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SELENIUM AND ITS EFFECTS ON GROWTH, YIELD AND TUBER QUALITY IN POTATO

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ABSTRACT

Selenium (Se) has been demonstrated to be an essential trace element for maintenance of animal and human health. Although it has not been confirmed to be an essential micronutrient in higher plants, there is increasing evidence that Se functions as an antioxidant in plants. Selenium has been shown to exert a positive effect on crop growth and stress tolerance at low concentrations. However, the specific physiological mechanisms that underlie the positive effects of Se in plants have not been clearly elucidated.

The aims of this study were to determine the Se concentration in potato (*Solanum tuberosum* L.) and the effects of Se on the accumulation of carbohydrates, growth and yield in potato plants. An additional aim was to study the impact of Se on the total glycoalkaloid concentration in immature potato tubers. The distribution of Se in different biochemical Se fractions and the effect of storage on the Se concentration were studied in Se-enriched tubers. Furthermore, the effect of Se on raw darkening and translocation of Se from seed tubers to the next tuber generation was investigated. Due to the established anti-ageing properties of Se, it was of interest to study if Se affects physiological age and growth vigour of seed tubers.

The Se concentrations in the upper leaves, roots, stolons and tubers of potato increased with increasing Se supplementation. The highest Se concentration was reached in young upper leaves, roots and stolons, indicating that added selenate was efficiently utilized and taken up at an early stage. During the growing period the Se concentration declined in the aerial parts, roots and stolons of potato plants whereas an intensive accumulation took place in immature and mature tubers. Selenium increased carbohydrate accumulation in the young upper leaves and in stolons, roots and tubers at maturity. This could not be explained by increased production of photoassimilates as net photosynthesis did not differ among Se treatments. The Se treated plants produced higher tuber yields than control plants, and at the highest Se concentration (0.3 mg kg^{-1}) lower numbers of larger tubers were harvested. Increased yield of Se treated plants suggested that Se may enhance the allocation of photoassimilates for tuber growth, acting as a strong sink for both Se and for carbohydrates. The positive impact of Se on the yield of potato plants could be related to its antioxidative effect in delaying senescence. The highest Se supplementation (0.9 mg kg^{-1}) slightly decreased the glycoalkaloid concentration of immature tubers. At this level the Se concentration in tubers was about 20 µg g^{-1} DW. The low Se applications (0.0035 and 0.1 mg kg^{-1}) diminished and retarded the degree of raw darkening in tubers stored for one and eight months, which can be attributed to the antioxidative properties of Se. The storage for 1 to 12 months did not affect the Se concentrations of tubers. In the Se enriched tubers Se was allocated to the organic Se fraction, indicating that it was incorporated into organic compounds in tubers. Elevated Se concentration in the next-generation tubers produced by the Se enriched seed tubers indicated

that Se could be translocated from the seed tubers to the progeny. In the seed tubers stored for 8 months, at high levels, Se had some positive effects on the growth vigour of sprouts, but Se had no consistent effect on the growth vigour of seed tubers at the optimal physiological age.

These results indicate that Se is a beneficial trace element in potato plants that exerts a positive effect on yield formation and improves the processing and storage quality of table potato tubers. These positive effects of Se are, however, dependent on the Se concentration and the age of the potato plant and tuber.

LIST OF ORIGINAL PUBLICATIONS

The thesis consists of the following journal articles, which are referred to by their Roman numerals in the text. The papers are reprinted with the permission of the publishers.

I **Turakainen, M., Hartikainen, H. and Seppänen, M. M.** 2004. Effects of selenium treatments on potato (*Solanum tuberosum* L.) growth and concentrations of soluble sugars and starch. Journal of Agricultural and Food Chemistry 52: 5378–5382.

II **Turakainen, M., Väänänen, T., Anttila, K., Ollilainen, VM., Hartikainen, H. and Seppänen, M.** 2004. Effects of selenate supplementation on glycoalkaloid content of potato (*Solanum tuberosum* L.). Journal of Agricultural and Food Chemistry 52: 7139–7143.

III **Turakainen, M., Hartikainen, H., Ekholm, P. and Seppänen, M.** 2006. Distribution of selenium in different biochemical fractions and raw darkening degree of potato (*Solanum tuberosum* L.) tubers supplemented with selenate. Journal of Agricultural and Food Chemistry 54: 8617–8622.

IV **Turakainen, M., Hartikainen, H., Sarjala, T. and Seppänen, M. M.** 2007. Physiological age and post-harvest physiology of selenium-enriched potato seed tubers. Manuscript.

LIST OF ABBREVIATIONS

cv	cultivar
dd	accumulated day-degrees
DW	dry weight
FW	fresh weight
GSH-Px	glutathione peroxidase
HPLC	high pressure liquid chromatography
MDA	malondialdehyde
PPO	polyphenol oxidase
Se	selenium
SOD	superoxide dismutase
SPE	solid-phase extraction
WAP	weeks after planting

1 INTRODUCTION

The element Se was discovered by Swedish chemists J. J. Berzelius and J. G. Gahan in 1817, who named it after the Greek moon goddess *Selene*. It is a metalloid: its chemical and physical properties lie between those of metals and non-metals. The major use of Se is in the electronics industry and its use is based on its photoelectric and semiconductor properties. It is used either as elemental Se or as cadmium selenide in electronics and photography especially in semiconductors, medical imaging equipment, photocopiers and solar cells. Thus, it finds applications that range from light sensors to xerography. In the glass industry, the addition of Se (sodium selenate and sodium selenite) to glass results in a beautiful ruby red colour. With iron, it can generate a green colour (Haygarth 1994 and references therein, Barceloux 1999 and references therein, Dhillon and Dhillon 2003).

Selenium is unevenly distributed in the earth's crust. Its concentration in soil varies markedly between geographic regions. The inorganic Se species most frequently found in soils are selenite and selenate (Rosenfeld and Beath 1964, McNeal and Balistrieri 1989, Barceloux 1999 and reference therein, Dhillon and Dhillon 2003). Interest in our study was focused on selenate, because multinutrient fertilizers in Finland are supplemented with selenate. Lower bioavailability of selenite in soils, and a higher supplementation requirement compared with that for selenate, has reduced interest in its use in fertilizer manufacture.

Selenium has been recognized as an essential trace element for humans and animals based on its presence in antioxidative defence systems (Schwartz and Foltz 1957, Flohe et al. 1973, Rotruck et al. 1973) and in hormone balance (Arthur et al. 1990, Pallud et al. 1997). Selenium is a complex trace element. Extremely low and extremely high Se concentrations are detrimental to human and animal health (Combs and Combs 1984, Combs 2001). In plants Se can be found both in inorganic and organic Se forms, including selenoamino acids and methylated compounds Current interest in Se is focused on the health benefits of high-Se plants as a source of cancer-preventative Se compounds (Finley et al. 2000, 2001), and the metabolism of Se in plant species that accumulate Se and are able to remediate Se-polluted soils and prevent Se from entering into the food chain (Berken et al. 2002).

Although Se is not classified as a micronutrient for higher plants, numerous studies have shown that at low concentrations, Se exerts a beneficial effect on growth and stress tolerance of plants by enhancing their antioxidative capacity (Hartikainen and Xue 1999, Xue and Hartikainen 2000, Xue et al. 2001, Djanaguiraman et al. 2005, Kong et al. 2005). Similarly, as in human and animal cells, Se increases plant resistance against oxidative stress caused by free oxygen radicals. However, agricultural crops are sensitive to high tissue Se concentrations, but

sensitivity varies among plant species (Hartikainen et al. 2000, Hartikainen et al. 2001, Rani et al. 2005, Lyons et al. 2005).

Plants play a unique role in recycling and delivering Se from the soil to the food chain. The concentration of Se in agricultural products and fodder depends on the content of Se in the soil and its bioavailability (Sippola 1979, Koivistoinen 1980, Yläranta 1983a, Yläranta 1985). Availability of Se is restricted in Finnish soils, and its content is relatively low as a result of reduced weathering status and acidity (Koljonen 1975, Sippola 1979, Yläranta 1983b). In humans this leads to low Se intake, increasing risk of cardiovascular disease, coronary heart disease and cancer (Salonen et al. 1982, 1984) and results in nutritional disorders in animals (Oksanen 1965). In order to increase the Se intake of the population and domestic livestock, Finnish fertilizers have been supplemented with sodium selenate since 1984. The aim of Se fertilization is to increase Se contents of spring cereals to a target level of 0.1 mg kg^{-1} dry matter (Yläranta 1985). From the beginning of 1998, the supplementation level of Se in Finnish fertilizers has been 10 mg kg^{-1}. Before introduction of Se fertilization the daily Se intake of the Finnish population was only about 25-30 µg per day (Varo and Koivistoinen 1980, Mutanen 1984). The average daily Se intake of the Finnish population is now approximately 80 µg (Eurola et al. 2003).

1.1 Potato

In 2005 potato was cultivated in Finland on 29 000 hectares (Forkful of Facts 2006). Potato is an import item in the Finnish diet, the consumption being 62 kg per year per capita (Forkful of Facts 2006). Potato is grown for food, animal feed and industry as well as for seed tuber production (Struik and Wiersema 1999). Potato tubers are a good source of energy, and contain high quality proteins, vitamin C and minerals including calcium and magnesium (Burton 1989). The development of potato plants can be divided into five different growth stages, which include sprout development, vegetative growth, tuber initiation/set, tuber bulking, and tuber maturation (Ewing and Struik 1992).

