facility

In the past some new devices were designed to germinate seeds. These include the bell jar or Jacobsen-apparatus, the germination cabinet, the room germinator and the Rodewald-apparatus (International Seed Testing Association, 1976). Recently, however, a new and unique device (named germination table) was designed with 100 temperature cells to study seed germination. We used this device to incubate potato explants in two separate experiments investigating the effects of temperature regimes. With this facility the effects of large numbers of temperatures regimes can be evaluated. The researcher can select different temperature regimes which are precisely controlled and monitored.

These 100 individually temperature-controlled cells are mounted on a table arranged in a 5 × 20 array, each cell is designed for one standard petri dish (96 × 16 mm) to contain seeds (or as in our case: cuttings). The cell is insulated with a rigid material to reduce heat transfer among cells and to surrounding air; power for cells is provided from a commercial power supply, temperature regimes are computer controlled.

This device was established in a special condition: a dark room without normal light, green light was provided with a green tube light placed above the table for survey and observation**.**

Temperature treatments

In Experiment 1, for each treatment, the temperature was lowest at 00:00 h (midnight) then gradually increased and it reached its highest value at 12:00 h (midday), it was then gradually decreased and at 00:00 h it was at its lowest value again. This process was routinely repeated every 24 hours. Each treatment was replicated four times.

Two different thermal phases per day (per 24 hour period) were set: Phase 1 and Phase 2. Phase 1 was the period from 00:00 h (midnight) till 12:00 h (midday) and Phase 2 was the period from 12:00 h (midday) till 24:00 h (midnight). Different temperatures were set for Phases 1 and 2.

Temperatures during Phase 1 included 12, 14, 16, 18 and 20 ^{o}C and temperatures during Phase 2 were 17, 19, 21, 23, and 25 ^{o}C. Temperatures during Phase 2 were higher to mimic diurnal temperature fluctuations with a higher day temperature than the night temperature, especially at the lower range of temperatures. All 25 possible combinations were included in the experiment. These treatments resulted in differences in temperature during the two phases of 1, 3, 5, 7, 9, 11 and 13 ^{o}C. Average temperatures were also different (see Table 1).

In Experiment 2, three different types of temperature trends were programmed: temperatures fluctuated over a 24 h period by either a sinusoidal trend (called sinusoidal regime), a linear increase followed by a linear decrease (called gradual regime) or by a sudden switch (called square regime). These trends were applied to six combinations of maximum and minimum temperatures: 30/10, 28/12, 26/14, 24/16, 22/18 and 20/20 ºC. These six combinations resulted in the same average temperature of

20 ºC, but with temperature amplitudes of 0, 4, 8, 12, 16 or 20 ºC. There were five replicates per temperature treatment.

Observations

Measurements included the proportion of explants that produced microtubers, the number of microtubers per explant, the size of each microtuber, the status of the bud, the length of the sprout that was formed when the microtuber did not remain dormant and the weight of the different structures. The proportion of explants that produced microtubers and the number of microtubers per explant were assessed to record the success rate of microtuber formation. These two parameters are usually closely linked as the number of microtubers per successful explant was usually 1. The size and weight of microtubers were assessed to investigate additional effects on growth after tuberization. Again, size and weight should be closely linked, but the records on size are likely to be more accurate than the records on weight. The status of the bud and the length of the sprout on the microtuber (when formed) were assessed to indicate how strong and continuous the level of induction was during the whole production cycle. Finally the weight of the different structures was also assessed to get an impression about total productivity of the explants and thus their efficiency to utilize the substrate available.
Non-destructive measurements were done after 35 and 45 days after cutting (Experiment 1) and 35, 45 and 60 days after cutting (Experiment 2). Destructive measurements were done 45 days after cutting (Experiment 1) or 60 days after cutting (Experiment 2). For Experiment 2 we will only report data on the last date of observation.

The status of the buds (to evaluate the degree of induction to tuberize of the explant) was quantified by using the following scale: 5 for non tuberization (without microtuber), 2 for a tuberized stolon and 1 for a sessile tuber (based on Ewing, 1981; McGrady et al., 1986). This scoring system means that for the parameter status of bud (Table 2) lower values indicate a higher level of induction and higher values illustrate a lower level of induction.

Experimental design

Experiment 1 was set up in a split-plot design with two main factors: temperature during Phase 1 with five levels (12, 14, 16, 18 or 20 °C) and temperature during Phase 2 with five levels (17, 19, 21, 23 or 25 °C). The different varieties (three levels: Gloria, Marfona and Agria) were included as a split factor; batch duration (days after cutting) with two levels (35 and 45 days after starting the experiment) was included in the analysis as well. The 25 temperature treatment combinations were randomly assigned in four replications; thus, a total of 1200 nodal cuttings were used: 5 temperatures of Phase 1 × 5 temperatures of Phase 2 × 3 cultivars × 4 replications × 4 nodal cuttings for each cultivar per petri dish = 1200 nodal cuttings.

Experiment 2 was also set up in a split-plot design with two main factors: temperature regime with three levels (sinusoidal, gradual and square regime) and diurnal temperature fluctuations with six levels (30/10, 28/12, 26/14, 24/16, 22/18 or 20/20 °C). The three types of temperature regimes were included to assess whether effects of diurnal temperature fluctuations are associated with sudden temperature switches or related to the magnitude of the temperature differential. The different varieties (three levels: Gloria, Marfona and Agria) were again included as a split factor. The 18 temperature treatment combinations were randomly assigned in four replications; thus, a total of 1350 nodal cuttings were used: 3 temperature regimes × 6 temperature amplitudes × 3 cultivars × 5 replications × 5 nodal cuttings for each cultivar per petri dish = 1350 nodal cuttings.

Data were analysed by the MSTATC statistical computer program and Duncan's Multiple Range Test were used to compare the means.

Table 1. Overview of temperature treatments in Experiment 1. Numbers give the average temperature and the absolute temperature differential over a 24 h period, all in ºC.

	Temperatures Phase 2 (from 12:00h – 24:00h)				
Temperatures Phase 1 (from 00:00 h – 12:00h)	17	19	21	23	25
12	14.5/5	15.5/7	16.5/9	17.5/11	18.5/13
14	15.5/3	16.5/5	17.5/7	18.5/9	19.5/11
16	16.5/1	17.5/3	18.5/5	19.5/7	20.5/9
18	17.5/1	18.5/1	19.5/3	20.5/5	21.5/7
20	18.5/3	19.5/1	20.5/1	21.5/3	22.5/5

Results and discussion

Experiment 1: Effect of temperature during Phase 1

Temperatures during the period 0 h – 12 h (Phase 1) significantly affected the percentage of explants that produced microtubers, the number of microtubers per cutting, the size and status of the bud, the length and weight of the sprouts and also the total propagule weight. There was no significant effect on the microtuber weight (Tables 2 and 3).

The lowest percentage of explants that produced microtubers and the lowest number of microtubers per explant were associated with cuttings grown at the highest temperature during Phase 1 (20 ^{o}C). The other temperatures during Phase 1 (all below 20 ^{o}C) gave similar results.

Microtubers were significantly larger at temperatures below 20 ^{o}C during Phase 1 than at 20 ^{o}C during this phase, but microtubers produced at 12 ^{o}C during Phase 1 were also significantly larger than those produced at 18 ^{o}C during Phase 1. Lowest level of induction (non tuberization) was more often observed with 20 ^{o}C during Phase 1 and the highest level of induction (highest frequency of sessile

tubers) was found with 14 ^{o}C (Table 2). Shortest sprouts were observed with lowest temperature during Phase 1 (Table 2). Highest values for length of sprout were obtained at 16 and 20 ^{o}C (Table 2). Although the differences observed for microtuber weight were not statistically significant they were in agreement with the temperature effects on microtuber size (Table 3). Lowest value for weight of sprouts was observed at a Phase 1 temperature of 12 ^{o}C; cuttings exposed to the highest Phase 1 temperatures obtained highest values for weight of sprouts (Table 3). The overall weight of the developed bud was lowest at the lowest temperature (Table 3). This information is included separately in Table 3 as an indicator of the overall performance of the propagules and as the statistical analysis yielded different results for Phase 2 compared to the statistical analysis of the weight of microtubers and sprouts (see below).

Experiment 1: Effect of temperature during Phase 2

Temperature during the period 12 h – 24 h (Phase 2) significantly affected the percentage of explants that produced microtubers, the number of microtubers, the size of microtuber and the status of the bud, the length and weight of the sprout originating from the microtuber and total propagule weight, but the effect on the weight of the microtubers was not significant (Tables 2 and 3).

The highest percentage of explants that produced microtubers and the highest number of microtubers per explant were associated with cuttings grown at the lowest temperatures during Phase 2 (17 and 19 ^{o}C). Temperatures above 20 ^{o}C during Phase 2 gave poorer results. However, there were also significant differences between the treatment with 25 ^{o}C during Phase 2 and the treatments with either 21 or 23 ^{o}C during this phase (Table 2). The difference with the temperature response in Phase 1 is probably associated with the different range of temperatures installed during the two phases and shows the relevance of our approach.

There was a gradual effect of Phase 2 temperature on size of microtubers: the lower the Phase 2 temperature the larger the size (Table 2). A significant effect of Phase 2 temperature on the status of the bud was also observed: a Phase 2 temperature of 17 ^{o}C caused more induction than Phase 2 temperature 21 ^{o}C, whereas 19, 21 and 23 ^{o}C during Phase 2 resulted in better induction than the

highest Phase 2 temperature (Table 2). Phase 2 temperatures also affected the length of the sprout, which developed on the microtubers. Increasing temperature promoted the length of the sprout: it was lowest with the lowest two temperatures, higher with 21 ^{o}C, and highest with the two highest temperatures (23 and 25 ^{o}C) (Table 2).

There was no significant effect of Phase 2 temperature on the microtuber weight (Table 3) but the trend matched the trend observed for size (Table 2). The effect of Phase 2 temperature on the weight of the sprout was also prominent. Cuttings treated with the highest Phase 2 temperature obtained highest values for sprout weight while it was lowest with lower temperatures and intermediate for the intermediate temperature (Table 3). The weight of the total propagule was significantly higher for 23 and 25 ^{o}C than for the lower three temperatures (Table 3).

Experiment 1: Effect of diurnal fluctuation

The difference between Phase 1 and Phase 2 temperatures showed a consistent effect on percentage of cuttings that produced microtubers, the number, size and weight of microtubers and the status of the bud. Fig. 1 shows this effect for number of microtubers, size and weight of microtubers (Fig. 1). Although the relation was not always statistically significant due to outliers, the overall trend was similar.

The number of microtubers became lower with a temperature differential above 9 ºC (Fig. 1). This suggests that combinations of too low temperatures of Phase 1 with too high temperatures of Phase 2 hampered microtuber formation and moderate temperatures throughout the 24 hours period during the in vitro phase are required for microtuber formation. Smallest microtubers were produced when temperature differential was 5 ºC (an outlier) and when the diurnal fluctuation was above 11 ºC (Fig. 1). Lowest level of induction was observed when the temperature difference between Phase 1 and Phase 2 was 13 ºC while highest level was observed with a difference of 5 ºC (data not shown), thus confirming the results obtained for number of microtubers. Lowest weight of microtubers was obtained with temperature differences of 11 and 13 ºC (Fig. 1).

Table 2. The effect of Phase 1 and Phase 2 temperatures, cultivar and batch duration on the number of explants that produced microtubers, the number of microtubers per cutting, the size of microtubers and the length of sprouts.

Abbreviations: A = Phase 1 temperature, B = Phase 2 temperature, C = Cultivar D = Batch duration, NS = not significant.

	Percentage of explants that produced microtubers	Number of microtubers per explant	Size of microtuber (mm)	Status of bud	Length of sprout (mm)
Phase 1 temperature (^{o}C)					
12	81 a	0.81 a	2.1 a	1.35 b	10.0 c
14	79 a	0.79 a	2.0 ab	1.02 c	15.0 ab
16	83 a	0.84 a	2.0 ab	1.29 b	17.7 a
18	81 a	0.81 a	1.9 bc	1.24 bc	12.8 bc
20	68 b	0.69 b	1.7 c	1.57 a	17.8 a
P	**	**	**	**	**
Phase 2 temperature (^{o}C)					
17	88 a	0.88 a	2.2 a	1.03 c	8.1c
19	87 a	0.86 a	2.1 ab	1.26 bc	9.8 c
21	76 b	0.76 b	1.9 bc	1.29 b	14.1 b
23	77 b	0.77 b	1.8 c	1.24 bc	20.0 a
25	64 c	0.66 c	1.6 d	1.64 a	21.2 a
P	**	**	**	**	**
Cultivar					
Gloria	61 b	0.61 b	1.7 b	1.62 a	12.9 b
Marfona	88 a	0.89 a	2.6 a	1.12 b	11.3 b
Agria	86 a	0.86 a	1.5 c	1.15 b	19.7 a
P	**	**	**	**	**
Batch duration					
35	76 b	0.76 b	1.9	1.28	8.0 b
45	80 a	0.81 a	1.9	1.31	21.3 a
P	*	**	NS	NS	**
Interactions					
AB	**	**	**	**	NS
AC	**	**	NS	**	**
BC	**	**	NS	**	**
ABC	**	**	**	**	NS
AD	NS	NS	NS	NS	NS
BD	NS	NS	NS	NS	**
CD	NS	NS	NS	NS	NS
ABD	NS	NS	NS	NS	NS
BCD	NS	NS	NS	NS	NS
ACD	NS	NS	NS	NS	NS
ABCD	NS	NS	NS	NS	NS

** : significant at P ≤0.01, *: significant at P ≤0.05. Values followed by a similar letter are not statistically significantly different.

Experiment 1: Cultivar effects

The percentage of explants that produced microtubers, the number of microtubers, the size and weight of microtubers, the status of the bud, the length and weight of sprouts and also the total propagule weight strongly differed among the three various cultivars (Tables 2 and 3).

Gloria (the very early cultivar) produced the lowest number of microtubers. The largest and heaviest microtubers were found for Marfona while the smallest and lightest microtubers were obtained with Agria (Tables 2 and 3). Despite its earliness, Gloria showed the poorest level of induction (Table 2). Agria produced the longest sprouts, but Gloria produced the heaviest sprouts (Table 3).

Experiment 1: Effect of batch duration

No effect of batch time was found on the size of microtubers or the status of the bud but longer batch time increased the percentage of explants that produced microtubers, the number of microtubers and the length of sprouts (Table 2). Increasing batch time increased the percentage of explants that produced microtubers, number of microtubers per explant and the sprout length as well.

Experiment 1: Interaction effects

Interaction between Phase 1 and Phase 2 temperatures. The two-way interaction Phase 1 temperature × Phase 2 temperature was significant for the percentage of cuttings that produced microtubers, the number, size and weight of microtubers and the status of the bud (Tables 2 and 3).

Effects of Phase 1 temperatures on number of microtubers were small at low Phase 2 temperature and considerably larger at Phase 2 temperatures above 21 ºC. Phase 2 temperatures hardly had an effect when Phase 1 temperatures were 14 – 18 ºC, but affected number of microtubers when Phase 1 temperatures were 12 or 20 ºC (data not shown). This suggests that moderate temperatures during the thermophase are required for proper microtuber formation and that too large differences in temperature during the thermophase are not beneficial (cf. Fig. 1). Similar observations on the

Table 3. Effects of Phase 1 and Phase 2 temperatures and cultivar on the weight of microtubers, the weight of sprouts and the weight of the total propagule.

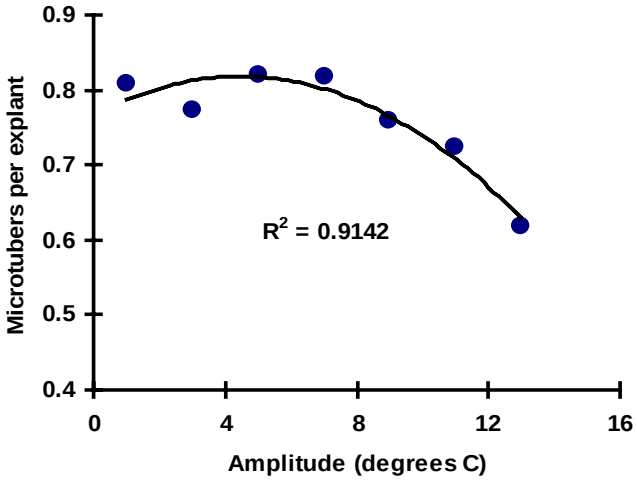
Abbreviations: A = Phase 1, B= Phase 2, C = Cultivar, NS = not significant.

	Weight of microtubers (mg)	Weight of sprouts (mg)	Weight of total propagule (mg)
Phase 1 temperature (^{o}C)			
12	7.5	10.5 b	18.0 b
14	7.1	15.6 ab	22.7 ab
16	7.3	19.0 a	26.3 a
18	6.7	15.9 ab	22.6 ab
20	6.5	20.4 a	26.9 a
P	NS	**	*
Phase 2 temperature (^{o}C)			
17	8.1	9.2 c	17.3 b
19	7.6	10.4 bc	18.0 b
21	6.9	14.8 b	21.7 b
23	6.3	23.6 a	29.9 a
25	6.3	23.2 a	29.5 a
P	NS[1]	**	**
Cultivar			
Gloria	6.0 b	21.0 a	27.0 a
Marfona	11.1 a	14.4 b	25.5 a
Agria	3.9 c	13.3 b	17.3 b
P	**	**	**
Interactions			
AB	*[2]	NS	NS
AC	NS	NS	NS
BC	NS	NS	NS
ABC	NS	NS	NS

** : significant at P ≤0.01, *: significant at P ≤0.05. Values followed by a similar letter are not statistically significantly different.

[1] P = 0.0760

[2] P = 0.0231


0.9
0.8
0.7
0.6
0.5
0.4
Microtubers per explant
R2 = 0.9142
0
4
8
12
16
Amplitude (degrees C)

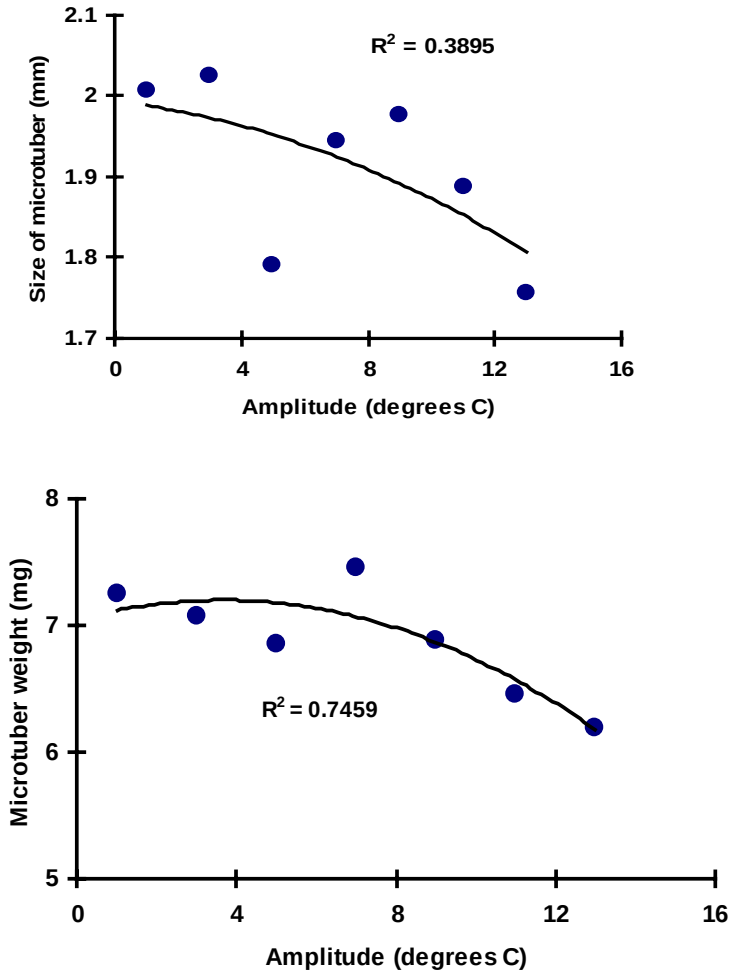


Fig. 1. The relationships between temperature amplitude and the number of microtubers (top), their size (middle) and weight per explant (bottom) (Experiment 1).

interaction between temperatures were made for the percentage of explants that produced microtubers (data not shown).

Largest microtubers were obtained with temperature combinations in which the Phase 2 temperature was 17, 19 or 21 ^{o}C. With high Phase 1 temperatures the effects of Phase 2 temperature were large, but did not show a consistent trend (data not shown). Moreover with a Phase 2 temperature of 21 ^{o}C, effects of the Phase 1 temperature on size of microtubers were relatively large.

Temperature × cultivar interaction effects. The interaction temperature × cultivar for the percentage of explants that produced microtubers, the number of microtubers per cutting, the status of the bud and

the length of sprout were all highly significant, for both Phase 1 temperature and Phase 2 temperature (Table 2). Figures 2 and 3 illustrate that for the parameters number of microtubers and length of sprout. Gloria proved to be more sensitive to higher Phase 1 and Phase 2 temperatures than the other two cultivars. This sensitivity was expressed by a stronger reduction of the number of microtubers and a much reduced expression of induction at the highest temperatures (data not shown). Yet, the Phase 1 and Phase 2 temperatures enhanced sprout growth much more in Agria than in the other cultivars (Figs 2 and 3).

Two-way interactions including the factor batch duration. The only significant two-way interaction including the factor batch duration was observed for length of sprout (Table 2; interaction with Phase 2 temperature). The effects of Phase 2 temperature were better expressed after a long batch duration.

Three- and four-way interactions. The three-way interaction Phase 1 temperature × Phase 2 temperature × cultivar was highly significant for all parameters measured except for the length of the sprout (Table 2), the weight of microtubers, the weight of sprouts and the total weight of propagule (Table 3). Other three- or four-way interactions were not statistically significant.

Experiment 2: Effect of temperature regime

There was a highly significant effect of the temperature regime ($P < 0.001$). The gradual regime had on average 0.670 microtubers per explant, much more than in the case of the sinusoidal regime (0.463) or the square regime (0.515). A physiological explanation of this phenomenon cannot be given. This might have been associated with a better temperature control caused by the gradual regime. The gradual regimes probably used much less energy controlling the temperature and thus would also have a better temperature control. As there was no interaction between regime and diurnal temperature fluctuation levels, this effect did not interfere with the conclusions on the most relevant aspect of the experiment.

Experiment 2: Effect of diurnal temperature fluctuations

The average temperature of all treatments in Experiment 2 was 20 ^{o}C. This is similar to treatments present in Experiment 1. However, the average treatment is chosen in such a way that it matches the critical temperatures of both Phase 1 and Phase 2 of Experiment 1. The average values of microtubers per plant were lower in Experiment 2 than in Experiment 1.

Both Fig. 4 and Fig. 5 indicate that there is only a weak correlation between the diurnal temperature fluctuation and the response. Nevertheless, the response is consistent for both parameters and is also consistent with the response found in Experiment 1. However, the range over temperature amplitudes of Experiment 2 is much wider than that of Experiment 1 and its effect is assessed at the same average temperature of 20 ^{o}C.

It is surprising how little effect we have observed for such a wide range of temperature amplitudes at exactly the same average temperature. This lack of response could be due to the insensitivity of the system used (see also introduction and Wang & Hu, 1985). On the other hand, the exclusion of light in our experiments would allow a much better assessment of the temperature effect (see Wang & Hu, 1985). Based on the current results it cannot be concluded that part of the effects of high temperatures can easily be compensated by a temporarily low temperature or by a large temperature differential.

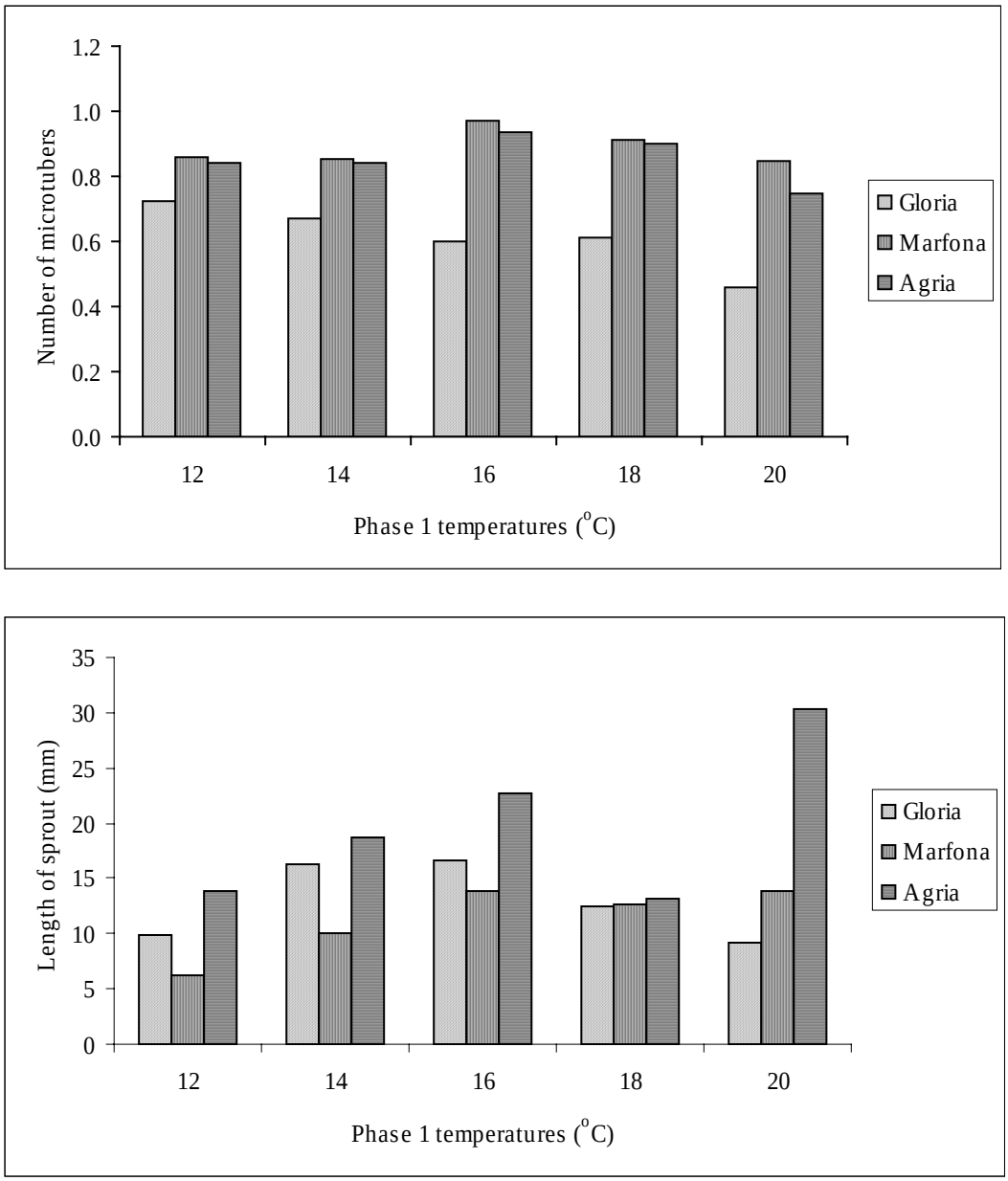
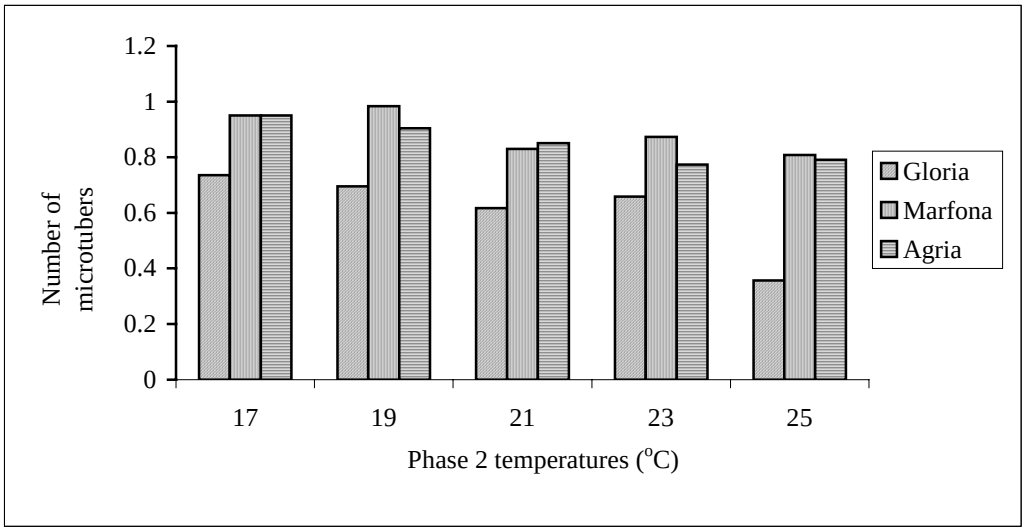


Fig. 2. The interaction between Phase 1 temperature and cultivar for the number of microtubers (above) and length of the sprout (below).



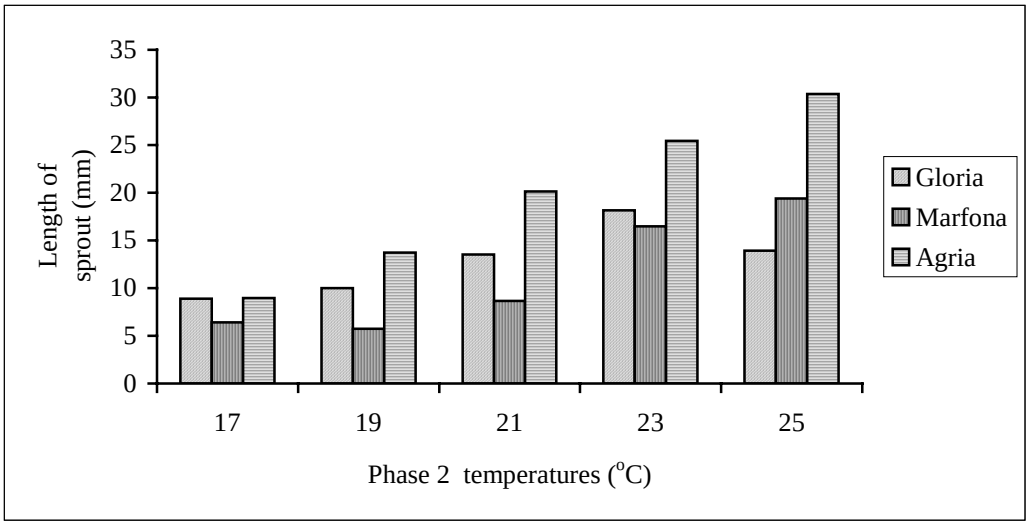


Fig. 3. The interaction between Phase 2 temperature and cultivar for the number of microtubers (above) and length of sprout (below).

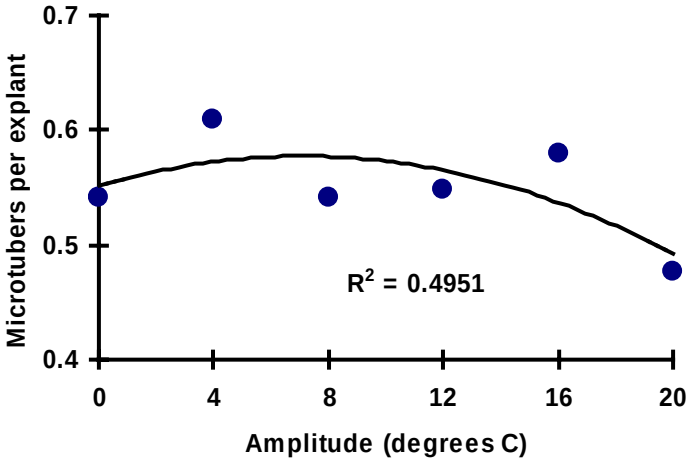


Fig. 4. The relationship between temperature amplitude and number of microtubers per explant (Experiment 2).

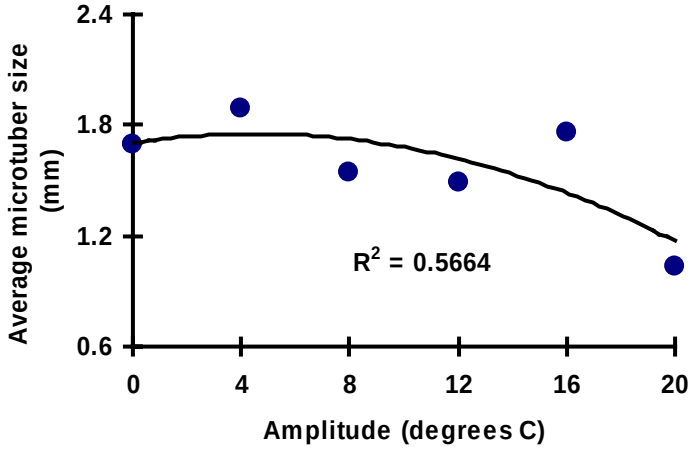


Fig. 5. Relationship between temperature amplitude and size of microtubers (Experiment 2).

Conclusions

We observed two critical temperatures for microtuber production. In Experiment 1, 20 ^{o}C was a clear threshold temperature. Crossing that threshold for the temperatures during Phase 1 or Phase 2 affected microtuber production. Temperatures during any of the two phases above 20 ^{o}C gave a lower value for percentage of explants that produced microtubers or number of microtuber per explant. The second critical temperature could only be observed during Phase 2 (Experiment 1). A temperature of 25 ^{o}C gave significantly fewer and smaller microtubers than 23 ^{o}C. Small diurnal temperature fluctuations gave slightly better performance than large diurnal fluctuations in temperature. Large fluctuations are definitely not beneficial compared to constant temperatures.

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Chapter 3

Effects of two contrasting growth regulators on the quality of in vitro plantlets and microtubers in three cultivars of potato in a seed tuber production scheme

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Chapter 4

Effects of different photoperiod and temperature combinations during in vitro potato plantlet production on the yield and quality of the progeny minitubers produced in the greenhouse

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Effects of different photoperiod and temperature combinations during in vitro potato plantlet production on the yield and quality of the progeny minitubers produced in the greenhouse

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Abstract

Environmental conditions during production of in vitro plantlets can affect the quality of the transplants used to produce minitubers in the greenhouse. Especially their effects on the size of the leaf apparatus and their carry-over effect on the tuber formation are important. In vitro plantlets of cultivars Gloria (very early), Marfona (mid-early) and Agria (late) were produced at all combinations of three temperatures (T; 16, 20 or 24 °C) and three photoperiods (P; 12, 16 or 20 h) and subsequently planted in a greenhouse to produce minitubers. These minitubers were then tested for their vigour after long storage and their ability to produce early progenies.

Lower temperatures during the in vitro phase gave greenhouse plants with more shoot mass than higher temperatures, whereas longer photoperiods gave more shoot mass than the shortest photoperiod. Temperature during the in vitro phase did not affect the number of minitubers produced in the greenhouse but lower T in vitro gave larger and heavier minitubers. Short P in vitro gave fewer, smaller and lighter tubers than longer Ps. Effects of conditions during the in vitro phase on minituber yields were correlated with effects on shoot mass: yields were higher for lower temperatures than for higher temperatures and higher for longer photoperiods than for the shortest photoperiod.

Many interactions between temperature and photoperiod were significant for factors related to shoot or minituber yield. Minituber yields were highest when 16 °C was combined with 16 (Marfona, Agria) or 20 h (Gloria).

The T and P treatments during the in vitro phase did not affect the number of sprouts or the vigour of the minitubers after 195 days of storage, although there was a significant effect of temperature during the in vitro phase on sprout weight, with the lowest temperature giving the highest sprout weight, probably because it also produced the largest minitubers. As the minitubers were de-sprouted and graded before assessing the growth vigour, the effect of temperature on average minituber size was not expressed in an effect of temperature on the growth vigour of the minitubers.

Results indicate that in vitro temperature and photoperiod have a significant after-effect on the plant growth, above ground dry matter and minituber production in the greenhouse phase. The effects on minituber yield were associated with increased above ground biomass, and more and heavier tubers. In vitro temperature can even influence sprout weight of minitubers after long storage. There were no after-effects of in vitro temperature or photoperiod on the vigour of the minitubers produced in the greenhouse. Our findings suggest that the greenhouse production of minitubers can be made more efficient by optimizing the conditions during in vitro plantlet production.

Keywords: Temperature, Photoperiod, In vitro plantlets, Minituber, After-effects, Vigour, *Solanum tuberosum*.

Abbreviations: T= Temperature, P= Photoperiod, C= Cultivar, DAP= days after planting; NS = not significant

Introduction

Potato can be rapidly multiplied using nodal cuttings produced in vitro. Methods, protocols and conditions to produce in vitro plantlets vary across laboratories (e.g. Goodwin & Brown, 1980; Miller et al., 1985; Mastenbroek & Eising, 1987; Seabrook, 1987; Sipos et al., 1988; Cournac et al., 1991). By this technique a large number of disease free in vitro plantlets with high quality and maximum health can be produced in a short period in a small facility year round. According to Beukema & Van der Zaag (1990) rapid multiplication systems make it possible for one bud to produce 3^6 to 10^6 plantlets during 6 months. In this way in vitro plantlets are produced that can be used for further rapid multiplication (in vitro), microtuber production (in vitro), minituber production (in the greenhouse; Struik & Lommen, 1990), or transplant production (followed by transfer to the field; Cole & Wright, 1967).