Potato is a vegetatively-propagated crop, producing harvestable tubers below ground. A potato tuber is a greatly shortened and swollen part of an underground stem (Ewing and Struik 1992, Vos 1999). During its life cycle, four different physiological tuber stages can be distinguished: dormancy, apical dominance, sprouting, and senescence (Ewing and Struik 1992, Struik and Wiersema 1999). Potato tubers have high water content and are sensitive to environmental impacts during their production and storage. Cultivation practices, growth conditions, crop management and storage conditions influence tuber quality (Burton 1989, Struik and Wiersema 1999). Similarly, the yield of a potato crop is affected by several factors, the most important of

which are growth conditions, cultivation practices, cultivar, and physiological quality of the seed tuber, i.e. its ability to produce a vigorous plant (Burton 1989, Struik and Wiersema 1999).

1.1.1. Quality parameters of table potato tubers

The maintenance of tuber quality during storage is important for potatoes destined for direct consumption, processing and propagation. There are two important classes of tuber quality: sensory and chemical (Storey and Davies 1992) or external and after-cooking quality (Kangas et al. 2006). The sensory quality characteristics include tuber size, physical characteristics such as shape and wounds, appearance, defects and health. The chemical quality includes the flesh texture, flavour, enzymatic and non-enzymatic darkening, glycoalkaloid content as well as nutritive value (Storey and Davies 1992, Kangas et al. 2006).

In Scandinavian countries, besides mature tubers, immature potatoes are harvested in early summer and consumed as young potatoes. The early harvested tubers contain high levels of soluble sugars, and the dry matter content is low. Thus, they are not considered to be chemically mature. Immature tubers are usually not stored for a long period, and thus storage does not create problems regarding quality. These potatoes have characteristic quality parameters, one being a generally high glycoalkaloid content (Hellenäs et al. 1995, Papathanasiou et al. 1998).

1.1.1.1 Starch

Starch is the major component of potato tubers, accounting for 75–80% of the tuber dry weight (Burton 1989). It is synthesized in plastids known as amyloplasts. Starch is composed of two types of glucose polymers: amylose and amylopectin. Amylose consists of linear chains of glucose monomers linked by α-1,4-glycosidic bonds, and in amylopectin the chains are branched and joined together by α-1,6-glycosidic bonds. Amylose and amylopectin form three-dimensional and semi-crystalline starch granules (Burton 1989, Davis et al. 2003).

Starch grains are small and of uniform size in young tubers. Towards tuber maturity the filling of starch into granules results in increased size and larger size distribution of granules (Burton 1989, Viola 2000). In growing potato tubers, starch concentration increases towards maturity, and thus, mature tubers have high starch and protein concentrations, but are low in sugar (Burton 1989). Therefore, the length of growing period of tubers has an important effect on the starch concentration of harvested tubers. Starch concentration increases from the pith towards the cortex, and it is lower at the stem-end than at the bud-end of the tuber. The concentration depends on cultivar, growth conditions and cultivation practices (Burton 1989). Increased

application levels of nitrogen, as well as phosphorus and potassium deficiency can lead to lower starch content in tubers (Burton 1989). In physiologically older tuber, the amyloplast membrane becomes more permeable due to oxidative damage and thus it is not able to protect starch against enzymatic degradation (Knowles and Knowles 1989). In additon, starch reserves in tubers are mobilized to support sprout growth, which in turn promotes further ageing of tubers (Kumar and Knowles 1993, 1996).

1.1.1.2 Glycoalkaloids

Glycoalkaloids are secondary plant metabolites that usually occur only in specialized, differentiated cells and are not required for cell metabolism or plant growth (Taiz and Zeiger 2002). These natural toxins are present in plants of the Solanaceae family, including potato, tomato (*Lycopersicon esculentum* Mill.) and eggplant (*Solanum melongena* L.) (Friedman and McDonald 1997). They are not translocated within plants (Valkonen et al. 1996 and references therein). Their natural function is probably to act as stress metabolites or mediate resistance to insects and plant pathogens (Maga 1994). They are not destroyed during heating, cooking, frying, baking or in a microwave oven (Friedman and McDonald 1997). At low concentrations they enhance flavour, but concentrations exceeding 150 mg kg^{-1} FW can result in a bitter taste (Friedman and McDonald 1997). An upper safety level in tubers of 200 mg total glycoalkaloids kg^{-1} FW is generally recommended.

The principal glycoalkaloids in cultivated potato are α–solanine and α–chaconine, which account for approximately 95% of the total glycoalkaloid content (Friedman and McDonald 1997). Their ratio is usually about 40:60 in potato, but it is not constant (Friedman and Dao 1992, Percival et al. 1997). α-Chaconine and α-solanine are triglycosylated products of the same aglycone, solanidine, but they have different sugar moieties. α-Chaconine is composed of solanidine-glucose-rhamnose-rhamnose and α-solanine of solanidine-galactose-glucose-rhamnose (Friedman and McDonald 1997). Glycoalkaloids are present in different concentrations in different parts of potato plants. Their level is usually low in cultivated potato tubers ranging on average from 30 to 100 mg kg^{-1} FW. In contrast, the high levels accumulate in leaves, stems, roots and sprouts (Friedman and Dao 1992, Sotelo and Serrano 2000, Friedman 2006). Glycoalkaloids are not evenly distributed within tubers. The highest concentration is found in the peel, decreasing towards the pith (Sotelo and Serrano 2000).

Glycoalkaloid biosynthesis in tubers is genetically controlled (Sanford and Sinden 1972). Therefore, the content of glycoalkaloids can vary greatly in different potato cultivars (Hellenäs et al. 1995). Glycoalkaloid levels are high in small tubers early in development, decreasing towards tuber maturation (Papathanasiou et al. 1998, 1999, Hellenäs et al. 1995). In addition, growing

and post-harvest conditions affect glycoalkaloid production level. Various stresses, including physical damage (i.e. bruising) (Dale et al. 1998), light exposure (i.e. greening) (Percival et al. 1996, Griffiths et al. 1998), a high growth temperature (Nitithamyong et al. 1999, Papathanasiou et al. 1999) and a high level of nitrogen fertilization (Mondy and Munshi 1990a) can elevate glycoalkaloid levels. However, greening and glycoalkaloid accumulation in tubers can occur independently of each other (Griffiths et al. 1998).

1.1.1.3 Enzymatic and non-enzymatic darkening

Enzymatic darkening in freshly cut tubers lowers product acceptability by consumers. However, the synthesis of the dark pigment, melanin, is thought to be a defence mechanism of the tuber tissue against entry of pathogens. Enzymatic darkening reactions in tubers result from enzymatic oxidation of phenolic compounds by polyphenol oxidase (PPO) to quinones, followed by non-enzymatic formation of melanin (Burton 1989, Martinez and Whitaker 1995, Friedman 1997). The cellular damage leads to release of amyloplast-located PPO, which then oxidizes monophenolic substrates such as tyrosine and chlorogenic acid. This series of reactions is oxygen-dependent and initially produces red-brown dihydroxy-phenol intermediates, followed by the formation of blue-black melanin pigments that can polymerize to form water-insoluble complexes (Burton 1989, Martinez and Whitaker 1995, Friedman 1997). The synthesis of melanin is cultivar-dependent and influenced by environmental factors such as climatic conditions, crop management and length of storage period (Burton 1989, Mondy and Munshi 1993a, b). The rate of darkening is mainly affected by the concentration of PPO and the oxygen available to the tissue. The degree of darkening is related to the concentration of phenolic compounds (Burton 1989, Friedman 1997). Cantos et al. (2002) showed that in fresh-cut potatoes, phenolic compounds and PPO activity were not rate-limiting.

Although a low storage temperature prevents tubers sprouting it may lead to a gradual breakdown of starch and accumulation of reducing sugar (glucose and fructose). Cottrell et al. (1993) showed that the reducing sugar content and activities of hydrolytic enzymes increased in tubers during the first weeks of storage at 4ºC. They also showed that sensitivity of starch degradation to storage temperature differs among cultivars. The phenomenon is termed cold-induced sweetening. The accumulation of reducing sugars is problematic for the potato processing industry, where high sugar levels cause unfavourable browning of chips and fries and dehydrated potato products (Burton 1989). In the Maillard reaction, the intensity of which is highly dependent on the reducing sugar content of processed tubers, the reducing sugars react with proteins during heating to produce dark colours (Burton 1989).

1.2.1 Quality parameters of seed tubers

The physiological age, shape, size and health of a seed tuber is an important determinant of its quality (Burton 1989, Struik and Wiersema 1999). 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 and Wiersema 1999). However, there is no practical indicator for rate of physiological ageing in seed tubers although it has been assessed using some physiological and chemical indicators (Allen and O'Brien 1986, Hartmans and van Loon 1987, van Ittersum et al. 1990, Struik and Wiersema 1999). Physiological age has been gauged using accumulated day-degrees from breakage of dormancy (Allen and O'Brien 1986), storage temperature sum (Struik and Wiersema 1999) and the physiological age index (PAI) (Caldiz et al. 2001). Moreover, the concentration of malondialdehyde (MDA), a lipid oxidation product, is used as a stress and an ageing indicator of plant membranes (Dhindsa et al. 1981, Du and Bramlage 1992, Kumar and Knowles 1993). This is related to increased concentration of reducing sugars during prolonged storage due to oxidation-induced damage of the amyloplast membrane (Knowles and Knowles 1989, Spychalla and Desborough 1990).

Polyamines occur as free polyamines in plant tissue, conjugated into small molecules or bound to macromolecules such as proteins (Galston and Sahwney 1990). Polyamines, spermidine and spermine and their diamine obligate precursor putrescine, are reported to be involved in plant growth, development and stress responses (Galston and Sawhney 1990, Bouchereau et al. 1999). *S*-adenosylmethionine decarboxylase (SAMDC) is a key enzyme in polyamine biosynthesis for biosynthesis of spermidine and spermine from putrescine (Pedros et al. 1999, Bouchereau et al. 1999). Furthermore, polyamine biosynthesis may interact with both ethylene biosynthesis, since they share the common precursor, S-adenosyl methionine (SAM) (Bouchereau et al. 1999). Polyamine biosynthesis has been shown to be linked to sprouting and tuberization. Mader (1995) reported that it is likely that the balance of free and bound polyamines is needed for optimal tuberization. Taylor et al. (1993) showed that during the initial tuberization stages, the activity of SAMDC and the levels of spermidine and spermine increased. Kaur-Sawhney et al. (1982) demonstrated that in the apical and lateral buds of dormant tubers the polyamine biosynthetic enzyme activity and polyamine concentration remained unchanged, whereas a large increase in both was observed with progressive sprouting.