Recently, especially minituber production has become popular world wide. Minitubers are small tubers with a size range of 5 – 20 mm in diameter or often even larger. As indicated above, this system involves two stages: 1) in vitro multiplication and production of in vitro plantlets, 2) production of minitubers in the greenhouse. There are different ways to produce minitubers, including minituber production in hydroponic systems and minituber production on solid media. Both ways can involve single or repetitive harvesting. The most common and practical way to produce minitubers is to produce in vitro plantlets and plant them in normal potting soil in the greenhouse, glasshouse or screenhouse (Struik & Wiersema, 1999). In this way, per in vitro plantlet two to more than ten minitubers can be produced (Struik & Lommen, 1990; Struik & Wiersema, 1999). Many factors operating during the second stage, including variety, size of containers, growth regulators, plant density etc., can affect minituber yields (Hagman, 1990). Not only yield, but also size distribution of minitubers is important, as this affects the growth vigour, number of stems of the plants grown from them, and number of tubers per stem, and thus their commercial value in different stages of the seed tuber production scheme (Seabrook et al., 1995).

In this paper, we focus on the conditions during the first phase. Environmental factors have *direct* effects on the growth of the buds from the nodal segments when transferred to the same medium after cutting for further multiplication or when transferred to a special medium for further multiplication and preparation for use in the greenhouse (the normalization phase).

Higher temperatures during the in vitro phase increase the rate of leaf appearance (Tadesse, 2000) and the final number of internodes (Hussey & Stacey, 1981; Caligari & Powell, 1989), and result in the production of in vitro plantlets with a normal habitus with leafy shoots in contrast to lower temperatures where the formation of stoloniferous structures with rudimentary leaves can occur (Hussey & Stacey, 1981). Tadesse (2000), however, showed that an in vitro temperature of 20 °C resulted in a larger leaf area per plantlet than 26 °C.

Usually, in vitro plantlets of potato are grown in a 16 h photoperiod (Seabrook, 2005) as such a photoperiod is required to maintain vegetative growth in vitro under the low light conditions commonly used (Dodds et al., 1992; Seabrook, 2005). Hussey & Stacey (1981) reported that the *direct* effects of temperature and photoperiod on in vitro potato plantlets are not always similar to the effects of these factors on whole plants grown from seed tubers. These statements were later confirmed by Dodds et al. (1992) and Seabrook (2005).

Partly through their *direct* effects on the growth of the in vitro plantlets (e.g. Tadesse, 2000), but also partly through other mechanisms such as induction to tuberize (Lommen, 1999), environmental factors have *indirect* effects that express themselves during the subsequent use of these in vitro plantlets for later stages of the multiplication scheme. The in vitro phase conditions can influence the initial quality of plantlets (Tadesse, 2000), their capacity for re-growth after transplanting (Grout, 1988) and storage (Pruski, 2001), and their performance as mother plants for minituber production (Hussey & Stacey, 1981; Marinus, 1985; Mastenbroek & Eising, 1987; Kozai et al., 1988; Hagman, 1990; Tadesse, 2000). The mechanisms of these effects are not always fully understood, but some efforts have been made to unravel the after-effects of temperature (Tadesse, 2000) and photoperiod (Seabrook et al., 1995).

However, it is known that important quality characteristics of in vitro potato plantlets (such as their leaf area and their level of induction to tuberize) can be affected by various treatments during the in vitro phase, including temperature and photoperiod, resulting in a better performance of the transplant derived from the in vitro plantlet. Positive after-effects of increased temperature during the normalization phase in vitro on transplant growth and minituber production have also been observed (Tadesse, 2000). Similar effects have been found for photoperiod: photoperiod during the in vitro phase influenced growth of in vitro plantlets and tuber yield in vitro (microtubers) and in the greenhouse (minitubers) (Seabrook et al., 1993, 1995).

In normal plants there are very strong interactions between photoperiod and temperature and these are well investigated (Ewing & Struik, 1992). Very few studies have been done on the interactions between photoperiod and temperature during the in vitro phase in relation to the direct and indirect effects on in vitro plantlets and their consequences when these in vitro plantlets are used for minituber production in the greenhouse. However, it is likely that the interactions in in vitro plantlets will be different from those found in normal plants (Hussey & Stacey, 1981). Identifying and analyzing the interactions between the effects of photoperiod and temperature during the in vitro phase as they affect the growth of the in vitro plantlets, the transplants derived from them, and the minitubers produced by these transplants is highly relevant. The different types of effects mentioned before (including the direct effects, the indirect effects and the after-effects) are relevant because they can be induced in a relatively cheap way and yet may have a pronounced effect on the efficiency of the production of minitubers which takes place under conditions which are more difficult and expensive to control. It is even not unlikely that also the vigour of minitubers may be affected as many aspects of minituber vigour are associated with their size (Struik & Lommen, 1990).

This study, conducted with three potato cultivars, therefore aimed to assess the effect of various temperatures and different photoperiods during the in vitro phase on the minituber production and minituber storage behaviour.

Materials and Methods

Starting material and subculturing

Disease-free, tissue cultured plantlets used in this experiment were obtained from Plant Research International (Wageningen, the Netherlands) and these were propagated by nodal cuttings. Each explant consisted of a bud and a part of a stem (approximately 3 mm) and was cultured in a vessel on 15 ml Murashige and Skoog medium with standard vitamins and 30 g/l sucrose. Agar 8 g/l was used to solidify the medium; the pH was adjusted to 5.8 before autoclaving.

Treatments during in vitro phase

In vitro plantlets of the cultivars (CV) Gloria (a very early cultivar), Marfona (a mid-early cultivar) and Agria (a late cultivar) were put in test tubes, which were sealed with household plastic foil and randomly placed in growth chambers set at different temperatures (T): 16, 20 or 24 °C, and various photoperiods (P): 12, 16 or 20 h supplied by fluorescent tubes. In vitro plantlets were grown in these growth chambers for 4 weeks.

Minituber production

After 4 weeks in vitro plantlets were transferred directly (without hardening) to the greenhouse to produce minitubers. Agar particles were removed from the roots. The in vitro plantlets were planted in a deep plant hole (allowing one third of the stem to be buried) in 30 × 30 × 13 cm pots filled with standard, nutrient rich potting soil at a density of 11 pots per m^2. Pots were randomly placed in a greenhouse set at day (12 h) temperature 18 ^oC, night (12 h) temperature: 12 ^oC, relative humidity 85%. Daylight was supplemented with 0.8 Philips Son-T-Agro lamps 400 W per m^2 and with incandescent illumination. Immediately after planting the plantlets were irrigated and adequate soil moisture was maintained through daily watering.

Nutrients in the growing medium were abundant for the first 2 months of growth. Two month after planting plants were fertilized once a week. In this respect we followed standard procedures developed by Lommen (1995).

Data collection after minituber production

At 96 days after transplanting (a normal production cycle for minitubers), numbers of stems, length of longest stem (plant height) and above ground dry matter were measured. One hundred days after planting, minitubers were harvested by hand and were left in the greenhouse for 2 more weeks at 18 ^{o}C, 85% RH and full darkness for skin set and hardening. At this stage, number, size and weight of all minitubers were individually recorded.

Quality assessment of minitubers

The minitubers were then transferred to the cold store where the temperature was 4 ^{o}C and the relative humidity 85%. After 195 days of storage (a normal storage duration when minitubers were to be used in the field), number of sprouts, length of longest sprout, weight of sprouts and vigour were measured. This involved removing the sprouts, which usually enhances the expression of differences in physiological status of mother tubers (Struik & Wiersema, 1999). Sprout initials at least 2 mm in length were considered a sprout (Van Ittersum, 1992). Length of the longest sprout was measured by taking the distance from the tip of longest sprout to the base of sprout. To assess vigour, first minitubers were graded into three size classes: 13-25 mm, 25-35 mm, 35-50 mm; each size class was assigned to a replication; within a size class, the de-sprouted minitubers were randomly selected and planted in the greenhouse. Conditions were as stated for the minituber production phase of the experiment. Date of emergence and number and length of longest stem were recorded. After 6 weeks, plants were harvested and above ground dry matter and dry matter of progeny tubers were recorded to assess the vigour of the minitubers, in agreement with standard procedures for assessment of growth vigour (see e.g. Lommen, 1995). The experimental design at this stage was a completely randomized factorial design with three factors: A= temperature during in vitro plantlet production with three levels

(16, 20 or 24 °C), B: photoperiod during in vitro plantlet production with three levels (12, 16 or 20 h); and C: cultivar with three levels (Gloria, Marfona and Agria), each size class of minitubers was used as a replication.

Statistical analysis

All analyses were carried out using the MSTATC statistical computer programme. When the ANOVA indicated significant treatment effects (5 or 1%) based on the F-test, the Duncan's Multiple Range Test ($P \leq 0.05$) was used as a method to determine which treatments were significantly different from others treatments.

Results

Influence of temperature

Plantlets grown at the lowest temperature during the in vitro phase produced highest above ground dry matter after planting in the greenhouse, but temperature during the in vitro phase did not affect plant height or number of minitubers per plant (Table 1). The lowest temperature enhanced tuber size and individual tuber weight, in comparison with the two other temperature treatments. Through these positive effects on size and individual weight the lowest temperature also gave the highest yield of minitubers (Table 1). Temperature did not have any significant effect on the number of sprouts or the length of the longest sprout after 195 days of storage. However, there was a significant effect of temperature during the in vitro phase on the weight of sprouts of the minitubers after 195 days of storage. This effect was associated with the positive effect of the lowest temperature on minituber size. Weight of sprouts of tubers produced on plantlets produced at the lowest temperature was significantly higher than the weight of sprouts on tubers from the other two temperature treatments (Table 1).

Table 1. Main effects of temperature, photoperiod and cultivar during the in vitro phase on above ground dry matter, plant height and the number, size, weight and yield of minitubers of potato plants grown in the greenhouse, and on the number of sprouts, length of longest sprout and weight of sprouts of minitubers after storing minitubers in the cold store for 195 days.

Abbreviations: T= Temperature, P= Photoperiod, C= Cultivar, NS = not significant.

	Above ground dry matter of greenhouse grown plants (g)	Height of greenhouse grown plants (mm)	Number of minitubers per plant	Size of minitubers (mm)	Weight of minitubers (g)	Yield of minitubers (g) [2]	Number of sprouts	Length of longest sprout (mm)	Weight of sprouts (g)
Temperature (^oC)									
16	1.69 a	130	9.0	28.2 a	7.7 a	68.2 a	1.74	13.6	0.052 a
20	1.25 b	120	8.6	25.6 b	5.7 b	47.6 b	1.69	11.8	0.037 b
24	1.23 b	129	9.0	24.6 b	5.5 b	44.7 b	1.67	12.0	0.036 b
P	**	NS	NS	**	**	**	NS	NS	**
Photoperiod (h)									
12	1.08 b	108 b	8.1 b	24.8 b	5.1 b	36.9 b	1.62	12.5	0.041
16	1.46 a	132 a	9.1 a	27.0 a	7.0 a	60.8 a	1.76	12.9	0.045
20	1.62 a	140 a	9.4 a	26.6 a	6.9 a	62.8 a	1.71	12.0	0.042
P	**	**	**	**	**	**	NS	NS	NS
Cultivar									
Gloria	1.65 a	134 a	9.7 a	27.1 a	6.2	57.0 a	1.32 c	19.2 a	0.066 a
Marfona	1.41 b	104 b	9.9 a	25.6 b	6.3	57.9 a	1.65 b	13.1 b	0.046 b
Agria	1.10 c	142 a	7.0 b	25.7 b	6.4	45.5 b	2.13 a	5.1 c	0.013 c
P	**	**	**	**	NS	**	**	**	**
Interactions									
T × P	**	**	NS	**	**	**	NS	NS	NS
T × C	NS	**	NS	**	NS	**	NS	NS	**
P × C	**	**	**	**	**	**	NS	NS	NS
T × P × C	**	**	NS[1]	**	**	**	NS	NS	NS

** : significant at P ≤0.01, ∗: significant at P ≤ 0.05. Values followed by a similar letter are not statistically significantly different.

[1] P = 0.0532; [2] Note that this yield may deviate from the product of number of minitubers and weight of minitubers as a result of missing values.

Table 2. Effects of temperature and photoperiod during the production of in vitro plantlets on the vigour of the minitubers (assessed on the basis of date of emergence, number of stems per minituber, length of longest stem after 6 weeks of growth, above ground dry matter after 6 weeks of growth and dry matter of tubers after 6 weeks of growth following 195 days of storage) produced by these plantlets in the greenhouse for three cultivars.

Abbreviations: T= Temperature, P= Photoperiod, C= Cultivar, DAS= days after planting; NS = not significant

	Date of emergence (days after planting)	Number of stems	Length of longest stem (cm)	Above ground dry matter (g)	Dry matter of progeny tubers (g)
Replication (size of minitubers)					
13 – 25 mm	15.0	1.6 c	15.0 ab	1.24 b	3.40 c
25 – 35 mm	16.6	2.2 b	13.3 b	1.68 a	4.68 b
35 – 50 mm	15.5	3.1 a	16.1 a	1.92 a	5.94 a
P	NS	**	**	**	**
Temperature (°C)					
16	15.4	2.4	15.5	1.67	5.07
20	16.2	2.2	14.4	1.52	4.58
24	15.5	2.3	14.5	1.64	4.38
P	NS	NS	NS	NS	NS[1]
Photoperiod (h)					
12	15.9	2.3	14.6	1.55	4.56
16	15.9	2.4	14.9	1.57	4.71
20	15.4	2.3	14.9	1.72	4.75
P	NS	NS	NS	NS	NS
Cultivar					
Gloria	13.2 b	2.7 a	13.8 b	1.93 a	5.47 a
Marfona	16.6 a	1.9 b	8.5 c	1.45 b	3.92 c
Agria	17.2 a	2.3 a	22.1 a	1.46 b	4.64 b
P	**	**	**	**	**
Interaction					
T × P	NS	NS	NS	NS	NS
T × C	NS	NS	NS	NS	NS
P × C	NS	NS	NS	NS	*
T × P × C	NS	NS	NS	NS	NS

** : significant at $P \leq 0.01$, *: significant at $P \leq 0.05$. Values followed by a similar letter are not statistically significantly different.

[1] P = 0.0666

The vigour of the minitubers was not affected by temperature during the in vitro plantlet production (Table 2): date of emergence, number of stems, length of the longest stem, above ground dry matter and dry matter of progeny tubers were not affected by temperature during in vitro plantlet production (Table 2). This is in contrast with the effect on the weight of sprouts after the storage period because the vigour tests were carried out using de-sprouted, graded minitubers.

Influence of photoperiod

There were significant effects of photoperiod during the in vitro phase on the dry matter of aerial plant parts and plant height after cultivation in the greenhouse (Table 1). These parameters showed higher values after plantlet production under longer photoperiod (16 or 20 h) than after a short photoperiod (12 h).

The effects of in vitro photoperiod on the number, size, individual weight and yield of minitubers were also statistically significant (Table 1); in all cases lowest values were reached after in vitro culturing in the shortest photoperiod. No significant differences among photoperiod treatments were obtained for the number, length and weight of sprouts after 195 days of storage of the minitubers (Table 1).

There were no significant effects of photoperiod during the in vitro phase on any of the vigour parameters assessed after 195 days of storage of the minitubers (Table 2).

Cultivar effects

Comparisons between cultivars showed that the highest amount of above ground dry matter was found for Gloria, whereas Agria produced the lowest amount (Table 1). Also plant height was affected by cultivar: shortest plants were found for Marfona whereas plant length was highest for Gloria and Agria.

The effect of cultivars on the number of minitubers was highly significant. The earlier cultivars Gloria and Marfona produced more minitubers than the late cultivar Agria. Largest minitubers were recorded for the very early cultivar Gloria while Marfona and Agria produced

smallest minitubers. There was no significant effect of cultivar on the individual weight of minitubers but yield of minitubers was highly affected by cultivar, Agria producing the lowest yield. This effect was associated with the low number of minitubers in Agria (Table 1). The number of sprouts after 195 days of storage of the minitubers was highest for Agria and lowest in Gloria, whereas sprouts were longest with Gloria and shortest in Agria (Table 1). Cultivar showed significant effects on the weight of sprouts, the ranking order for the weight of sprouts was Gloria > Marfona > Agria (Table 1).

Minitubers of Gloria emerged earlier (13 days after planting (DAP) and the emergence date for Marfona and Agria was about 17 DAP (Table 2). Number of stems produced by the planted minitubers was significantly affected by cultivar; it was higher for Gloria and Agria than for Marfona. Length of plants grown from planted minitubers was longest for Agria while Marfona produced shortest plants. The factor cultivar had a significant effect on the dry matter of aerial plant parts produced from minitubers planted in the greenhouse to evaluate the vigour of the minitubers. This effect was in line with the effect on the date of emergence, but not in line with the effects on number of stems or length of the longest stem, suggesting that the duration of above ground growth was the most important factor determining the above ground dry matter of the cultivars. The factor cultivar also affected the dry matter yield of progeny tubers. The very early cultivar Gloria not only produced more above ground dry matter but also more dry matter of progeny tubers than the cultivars of Agria and Marfona. The ranking order for the dry matter of progeny tubers produced from planted minitubers was Gloria > Agria > Marfona (Table 2). This means that the difference in dry matter yield of progeny tubers between the cultivars Marfona and Agria was significant, whereas they produced similar amounts of above ground dry matter. Probably, the difference in number of stems between the three cultivars had consequences for the dry matter yield of progeny tubers. All three cultivars produced 2.0 g dry matter of progeny tubers per stem.

Minituber size

The size of minitubers had no significant effect on the date of emergence, but significantly affected number of stems, length of longest stem, dry matter of aerial plant parts and dry matter of progeny

tubers (Table 2). As expected, the number of stems increased with an increase in minituber size. Largest minitubers also produced the longest stem, largest quantities of dry matter of aerial plant parts and highest yields of progeny tubers. Per individual stem, the two smaller minituber sizes produced the same amounts of above ground dry matter and dry matter of progeny tubers, but the largest minituber size had slightly lower values for above ground dry matter per stem and dry matter of progeny tubers per stem (data not shown).

Due to the methodology and statistical design, it was not possible to assess the interaction between minituber size and the factors temperature, photoperiod and cultivar. Possible differences in after-effects of in vitro treatments through effects on minituber size were therefore excluded.

Interactions

The two-way interactions Temperature × Photoperiod, Temperature × Cultivar and Photoperiod × Cultivar were highly significant for some parameters (data not shown). This was especially true for the individual minituber weight and the yield of minitubers. However, also the three-way interaction Temperature × Photoperiod × Cultivar was significant for above ground dry matter, plant height, size, weight and yield of minitubers (Table 3), but it was not significant for number of minitubers ($P = 0.0532$). We focus the description of the results on the effects on yield of minitubers.

Ranking of the cultivars based on the yield of minitubers was different for different in vitro temperatures and photoperiods. Within five of the nine Temperature × Photoperiod combinations, yields of tubers of the three cultivars were not statistically significant. In two Temperature × Photoperiod combination (temperature 16 ^{o}C/photoperiod 20 h and temperature 24 ^{o}C/photoperiod 12 h), minituber yield was higher when the cultivar was earlier, with Gloria being the highest yielding cultivar. In the other two Temperature × Photoperiod combinations with significant differences among cultivars (T 16 ^{o}C/P 16 h and T 20 ^{o}C/P 20 h) effects were different: Gloria showed lowest yield in T 16 ^{o}C/P 16 h, whereas the highest yield was found for Marfona in T 20 ^{o}C/P 20 h. However, the effects of the different environmental treatment combinations on the progeny yield of the different cultivars were in line with the effects observed for above ground dry matter. There was a close relationship

Table 3 Three-way interaction between temperature and photoperiod during the in vitro phase and cultivar for the above ground dry matter, plant height, size, weight and yield of minitubers after planting in vitro plantlets in the greenhouse. The number of minitubers is not given as the three way interaction was not statistically significant for this parameter (P = 0.0532).

Temperature (^{o}C)	Photoperiod (h)	Cultivar	Above ground dry matter (g)	Plant height (mm)	Size of minitubers (mm)	Weight of minitubers (g)	Yield of minitubers (g)
16	12	Gloria	1.62 cdefg	135 g	27.7 bcde	6.8 def	47.67 defghi
		Marfona	1.04 hijklm	72 r	23.6 def	4.6 lmn	38.57 efghi
		Agria	0.80 lm	95 p	24.8 def	5.8 fghij	31.92 fghi
	16	Gloria	1.48 defghi	109 mn	26.2 def	6.2 efghi	55.41 def
		Marfona	2.09 bc	168 c	33.1 a	11.3 a	103.3 b
		Agria	2.31 b	187 b	31.3 abc	10.9 ab	103.1 ab
	20	Gloria	2.96 a	184 b	31.5 ab	10.1 b	125.6 a
		Marfona	1.87 bcde	104 o	31.2 abc	8.5 c	72.54 cd
		Agria	1.00 ijklm	112 lm	24.2 def	5.3 hijkl	35.58 efghi
20	12	Gloria	1.00 ijklm	110 lmn	25.1 def	4.6 lmn	33.71 fghi
		Marfona	0.81 klm	72 r	22.1 f	3.6 n	27.77 hi
		Agria	0.69 m	114 kl	23.5 ef	4.5 lmn	23.33 i
	16	Gloria	1.33 fghijk	107 no	27.3 bcde	5.3 hijkl	41.90 efghi
		Marfona	1.27 fghijkl	74 r	24.3 def	5.1 ijkl	55.43 def

		Agria	0.99 ijklm	152 d	27.4 bcde	7.3 d	54.94 defg
	20	Gloria	1.54 defgh	131 hi	26.1def	5.0 jklm	50.27 defghi
		Marfona	1.96 bcd	116 jk	26.5 def	9.0 c	85.62 bc
		Agria	1.63 cdef	205 a	28.2bcd	7.1 de	55.12 def
24	12	Gloria	1.70 cdef	147 cf	27.8 bcde	6.3 defgh	61.33 de
		Marfona	1.36 efghij	89 q	22.1 f	3.9 mn	39.57 efghi
		Agria	0.68 m	134 gh	26.4 def	5.7 ghijk	27.95 ghi
	16	Gloria	1.62 cdefg	128 i	27.0 cde	6.5 defg	51.65 defgh
		Marfona	1.18 ghijklm	117 jk	23.3 ef	4.6 lmn	43.83 efghi
		Agria	0.88 jklm	144 f	23.2 ef	5.3 hijkl	37.51 efghi
	20	Gloria	1.57 defg	151 de	25.4 def	4.7 klmn	45.86 efghi
		Marfona	1.10 ghijklm	120 j	24.0 def	6.1 efghij	54.78 defgh
		Agria	0.92 jklm	134 gh	22.0 f	5.9 fghij	40.16 efghi

Values followed by a similar letter are not statistically significantly different.

between above ground dry matter and yield of minitubers for each cultivar which was consistent across all cultivars (Fig. 1). This figure suggests that the in vitro conditions influence the haulm growth and therefore yield of minitubers. When highest above ground dry matter was produced highest yield of minitubers was obtained.

Discussion

Minituber number, size, individual weight and yield of minitubers could all be strongly affected by in vitro photoperiod. Such effects are consistent with findings in the literature (e.g. Seabrook et al., 1993, 1995). Seabrook et al. (1995), for example, found that plantlets grown under longer photoperiod (16 h) during the plantlet production phase resulted in taller plants in the greenhouse with more nodes than plantlets exposed under shorter photoperiod in vitro (12 h). Seabrook et al. (1995) also reported that fresh weight of minitubers produced on plants developed from tissue culture plantlets grown under 16 h photoperiod during the in vitro phase was higher than that of minitubers produced with plants developed from plantlets grown under 12 h photoperiod during the in vitro phase. Our experiment confirms these results. In our study, higher numbers of minitubers and larger sizes, higher individual weights and higher yields could be obtained with longer in vitro photoperiod. The largest differences were observed between the shortest photoperiod (12 h) and the two longer photoperiods (16 and 20 h), which is consistent with the critical photoperiod (around 15 h) of the cultivars used. Effects of photoperiod are, however, not only related to its effect on induction to tuberize, but certainly also to the vigour of the in vitro plantlets produced. The positive effects of the photoperiods 16 and 20 h compared to the photoperiod of 12 h are also consistent with the suggestion that for vigorous in vitro plantlets a long photoperiod during the in vitro phase is required (Dodds et al., 1992) to provide enough photosynthetically active radiation to sustain growth (Dodds et al., 1992; Seabrook, 2005). Long photoperiods thus create an in vitro plantlet which is more suitable for use as a transplant used in greenhouse production of minitubers.

No photoperiod effects on minituber quality after storage were observed. Apparently, the effects of in vitro photoperiod on the size and individual weight of the minitubers were too small to induce effects on weight of sprouts and any possible effects on dormancy and vigour were lost during the long storage or overruled by the de-sprouting or grading carried out before the vigour test. Large effects of photoperiod on dormancy and growth vigour independent of the timing of tuber formation were not expected anyway on the basis of the literature (Van Ittersum, 1992).

In vitro temperature had a strong effect on above-ground dry matter, minituber size, individual weight and yield of minitubers, but not on minituber number. When effects were observed, the lowest in vitro temperature resulted in larger minitubers and higher minituber yields than the other two in vitro temperatures, with no differences between the higher two temperatures. Apparently this lowest temperature resulted in a more vigorous transplant which performed better in the greenhouse. A similar after-effect was observed by Tadesse (2000). He indicated that the effect of in vitro temperature was mainly through a positive effect on leaf area of the transplants, resulting in more light being intercepted during the in vitro phase and a higher total in vitro plantlet weight. These effects also result in a faster initial growth due to better light interception immediately after transplanting. However, Tadesse (2000) found the largest differences in the range of 20 to 26 °C, growing the in vitro plantlets at a photoperiod of 16 h.

The lack of effects of in vitro temperature on the number of microtubers as observed in our experiment is also consistent with the results of Tadesse (2000; after-effect of in vitro conditions in greenhouse and field). Consequently our effects on minituber yield were mainly brought about by effects on size and weight of minitubers (Tables 1 and 3).

There was no after-effect of in vitro temperature on the number of sprouts or length of longest sprout of the minitubers but there was an after-effect of in vitro temperature on weight of sprouts. The lowest temperature resulted in a significantly higher sprout weight than the other two in vitro temperatures, with no differences between the higher two temperatures. This effect may be associated with thicker sprouts made possible by the larger size of the minitubers produced.

No effects of in vitro temperature on the growth vigour of the minitubers after storage were observed, although the temperature effect on the dry matter of the progeny tubers was almost significant, with the lowest in vitro temperature giving more yield than the other two in vitro temperatures. Apparently, any possible effects on dormancy and vigour were lost during the long storage or overruled by the de-sprouting or grading carried out before the vigour test. Within the range of in vitro temperatures imposed, large effects of temperature on dormancy and growth vigour independent of the timing of tuber formation were not expected. Even with a large range of temperatures during tuber growth, Van Ittersum (1992) could not induce large differences in dormancy and vigour.

Cultivars were significantly different for above ground dry matter, plant height, number, size and yield of minitubers but not different for individual tuber weight. The very early cultivar Gloria produced more above ground dry matter, more minitubers with larger size and higher yield than the late cultivar Agria. Sprout behaviour was completely different among cultivars, the very early cultivar Gloria producing fewer sprouts with longer length and higher weight than the late cultivar Agria, with Marfona being intermediate. These cultivar effects persisted during storage and resulted in clear differences in growth vigour.

Effects of minituber size and cultivar on the dry matter of the progeny tubers were mainly accounted for by the differences in number of stems produced by the minitubers. This suggests a direct relationship between number of stems and number of tubers (as also observed in field-grown crops; Struik & Wiersema, 1999), with – during early stages of tuber bulking – not much inter-stem competition for light affecting the tuber set.

There were significant interactions between temperature, photoperiod and cultivar, but almost exclusively only on the performance of the transplants and hardly on the characteristics after long storage of the minitubers produced. Within some regimes during the in vitro phase, cultivars responded according to their earliness, but with other combinations of temperature and photoperiod, earliness seemed to be overruled by other specific responses of the cultivar to the in vitro conditions.

Despite this variation in behaviour of the different cultivars there were some overall and consistent trends in the interaction between above ground and below ground performance. Above ground dry matter after greenhouse cultivation of in vitro plantlets was strongly influenced by the conditions during the in vitro phase, longer photoperiod and low temperature promoting above ground dry matter. Enhanced haulm growth of greenhouse plants increased minituber yield as suggested by the close relationship between above ground dry matter and yield of minitubers (Fig. 1). There were also significant relationships between above ground dry matter and the yield components number, size

and individual weight of minitubers, but these were not so close (not shown). Higher yields were realised by producing both more and heavier tubers.

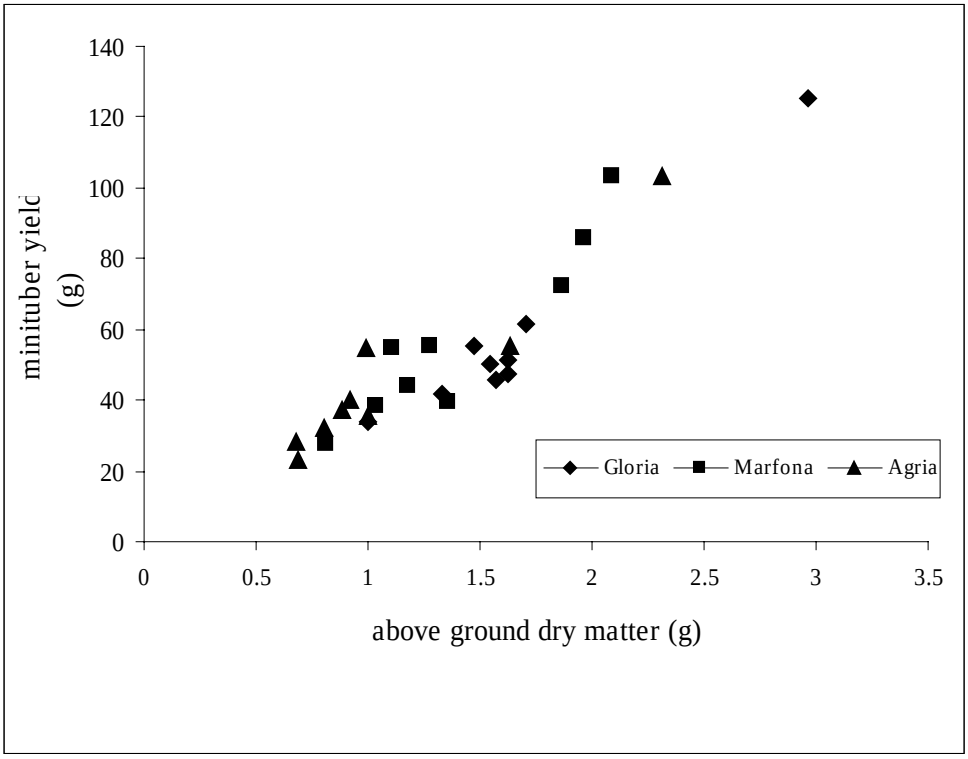

Fig. 1. Relationship between above ground dry matter and fresh minituber yield.

Conclusions

In vitro photoperiod and temperature have a significant after-effect on the plant growth, above ground dry matter and minituber production in the greenhouse phase. The effects on minituber yield were associated with increased above-ground biomass, more and heavier tubers. There were no or hardly any after-effects of in vitro photoperiod or temperature on the vigour of the minitubers. These results suggest that it is possible to adjust the conditions during the last step of in vitro multiplication in such a way that the minituber production is enhanced. However, optimal conditions and benefits are cultivar-specific.

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Chapter 5

Effects of storage temperature, size of minitubers and growth regulator application on the dormancy, sprout behaviour, growth vigour and quality of minitubers of different cultivars of potato

Otroshy, M., & P.C. Struik, 2006

Effects of storage temperature, size of minitubers and growth regulator application on the dormancy, sprout behaviour, growth vigour and quality of minitubers of different cultivars of potato

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Abstract

Minitubers are usually more sensitive to water loss and have deeper dormancy than seed tubers of normal sizes. Whereas minitubers can produce many sprouts, the amount of resources available per sprout is much lower than in normal seed tubers. These aspects strongly depend on the size of the minitubers. The control of the behaviour of minitubers of different sizes by storage treatments, physical treatments and application of growth regulators is therefore essential. Sprout behaviour and vigour of minitubers of different sizes were studied in three potato cultivars after storage at different temperatures, and applying different growth regulators (GR: gibberellic acid and Chlormequat (CCC)) when tubers were cut after storage.

Storage at 8 ^{o}C resulted in more weight loss than at 4 or 12 °C. Absolute weight losses were highest but relative weight losses were lowest for largest minitubers. Before applying GRs increasing temperature increased number, length and weight of sprouts. After GR application optimal sprout behaviour was observed after storage at 8 ^{o}C. Number, length and weight of sprouts were higher with larger minitubers. Gibberellic acid enhanced sprout growth.

Storage temperature did not affect date of emergence, number or length of stems or vigour, but did affect shoot mass and early tuber yield. Minituber size significantly affected all parameters relating to seed tuber performance except date of emergence. GR treatments influenced seed tuber performance in a complex manner.

Results indicate that it is possible to optimize the performance of minitubers by selecting the right conditions for storage. Further enhancing the performance of the minitubers by treating them with growth regulators, however, is difficult.

Keywords: minitubers, storage temperature, growth regulator, gibberellic acid, Chlormequat, CCC, sprout behaviour, vigour.

Abbreviations: GA= Gibberellic acid, CCC= Chlorocholine chloride (or Chlormequat), DAP = Day after planting, GR = growth regulator; NS: not significant.

Introduction

The potato (*Solanum tuberosum* L.) crop is a very important food for people in developed and developing countries; it is used as a staple food, as a raw material for processed food (French fries, chips, etc.) and for the production of feed, starch and alcohol. It can be grown from sexual propagules (True Potato Seed) or vegetative propagules (seed tubers). Generally farmers use seed tubers for multiplication and cultivation. Using seed tubers has some advantages: they are easy to plant and provide high yields with large uniformity (Gopal, 2004). However, there are also many disadvantages: they are labour and time consuming, have high risks of pests and bacterial and fungal diseases, and a low rate of multiplication, resulting in many field multiplications (Beukema & Van der Zaag, 1990; Struik & Wiersema, 1999). Seed tuber production is therefore expensive and requires intensive pest and disease control.

The yield of the potato crop is highly affected by the quality of seed tubers. Yields are higher when seed tubers of higher quality are planted (Struik & Wiersema, 1999). Seed tuber quality has physical (size, absence of damage, resistance to water loss, ratio of number of eyes to size or weight), genetic (absence of off-types), biotic (absence of seed-borne pathogens) and physiological

(absence of dormancy; high vigour) aspects. We focus on the physical and physiological aspects of seed tuber quality.

The physical character size of the seed tubers is known as one of the most important properties of a seed tuber (Struik & Wiersema, 1999); it affects the number of eyes, sprouts and stems per tuber, but sometimes seed tubers with the same size have different numbers of eyes because of differences in growing conditions during production and because of genetic differences (Struik & Wiersema, 1999). Because of the variable effects of size on the number of eyes, also the ratio number of eyes: weight of the seed tuber is important, especially at smaller sizes.

Dormancy is one of the most important physiological properties of seed tubers (Rastovski et al., 1987; Harris, 1992; Van Ittersum, 1992). Generally, potato tubers are dormant at the time of harvest (Harris, 1992; Rowe, 1993) for a period of 1 – 15 weeks (Wiltshire & Cobb, 1996) or maybe even 18 – 33 weeks (Harris, 1992). Wiersema et al. (1987) reported that the dormant period of seed potato tubers is longer with small tubers than with large tubers. Lommen (1994) showed that the dormancy of minitubers is usually longer than the dormancy of normal seed tubers and that the dormancy depends on minituber size: the smaller the minitubers the longer the dormancy period. Leclerc et al. (1995) and Ranalli (1997) indicated that also for microtubers, the length of the dormancy depended on size of the microtubers: smaller microtubers had longer dormancy periods than larger microtubers. However, in normal seed tubers also these effects of size on dormancy depend on the cultivar (Van Ittersum, 1992). As a consequence, dormancy varies between and within potato seed lots, even when all tubers are of the same cultivar (Van Ittersum, 1992).

Usually, the dormant period is shorter in early cultivars than in later cultivars (Harris, 1992), although this relation is not very strict (Burton, 1968). Cutting and injuring seed tubers can break tuber dormancy and shorten the period of dormancy (Struik & Wiersema, 1999). Growth conditions during seed production (especially temperature, photoperiod, light intensity, and nitrogen fertilization) can also affect the duration of dormancy (Van Ittersum, 1992). However, the development of dormancy during storage is most strongly affected by the storage conditions.

Especially the temperature regime, the presence of light and the relative humidity have a strong impact (Struik & Wiersema, 1999).

Until breaking of the dormancy, changes in the physiological status of the seed tuber are only reflected by changes in the physiology and biochemistry of the seed tuber and not by morphological changes. Dormancy ends when visible sprouts have appeared (Davidson, 1958). This moment is usually defined as the time at which at least one sprout with a length of 2 mm (Van Ittersum, 1992) or 3 mm (Krijthe, 1962) has appeared. At this stage the seed tuber may start to sprout. Sprouting and growth vigour of normal seed tubers also depend on seed tuber size, larger sizes giving more vigorous sprouting and a higher growth vigour of these sprouts (Wiersema et al., 1987; Van Ittersum, 1992; Struik & Wiersema, 1999). This effect of size is also visible (and even stronger) among different minituber size classes (Lommen, 1994) and microtuber size classes (Bus et al., 1990). Also potato tuber sprouting can be affected by storage conditions (Burton, 1978; Rastovski et al., 1987; Struik & Wiersema, 1999). Storage temperature can affect the length of the period between harvesting and sprouting; higher temperatures shorten this period and lower storage temperatures prolong it (Davidson, 1958; Struik & Wiersema, 1999). Moreover, the longer the period at 2 – 4 °C the more sprouts will eventually develop (Struik & Wiersema, 1999).

Storage temperature can also affect the sprouting capacity (Hartmans & Van Loon, 1987) and the growth vigour of the seed when planted (Van der Zaag & Van Loon, 1987). The number of sprouts per seed tuber planted and the final number of stems per seed tuber are closely associated with the sprouting capacity. Shocks of short periods of heat or cold and the same accumulated day-degrees built up in different ways all have their specific effects on physiological behaviour after breaking of the dormancy, depending on cultivar (Struik et al., 2006). Struik & Wiersema (1999) gave an overview of the complex interactions between storage temperature regimes and cultivar in relation to growth vigour. Struik et al. (2006) showed that cultivars with a high rate of physiological ageing are very sensitive to the way the accumulated day-degrees are built-up during the storage season.

High level of storage losses, excessive sprouting and lack of tuber vigour are the most important problems for seed potato storage (International Potato Center, 1984). During the storage period water can be lost via (evapo)transpiration and respiration can cause loss of dry matter. Both transpiration and respiration are increased by sprouting and by the occurrence of diseases. Both physiological processes reduce the physiological quality of the seed tubers.

Conditions affecting dormancy and vigour also affect loss of dry matter and water. Optimal conditions for dormancy breaking are not the same as optimal temperatures for sprouting or for avoiding losses (Struik & Wiersema, 1999). Temperatures that will allow the shortest dormancy are in the range of 25 – 30 °C. A storage temperature of 18 ^{o}C is known as optimal for sprout growth and at higher temperature sprout growth will be arrested by sub-apical necrosis (McGee et al., 1986).

There are close interactions between genotype and storage conditions (Van Ittersum, 1992; Struik et al., 2006 and references therein) and between seed tuber size and storage conditions (Struik & Wiersema, 1999). These interactions can be manipulated to some extent by applying growth regulators just before haulm destruction (Van Ittersum, 1992) and during storage (Struik & Wiersema, 1999). Chemical compounds can affect sprouting (Marth & Schultz, 1952; Holmes et al., 1970; Li, 1985; Rastovski et al., 1987). Concentration, time and method of application all affect the response (Rappaport et al., 1957).

Gibberellic acid can stimulate sprouting and advance emergence (Van Hiele, 1961) and break dormancy (Rappaport et al., 1957). Effects of gibberellic acid applied to the tuber on sprout growth include more sprouts, more branching of sprouts, thinner sprouts, and longer sprouts (Van Hiele, 1961). Soil application (Struik et al., 1989) and foliar application of gibberellic acid (Hammes & Nel, 1975; Struik et al., 1999) have similar effects on shoot, stolon and tuber formation: they result in higher yield of the shoot, longer plants, smaller leaves, more branching, change in rate of development of the potato plant, longer stolons, and increased fresh yield of tubers. The number of tubers produced can also be strongly increased (Struik et al., 1989). However, application to the soil before 80 day after planting reduced tuber dry matter yield. Yet, there is lack of information about the effect of GA levels on the stolon tips (Struik et al., 1999).

Gibberellic inhibitors such as CCC (chlormequat) can induce opposite effects on sprouting and emergence. CCC usually prolongs dormancy, reduces sprouting capacity and may have an after-effect on the performance of the plant emerging from the sprouts, especially when applied to the haulm of the seed tuber crop to enhance uptake in the seed tubers (e.g. Struik & Wiersema, 1999).

There are many more growth regulators which can be applied to affect seed tuber dormancy or growth vigour of tubers. These include rindite (a mixture of ethylene chlorhydrin, ethylene dichloride and carbon tetrachloride), ethylene chlorhydrin, carbon disulphide, thiourea and benzyl adenine (Struik & Wiersema, 1999).

This paper deals with manipulating the physical and physiological aspects of seed tuber quality, when minitubers are used. Minitubers are small tubers grown in the greenhouse from in vitro plantlets. Their health standards are very high. As they are small, their physical quality (including the physical resistance to water loss, the amount of reserves and the ratio between number of eyes and the amount of reserves) is smaller than that of seed tubers of normal sizes. But also their physiological quality is often lower. As stated above, minitubers normally have a deeper dormancy than normal seed tubers and the increase in growth vigour with a prolongation of storage is also slower. Therefore, minitubers cannot be planted immediately after harvest time and it is necessary to store them at least until dormancy is broken (Lommen & Struik, 1993). Moreover, the water and dry matter losses in minitubers can be more significant and damaging than in seed tubers of normal sizes (Lommen, 1993) and are associated with the sprouting behaviour of the minitubers. Modifying the behaviour in response to storage conditions using the application of growth regulators, especially in relation to dormancy and vigour, is therefore crucial in minitubers.

However, there is little information available on the interactions between minituber size, storage temperature and growth regulators applied during the storage phase on dormancy, sprout behaviour and minituber growth vigour. The main objectives of this study were therefore to find out how minituber size, storage conditions and growth regulators could interact in affecting quality of minitubers, in order to find out a method to increase yield and quality of seed tubers in a tuber production scheme based on minitubers. As model substances we used the growth regulators

gibberellic acid (GA) and cycocel (chlormequat; 2-chloroethyltrimethylammonium chloride; CCC) as their effects are relatively well-known, thus improving the prospects of proper interpretation of the complex interactions to be expected.

Materials and methods

In vitro plantlet production

Potato (*Solanum tuberosum* L., cvs Gloria (very early), Marfona (mid-early) and Agria (late)) plantlets from virus-free stock plantlets were multiplied using single-node cuttings. Sixteen viable stem cuttings (discarding the top) with one leaf node were cultured in a culture jar (D76, 75 × 73 mm) on 50 ml standard medium containing 4.4 g/l MS salts (Murashige & Skoog, 1962) with vitamins, 30 g/l sucrose as a carbon source and 8 g/l agar to solidify the medium; pH was adjusted at 5.8 prior to the addition of agar and before autoclaving. The culture jars with media were autoclaved at 120 ^{o}C for 15 minutes. The sealed jars were incubated in a growth chamber at 24 ^{o}C and a photophase of 16 h under 4000-5000 lux light intensity provided by white fluorescent tube lamps, Philips 230 v for 4 weeks. The process was routinely repeated until the required numbers of plantlets for minituber production were achieved.

Minituber production

When required numbers of 4 weeks old plantlets were obtained, the plantlets were removed from the culture jars and roots were washed by water to remove the media. Cleaned plantlets were directly (without hardening) and carefully transplanted into 5 litre pots filled with potting soil mixed with required granule nutrient (3 g/l = 15 g per pot nutrient containing 13, 13, 13% NPK) at a density of 11 pots per square meter. One plantlet was planted per pot and one third of its stem was put in the soil.

The plantlets were grown in the greenhouse at a day/night temperature of 18/12 ^{o}C, a day length of 12 h (from 07:00 to 19:00) and a relative humidity of 85%. Temperature, photoperiod,

humidity and day length in the greenhouse were automatically controlled and 24 SON-T lamps 400 W (0.8 per m^2) provided additional photosynthetically active radiation and (together with incandescent lights) the appropriate photophase. Plantlets were daily and carefully irrigated starting immediately following planting. Pests and diseases were adequately controlled, either biologically or chemically, whenever needed.

Minituber handling and treatments

Minitubers of the three potato cultivars were hand-harvested after 100 days after planting the in vitro plantlets and were cleaned to remove the soil, then were left in the greenhouse for 2 weeks at the same greenhouse conditions and full darkness to harden and set skin. After 2 weeks minitubers were graded into three size classes: 13 – 25 mm, 25 – 35 mm and 35 – 50 mm, and stored at three different storage temperatures: 4 ^oC, 8 ^oC and 12 ^oC and a relative humidity of 90%. The individual weight of each minituber at the beginning and the end of the storage period was recorded to measure the absolute minituber weight loss. To assess the relative weight loss (in %) the difference between the individual initial and final minituber weight was divided by the initial tuber weight and expressed as percentage.

After 90 days of storage, the number of sprouts, length of longest sprout and total weight of sprouts were recorded to evaluate the effect of storage temperature and minituber size on the sprouting behaviour. The criterion for a sprout was a stem structure of at least 2 mm (Van Ittersum, 1992). These measurements involved de-sprouting of the minitubers so that the following experimental procedures were carried out with de-sprouted minitubers. De-sprouting enhances the expression of the effects of storage temperatures on growth vigour (Struik & Wiersema, 1999) and probably also the effect of the growth regulators.

All minitubers (except those meant for the uncut control) were hand cut (from apical end of the tuber to two thirds of its longitudinal length). A small piece of cardboard was placed in the minituber cut to prevent the cut from closing again and to keep the cut open to allow the plant growth regulator applied to penetrate easier.

To apply the growth regulators, minitubers were placed on a tray and were soaked in solutions of the growth regulators containing 2.5 or 5 mg gibberellic acid (GA3) per litre, or 10 or 100 mg CCC per litre for a period of 10 minutes; control tubers (untreated tubers, minitubers cut (control 1), or not cut (control 2), were soaked in pure water for the same time. The trays with minitubers were covered with household plastic to keep humidity at high level and were stored at 18 ^{o}C, full darkness and a humidity of 80%. After 5 days the plastic cover was removed and finally after a period of 11 days of storage number of sprouts, length of longest sprout and weight of sprouts were recorded to assess the effect of storage temperature and size of minitubers again and to record the effects of the growth regulators on the sprouting behaviour after the de-sprouting. For this, minitubers were transferred to a cold store at 4 ^{o}C and a relative humidity of 80% and collecting data for sprouting behaviour was started. Data collection only took a few days.

After these records, and thus after a second de-sprouting, minitubers were planted in the greenhouse under the same conditions as previously described for the minituber production and allowed to produce plants for a period of 8 weeks. Date of emergence was recorded during this period and after 8 weeks (a normal duration to assess vigour of tubers; Van Ittersum, 1992; Lommen, 1995) growth vigour of the minitubers was further assessed.

Experimental design, statistical analysis and measurements

For the evaluation of the effect of storage temperature and minituber size on the sprout behaviour (before applying the plant growth regulators), the experimental design was a completely randomized factorial with 3 factors: storage temperature (4, 8 or 12 ^{o}C), minituber size (13 – 25 mm, 25 – 35 mm and 35 – 50 mm) and cultivar (Gloria, Marfona and Agria) and 60 replicates. Traits were recorded at the end of storage, just before growth regulator application.

For testing the interactions with growth regulator application after storage, the experimental design was a factorial experiment based on a randomized complete design, including 4 factors: 3 potato cultivars (cvs) × 3 storage temperatures (T) (4 ^{o}C, 8 ^{o}C, 12 ^{o}C) × 3 sizes of minitubers (S) (13 – 25 mm, 25 – 35 mm and 35 – 50 mm) × 6 growth regulator treatments. The latter included two doses

of gibberellic acid (GA3) (2.5 or 5 mg/l), two doses of chlormequat (CCC) (10 or 100 mg/l) and two control treatments (without plant growth regulators, cut or uncut). There were 10 replicates. Sprout behaviour traits were recorded again after some storage after growth regulator application (see above) but before planting the minitubers in the greenhouse.

Growth vigour of the minitubers after 8 weeks of growth in the greenhouse was assessed by counting the number of stems, measuring the length of the longest stem, and determining the dry matter of the above ground plant parts and the dry matter yield of progeny tubers.

Randomization was done using excel software and all data were statistically analysed by the MSTATC statistical program. Means were compared using the Duncan's Multiple Range Test at 5% significance level. A few values were missing and the MSTATC statistical programme was used to estimate them.

Results and discussion

Main effects of storage temperature, minituber size and cultivar on tuber weight loss and sprout behaviour before and after growth regulator application

Storage temperature effects

Effects of storage temperature on relative and absolute tuber weight loss. In general, tuber weight losses were only minor, thanks to the high quality storage facilities used in the experiments. Nevertheless, storage temperature significantly affected the relative and absolute weight losses of the stored minitubers (Table 1). Weight losses were highest at a storage temperature of 8 ^{o}C while 4 ^{o}C showed lowest values (Table 1). The effect of the lowest storage temperature is consistent with effects observed in normal seed tubers (Hartmans & Van Loon, 1987). According to Rastovski et al. (1987) permeability of water through the periderm, degree of suberization, maturity of tubers, damaging and bruising of tuber, degree of sprouting, and vapour pressure deficit in the storage are the most important parameters affecting evaporation rate and weight losses during the storage. Materials are transferred from tubers to the sprout for sprout growth and synthesis of dry matter in sprouts increases respiration

(Harris, 1992). Increasing respiration increases tuber weight loss as it requires assimilates previously stored.

Effects of storage temperature on sprout behaviour. Storage temperature showed a highly significant effect on the number of sprouts, the length of the longest sprout and sprout weight: the higher the storage temperature the higher the values observed for these parameters after 90 days of storage (Table 1). This is consistent with the literature on normal (seed) tubers when assessments are carried out shortly after breaking of the dominance (Davidson, 1958; McGee et al., 1986; Hartmans & Van Loon, 1987; Van Ittersum, 1992; Struik & Wiersema, 1999). Higher storage temperatures advance the breaking of dominance.

However, the effect of storage temperature on the behaviour of sprouting of minitubers as reflected in the number, length and weight of sprouts was different before and after growth regulator application (Tables 1 and 2). Before growth regulator application, lowest numbers of sprouts with smallest size and lowest weight were obtained at lowest storage temperature and highest values were obtained at highest storage temperature. In contrast, after growth regulator application, lowest values were obtained at highest storage temperature and highest values were obtained at 8 ^{o}C (Tables 1 and 2). This difference in behaviour is due to three aspects of the methodology: de-sprouting, cutting and the longer duration of storage. The de-sprouting contributes to the development of new sprouts and more sprouts with a higher rate of sprout growth than without de-sprouting (Struik & Wiersema, 1999). De-sprouting after storage at the lowest temperature gave less loss of vigour of the minitubers than de-sprouting of the minitubers more advanced in sprouting in response to the higher storage temperature. Cutting contributes to the breaking of the dormancy (Rappaport & Sachs, 1967) and to the breaking of the apical dominance (Struik & Wiersema, 1999). It also stimulates the sprout growth (Struik & Wiersema, 1999). Cutting may also have had a larger effect on the minitubers stored at the lowest temperatures than on the minitubers stored at higher temperatures. Longer storage advances the physiological age and thereby increases the number of sprouts produced per seed tuber, although well beyond dormancy the number of sprouts becomes highly dependent on the storage temperature: a

longer storage at lower temperature increases the number of sprouts as apical dominance is stronger at higher storage temperatures (Struik & Wiersema, 1999). The combination of the effects of these three aspects of the methodology resulted in an optimal storage temperature of 8 °C.

Minituber size effects

Effects of size of minitubers on relative and absolute tuber weight loss. A negative relationship between size of minitubers and relative weight loss was observed; the relative weight loss was larger in smaller minitubers (Table 1). Increasing the size of minitubers also changed absolute minituber weight loss significantly; however, the absolute weight loss was higher with larger minitubers (Table 1). Lommen (1995) reported similar results; she indicated that the highest percent of fresh weigh loss during storage was associated with the smallest minitubers. Wiersema et al. (1987) also observed a higher weight loss in smaller seed potato tubers than in larger seed potato tubers. Higher tuber weight loss in smaller tubers compared to larger tubers can be explained by the difference in the ratio between the surface area and the weight of the minitubers. In addition to that the evaporation per unit of skin area is higher with smaller tubers than with larger tubers (Lommen, 1995; Wiersema et al., 1987). There is a negative relationship between rate of evaporation and suberization of the periderm; larger (and thus older) tubers have a periderm which is more advanced in suberization and thus have a higher periderm resistance and show lower evaporation (Rastovski et al., 1987). This may especially play a role with minitubers (Lommen, 1995) as these are still very young and the size differences probably reflect age differences very well.

Weight losses during the storage period not only result from water loss but also from respiration (Rastovski et al., 1987). According to Burton (1964) there is an inverse relationship between rate of respiration per kg and tuber size, because larger tubers require a smaller proportion of the total amount of assimilates available for maintenance and support of the sprouts. This is associated with the fact that the number of eyes per seed tuber does not show a linear increase with the size of the individual seed tuber. Also very small minitubers already have a relatively large number of eyes.

Effect of size of minitubers on sprout behaviour. Minituber size affected the sprout behaviour. Larger minitubers showed higher values for the number of sprouts, the length of the longest sprout and the weight of sprouts after 90 days of storage (i.e. before the de-sprouting and applying the growth regulators) (Table 1). Similar effects of minituber size on sprout behaviour were observed after the application of the growth regulators (Table 2). These effects are consistent with the effect of tuber size on number of eyes, the proportion of eyes producing a sprout and the tuber size related vigour (Struik & Wiersema, 1999). Similar results are also reported by Wiersema et al. (1987); they also observed that increasing tuber size increased the number and length of sprouts.

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Cultivar effects

Effects of cultivar on relative and absolute tuber weight loss. There was a highly significant difference between the three cultivars in the absolute and relative minituber weight losses. Both parameters were highest for Gloria (the very early cultivar), while Agria (the late cultivar) showed the lowest losses (Table 1). Genetic variation for skin permeability is a common phenomenon in potato (Burton, 1989) and this may well explain this cultivar effect.

Effects of cultivar on sprout behaviour. Before the de-sprouting and the application of the growth regulators there was no significant difference between cultivars in the number of sprouts (Table 1; P = 0.064), but after de-sprouting and growth regulator application cultivars showed a highly significant difference in number of sprouts (Table 2). The lowest number of sprouts was observed with Marfona while cvs Gloria and Agria were similar and produced highest sprout numbers.

Longest sprouts were observed with cvs Marfona and Gloria before and after applying the growth regulators, respectively (Tables 1 and 2). Cv. Marfona produced the longest sprouts before growth regulator application while after de-sprouting and applying the growth regulators it showed the shortest sprouts. Gloria produced shorter sprouts before applying the growth regulators but after de-sprouting and applying the growth regulators this cultivar produced the longest sprouts. Agria had the shortest sprouts before and after growth regulator application. There was no significant

difference in the length of sprouts between cvs Marfona and Agria after applying the growth regulators (Table 2). After de-sprouting, differences between cultivars in length of the longest sprout were small anyway.

The three cultivars showed different responses regarding the weight of sprouts before and after de-sprouting and growth regulator application (Tables 1 and 2). A highly significant difference in the sprout weight was observed among the three cultivars before using the growth regulators (and thus before de-sprouting): it was highest with Marfona and Agria had lowest sprout weight. There was no significant difference after growth regulator application ($P = 0.062$). The effect before de-sprouting was most likely associated with cultivar differences in dormancy (note that all cultivars were still in the phase of apical dominance), whereas de-sprouting broke the remaining dormancy and the apical dominance causing the number of sprouts to become more important for sprout weight and the size of the individual sprouts to become less important.

Main effects of growth regulator application on sprouting

Growth regulators had a strong effect on the sprout behaviour after de-sprouting; they affected the number of sprouts, the length of the longest sprout and sprout weight (Table 2).

Minitubers treated with the higher dose of gibberellic acid had the highest number of sprouts, but the lowest numbers of sprouts were found with both controls and the higher dose of CCC (Table 2).

Gibberellic acid was effective in increasing the length of sprouts. Length of sprouts was enhanced in a similar way by both doses of gibberellic acid. The lower dose of CCC enhanced the length of sprouts compared to both controls, the higher dose of CCC enhanced sprout length only compared to the cut control (Table 2).

Growth regulators also affected significantly the weight of sprouts. The two doses of gibberellic acid and also both doses of CCC increased the weight of sprouts compared to the cut

control; both controls (cut and not cut) showed the lowest values for sprout weight (Table 2). The difference between the higher dose of CCC and the uncut control, however, was not significant.

The observed effects on sprout number are in line with Holmes et al. (1970) who observed that gibberellic acid reduces apical dominance thus increasing the number of sprouts. Van Hiele (1961) observed that gibberellic acid (GA3) increased both the number and the length of sprouts. Similar effects were reported by Rappaport et al. (1957), Rastovski et al. (1981) and Li (1985). There was a significant difference between both doses of CCC on sprouting behaviour (number, length and weight of sprouts) so it can be suggested that the effect of CCC on sprouting behaviour of potato minitubers depends on its concentration.

The difference between the two control treatments was also significant for the sprouting parameter length of longest sprout, suggesting an effect of cutting on the vitality of the minitubers. Most likely, the cut control used energy to recover from the cut wounds leaving less energy for sprouting.

Main effects after planting (vigour)

Effects of storage temperature on vigour. Although no significant effects of storage temperature on the vigour parameters date of emergence, number of stems or length of the longest stem were found, strong effects on the above ground dry matter and progeny tuber dry matter were observed (Table 3). A higher storage temperature reduced the dry matter yield of shoot and of tubers even when no effects were observed on emergence date or stem numbers. The highest storage temperature resulted in the lowest above ground dry matter while both other storage temperatures had similar (high) values for above ground dry matter. Progeny tuber dry weight was higher after a storage temperature of 4 ^{o}C than after the highest storage temperature. These effects are consistent with the effects of storage temperature observed for normal seed tubers (Van der Zaag & Van Loon, 1987; Van Ittersum, 1992; Struik & Wiersema, 1999). These results also suggest that initial plant growth and subsequent plant vigour were affected by the amount of assimilates still available during the phase before

photoautotrophic growth of the emerged plants. These amounts most likely have been lower for the highest storage temperature than for the lower ones.

Effects of minituber size on vigour. All minituber sizes emerged at the same date, but there were highly significant effects of minituber size on number of stems, length of longest stem, above ground dry matter and dry matter of progeny tubers (Table 3). The largest minitubers produced the highest numbers of stems with longest stems and the highest above ground dry matter and dry matter of progeny tubers, while lowest values for these traits were observed with smallest minitubers (Table 3). The effects of minituber size observed on the above ground and below ground development are consistent with results observed or described by Lommen & Struik (1993), Lommen (1994), Ranalli (1997) and Struik & Wiersema (1999). But also in normal seed tubers vigour is strongly affected by seed tuber size (Struik & Wiersema, 1999). They confirmed that number of stems per seed tuber is affected by size of tuber; higher number of stems is associated to larger seed tubers and there is a linear relationship between tuber size and stem number; moreover aerial parts of plants grown from smaller tubers develop slower compared to larger tubers. Lower number of stems and slower development of aerial parts of plant grown from smaller tubers resulted in lower accumulated amount of radiation intercepted and in lower crop yield for smaller tubers. Wiersema et al. (1987) also observed more stems with larger plant foliage and higher tuber yield with plants grown from larger tubers compared to smaller tubers.

Table 1. Effects of storage temperature, size of minitubers and cultivar on minituber weight loss and sprout behaviour after 90 days of storage and before growth regulator application.

Symbols: A = Storage temperature, B = Size of minitubers, C = cultivar, NS: not significant (P ≥ 0.05).

	Relative weight loss (%)	Absolute weight loss (g)	Number of sprouts	Length of longest sprout (mm)	Weight of sprouts (mg)
Storage temperature (^oC)					
4	0.8 c	0.06 c	0.1 b	0.04 c	0.01 c
8	2.9 a	0.24 a	1.3 a	8.0 b	22 b
12	2.5 b	0.21 b	1.4 a	29 a	69 a
P	**	**	**	**	**
Size of minitubers (mm)					
13 – 25	2.6 a	0.08 c	0.6 c	9.5 b	11 c
25 – 35	1.9 b	0.16 b	0.8 b	14 a	30 b
35 – 50	1.7 c	0.27 a	1.3 a	13 a	50 a
P	**	**	**	**	**
Cultivar					
Gloria	2.6 a	0.23 a	1.0	9.9 b	30 b
Marfona	2.2 b	0.18 b	0.9	23 a	49 a
Agria	1.4 c	0.11 c	0.9	4.0 c	11c
P	**	**	NS[1]	**	**
Interactions					
AB	**	**	**	**	**
AC	**	**	**	**	**
BC	NS	**	**	**	**
ABC	**	**	NS	**	**

** : Significant at P ≤ 0.01; ∗: significant at P ≤ 0.05, Values followed by the same letter are not statistically significantly different.
[1] P = 0.064.

Table 2. The effect of storage temperature, size of minitubers and growth regulators on the sprout behaviour after growth regulator application in different cultivars of potato.

Symbols: A = Storage temperature, B = Size of minitubers, C = growth regulator, GA = Gibberellic acid, CCC = Chlorocholine chloride (or Chlormequat), D = cultivar. NS: not significant, $P \geq 0.05$.

	Number of sprouts	Length of longest sprout (mm)	Weight of sprouts (mg)
Storage temperature (^oC)			
4	2.2 a	10 b	30 b
8	2.3 a	14 a	37 a
12	1.9 b	10 b	22 c
P	**	**	**
Size of minitubers (mm)			
13 – 25	1.1 c	8.3 c	8.6 c
25 – 35	2.1 b	13 b	28 b
35 – 50	3.2 a	14 a	53 a
P	**	**	**
Growth regulator			
2.5 mg/l GA	2.7 b	19 a	40 a
5 mg/l GA	2.9 a	20 a	41 a
10 mg/l CCC	2.5 b	11 b	38 a
100 mg/l CCC	1.7 c	7.7c	25 b
Control (minituber-cut)	1.4 d	4.3 d	13 c
Control (minituber-uncut)	1.7 cd	7.2 c	21 bc
P	**	**	**
Cultivar			
Gloria	2.4 a	13 a	31
Marfona	1.6 b	11 b	26
Agria	2.4 a	11 b	32
P	**	**	NS[1]
Interactions			
AB	**	NS	**
AC	**	**	**
BC	NS	NS	**
ABC	NS	NS	NS
AD	**	**	**
BD	**	**	NS
ABD	NS	**	NS
CD	**	**	**
ACD	**	**	**
BCD	**	**	**
ABCD	**	**	**

**: significant at $P \leq 0.01$, *: significant at $P \leq 0.05$. Values followed by the same letter are not statistically significantly different.

[1] $P = 0.062$.

Effects of growth regulators on vigour. Growth regulator application significantly affected most vigour parameters, but no significant effect was observed on above ground dry matter (Table 3).

Minitubers treated with 5 mg/l GA emerged earlier and those treated with 100 mg/l CCC emerged later than the cut control (Table 3). The lowest number of stems was observed with the uncut control treatment (Table 3) whereas the other treatments had similar, higher values. Minitubers treated with a high dose of gibberellic acid produced longest stems while the shortest stems were produced by the uncut control (Table 3). Application of gibberellic acid resulted in the lowest dry matter of progeny tubers while both controls showed the highest values for dry matter production of progeny tubers (Table 3).

The positive effect of gibberellic acid on emergence date confirms earlier results by Rappaport et al. (1957), Timm et al. (1962) and Holmes et al. (1970) on normal seed tubers. No significant effect of growth regulators on the above ground dry matter was observed while a highly significant effect of growth regulator on the dry matter of progeny tubers was found: treatments with gibberellic acid and controls showed lowest and highest value for minitubers dry matter, respectively.

Effect of cultivar on vigour. All vigour parameters except the above ground dry matter were significantly affected by cultivar (Table 3). For the above ground dry matter the cultivar effect was almost significant ($P = 0.053$). Cvs Gloria and Marfona were similar for date of emergence and emerged later than Agria, but there was no difference between Marfona and Agria for number of stems and they produced fewer stems than Gloria. Differences in length of longest stem were correlated to differences in number of stems. No significant difference was observed among three cultivars for dry matter of aerial part of plants but for dry matter of minitubers cvs Gloria and Agria were similar and produced more dry matter than Marfona (Table 3).

Interactions before de-sprouting and growth regulator application

Two-way interactions storage temperature × minituber size, storage temperature × cultivar and minituber size × cultivar were significant for relative and absolute weight loss and sprout behaviour, with the exception that there was no significant interaction between minituber size and cultivar for the relative weight loss (Table 1). For all tested parameters except number of sprouts the three-way interaction storage temperature × minituber size × growth regulator was also significant (Table 1).

Storage temperature × minituber size interaction before de-sprouting and growth regulator application. Differences between minituber sizes in relative weight loss during storage were largest at 8 °C, i.e. when the average relative weight losses over sizes were also the largest (Fig. 1). For the absolute weight loss, the effect of minituber size was highest at 12 °C (Fig. 1). For the number of sprouts, the length of the longest sprout and the weight of the sprouts, the differences between minituber sizes increased with an increase in storage temperature (Fig. 2).

Storage temperature × cultivar interaction before de-sprouting and growth regulator application. Marfona had surprisingly low values for relative and absolute weight losses at 4 °C, but showed weight losses similar to Gloria (the cultivar with the strongest losses) at the other two storage temperatures (Fig. 3). Marfona may have been more dormant at the lowest storage temperature than the other cultivars, especially Gloria, but responded well to the higher storage temperatures in breaking its dormancy. Cultivar differences in weight losses were stronger at higher storage temperatures. Agria responded much less to an increase in storage temperature than Gloria and Marfona, with regard to the relative and absolute weight losses. This effect is associated with its much smaller effect of storage temperature on sprouting (Fig. 4). Indeed cultivars also differed in their response to storage temperature regarding the number of sprouts, but the differences in response were not large (Fig. 4). However, the response to an increase in storage temperature in the parameters length of longest sprout and sprout weight was much higher for cv. Marfona than for the

other two cultivars, and especially than for Agria (Fig. 4). Results from Figure 4, therefore explain to a large extent the differences in weight losses indicated in Figure 3.

Minituber size × cultivar interaction before de-sprouting and growth regulator application. A highly significant two-way interaction between size of minitubers and cultivar was observed for the absolute weight loss and sprout behaviour (data not shown). In all three cultivars, largest and smallest minitubers resulted in highest and lowest values for weight loss, respectively, but the size of this effect depended on the cultivar. Similarly, the effects of size on the number of sprouts and sprout weight were consistent over the three cultivars tested but were not of the same magnitude (data not shown). The effects of size on length of longest sprouts were not consistent over the three cultivars: Agria did not show a size effect, Marfona showed longest sprouts at the intermediate size, whereas Gloria showed the expected increase in length of longest sprout with an increase in size (data not shown).

Storage temperature × minituber size × cultivar interaction before de-sprouting and growth regulator application. For weight losses (relative weight loss and absolute weight loss) during the storage period, and for length and weight of sprouts, the three-way interaction storage temperature × minituber size × cultivar was highly significant, but this interaction was not significant for sprout number (Table 1). Ranking of the cultivars were different at different combinations of storage temperature and minituber sizes for relative and absolute weight losses and for length and weight of the sprouts. This is not surprising as the effects of storage temperatures were assessed when the tubers at the lowest storage temperature were still partly dormant and cultivar differences in duration of dormancy were clearly expressed (Fig. 4). These differences in ranking for weight losses may therefore have been associated with differences in the age of the minitubers, but also with cultivar-specific responses to storage temperature (see above) and minituber size, partly associated with differences in skin characteristics and partly associated with differences in duration of dormancy and sprouting behaviour. Marfona showed the lowest relative and absolute weight losses at the lowest storage

Table 3. The effect of storage temperature, size of minitubers and growth regulators on the vigour in different cultivars of potato.

Symbols: A = Storage temperature, B = Size of minitubers, C = growth regulator, GA = Gibberellic acid, CCC= Chlorocholine chloride (or Chlormequat), D = cultivar, DAP = Day after planting, NS: not significant, P ≥ 0.05.

	Date of emergence (DAP)	Number of stems	Length of longest stem (cm)	Dry matter of aerial part of plants (g)	Dry matter of progeny tubers (g)
Storage temperature (^oC)					
4	15	2.0	32.5	5.3 a	3.0 a
8	15	1.9	34.8	5.1 a	2.6 ab
12	13	1.8	30.9	4.1 b	2.2 b
P	NS	NS	NS	**	**
Size of minitubers (mm)					
13 – 25	14	1.2 c	22.5 c	3.4 c	1.5 c
25– 35	14	1.5 b	33.8 b	5.0 b	2.7 b
35 – 50	15	3.0 a	41.9 a	6.1 a	3.6 a
P	NS	**	**	**	**
Growth regulator					
2.5 mg/l GA	14 bc	2.0 a	32.5 b	4.8	0.9 c
5 mg/l GA	12 c	2.2 a	38.8 a	5.7	1.5 c
10 mg/l CCC	15 bc	1.9 a	32.2 b	4.8	2.9 b
100 mg/l CCC	18 a	2.0 a	35.4 ab	4.6	2.3 b
Control (minituber-cut)	15 b	1.9 a	31.3 b	4.8	3.8 a
Control (minituber-uncut)	13 bc	1.3 b	25.9 c	4.4	4.2 a
P	**	**	**	NS[1]	**
Cultivar					
Gloria	15 a	2.4 a	35.4 a	5.2	3.4 a
Marfona	15 a	1.6 b	30.1 b	4.8	1.1 b
Agria	13 b	1.7 b	32.6 ab	4.5	3.3 a
P	*	**	**	NS[2]	**
Interactions					
AB	NS	NS	NS	NS	NS
AC	NS	NS	NS	NS	NS
BC	NS	*	NS	NS	**
ABC	NS	NS	NS	NS	NS
AD	NS	NS	*	NS	NS
BD	NS	NS	NS	NS	NS
ABD	NS	NS	NS	NS	NS
CD	NS	NS	NS	NS	**
ACD	NS	NS	NS	NS	*
BCD	NS	NS	NS	NS	NS
ABCD	NS	NS	NS	NS	NS

**: significant at P ≤ 0.01, *: significant at P ≤ 0.05, Values followed by the same letter are not statistically significantly different.

[1] significant at P = 0.059, [2] significant at P = 0.053.

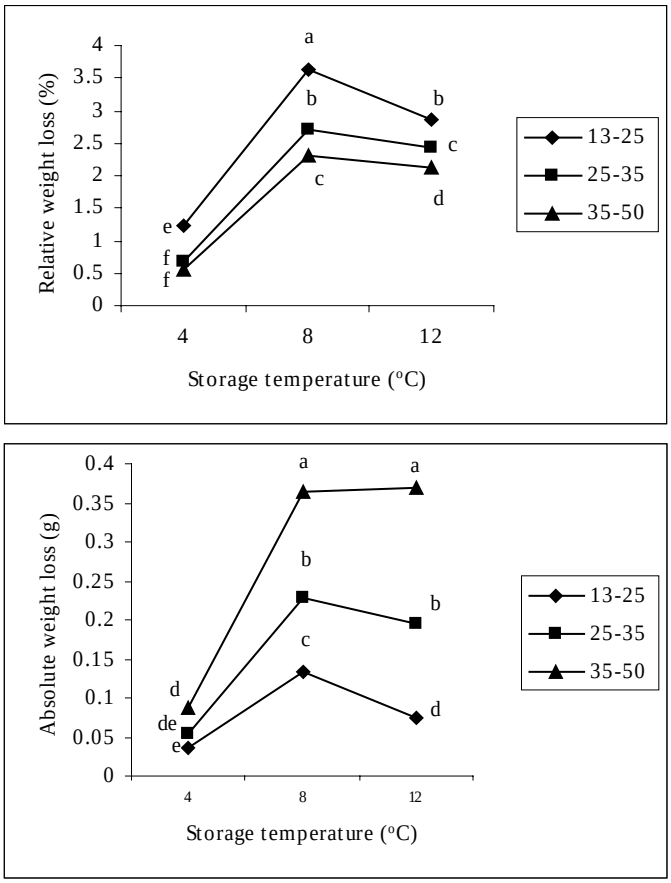


Fig. 1. The storage temperature × minituber size interaction for the relative weight loss and absolute weight loss. Letters a to e show highest and lowest values respectively and data points with the same letter are not statistically significantly different; these letters are based on Duncan's Multiple Range Test.

temperatures for all size classes, whereas at the higher storage temperatures one of the other cultivars showed the lowest relative and absolute weight losses, depending on minituber size. The differences in cultivar ranking for some sprout characteristics among storage temperature and minituber size

combinations also suggest that breaking of dormancy and initial sprout growth were affected by cultivar-specific responses to storage temperature and minituber size. Marfona indeed is a cultivar with a relatively long dormancy (Van Ittersum, 1992).

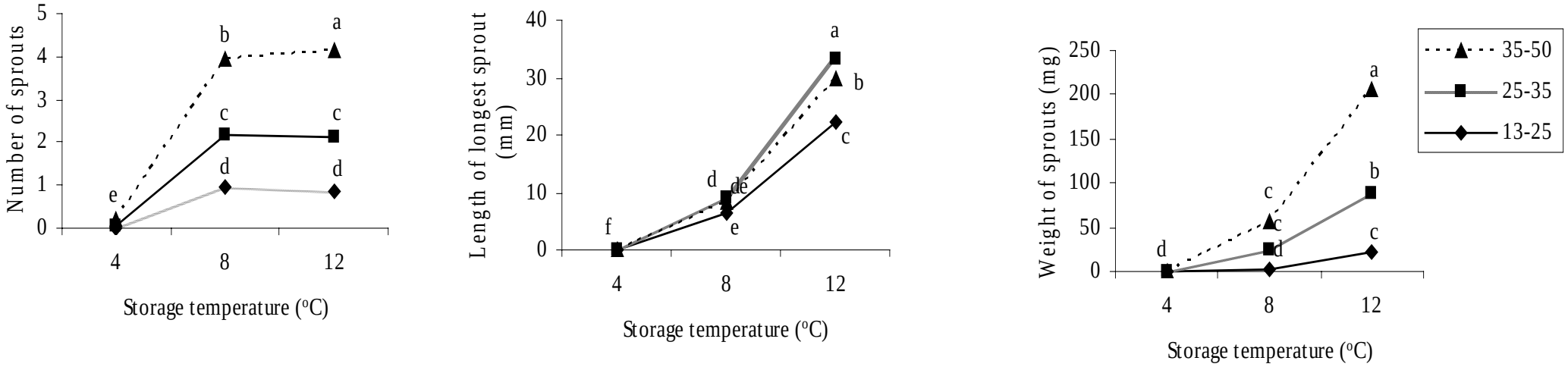


Fig. 2. The storage temperature × minituber size interaction for the number of sprouts, length of longest sprouts and weight of sprouts before growth regulator application. Letters a to e show highest and lowest values respectively and data points with the same letter are not statistically significantly different; these letters are based on the Duncan's Multiple Range Test.