1.3 Selenium

1.3.1 Selenium in humans and animals

Selenium has long been recognized as an essential trace element for animals (Schwarz and Foltz 1957) and humans (Flohe et al. 1973, Rotruck et al. 1973). Flohe et al. (1973) and Rotruck et al. (1973) established the biological function of Se as an integral component of GSH-Px, an important antioxidative enzyme that catalyzes the destruction of hydrogen peroxide generated during oxidative metabolism in human and animal cell. Since then, Se was identified as an essential component in human and animal cells of thioredoxin reductase (Tamura and Stadman 1996), Type I, II, III iodothyronine deiodinases (Arthur et al. 1990, Pallud et al. 1997) and for a number of selenoproteins (Gladyshev et al. 1998, Allan et al. 1999, Kryukov et al. 2003). More recently, the interest has focused on specific methylated forms of Se, such as Se-methylselenocysteine, which have been shown to provide chemoprotective effects against certain types of cancer in humans (Finley et al. 2000, 2001, Whanger 2002, Ellis and Salt 2003). In the 1930s Se was found to be an environmental toxin responsible for health problems in livestock grazing on soils with a high Se content (Brown and Shrift 1982 and references therein). At Se concentrations of 3–15 mg kg^{-1} DW in tissues, some plants can cause toxic symptoms in animals. The range between requirement and toxic doses of Se is narrow. The Se requirement for most farm animals is between 0.1 and 0.3 mg kg^{-1} of feed (Mayland 1994). The recommended daily intake of Se is for human adults 50–70 μg and for children 20–30 μg. The minimum Se dietary requirement is approximately 20 μg Se per day for adults (RDA, Recommended Dietary Allowances 1989). The Food and Nutrition Board has set the Tolerance Upper Intake Levels (UL) for Se at 400 μg per day for adults (Food and Nutrition Board and Institute of Medicine 2005). However, Se intakes greater than those set by RDA may be beneficial to human. Clark et al. (1996) demonstrated that dietary supplementation of 200 μg of Se per day significantly reduced lung, prostate, and colorectal cancer in humans. Chronic Se toxicity is caused by intakes of 2–4 mg per day or prolonged intakes of 1 mg per day. Chronic symptoms of excessive consumption of Se include morphological changes in fingernails, nail brittleness and loss of hair, as well as nausea, vomiting and skin lesions. Symptoms of Se deficiency include muscle pain, weakness, and loss of pigment in hair and skin, and whitening of nail beds (Subcommittee of Selenium, Committee on Animal Nutrition, Board of Agriculture National Research Council 1983, Haygarth 1994 and references therein).

1.3.2 Selenium addition of fertilizers in Finland

The results of a large study on mineral composition of Finnish foods during the 1970s showed that the Se content of plant and animal products in Finland was very low (Varo and Koivistoinen

1980, Varo et al. 1980). Moreover, Mutanen (1984) reported that the Se intake in the Finnish population was very low (20–30 mg per day). However, diseases directly related to low Se intake in humans were not detected. In contrast, insufficient Se intake in young domestic animals caused muscular dystrophy and disorders together with vitamin-E deficiency (Oksanen 1965, Bengtsson et al. 1978). To prevent Se deficiency in Finnish livestock, in 1969 mineral mixtures used in animal feed were supplemented with selenium, mainly as sodium selenite. In 1984, the resolution of the Ministry of Agriculture and Forestry initiated supplementation of multinutrient fertilizers with selenate to increase and ensure the adequate Se intake in the Finnish population. The original target was to raise the Se concentration of spring cereals to about 0.1 mg Se kg^{-1} dry matter. The selenium working group regularly monitored the effects of Se concentration in fertilizers on soils, basic foods, feeds, human serum and Se intake. Initially, the fertilizers were supplemented at two Se levels: for forage production at 6 mg and for cereal production at 16 mg Se per kg fertilizer. In 1990 the Se level was reduced to 6 mg Se kg^{-1}, to avoid any risk of too high a Se intake and environmental risk from additional Se. However, the reduced Se level in fertilizers resulted in decreased Se in food (Ekholm et al. 1997), and thus reduced intake of Se by humans and domestic animals. This came about through reduced application of fertilizers by farmers (Eurola and Hietaniemi 2000). For these reasons, in 1998 the Se level in all fertilizers was raised to 10 mg kg^{-1} (0.001%). The application of 500 kg Se-supplemented fertilizer ha^{-1} corresponds to 5 g Se ha^{-1}. The regulations governing organic farming do not allow Se to be added to organic fertilizers. As a result, in organically-cultivated cereals the Se concentration is low, 0.01 to 0.02 mg kg^{-1} DW (Eurola et al. 2005). Also, Se-supplemented fertilizers are not yet allowed in greenhouse cultivation.

In Finland, the Se concentration in wheat flour is now at its orginal target level (0.1 µg g^{-1} DW) (Table 1). In comparison with wheat flour, the Se concentration in potatoes is still at a low level in Finland (Table 1) and thus potatoes are considered to be a poor source of Se in the human diet.

Table 1. Se concentration (µg g^{-1} DW) of wheat flour and potato tubers before (1975/1977) and after the Se supplementation in fertilizers in Finland

	1975/1977[a]	1986/1987[b]	1990/1991[c]	1996[c]	2001[e]	2004[e]
			µg Se g^{-1} DW			
Wheat flour	0.02	0.16	0.23	0.12	0.12	0.11
Potato tubers	< 0.01	0.07	0.11	0.03	0.03	0.03

[a] Koivistoinen 1980, [b]Ekholm 1997, [b]Eurola et al. 1991, [c]Eurola et al. 2003, [e]Eurola et al. 2005.

1.3.3 Selenium in soil

Selenium content of most soils varies between 0.1 and 2.0 mg kg^{-1} depending on geographical area (Mayland 1994, Dhillon and Dhillon 2003). In some countries, such as New Zealand, Finland and some parts of China, the availability of Se in soils is naturally low. The Se concentration of soil depends on the composition of bedrock from which the soil component derives and the geochemical processes that produce the soil. In Finland, the mean Se concentration in most common rocks varies between 0.025 and 9.9 mg kg^{-1} (Koljonen 1975) bedrock is low and ranges between 0.025 and 0.10 mg kg^{-1} (Koljonen 1975, Yläranta 1983b). The total amount of Se in Finnish soils is not exceptionally low, but the light weathering processes of rock lead to the low concentration and availability of Se. The Se concentration in the Finnish agricultural mineral soils ranges from 0.04 to 0.7 mg kg^{-1} dry soil taken from the plough layer (Koljonen 1975, Yläranta 1983b). In clay soils, Se content was generally higher than in coarse mineral soils. The mean Se content in clay soils was 0.29 mg kg^{-1} and in coarse mineral soils 0.17 mg kg^{-1}. The highest Se concentration was measured from organic soils (0.46 mg kg^{-1}) (Koljonen 1975, Sippola 1979, Yläranta 1983b).

In the soil, Se may be present in four different oxidation states: selenate (+6), selenite (+4), elemental Se (0), and as inorganic and organic selenide (–2). The chemical form, the soil redox potential, pH and clay content determine the bioavailability of Se in the soil (Gissel-Nielsen 1971, Mikkelsen et al. 1989, McNeal and Balistrieri 1989). The predominant Se inorganic forms in cultivated soils are selenate and selenite. Selenate is more soluble and available for plants under oxidising and alkaline soil conditions (Mayland 1994, Masscheleyn et al. 1990). Selenite is less available to plants than selenate because it is absorbed more strongly by iron oxide surfaces and soil clays (Yläranta 1983a, Yläranta 1985, Mikkelsen et al. 1989). On the other hand, liming decreased the sorption of selenite by oxide surfaces and thus, increased the solubility in the soil and the uptake of Se (Gissel-Nielsen 1971, Yläranta 1983a, Mikkelsen et al. 1989, Garlston et al. 1991). Besides sulphate, interaction between Se and other nutrients such as phosphate decreases the availability of Se in soils and therefore decreases uptake and accumulation of Se (Rosenfeld and Beath 1964, Singh at al. 1980, Yläranta 1985, Hopper and Parker 1999). Because of the reduction and sorption reactions, Se must be applied in fertilizers every year and to each crop separately, in order to maintain a sufficient Se concentration in agricultural products. Utilization of fertilizer Se by plants is usually 5–20%; the rest is absorbed by soil in an insoluble form that is not available to plants (Sippola 1979, Yläranta 1983a, Yläranta 1985). However, there is concern that Se accumulates in soils due to use of Se-supplemented fertilizers. However, studies demonstrated that after the onset of use of Se fertilizers, the Se concentration in Finnish agricultural soils has not increased (Yläranta 1990, Mäkelä-Kurtto and Sippola 2002, Eurola et al. 2003).

1.3.4 Selenium in plants

The Se concentration in plants depends on the chemical form of Se, its concentration and bioavailability in soils and the accumulation capacity of the plant. In higher plants metabolism of Se is closely related to that of sulphur due to their chemical similarity. The non-specific incorporation of the selenoamino acids (selenomethionine and selenocysteine) into proteins is thought to be the major cause of Se toxicity in non-accumulator plants supplied with a high Se dose (Brown and Shrift 1981, 1982). Lyons et al. (2005) suggested that one explanation for higher toxicity of selenite compared to selenate is that after uptake selenite is incorporated faster than selenate into selenoamino acids in roots. High Se concentrations were shown to provoke oxidative stress responses such as increased lipid peroxidation in plants (Hartikainen et al. 2000). The ability to accumulate and tolerate high Se levels is related to differences in Se metabolisms between accumulator and non-accumulator plant species. Se accumulators and some secondary accumulators limit the integration of selenoamino acids into proteins by converting Se into soluble non-protein selenoamino acids like Se-methylselenocysteine, γ-glutamyl-Se-methylselenocysteine, and selenocystathionine (Brown and Schift 1981, Terry et al. 2000, Whanger 2002). The Se-methylselenocysteine is the most predominant selenoamino acid in the Se-accumulators such as garlic (*Allium sativum* L.), onion (*Allium cepa* L.), broccoli (*Brassica oleracea* L.) and wild leek (*Allium tricoccum* L.) (Neuhriel et al. 1999, Whanger 2002). Selenium has been shown to act as a cancer-preventing agent (Clark et al. 1996, Whanger 2002, Ellis and Salt 2003) and Se-methylselenocysteine in particular has been shown to have chemoprotective effects against cancer **(**Finley et al. 2000, 2001). In non-accumulator plants, selenomethione has been found to be the main Se species in seeds of cereals (Stadlober et al. 2001) and in seed and leaves of pea (*Pisum sativum* L.) plants (Smrkolj et al. 2006).

1.3.4.1 Selenium uptake, assimilation and volatilization

The uptake of selenate into roots and its distribution in plants is much faster than that of selenite (Shrift and Ulrich 1969, Asher et al. 1977, Arvy 1993, de Souza et al. 1998, Pilon-Smits et al. 1998, Cartes et al. 2005). De Souza et al. (1998) reported that total Se accumulation in a plant was about 10-fold higher from selenate than from selenite. It was proposed that selenate, chemically analogous to the sulphate ion, is actively transported into roots via sulphate transporters and subsequently quickly transported into shoots (Asher et al. 1977, Arvy 1993, Terry et al. 2000). The mechanism of selenite uptake is not fully understood, but it is assumed to be passive (Asher et al. 1977, Arvy 1993, Zhang et al. 2003, Terry et al. 2000, White et al. 2004). In addition, plants can actively take up organic forms of Se such as selenomethionine (Zayed et al. 1998, Terry et al. 2000). The transport of Se from roots to shoots is thought to occur via xylem (Asher et al. 1977, Arvy 1993).

Higher plants have differing capacities to accumulate and tolerate Se. They are classified into non-accumulators, indicators and accumulators (Rosenberg and Beath 1964, Terry et al. 2000, Dhillon and Dhillon 2003, White et al. 2004). Some particular plant species are termed Se hyperaccumulators. The largest group of hyperaccumulators belongs to the genus *Astragalus* and *Stanleya* (Rosenfeld and Beath 1964, Neuhierl et al. 1999, Terry et al. 2000 and reference therein). The Se hyperaccumulators are placed into two groups: the primary Se accumulators are able to accumulate thousands of milligrams of Se kg^{-1} (> 4000 mg kg^{-1}), and the secondary accumulators hundreds of milligrams Se kg^{-1}. Brassicaceae species including Indian mustard (*Brassica juncea* L.), broccoli (*Brassica oleracea botrytis* L.) and canola (*Brassica napus* spp. *oleifera* L.) have been classified as primary accumulators. Plant species with a high Se capacity to accumulate and tolerate Se could be used in the phytoremediation of Se-contaminated sites (Terry et al. 2000, Berken et al. 2002). However, most cultivated crop plants have a low tolerance of high Se levels. Generally, they contain less than 25 µg Se g^{-1} DW and are considered to be non-accumulators. Therefore, potato is classified as a Se non-accumulator (White et al. 2004). Although non-accumulators are sensitive to high Se concentration, they can tolerate as well as accumulate even high concentrations of Se without growth reduction when grown in Se-enriched soils (Rani et al. 2005). The critical Se concentration in plant tissues, which decreased the yield in Indian mustard was 105 µg g^{-1} DW, in maize (*Zea mays* L.) 77 µg g^{-1} DW, in rice (*Oryza sativa* L.) 42 µg g^{-1} DW and in wheat 19 µg g^{-1} DW, a levels attained by Se addition as selenite of 5 µg g^{-1} soil for Indian mustard and maize, 4 µg g^{-1} soil for wheat and 10 µg g^{-1} soil for rice (Rani et al. 2005).

Se is evidently metabolized via the same enzymes used in sulphur assimilation and can be incorporated into amino acids in the place of sulphur (Brown and Shrift 1981, Anderson and Scarf 1983, Pilon-Smits et al. 1999, Terry et al. 2000). *In vitro* studies of Burnell (1981) suggested that ATP sulfurylase is the first enzyme in the reduction of selenate and sulphate in plants. ATP sulfurylase is thought to be primarily localized in plastids, but a minor cytosolic form was detected by Leustek et al. (2000). The reduction of selenate to organic Se is thought to occur in chloroplasts (de Souza et al. 1998, Pilon-Smits et al. 1998, 1999). Roots were unable to reduce selenate when shoots were removed (Pilon-Smits et al. (1999). This result indicates that organic Se forms are probably transported into roots in the phloem. The reduction of selenate by ATP sulfurylase is a rate-limiting step in higher plants (de Souza et al. 1998), whereas selenite can be relatively quickly incorporated into selenoamino acids (Shrift and Ulrich 1969, Arvy 1993, de Souza et al. 1998, Pilon-Smits et al. 1999, Zhang et al. 2003). More evidence of Se assimilation is provided by transgenic phytoremediator plants overexpressing enzymes of sulphur metabolism (de Souza et al. 1998, Pilon-Smits et al. 1999). An overexpression of plastidic ATP sulfurylase in a selenate-supplied Indian mustard (*Brassica juncea*) led to 2–3-fold higher shoot Se concentration and enhanced Se tolerance. Moreover, results of de Souza et al.

(1998) indicated that when wild-type Indian mustard plants were supplied with selenate, they accumulated mainly selenate, whereas transgenic ATP sulfurylase plants accumulated an organic Se form. The proposed non-enzymatic and enzymatic pathway of Se in higher plants is shown in Figure 1.

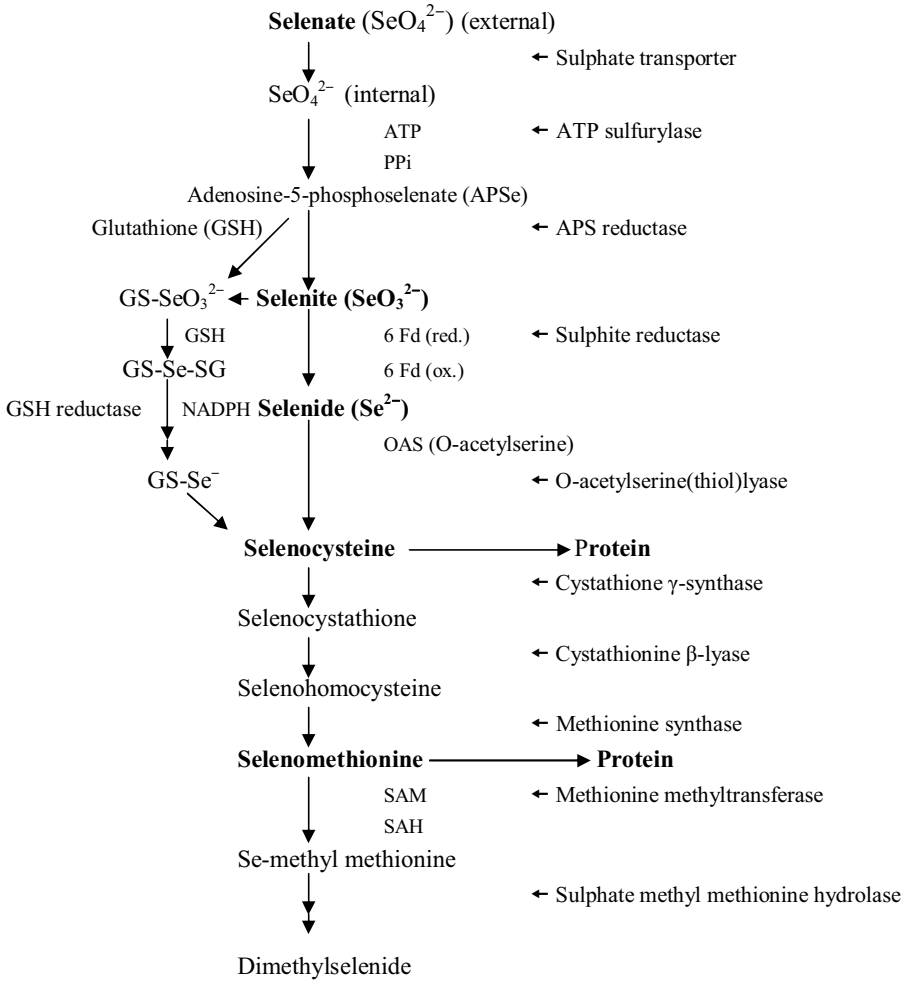

Figure 1. Simplified scheme summarizing a proposed Se assimilation and volatilization pathway in non-accumulator plants (modified from Terry et al. 2000 and Berken et al. 2002). Selenate is taken up via sulphate transporters and assimilated by enzymes of the sulphur assimilation pathway or through non-enzymatic reduction. Selenate is activated by ATP sulfurylase and reduced to selenite and further to selenide, which can be incorporated into selenocysteine and then converted to selenomethionine. Selenomethionine is methylated to methylseleno methionine, which is converted to volatile dimethylselenide. S-adenosyl methionine (SAM), S-adenosyl homocysteine (SAH).