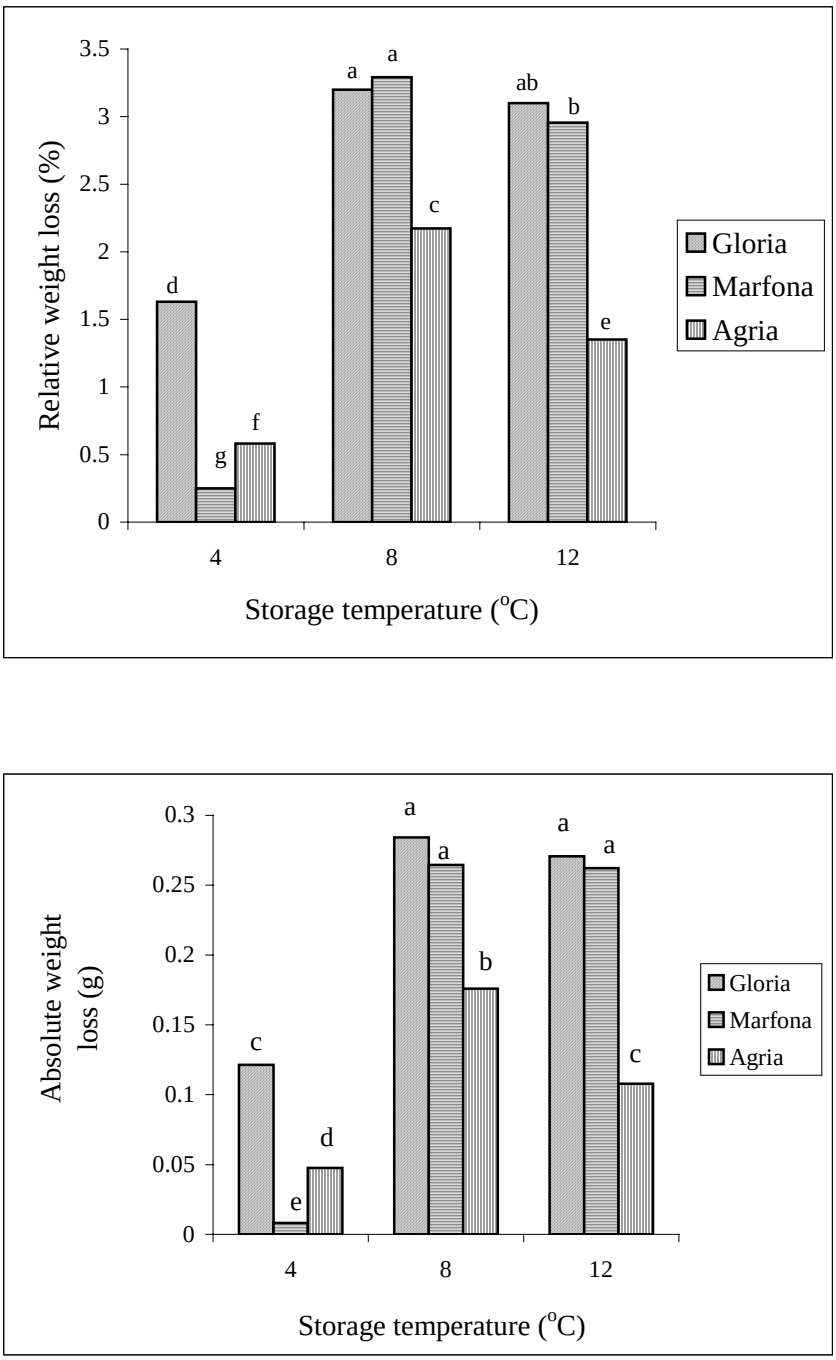


Fig. 3. The storage temperature × cultivar interaction for the minituber weight loss before growth regulator application. Letters a to e show highest and lowest values respectively; data points with the same letter are not statistically significantly different; these letters are based on the Duncan's Multiple Range Test.

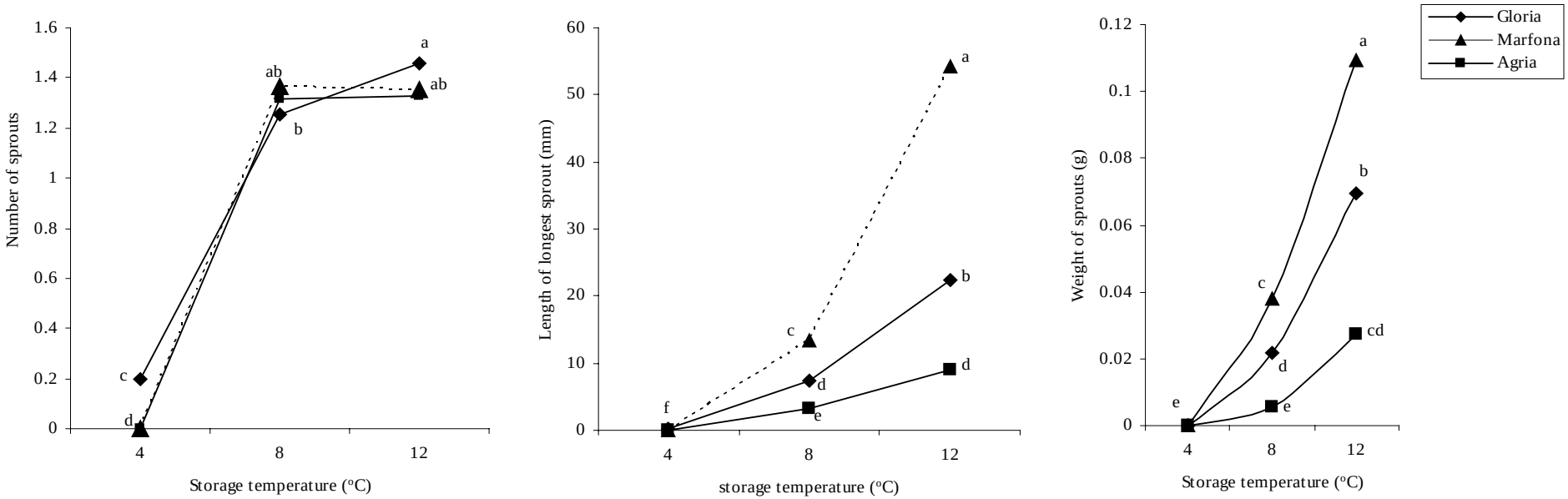


Fig. 4. The storage temperature × cultivar interaction for the sprout number, length of longest sprout and sprout weight before growth regulator application. Letters a to e show highest and lowest values respectively and data points with the same letter are not statistically significantly different; these letters are based on the Duncan's Multiple Range Test.

Interactions after growth regulator application

Highly significant two-way, three-way and four-way interactions were observed after growth regulator application (Table 2).

Storage temperature × minituber size interaction after growth regulator application. The interaction of storage temperature × minituber size was not significant for the length of longest sprout but it was significant for number and weight of sprouts (Table 2). There was no difference among storage temperatures in number of sprouts with intermediate size of minitubers (25 – 35 mm), for the largest minitubers the number of sprouts differed greatly among storage temperatures: the higher the storage temperature the fewer sprouts were observed (data not shown). Similarly, the weight of sprouts showed a larger temperature effect for the larger minitubers (data not shown). Struik & Wiersema (1999) indicated that well beyond the end of dormancy higher storage temperatures usually cause fewer sprouts to develop than lower storage temperatures: the number of sprouts produced by a single seed tuber depends on the period of storage at low temperatures for all cultivars they analysed. This is consistent with the effect we observed for the larger minituber sizes. However, small tubers may have a longer dormancy period and increasing storage temperature will advance the breaking of dormancy. Therefore, with the smaller tuber sizes, the negative effect of the higher storage temperature on sprout number may have been compensated by the advancing effect on dormancy breaking by the higher storage temperatures, as our assessments took place shortly after breaking of the dormancy, at least for the lowest storage temperature.

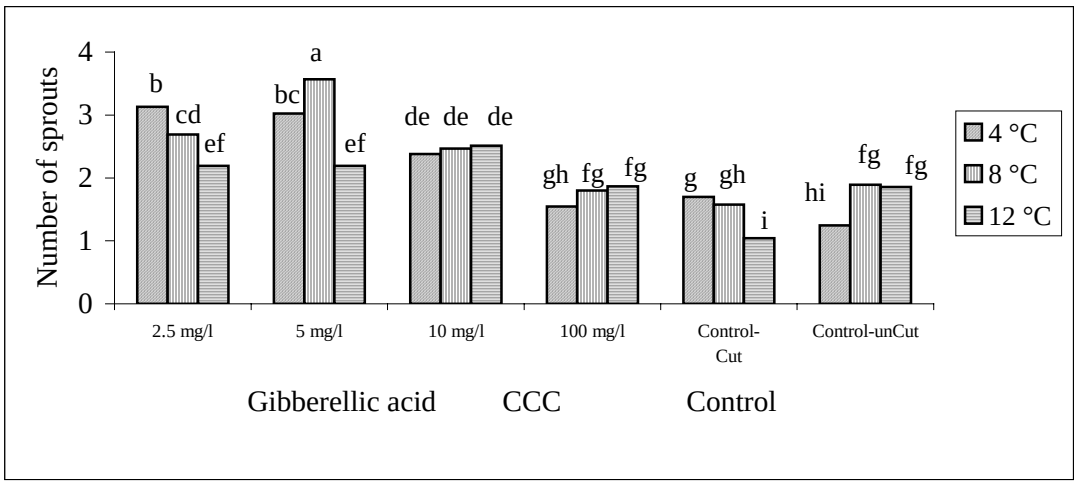
Storage temperature × growth regulator interaction after de-sprouting and growth regulator application. The two-way interaction between storage temperature and growth regulator application was significant for number, length and weight of sprouts (Table 2; Fig. 5). There was no difference among the three storage temperatures for number of sprouts when CCC was applied at either concentration. However, the storage temperature effect depended on the concentration of GA applied.

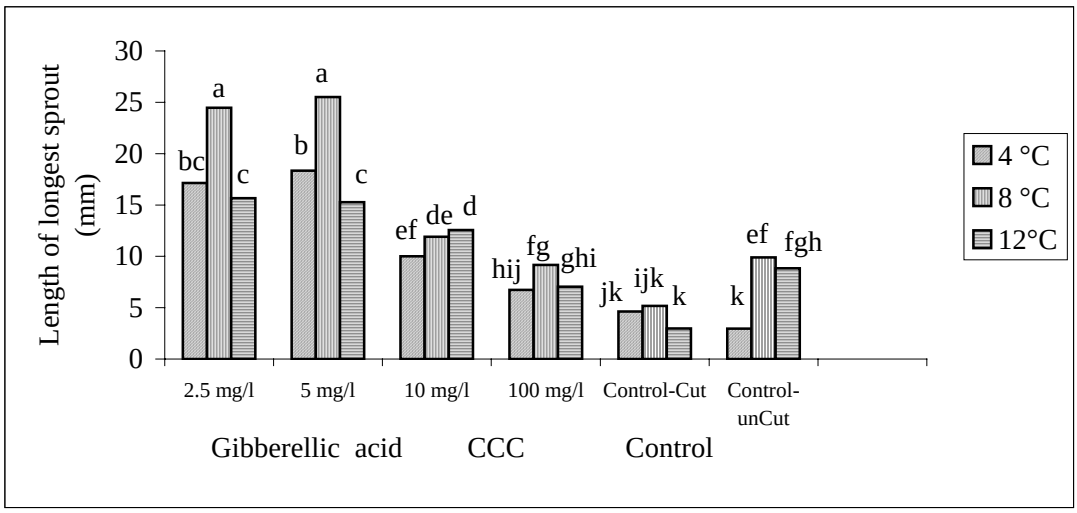
At the highest GA concentration, there was an optimal storage temperature, resulting in most sprouts observed with a combination of T 8 °C / GA 5 mg/l (Fig. 5). At the lower concentration of GA, the number of sprouts decreased with an increase in storage temperature as commonly observed (Struik & Wiersema, 1999). However, the cutting treatment also played an important role. The two control treatments (cut and uncut) also showed a different response to storage temperature: with the cut control, the highest storage temperature gave the lowest number of sprouts (as expected), but with the uncut treatment the lowest storage temperature gave the lowest number of sprouts (Fig. 5), probably because in this treatment (partial) dormancy still played a role. It is well known that cutting seed tubers contributes to the breaking of the dormancy and to the breaking of the apical dominance (Rappaport & Sachs, 1967; Struik & Wiersema, 1999).

For the length of longest sprouts, there was a clear optimum temperature at 8 °C when GA or the high dose of CCC was applied. Also with no chemicals applied this storage treatment gave the longest sprouts, but the temperature effects were much smaller. However, after a low dose of CCC there was an increase in sprout length with an increase in storage temperature over the entire range of storage temperatures (Fig. 5).

The interaction between storage temperature and growth regulator for weight of sprouts was very similar as the interaction effect on length of longest sprouts except for the low dose of CCC (Fig. 5). There seemed to be a decline in sprout weight with an increase in storage temperature for the low dose of CCC but the differences were not statistically significant.

Minituber size × growth regulator interaction after de-sprouting and growth regulator application. Number and length of sprouts did not show a significant interaction between minituber size and growth regulator but a highly significant interaction was observed for sprout weight (Table 2; Fig. 6). This interaction reflected the difference in size of the minituber size effect after different growth regulator applications: minituber size had the strongest effect after GA application (both doses)





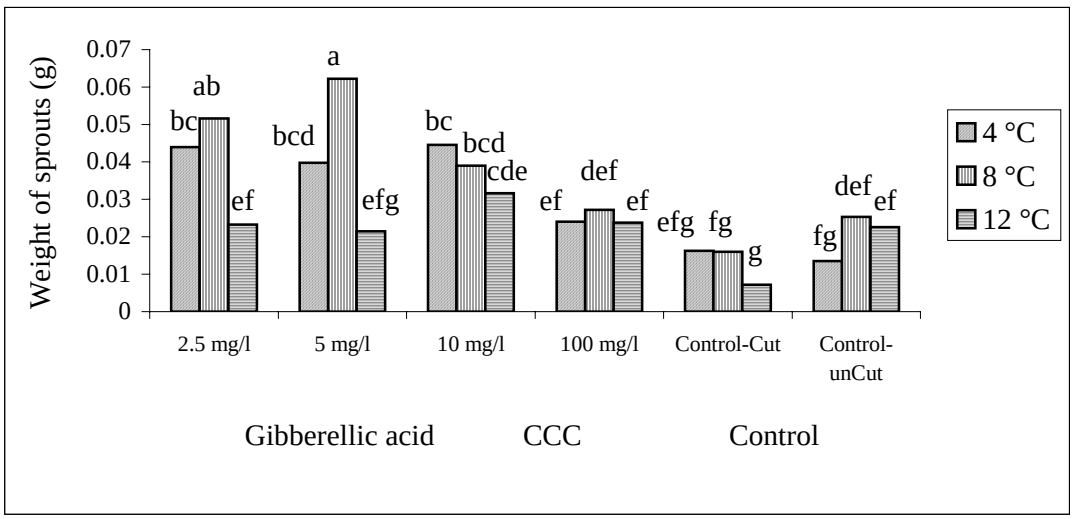


Fig. 5. The storage temperature × growth regulator interaction for the number of sprouts, length of longest sprout and sprout weight. Letters a to e show highest and lowest values respectively and data points with the same letter are not statistically significantly different; these letters are based on Duncan's Multiple Range Test.

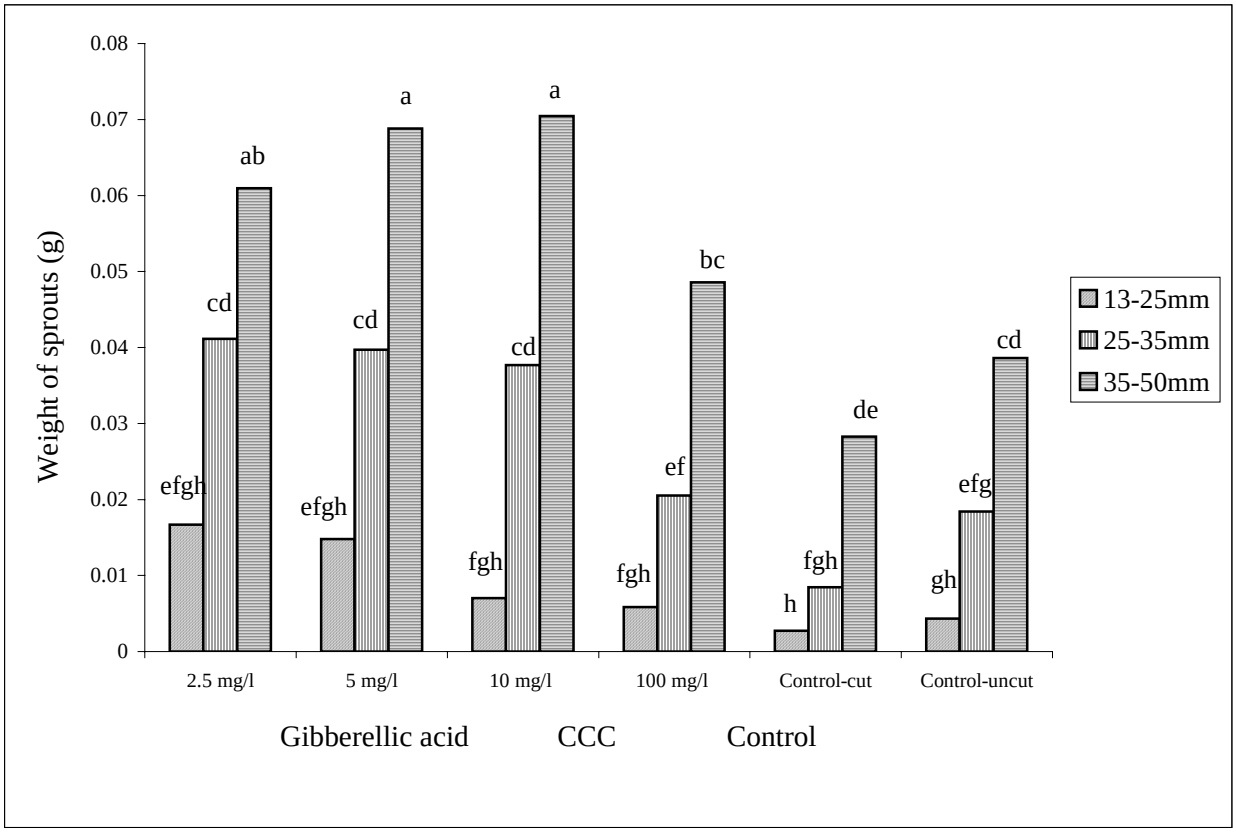


Fig. 6. The minituber size × growth regulator interaction for the weight of sprouts. Letters a to e show highest and lowest values respectively and data points with the same letter are not statistically significantly different; these letters are based on Duncan's Multiple Range Test.

or after a low dose of CCC (Fig. 6), probably because with the help of GA it was possible to make available the larger quantities of resources from the larger mother tubers to the growing sprouts.

Storage temperature × cultivar interaction after de-sprouting and growth regulator application. A meaningful interaction between storage temperature and cultivar for sprout behaviour was observed (Table 2; Fig. 7). Gloria did not show a strong response to storage temperature for number of sprouts whereas Agria showed a relatively strong response with most sprouts observed after storage at 8 °C (Fig. 7). At 8 ^{o}C the cultivars had similar and highest values for length of longest sprout while larger differences among three cultivars existed in length of longest sprout at 4 and 12 ^{o}C (Fig. 7). There was no difference among the three cultivars in weight of sprouts at 12 ^{o}C but at the lower temperatures cultivars differed significantly. Gloria showed a declining trend with an increase in storage temperature whereas the other two cultivars showed a clear optimum sprout weight at 8 °C (Fig. 7).

Interactions between storage regime and cultivar are common for normal sized seed tubers, especially after the break of dormancy (Van Ittersum, 1992; Struik & Wiersema, 1999; Struik et al., 2006). Interactions between storage regime and cultivar have also been observed for minitubers (Lommen, 1995).

Minituber size × cultivar interaction after de-sprouting and growth regulator application. The two-way interaction between minituber size and cultivar was highly significant for the number and length of sprouts, but not for weight of sprouts (Table 2). The three cultivars were similar in number of sprouts with size 13 – 25 mm, but with the other minituber sizes cultivar differences were larger (data not shown). The three cultivars were similar in length of longest sprout with size 35 – 50 mm. Marfona and Agria were also similar in length of longest sprout with the other two sizes but for those sizes they showed a significant difference with Gloria (data not shown).

Growth regulator × cultivar interaction after growth regulator application. Sprout behaviour showed highly significant interactions between growth regulator treatment and cultivar (Table 2; Fig. 8). Cvs Gloria and Agria seemed more responsive to GA for number of sprouts and length of the longest sprout than Marfona (Fig. 8). Gloria showed a stronger positive response to GA and CCC for the length of the longest sprout than the other cultivars (Fig. 8). For the weight of sprouts cv. Gloria and especially cv. Agria showed significant chemical treatment effects compared to the uncut control, whereas the differences between the uncut control and the chemically treated minitubers were not significant for cv. Marfona (Fig. 8). In Marfona, the cut control showed a significantly lower value for sprout weight than the uncut control. This difference was not visible in the other two cultivars. It was obvious that interactions between growth regulator and cultivar for sprout behaviour very much depended on the parameter used to describe the effect.

Storage temperature × minituber size × cultivar interaction after de-sprouting and growth regulator application. The length of the longest sprout showed a significant three-way interaction storage

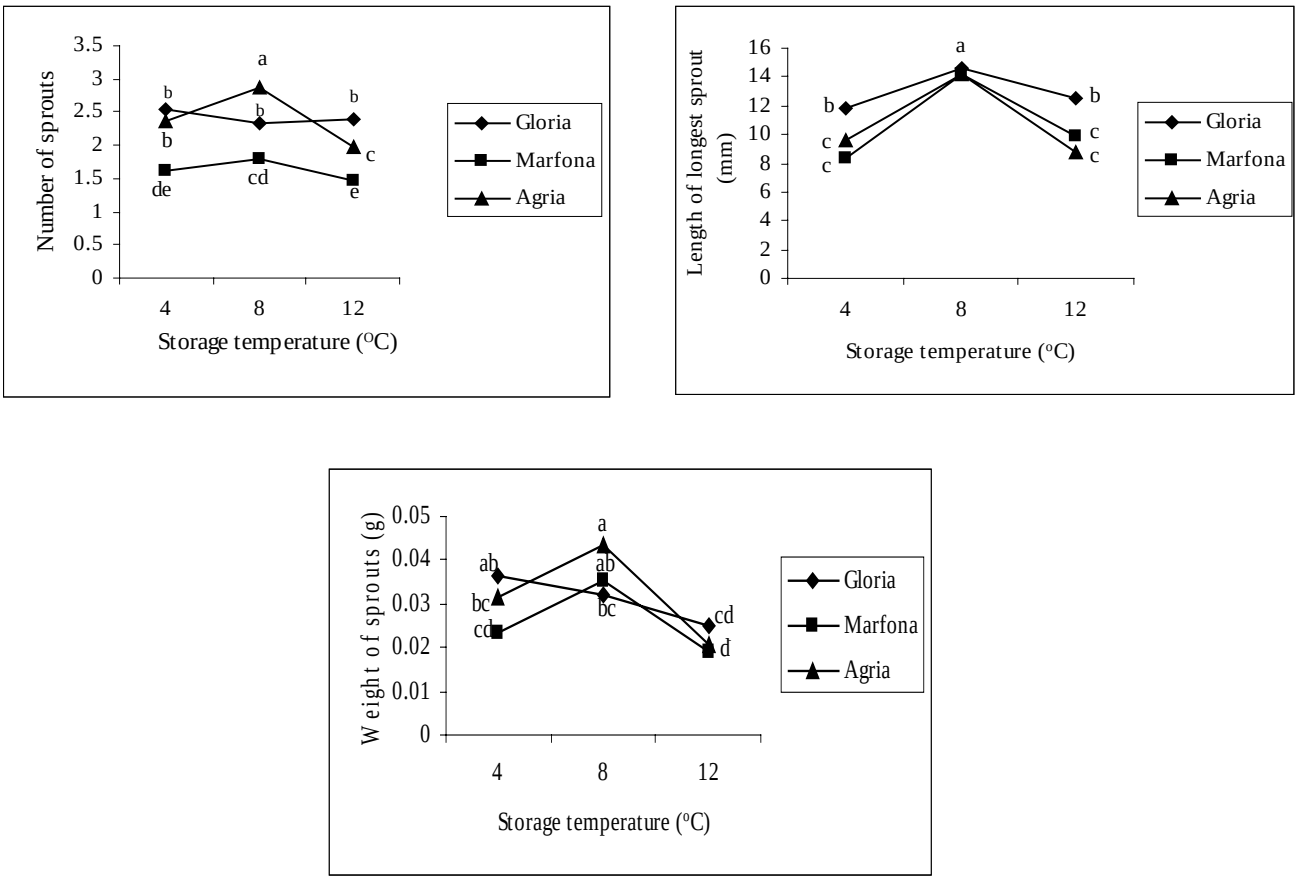


Fig. 7. The storage temperature × cultivar interaction (after growth regulator application) for the number, length and weight of sprouts. Letters a to e show highest and lowest values respectively and data points with the same letter are not statistically significantly different; these letters are based on Duncan's Multiple Range Test.

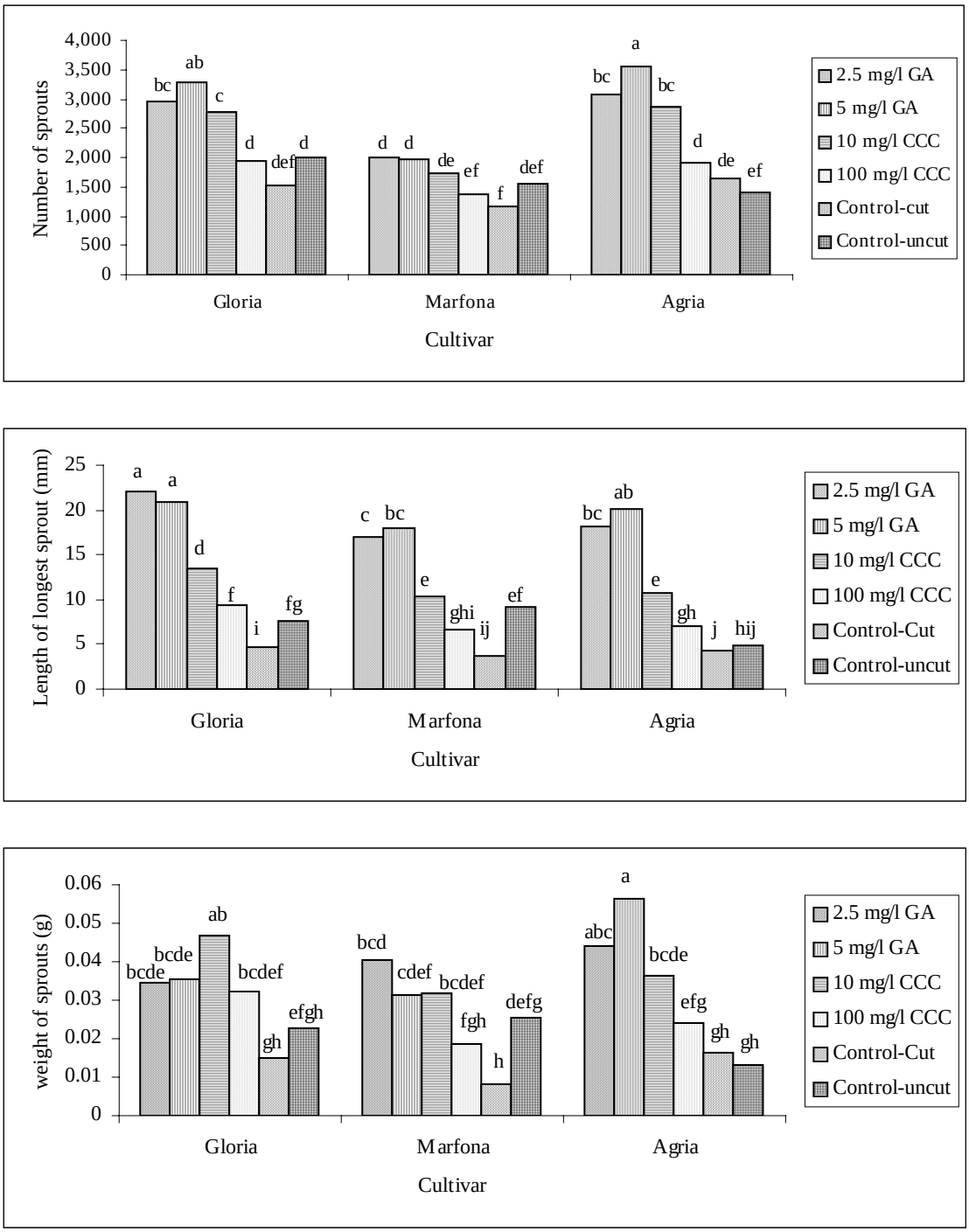


Fig. 8. The growth regulator × cultivar interaction for the number of sprouts, length of longest sprout and sprout weight. Letters a to e show highest and lowest values respectively and data points with the same letter are not statistically significantly different; these letters are based on Duncan's Multiple Range Test.

temperature × minituber size × cultivar, whereas for the number and weight of sprouts this three-way interaction was not significant (Table 2).

In some storage temperature × minituber size combinations, the length of sprouts of the three cultivars was similar. In three combinations of temperature × minituber size (T 8 ^{o}C/ S 35 – 50 mm, T 8 ^{o}C/ S 25 – 35mm and T 12 ^{o}C/ S 25 – 35 mm) Gloria showed largest sprout length while it was shortest with T 4 ^{o}C/ S 13 – 25 mm. Largest length of sprout for Marfona was observed with two temperature × minituber size combinations (T 8 ^{o}C/ S 25 – 35mm, T 8 ^{o}C/ S 35 – 50 mm) while Marfona showed smallest length of sprout with a combination of T 4 ^{o}C/ S 13 – 25 mm. In one combination (T 8 ^{o}C/ S 35 – 50 mm) Agria produced largest sprout whereas in T 4 ^{o}C/ S 13 – 25 mm combination Agria produced smallest sprout length.

At lowest storage temperature (4 ^{o}C) largest tuber (35 – 50 mm) in three cultivars produced longer sprouts than other tuber sizes especially than smallest minitubers. At higher storage temperatures (8 and 12 ^{o}C) larger tubers (25 – 35 and 35 – 50 mm) in three cultivars resulted in larger sprouts than smaller tubers (data not shown).

Three-way interactions after growth regulator application. The three-way interactions storage temperature × growth regulator × cultivar and size of minituber × growth regulator × cultivar were significant for number, length and weight of sprouts (Table 2). These three-way interactions showed that the effects of the growth regulators on sprout behaviour depended on the effects of storage temperature, size of minitubers and cultivar. The differential responses of the different cultivars to combinations of storage temperature, size of minitubers and growth regulator treatments are especially noteworthy, because they may be useful in efforts to create cultivar-specific protocols for optimizing seed tuber production schemes. However, in all cases the effects of growth regulator application on progeny yield was negative, suggesting that growth regulator applications may not be useful. It would make more sense to optimize the use of the interaction between storage temperature, minituber size and cultivar. However, this three-way interaction was not significant for the dry matter yield of progeny tubers.

Conclusions

1. Tuber weight loss is affected by tuber size: larger minitubers lose more weight but a lower percentage of their weight.

2. In general, larger minitubers gave more sprouts, longer sprouts, more sprout weight and more vigour. Agria showed a smaller response to minituber size than the other two cultivars.

3. Effects of storage temperature on the sprout behaviour were different before and after de-sprouting or growth regulator application. This difference was caused by the combined effect of de-sprouting, the cutting of the minitubers and the longer storage period on the expression of the storage temperature effect.

4. Gibberellic acid increased number, length and weight of sprouts.

5. For sprouting behaviour, there were significant three-way interactions between storage temperature, growth regulator application and cultivar and between growth regulator, minituber size and cultivar. The de-sprouting, cutting and longer duration of the storage period may have interfered with the effects of the chemicals.

6. Low storage temperature, large minituber sizes and refraining from applying growth regulators gave the highest vigour of the minitubers when used to produce progeny in a short time.

7. It is possible to optimize the performance of minitubers by selecting the right conditions for storage. Further enhancing the performance of the minitubers by treating them with growth regulators, however, is difficult.

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Chapter 6

Effects of size of normal seed tubers and growth regulator application on the dormancy, sprout behaviour, growth vigour and quality of normal seed tubers of different potato cultivars

Otroshy, M., & P.C. Struik, 2006

Effects of size of normal seed tubers and growth regulator application on dormancy, sprout behaviour, growth vigour and quality of normal seed tubers of different potato cultivars

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Abstract

Physiological behaviour of normal seed tubers of potato (*Solanum tuberosum* L.) can be manipulated by storage treatments, but also by selecting the proper seed tuber size and treating the seed tubers with growth regulators. When the proper combination of size and chemical treatment is chosen, the vigour can be enhanced, resulting in a higher rate of multiplication or in higher yields. A greenhouse experiment was carried out to investigate the effects of size of normal seed tubers and application of growth regulators (gibberellic acid (GA) and chlormequat (CCC)) to the seed tubers on the dormancy, sprouting behaviour and growth vigour of normal-sized potato seed tubers. These growth regulators were selected because the interaction with seed tuber size can be interpreted easily as their main effects are well known.

Three tuber sizes (35 – 45 mm, 45 – 55 mm and 55 – 65 mm) of three potato cultivars (Frieslander, Marfona and Santé) were treated with GA (2.5 mg/l and 5 mg/l) or CCC (10 mg/l and 100 mg/l); two controls were included: cut seed tubers and uncut seed tubers.

Larger seed tubers gave more sprouts, longer sprouts, a higher spout weight and more stems. Seed tuber size did not affect the date of emergence, the time of plant senescence, the duration of the life cycle (except for Santé), the plant height, the above ground dry mater, the number of progeny tubers or the dry weight of the tubers.

GA effects on sprouting depended on cultivar. Frieslander produced more sprouts, but not longer sprouts, while Marfona produced longer sprouts, but not more sprouts in response to GA. The

growth vigour was also affected by the growth regulators, but also this effect depended on cultivar. GA increased the days of growth with Marfona and Santé, but not with Frieslander. GA let Frieslander produce more stems, but not longer stems, while Marfona produced longer stems, but not more stems. Only Marfona produced more foliage dry matter with GA. Within the seed tuber treatments with GA Frieslander produced more tubers and a higher tuber yield than Marfona and Santé.

CCC did not have a large effect on the growth vigour. Frieslander got fewer days of growth, Marfona got more tuber yield and Santé got a better emergence percentage in response to CCC.

There were many significant two-way interactions between seed tuber size, cultivar or growth regulator application. Many of these interactions, however, were not only associated with the fact that Frieslander responded better to GA treatments than the other two cultivars, but also because it gave much better performance in terms of emergence of uncut seed tubers than Marfona and Santé, resulting in more stems, more tubers and higher tuber yields for this cultivar in that particular seed tuber treatment than for the other two cultivars.

Keywords: *Solanum tuberosum* L., growth regulator, gibberellic acid, CCC, sprout behaviour, vigour.

Abbreviations: GA = gibberellic acid, CCC = Chlorocholine chloride (or chlormequat chloride), Cv. = cultivar, GR = growth regulator, DAP = day after planting, DAE = day after emergence; NS: not significant.

Introduction

Potato (*Solanum tuberosum* L) is the most widely grown and the most important non-cereal crop in the world (Van der Zaag & Horton, 1983). The total world wide area that is cropped to potato is over 19 million ha per year, resulting in a world wide total production of 311 million tons per year (FAO, 2002).

Generally, the propagation material used to grow the crop is the seed tuber. A tuber is a greatly shortened and thickened stem that bears scale leaves with axillary buds (Van Ittersum, 1992). The vegetative propagation is the cheapest and easiest way of potato propagation yet, but it has some disadvantages. One of these disadvantages is the low multiplication rate that can be achieved with this way of vegetative multiplication. Therefore, 15% of the total potato area is needed for seed tuber production. Cereals, for example, need only 1/30 of their area for multiplication. Another disadvantage is the degeneration, when propagated via seed tubers. When potatoes are grown in the field, diseases and infections can more easily occur and infect the progeny than when grown in the laboratory or in vitro (Struik & Wiersema, 1999). If this progeny from the field is used for seed tuber multiplication again, an accumulation of diseases will occur.

The physiological age of the seed tuber is crucial for its quality as planting material. Physiological age is defined as the stage of development of a tuber, which is modified progressively by increasing chronological age, depending on growth history and storage conditions (Struik & Wiersema, 1999). The physiological age influences the productive capacity of the seed tuber (Reust, 1986), but also changes the quality of the produce (e.g. by influencing the tuber size distribution; Struik & Wiersema, 1999).