Inorganic Se compounds can be transformed into volatile compounds such as dimethylselenide and dimethyldiselenide through plants (Terry et al. 1992, de Souza et al. 1998). The Se assimilation and volatilization processes in accumulator plants have been studied extensively for phytoremediation applications (de Souza et al. 1998, Pilon-Smith et al. 1999, Berken et al. 2002). Lewis et al. (1974) demonstrated that the rate of Se volatilization from cabbage (*Brassica oleracea* L.) leaf homogenates decreased with a decreasing temperature. Based on these results, it can be concluded that low storage temperature prevents loss of Se through volatilization from various plant parts. The rate of Se volatilization differs among plant species. Sugar beet (*Beta vulgaris* L.), bean (*Phaseolus vulgaris* L.) and lettuce (*Lactuca sativa* L.) have low Se volatilization, whereas carrot (*Daucus carota* L.), barley (*Hordeum vulgare* L.), alfalfa (*Medicago sativa* L.), tomato and cucumber (*Cucumis sativus* L.) are intermediate. Rice, broccoli and cabbage have been shown to accumulate Se very efficiently and thus, volatilize Se at the highest rates (Terry et al. 1992, Zayed et al. 1998). However, there have been no studies on the volatilization rate of Se in potato. Previous studies indicated that selenate is less readily available for volatilization due to its lower metabolism in plants than the more reduced Se forms selenite and selenomethionine (de Souza et al. 1998, Pilon-Smits et al. 1998, Zayed et al. 1998). The inorganic and organic Se in soils can be transformed into volatile forms by microorganisms (Frankenberger and Karlson 1994).

1.3.4.2 Selenium effects on plants

Whether Se is essential in higher plants is still a controversial issue. However, there are indications that Se might be an essential micronutrient for accumulator plants species such as *Astragalus* (Trelease and Trelease 1938). In addition, it was shown that Se is required for a growth of a green alga (*Chlamydomonas reinhardtii*) from which Se-dependent GSH-Px was identified (Yokota et al. 1988, Fu et al. 2002). In contrast, Se non-accumulator plants, including most crop species, do not appear to require Se for their growth (Terry et al. 2000). Broyer et al. (1966) found that applied selenite had no beneficial effect on the growth of the non-accumulator plants alfalfa (*Medicago sativa* L.) and subterranean clover (*Trifolium subterraneum* L.). Probably the first positive effect of Se on plant growth was reported by Singh et al. (1980), who showed that the application of 0.5 mg kg^{-1} Se as selenite stimulated growth and dry-matter yield of Indian mustard (*Brassica juncea* L.). More recently, it was revealed that Se, applied at low concentrations, enhanced growth and antioxidative capacity of both mono- and dicotyledonous plants. The growth-promoting response to Se was demonstrated in lettuce and ryegrass (*Lolium perenne* L.) (Hartikainen et al. 1997, Hartikainen and Xue 1999) and in soybean (*Glycine max* L.) (Djanaguiraman et al. 2005). Pennanen et al. (2002) also observed that Se induced starch accumulation in chloroplasts of young leaves. Addition of Se at low concentrations alleviated the oxidative stress caused by UV-irradiation in lettuce and ryegrass (Hartikainen and Xue 1999,

Hartikainen et al. 2000) and in strawberry (*Fragaria* × *ananassa* Duch.) (Valkama et al. 2003). Furthermore, at an optimal level Se was able to increase the antioxidative capacity of senescing plants and delay senescence in lettuce, ryegrass (Xue et al. 2001) and soybean (Djanaguiraman et al. 2005), to improved the recovery of potato plant from light and chilling stress (Seppänen et al. 2003), and to enhance salt-resistance in sorrel (*Rumex patientia* X *R. tianshanicus*) seedlings (Kong et al. 2005). In combination with UV-B, Se even promoted growth of lettuce subjected to short UV-B episodes (Hartikainen and Xue 1997, Hartikainen and Xue 1999). Authors suggested that Se might improve the utilization of short-wavelength light by plants. Furthermore, Pennanen et al. (2002) reported that in addition to Se increasing the growth of plants, it was also able to delay the death of plants subjected to severe UV-stress. Several studies have shown that a protective role of Se against the oxidative stress in higher plants coincided with enhanced GSH-Px activity and decreased lipid peroxidation (Hartikainen and Xue and Hartikainen 2000, Djanaguiraman et al. 2005, Djanaguiraman et al. 2005, Cartes et al. 2005). Cartes et al. (2005) demonstrated that selenite was more efficient than selenate as an inductor of GSH-Px activity in ryegrass plants. In addition, Se affected the activity of catalase, superoxide dismutase and glutathione S-transferase (Xue and Hartikainen 2000). In potato, Moreover, Xue et al. (2001) and Pennanen et al. (2002) showed that Se retarded the decline of tocopherols, especially the more biologically active form, α-tocopherol. In addition, Se was demonstrated to have an effect in leaf mesophyll and roots tip cells by affecting membrane integrity of chloroplasts and mitochondria (Kong et al. 2005).

Moreover, Se supplementation (applied as selenite) was shown to decrease the glycoalkaloid and the nitrate concentration of mature potato tubers (Mondy and Munshi 1990b, Munshi and Mondy 1992) while it increased the total and protein amino acid contents (Munshi et al. 1990). Se has also had a demonstrated effect on germination. Carvalho et al. (2003) reported that at higher supplementation level than 29 mg kg^{-1} soil, Se inhibited the growth and germination of tomato, lettuce and radish (*Raphanus sativus* L.) seeds. In contrast, priming of seeds with selenite promoted germination of bitter gourd (*Momordica charantia* L.) seeds at sub-optimal temperatures (Chen and Sung 2001). The positive effect on germination was linked to antioxidative activity manifested as increased GSH-Px and ascorbate-glutathione cycle activity.

There are indications that Se might affect biosynthesis of glycoalkaloids (Munshi and Mondy 1992), chlorophylls (Padmaja et al. 1989) as well as nitrogen assimilation (Aslam et al. 1990). Konze et al. (1978) showed that selenomethionine enhanced ethylene biosynthesis in plants; it was a better substrate than methionine for SAMDC. S-adenosylmethionine is required in the synthesis of selenomethionine as well as in polyamine biosynthesis (Terry et al. 2000, Martin-Tanguy 2001).

In Se hyperaccumulator plant species, a high Se accumulation capacity was proposed as an evolutionary protection mechanism against insect feeding. Hanson et al. (2004) showed that Se protected Indian mustard plants against feeding by green peach aphids (*Myzus persicae* Sulzer). In addition, Se protected Indian mustard plants from white cabbage caterpillars (*Pieris rapae* L.). (Hanson et al. 2003). Moreover, Se-enriched Indian mustard plants were more tolerant than controls to a root and stem fungal pathogen (*Fusarium* sp.) and a leaf pathogen (*Alternaria brassicicola* [Schweitnitz] Wiltshire) infection (Hanson et al. 2003).

1.4 AIMS OF THE PRESENT STUDY

The question of whether Se is an essential micronutrient for higher plants remains unanswered. However, it is worth questioning if it has beneficial effects in potato. In the present study, we carried out systematic experiments in attempt to answer this question. The aim of the study was to analyse Se concentration and its effects on the accumulation of carbohydrates, growth and yield in potato plants (I). An additional aim was to study the effect of Se on the total glycoalkaloid concentration in immature potato tubers (II). The changes in different biochemical fractions and Se concentration were studied in Se-enriched tubers. These included the effect of Se on the degree of raw darkening. Translocation of Se from seed tubers to progeny was also investigated (III). According to the anti-ageing properties of Se, it was of interest to study if Se affects the rate of physiological ageing and post-harvest physiology of seed tubers (IV).

The main working hypotheses tested were:

1) Selenium supplementation increases Se concentration, carbohydrate accumulation and growth as well as total yield of potato plants (I).
2) Selenium supplementation decreases the glycoalkaloid concentration of immature tubers (II).
3) Selenium supplementation affects potato processing quality, possible changes in Se concentration and chemical form during storage and translocation from seed tubers to the next-generation tubers (III).
4) Selenium is able to retard the ageing-induced changes in seed potato tubers through its antioxidative functions which have consequent effects on growth vigour (IV).

2 MATERIAL AND METHODS

The experimental part of the work is described here as a general outline. It is presented more thoroughly in the original publications (I–IV).

2.1 Plant material and experimental designs (I–IV)

In the greenhouse experiments certificated seed potato tubers of two Finnish potato cultivars were used: Satu (I-IV) and Sini (II). Plants were grown in quartz sand in all experiments (I-III) except those in paper IV, where the plants were grown in peat. Tubers were harvested 10 (II), 15 (II), and 16 (III-IV) weeks after planting and stored at 4°C, at 75% relative humidity prior to analyses or transfer to sprouting conditions. Experiments carried out for each paper are detailed in Table 2.

Table 2. Plant parts, selenium treatments, and measurements presented in the original papers.

Paper no.	Exp. no	Plant part	Se added mg kg^{-1} soil	Growing, storage and sprouting time	Measurements n=3-10
I	1	Upper leaves, stolons, roots	0, 0.075 and 0.3	4 and 8 WAP[1)] 4, 8, 13 and 15 WAP	Se concentration, soluble sugar and starch concentration
	1	Tubers		15 WAP	yield, tuber weight and number per plant
	1	Leaves		4 and 8 WAP	photosynthesis, dry weight
	1	Roots, stolons		4, 8, 13 and 15 WAP	dry weight
II	1	Tubers	0, 0.01 and 0.075	immature tubers, 10 WAP	glycoalkaloid and Se concentration
	2	Tubers	0, 0.0035, 0.01, 0.075 and 0.9	immature tubers 10 WAP	glycoalkaloid and Se concentration
III	1	Tubers	0, 0.0035, 0.01, 0.075 and 0.9	1-, 3-, 6- and 12-month storage	Se concentration
	1	Tubers		1-, 6- and 12-month storage	Distribution of Se in different biochemical fractions
	2 3	Tubers Tubers		2- and 8-month storage 2-, 3- and 8-month storage	raw darkening, n=20 translocation of Se to the next-generation tubers

Continues on the next page

Paper no.	Exp. no	Plant part	Se added mg kg^{-1} soil	Growing, storage and sprouting time	Measurements
IV	1	Tubers	0, 0.0035, 0.01, 0.075 and 0.9	3-, 6- and 8-month storage, sprouting 88 days 6- and 8-month storage sprouting 88 days	MDA concentration starch concentration
	1	Spouts		8-month storage, sprouting 88 days	concentration of polyamines
	1	Sprouts		2-, 3-, 4-, 6- and 8-month storage, sprouting 80 days	sprouting capacity, number of sprouts < 1 cm and ≥1 cm $tuber^{-1}$
	2	Tubers, stems		2-, 3-, 4-, 6- and 8-month storage, sprouting 60 days, harvested 16 WAP	main stems number $tuber^{-1}$, yield, tuber weight and tuber number $plant^{-1}$, tuber number $main\ stem^{-1}$

[1)] WAP, weeks after planting

2.2 Methodology

2.2.1 Sample preparation (I-IV)

For chemical analysis, leaves, roots, stolons and chopped tubers were immediately immersed in liquid nitrogen and stored at -20°C until lyophilization. After lyophilization, leaf, root and tuber samples were weighed and ground into a fine powder using an electric mill (1093 Cyclotec Sample Mill, Foss Tecator, Högnäs, Sweden). Stolons were ground with IKA A10 (Janke & Kunkel GmbH & Co., Staufen, Germany). The fresh sprouts were ground in liquid nitrogen using a mortar and pestle.

2.2.2 Selenium analysis (I-IV)

Selenium in plant samples was analysed by an electrothermal atomic absorption spectrometry method (Kumpulainen et al. 1983, Ekholm 1997). Briefly the lyophilised samples were digested in an acid mixture HNO_3, $HClO_4$ and H_2SO_4. Se (+6) was reduced to Se (+4) by 4 M HCl, chelated with ammonium pyrrolidine dithiocarbamate, and extracted into methyl isobutyl ketone. Se concentration was determined by atomic absorption spectrophotometer (Perkin Elmer Model 5100) at 196.1 nm equipped with HGA-600 graphite furnace. Three in-house reference samples were included in each sample batch to check the accuracy of the analytical method.

2.2.3 Potato growth and yield parameters (I, IV)

The dry biomass of upper leaves, roots and stolons was determined. Fresh yield, weight and number of tubers per plant were recorded. Only tubers weighing ≥ 2 g were included.

2.2.4 Photosynthesis (I)

Photosynthesis was measured from the youngest uppermost leaflets 4 and 8 WAP using an open gas-exchange system (Li-6400; LI-COR Inc., Lincoln, NE). Measuring conditions were 500 μmol photons m^{-2} s^{-1} at 24°C. A CO_2 reference target was set at 400 μmol mol^{-1}. Flow rate in the chamber was set at 500 μmol s^{-1}.

2.2.5 Quality parameters of potato tubers

2.2.5.1 Carbohydrate analyses (I, IV)

Soluble sugars were extracted in deionised water and determined using the Anthrone method (Yemm and Willis 1954). Absorbance of solutions was measured at 630 nm with spectrophotometer (Shimadzu UV-160A, Shimadzu Co., Kyoto, Japan). The starch concentration was determined enzymatically using a method described by Volenec et al. (1991) and modified by Palonen (1999). Soluble sugars were extracted from a sample three times with deionised water followed by centrifugation. The sugar-free residue was resuspended in acetate buffer following enzymatic hydrolysis of amyloglucosidase. Trinder reagent was used to quantify the glucose in the supernatant. Absorbance of sample solutions was measured at 505 nm with a spectrophotometer (Shimadzu UV-160A, Shimadzu Co., Kyoto, Japan).

2.2.5.2 Determination of glycoalkaloids (II)

For potato tuber glycoalkaloid analysis, α-solanine and α-chaconine were determined using reverse-phase high pressure liquid chromatography (RP-HPLC) according to Kuronen et al. (1999). The lyophilized sample was extracted twice with 5% (v/v) acetic acid. For purification, the solid-phase extraction (SPE) method with Sep-Pak C_{18} (500 mg) SPE cartridges (Waters Corp.) was used. Glycoalkaloids were eluted from cartridges with methanol. The analysis was carried out in a HPLC (Agilent Technologies 1100A) apparatus equipped with an autoinjector and a diode-array detector at 208 nm controlled by Chemstation software. The elution with

acetonitrile-triethylammonium phosphate (TEAP) buffer followed a stepwise gradient. The total glycoalkaloid concentration was calculated as the sum of α-solanine and α-chaconine.

2.2.5.3 Separation of biochemical selenium fractions (III)

The distribution of added Se in various fractions (free amino acids, residue, inorganic and soluble protein fraction) was assessed by modifiying the methods of Lazarus (1973) and Gissel-Nielsen (1987) described in Hartikainen et al. (1997). In brief, 5 g of dry tuber sample was extracted twice with 50 mL and once with 25 mL of deionised water followed by centrifugation. The insoluble residue was dried for 2 days at 70ºC. Soluble proteins in the supernatant were precipitated with 30 mL of 30% (v/v) trichloroacetic acid. After centrifugation, the precipitate containing soluble proteins was dried for 2 days at 70°C. A moist ion-exchange resin (Dowex 50W-8, Sigma-Aldrich Chemies GmbH, Steinheim, Germany) column was used to separate inorganic and free amino acid fractions in the supernatant. The inorganic Se fraction was eluted from the column with 1 M HCl and deionised water. The free amino acids fraction containing Se was eluted with 0.2 M NaOH. The Se concentration of each fraction was determined as described before. The relative distribution of Se between different fractions in the tubers was expressed as a percentage (%) of the total Se.

2.2.5.4 Evaluation of raw darkening (III)

Sensory analyses of potato tubers were carried out using the standard method of the Potato Research Station, Lammi, Finland: twenty tubers per Se treatment were split longitudinally in half. Three panellists evaluated the degree of the total discoloured area on the surface of each tuber 30 and 60 min after splitting. The darkening was rated on a scale of 9–1, with 9 standing for no darkening (0%), 7 for less than 10%, 5 for less than 30%, 3 for less than 70%, and 1 for unacceptable discolouration (100%).

2.2.5.5 Determination of growth vigour of seed tubers (IV)

The depth on endodormancy was assessed by transferring seed tubers to sprouting conditions (14ºC, light 30 μmol m^{-2} s^{-1}, 70% relative humidity). The tubers were considered as being in a state of dormancy unless sprout growth of 2 mm was observed after 2 weeks in the sprouting conditions. Sprouting capacity of seed tubers was estimated by calculating developed sprouts as a percentage of total sprout number per tuber (%), and by calculating the number of developed sprouts. Sprouts were classified in two categories according to their length, < 1 cm and ≥ 1 cm.

In greenhouse experiments, the impact of Se enrichment on the early growth vigour of seed tubers was estimated by calculating days from planting to shoot emergence, from planting to beginning of flowering, and by assessing the main stem number and yield parameters: tuber yield, tuber weight and number per plant.

Free putrescine, spermidine and spermine in frozen sprout samples were analysed by using a modified procedure based on that described by Smith and Davies (1985) and Sarjala and Kaunisto (1993). Polyamines were separated using HPLC (Merck Hitachi Model, Japan) as described by Sarjala and Kaunisto (1993).

Lipid peroxidation in potato tubers during storage and sprouting was determined by measuring the amount of malondialdehyde (MDA) formation using the thiobarbituric acid (TBA) method described by Dhindsa et al. (1981) and Kumar and Knowles (1993). The absorbance of the sample was measured at 532, 600 and 440 nm with a spectrophotometer (Shimadzu UV-160A, Shimadzu Co., Kyoto, Japan) in order to correct the interferences of carbohydrates.

2.3 Statistics

The statistical analyses were carried out using SAS System for Windows, versions V6.12 and V8.2 (SAS Institute Inc. Cary, NC, USA). Data were tested using analysis of variance in the GLM procedure. Significantly different mean values for Se treatments were separated with Duncan's Multiple-range test. Differences at $P \leq 0.05$ were considered to be statistically significant (I-IV). In addition, differences in the Se concentrations between two storage times (1 month and 12 months) at various Se treatments were tested by the procedure using PROC NPAR1WAY and the Wilcoxon signed rank-test (SAS Institute Inc., Cary, NC, USA).

3 RESULTS AND DISCUSSION

3.1 Selenium concentration in potato plants (I–IV)

During the course of this study, Se concentration in all analyzed plant parts, i.e. upper leaves, roots and stolons (I) as well as in potato tubers (II–III) increased in proportion to the level of Se added, indicating that the added selenate was efficiently taken up by plants. In all experiments, the highest Se concentration was measured in young plants (4 WAP), especially in the upper leaves (90 μg g^{-1} DW) (I). The Se concentration was high also in the roots (33 μg g^{-1} DW) and in the stolons (40 μg g^{-1} DW) of the young plants (I). However, plants growing in soil or peat may have a lower Se content than in the present study due to sorption of Se in soil. In our study, the

efficient translocation of selenate to various potato plant parts indicates that only a fraction of selenate in a plant is incorporated as organic Se compounds that are not as easily transported (de Souza et al. 1998, Zayed et al. 1998). The results of the present study agree with those of previous studies showing that selenate is easily translocated from roots to shoots and is more mobile in plants and more slowly assimilated in the plant than selenite (de Souza et al. 1998, Hopper and Parker 1999, Pilon-Smith et al. 1999, Terry et al. 2000). In the case of potato plants, an intensive accumulation of Se in the tubers indicates that the target or sink for Se could be the growing tubers. Kahakachchi et al. (2004) and Smrkolj et al. (2006) reported that actively growing plant parts such as young leaves and seeds accumulated the largest amounts of Se.