In temperate regions, potatoes can only be grown once a year, in contrast to tropical and sub-tropical regions where potatoes can be grown year round. In the temperate regions, there are almost no problems with the physiological age of seed tubers, because of the natural dormancy period during the winter when the harvested seed potatoes are stored in good cold storage facilities. However, in tropical and sub-tropical regions large problems with physiological age of seed tubers can occur. This problem

is caused by the short period between two potato crop cultivations and by the high storage temperatures when no cold stores are available. The seed tubers can be physiologically too young (when taken from the previous crop: short storage) or too old (when taken from last year's crop: long storage at high temperatures) for planting (Van Ittersum, 1992; Struik & Wiersema, 1999). The physiological age of seed tubers is very important for the productivity of the crop. Van der Zaag & Van Loon (1987) found that the physiological age of a planted seed tuber affects the growth pattern of the plant. They indicated that the growth vigour of seed tubers is a reflection of its physiological age. Growth vigour is defined as the potential of a seed tuber to produce a well developed plant within a relatively short period of time. During the dormancy period, the tuber's vigour is zero. At the end of the dormancy period the vigour of the tuber increases. After some time the increase will stop and growth vigour is steady at a maximum. This maximum vigour only exists for a short period of time and then the vigour declines until it is zero again and sprouting of the tuber is not possible anymore. Crops from physiologically young seed tubers grow longer and are more yielding than those from physiologically old seed tubers. Wiersema (1985) found that crops from physiologically young seed tubers produce fewer stems, have later tuberization, more foliage and more tubers per stem than crops from physiologically old seed tubers.

Not only physiological age, but also other factors affect the vigour of the potato plant. One of these factors is the seed tuber size. According to Wiersema (1989), seed tubers below 20 g are less yielding per stem and have less foliage production per stem than seed tubers heavier than 20 g. Seed tubers heavier than 20 g showed no differences in yield per stem when different sizes were compared. When the tuber yields of whole plants with different seed tuber sizes were compared, the heaviest seed tubers were most yielding and gave the best ground cover. Seed tubers heavier than 100 g showed more sprouts with longer length compared to small potato seed tubers of 2.5 g and gave a steady ground cover and yield (Wiersema et al., 1987). Van Ittersum (1992) found a relation between the size of the seed tuber and its physiological age. Small seed tubers had a longer dormancy, so they were physiologically younger than bigger seed tubers. However this effect depended on cultivar, within some cultivars hardly an effect of size over a wide size range, but in other cultivars a significant

response over a wide range of sizes. Lommen & Struik (1993), Lommen (1994) and Ranalli (1997) showed that size effects on dormancy and vigour are even more important for minitubers.

Physiological ageing can be controlled by storage conditions. With higher temperatures during storage, ageing goes faster than with lower temperatures (Struik & Wiersema, 1999). However, the way the temperature sum is built up during the storage season is also important, especially for cultivars with a high rate of natural ageing (Struik et al., 2006).

Another factor that affects the growth vigour of potato seed tubers is the cultivar. The trend of vigour after the dormancy period varies among cultivars (e.g. Van der Zaag & Van Loon, 1987; Van Ittersum, 1992; Struik & Wiersema, 1999; Struik et al., 2006). Important cultivar-specific variables are the rate of increase, time of increase and duration of increase (Van der Zaag & Van Loon, 1987). The time, level and length of maximum growth vigour, and also the time at which vigour starts to decline and the rate of decline are also all different between cultivars (Bodlaender et al., 1985; Van der Zaag & Van Loon, 1987). The optimum growth vigour of a cultivar at a certain time can be reached by programming storage conditions properly. These optimal storage conditions are also different among cultivars (Struik & Wiersema, 1999).

Cutting of the seed tuber can influence the early growth vigour in a positive way, because cutting may break dormancy. Also apical dominance will be broken, the sprouts will grow faster and more stems will grow out from one seed tuber (Struik & Wiersema, 1999). This can be explained by the wound induced production of gibberellins (Rappaport & Sachs, 1967). De-sprouting of relatively young seed tubers may contribute to the development of new sprouts and more sprouts with a higher rate of sprout growth than without de-sprouting (Struik & Wiersema, 1999).

Plant growth regulators like gibberellic acid (GA3), but also the anti-gibberellin CCC (2-chloroethyltrimethylammonium chloride, Chlormequat, Chlorocholine chloride) have been tested on the potato plant many times. It has been shown that plant growth regulators affect many potato plant characteristics. GA is a growth stimulating plant hormone, which naturally exists in plants. The GA hormone was discovered in 1926. It concerned the real gibberellic acid better known as GA3 isolated from the fungus *Gibberella fujikuroi*. Later it was discovered that gibberellins play a very important

role in the regulation of growth and development in higher plants (Mohr & Schopfer, 1995), but that GA3 was not the only gibberellin in plants. There are more than 80 different gibberellins in higher plants (Mohr & Schopfer, 1995). In the potato plant, GA plays an important role in different developmental events of the plant, but especially in tuber formation (Jackson, 1999; Struik et al., 1999). GAs affect most steps in tuber formation and they are influenced by inducing or non-inducing conditions in a manner consistent with the effects of these conditions on tuber induction (Struik et al., 1999). Jackson (1999) showed that potato mutants that block GA formation could produce tubers under long day photoperiod. Normally GA levels in the potato plant are much higher during long day than during short day. So Jackson (1999) concluded that GA inhibited tuber formation. Okazawa (1967) and Koda & Okazawa (1983) observed that the GA level is high in elongating stolons and this level decreases when the stolons begins to swell; they reported that GA enhances stolon elongation and inhibits stolon swelling. GA plays an important negative role in tuberization, shifting growth away from tubers towards stolons (Ewing & Struik, 1992). Krauss (1981) looked for a relation between the level of GA and the growth rate of individual tubers. No correlation between these two parameters was found, so he suggested that GA did not regulate tuber growth.

More is known about the effect of GA when it is exogenously applied. Shoot growth is promoted by an exogenous GA3 treatment and plant height is increased but tuber weight is decreased (Menzel, 1980). A foliar spray of GA also results in long stolons (Hammes & Nel, 1975). Gibberellin is a dominant factor in tuber initiation and tuber formation can be stimulated by CCC because of its anti-gibberellin action (Ewing & Struik, 1992; Struik et al., 1999).

Most important in the potato crop is the tuberization process. When GA will be applied to a potato crop by foliar application, tuber initiation will be delayed and partially grown tubers may respond by ceasing to bulk and by growing out as stolons (Lovell & Booth, 1967). Other experiments showed the same and also showed that tuber formation could even be prevented (Lippert et al., 1958; Okazawa, 1960; Menzel, 1980; Sharma et al., 1999). However, under short day conditions normally GA does not prevent tuber formation in whole plants, although the number and dry mass of tubers per plant decreases (Hammes & Nel, 1975; Sharma et al., 1999).

Another effect of GA is the effect on the dormancy period of seed tubers. Van Ittersum & Scholte (1993) showed that a foliar application of GA just before haulm destruction, shortened the dormancy period of the harvested seed tubers up to three months. This treatment increased vigour of the seed tubers, especially at early plantings (Van Ittersum, 1992). Suttle (2004), on the other hand, observed that continued exposure of developing tubers to inhibitors of GA biosynthesis did not extend tuber dormancy but rather hastened dormancy release. There is no role for endogenous GA in potato tuber dormancy release, but there is a role for GA in the regulation of sprout growth (Suttle, 2004).

The use of the growth retardant CCC has its roots in the 1960s. CCC became widely used to control lodging in wheat, but is also applied in other crops for various reasons. Different studies, for example, showed that CCC had a positive effect on various vegetables crops: CCC promoted growth of root systems in tomato and bean plants (Tognoni et al.., 1966). The basic, direct physiological effect of CCC is that of blocking the biosynthesis of GA in a very early stage (Rademacher, 1999). The absence of GA will change the whole hormone balance in the plant and as a consequence the growth behaviour of the plant will change as well.

Because of the blocking of the biosynthesis of GA it seems logical that the effects of GA and of CCC in potato are opposite. Indeed, sprouting is inhibited by CCC (Majeed & Bano, 2004). Shoot growth is reduced by a foliar application of CCC: CCC reduces stem length, leaf area and internode length (Dyson, 1965; Digby & Dyson, 1973). The number of internodes is not affected by CCC, so CCC only affects the elongation in stems (Digby & Dyson, 1973). The growth of stolons is also reduced by CCC (Dyson, 1965) and this may influence tuberization.

CCC has a positive influence on tuber formation. The start of tuberization was earlier when crops received CCC than when crops did not receive CCC (Dyson, 1965). CCC can also increase the tuber number by foliar application (Sekhon & Singh, 1985; Rex, 1992; Sharma et al., 1999). For the tuber yield the opinions of different researchers differ. Some researchers claim that a foliar application of CCC will increase tuber yield (Radwan et al., 1971; Sharma et al., 1999), while other researchers state that a foliar application of CCC reduced tuber yield (Sekhon & Singh, 1985; Rex, 1992). Menzel

(1984) observed substantial yield increases by application of CCC in only two out of nine cultivars and suggested that the effect of CC on tuber yield was cultivar and plant age dependent.

Information on the effects of CCC on sprouting and vigour of seed tubers when applied directly to the seed tubers is scarce.

In the previous chapter of this thesis (Chapter 5), we observed that minituber size significantly affected all parameters relating to seed tuber performance except date of emergence and that treating these minitubers with growth regulators influenced seed tuber performance in a complex manner. However, optimizing the performance of minitubers by treating them with growth regulators did not seem to be a good idea, as most of the parameters associated with growth vigour of seed tubers were not positively influenced by growth regulator application. The lack of positive effect could be due to the small size or the young physiological age of the minitubers used.

In this chapter, the effects of two different doses of GA and CCC applied directly on the seed tuber, on the sprouting behaviour and growth vigour of seed tubers will be described, using different sizes of normal seed tubers of normal to relatively high physiological age and different cultivars. Moreover, the effect of cutting of the seed tubers (necessary to effectively apply the growth regulator) will be assessed.

Materials and methods

The experiment included three factors: size of normal seed tubers (35 – 45 mm, 45 – 55 mm and 55 – 65 mm), growth regulator (GA and CCC, including two controls) and potato cultivars (Frieslander (early), Marfona (mid-early; also used in Chapter 5) and Santé (late). The factor growth regulator consisted of six levels: two doses of GA3 (2.5 mg/l and 5 mg/l), two doses of CCC (10 mg/l and 100 mg/l) and two controls (seed tubers cut and seed tubers uncut). The experiment consisted of two parts. The first part was a sprout behaviour test and the second part was a vigour experiment.

The seed tubers were delivered by Unifarm of Wageningen University. The cultivars Marfona and Santé were bought from Agrico, a Dutch potato breeding company. The cultivar Frieslander was from Unifarm. All the seed tubers were NAK certified and placed in the A-class.

First, seed tubers were accurately sorted out by a grader into the three sizes (35 – 45 mm, 45 – 55 mm and 55 – 65 mm). The seed tubers were stored in a cold store at 4 °C and a relative humidity of 80%.

For the sprout behaviour test, 10 seed tubers per treatment combination were selected. All seed tubers (except those for the control treatment with uncut seed tubers) were hand cut and an incision was made to two thirds of the longest axis of the seed tubers, to allow growth regulators to penetrate. To prevent the incision from closing a piece of paper was inserted in the cut. After all incisions were made, growth regulators were applied by soaking the seed tubers in the appropriate solution for 10 min. Treatments included 2.5 or 5 mg/l gibberellic acid, 10 or 100 mg/l CCC, or demi-water. Seed tubers were then stored for a period of 11 days at 18 °C, full darkness and a very high humidity to allow wound healing and sprouting. After 11 days, the seed tubers had produced sprouts and were transferred to the cold store (4 °C; 80% relative humidity) to allow recording of data on sprouting at the same stage of development. Data were collected on number of sprouts, length of longest sprout, and weight of sprouts to assess the effects of seed tuber size and growth regulators on sprouting capacity. As also the weight of the sprouts was recorded, the seed tubers were de-sprouted and the second part of the experiment was therefore carried out with de-sprouted seed tubers. De-sprouting enhances the expression of the effects of storage temperatures on growth vigour (Struik & Wiersema, 1999) and may also enhance the effects of the growth regulators (see also Chapter 5).

When measurements on sprout behaviour were completed, the second part of experiment was started. This part was designed to assess the effects of the treatments on the growth vigour of the seed potato tubers. Seed tubers were planted into 5 litre pots (filled with potting soil without fertilizer) at a depth of 8 cm. Pots were randomly placed in a greenhouse at Wageningen Unifarm, Haarweg 333 under the following conditions: day temperature 18 °C (12 h), night temperature 12 °C (12 h), relative

humidity 80%. Daylight (12 h) was supplemented with 0.8 Philips SON-T-Agro lamps 400 W per m^2 and with incandescent illumination.

The planting date was 24 June 2003. The harvest date was plant-specific: it was done when the plant had fully died.

Methodology and observations

For part one of the experiment (the sprout behaviour test) records were taken on number of sprouts, length of longest sprout and weight of sprouts. This set of parameters provides a good characterization of the sprouting capacity of the seed tubers (see Struik & Wiersema, 1999). The criterion for a sprout was a structure of at least 2 mm length (Van Ittersum, 1992). Length of the longest sprout was measured by taking the distance from the tip of the longest sprout to the base of sprout.

For part two (the vigour test) records taken included date of emergence (days after planting (DAP)), number of stems, length of the longest stem, date of leaf death (or date of senescence; days after planting), duration of growth (date of senescence minus date of emergence; days), number of progeny tubers, dry weight of tubers and dry weight of aerial parts of plant. This set of parameters provides a characterization of both the above ground shoot development and the rate of multiplication and yield of tubers. The date of emergence was assessed as the day when the top of the first stem broke the soil surface. Not all seed tubers produced an emerged plant. When no plant was produced, the experimental unit was either recorded as one with a missing value or the value of 126 DAP (the end of the experiment) was assigned. The latter procedure not only allowed for a more reliable statistical analysis, it also gave a better reflection of the actual effect of the treatments.

The number of stems was counted at 72 days after planting (DAP). The length of the longest stem was measured at 73 DAP. The date of senescence (date of leaf death) was defined as the day when all leaves were yellow. Because some seed tubers did not produce an emerging plant, the date of senescence for these cases was either recorded as a missing value or set at 126 days (the end of the experiment). The days of growth were calculated as the difference between day of senescence and day

of emergence. For seed tubers that did not produce an emerging plant, the duration of growth was recorded as a missing value or set at 0 (126 DAP minus 126 DAP).

To assess above ground dry matter, the aerial part of the plants were cut and dried in a forced ventilated oven at 105 °C for at least 24 hours. Progeny tubers were harvested and number and weight of progeny tubers were recorded. Tubers were chopped and dried in an oven at 105 °C for at least 24 hours to assess the dry weight of the progeny tubers.

Statistical analysis

The entire experiment was executed in 10 replications and included 540 experimental units (3 tuber sizes × 3 cultivars × 6 growth regulator treatments × 10 replications). The experimental design was a completely randomized factorial experiment with three factors: A= tuber size with 3 levels (35 – 45 mm, 45 – 55 mm and 55 – 65 mm); B: Cultivar with 3 levels (Frieslander, Marfona and Santé); and C: growth regulator treatments with 6 levels (2.5 and 5 mg/l GA, 10 and 100 mg/l CCC, two controls: seed tubers cut and seed tubers uncut).

Analysis of variance was applied on the variables using the MSTATC statistical package. Duncan's Multiple Range Test was used to compare the means. As a number of seed tubers did not produce an emerging plant, some of the ANOVAs were run twice: once with the experimental units without an emerging plant included as missing values (through which the statistical package then makes an estimate of the missing value) and once with specific values assigned to the missing value to account for the fact that a seed tuber which did not produce an emerging plant, most likely has a very low seed tuber quality and should be given a value that reflects that poor quality.

Results

Sprouting behaviour

Tuber size, cultivar and growth regulator all had a highly significant effect on the sprouting behaviour, as illustrated by the length, number and weight of sprouts (Table 1).

Length of longest sprout

There was a significant effect ($P = 0.0013$) of seed tuber size on the length of the longest sprout. The longest sprouts were produced by the size 55 – 65 mm. The sizes 35 – 45 mm and 45 – 55 mm produced the shortest sprouts and were not significantly different (Table 1). The effect of cultivar on the length of the longest sprout was highly significant ($P < 0.0001$). All cultivars differed significantly from one another. Marfona produced the longest sprouts and Santé the shortest ones. The effect of growth regulator on the length of the longest sprout was also highly significant ($P < 0.0001$). The seed tubers treated with GA produced the longest sprouts. There was also a significant difference between the two GA doses, where the highest dose produced the longest sprouts. There was no significant difference between the two CCC doses and the control cut. The control uncut produced the shortest sprouts. There was a significant interaction between seed tuber size × cultivar (Table 1; Fig. 1). Marfona produced the longest sprouts with the largest seed tubers. Santé produced the shortest sprouts compared to the other cultivars, but the size 45 – 55 mm was the shortest. There was no difference between seed tuber sizes in cv. Frieslander.

There was also a highly significant interaction effect of cultivar × growth regulator on the length of the longest sprout (Table 1; Fig. 2). Marfona produced the longest sprouts with GA. Frieslander had the same length for all growth regulator treatments except the control no cut, which was shorter. Santé showed the longest sprouts with the higher GA concentration. Different doses of CCC had no effect on the length of sprouts of the three cultivars and these treatments gave the same results as the control cut. The uncut control of all cultivars had the shortest sprouts among all growth regulator treatments. The uncut control of Frieslander had the longest sprouts of all uncut seed tubers.

Table 1. The effect of tuber size and growth regulators on the sprout behaviour after growth regulators application in different cultivars of potato.

Abbreviation: GA = gibberellic acid, CCC = Chlorocholine chloride (or chlormequat chloride), cv. = cultivar, GR = growth regulator. NS: not significant, $P \geq 0.05$.

	Length of longest sprout (mm)	Number of sprouts	Weight of sprouts (g)
Size of seed tuber (mm)			
35-45	19.1 b	12.3 b	0.339 c
45-55	18.3 b	12.2 b	0.412 b
55-65	21.1 a	15.9 a	0.620 a
P	**	**	**
Cultivar (cv.)			
Frieslander	21.8 b	23.8 a	0.687 a
Marfona	24.8 a	9.6 b	0.435 b
Santé	11.9 c	6.9 c	0.250 c
P	**	**	**
Growth regulator (GR)			
2.5 mg/l GA	23.0 b	16.5 b	0.393 b
5 mg/l GA	27.0 a	19.2 a	0.500 a
10 mg/l CCC	19.4 c	13.2 c	0.531 a
100 mg/l CCC	19.1 c	12.6 c	0.558 a
Control			
Control (tuber cut)	18.9 c	12.8 c	0.548 a
Control (tuber not cut)	9.7 d	6.3 d	0.214 c
P	**	**	**
Interactions			
Size × cv.	*	*	*
Size × GR	NS	**	*
Cv. × GR	**	**	NS
Size × cv. × GR.	**	NS	NS

**: significant at $P \leq 0.01$, *: significant at $P \leq 0.05$. Values followed by a similar letter are not statistically significantly different.

Santé had the shortest sprouts with the uncut seed tubers. The description suggests that is was mainly the cultivar dependent effect of the GA applications which was responsible for the cultivar × growth regulator interaction.

The statistical analysis also shows that there was a highly significant three-way interaction (P = 0.0002). This three-way interaction was not easy to interpret.

Number of sprouts

The main effects of the three factors were all significant for the parameter number of sprouts. For the factor seed tuber size (P < 0.0001), the biggest size (55 – 65 mm) had significantly more sprouts than the other sizes, which were the same. For the factor cultivar (P < 0.0001), Frieslander had significantly more sprouts than Santé and Marfona. For the factor growth regulator (P < 0.0001), the highest dose of GA (5 mg/l) produced the highest number of sprouts, followed by 2.5 mg/l of GA. Different doses of CCC and the cut control were similar. The uncut control produced the lowest number of sprouts.

The two-way interactions seed tuber size × cultivar (P = 0.0184) (Table 1; Fig. 1) and cultivar × growth regulator (P < 0.0001) (Table 1; Fig. 2) were significant. Frieslander and Santé produced the highest number of sprouts with the biggest size, while Marfona showed no significant difference between its two biggest sizes. Marfona produced the lowest number of sprouts with the smallest size, while the other two cultivars showed no significant difference between the two smallest sizes. In sizes 35 – 45 mm and 55 – 65 mm Frieslander produced the highest number of sprouts, while the other two cultivars showed no significant difference. In the size 45 – 55 mm, Frieslander produced the highest number of sprouts and Santé produced the lowest number of sprouts.

The interaction cultivar × growth regulator showed that the effect of cutting was significant for all cultivars (Fig. 2). Frieslander produced the highest number of sprouts in combination with GA. Different doses of GA showed no significant difference in the cultivars Frieslander and Marfona. With Santé the highest dose of GA gave more sprouts than the lowest dose. Different doses of CCC and the

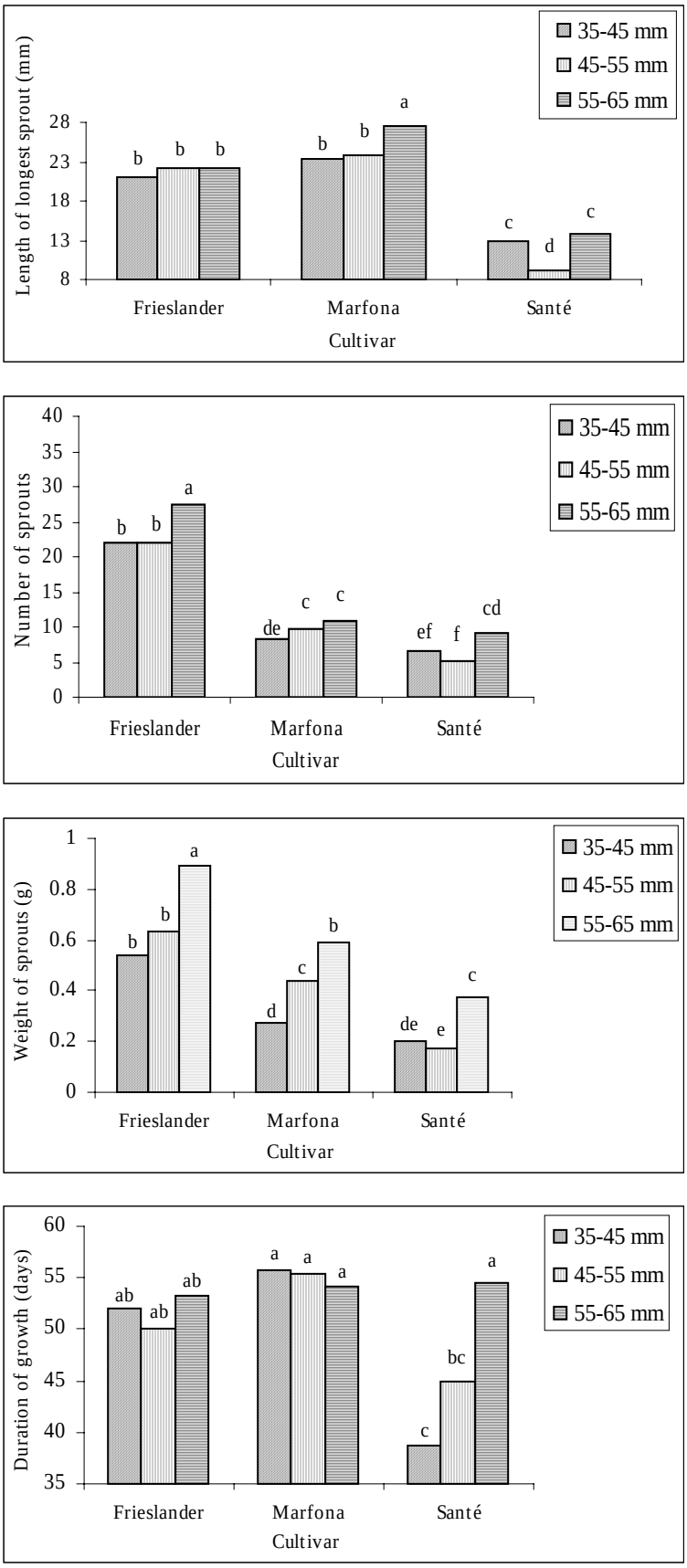

Fig. 1. Seed tuber size × cultivar interaction for length of the longest sprout (top), number of sprouts (second from above), weight of sprouts (second from below) and duration of growth (below). Letters a to f show highest and lowest values respectively and bars with a similar letter are not statistically significantly different based on the Duncan's Multiple Range Test.

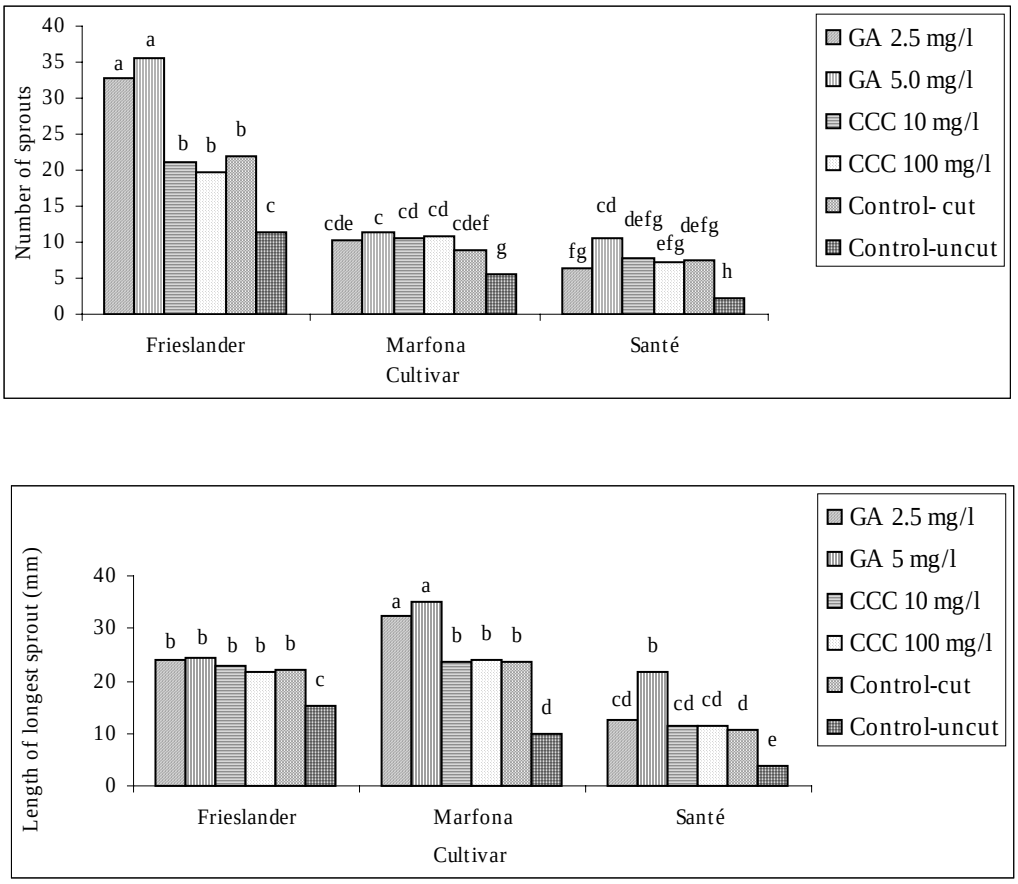

Fig. 2. The cultivar × growth regulator interaction for the number of sprouts (upper) and length of the longest sprout (lower). Letters a to h show highest and lowest values respectively and bars with a similar letter are not statistically significantly different based on the Duncan's Multiple Range Test.

cut control showed no significant difference within the same cultivar. Frieslander produced more sprouts with CCC and the control cut than the other cultivars. Marfona had no significant difference between different treatments at all, except that its uncut control had fewer sprouts. The combination of Santé and uncut control produced the lowest number of sprouts (Fig. 2).

There was also a highly significant interaction between seed tuber size and growth regulator treatment (data not shown): GA effects were larger with larger seed tubers as these larger seed tubers had more eyes and thus more potential sprouts than the smaller seed tubers.

Weight of sprouts

All main factors showed a highly significant effect on the weight of sprouts ($P < 0.0001$). The bigger the seed tuber size, the higher the sprouts weight. Frieslander produced more sprout weight, especially

than Santé. CCC (100 mg/l) produced the highest sprout weight, but showed no significant difference with GA 5 mg/l, both doses of CCC 10 mg/l and the cut control. GA 2.5 mg/l produced less sprout weight and the uncut control produced the lowest amount.

The two-way interactions seed tuber size × cultivar (P = 0.0157) and seed tuber size × growth regulator (P = 0.0410) were significant for the weight of sprouts (Table 1). For cultivars Frieslander and Santé there was no significant difference between seed tuber sizes 35 – 45 mm and 45 – 55 mm while Marfona showed significantly different sprouts weights for each of the three different sizes. The biggest size produced the highest sprout weight. In other words: Santé and Frieslander produced the lowest sprout weight in the two smallest sizes but Marfona showed lowest value for sprouts weight with smallest seed tubers (Fig. 1).

With the smallest seed tubers, all growth regulator treatments had the same sprout weight, except for the uncut control, which was significantly lower (Fig. 3). For the medium size seed tubers, GA 2.5 mg/l produced less sprout weight than both doses of CCC and produced the same sprout weight as the uncut control. For the largest seed tuber sizes GA 2.5 mg/l also produced less sprout weight than all other growth regulator treatments and the cut control, but here the uncut control was even lower. For all growth regulator treatments and the cut control, the biggest seed tuber size produced the highest sprout weight. Only the uncut control showed no significant effect of seed tuber size (Fig. 3).

Despite the significant two-way interaction cultivar × growth regulator for length of sprout and number of sprouts, this two-way interaction was not statistically significant for weight of sprouts.

Vigour

Of all vigour parameters measured, size of seed tubers only affected the number of stems while highly significant effects of cultivar and growth regulators on all vigour parameters assessed were observed (Tables 2 and 3).

Date of emergence

We discuss here the results of the analysis in which the seed tubers which did not produce an emerging plant were assigned the value of 126 days (see Materials and methods). The main effect of cultivar on the date of emergence was highly significant ($P < 0.0001$). Santé had the latest emergence. Frieslander had the earliest emergence. All cultivar differences were significant. The main effect of growth regulator was also highly significant ($P < 0.0001$). The uncut control gave the latest emergence. CCC 100 mg/l gave the earliest emergence, but there was no significant difference with GA 2.5 mg/l, CCC 10 mg/l and the cut control.

There was a significant seed tuber size × growth regulator interaction ($P = 0.0463$) for the date of emergence (Table 2; Fig. 3). The latest emergence was reached for the uncut controls in all sizes. There was no seed tuber size effect among the uncut controls. Growth regulators only had an effect compared to the cut control in the smallest size. Here GA 2.5 mg/l and CCC 100 mg/l significantly emerged earlier. Within the seed tuber sizes, only the smallest size showed significant differences between different growth regulators. GA 2.5 mg/l and CCC 100 mg/l had a significantly earlier emergence than GA 5 mg/l. Among the different seed tuber sizes, CCC 10 mg/l and the cut control showed a significant difference between 35 – 45 mm and 45 – 55 mm, where the smallest size emerged later.

The second important interaction was the one between cultivar and growth regulator ($P < 0.0001$) (Table 2 and Fig. 4). Cutting resulted in a significantly earlier emergence for all cultivars compared to the uncut seed tubers. Usually the uncut control gave the latest emergence of all treatments, but for Frieslander there was no significant difference between the uncut control and GA 5 mg/l. The emergence of the uncut control of Frieslander was also significantly earlier than the emergence of the uncut treatment of the other two cultivars. Within the cultivars, different chemical seed tuber treatments showed no significant differences. Within the various treatments no significant differences were present between Frieslander and Marfona, except for the uncut control, where Marfona emerged later than Frieslander. But within GA 2.5 mg/l and the cut control, Santé showed a significantly later emergence than Frieslander and Marfona.

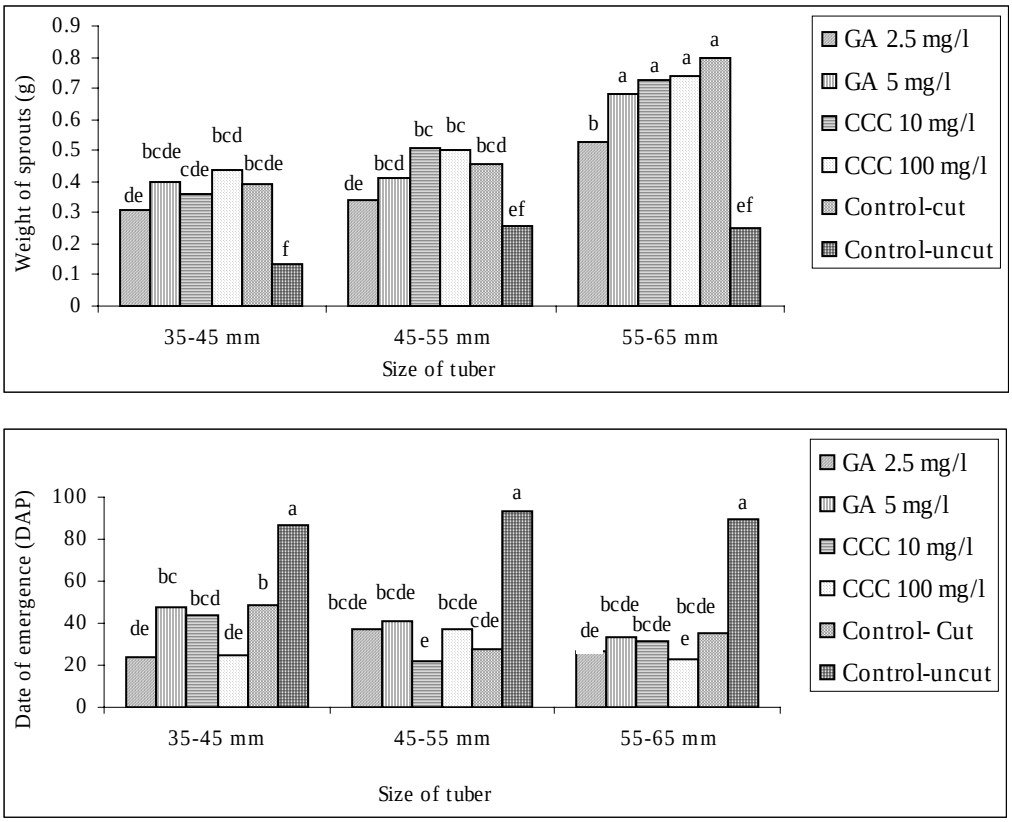

Fig. 3. Tuber size × growth regulator interaction for weight of sprouts (above) and date of emergence (below). Letters a to f show highest and lowest values respectively and bars with a similar letter are not statistically significantly different based on the Duncan's Multiple Range Test.

When non-emerged seed tubers were included in the analysis as missing values there were no significant differences between seed tuber treatments at all.

Date of leaf senescence

We discuss here the results of the analysis in which the seed tubers which did not produce an emerging plant were assigned the value of 126 days (see Materials and methods). The main effects of cultivar and growth regulator on the day of leaf senescence were highly significant ($P < 0.0001$). All differences among cultivars were significant for the day of leaf senescence. The earliest senescence was with the early cultivar Frieslander. The latest senescence was with the late cultivar Santé. The uncut control had the latest leaf senescence among the seed tuber treatments. When comparing the seed tubers treated with growth regulators or cut, only GA 5 mg/l showed a significantly later leaf senescence than the other treatments. The other treatments showed no significant difference at all

Table 2. The effect of seed tuber size and growth regulators on the vigour in different cultivars of potato.

Abbreviation: DAP = day after planting, DAE = day after emergence, GA= gibberellic acid, CCC= Chlorocholine chloride (or chlormequat), cv. = cultivar, GR = growth regulator; NS: not significant, $P \geq 0.05$.

	Date of emergence (DAP)	Date of leaf death (DAP)	Duration of growth (days)
Size of seed tuber (mm)			
35 – 45	46.0	94.9	48.9
45 – 55	43.1	93.3	50.2
55 – 65	40.0	94.1	54.1
P	NS	NS	NS
Cultivar (cv.)			
Frieslander	26.8 c	78.6 c	51.8 a
Marfona	42.3 b	97.4 b	55.1 a
Santé	59.6 a	106.2 a	46.5 b
P	**	**	**
Growth regulator (GR)			
2.5 mg/l GA	28.9 c	90.5 c	61.6 a
5 mg/l GA	40.8 b	97.9 b	57.2 ab
10 mg/l CCC	32.3 bc	87.4 c	55.1 ab
100 mg/l CCC	28.2 c	87.5 c	59.2 ab
Control			
Control (tuber cut)	37.2 bc	90.4 c	53.2 b
Control (tuber not cut)	89.9 a	110.5 a	20.6 c
P	**	**	**
Interactions			
Size × cultivar	NS	NS	*
Size × growth regulator	*	NS	NS
Cv. × growth regulator	**	NS	**
Size × cv. × GR	NS	NS	NS

**: significant at $P \leq 0.01$, *: significant at $P \leq 0.05$. Values followed by a similar letter are not statistically significantly different.

Table 3. The effect of seed tuber size and growth regulators on the vigour in different cultivars of potato.

Abbreviation: DAP = day after planting, GA= gibberellic acid, CCC= Chlorocholine chloride (or chlormequat), cv. = cultivar, GR = growth regulator; NS; not significant, P ≥ 0.05.