In the present study, during the growing period, the Se concentration declined in the aerial parts, roots and stolons (I). This may also be attributable to an increase in shoot biomass, reduced Se content in soil or translocation of Se into the developing tubers. Xue et al. (2001) reported that a decrease in the Se concentration of lettuce leaves was due to a dilution effect caused by an increase in the biomass. The lower Se concentration in the roots at 8 WAP demonstrated that the amount of available Se had decreased. In comparison with the upper leaves, roots and stolons, the Se concentration of tubers did not decline during the growing period, indicating that translocation of Se continues throughout tuber growth and thus potato tubers can accumulate high levels of Se (I-IV). Our results showed that the Se concentration in immature and mature tubers was almost the same although the total yield harvested from mature plants was 2.5-times higher than from immature plants (I-III). This shows that the Se concentration in the mature tubers was not affected by dilution due to biomass accumulation.

3.2 Response of growth, yield and carbohydrates to selenium application (I, II)

Se addition at 0.075 and 0.3 mg kg^{-1} did not promote the growth of potato shoots, roots or stolons as assessed by the dry matter production (I). Selenate did, however, increase shoot and root biomass production in lettuce (Xue et al. 2001, Simojoki et al. 2003) and ryegrass fertilized with 0.1 mg Se kg^{-1} (Hartikainen et al. 2000, 2001). It also increased the shoot dry matter production of soybean sprayed with 50 mg Se L^{-1} (Djanaguiraman et al. 2005).

Selenium applications of 0.01 and 0.075 mg kg^{-1} had no effect on the yield of immature tubers of cultivars Satu or Sini (II). However, the highest yields of cv Satu were harvested from the mature plants treated with Se at 0.075 and 0.3 mg kg^{-1} (II). The final number of tubers was reduced, but tuber size was increased in the Se-supplemented plants. This suggests that Se may alter the allocation pattern of assimilates. At an optimal level Se appears to promote tuber growth; potato tubers and rapidly expanding leaves in lettuce and ryegrass leaves are strong sinks for carbohydrates (Xue et al. 2001, Hartikainen et al. 2000, Pennanen et al. 2002). Similarly as in

other plant species, the impact of Se on tuber yield of potato plants could be attributable to its antioxidative effects. According to results from previous studies, increased growth of Se-treated plants was associated with a decrease in lipid peroxidation that coincided with increased activity of GSH-Px (Hartikainen et al. 2000, Xue et al. 2001, Djanaguiraman et al. 2005) and with halting decline in tocopherol concentration (Xue et al. 2001). Additionally, a growth-promoting effect of Se was recorded for senescing plants (Xue et al. 2001, Djanaguiraman et al. 2005). Similarly, in the present study it can be suggested that Se led to retarded senescence of potato plants as demonstrated by elevated carbohydrate concentration in aged roots and stolons.

The starch concentration in the upper leaves of young plants (4 WAP) increased with increasing Se application levels. Also, the concentration of soluble sugars at the Se application rate of 0.3 mg Se kg^{-1} was elevated (I). We hypothesized that the increased starch accumulation in the upper leaves of the Se-treated plants resulted from enhanced starch synthesis, reduced sucrose translocation from the chloroplasts, and enhanced photosynthetic efficiency. The increased starch accumulation in leaves cannot be explained by the increase in net photosynthesis because photosynthesis did not differ between the Se-supplemented and control plants. It also appears that accumulation of photoassimilates in leaves was not due to reduced sucrose export from leaves because it was transported to below-ground plants parts. Pennanen et al. (2002) reported that Se enhanced production and accumulation of starch in granules in young lettuce plants. In Se-treated plants the build-up of energy reserves in leaves became evident as increased shoot yields. The authors concluded that Se-induced increase in growth was related to the inducible role of Se in chloroplast enzymes and carbohydrate metabolism. Similarly, Mazzafera (1998) showed that in coffee plants (*Coffea arabica* L.) selenite applied to the soil caused increased soluble sugar concentration in the first pair of leaves. Similarly, foliar application lead to increase in soluble sugar concentration in the beans. However, the mechanism through which Se acts on carbohydrate metabolism of plants is not fully understood. In the present study, the Se applications appeared to elevate the concentration of carbohydrates in roots and stolons of mature plants (15 WAP) (I), suggesting that Se maintained the metabolic activity in roots and stolons or that Se delayed their senescence. In contrast to roots, stolons continued to accumulate soluble sugars and starch up to maturity. Elevated starch concentration in aged stolons may mean that Se was able to retard their senescence.

As for young leaves, higher starch concentration was recorded in the tubers grown at a Se fertilization level of 0.075 mg kg^{-1} than in the control (I). The increased starch accumulation in the Se-supplemented tubers compared with control ones could be explained either by increased assimilate production in leaves or the stronger sink demand. It can be suggested that Se may also delay senescence of potato plants, thereby significantly affecting starch accumulation in tubers.

3.3 Impact of selenium on the quality of table potato tubers

3.3.1 Glycoalkaloid concentration in immature tubers (II)

The total glycoalkaloid concentration ± standard error in control immature tubers of cv Sini was higher than that of cv Satu; 228 ± 10 mg kg^{-1} FW and 105 ± 9 mg kg^{-1} FW, respectively (II). In the tubers of Sini the total glycoalkaloid concentration exceeded the recommended upper safe level of 200 mg kg^{-1} FW. A high glycoalkaloid level was also a characteristic feature of mature Sini tubers (approximately 149 mg kg^{-1} FW) (Pietilä 2000), whereas Satu had a reduced tendency to accumulate glycoalkaloids (approximately 50 mg kg^{-1} FW) (Blomberg and Penttilä 2000). Significant differences in glycoalkaloid concentrations among commercial potato cultivars have been well documented (Griffiths et al. 1998, Hellenäs et al. 1995, Sotelo and Serraro 2000). Glycoalkaloid concentrations of young tubers have been shown to be high (Papathanasiou et al. 1999, Hellenäs et al. 1995, Griffiths et al. 1998). In the present study, the Se applications (0.01 and 0.075 mg kg^{-1}) had no statistically significant effect on the total glycoalkaloid concentration in tubers of Sini. However, they appeared to raise the total glycoalkaloid concentration in tubers of Satu in the first experiment, although a similar effect of Se was not observed in the second experiment. A high temperature period during greenhouse cultivation (28 ºC for 3 to 4 weeks) probably increased the total glycoalkaloid concentration in the tubers of Satu in the first experiment.

In the second experiment, the total glycoalkaloid concentration in immature tubers of control plants of cv Satu was 92 mg kg^{-1} FW (II). The highest Se application of 0.9 mg Se kg^{-1} decreased glycoalkaloids to 75 mg kg^{-1} FW, but the decrease was not statistically significant. At this level of Se application, the Se concentration of tubers was 20 µg g^{-1} DW. The upper safe daily intake level of 400 µg for human dietary would be exceeded if 100 g of these fresh potatoes are consumed (total 500 µg). In other studies, Mondy and Munshi (1990b) showed that selenite application decreased glycoalkaloids in mature tubers significantly from about 125 mg kg^{-1} FW to 100 mg kg^{-1} FW in the cortex, and in the pith from about 10 mg kg^{-1} FW to 9 mg kg^{-1} FW. In their study, Se concentration of tubers did not exceed 2.8 µg Se g^{-1} DW, although the Se application was as high as 0.7–3.4 mg Se kg^{-1}. Munshi and Mondy (1990b) proposed that added selenite leads to decreased activities of enzymes involved in the biosynthesis of glycoalkaloids due to the incorporation of selenoamino acids into proteins and thereby changing their activity. According to results from previous studies, it is apparent that selenite can be more active in plant metabolism than selenate because selenite is more readily and rapidly incorporated into plant proteins, despite the lower uptake rate (de Souza et al. 1998, Zayed et al. 1998). Selenate is assimilated more slowly into organic Se forms because it must be first reduced to selenite by ATP sulfurylase, an enzyme shown to be a rate-limiting factor in selenate assimilation (de Souza et al. 1998, Pilon-Smits et al. 1999). Moreover, Cartes et al. (2005) demonstrated that in ryegrass

plants treated with selenate or selenite the effect of Se on the GSH-Px activity was related to the chemical form of applied Se rather than to the shoot Se concentration. According to our results and the literature, the effect of Se on the glycoalkaloid concentration in potato tubers depends on the chemical form of added Se and on the stage of tuber maturation.

In the present study, the ratio of α-solanine to α-chaconine varied in the tubers of cv Satu between the first and second experiment. The elevated temperature of 28 ºC for 3 to 4 weeks during greenhouse cultivation could explain the difference in the ratio of α-solanine to α-chaconine in tubers.

3.3.2 Selenium stability during storage (III)

It is possible that Se is volatilized or metabolized during storage of potato tubers. Our results showed, however, that Se concentration in the Se-enriched tubers remained constant during storage for 1 to 12 months, indicating that maintenance of Se in tubers is possible at low temperature (III). Evidently, the low biochemical activity in tubers prevented losses due to the volatile form of Se. Previous results by Lewis et al. (1974) support this assumption, demonstrating that a high temperature stimulated the volatilization of Se from cabbage leaf homogenates. There is no experimental evidence to suggest that volatilization of Se occurs in potato tubers. The stability of Se concentration during storage means that the nutritive value of Se-enriched potato tubers, as a Se source in human diet, can be maintained under proper storage conditions.

3.3.3 Selenium in biochemical fractions (III)

In this study, Se fractionation results showed that most of the added Se (approximately 66 %) was allocated in tubers into the organic fraction (soluble proteins, residue and amino acids) (III). However, the allocation of Se into organic fraction was lower than reported by Hartikainen et al. (1997) in leaves of lettuce (82%) and ryegrass (78%) supplied with selenate. Cartes et al. (2006) found that in the shoots of selenite-supplied ryegrass, over 73% of Se was found in the organic fraction. The lower organic-bound proportion of Se in potato tubers than in leaves of other species in previous studies might be attributable to lower protein content in tubers than in leaves. In contrast, there is no previous evidence of Se being converted into an organic Se form in tubers. In the present study, for the Se treatment of 0.9 mg kg^{-1}, Se declined in the inorganic and free amino acid fractions, which provided evidence that the conversion of Se to organic form occurs in tubers (III). On the other hand, selenate reduction in tubers may be dependent on the metabolites supplied by the shoot. The translocation of selenoamino acids from shoots to tubers

is one possibility. Stadlober et al. (2001) showed that in cereal grains between 70% and 83% of the Se was found in the form of selenomethionine, demonstrating that a metabolic pathway for Se assimilation exists in seeds. Furthermore, our results showed that the high addition Se levels (0.075 and 0.9 mg kg^{-1}) lead to decline in the proportion of Se in the free amino acid and inorganic fractions, suggesting that some biochemical processes continued in the tubers during storage despite of low temperature. The current study is the first one to report that in Se-enriched potato tubers Se was converted into organic compounds. Our results indicated that the Se enrichment improved the nutritive quality of potato by increasing the amount of organic Se in tubers.

3.3.4 Raw darkening of potato tubers (III)

Raw darkening of peeled potatoes decreases their processing quality and the acceptability by consumers. Darkening is an enzymatic reaction caused by the oxidation of phenolic compounds catalyzed by PPO following cell injury (Friedman 1997, Stevens and Davelaar 1997, Cantos et al. 2002). Therefore, raw darkening of fresh-cut potatoes has been prevented by using antibrowning agents (inhibitors), such as sulphites, citric acid and ascorbic acid (Friedman 1997). In this study, Se application lead to diminished raw darkening: in the tubers stored for 1 month the positive effect was recorded at 0.01 mg Se kg^{-1} and in those stored for 8 months at 0.0035 and 0.01 mg Se kg^{-1} (III). This beneficial effect of Se can be ascribed to its antioxidative function, delaying the oxidation reaction in the enzymatic discolouration process. It is well documented that at low concentrations Se acts as an antioxidant (Hartikainen and Xue 1997, Xue and Hartikainen 2000, Xue et al. 2000, Seppänen et al. 2003, Kong et al. 2005). On the other hand, it is suggested that a low Se concentration can directly affect the activity of PPO in tubers. This assumption is supported by the recent results of Nowak et al. (2004), demonstrating that the selenite at 0.45 mmol Se kg^{-1} caused a slight decrease in the activity of PPO. Alternatively, Se may reduce the degree of darkening through decreasing the activity of PPO by lowering the availability of the substrates tyrosine and chlorogenic acid. On the other hand, Cantos et al. (2002) found no correlation between the rate and degree of darkening, PPO and the chlorogenic acid content. In our study, for all Se treatments, prolonged storage (8 months) resulted in a decline in the degree of raw darkening of tubers. This is in disagreement with the findings of Mondy and Munshi (1993b), who showed that darkening of tubers increased during storage.

3.4. Impact of selenium on the growth vigour of seed tubers (III-IV)

3.4.1 Selenium translocation from selenium-enriched seed tubers (III)

The next-generation tubers produced by the selenate-enriched seed tubers exhibited elevated Se concentration, providing evidence that Se can be, at least partly, translocated from the seed tuber to the next generation: the higher Se concentration was in the seed tuber, the higher it got in the next-generation tubers (III). Moreover, at the highest supplementation level (0.9 mg Se kg^{-1}) the translocation of Se from the 8-month stored seed tubers was less efficient than from the tubers stored for 1 month. This could result from a decrease in the easily mobilised inorganic Se recorded in the seed tubers when the storage was prolonged from 1 to 12 months. Another possible explanation is the dilution of Se due to higher tuber biomass of the progeny crop produced by the seed tubers of advanced age. The seed tubers stored for 8 months produced 42% higher yield than those stored for 1 month due to the release from dormancy and their increased physiological age. This is in accordance with the results of de Souza et al. (1998) who showed that in selenite-supplied plants, Se was incorporated into organic Se compounds that accumulated in roots, and only a small proportion of Se was translocated to shoots.

3.4.2 Effect of selenium on the physiological age of seed tubers (IV)

In the present study, the Se treatments did not affect deep of dormancy of tubers stored for 2 months (IV). Dormancy was lost in the tubers stored for 3 months. The number of long sprouts ($\geq$ 1 cm) per tuber and their sprouting capacity increased for all Se treatments, indicating increased loss in apical dominance during prolonged storage time from 2 to 8 months. The sprouting capacity reached the peak value (100%) at Se levels of 0.075 and 0.9 mg kg^{-1}. The Se level of 0.9 mg kg^{-1} also significantly increased free putrescine concentration in the sprout of the seed tubers stored for 8 months, indicating that Se may have promoted synthesis. Therefore, the high free concentration of polyamines can be considered as growth vigour indicator of sprouts. However, the better growth vigour of sprouts was not consistent with the growth vigour of the seed tubers and the next yield produced.

An increased accumulation of lipid peroxidation products such as malondialdehyde (MDA) in the seed tubers has considered a relevant ageing indicator of tubers stored for long periods (Kumar and Knowles 1993, 1996). In the present study, although MDA increased during storage and after sprouting, the Se enrichment in the seed tubers had no consistent effect on the accumulation of MDA. Only a slight increase in the level of MDA was recorded during storage indicating that the rate of ageing of the seed tubers in the optimum age was low and thus, indicating that the tubers were physiologically young. Evidence provided by Kumar and

Knowles (1993b) showed that lipid peroxidation and thus MDA content in tuber tissue can be used to analyze of physiological ageing of tubers when they have past their optimum age.

In the present study, starch concentration remained and no consistent effect of Se on the starch concentration was recorded during storage and after sprouting. However, in the sprouted tubers starch concentration was slightly diminished. Even though this provides evidence that some starch was degraded, the contribution of this process to sprouts growth was not seen. Irrespective of the level of Se added, soluble sugars decreased during storage for 3 to 8 months storage, which agrees with the results of Kumar and Knowles (1993).

In the present study, the tuber yield doubled when storage time of seed tubers increased from 2 to 6 months. Although Se did not affect the next progeny yields, our results showed that a storage period of 6 months was optimal in terms of growth vigour. However, the accumulated day-degrees during greenhouse cultivation were approximately 100 dd higher for the tubers stored for 6 months than for the other storage periods. Seed tubers stored for 8 months lost some of their growth potential, which was reflected as lower yields and smaller tuber sizes. However, Se enrichments of seed tubers did not result in decreases in the yields.

In contrast to earlier results (Xue et al. 2001, Djanaguiraman et al. 2005) showing an effective antioxidative role of Se in delaying the senescence of lettuce and soybean leaves, no similar antioxidative effects of Se could not yet be found in potato seed tubers. It should be stressed that to analyze the effects of Se on age-induced decline in the growth vigour and accelerated ageing, the physiologically older tubers should be used than those investigated in the present study. Furthermore, potato tubers cultivated under greenhouse conditions are maybe physiologically younger than those grown in the fields.

In conclusion the better growth vigour of sprouts was not consistent with the growth vigour of the seed tubers. No significant effect of Se on the early growth and yield parameters was observed. Irrespective the Se treatment, the highest yield was harvested from plants produced with seed tubers stored for 6 months. This study further demonstrated that high Se levels had some positive effects on the growth vigour of sprouts, but it had no consistent effect on the growth vigour of seed tubers of optimal physiological age. In order to unravel the effects of Se on growth vigour related to ageing processes in potato seed tubers further studies with physiologically older tubers than those investigated in the present study are needed.

4 CONCLUDING REMARKS

The results of this study demonstrated that Se, added as selenate, is taken up by potato plants at an early stage. The high Se accumulation capacity of immature tubers must be taken into account when young tubers are to be consumed.

This study provided further evidence on the positive effects of Se on carbohydrate accumulation and yield formation in crops. In young potato plants Se promoted starch accumulation in the upper leaves and later in mature roots and stolons, as well as in tubers. Therefore, further studies are needed to investigate the regulating role of Se in carbohydrate metabolism in plants. The increased tuber yields of Se-treated potato plants suggested that Se can alter the allocation pattern of assimilates. It appears that at an appropriate level Se promotes the growth of plant parts that are strong sink for carbohydrates and Se, such as growing tubers in potato and rapidly expanding leaves. Similarly as for other plant species, the positive impact of Se on the yield of potato plants could be related to its antioxidative effect in delaying senescence.

This study also demonstrated that the Se enrichment improved both processing and nutritional quality of stored potato tubers. The improved nutritional value of potato tubers obtained following Se fertilization can be maintained under optimal storage conditions. In terms of human nutrition, Se fertilization may improve the nutritive value of potato tubers by increasing the content of organic Se compounds in tubers. A high rate of Se application (0.9 mg kg^{-1}) resulted in a decrease in total concentration of glycoalkaloids in immature tubers. At this level the Se concentration in tubers was about 20 μg g^{-1} DW. The upper safe daily intake level of 400 μg for human dietary would be exceeded if 100 g of these fresh potatoes are consumed (total 500 μg).

In this study the elevated Se concentration of the next-generation tubers provided evidence that Se can be, at least partly, translocated from the seed tuber to the next generation. In the seed tubers stored for 8 months, the high Se levels had some positive effects on the growth vigour of sprouts during ageing, but Se had no consistent impact the growth vigour of seed tubers at the optimal physiological age. In order to unravel the effects of Se on the growth vigour related to ageing processes in potato seed tubers, further studies with tubers of increased physiological age than those investigated in this study are needed.

These results indicate that at an appropriate level Se is a beneficial trace element in potato plants. The positive effects of Se are dependent on Se concentration and the age of the potato plants and tubers.

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