	Number of stems	Length of longest stem (cm)	Dry matter of aerial part of plants (g)	Number of tubers	Dry weight of tubers (g)
Size of seed tuber (mm)					
35 – 45	3.1 b	18.3	4.78	11.6	34.7
45 – 55	3.2 b	18.3	4.93	12.1	36.2
55 – 65	3.8 a	19.7	5.26	12.7	39.2
P	**	NS	NS	NS	NS
Cultivar (cv.)					
Frieslander	5.4 a	15.4 b	4.62 b	18.0 a	42.4 a
Marfona	2.8 b	23.5 a	6.45 a	8.6 b	39.9 a
Santé	1.8 c	17.2 b	3.92 b	9.7 b	27.9 b
P	**	**	**	**	**
Growth regulator (GR)					
2.5 mg/l GA	4.5 a	23.6 a	6.14 a	16.0 a	41.8 a
5 mg/l GA	4.1 ab	24.0 a	5.99 a	14.1 ab	34.5 b
10 mg/l CCC	3.8 abc	19.5 b	5.52 ab	13.2 bc	42.4 a
100 mg/l CCC	3.5 bc	20.3 b	5.50 ab	12.8 bc	44.4 a
Control					
Control (tuber cut)	3.1 c	18.6 b	5.01 b	11.8 c	41.2 a
Control (tuber not cut)	1.2 d	6.1 c	1.79 c	4.7 d	16.1 c
P	**	**	**	**	**
Interaction					
Size × cv.	NS	NS	NS	NS	NS
Size × GR	NS	NS	NS	NS	NS
Cv. × GR	NS	**	**	**	**
Size × cv. × GR	NS	NS	NS	NS	NS

**: significant at P ≤ 0.01, *: significant at P ≤ 0.05. Values followed by a similar letter are not statistically significantly different.

among them. Interactions between the three factors were not statistically significant. If the not emerged seed tubers were analysed as missing values, GA delayed senescence.

Duration of growth

The duration of growth is the date of leaf senescence minus the date of emergence (also valid for the non-emerged seed tubers). The main effects of cultivar and growth regulator treatments were both highly significant ($P < 0.0001$). Cv. Santé had a significantly shorter growth period than Frieslander and Marfona, which were statistically the same. Seed tubers treated with GA 2.5 mg/l had significantly more growing days than the controls. The uncut control had by far the lowest number of growing days. The different chemical treatments showed no significant differences among themselves. The interactions size × cultivar ($P = 0.0451$) (Table 2; Fig. 1) and cultivar × growth regulator ($P < 0.0001$) (Table 2; Fig. 4) were highly significant.

The interaction size × cultivar showed that only the cultivar Santé had significant differences among different sizes (Fig. 1). With this cultivar, the biggest seed tubers significantly had the longest growing period. The other two sizes did not differ significantly with each other. The cultivars Frieslander and Marfona showed no significant differences among different sizes. In the size 35 – 45 mm Frieslander and Marfona had significantly more growing days than Santé. In the size 45 – 55 mm only Marfona had significantly more growing days than Santé. In the biggest size all cultivars had statistically the same number of growing days.

The interaction cultivar × growth regulator showed that the effect of cutting was significant with the cultivars Marfona and Santé, where cut seed tubers had more growing days than uncut seed tubers (Fig. 4). Santé was the only cultivar that showed a significant difference between different growth regulators and the cut control. The seed tubers of Marfona treated with 5 mg/l GA had a significantly longer growth than seed tubers of Frieslander with the same treatment. The cut control showed a significant difference between Santé and Marfona, where Marfona had the most growing days. The uncut control from the cultivar Frieslander had significantly more growing days than that control of the cultivars Marfona and Santé; cultivars Marfona and Santé showed no significant difference for duration of growth (Fig. 4). For these cultivars, and especially for cultivar Santé, many

of the uncut seed tubers did not produce an emerging plant. Therefore their duration of growth was close to 0 days.

When the not emerged tubers were analysed as missing values, different cultivars had different days of growth. Frieslander now had fewer growing days than Marfona and Santé. This again was caused by the earliness of the cultivar Frieslander. There was also an effect of growth regulators if not emerged seed tubers were analysed as missing values. GA increased the number of days of growth with the cultivars Marfona and Santé. The cultivar Frieslander did not show more growing days. As mentioned before, Frieslander responds to GA in a different way than the other cultivars. Cutting and treating with CCC even decreased the number of growing days for the cultivar Frieslander. Seed tuber size effects on the days of growth were not shown.

Number of stems

All main effects on the number of stems were significant (Table 3). The seed tuber size effect ($P = 0.0017$) showed that the biggest seed tuber size produced significantly more stems than the lower two seed tuber sizes. Frieslander produced most stems. Santé produced the lowest number of stems. Seed tubers treated with GA 2.5 mg/l produced the highest number of stems when the seed tuber treatments were compared. The uncut control produced the lowest number. There was no significant difference between GA 2.5 mg/l, GA 5 mg/l and CCC 10 mg/l.

None of the interactions were statistically significant.

Length of the longest stem

The main effects of cultivar and growth regulator were both highly significant for the length of the longest stem ($P < 0.0001$). Marfona produced longer stems than Frieslander and Santé. There was no significant difference between Frieslander and Santé. The growth regulator effect showed that the seed tubers treated with GA (either 2.5 or 5 mg/l) produced the longest stems. The uncut control produced the shortest stems. There was an interaction effect of cultivar × growth regulator ($P < 0.0001$) (Table 3

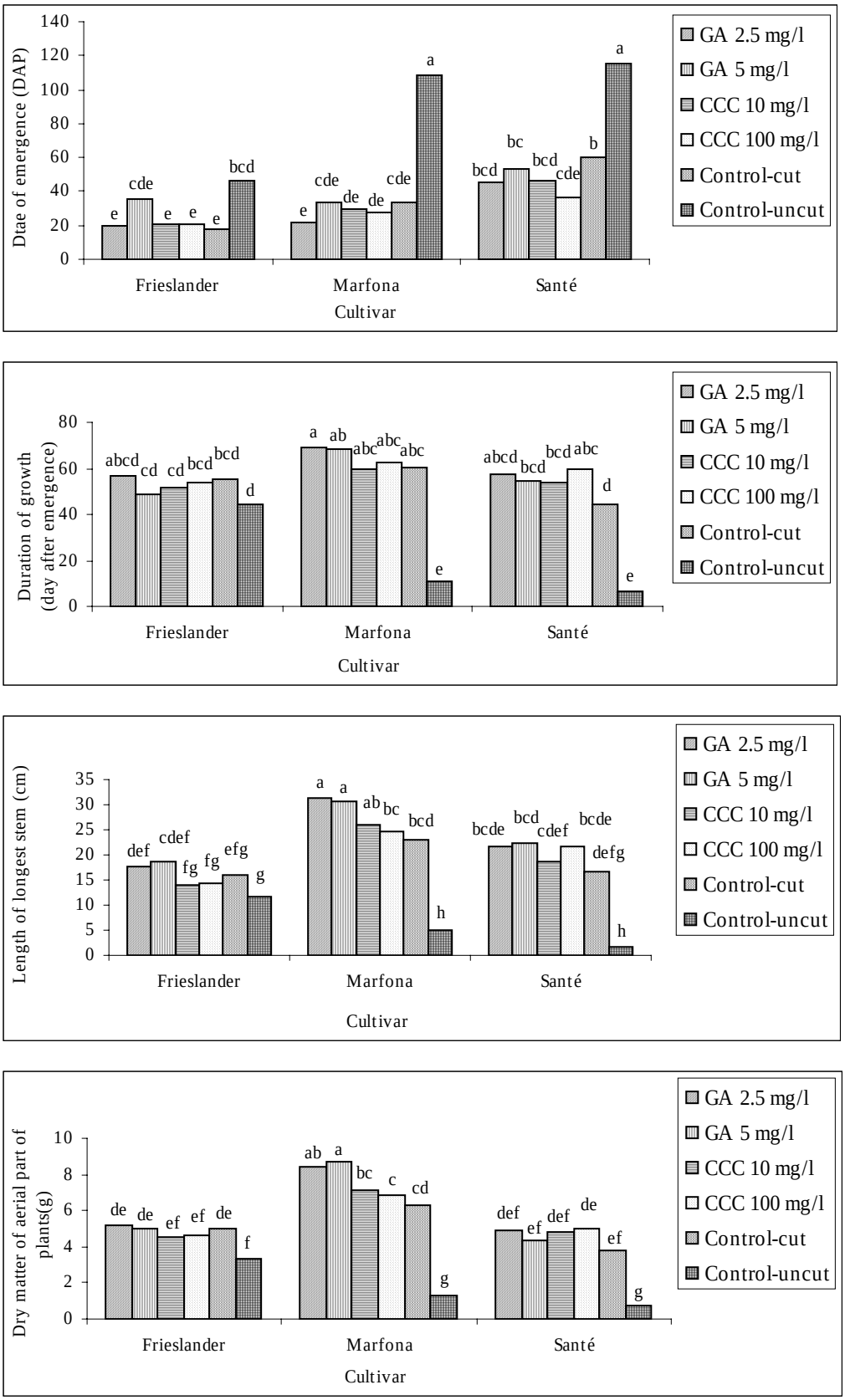

Fig. 4. Cultivar × growth regulator interaction for date of emergence (top), duration of growth (second highest), length of longest stem (second lowest) and dry matter of aerial parts (bottom). Letters a to h show highest and lowest values respectively and bars with a similar letter are not statistically significantly different based on the Duncan's Multiple Range Test.

and Fig. 4). The effect of cutting was significant with the cultivars Marfona and Santé, where the uncut seed tubers produced shorter stems than the cut seed tubers, associated with the much shorter duration of growth (Fig. 4) and partly caused by the poor emergence of these two cultivars. In the cultivar Frieslander, where emergence was good for all seed tuber treatments, this difference between the two controls was absent. The cultivar Frieslander only showed a significant difference between GA and the uncut control, where the two treatments with GA produced longer stems than the uncut control. With Marfona, different treatments of GA produced significantly longer stems than when seed tubers treated with 100 mg/l of CCC or not treated. Marfona treated with 10 mg/l of CCC only showed a significant difference with the uncut seed tubers. With Santé, only the uncut seed tubers produced significantly shorter stems than the other treatments. Within seed tuber treatment CCC 100 mg/l Marfona and Santé produced significantly longer stems than Frieslander. Within the cut control Marfona produced significantly longer stems than Frieslander. Within the uncut control, Frieslander produced the longest stems.

When the not emerged seed tubers were analysed as missing values, the positive effect of the GA treatment on the length of the longest stem was clearer than when these seed tubers were assigned the value zero.

Dry matter of aerial part of plants

Main effects of cultivar and growth regulator were significant for the dry weight of aerial parts ($P < 0.0001$; Table 3). Marfona produced the highest yield of aerial parts. The growth regulator effect showed that seed tubers treated with GA significantly produced more foliage than the controls. There was also a significant difference between the two controls: the uncut seed tubers yielding much less dry matter than the cut seed tubers.

There was a significant cultivar × growth regulator interaction ($P < 0.0001$) (Table 3 and Fig. 4). Cutting the seed tubers had a significant effect in all cultivars, where the cut seed tubers produced more foliage than the uncut seed tubers. However, the difference between cut and uncut was much smaller for Frieslander than for Marfona and Santé, associated with the large number of non-emerged plants in the uncut treatment of these two cultivars. All treatments of Frieslander and Santé where the

seed tubers were cut were statistically the same. For Marfona, however, seed tubers treated with GA 5 mg/l produced significantly more aerial parts than seed tubers treated with CCC or not treated. Marfona seed tubers treated with 2.5 mg/l of GA significantly produced more foliage than Marfona seed tubers treated with CCC 100 mg/l or not treated. Within the seed tuber treatments with GA or CCC in both doses Marfona produced the largest amount of aerial parts. With the cut control, Marfona significantly produced more foliage weight than Santé. Within the uncut control, Frieslander had the highest production of aerial parts as this cultivar was most successful in producing emerging plants.

When not emerged seed tubers were analysed as missing values, Frieslander also showed a little increase in weight of aerial parts.

Number of tubers

The main effects of cultivar and growth regulator were significant for number of tubers ($P < 0.0001$) (Table 3). Frieslander produced the highest number of tubers, probably associated with the highest number of stems present in this cultivar. The main effect of growth regulator showed that seed tubers treated with GA 2.5 mg/l produced more tubers than seed tubers treated with CCC or not treated. The uncut control produced the lowest number of tubers, significantly fewer even than the cut control.

There was a significant cultivar × growth regulator interaction ($P < 0.0001$) (Table 3; Fig. 5).The effect of cutting seed tubers was significant for all cultivars, most likely associated with the very strong effect of cutting on the number of stems per plant. GA in both doses significantly produced more tubers than the other seed tuber treatments with Frieslander. Different doses of CCC and the cut control were not significantly different with Marfona, but the higher dose of GA gave significantly fewer tubers than the lower dose of CCC. Santé showed no significant differences at all, except that the uncut control was much lower. Within the GA treatments, Frieslander produced more tubers than the other cultivars, which did not differ between each other. Marfona and Santé treated with GA showed no significant difference. Within the seed tuber treatment CCC 10 mg/l Frieslander and Santé produced the highest number of tubers. Within the seed tuber treatment CCC 100 mg/l Frieslander produced the highest number of tubers, but this was only significantly different with Marfona. Within the cut and uncut controls Frieslander also significantly produced more tubers than

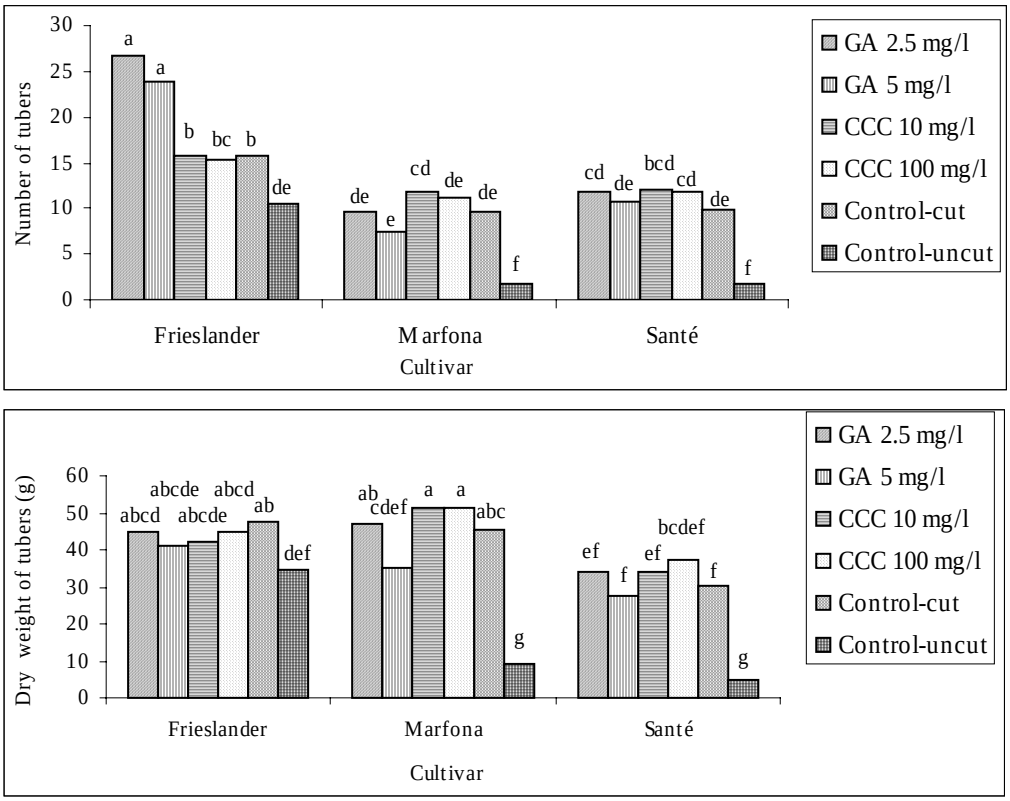

Fig. 5. The cultivar × growth regulator interaction for number of tubers (top) and dry weight of tubers (bottom). Letters a to g show highest and lowest values respectively and bars with a similar letter are not statistically significantly different based on the Duncan's Multiple Range Test.

the other two cultivars. This fecundity of Frieslander is partly associated with its high success rate in producing emerging plants.

Dry weight of tubers

The main effects on the dry weight of the tubers were significant for cultivar and for growth regulator (Table 3). The cultivar effect ($P < 0.0001$) showed that the cultivars Frieslander and Marfona produced

higher tuber yields than the cultivar Santé. Seed tubers treated with GA 5 mg/l had significantly more yield than the uncut seed tubers, but a lower yield than the other treatments. The other treatments showed no significant differences among each other.

Only the cultivar × growth regulator interaction was significant ($P < 0.0001$) (Table 3; Fig. 5). The effect of cutting was significant for all cultivars, but with Frieslander, this effect was small and only significant when the seed tubers were not treated with any growth regulator. Frieslander further showed no significant differences between different treatments. Seed tubers of Marfona treated with CCC and GA 2.5 mg/l, significantly produced more tuber weight than when treated with GA 5 mg/l. The cut control of Marfona showed no significant differences with any of the growth regulator treatments. All treatments of Santé with cut tubers were similar in tuber yield. Within the seed tuber treatment GA 2.5 mg/l Santé produced the lowest tuber weight; the other cultivars showed no significant difference. Within the seed tuber treatment GA 5 mg/l, Frieslander significantly produced more tuber weight than Santé. Within the seed tuber treatment with CCC (both doses), Marfona significantly produced more tuber weight than Santé. Within the cut control Santé produced the lowest tuber yield. Within the uncut control Frieslander had the highest yield.

When the data were analysed with not emerged seed tubers included as missing values, Marfona and Santé still had a much lower yield when treated with a high dose of GA, but the tuber yield of Frieslander was higher with a high dose of GA. On the other hand CCC increased tuber yield of Marfona, but decreased tuber yield of Frieslander.

Discussion

Methodology

From the Results section it becomes clear that there are four methodological aspects that have significantly contributed to the way the seed tuber treatments have been able to express themselves. First of all, the selection of seed tuber sizes was important. Larger seed tubers gave more sprouts, longer sprouts, a higher spout weight and more stems. Seed tuber size did not affect the date of

emergence, the time of plant senescence, the duration of the life cycle (except for Santé), the plant height, the above ground dry mater, the number of progeny tubers or the dry weight of the tubers. This means that the selection of tuber sizes was such that during the vigour test (part 2 of the experiment described) only the difference in number of stems between the largest size and the smaller two sizes was statistically significant. This general lack of seed tuber size effect is in contrast with the findings with minituber sizes in Chapter 5, where minituber size effects on yields of the aerial parts and the tubers were significant. This makes it obvious that the range of sizes is very important for creating differences in response. Despite this lack of main effect of size of normal seed tubers there were a few statistically significant interactions in which seed tuber size was involved, but these did not contribute much to our insight in the physiological aspects of the seed tuber vigour.

Secondly, the seed tubers have been de-sprouted before they were cut, treated and planted. The de-sprouting enhances the formation of new sprouts, which may result in more sprouts and sprouts with a higher rate of sprout growth than before de-sprouting (Struik & Wiersema, 1999). In Chapter 5 we stated that de-sprouting after storage at the lowest temperature gave less loss of vigour of the minitubers than de-sprouting of the minitubers more advanced in sprouting in response to the higher storage temperature. The minitubers used in Chapter 5 were, however, young. In the experiment described in the current chapter, de-sprouting has been applied on seed tubers which were already advanced in their ageing process (as shown by the sometimes very high numbers of stems per individual seed tuber observed in single cases) and the vigour loss could be substantial. The planting of the seed tubers in the greenhouse was much later than normally done in the field in the Netherlands for which the seed lots were originally meant. De-sprouting probably has contributed to the ageing of these old seed tubers (see also Struik & Wiersema, 1999) and thus to the poor emergence of some of the material, especially when cutting did not contribute to sprouting.

Thirdly, cutting may contribute to the breaking of the dormancy (Rappaport & Sachs, 1967) and to the breaking of the apical dominance (Struik & Wiersema, 1999). These aspects were probably not relevant in our experiment, as the seed tubers were well beyond the phases of dormancy and apical dominance. Cutting may also stimulate sprout growth (Struik & Wiersema, 1999). In Chapter 5, we

stated that cutting might have had a larger effect on the minitubers stored at the lowest temperatures than on the minitubers stored at higher temperatures, suggesting that younger seed tubers may benefit more from such a treatment than older seed tubers. This would mean that in our case, with old seed lots, positive effects of cutting are unlikely. However, we found a very large, positive effect of the cutting, both in our sprout behaviour test and in our vigour test. Cutting apparently rejuvenated the seed tubers, especially in cultivars which had advanced more in age.

Fourthly, the choice of cultivars is always crucial when one wants to identify or understand physiological mechanisms. Especially in relation to seed tuber behaviour there are many interactions between seed tuber treatments and cultivar (see e.g. Struik & Wiersema, 1999). We used in our experiment three cultivars differing in maturity type, but the cultivars also differed in duration of dormancy, rate of physiological ageing of the seed tubers, shape of the seed tubers, sensitivity to some of the growth regulators, earliness of tuber formation, intensity of tuber set, etc. The cultivar Marfona was present in the research described here and in the research described in Chapter 5. The responses of Marfona to growth regulator applications seem rather consistent for the sprout behaviour test: especially the sensitivity of the length of the longest sprout for GA is clearly visible in both cases. The same is true for the generally small effects of growth regulator application to seed tubers on the number of sprouts. However, for the vigour test it is striking that in Chapter 5 hardly any interaction between cultivar and growth regulator application was reported significant, whereas in the current chapter a considerable number of interactions proved significant. The response of Marfona to the seed tuber treatments were more pronounced in the experiment with the normal seed tubers than in the experiment with the minitubers, probably because of the younger age of the minituber in this cultivar with a relatively long dormancy.

Note that the duration of the vigour test was very different in the two chapters.

Length of the longest sprout

Seed tuber size had an effect on sprout elongation. The largest seed tuber size had longer sprouts than the other seed tuber sizes. The effect, though not large, is consistent with the generally observed effect of seed tuber size on vigour of each individual bud on the seed tuber (Struik & Wiersema, 1999). The size of the effect depends on the range of seed tuber sizes investigated (see above). There was also a significant interaction between seed tuber size and cultivar for this parameter. Such interactions can occur when there are differences between cultivars in seed tuber shape or when the effect of size on the physiological behaviour (such as reflected by duration of dormancy or vigour, Van Ittersum, 1992) is not the same for each cultivar.

Suttle (2004) showed that sprout length on seed tubers increases by exogenously applied GA. This was only investigated for the cultivar Russet Burbank. Our experiment confirms this. He also found that the endogenous GA levels became higher when the potato tuber reached dormancy release. After dormancy release the GA levels were still increasing. The seed tubers in this experiment were past their dormancy release point for a long time. This means that the endogenous GA levels were high. The different cultivars included in the experiment, however, may have differed in the temperature sum required to break dormancy and in the rate of physiological ageing after dormancy. For example, Marfona is known to have a long period of dormancy (Van Ittersum, 1992).

The effect of GA on the length of the longest sprout was indeed different among cultivars. Frieslander did not react on GA in contrast to cultivar Marfona, which showed a strong response to both doses of GA by producing longer sprouts. Santé on the other hand only responded to the high dose of GA. These differences among cultivars could be explained by different sensitivities to GA. Since Frieslander is an early cultivar, which reached its dormancy release point sooner than the other cultivars, it is likely that the GA level in this tuber is so high that exogenous GA does not work anymore. Cultivar Marfona has a long dormancy and thus the endogenous levels of GA may have been much lower, which will enhance the effect of exogenous GA. Santé only responded to the highest concentration applied, suggesting that it has the right stage to be responsive but requires a higher dose as it is not sensitive to lower doses.

El-Sayed et al. (1988) investigated the effect of CCC on sprout length in potato. They observed that CCC reduced sprout length. This is consistent with the positive effect of GA on sprout length as we observed in the current experiment. However, it is not consistent with the lack of effect of CCC itself in our experiment. We made the observations on sprout length 11 days after seed tuber treatment, whereas El-Sayed et al. (1988) measured their sprouts after 4 months. It is possible that the CCC effect required more time than the 11 days given by us to come to expression.

Cutting stimulated sprout elongation for all seed tuber sizes. This effect can be explained by the GA produced for wound healing (Rappaport & Sachs, 1967).

Number of sprouts

The effect of size of the seed tuber on the number of sprouts showed that larger seed tubers produce more sprouts than small seed tubers. This is caused by the increase in number of eyes in the seed tubers, which is higher with large seed tubers, but also by the amount of reserves available per eye (Struik & Wiersema, 1999). However, as observed for the length of the longest sprout, in our experiment only the difference between the largest seed tubers and the two smaller seed tuber classes was significant. Effects on number of sprouts were consistent with the effects on length of the longest sprout.

Cultivar effects on number of sprouts were very large, especially Frieslander produced many sprouts per individual seed tuber. Among the seed tuber treatments, the uncut seed tubers produced by far the lowest number of sprouts in all cultivars. Despite the energy required to heal the wounds caused by the cut, the cut seed tubers performed better, as the cutting procedure seemed to rejuvenate the seed tuber. For cultivar Marfona, seed tuber treatments with growth regulators after cutting had no effect on number of sprouts, although Marfona did show growth regulator effects for length of the longest sprout, GA enhancing the length. Frieslander on the other hand, which cultivar did not respond to growth regulators for the length of longest sprout, did respond to GA for the number of sprouts. Seed tubers of Frieslander, treated with GA, produced many more sprouts than when not treated. This

large number of sprouts was not caused by more eyes producing sprouts, because with cutting normally all eyes will produce sprouts, but because many more sprouts emerged from one single eye. Santé expressed a positive effect of the higher concentration of GA compared to the lower concentration for both the length of the longest sprout and the number of sprouts, showing a consistent behaviour for both parameters. This shows that Frieslander, Marfona and Santé were all sensitive to GA, but that they showed their sensitivity in different ways. This is most likely associated with the difference in apical dominance within one eye or stage of ageing.

Weight of sprouts

The weight of the sprouts depends upon the number of sprouts, the length of the sprouts, and the thickness of the sprouts. The thickness of the sprouts was not measured. The results showed that the larger the seed tubers the higher the sprout weight, despite the fact that for length of the longest sprout and the number of sprouts only the difference between the largest seed tuber size and the two smaller seed tuber sizes were significantly different.

The effects of cultivar on sprout weight partly reflected the cultivar effects on length of longest sprout and partly the cultivar effect on number of sprouts.

Cutting had a strong effect on the weight of sprouts, which is consistent with the effects on length of the longest sprout and number of sprouts. GA in a low dose had a large negative effect on the sprout weight. During observations, it was obvious that GA treated seed tubers had thinner sprouts and this could explain a greater portion of the GA effect on weight. This effect was only visible in the lower concentration as for the higher concentration the lower sprout thickness was better compensated by longer sprouts and more sprouts per individual seed tuber. Different aspects of sprout behaviour show different sensitivities for GA.

Date of emergence after planting

Cultivar differences in date of emergence were large, as Frieslander performed much better (especially in the uncut seed tuber treatment) than the other two cultivars. From the different treatments only

cutting had a big effect on the date of emergence. Cutting a seed tuber made it emerge much sooner. This is because uncut seed tubers had a lot seed tubers which did not produced an emerging plant and these seed tubers were assigned an emergence date of 126 DAP (the end of the experiment). When these non-emerged seed tubers were included in the analysis as missing values there were no differences between seed tuber treatments at all. With the analysis illustrated in Table 2, there was also an effect of GA concentration: the higher GA concentration gave significantly later emergence. An explanation might be that the higher GA concentration resulted in weaker individual sprouts. However, this is not confirmed by calculations on the ratio between weight of sprouts and number of sprouts, so this possible effect on individual sprouts must have arisen after the de-sprouting.

Date of senescence after planting

As stated before, the day of senescence after planting was also difficult to determine for the not emerged seed tubers. In the results, the presented data includes not emerged seed tubers, for which the values for date of senescence were also set at 126 days. Since most not emerged seed tubers were with the uncut control, it seems logical that this treatment had the highest values for date of senescence. If the not emerged seed tubers were analysed as missing values, GA delayed senescence, which is in agreement with the enhancing effect of GA on overall shoot development and the delaying effect on tuber formation (Ewing & Struik, 1992). Singh et al. (1992) observed the same effect of GA on senescence in tobacco and Noodén (1986) showed this effect for soybean. Frieslander and Marfona showed no effects of size of the seed tubers on date of senescence, but Santé showed that the biggest seed tubers gave plants that senesced later, probably because of a more vigorous shoot, resulting in more sympodial layers.

The results show that the early cultivar Frieslander also had the earliest senescence and that the cultivar Santé which is a late cultivar, was the latest in senescence.

Duration of growth

The number of growing days was determined from the date of emergence to the day of senescence. Different cultivars did not show large differences in growing days, except Santé, which cultivar had fewer growing days, especially with the smaller sized tubers. The fact that Santé had a lot of not emerged seed tubers, which had zero days of growth, caused a low average value for days of growth of Santé. Different treatments with growth regulators also had no effect on the days of growth. Only the control that was not cut showed fewer days of growth. It was especially this seed tuber treatment that gave seed tubers that did not produce an emerging plant. When these non emerged plants were treated as missing values, the picture changed drastically. Especially this parameter of number of days of growth was very sensitive to the method of analysis.

Number of stems

The largest seed tubers produced more stems than the smaller size classed. Here, the same applies as for the number of sprouts. The bigger the seed tuber, the more eyes are present in the seed tuber and the more reserves are available per eye. However, it was the only significant main effect of seed tuber size after planting.

Frieslander gave by far the highest number of stems. This was already visible in the number of sprouts. This was especially true when treated with GA, as this increased the number of stems of Frieslander. Other seed tuber treatments did not show an effect on the number of stems. No direct correlation was found between number of sprouts and number of stems. When there were 40 sprouts, some seed tubers produced one stem and some seed tubers produced 15 stems. Only a small portion of the sprouts were transformed into a stem, but it is likely that if a seed tuber produces many sprouts, many stems will be produced too. The de-sprouting that was carried out might have reduced the relation between sprout number and stem number.

Length of the longest stem

GA increased the length of the longest stem in Marfona. This effect was clearer when the not emerged seed tubers were analysed as missing values, than when these seed tubers were assigned the value zero, and is consistent with the effect on the length of the longest sprout. For Frieslander there was no GA effect on either the length of the longest sprout or the length of the longest stem, when compared to the cut control. In Santé, the effects of GA on the longest sprout and on the longest stem, did not match, probably because the de-sprouting affected the persistence of the GA effect. CCC showed no retardant effect on the length of the longest stem. This is not as expected, because in wheat, CCC is used to retard stem elongation and Sharma et al. (1999) and Digby & Dyson (1973) showed that CCC applied to a potato crop in a foliar application did reduce shoot growth. This could mean that CCC applied to a seed tuber which is subsequently de-sprouted is not as effective as when sprayed on the foliage. The transport from the tuber to the new sprouts might not be efficient enough to establish a large effect.

Marfona produced the longest stems in all treatments. This should be a cultivar characteristic.

Number of tubers

GA inhibits tuberization (Ewing & Struik, 1992; Struik et al., 1999; Vreugdenhil & Sergeeva, 1999). In the experiment of Sharma et al. (1999), there were also fewer tubers per plant when treated with GA. However, in our experiments GA either had a positive effect on the number of tubers (Frieslander) or no effect at all (Marfona and Santé) compared to the cut control. In the cultivar Frieslander the positive effect was very large: many more tubers per plant were produced when the seed tubers had been treated with GA. The number of tubers almost doubled when treated with GA. Although GA might impede or delay tuberization, it can enhance the production of tuber sites, by increasing the number of stems, stimulating stolonization, and enhancing stolon branching. Although

probably all these effects occurred, the increased stolonization is most likely the dominant effect as appeared during the harvesting of the plants (Cees van den Hoek, personal communication).

CCC did not increase the number of tubers per plant compared to the cut control. This was true for all cultivars. This is not in line with the results of Radwan et al. (1971) and Sharma et al. (1999), who found that CCC increases the number of tubers. However, this was a foliar application and it differs with this experiment where the CCC was applied on seed tubers which were subsequently de-sprouted before planting (see above).

Dry weight of tubers

The results showed that the cultivar Marfona produced the highest tuber yield. The difference with Santé is remarkable and this may be associated with reports from practice that Santé is sensitive to relatively low storage temperatures and needs to be stored at higher temperatures than other cultivars (Cees van den Hoek, personal communication). The results also showed that there were no big differences between the different treatments with growth regulators. The only difference was with GA at a high dose, which produced less tuber weight when applied to Marfona and Santé. This is in accordance with the research of Sharma et al. (1999). However, it is also in accordance with data presented in Chapter 5 for minitubers. Also in that chapter there were no positive effects of application of growth regulators on dry weight of progeny tubers.

When the data were analysed with not emerged seed tubers included as missing values, Marfona and Santé still had a much lower yield when treated with a high dose of GA, but the tuber yield of Frieslander was higher with a high dose of GA. Effects of CCC on tuber yield became inconsistent across cultivars.

Dry weight of aerial part of plants

GA only increased the dry weight of aerial parts when applied to cultivar Marfona. When not emerged seed tubers were analysed as missing values, Frieslander also showed a little increase in weight of

aerial parts. Santé did not show any differences in dry weight of aerial parts when treated with different growth regulators. This can be partly ascribed to the effect of cold storage again.

CCC did not decrease the dry weight of the foliage. This was not as expected, because in a previous study CCC did decrease foliage dry weight (Sharma et al., 1999). In the experiment of Radwan et al. (1971), CCC decreased foliage dry weight only in high concentrations (2000 g/ha). These concentrations were in g per hectare, so this is not comparable with this experiment.

Conclusions

The interpretation of the results of this experiment is strongly complicated by the methodology used and the relatively old age of the seed tubers used. However, the most striking findings are the following:

- Cutting of relatively old seed tubers seemed to improve their performance.
- GA applied to seed tubers affected sprout behaviour and growth vigour after de-sprouting in various ways. The effects strongly depended on cultivar. In Frieslander, GA treated seed tubers produced more sprouts with GA, but of the same length. In Marfona GA treated seed tubers produced similar numbers but longer sprouts compared to the control.
- CCC did not affect sprouting behaviour.
- Although many significant effects on various parameters reflecting part of the growth vigour of seed tubers were recorded, none of the seed tuber treatments was able to increase the dry weight of the tubers, the ultimate test for the efficacy of a growth regulator. The only positive response relevant for a seed production scheme was observed in the cultivar Frieslander: it

produced more tubers in response to GA, but this effect was mainly brought about by an increase in the number of stems per seed tuber.

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General discussion

Introduction

In conventional seed potato systems, farmers use seed potato tubers for the multiplication and production. This method has some major disadvantages (Chapter 1; Beukema & Van der Zaag, 1990; Struik & Wiersema, 1999). Over the last three decades rapid multiplication systems became an important technique to provide disease-free propagules. These techniques yield in vitro plantlets, transplants, microtubers and minitubers, which are used in the initial phases of a seed tuber production scheme (Murashige, 1974; Roca et al., 1978; Hussey & Stacey, 1981; Wang & Hu, 1982; Jones, 1988).

The seed tuber production system used in this research consists of four phases (Chapter 1: Fig. 5):

1. The phase of rapid multiplication; in this phase in vitro potato plantlets are produced and propagated routinely from explants by using single-node cutting in a culture medium.
2. The phase of microtuber production, during which microtubers are produced under in vitro conditions from explants; these microtubers could serve as an alternative starting material for the production of minitubers.
3. The minituber production phase, during which minitubers are produced from rooted plantlets cultured in the greenhouse.
4. The phase of seed tuber production, during which normal seed tubers are produced in the greenhouse or under field conditions by planting minitubers or microtubers.

In vitro plantlet production and microtuber production can be considered as similar stages of the seed production scheme. The need for further multiplication, the period until use, the need for storage and transport, the desired vigour, the use of the progeny material, the availability of labour and

special facilities and other economic factors determine which one of the two will be most appropriate. In general, production of in vitro tubers does not give (much) additional multiplication, but changes the type of propagule.

Moreover, the storage phase, i.e. the period between the harvest of microtubers, minitubers, or normal tubers and planting them, can be seen as an additional phase during which management can affect the vigour, but not the number of propagules.

In the research described in this thesis, the influence of five main factors on the production and quality of progeny material and possible after-effects on the next generation of propagules was investigated. These factors included temperature, photoperiod, growth regulator application, seed size and cultivar. First these factors will be briefly discussed.

Temperature

Temperature is one of the main physical factors affecting growth, development and yield of plants and crops but also behaviour of propagules during storage. For most processes there is a base temperature (below which a certain process cannot take place), an optimal temperature (at which the process reaches its maximum rate) and a maximum temperature (above which the process does not take place). Between the minimum and optimum temperature the rate of a process increases with an increase in temperature. Often the rate of a process doubles with an increase in temperature of 10 °C, but the typical increase in rate depends on the process and on the range of temperatures over which the increase is assessed. Above the optimum temperature the rate of the process decreases with an increase in temperature. In potato, as in many other crops, there are clear differences in optimum temperature for the different processes. During storage, the breaking of the dormancy has an optimum temperature of about 28 °C, whereas the optimum temperature for sprout growth is about 18 °C (Van Ittersum, 1992). The optimum temperature for obtaining the maximum vigour of a seed tuber is also different and very much depends on the variety (Struik & Wiersema, 1999). During the plant phase, the optimum temperature for shoot development is higher than the optimum temperature for tuber formation (Ewing & Struik, 1992). An optimum temperature does not mean that the highest

production is realized. When the growth period is long enough, yield not only depends on the maximum rate of a process, but also on the duration of the process and the amount of assimilates produced during the completion of a process (see for potato tuber production e.g. Van Dam et al., 1996). In addition to the effect of temperature *per se*, there are also effects of diurnal temperature fluctuations. These effects of the temperature fluctuations are at least partly caused by the fact that many processes have specific diurnal patterns of their intensities. Some processes mainly occur during the night (e.g. elongation of organs, transport of certain assimilates), whereas other processes mainly occur during the day (e.g. photosynthesis, transpiration).

In this thesis the following investigations on effects of temperature were carried out:

1. The effects of temperature regime on microtuber production in the dark (Chapter 2). Different temperatures were set for different phases during a 24 h period and the effects of temperature in each phase but also the effect of the temperature difference between the two phases were studied over a wide range of temperatures, both above and below the optimum temperature for tuber formation in the dark.
2. The effects and after-effects of temperature (either as a main factor or in interaction with photoperiod) during in vitro plantlet production on the production and quality of minitubers produced with these in vitro plantlets in the greenhouse (Chapter 4). The range of temperatures selected was such that the rate of growth was supposed to increase over the entire range, but that the vigour of the resulting in vitro plantlet (given the low light intensity at which these plantlets were produced) was supposed to decrease with an increase in temperature.
3. The effect of temperature during storage of minitubers on the sprouting capacity and vigour of minitubers (Chapter 5). These effects were assessed either before or after growth regulator application. The range of experiments tested was selected to assess the differential effect of storage on the rate of breaking the dormancy, the rate of breaking the apical dominance and the rate of change in growth vigour.

Photoperiod

Potato is a quantitative short-day plant for flowering and tuberization, meaning that these processes start earlier when the plants are grown under a day length which is shorter than the critical photoperiod (i.e. the longest photoperiod that will permit the process to occur within a reasonable period of time) (Almekinders & Struik, 1996). However, flowering is more abundant under long days than under short days (Almekinders & Struik, 1994). Under natural conditions longer photoperiods are associated with larger quantities of incoming radiation. Extension of photoperiod with full light has a smaller effect than extension of photoperiod with low light intensities (Ewing & Struik, 1992). During in vitro production of microplantlets, photoperiod is usually regulated with full light. This means that a long photoperiod also means an increase in the amount of incoming light per day. With the low light intensities common in in vitro laboratories and with the effect of the medium and other conditions on the development of the in vitro plantlets, this increase in amount of light on growth and vigour might be equally or even more important than the effect of photoperiod on development (see, e.g. Wang & Hu, 1985).

In this thesis only one experiment on the effects of photoperiod was carried out. This experiment involved the effects of photoperiods in the range of 12 – 20 h (i.e. from well below to well above the critical photoperiod), in association with the effects of temperature, on the vigour of in vitro plantlets when used as propagules for minituber production in the greenhouse, the resulting rate of multiplication and yield of minitubers and their quality (Chapter 4).

Growth regulators

Growth regulators are substances modifying plant growth and development and are widely used in field production, during storage of vegetative propagules and in in vitro multiplication to change the behaviour of the propagules or plants, thus optimizing the quality or quantity of the produce obtained. Also in potato, growth regulators are widely used to change the behaviour of the crop (manipulating tuber number, secondary growth, sprouting behaviour of tubers produced), change the behaviour of the stored seed or ware tubers (influence duration of dormancy and sprouting) or during in vitro

plantlet or microtuber production (e.g. to create a more sturdy in vitro plantlet to obtain a stronger transplant, induce tuberization or increase the number or size of the propagules) (e.g. Struik & Wiersema, 1999). The effects of growth regulators vary depending on the sensitivity of the plant or organ to which they are applied, on the conditions after application, on their concentration or method of application and many other factors.

In this thesis, experiments are described using two growth regulators:

- GA3, which stimulates cell division and elongation. It enhances stem length, stolon growth, breaking of dormancy, sprout growth, and flowering, but impedes tuber formation and starch accumulation; and
- CCC (Cycocel), which inhibits shoot development and growth, reduces sprout growth and enhances tuberization.

The effects of these growth regulators are reasonably well described in literature (see overviews in individual chapters) and the focus in this thesis was on the after-effects of their application during different phases of a seed production scheme. The following experiments were carried out:

- Effects of growth regulators applied in vitro on in vitro plantlet production (Chapter 3);
- Effects of growth regulators applied in vitro on microtuber production, either directly on the in vitro plantlets treated or indirectly by subculturing the explants taken from the treated in vitro plantlets (Chapter 3);
- Effects of growth regulators applied after some period of storage at various storage temperatures on the sprouting behaviour and vigour of minitubers (Chapter 5); growth regulators were applied shortly after breaking of dormancy.
- Effects of growth regulators applied after some period of storage on the sprouting behaviour and vigour of normal seed tubers (Chapter 6). Normal sized seed tubers were treated long after their dormancy was broken.

Growth regulators were applied in different concentrations aiming at one below-optimal, but safe concentration and one concentration with a more or less maximum positive effect, but with a higher chance of negative side effects.

Seed size

In microtubers and minitubers, the size of the propagule is of essence for their behaviour during storage and after field planting (Struik & Lommen, 1990; Struik & Lommen, 1999). Larger sizes give better emergence and a better early vigour, and produce a higher yield and more tubers per plant.
Some of these effects can also be found in ranges that are common for normal seed tubers (Van Ittersum, 1992; Struik & Wiersema, 1999), although these effects depend on the variety used. Usually, larger seed sizes have more eyes per seed tuber, a higher proportion of eyes producing a sprout, a larger proportion of the sprouts producing a stem, a larger number of tubers per stem and often also a larger individual weight of the progeny tubers (Struik & Wiersema, 1999). There is also an effect of size on physiological behaviour of the minitubers and normal seed tubers as reflected in the duration of dormancy and the growth vigour (Van Ittersum, 1992; Lommen, 1995; Struik & Wiersema, 1999).

In this thesis the effects of seed tuber size were tested both for minitubers (Chapter 5) and normal seed tubers (Chapter 6).

Cultivar

There are numerous varieties of the potato plant. More than 4,000 have been described in detail (Hils & Pieterse, 2005). These varieties differ in foliage maturity type, earliness and intensity of tuber formation, their response to abiotic environmental conditions both during the field phase and during storage, in their tolerance to or resistance against many diseases and pests, rate of multiplication in different systems of rapid in vitro or field multiplication, quality for various uses and in many other aspects (including their response to cultural practice).

In Chapters 2, 3, 4 and 5, three cultivars were used: Gloria, Marfona and Agria. These were selected on the basis of differences in maturity type, Gloria being very early, Marfona being mid-early and Agria being late. Moreover, Marfona and Agria were selected on the basis of their actual use in Iran (see Chapter 1).

In Chapter 6, the following cultivars were used: Frieslander (early), Marfona (mid-early) and Santé (late). They were selected on the basis of differences in maturity type and on the basis of availability of seed tubers of similar physiological age.

All these cultivars are described in Hils & Pieterse (2005).

In the following section, some comments on the general methodology used in the research described in this thesis will be given.

Some comments on the general methodology

The phase of rapid multiplication through in vitro plantlet production was carried out in the growth chamber at 24 ^{o}C and a photophase of 16 h provided with fluorescent tube lamps (Philips TL 84) and with a photosynthetic photon flux density of 30 μmol m^{-2} s^{-1} for a period of 5 weeks. Nodal cuttings were taken from 5 weeks old in vitro plantlets and cultured on a standard medium containing 4.4 g/l MS salts (Murashige & Skoog, 1962) with vitamins, 30 g/l sucrose (as source of energy) and 8 g/l agar (to solidify the medium), pH was adjusted at 5.8 before using agar and before autoclaving (at 120 ^{o}C, pressure 0.11 MPa = 16 PSI for 15 minutes). The multiplication phase was regularly repeated every 5 weeks by single-node stem cutting until the required numbers of in vitro plantlets for experiments were achieved and also to keep some plantlets as a stock. This method of subculturing in vitro plantlets can be considered as a standard method of in vitro plantlet production, with a normal medium and normal environmental conditions (Wang & Hu, 1985; Struik & Wiersema, 1999; Tadesse, 2000). This also means that the light intensity was rather low and that large effects can be expected of expanding the photoperiod (Dodds et al., 1992; Seabrook, 2005).

For microtuber production, sucrose was increased to 80 g/l, agar was decreased to 6 g/l and 500 mg/l CCC was added to the medium after autoclaving, pH was adjusted at 5.7 before autoclaving. Microtuber production was carried out at various temperatures (Chapter 2) or at 18 °C (Chapter 3). In both cases, however, microtuber production took place in the dark. For the experiment in Chapter 2, this condition was dictated by the equipment used. Darkness, although favourable for tuber formation

in field grown plants (Ewing & Struik, 1992), is not favourable for microtuber production. Usually, it is recommended to produce microtubers in light, as this will provide more and heavier microtubers than in the dark (Ranalli, 1997; Pruski, 2001). Short photoperiods (8 h) result in earlier microtuber formation, more microtubers and heavier microtubers than long photoperiods (16 h) (Wang & Hu, 1985; Garner & Blake, 1989; Seabrook et al., 1993). The lack of light might have contributed to the low numbers of microtubers produced and the strong secondary growth phenomena observed in Chapters 2 and 4, compared to results in the literature.

For minituber production basically 4 week-old rooted in vitro plantlets were planted in 5 litre pots (30 × 30 × 13 cm) filled with potting soil. The plantlets were grown in the greenhouse under day/night temperatures of 18/12 ^{o}C, and a relative humidity of 85%, for a period of about 100 days. This methodology can be considered as standard (Struik & Wiersema, 1999), although usually during the last phase of multiplication (also called the normalization phase; Tadesse, 2000) the growth regulator alar is added to get more sturdy and robust transplants. For the specific effects of alar on the transplant quality, see Tadesse et al. (2000).

The storage period can be divided into two stages:

a. A curing period during which harvested tubers are left at a temperature of 18 ^{o}C and high humidity of 80% for a period of 2 weeks for hardening and skin set.

b. A period of cold storage during which tubers are stored at a temperature of 4 ^{o}C and complete darkness. This period lasts 4 months or longer.

In Chapters 5 and 6, where stored seed tubers (either minitubers (Chapter 5) or normal seed tubers (Chapter 6)) were used, these storage phases have also been included. However, the minitubers used in Chapter 5 were produced under controlled conditions in our own glasshouses and subsequently stored during the first phase under controlled conditions in the storage facilities of Unifarm until use. The seed tubers used in Chapter 6 were either from commercial sources or commercially produced by Unifarm and therefore the conditions during the both storage phases were not so well controlled as for the minitubers.

In Chapters 5 and 6 various growth regulators (gibberellic acid and CCC) were applied to examine the effects of storage temperature and growth regulator application during the storage period on the dormancy, sprout behaviour and growth vigour of minitubers (Chapter 5) and the effects of growth regulators on ordinary tubers (Chapter 6). These growth regulators were just examples of contrasting ones to show different results on dormancy, sprout behaviour and vigour in association with temperature treatments. For growth regulator application first tubers were cut and placed on a tray and were soaked in the solution of the growth regulators for a period of 10 minutes. There were two controls (no growth regulator application; tubers cut or uncut) and they were soaked in the pure water for the same period. A small piece of cardboard was used in the tuber cut to keep the gap open because in this case tuber cut cannot be closed and growth regulators can penetrate easier. The tray with tubers were covered with household plastic and stored at a storage temperature of 18 ^{o}C, relative humidity 80% and full darkness. After 5 days plastic cover was removed and 11 days after storage tubers were transferred to the cold store at 4 ^{o}C and a relative humidity of 80% then collecting data was started. Applying growth regulators therefore included a cutting treatment of the seed sources, a period of wound healing and a step of de-sprouting. Cutting, wound healing and de-sprouting all had a strong influence on the outcome of the experiments described in the Chapters 5 and 6. These influences have been discussed in detail in these two chapters.

This following parts of the general discussion discuss

- the effects of temperature during microtuber production (including the temperatures during the different phases and their interactions);
- the effects of temperature during the in vitro production of microplantlets on the subsequent minituber production in the greenhouse;
- the effects of temperature during storage of minitubers;
- the effects of photoperiod during the in vitro production of microplantlets on minituber production in the greenhouse;

- the interactions between temperature and photoperiod during the in vitro production of microplantlets on the minituber in the greenhouse;
- the effects of growth regulators during the in vitro production of microplantlets on the in vitro plantlet;
- the effects of growth regulators during the in vitro production of microplantlets on microtuber production;
- the effects of growth regulators applied on minitubers and normal seed tubers after storage;
- the effects of minituber size on storage losses and minituber performance;
- the effects of tuber size of normal tubers on seed tuber performance;
- the effects of cultivar.

The general discussion will end with a few closing remarks.

Temperature effects

Effects of temperature during Phase 2 (phase with higher temperature range) on microtuber production

There are numerous reports on the effects of temperature on microtuber production of potato. However, the literature is not very consistent regarding optimal temperatures. Optimal temperatures or temperature ranges for in vitro tuberization differ from 20 – 25 ^{o}C (Hussey & Stacey, 1981), 20 ^{o}C (Wang & Hu, 1982), 15 – 18 ^{o}C (Akita & Takayama, 1994), or even 15 ^{o}C (Wattimena, 1984; cited by Wang & Hu, 1985). Wattimena (1984) even found that there was only a slight reduction in tuberization at 10 ^{o}C compared to the tuberization at 15 ^{o}C. In contrast, Okazawa (1967) observed that temperatures below 12 ^{o}C strongly inhibited tuber formation. The optimal values and ranges indicated vary very much. Wang & Hu (1985) indicated that this variation could be associated with differences in light intensity (high light intensity increasing the temperature inside the vessels compared to the temperature environment) and to the different use of growth regulators. In our experiments no light

was supplied, which means that the light could not have contributed to the temperature in the Petri dishes.

In the experiments described in the Chapter 2 the effect of diurnal temperature fluctuations on the number, size and weight of microtubers was tested. As the experiments were necessarily carried out in full darkness (given the facility used) there were not really a day and night period in the experiment. In Chapter 2 therefore the terms Phase 1 and Phase 2 were introduced. Phase 1 was the period from 00:00 h (midnight) till 12:00 h (midday) and Phase 2 was the period from 12:00 h (midday) till 24:00 h (midnight). Different temperatures were set for Phases 1 and 2. Temperatures during Phase 1 included 12, 14, 16, 18 and 20 ^{o}C (the lower temperature range) and temperatures during Phase 2 were 17, 19, 21, 23, and 25 ^{o}C (the higher temperature range).

Many microtubers formed in the experiments of Chapter produced structures that indicated that the induction to tuberize was not very strong. The Petri dishes used in the experiments of Chapter 2 were very tightly sealed, and the lack of gas exchange could have contributed to this behaviour of the explants.

In Experiment 1 of Chapter 2, number and size of microtubers were significantly affected by Phase 2 temperature but no effect on the weight of microtubers was observed (Tables 1 and 2, Chapter 2). Lower Phase 2 temperatures resulted in more microtubers while a higher Phase 2 temperature resulted in fewer microtubers. For normal potato plants Marinus & Bodlaender (1975) obtained more tubers at a temperature of 16 ^{o}C than at 22 or 28 ^{o}C. According to Bushnell (1925) tuber formation fails at temperature of 26 – 29 ^{o}C because increasing temperature would increase the rate of respiration much more than the rate of photosynthesis and therefore there would not be enough carbohydrate available for tuber formation. At lower temperatures stolonization is more intense (Borah & Milthorpe, 1962), whereas tuberization is earlier (Borah & Milthorpe, 1962; Scaramella-Petri, 1968) and tuber bulking more rapid, especially at higher net assimilation (Borah & Milthorpe, 1962). In the present study lower temperature also resulted in larger microtubers than higher temperatures (Table 1, Chapter 2). A Phase 2 temperature of 17 ^{o}C was found to be optimal for number, size and weight of microtubers. Bushnell (1925) reported that 17 ^{o}C is an optimal temperature for potato yield in the

field. A negative effect of high temperature on the potato yield was reported by Ewing (1981), he indicated that high temperatures increase moisture stress, decrease plant growth and probably affect interrelationships among hormones and enzymes; maybe also membranes can be affected by high temperatures. Moreover, less photosynthate is provided for growth. The proportion of dry matter partitioned to tubers is also higher at lower temperature than at higher temperature (Tadesse, 2000).

Effects of temperature during Phase 1 (phase with lower temperature range) on the microtuber production

The effects of Phase 1 temperature were similar to the ones of the Phase 2 temperature (Experiment 1, Chapter 2). This was significant for number and size of microtubers. However, no effect of Phase 1 temperature on the weight of microtuber was observed (Chapter 2). As there was no light phase the similarity of the two types of effects is logical. However, the ranges of temperatures were different for the two phases. Cuttings cultured at highest Phase 1 temperature produced lowest number of microtubers whereas cuttings cultured at other Phase 1 temperatures showed highest number of microtubers. Lower Phase 1 temperatures resulted in larger microtubers and lowest induction was observed at highest Phase 1 temperature. Phase 2 temperatures 17 and 19 ^{o}C were similar and resulted in the highest value for percentage of explants that produced microtubers and also for number of microtubers while Phase 1 temperatures of 12, 14, 16, 18 ^{o}C affected percentage of explants that produced microtubers and also number of microtubers per explant similarly and gave higher values than 20 ^{o}C. Already in 1957 Went described that especially night temperature strongly affects potato production in the field; at night temperature higher than 20 ^{o}C top development is good, but no tubers are formed, while at night temperature between 10 and 14 ^{o}C the highest total production (top and tubers) will be obtained. In vitro these effects are different because of the overriding effect of the culture medium. Moreover, as explained earlier in this general discussion, the lack of light during microtuber production in our experiments might have played a significant role.

Interaction between Phase 1 and Phase 2 temperatures during the in vitro phase for microtuber production

High temperatures throughout the 24 h period were not suitable for microtuber formation (Chapter 2). Microtuber production was most successful when low Phase 1 temperatures were combined with low Phase 2 temperatures (Experiment 1, Chapter 2). There was also a significant Phase 1 temperature × Phase 2 temperature × cultivar interaction for percentage of explants that produced a microtuber, number, size and status of microtubers (Table 1, Chapter 2). This significant three-way interaction suggests that optimal combinations of temperatures during the different phases differ for each cultivar or that the effects of different temperature combinations depend on the cultivar. The very early cultivar Gloria proved more sensitive to high temperatures during one of the two phases than the other two cultivars.

In Experiment 1, where the average temperatures differed for the different combinations of Phase 1 and Phase 2 temperatures, the microtuber formation was less successful when the diurnal fluctuations were 9 °C or more. Only treatment combinations with Phase 2 temperatures of 21 °C and above could reach such large fluctuations. In Experiment 2, the average temperatures were kept constant over all treatments but the diurnal fluctuations differed very much. In that experiment the temperature amplitude had surprisingly little effect on microtuber formation. Only the treatment combination with the highest Phase 2 temperature (and thus the lowest Phase 1 temperature) seemed to have a slightly poorer performance, indicating that a strong fluctuation had a negative effect, probably because it involved heat stress during Phase 2. Similar results for normal plants have been reported (Bushnell, 1925; Borah & Milthorpe, 1962; Scaramella-Petri, 1968; Marinus & Bodlaender, 1975; Ewing, 1981). The conclusion from our work that there is little effect of diurnal temperature fluctuations and that if there is any effect to be found, large fluctuations will have a negative effect is in agreement with literature: Wang & Hu (1982) also suggested that with equal average temperatures, temperatures combinations with little fluctuation gave better microtuber formation than temperature combinations with large fluctuations. A negative effect of fluctuations in temperature is, however, in strong contrast with findings in whole plants: Steward et al. (1981) and Bennett et al. (1991) found

that diurnal temperature fluctuations were generally beneficial in whole plants (Ewing & Struik, 1992).

Temperature effects during the in vitro phase on the minituber production in the greenhouse

In this section the effect of temperature during the in vitro phase on the minituber production in the greenhouse after transplanting in vitro plantlets into the greenhouse will be discussed.

Temperature during the phase of in vitro plantlet production did not significantly affect the number of minitubers produced in the greenhouse (Table 1, Chapter 4). A similar result, but over a different range of temperatures, was reported by other studies on temperature during the in vitro phase (Tadesse, 2000). Effects of temperature on normal plants, however, are often different from those on in vitro plantlets. Increasing temperature decreases tuber yield and total dry weight for normal plants (Prange et al., 1990). Marinus & Bodlaender (1975) observed in a greenhouse study that for normal potato plants mostly the highest number of tubers was obtained at the lowest temperature (16 ^{o}C in their case); such effects were clearly observed for the early cultivar (Eersteling) and a late cultivar (Eba), while another late cultivar (Alpha) produced most tubers at a higher temperature. An adverse effect of temperature on the number of tubers was also observed by Nagarajan & Bansal (1990); they found that at a very high temperature of 40/22 ^{o}C the number of tubers was reduced.

In the research reported in this thesis, a negative effect of increasing temperature in vitro on the size and weight of minitubers produced in the greenhouse was found (Table 1, Chapter 4). This finding supports the results of other studies on in vitro temperature (Tadesse, 2000) and is most likely associated with the temperature effects on the vigour of the transplant. A more vigorous transplant can be produced by relatively lower temperatures during in vitro production of microplantlets, especially when the light conditions are limiting growth. More vigour results in larger progeny tubers. In this case the results are similar as in normal plants. Marinus & Bodlaender (1975) and Tadesse (2000) also found that increasing temperatures reduced tuber yield; they also observed a reduction in the yield of tubers when temperature increased above 16 ^{o}C and especially above 22 ^{o}C. High temperatures also reduce tuber growth rate (Van Dam et al., 1996; Burton, 1972; Midmore, 1984). Optimum tuber

production occurs with day temperatures up to 23 ^{o}C and night temperatures of 10 – 14 ^{o}C (Went, 1959). According to Struik (1987) the after-effect of a short period of high temperatures on the development, yield and tuber-size distribution of potato depends on the treatment initiation time: short periods of high temperatures before tuberization increased elongation and branching of stolons and also could reduce tuber number in some cases whereas treatments after tuberization only delay tuber set and growth of tuber. High temperatures (30 – 35 ^{o}C) completely inhibit tuber production (Menzel, 1983). Higher temperature affects total dry weights of plants adversely and also decreases dry matter contents of tubers (Marinus & Bodlaender, 1975). Tuber dry matter content is also lower at high temperature (Ben Khedher & Ewing, 1985).

Temperature effects during the storage on weight loss, sprout behaviour and vigour of minitubers

The effects of storage temperature on sprout behaviour were different during early storage compared to late storage stage (Tables 1 and 2, Chapter 5), associated with the de-sprouting and the cutting (see elsewhere in this General discussion). Increasing temperature caused an increase in weight losses (Table 1, Chapter 5). This finding is in agreement with the results of other studies (Hartmans & Van Loon, 1987; Harris, 1992). Increasing respiration and evaporation with higher storage temperature are known as the main reasons for higher weight losses at higher temperature. According to Harris (1992) storage temperature affects water vapour deficit of the storage air and finally affects water loss. Hartmans & Van Loon (1987) reported a higher rate of respiration, evaporation, and sprouting and finally higher weight losses at a storage temperature of 12 ^{o}C than at 4 ^{o}C. Lower evaporation was observed with tubers with more strongly suberized periderm, because higher periderm resistance is related to higher suberized tubers (Lommen, 1995). It is likely that storage temperature effects on weight losses are larger in minitubers than in normal seed tubers, as minitubers are younger (less mature) and have less advanced periderm. Evaporation rate and subsequently weight losses during the storage period can also be affected by some other parameters such as degree of sprouting (with minitubers being dormant for a longer time than normal seed tubers); damaging and bruising of tubers (not relevant in our experiments as all tubers were hand-harvested); permeability of water through the

periderm (probably higher through the thin skins of the minitubers); and vapour pressure deficit in storage (which was well controlled in our case) (Rastovski, Van Es et al., 1987). Moreover, the minitubers were placed in trays to protect them from any pressure by other tubers and covered with wet paper tissue when needed. Respiration and evaporation weight losses can also be affected by sprouting capacity, number, size and shape of sprouts, and extent of sprout branching (Hartmans & Van Loon, 1987). As sprouting was affected by the storage temperature treatment, this could also have played a significant role in our research. Within a cultivar, the weight losses correlated well with the number of sprouts.

In the study described in this thesis also a positive effect of storage temperature on sprout behaviour at the early stage of storage was found; tubers stored at higher storage temperature showed more sprouts with longer lengths and higher weight (Table 1, Chapter 5). After de-sprouting, cutting and prolonged storage, a storage temperature of 8 ^{o}C resulted in the highest number of sprouts with longest length and highest weight (Table 2, Chapter 5); storage temperatures 4 and 8 ^{o}C affected sprouts number similarly, 4 and 12 ^{o}C caused similar length of sprout. According to Davidson (1958) sprouts had minimum growth at a cool temperature of 2 – 4 ^{o}C. Schippers (1977) reported that increasing storage temperature increases length of sprout. There are also effects of temperature before storage: more sprouts per tuber were observed when seed potato production took place at a higher day and night temperature than at lower temperatures (Van Ittersum, 1992).

Application of growth regulators changed the response to storage temperature in relation to number, length and weight of sprouts (see below).

Storage temperature strongly affected both dry matter of aerial parts of the plant and dry matter of progeny tubers measured in a vigour test, but had no significant effect on the date of emergence, or number and length of stems (Table 3, Chapter 5). This suggests that storage temperature did have an effect on the vigour of the minitubers planted, but that this effect was only expressed by altering the performance of individual stems in terms of their dry matter growth above ground (e.g. by changing the leaf area of the individual stem) and below ground (by affecting the time of tuber

formation and / or influencing the proportion of dry matter partitioned to the tubers during the short period of growth during the vigour test.

Photoperiod effects during the in vitro phase on the minituber production in the greenhouse

Photoperiod during the in vitro phase significantly affected the above ground dry matter, plant height, and number, size and weight of minitubers produced in the greenhouse when in vitro plantlets were planted in the greenhouse (Table 1, Chapter 4). This effect is partly caused by the direct and indirect effects of duration of the photophase on the development of the in vitro plantlet which is used as a transplant, although this effect is strongly modified by the typical in vitro conditions. There can also be after-effects associated with the effect of photophase on the readiness of the transplant to tuberize. The effects of photoperiod during in vitro production of microplantlets are also brought about by the effect of photoperiod on the total amount of light received by the plant (Seabrook, 2005). Also these effects can be direct or indirect effects or even after-effects.

Positive effects of in vitro photoperiod on greenhouse plant height, above ground dry matter number, size and weight of minitubers produced in the greenhouse were observed (Table 1, Chapter 4). Potato plants derived from plantlets grown under longer in vitro photoperiod had more minitubers with larger size and higher weight than potato plants derived from plantlets developed under shorter photoperiod. Similar results for fresh weight of minitubers were also observed by Seabrook et al. (1995). Seabrook et al. (1995) also reported that potato plants exposed to 16 hours in vitro photoperiod were taller and also had more nodes than plants treated with 12 hours in vitro photoperiods. Under longer photoperiod in vitro plantlets receive more light per day, total light intercepted by in vitro plantlets increases under longer photoperiod and subsequently the height of greenhouse plants can be increased (Seabrook et al. (1995). Longer day (16 hours) during the in vitro period resulted in greenhouse minitubers with higher weight than obtained from in vitro plantlets exposed to 12 hours photoperiod (Seabrook et al., 1995).

For normal plants similar results were reported by others (Edmundson, 1941; Demagante & Vander Zaag, 1988). Plants are taller under 16 h than under shorter photoperiod (Demagante & Vander Zaag, 1988) and under shorter photoperiod stems are shorter (Edmundson, 1941).

In our study no effects of photoperiod during the in vitro phase on the sprout number, size and weight of sprouts of greenhouse minitubers after minitubers were stored at low temperatures for 195 days. There was also no effect on the vigour of minitubers (Chapter 4). Apparently, the effects of the photoperiod during the in vitro phase did not affect the timing of tuber formation, the subsequent tuber growth and development, the senescence of the plants and the relation between above ground and below ground growth and development to such an extent that the physiology of the tubers after harvesting and storage were still affected. It is a common phenomenon that the small differences that are induced in the life history of tubers do not persist after long storage.

Interactions between temperature and photoperiod in vitro

In the present study there were significant interactions between temperature and photoperiod for above ground dry matter, plant height, size and weight of minitubers (Chapter 4). Taller potato plants with higher above ground dry weight, larger minitubers and also higher weight of minitubers were from plantlets grown under a combination of 16 ^{o}C and 16 h photoperiod, i.e. plantlets with a strong vigour because they could profit form the positive effects of the low temperature on their development by intercepting the additional light provided by the long photoperiod. Plants from plantlets grown under conditions of 20 ^{o}C and 12 h were shortest and produced smallest minitubers with lowest weight and also showed lowest values for above ground dry matter. Also in normal plants an interaction between temperature and photoperiod is common. Usually the delaying effects of long days on tuberization are stronger at high temperatures than at moderate temperatures (Ewing & Struik, 1992; Almekinders & Struik, 1994).

Effects of growth regulators

In this part of the General discussion, first the effect of growth regulators during the in vitro phase on the in vitro plantlets and microtuber production will be discussed. Then we will focus on how growth regulators during the storage phase can affect dormancy, sprout behaviour and quality of tubers.

Effects of growth regulators during the in vitro phase on the in vitro plantlet

Generally it is accepted that presence of CCC (Chloromequate chloride) in the medium decreases plantlet height; this was also observed in the present study: in our study also a higher dose of CCC in the in vitro medium resulted in the shortest length for in vitro plantlets. Gibberellic acid also reduced plant height compared to the control. Presence of CCC in the in vitro medium (with higher doses; 500 and 1000 mg/l) also resulted in lowest numbers of leaves for in vitro plantlets. However, the highest concentration of gibberellic acid also reduced leaf number and leaf area compared to the control. Gibberellic acid in the medium increased the number of roots, but CCC did not affect this parameter. The highest concentration of CCC (1000 mg/l) resulted in the shortest length of plantlet roots and no growth regulator showed a positive effect on longest length of roots. Lower concentrations of CCC (100 and 500 mg/l) and the three concentrations of gibberellic acid affected length of roots similarly. The positive effect of GA on root number may have been responsible for its negative effect on root length. Treatments with CCC and also the highest concentration of gibberellic acid decreased leaf area (Table 1, Chapter 3).

One of the striking observations in this experiment is that, although selected for their contrasting effects, higher concentrations of CCC often had similar effects compared to the higher concentrations of gibberellic acid. This was for example true for plant height, number of leaves and leaf area.

The studies of Vecchio et al. (2000) and Hussey & Stacey (1984) support most of the results observed. Harvey et al. (1991) indicated that chlormequat causes only a transient inhibition of in vitro elongation. In vitro results are consistent with results in the field. The effect of CCC on cereal growth

is well known and widely used. A treatment with CCC also decreased shoot size in cultivars of carrot (Thomas et al., 1973) and in linseed (*Linum usitatissimum*) it shortened the length of main stem. Reduction in the potato stem length by CCC application was found by Dyson (1965).

Effects of growth regulators during the in vitro phase on microtuber production

A treatment with CCC or no growth regulator caused more microtubers with larger size than a treatment with gibberellic acid. However, the effect on the microtuber weight was not significant due to the large variation and the relatively large error in assessing the very small weights. Also for size of microtubers the *lowest* concentration of gibberellic acid in the in vitro media showed the same result as CCC and no growth regulator (Table 2, Chapter 3). Treatment of gibberellic acid with a concentration of 0.5 mg/l resulted in the highest number of microtubers with larger size and highest weight, although the control treatment (no growth regulator) showed similar results (Table 4 and Fig. 3, Chapter 3). The other treatments, i.e. the three doses of CCC and the other two doses of GA resulted in the lowest values for number, size and weight of microtubers. Harvey et al. (1991) stated that microtuber formation was stimulated by chlormequat with a recalcitrant cultivar of potato but in a cultivar that tuberized readily in its absence fresh weight of microtubers was reduced. They also showed that concentrations of chlormequat in the media to stimulate tuber initiation by recalcitrant cultivars may have deleterious effects on the growth of microtubers. CCC may also be persistent in tubers (K.B.A. Bodlaender, personal communication), deepening the dormancy and therefore may impede their timely use as propagules. This is especially relevant for microtubers, which have a long dormancy anyway. The use of CCC in microtuber production should therefore be considered with care. However, after-effects of gibberellic acid may be useful: it may reduce the depth of the dormancy or shorten the dormancy period. This effect can only be used if the gibberellic acid can be applied late, i.e. shortly before harvesting the microtuber batch, to avoid the negative effect of gibberellic acid on microtuber growth.

Effect of growth regulators during storage on weight loss, sprout behaviour and vigour of minitubers

The storage phase is a rest phase between harvesting tubers and planting them. This last phase before planting of tubers in a seed tuber production scheme has an important role in the dormancy, sprouting behaviour and quality of tubers. In this part of the thesis, the effect of growth regulator application and its interaction with storage temperature and size of minitubers on the storage losses, sprout behaviour and vigour of minitubers before their use as seed tubers to produce ordinary seed tubers will be discussed.

Gibberellic acid proved to be an effective growth regulator to enhance sprouting, and to increase number, length and weight of sprouts of minitubers (Table 2, Chapter 5). To achieve this effect, the minitubers were cut to improve the uptake of the growth regulator applied. Moreover, they had been de-sprouted before growth regulator application (see before in this General discussion). These pre-treatments may have affected the magnitude of the effects obtained. Nevertheless, the findings in Chapter 5 are in agreement with the results of Van Hiele (1961) for normal seed tubers; according to him number and length of sprouts are increased by gibberellic acid (GA3).

In the present study it was obvious that minitubers treated with gibberellic acid (especially with higher doses) gave an earlier emergence and caused an increase in the length of the stem whereas a higher dose of CCC caused very late emergence (Table 3, Chapter 5). These results are in agreement with other studies using normal tubers (Rappaport et al., 1957; Van Hiele, 1961; Timm et al., 1962; Holmes et al., 1970).

Gibberellic acid affects apical dominance and increases number of sprouts and also results in earlier emergence for normal plants (Rappaport et al., 1957; Timm et al., 1962; Holmes et al., 1970). Many of these results were obtained without cutting the seed tubers before applying the growth regulators. According to Rappaport et al. (1957) gibberellic acid stimulates sprouting and resulted in longer stems; they also confirmed that in tubers dipped in water only the apical eye produced a single sprout, whereas in tubers treated with gibberellic acid almost all eyes produced sprouts and these tubers also produced branched sprouts and emerged earlier; they also indicated that tubers treated with gibberellic acid produced longer stems than those tubers dipped in water.

The increase in the number of sprouts by a treatment with gibberellic acid is apparently caused by overruling apical dominance. Timm et al. (1962) suggested that a treatment with gibberellic acid caused earlier and more rapid emergence in treated tubers. Van Hiele (1961) showed that tubers treated with a higher concentration of GA3 showed earlier emergence. Holmes et al. (1970) confirmed that earlier emergence was observed with tubers treated with gibberellic acid; according to their study longer sprouts and faster elongation of sprouts on tubers treated with gibberellic acid could result in the earlier emergence. The rate of stem emergence depends on the concentration of gibberellic acid (Timm et al., 1962).

In our study on minitubers, lowest number of stems was associated with the treatment control no-cut but other treatments (especially the higher dose of gibberellic acid) resulted in more stems (Table 3, Chapter 5). Increases in number of stems by gibberellic acid are confirmed by other studies using normal tubers (Van Hiele, 1961; Holmes et al., 1970; Rastovski, Van Es et al., 1987). Increasing the concentration of gibberellic acid affects stem number and increases number of stems per hill (Timm et al., 1962).

A treatment with gibberellic acid also resulted in a lower dry matter content of progeny tubers (Table 3, Chapter 5). Van Hiele (1961) observed this effect also in normal tubers.

Effect of growth regulators during storage on sprout behaviour and vigour of normal tubers

To compare the effects of growth regulator applications on relatively young minitubers (Chapter 5) with the effects of normal seed tubers of more advanced age, a similar experiment as described in Chapter 5 was carried out with the cultivars Frieslander, Marfona and Santé (Chapter 6). A major difference, however, was that there were no different storage regimes before applying the growth regulators. Three sizes (35-45 mm, 45-55 mm and 55-65 mm) of normal tubers of three potato cultivars (Frieslander, Marfona and Santé) were treated with GA (2.5 mg/l and 5 mg/l) and CCC (10 mg/l and 100 mg/l). There were also two controls included: tubers cut and tubers uncut.

There were more interactions between cultivar and growth regulator for the growth vigour assessments in the experiment with the normal seed tubers than in the experiment with the minitubers.

GA had different effects on sprouting behaviour with different cultivars. Frieslander produced more sprouts, but not longer sprouts, while Marfona produced longer sprouts, but not more sprouts. The growth vigour was also affected by the growth regulators, but also in a different way for the different cultivars. GA increased the days of growth but the effects were different for the different cultivars. The same what was true for the sprouts, was true for the stems: GA let Frieslander produce more stems, but not longer stems, while GA let Marfona produce longer stems, but not more stems. Only Marfona produced more foliage dry matter with GA. Tuber yield also showed an interaction between GA treatment and cultivar. Frieslander produced more tubers and a higher tuber yield, while Marfona and Santé produced fewer tubers and lower tuber yield. CCC did not have a big influence on the growth vigour. Only Frieslander got fewer days of growth, Marfona got more tuber yield and Santé got a better emergence percentage.

The interpretation of the results of the experiment with the normal seed tubers was very much complicated by the fact that not all seed tubers produced an emerging plant. This problem was solved by analyzing relevant quantitative data both with non-emerged plants set at a relevant value and by analyzing the data set considering non-emerged plants as missing plots.

Effect of minituber size on weight loss, sprout behaviour and vigour

In the study carried out at the storage phase, the rest phase between harvesting of tubers and planting them, the effect of minituber size on weight loss, dormancy, sprouting behaviour and vigour was examined and the result of this experiment will be explained in this part.

Higher absolute weight losses were found for larger tubers; however, smaller tubers showed higher relative weight losses. This is caused by the fact that in smaller tubers, the ratio between surface area and volume is higher than in larger tubers (Lommen, 1995). Moreover, smaller tubers are less mature than larger tubers; therefore the resistance of the periderm in smaller tubers is lower than in larger tubers (because of an incomplete or less suberized periderm). Finally, the density of lenticels may be higher in small tubers compared to large tubers. So in smaller tubers, the evaporation is higher

than in larger tubers and consequently relative weight loss through evaporation is higher in smaller tubers than in larger tubers.

Such effects were also observed for normal tubers. Degree of suberization, resistance of tuber periderm, damaging and bruising of tuber, surface area of tubers, and maturity of tubers can all affect tuber evaporation, and a negative relationship between periderm resistance and rate of evaporation and a positive relationship between surface area of tuber and evaporation were reported in some studies (Rastovski, Van Es et al. 1987). That is why the weight loss in percent is higher in smaller tubers than in larger tubers. Higher degree of suberization causes higher periderm resistance and results in lower evaporation and lower water weight loss (Lommen, 1995). But not only for water loss, also for respiration losses there are tuber size effects. An inverse relationship between rate of respiration per kg and tuber size was found by Burton (1964). Sprouting, respiration and evaporation were all reported as factors influencing weight losses during storage (Rastovski, Van Es et al., 1987).

In the present study a positive relationship between minituber size and sprouting was observed: larger tubers showed higher numbers of sprouts with larger length and higher weight (Tables 1 and 2, Chapter 5). Some studies using normal tubers confirmed this result and reported that larger tubers had more eyes (Harris, 1992; Struik & Wiersema, 1999). Tuber size can also affect the number of sprouts per eye (Struik & Wiersema, 1999).

As expected, larger minitubers resulted in higher number of stems, increased length of stem and produced higher tuber and haulm dry matter (Table 3, Chapter 5). In this study a positive relationship was found between size of minitubers and some parameters reflecting growth vigour such as number of stems, length of stem and above ground dry matter and dry matter yield of tubers produced within a short period of time. However, there was no effect of tuber size on the date of emergence. Other studies using normal seed tubers support this result (Werner, 1954; Toosey, 1962; Struik & Wiersema, 1999). Size of tubers strongly influences vigour (Struik & Wiersema, 1999). Toosey (1962) also reported that larger tubers produced more plants and also more tubers than smaller tubers. Struik & Wiersema (1999) also reported a linear relationship between number of stems and size of tuber; according to them larger tubers produce more stems. In our study, smaller tubers

produced lower dry matter for aerial part of plants and also dry matter of progeny tubers. According to Struik & Wiersema (1999) smaller seed tubers produce fewer stems (and consequently small tubers produce lower above ground dry matter). These can be the main reasons to produce lower above ground dry matter and lower dry matter of tubers by smaller tubers than larger tubers.

Effects of tuber size of normal tubers

When normal tubers were used, larger tuber sizes resulted in more sprouts, longer sprouts, higher sprout weights and more stems. This is consistent with what was found in the literature (see previous section). However, mainly the differences between the largest seed tuber size and the two smaller sizes

were significant. There were many interactions between cultivar and seed tuber size, probably caused by differences in seed tuber shape.

Effects of cultivar

Our study confirmed that large differences exist between potato cultivars: different cultivars showed different responses to various environmental conditions during the in vitro phase and during storage, chemical treatments, either during the in vitro phase or during storage and tuber size. This was obvious during the different stages of the seed tuber production scheme.

Concluding remarks

On the basis of the research described in this thesis, the following observations can be made:

1. It is possible to produce large numbers of in vitro plantlets, microtubers and minitubers with high quality throughout the year by utilization of rapid multiplication in a seed tuber production scheme.
2. In some countries such as Iran there are suitable areas with skillful farmers to produce ware potatoes but lack of high quality seed potatoes is a major limiting factor for potato production.
3. Under such conditions rapid multiplication techniques and some of the aspects of seed potato production described in this thesis can become important.
4. The storage phase is one of the most important phases in a seed tuber production scheme as it can affect dormancy, sprouting behaviour and quality of seed tubers. Under suitable storage conditions proper seed potatoes with high quality can become available at the time they are needed.
5. In countries like Iran the potato crop can be grown in different areas and in different seasons. This will help to make the produce available throughout the year without costly, long-term storage, provided suitable seed potatoes are available whole year round.
6. Having available non-dormant tubers at planting of all the agro-ecological zones during the entire year is very difficult. Deficiency of suitable seed potato tubers at planting time will remain one of the main problems for potato production in Iran, unless with the proper combination of storage conditions and perhaps chemical treatments sprouting tubers become available at the appropriate time.
7. In vitro plantlet, microtuber and minituber production can be manipulated by in vitro conditions.
8. In vitro conditions can influence the quality of plantlets and affect the establishment of the in vitro plantlets in the soil after transplanting. The performance of plantlets in the greenhouse can be affected by the status of the plantlet during the in vitro phase.
9. Sprouting behaviour, storage losses, vigour of tubers and quality of tubers can be affected by storage conditions.
10. Larger tubers have more vigour and can produce more sprouts with longer length and higher weight, resulting in a more vigorous plant.

Finally it is concluded that with the utilization of the proper rapid multiplication techniques, manipulating in vitro and storage conditions, the availability of high quality seed tubers can be enhanced. Even in countries like Iran, with its complex agro-ecology, it is possible to have high quality seed tubers available when needed. If this is realized, Iran can become an important exporter for ware and seed potato tubers.

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Summary

Potato can be multiplied sexually or asexually (vegetatively) but generally it is propagated vegetatively: farmers use seed tubers for multiplication and propagation. The main disadvantages of the traditional potato seed tuber production system are: high cost, labour and time consuming, low multiplication rate, high risk of spreading pathogens and high number of field multiplications. Since recent years micropropagation is widely used in seed tuber production systems to produce large amounts of disease-free potato seed tubers with high quality in a short period of time and to solve the problems associated with traditional system.

The micropropagation system often consists of a combination of the following phases: 1) the in vitro multiplication phase in which in vitro plantlets are produced, 2) in vitro microtuber production, 3) minituber production phase in the greenhouse or in the field condition, using in vitro plantlets or microtubers as starting material.

In vitro plantlets are small plants produced by using single-node cuttings under in vitro conditions (fully aseptic and artificial). These plantlets are used to produce transplants by culturing them under semi in vitro conditions or in vivo (ex vitro) conditions, such as a greenhouse.

Microtubers are very small in vitro potato tubers (3 – 10 mm or larger in diameter and 0.2 – 0.7 g or heavier). They are produced on in vitro plantlets or on cuttings under light or dark conditions. Usually only one microtuber is produced per plantlet or explant. Generally microtubers are very dormant. Microtubers are not large enough to directly plant them in the field. They are usually planted in the greenhouse to produce progeny tubers of larger size.

Minitubers are small tubers produced under semi in vitro or in vivo conditions. The size of minitubers is 5 – 25 mm or larger and their weight ranges from 0.1 to 10 g. The number of minitubers produced per in vitro plantlet ranges from 2 – 10 to over 40. Minitubers can be planted in the greenhouse and also are suitable for direct planting in the field.

This research project aimed to investigate the following research questions:

- How can in vitro conditions affect the production and quality of the different types of propagules?

- How can the production and quality of the different types of propagules be influenced by growth regulators either applied in vitro or during storage?
- How can the sprout behaviour and vigour of tubers be affected by storage conditions?
- Is there a relationship between size of tubers and their sprouting capacity and vigour?

Microtuber and in vitro plantlet production

Microtuber production can be affected by day and night temperatures and by their differential. Experiments were carried out with three different potato cultivars: Gloria (very early), Marfona (mid-early) and Agria (late). Experiments were carried out in the dark and different temperatures were set during two thermal phases per period of 24 hours: Phase 1 from 0:00 h till 12:00 h and Phase 2 from 12:00 h till 24:00 h. In Experiment 1, explants were cultured at 5 different Phase 2 temperatures (17, 19, 21, 23 and 25 ^{o}C) and 5 different Phase 1 temperatures of 12, 14, 16, 18, or 20 ^{o}C (Chapter 2). Phase 2 temperature showed significant effects on the number, size, weight and status of the buds. More microtubers with larger size were produced at a Phase 2 temperature of 17 ^{o}C and 19 ^{o}C, whereas a Phase 2 temperature of 25 ^{o}C resulted in the lowest number of microtubers with smallest size. A highest Phase 2 temperature of 25 ^{o}C caused lowest level of induction as reflected by the status of the buds. Cuttings grown at the highest Phase 1 temperature (20 ^{o}C) showed the lowest number of microtubers with smallest size and lowest level of induction; other Phase 1 temperatures gave similar numbers, but there were differences in size and status among them. Increasing the temperature amplitude during the in vitro phase reduced the number, size and weight of microtubers in Experiment 1, but treatments with the same temperature amplitude did not always share the same average temperature. In Experiment 2 cuttings were grown with the same average temperature, but a wide range of temperature amplitudes. The effects of temperature amplitude on microtuber production in this second experiment were surprisingly small. However, very large differences between Phase 2 and Phase 1 temperatures tended to reduce the microtuber production.

In Chapter 3 the direct effects and after-effects of growth regulators during the in vitro phase were investigated. Three different potato cultivars (Gloria, Marfona and Agria) were exposed to three doses of gibberellic acid (GA; 0.1, 0.5 or 1 mg/l) and three doses of CCC (100, 500 or 1000 mg/l). Direct effects of growth regulator application during the in vitro phase on plantlet characteristics were observed: the number of branches, plantlet height, and number of leaves, leaf area, number and length of roots were significantly affected compared to the untreated control. The number of branches decreased with an increasing dose of CCC, whereas GA did not affect this characteristic. Increasing the dose of CCC also reduced plantlet height and number of leaves. GA enhanced the number of roots, but the highest dose of CCC reduced root length. Growth regulator application also had a direct effect on microtuber production. GA reduced the number and size of microtubers produced significantly.

Highly significant after-effects of growth regulator application on the number, size and weight of microtubers were observed: cuttings from plantlets treated with CCC gave lowest numbers of microtubers with smallest size and lowest weight. A high dose of GA also reduced the number of microtubers significantly.

Minituber production

The effects of temperature and photoperiod during in vitro plantlet production on the production and quality of minitubers produced from these in vitro plantlets in the greenhouse were studied. Three potato cultivars (Gloria, Marfona and Agria) were exposed to all combinations of three temperatures (16, 20 or 24 ^{o}C) and three photoperiods (12, 16 or 20 h) during the in vitro phase. In vitro potato plantlets produced were then planted in the greenhouse to produce minitubers; the quality of these minitubers was also assessed (Chapter 4).

Low temperature during the in vitro phase gave higher above ground dry matter in the greenhouse, but there was no effect of temperature on the plant height and number of minitubers produced. However, size of minitubers was larger when in vitro plantlets had been exposed to low temperatures in vitro. The same effect was observed for average minituber weight resulting in higher

minituber yields for plants exposed to low temperatures in vitro. The minitubers from in vitro plantlets produced under low temperature in vitro also showed the highest sprout weight after a long cold storage period. However, after planting the minitubers no effects on plant vigour were observed. Yet, larger minitubers produced more stems, more above ground dry matter and more tuber dry matter in a vigour test. Growth vigour of tubers of different potato cultivars was also completely different.

Long photoperiod during the in vitro phase resulted in greenhouse plants that produced more above ground dry matter, taller plants, and more, larger and heavier minitubers. However, the quality of the minitubers as assessed after long storage was not affected. The interactions between temperature and photoperiod during the in vitro phase were highly significant for above ground dry matter, plant height, size and weight of minitubers. The three cultivars also responded differently to the temperature and photoperiod treatments.

Effects of storage conditions and growth regulators applied to tubers

Minitubers of three potato cultivars (Gloria, Marfona and Agria) were exposed to three different storage temperatures: (4, 8 and 12 ^{o}C) for 3 months and then de-sprouted and cut to treat them with various doses of different growth regulators (2.5 and 5 mg/l GA, 10 and 100 mg/l CCC with two controls: minitubers cut and minitubers not cut). Three minituber sizes were used (13 – 25 mm, 25 – 35 mm and 35 – 50 mm). Tubers were then tested for weight loss, sprout behaviour and growth vigour (Chapter 5).

A low storage temperature of 4 ^{o}C caused the lowest weight losses whereas minitubers stored at a temperature of 8 ^{o}C lost most weight. A positive relationship between size of minituber and weight loss was found: larger minitubers lost more weight than smaller tuber. However, relative weight loss was smaller the larger the minitubers. Storage temperature affected sprout behaviour differently before and after growth regulator application. Before applying the regulators, the highest storage temperature (12 ^{o}C) resulted in the highest number of sprouts with longest length and highest weight, with the lowest temperature maintaining the dormancy much longer. After growth regulator

application a storage temperature of 8 ^{o}C caused highest numbers of sprouts with longest length and highest weight (Chapter 5). This different response to storage temperature was associated with the influence of cutting, de-sprouting and the chronological age of the minitubers.

Size of the minitubers significantly affected sprouting behaviour. A positive relationship between size of tubers and sprout number and also length and weight of sprouts was found, larger tuber produced more sprouts, longer sprouts and higher sprout weights.

Growth regulator application significantly affected sprouting behaviour; GA enhanced sprouting while number, length and weight of sprouts were lowest with the control.

Both before and after growth regulator application interactions between factors were sometimes highly significant and complex for weight loss and sprouting behaviour.

Storage temperature could not affect date of emergence, stem number and length of stem, but significantly affected above ground dry matter and dry matter of tubers in vigour tests. In the vigour tests, growth regulators applied on seed tubers after storage appeared to significantly affect date of emergence, number of stems, length of longest stem and dry matter of tubers; no significant effects were observed for above ground dry matter. Minitubers treated with the highest dose of GA emerged earlier and those treated with the highest dose of CCC emerged later. GA resulted in the longest stems. Growth regulators reduced the dry matter of tubers produced in the vigour test; lowest dry matter of tubers was produced by minitubers treated with a dose of gibberellic acid. Growth regulators did not significantly affect above ground dry matter in the vigour test. Larger minitubers did not result in earlier emergence, but produced more stems with longer length and more dry matter in the vigour test.

Storage and growth regulator treatments were also applied to normal tubers of relatively old (physiological) age (Chapter 6). Three tuber sizes (35 – 45 mm, 45 – 55 mm and 55 – 65 mm) of three potato cultivars (Frieslander, Marfona and Santé) were treated with GA (2.5 mg/l and 5 mg/l) and CCC (10 mg/l and 100 mg/l); also two controls, tubers cut and tubers uncut, were included. GA had different effects with different cultivars. Frieslander produced more sprouts, but not longer sprouts, while Marfona produced longer sprouts, but not more sprouts in response to GA. GA increased the days of growth with Marfona and Santé, but not with Frieslander. GA let Frieslander produce more

stems, but not longer stems, while Marfona produced longer stems, but not more stems in response to GA. Only Marfona produced more foliage with GA. Frieslander produced more tubers and a higher tuber yield, while Marfona and Santé produced fewer tubers and a lower tuber yield after GA application.

CCC did not have a big influence on the growth vigour. Only Frieslander got fewer days of growth, Marfona got more tuber yield and Santé got a better emergence percentage.

Effects observed were strongly affected by the treatment effects on the emergence of the cultivars Marfona and Santé.

Concluding remarks

This thesis has illustrated that it is important to test after-effects of treatments applied during in vitro production of plantlets on the performance of the propagules produced and their progenies. It is also important to realize that the response of in vitro propagules to environmental conditions is not always similar to what can be found under field conditions. Moreover, this thesis has shown that many of the differences induced by differences in in vitro conditions are lost during storage of the minitubers harvested from in vitro plantlets. To further optimize the quality of seed tubers, storage treatments and growth regulator applications can be useful.

With the utilization of the proper rapid multiplication techniques, manipulating in vitro and storage conditions, and growth regulator applications the availability of high quality seed tubers can be enhanced. Even in countries like Iran, with its complex agro-ecology, it is possible to have high quality seed tubers available when needed. If this is realized, Iran can become an important exporter for seed potato tubers.

Samenvatting

De aardappel kan zowel generatief als vegetatief worden vermeerderd. In de praktijk is vegetatieve vermeerdering gebruikelijk: boeren gebruiken pootgoed voor vermeerdering en als uitgangsmateriaal in de commerciële teelt. Er zijn echter nadelen aan de vegetatieve vermeerdering verbonden. Het brengt hoge kosten met zich mee, vergt veel arbeid en tijd, de vermenigvuldigingsfactor is laag, er zijn grote risico's voor de verspreiding van ziekten en plagen en het vergt een groot aantal vermeerderingen in het veld. Gedurende de laatste jaren wordt daarom in pootgoed-productieprogramma's veel gebruik gemaakt van snelle vermeerdering. Hiermee kunnen in korte tijd grote hoeveelheden ziektevrij pootgoed worden geproduceerd van hoge kwaliteit. Op deze wijze kunnen de problemen die kleven aan het traditionele systeem worden ondervangen.

Snelle vermeerdering van aardappel bestaat vaak uit de volgende stadia: 1. een vermeerderingsstap in vitro, waarin in vitro plantjes worden geproduceerd, 2. in vitro productie van microknollen, 3. productie van miniknollen in de kas of in het veld, waarbij de in vitro plantjes of de microknollen als uitgangsmateriaal worden gebruikt.

In vitro plantjes zijn kleine plantjes; ze worden geproduceerd onder in vitro omstandigheden (dat wil zeggen onder aseptische en kunstmatige condities) met behulp van stekken bestaande uit een enkele knoop. Met behulp van deze in vitro plantjes worden vervolgens transplants geproduceerd door ze op te kweken onder semi in vitro of in vivo (ex vitro) omstandigheden, bijvoorbeeld in een kas.

Microknollen zijn zeer kleine in vitro knolletjes van de aardappel. Ze hebben in het algemeen een diameter van 3 – 10 mm of groter en wegen 0.2 – 0.7 g of meer. Deze microknollen worden geproduceerd aan in vitro plantjes of aan stekken, hetzij in het licht hetzij in het donker. Meestal ontstaat er niet meer dan een microknol per in vitro plantje of stek. Deze microknollen hebben gewoonlijk een diepe kiemrust. Microknollen zijn te klein om direct uitgepoot te worden in het veld. Ze worden dan ook meestal uitgeplant in een kas om zo eerst dochterknollen te produceren die groter zijn.

Miniknollen zijn kleine knollen die worden geproduceerd in semi in vitro of in in vivo condities. De diameter van miniknollen is 5 – 25 mm of groter en hun gewicht varieert van 0.1 tot 10 g en meer. Per in vitro plantje kunnen 2 – 10 miniknollen worden geproduceerd. Er zijn tegenwoordig echter systemen waarbij de productie 40 miniknollen per in vitro plantje bedraagt. Miniknollen kunnen worden uitgeplant in een kas, maar ze zijn ook geschikt om rechtstreeks in het veld te worden gepoot.

Het onderzoeksproject beoogde de volgende onderzoeksvragen te bewerken:

- Hoe beïnvloeden in vitro condities de productie en kwaliteit van verschillende typen uitgangsmateriaal?
- Hoe kan de productie en kwaliteit van de verschillende typen uitgangsmateriaal worden beïnvloed met behulp van groeiregulatoren, hetzij toegediend tijdens de in vitro fase, hetzij tijdens de bewaring?
- Hoe kan het spruitgedrag en de groeikracht van knollen worden beïnvloed door de omstandigheden tijdens de bewaring?
- Is er een relatie tussen knolgrootte en het spruitgedrag of de groeikracht?

Productie van microknollen en in vitro plantjes

De productie van microknollen wordt beïnvloed door de dagtemperatuur, de nachttemperatuur en door het verschil tussen die twee. Twee proeven werden uitgevoerd met de rassen Gloria (zeer vroeg), Marfona (middelvroeg) en Agria (laat). Er werden twee proeven in het donker uitgevoerd waarin verschillende temperaturen werden ingesteld gedurende twee fasen per etmaal: Fase 1 van 0.00 uur tot 12.00 uur en Fase 2 van 12.00 uur tot 24.00 uur. In proef 1 werden stekken van deze rassen opgekweekt bij vijf verschillende temperaturen gedurende Fase 2 (17, 19, 21, 23 en 25 ºC) en bij vijf verschillende temperaturen gedurende Fase 1 (12, 14, 16, 187 en 20 ºC) (Hoofdstuk 2). De temperatuur gedurende Fase 2 had een significant effect op het aantal microknollen, hun grootte en gewicht, alsmede hun fysiologische toestand. Temperaturen gedurende Fase 2 van 17 en 19 ºC leidden tot meer en grotere microknollen, terwijl bij een Fase 2 temperatuur van 25 ºC het geringste aantal en

de kleinste microknollen werden geproduceerd. Bovendien waren de knollen bij een temperatuur gedurende Fase 2 van 25 ºC het minst geïnduceerd, zoals bleek uit de ontwikkeling van de knoppen. Bij een temperatuur gedurende Fase 1 van 20 ºC werden de minste en kleinste microknollen geproduceerd, die bovendien de minst diepe inductie tot knolaanleg vertoonden. De overige temperaturen gedurende Fase 1 gaven vergelijkbare aantallen microknollen, maar verschilden wel in grootte en fysiologische toestand van de microknollen. Een toename van het verschil tussen de beide temperaturen gedurende de in vitro fase leidde tot een afname van het aantal, de grootte en het gewicht van de microknollen. In proef 1 hadden de behandelingen met hetzelfde temperatuurverschil echter niet altijd dezelfde gemiddelde etmaaltemperatuur. In proef 2 werden de stekken opgekweekt bij een gelijke gemiddelde etmaaltemperatuur, maar met een grote variatie in temperatuurverschillen tussen Fase 1 en Fase 2. De effecten van het verschil tussen Fase 1 temperatuur en Fase 2 temperatuur op de productie van microknollen waren verrassend klein. Echter, grote temperatuurfluctuaties leken de productie van microknollen te hinderen.

In Hoofdstuk 3 werd onderzoek gedaan naar de directe effecten en de na-effecten van het toedienen van groeiregulatoren tijdens de in vitro fase. Drie verschillende rassen (Gloria, Marfona en Agria) werden blootgesteld aan drie verschillende concentraties gibberellinezuur (GA; 0,1, 0,5 of 1 mg/l) en drie concentraties CCC (100, 500 of 1000 mg/l). Er werden directe effecten van het toedienen van groeiregulatoren gedurende de in vitro fase op uiteenlopende eigenschappen van de plantjes waargenomen. Het aantal zijassen, de hoogte van het plantje, het aantal bladeren, de bladoppervlakte, het aantal wortels en de lengte van de wortels werden alle significant beïnvloed ten opzichte van de (onbehandelde) controle. Het aantal zijassen nam af met een toename van de concentratie CCC, maar GA beïnvloedde deze eigenschap niet. Een toename in de concentratie CCC leidde ook tot een afname van de planthoogte en het aantal bladeren. Toediening van GA leidde tot een toename van het aantal wortels, maar de hoogste dosis GA gaf wel kortere wortels. Het toedienen van groeiregulatoren had ook een rechtstreeks effect op de productie van microknollen. Toedienen van GA leidde tot minder en kleinere microknollen.

Er was ook sprake van zeer significante na-effecten van het toedienen van groeiregulatoren op het aantal, de grootte en het gewicht van microknollen. Als stekken werden gebruikt van met CCC behandelde planten dan werden er minder, kleinere en lichtere microknollen geproduceerd. Een hoge dosis GA leidde ook tot significant minder miniknollen.

Productie van miniknollen

In Hoofdstuk 4 werden de effecten onderzocht van temperatuur en daglengte tijdens de productie van in vitro plantjes op de productie en kwaliteit van miniknollen die met behulp van deze in vitro plantjes werden geproduceerd in de kas. Drie aardappelrassen (Gloria, Marfona en Agria) werden gedurende de in vitro fase blootgesteld aan alle combinaties van drie temperaturen (16, 20 of 24 ºC) en drie daglengtes (12, 16 of 20 uur). De op deze wijze geproduceerde in vitro plantjes werden vervolgens uitgeplant in een kas om miniknollen te produceren; met deze miniknollen werden kwaliteitstesten uitgevoerd.

Lage temperaturen tijdens de in vitro fase leidden in de kas tot een grotere bovengrondse droge massa, maar niet tot hogere planten of een verschil in het aantal miniknollen. Miniknollen waren echter groter als ze werden geproduceerd met behulp van planten die tijdens de in vitro fase waren blootgesteld aan lagere temperaturen. De miniknollen die met behulp van in vitro plantjes opgekweekt onder lage temperaturen werden geproduceerd, bleken na een lange periode bij koude bewaring ook de hoogste spruitgewichten op te leveren. Na het uitplanten van de miniknollen werd echter geen temperatuureffect op de groeikracht gevonden. Grotere miniknollen gaven evenwel wel meer stengels, een grotere bovengrondse droge massa en een groter drogestofopbrengst aan knollen in een groeikrachttest. De rassen verschilden sterk in de groeikracht van de knollen.

Een lange daglengte gedurende de in vitro fase resulteerde in planten die in de kas meer bovengrondse droge massa produceerden. Bovendien waren dergelijke planten langer en produceerden ze meer, grotere en zwaardere knollen. De kwaliteit van deze knollen na een lange bewaarperiode werd niet beïnvloed. De interacties tussen temperatuur en daglengte tijdens de in vitro fase bleken zeer

significant voor bovengrondse droge massa, planthoogte, grootte en individueel gewicht van de miniknollen. De drie rassen reageerden verschillend op de temperatuur- en daglengtebehandelingen.

Effecten van bewaarcondities en groeiregulatoren toegediend aan de knollen

Miniknollen van drie rassen (Gloria, Marfona en Agria) werden gedurende 90 dagen blootgesteld aan drie verschillende bewaartemperaturen en vervolgens afgesproten en gesneden teneinde ze te kunnen behandelen met verschillende concentraties van de groeiregulatoren GA en CCC. Voor GA werden de concentraties 2,5 en 5 mg/l gebruikt, voor CCC 10 en 100 mg/l. Er werden twee controles in de proef opgenomen: een controle zonder snijden en een controle met een snijdbehandeling. De proef werd uitgevoerd met drie verschillende miniknolgroottes, te weten 13 – 25 mm, 25 – 35 mm en 35 – 50 mm. De knollen werden vervolgens getest op gewichtsverlies, spruitgedrag en groeikracht (Hoofdstuk 5).

Een lage bewaartemperatuur van 4 ºC leidde tot de laagste bewaarverliezen, terwijl miniknollen bewaard bij 8 ºC de hoogste bewaarverliezen vertoonden. Er werd een positief verband gevonden tussen grootte van de miniknollen en hun gewichtsverlies: grotere knollen verloren meer gewicht dan kleinere knollen. Het relatieve gewichtsverlies was echter juist het kleinst bij de grotere knollen. Bewaartemperatuur had een effect op het spruitgedrag, maar dit effect was voor en na de behandeling met groeiregulatoren verschillend. Voordat de groeiregulatoren werden toegediend had de hoogste bewaartemperatuur (12 ºC) de hoogste waarden voor het aantal spruiten, de spruitlengte en het spruitgewicht. Bij de laagste temperaturen werd de kiemrust langer aangehouden. Na de toediening van groeiregulatoren bleek een bewaartemperatuur van 8 ºC te leiden tot de hoogste waarden voor het aantal spruiten, de spruitlengte en het spruitgewicht (Hoofdstuk 5). Het verschil in reactie op de bewaartemperatuur voor en na de behandeling met groeiregulatoren hing samen met de effecten van het afspruiten, het snijden en de chronologische leeftijd van de miniknollen.

De grootte van de miniknollen had ook een significant effect op het spruitgedrag. Er werd een positief verband gevonden tussen grootte van de miniknollen en het aantal spruiten, de lengte van de

spruiten en het spruitgewicht. Dat wil zeggen dat grotere miniknollen meer spuiten, langere spruiten en een hogere spruitopbrengst gaven.

De groeiregulatoren beïnvloedden het spruitgedrag ook sterk. GA bevorderde de spruitvorming, terwijl de controle de laagste waarden gaf voor aantal, lengte en gewicht van de spruiten.

Zowel voor als na de behandeling met groeiregulatoren bleken de interacties tussen de verschillende factoren soms significant. Dit leidde tot een complex beeld van de verschillende invloeden bij de parameters gewichtsverlies en spruitgedrag.

De bewaartemperatuur had geen effect op de opkomstdatum, het aantal stengels en de lengte van de stengels. Er was echter een significant effect op de bovengrondse droge massa en de drogestofopbrengst aan knollen in de groeikrachttest. In de groeikrachttest bleek het effect van de toediening van groeiregulatoren na bewaring aan de miniknollen significant. Er werden significante effecten waargenomen op datum van opkomst, aantal stengels, lengte van de langste stengel en droge stof van de knollen. Er werd echter geen effect gevonden voor de droge stof van de bovengrondse massa. De miniknollen die met de hoogste concentratie GA waren behandeld bleken eerder op te komen, terwijl degenen die met de hoogste dosis CCC waren behandeld het laatst opkwamen. Bij de GA behandeling waren de stengels het langst. Beide groeiregulatoren gaven een lagere opbrengst aan knollen in de groeikrachttest; de laagste knolopbrengsten werden verkregen na behandeling met GA. De groeiregulatoren gaven echter geen effect op de bovengrondse droge massa in de groeikrachttest. Grotere miniknollen gaven geen vervroeging van de opkomst, maar produceerden wel meer stengels, die ook langer waren en meer bovengrondse massa produceerden in de groeikrachttest.

De bewaar- en groeiregulatorbehandelingen werden ook toegepast op knollen van normale grootte, maar met een relatief hoge fysiologische leeftijd (Hoofdstuk 6). Het materiaal bestond uit drie knolgroottes (35 – 45 mm, 45 – 55 mm en 55 – 65 mm) van de rassen Frieslander, Marfona en Santé. Dit materiaal werd behandeld met 2,5 of 5 mg/l GA en 10 of 100 mg/l CCC. De proef bevatte twee controlebehandelingen: ongesneden en gesneden knollen. Het effect van GA bleek te verschillen bij de verschillende rassen. In reactie op GA bleek Frieslander meer, maar niet langere spruiten te

produceren, terwijl Marfona juist langere, maar niet meer spruiten aanmaakte in reactie op GA. GA verlengde de groeiduur van Marfona en Santé, maar niet van Frieslander. GA leidde bij Frieslander tot meer stengels, maar deze werden niet langer. Bij Marfona, daarentegen leidde GA juist tot langere stengels, maar niet meer. Slechts bij Marfona was er sprake van een effect van GA op de hoeveelheid bovengrondse massa. Bij Frieslander leidde een GA-behandeling tot meer knollen en een hogere knolopbrengst, terwijl bij Marfona en Santé juist minder knollen werden gevormd, wat ook resulteerde in een lagere knolopbrengst.

CCC had geen groot effect op de groeikracht. Bij Frieslander gaf CCC een kortere groeiperiode, bij Marfona leidde CCC tot een hogere knolopbrengst en bij Santé werd een hoger opkomstpercentage waargenomen als gevolg van een CCC behandeling.

De effecten die werden gevonden, bleken sterk samen te hangen met de behandelingseffecten op de opkomst van de rassen Marfona en Santé.

Slotopmerkingen

Dit proefschrift heeft aangetoond dat het belangrijk is na-effecten te onderzoeken van behandelingen die gedurende de in vitro productie van plantjes worden gegeven op de prestaties van dit uitgangsmateriaal en zijn nakomelingschap. Het is ook belangrijk te realiseren dat de reactie van in vitro uitgangsmateriaal op omgevingsfactoren niet altijd gelijk is aan de reactie die onder veldomstandigheden wordt waargenomen. Bovendien heeft dit proefschrift aangetoond dat veel van de verschillen die worden geïnduceerd door verschillen tijdens de in vitro fase verloren gaan tijdens de bewaring van de miniknollen die met dergelijk in vitro materiaal zijn geproduceerd. Om de kwaliteit van pootgoed verder te optimaliseren kan gebruik gemaakt worden van behandelingen tijdens de bewaring en van toediening van groeiregulatoren.

Met het gebruik van de juiste technieken van snelle vermeerdering, en door het manipuleren van in vitro omstandigheden en omstandigheden tijdens de bewaring en via toedienen van groeiregulatoren kan de beschikbaarheid van kwalitatief hoogstaand pootgoed worden bevorderd.

Zelfs in landen als Iran, met zijn complexe agro-ecologie, is het mogelijk om kwalitatief hoogstaand pootgoed beschikbaar te hebben op elk moment waarop het nodig is. Indien dit wordt gerealiseerd, kan Iran een belangrijke exporteur van aardappelpootgoed worden.

Publications of the author

Otroshy, M., 1997. Report on potato in Iran. International Potato Course, International Agricultural Centre, (IAC), Wageningen, The Netherlands: 64-72.

Otroshy, M. & P. C. Struik, 2002. The effect of photoperiod and temperature during the in vitro phase on the quality of minitubers in the greenhouse in different cultivars of potato in a seed tuber production scheme. Proceeding of 15th triennial conference of the European Association for Potato Research, Hamburg, Germany, 276 pp.

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Otroshy, M. & P.C. Struik, 2006. Effects of temperature regime on microtuber production in different cultivars of potato. Potato Research. *Submitted.*

Otroshy, M. & P.C. Struik, 2006. Effects of two contrasting growth regulators on the quality of in vitro plantlets and microtubers in three cultivars of potato in a seed tuber production scheme. Potato Research. *Submitted.*

Otroshy, M. & P.C. Struik, 2006. Effects of different photoperiod and temperature combinations during in vitro potato plantlet production on the yield and quality of the progeny minitubers produced in the greenhouse. Potato Research. *Submitted.*

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Curriculum vitae

Mahmoud Otroshy was born in Shahreza (Iran) on 4th of August 1954. He obtained his BSc in Agronomy and Plant Breeding from the Tehran University in 1977. He ranked first among all graduated students in his field.

In 1979 Mahmoud joined the Ministry of Agriculture of Iran, where he was Director of Agriculture in Shahreza for 17 years, and then he was re-appointed as Manager of Agronomy in the Agricultural Organisation of the Isfahan Province. In 1992, Mahmoud was recognized as "Sample Expert" by the Ministry of Agriculture of Iran because of his valuable efforts in Agricultural Propagation and Production. This recognition showed the appreciation of the President of Iran, the Minister of Agriculture of Iran, the General Governor of Isfahan Province and the General Director of Isfahan Agricultural Organisation.

He completed his MSc studies in Agriculture (Agronomy) in 1996 from Azad University-Khorasgan Branch; he ranked first among all MSc students in the above field.

During his work in the Ministry of Agriculture of Iran Mahmoud attended many specialised training courses in various institutions and in different countries to upgrade his knowledge related to his responsibility and his job. These courses lasted from 1 month to 4 months or longer. He could take part in the 26th International Potato Course organized by the International Agricultural Centre (IAC) of Wageningen in The Netherlands. During this international course Professor Dr Paul C. Struik, chairman of the supervisory board of this international course, awarded him an admission letter to pursue a PhD study in the Department of Crop Science of Wageningen University and Research Centre.

Mahmoud started his PhD study at Wageningen University and Research Centre, with a research project titled: Utilization of Tissue Culture Techniques in a Seed Potato Tuber Production Scheme.

He actively attended various congresses and conferences in different countries such as Germany, the Netherlands, Morocco and Spain.

Mahmoud has been a member of different Committees including the Potato Committee. He is a member of several international agricultural associations, including the European Association for Potato Research (EAPR) and the Netherlands Society for Plant Biotechnology and Tissue Culture (NVPW). He finished his PhD study in 2006 and currently has a position in the Ministry of Agriculture of Iran.

Mahmoud is married and he is the father of two sons, Amin and Adib, 20 and 18years old.

His e-mail address is: Otroshy@yahoo.com.

PE&RC PhD Education Statement Form

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 22 credits (= 32 ECTS = 22 weeks of activities)

Review of Literature (4 credits)

- Utilization of Tissue Culture in a Seed Potato Tuber Production Scheme (2000)

Writing of Project Proposal (4 credits)

- Utilization of Tissue Culture in a Seed Potato Tuber Production Scheme (2000)

Post-Graduate Courses (1.6 credits)

- How to Manage Diversity in Living Systems (2001)
- Biological Diversity in Agricultural Landscape: the Role of Weed (2002)
- Integrated Crop Management and Biodiversity (2002)
- Virtual Plant Modeling (2003)

Deficiency, Refresh, Brush-up and General Courses (4 credits)

- Basic Statistics Course (2000)
- Techniques for Writing and Presenting a Scientific Paper (2001)
- Written English (2001)
- Scientific Writing (2003)

PhD Discussion Groups (4 credits)

- Crop and Weed Ecology (2000-2005)

PE&RC Annual Meetings, Seminars and Introduction Days (3.1 credits)

- Seminar about Ghost Forest, Global Warming and Mountain Pine Beetles (2001)
- Ecology of Oligotrophic Bacteria in Soil and Rhizosphere (2001)
- Trends and Challenges for Rural Transformation in a Globalised World (2001)
- Image Analysis in Agriculture and Environmental Studies (2001)
- C4 Grass Species and their Biochemical subtypes in the Okavango Delta (2001)
- Agriculture and Nature Day (2001)
- Aquatic Plants and Water Quality (2002)
- PE&RC annual meeting: Ethics in Science (2002)
- PE&RC annual meeting: Global Climate Change and Biodiversity (2003)
- PE&RC weekend (2003)
- Competition Models Outlook on Future (2003)
- Modeling the Spread of *Phytophtora infestans* in Potato Variety Mixture (2003)
- PE&RC annual meeting: Biological Disasters (2004)
- PE&RC annual meeting: The Truth of Science (2005)

International Symposia, Workshops and Conferences (6.2 credits)

- International Conference on Agricultural Science and Technology (2001)

- 15th Triennial Conference of European Association for Potato Research in Hamburg, Germany (2002)
- Mini symposium in WICC (2003)
- Potato Conference (2004)
- African Potato Association Congress in Morocco (2004)
- 16th Triennial Conference of European Association for Potato Research in Bilbao, Spain (2005)
- Potato 2005 Congress in Emmeloord, the Netherlands (2005)

Laboratory Training and Working Visits (4 credits)

- Plant Breeding Department. Tissue Culture (2000)
- Plant Breeding Department. Mediums and Growth Regulators (2000/2001)